Chittaranjan Kole *Editor*

Wild Crop Relatives: Genomic and Breeding Resources

Oilseeds

Springer

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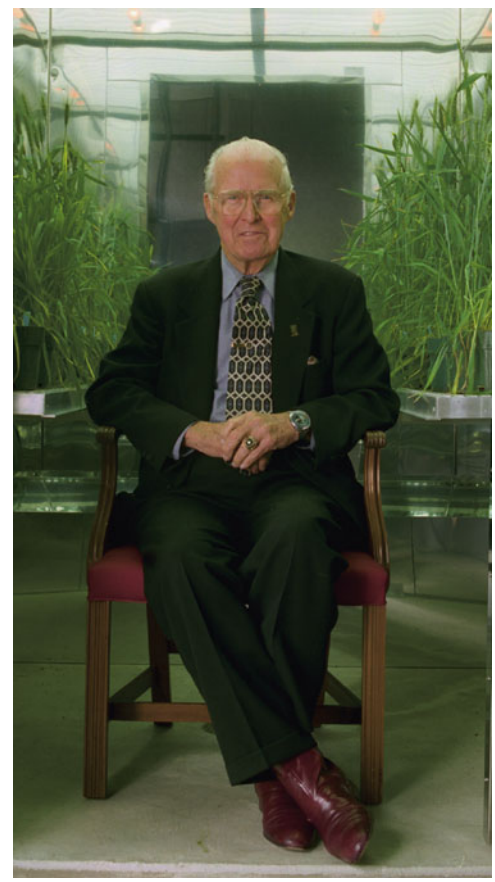
Dedication



Dr. Norman Ernest Borlaug,[1] the Father of Green Revolution, is well respected for his contributions to science and society. There was or is not and never will be a single person on this Earth whose single-handed service to science could save millions of people from death due to starvation over a period of over four decades like Dr. Borlaug's. Even the Nobel Peace Prize he received in 1970 does not do such a great and noble person as Dr. Borlaug justice. His life and contributions are well known and will remain in the pages of history of science. I wish here only to share some facets of this elegant and ideal personality I had been blessed to observe during my personal interactions with him.

It was early 2007 while I was at the Clemson University as a visiting scientist one of my lab colleagues told me that "somebody wants to talk to you; he appears to be an old man". I took the telephone receiver casually and said hello. The response from the other side was – "I am Norman Borlaug; am I talking to Chitta?" Even a million words would be insufficient to define and depict the exact feelings and thrills I experienced at that moment!

[1]The photo of Dr. Borlaug was kindly provided by Julie Borlaug (Norman Borlaug Institute for International Agriculture, Texas A&M Agriculture) the granddaughter of Dr. Borlaug.

I had seen Dr. Borlaug only once, way back in 1983, when he came to New Delhi, India to deliver the Coromandal Lecture organized by Prof. M.S. Swaminathan on the occasion of the 15th International Genetic Congress. However, my real interaction with him began in 2004 when I had been formulating a 7-volume book series entitled *Genome Mapping and Molecular Breeding in Plants*. Initially, I was neither confident of my ability as a series/book editor nor of the quality of the contents of the book volumes. I sent an email to Dr. Borlaug attaching the table of contents and the tentative outline of the chapters along with manuscripts of only a few sample chapters, including one authored by me and others, to learn about his views as a source of inspiration (or caution!) I was almost sure that a person of his stature would have no time and purpose to get back to a small science worker like me. To my utter (and pleasant) surprise I received an email from him that read: "May all Ph.D.'s, future scientists, and students that are devoted to agriculture get an inspiration as it refers to your work or future work from the pages of this important book. My wholehearted wishes for a success on your important job". I got a shot in my arm (and in mind for sure)! Rest is a pleasant experience – the seven volumes were published by Springer in 2006 and 2007, and were welcome and liked by students, scientists and their societies, libraries, and industries. As a token of my humble regards and gratitude, I sent Dr. Borlaug the Volume I on *Cereals and Millets* that was published in 2006. And here started my discovery of the simplest person on Earth who solved the most complex and critical problem of people on it – hunger and death.

Just one month after receiving the volume, Dr. Borlaug called me one day and said, "Chitta, you know I cannot read a lot now-a-days, but I have gone through only on the chapters on wheat, maize and rice. Please excuse me. Other chapters of this and other volumes of the series will be equally excellent, I believe". He was highly excited to know that many other Nobel Laureates including Profs. Arthur Kornberg, Werner Arber, Phillip Sharp, Günter Blobel, and Lee Hartwell also expressed generous comments regarding the utility and impact of the book series on science and the academic society. While we were discussing many other textbooks and review book series that I was editing at that time, again in my night hours for the benefit of students, scientists, and industries, he became emotional and said to me, "Chitta, forget about your original contributions to basic and applied sciences, you deserved Nobel Prize for Peace like me for providing academic foods to millions of starving students and scientists over the world particularly in the developing countries. I will recommend your name for the World Food Prize, but it will not do enough justice to the sacrifice you are doing for science and society in your sleepless nights over so many years. Take some rest Chitta and give time to Phullara, Sourav and Devleena" (he was so particular to ask about my wife and our kids during most of our conversations). I felt honored but really very ashamed as I am aware of my almost insignificant contribution in comparison to his monumental contribution and thousands of scientists over the world are doing at least hundred-times better jobs than me as scientist or author/editor of books! So, I was unable to utter any words for a couple of minutes but realized later that he must been too affectionate to me and his huge affection is the best award for a small science worker as me!

In another occasion he wanted some documents from me. I told him that I will send them as attachments in emails. Immediately he shouted and told me: "You know, Julie (his granddaughter) is not at home now and I cannot check email myself. Julie does this for me. I can type myself in type writer but I am not good in computer. You know what, I have a xerox machine and it receives fax also. Send me

the documents by fax". Here was the ever-present child in him. Julie emailed me later to send the documents as attachment to her as the 'xerox machine' of Dr. Borlaug ran out of ink!

Another occasion is when I was talking with him in a low voice, and he immediately chided me: "You know that I cannot hear well now-a-days; I don't know where Julie has kept the hearing apparatus, can't you speak louder?" Here was the fatherly figure who was eager to hear each of my words!

I still shed tears when I remember during one of our telephone conversations he asked: "You know I have never seen you, can you come to Dallas in the near future by chance?" I remember we were going through a financial paucity at that time and I could not make a visit to Dallas (Texas) to see him, though it would have been a great honor.

In late 2007, whenever I tried to talk to Dr. Borlaug, he used to beckon Julie to bring the telephone to him, and in course of time Julie used to keep alive all the communications between us when he slowly succumbed to his health problems.

The remaining volumes of the *Genome Mapping and Molecular Breeding in Plants* series were published in 2007, and I sent him all the seven volumes. I wished to learn about his views. During this period he could not speak and write well. Julie prepared a letter based on his words to her that read: "Dear Chitta, I have reviewed the seven volumes of the series on *Genome Mapping and Molecular Breeding in Plants*, which you have authored. You have brought together genetic linkage maps based on molecular markers for the most important crop species that will be a valuable guide and tool to further molecular crop improvements. Congratulations for a job well done".

During one of our conversations in mid-2007, he asked me what other book projects I was planning for Ph.D. students and scientists (who had always been his all-time beloved folks). I told him that the wealth of wild species already utilized and to be utilized for genetic analysis and improvement of domesticated crop species have not been deliberated in any book project. He was very excited and told me to take up the book project as soon as possible. But during that period I had a huge commitment to editing a number of book volumes and could not start the series he was so interested about.

His sudden demise in September 2009 kept me so morose for a number of months that I could not even communicate my personal loss to Julie. But in the meantime, I formulated a 10-volume series on *Wild Crop Relatives: Genomic and Breeding Resources* for Springer. And whom else to dedicate this series to other than Dr. Borlaug!

I wrote to Julie for her formal permission and she immediately wrote me: "Chitta, Thank you for contacting me and yes I think my grandfather would be honored with the dedication of the series. I remember him talking of you and this undertaking quite often. Congratulations on all that you have accomplished!" This helped me a lot as I could at least feel consoled that I could do a job he wanted me to do and I will always remain grateful to Julie for this help and also for taking care of Dr. Borlaug, not only as his granddaughter but also as the representative of millions of poor people from around the world and hundreds of plant and agricultural scientists who try to follow his philosophy and worship him as a father figure.

It is another sad experience of growing older in life that we walk alone and miss the affectionate shadows, inspirations, encouragements, and blessings from the fatherly figures in our professional and personal lives. How I wish I could treat my next generations in the same way as personalities like Mother Teresa and Dr. Norman Borlaug and many other great people from around the world treated me!

During most of our conversations he used to emphasize on the immediate impact of research on the society and its people. A couple of times he even told me that my works on molecular genetics and biotechnology, particularly of 1980s and 1990s, have high fundamental importance, but I should also do some works that will benefit people immediately. This advice elicited a change in my thoughts and workplans and since then I have been devotedly endeavoring to develop crop varieties enriched with phytomedicines and nutraceuticals. Borlaug influenced both my personal and professional life, particularly my approach to science, and I dedicate this series to him in remembrance of his great contribution to science and society and for all his personal affection, love and blessings for me.

I emailed the above draft of the dedication page to Julie for her views and I wish to complete my humble dedication with great satisfaction with the words of Julie who served as the living ladder for me to reach and stay closer to such as great human being as Dr. Borlaug and express my deep regards and gratitude to her. Julie's email read: "Chitta, Thank you for sending me the draft dedication page. I really enjoyed reading it and I think you captured my grandfather's spirit wonderfully. . .. So thank you very much for your beautiful words. I know he would be and is honored."

Clemson, USA Chittaranjan Kole

Preface

Wild crop relatives have been playing enormously important roles both in the depiction of plant genomes and the genetic improvement of their cultivated counterparts. They have contributed immensely to resolving several fundamental questions, particularly those related to the origin, evolution, phylogenetic relationship, cytological status and inheritance of genes of an array of crop plants; provided several desirable donor genes for the genetic improvement of their domesticated counterparts; and facilitated the innovation of many novel concepts and technologies while working on them directly or while using their resources. More recently, they have even been used for the verification of their potential threats of gene flow from genetically modified plants and invasive habits. Above all, some of them are contributing enormously as model plant species to the elucidation and amelioration of the genomes of crop plant species.

As a matter of fact, as a student, a teacher, and a humble science worker I was, still am and surely will remain fascinated by the wild allies of crop plants for their invaluable wealth for genetics, genomics and breeding in crop plants and as such share a deep concern for their conservation and comprehensive characterization for future utilization. It is by now a well established fact that wild crop relatives deserve serious attention for domestication, especially for the utilization of their phytomedicines and nutraceuticals, bioenergy production, soil reclamation, and the phytoremediation of ecology and environment. While these vastly positive impacts of wild crop relatives on the development and deployment of new varieties for various purposes in the major crop plants of the world agriculture, along with a few negative potential concerns, are envisaged the need for reference books with comprehensive deliberations on the wild relatives of all the major field and plantation crops and fruit and forest trees is indeed imperative. This was the driving force behind the inception and publication of this series.

Unlike the previous six book projects I have edited alone or with co-editors, this time it was very difficult to formulate uniform outlines for the chapters of this book series for several obvious reasons. Firstly, the status of the crop relatives is highly diverse. Some of them are completely wild, some are sporadically cultivated and some are at the initial stage of domestication for specific breeding objectives recently deemed essential. Secondly, the status of their conservation varies widely: some have been conserved, characterized and utilized; some have been eroded completely except for their presence in their center(s) of origin; some are at-risk or endangered due to genetic erosion, and some of them have yet to be explored. The third constraint is the variation in their relative worth, e.g. as academic model, breeding resource, and/or potential as "new crops."

The most perplexing problem for me was to assign the chapters each on a particular genus to different volumes dedicated to crop relatives of diverse crops grouped based on their utility. This can be exemplified with *Arabidopsis*, which has primarily benefited the Brassicaceae crops but also facilitated genetic analyses and improvement in crop plants in other distant families; or with many wild relatives of forage crops that paved the way for the genetic analyses and breeding of some major cereal and millet crops. The same is true for wild crop relatives such as *Medicago truncatula*, which has paved the way for in-depth research on two crop groups of diverse use: oilseed and pulse crops belonging to the Fabaceae family. The list is too long to enumerate. I had no other choice but to compromise and assign the genera of crop relatives in a volume on the crop group to which they are taxonomically the closest and to which they have relatively greater contributions. For example, I placed the chapter on genus *Arabidopsis* in the volume on oilseeds, which deals with the wild relatives of Brassicaceae crops amongst others.

However, we have tried to include deliberations pertinent to the individual genera of the wild crop relatives to which the chapters are devoted. Descriptions of the geographical locations of origin and genetic diversity, geographical distribution, karyotype and genome size, morphology, etc. have been included for most of them. Their current utility status – whether recognized as model species, weeds, invasive species or potentially cultivable taxa – is also delineated. The academic, agricultural, medicinal, ecological, environmental and industrial potential of both the cultivated and/or wild allied taxa are discussed.

The conservation of wild crop relatives is a much discussed yet equally neglected issue albeit the in situ and ex situ conservations of some luckier species were initiated earlier or are being initiated now. We have included discussions on what has happened and what is happening with regard to the conservation of the crop relatives, thanks to the national and international endeavors, in most of the chapters and also included what should happen for the wild relatives of the so-called new, minor, orphan or future crops.

The botanical origin, evolutionary pathway and phylogenetic relationship of crop plants have always attracted the attention of plant scientists. For these studies morphological attributes, cytological features and biochemical parameters were used individually or in combinations at different periods based on the availability of the required tools and techniques. Access to different molecular markers based on nuclear and especially cytoplasmic DNAs that emerged after 1980 refined the strategies required for precise and unequivocal conclusions regarding these aspects. Illustrations of these classical and recent tools have been included in the chapters.

Positioning genes and defining gene functions required in many cases different cytogenetic stocks, including substitution lines, addition lines, haploids, monoploids and aneuploids, particularly in polyploid crops. These aspects have been dealt in the relevant chapters. Employment of colchiploidy, fluorescent or genomic in situ hybridization and Southern hybridization have reinforced the theoretical and applied studies on these stocks. Chapters on relevant genera/species include details on these cytogenetic stocks.

Wild crop relatives, particularly wild allied species and subspecies, have been used since the birth of genetics in the twentieth century in several instances such as studies of inheritance, linkage, function, transmission and evolution of genes. They have been frequently used in genetic studies since the advent of molecular markers. Their involvement in molecular mapping has facilitated the development of mapping

populations with optimum polymorphism to construct saturated maps and also illuminating the organization, reorganization and functional aspects of genes and genomes. Many phenomena such as genomic duplication, genome reorganization, self-incompatibility, segregation distortion, transgressive segregation and defining genes and their phenotypes have in many cases been made possible due to the utilization of wild species or subspecies. Most of the chapters contain detailed elucidations on these aspects.

The richness of crop relatives with biotic and abiotic stress resistance genes was well recognized and documented with the transfer of several alien genes into their cultivated counterparts through wide or distant hybridization with or without employing embryo-rescue and mutagenesis. However, the amazing revelation that the wild relatives are also a source of yield-related genes is a development of the molecular era. Apomictic genes are another asset of many crop relatives that deserve mention. All of these past and the present factors have led to the realization that the so-called inferior species are highly superior in conserving desirable genes and can serve as a goldmine for breeding elite plant varieties. This is particularly true at a point when natural genetic variability has been depleted or exhausted in most of the major crop species, particularly due to growing and promoting only a handful of so-called high-yielding varieties while disregarding the traditional cultivars and landraces. In the era of molecular breeding, we can map desirable genes and polygenes, identify their donors and utilize tightly linked markers for gene introgression, mitigating the constraint of linkage drag, and even pyramid genes from multiple sources, cultivated or wild taxa. The evaluation of primary, secondary and tertiary gene pools and utilization of their novel genes is one of the leading strategies in present-day plant breeding. It is obvious that many wide hybridizations will never be easy and involve near-impossible constraints such as complete or partial sterility. In such cases gene cloning and gene discovery, complemented by intransgenic breeding, will hopefully pave the way for success. The utilization of wild relatives through traditional and molecular breeding has been thoroughly enumerated over the chapters throughout this series.

Enormous genomic resources have been developed in the model crop relatives, for example *Arabidopsis thaliana* and *Medicago truncatula*. BAC, cDNA and EST libraries have also been developed in some other crop relatives. Transcriptomes and metabolomes have also been dissected in some of them. However, similar genomic resources are yet to be constructed in many crop relatives. Hence this section has been included only in chapters on the relevant genera.

In this book series, we have included a section on recommendations for future steps to create awareness about the wealth of wild crop relatives in society at large and also for concerns for their alarmingly rapid decrease due to genetic erosion. The authors of the chapters have also emphasized on the imperative requirement of their conservation, envisaging the importance of biodiversity. The importance of intellectual property rights and also farmers' rights as owners of local landraces, botanical varieties, wild species and subspecies has also been dealt in many of the chapters.

I feel satisfied that the authors of the chapters in this series have deliberated on all the crucial aspects relevant to a particular genus in their chapters.

I am also very pleased to present many chapters in this series authored by a large number of globally reputed leading scientists, many of whom have contributed to the development of novel concepts, strategies and tools of genetics, genomics and breeding and/or pioneered the elucidation and improvement of particular plant

genomes using both traditional and molecular tools. Many of them have already retired or will be retiring soon, leaving behind their legacies and philosophies for us to follow and practice. I am saddened that a few of them have passed away during preparation of the manuscripts for this series. At the same time, I feel blessed that all of these stalwarts shared equally with me the wealth of crop relatives and contributed to their recognition and promotion through this endeavor.

I would also like to be candid with regard to my own limitations. Initially I planned for about 150 chapters devoted to the essential genera of wild crop relatives. However, I had to exclude some of them either due to insignificant progress made on them during the preparation of this series, my failure to identify interested authors willing to produce acceptable manuscripts in time or authors' backing out in the last minute, leaving no time to find replacements. I console myself for this lapse with the rationale that it is simply too large a series to achieve complete satisfaction on the contents. Still I was able to arrange about 125 chapters in the ten volumes, contributed by nearly 400 authors from over 40 countries of the world. I extend my heartfelt thanks to all these scientists, who have cooperated with me since the inception of this series not only with their contributions, but also in some cases by suggesting suitable authors for chapters on other genera. As happens with a mega-series, a few authors had delays for personal or professional reasons, and in a few cases, for no reason at all. This caused delays in the publication of some of the volumes and forced the remaining authors to update their manuscripts and wait too long to see their manuscripts in published form. I do shoulder all the responsibilities for this myself and tender my sincere apologies.

Another unique feature of this series is that the authors of chapters dedicated to some genera have dedicated their chapters to scientists who pioneered the exploration, description and utilization of the wild species of those genera. We have duly honored their sincere decision with equal respect for the scientists they rightly reminded us to commemorate.

Editing this series was, to be honest, very taxing and painstaking, as my own expertise is limited to a few cereal, oilseed, pulse, vegetable, and fruit crops, and some medicinal and aromatic plants. I spent innumerable nights studying to attain the minimum eligibility to edit the manuscripts authored by experts with even life-time contributions on the concerned genera or species. However, this indirectly awakened the "student-for-life" within me and enriched my arsenal with so many new concepts, strategies, tools, techniques and even new terminologies! Above all, this helped me to realize that individually we know almost nothing about the plants on this planet! And this realization strikingly reminded me of the affectionate and sincere advice of Dr. Norman Borlaug to keep abreast with what is happening in the crop sciences, which he used to do himself even when he had been advised to strictly limit himself to bed rest. He was always enthusiastic about this series and inspired me to take up this huge task. This is one of the personal and professional reasons I dedicated this book series to him with a hope that the present and future generations of plant scientists will share the similar feelings of love and respect for all plants around us for the sake of meeting our never-ending needs for food, shelter, clothing, medicines, and all other items used for our basic requirements and comfort. I am also grateful to his granddaughter, Julie Borlaug, for kindly extending her permission to dedicate this series to him.

I started editing books with the 7-volume series on Genome Mapping and Molecular Breeding in Plants with Springer way back in 2005, and I have since

edited many other book series with Springer. I always feel proud and satisfied to be a member of the Springer family, particularly because of my warm and enriching working relationship with Dr. Sabine Schwarz and Dr. Jutta Lindenborn, with whom I have been working all along. My special thanks go out to them for publishing this "dream series" in an elegant form and also for appreciating my difficulties and accommodating many of my last-minute changes and updates.

I would be remiss in my duties if I failed to mention the contributions of Phullara – my wife, friend, philosopher and guide – who has always shared with me a love of the collection, conservation, evaluation, and utilization of wild crop relatives and has enormously supported me in the translation of these priorities in my own research endeavors – for her assistance in formulating the contents of this series, for monitoring its progress and above all for taking care of all the domestic and personal responsibilities I am supposed to shoulder. I feel myself alien to the digital world that is the sine qua non today for maintaining constant communication and ensuring the preparation of manuscripts in a desirable format. Our son Sourav and daughter Devleena made my life easier by balancing out my limitations and also by willingly sacrificing the spare amount of time I ought to spend with them. Editing of this series would not be possible without their unwavering support.

I take the responsibility for any lapses in content, format and approach of the series and individual volumes and also for any other errors, either scientific or linguistic, and will look forward to receiving readers' corrections or suggestions for improvement.

As I mentioned earlier this series consists of ten volumes. These volumes are dedicated to wild relatives of Cereals, Millets and Grasses, Oilseeds, Legume Crops and Forages, Vegetables, Temperate Fruits, Tropical and Subtropical Fruits, Industrial Crops, Plantation and Ornamental Crops, and Forest Trees.

This volume "Wild Crop Relatives – Genomic and Breeding Resources: Oilseeds" includes 17 chapters dedicated to *Arabidopsis*, *Brassica*, *Capsella*, *Carthamus*, *Crambe*, *Cuphea*, *Diplotaxix*, *Eruca*, *Helianthus*, *Hirschfeldia*, *Linum, Moricandia, Orychophragmus, Pachycladon, Ricinus, Sesamum* and *Sinapsis*. The chapters of this volume were authored by 33 scientists from 11 countries of the world including Canada, China, France, Germany, India, Italy, New Zealand, Poland, Spain, UK and USA.

It is my sincere hope that this volume and the series as a whole will serve the requirements of students, scientists and industries involved in studies, teaching, research and the extension of oilseed crops with an intention of serving science and society.

Clemson, USA Chittaranjan Kole

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Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic acid
3MSOP	3-Methylsulfinylpropyl glucosinolate
3MTP	3-Methlythiopropyl glucosinolate
4MSOB	4-Methylsulfinylbutyl gucosinolate
7MSOH	7-Methylsulfinylheptyl glucosinolate
ACT	Acyl carrier thioesterase
ADH	Alcohol dehydrogenase
Adh	Alcohol dehydrogenase gene
AFLP	Amplified fragment length polymorphism
AICORPO	All India Coordinated Research Project on Oilseeds
Ala	Alanine
AMOVA	Analysis of molecular variance
AOP2	2-Oxoglutarate-dependent dioxygenase 2 gene
Arg	Arginine
AROS	Array-ready oligonucleotide set
ARS	Agricultural Research Service
As	Arsenic
Asp	Aspartic acid
atpA	Gene encoding the alpha subunit of the F(1) ATPase
*atp*B	Adenosine triphosphatase B gene
*atp*α	ATPase α gene
AWC	Allen Wilson Center
BAC	Bacterial artificial chromosome
BAP	Benzyl amino purine
BApNA	*N*α-benzoyl-DL-arginine-*p*-nitroaniline
BC	Backcross
BCgDFS	*Brassica* C-Genome Diversity Fixed Foundation Set Resources
BEAST	A cross-platform Bayesian Markov chain Monte-Carlo sequence analysis program
BGCI	Botanic Gardens Conservation International
BGV	Banco de Germoplasma Vegetal
BLAST	Basic local alignment search tool
BolDFS	*Brassica oleracea* Crop Genepool
BoS	*Brassica oleracea* SINE
Bras-EDB	European Brassica Database
C:12	Lauric acid
C:14	Myristic acid

CAD5	Cinnamyl alcohol dehydrogenase 5 gene
CaMV	Cauliflower mosaic virus
CAPS	Cleaved amplified polymorphic sequence
CCA1	Circadian clock associated
CCP	Comparative chromosome painting
cDNA	Complementary-DNA
CGH	Comparative genomic hybridization
CGRIS	Chinese Genetic Resources Information System
Chs	Chalcone synthase
CHS	Chalcone synthase gene
CISS	Chromosomal in situ suppression
CMS	Cytoplasmic male sterility
CNR	National Research Council
Col-0	Columbia ecotype
CP	Chromosome painting
cpDNA	Chloroplast-DNA
CSIR	Council of Scientific and Industrial Research (New Delhi, India)
C-value	Constant value (1C = Amount of DNA within a haploid nucleus)
CWR	Crop wild relatives
Cys	Cysteine
DAMD	Direct amplification of minisatellite DNA
DBT	Department of Biotechnology (New Delhi, India)
DFS	Diversity Foundation Set
DNAML	DNA maximum likelihood program
DOG	Delay of germination
DOR	Directorate of Oilseeds Research (Rajendranagar, India)
DRDO	Defence Research and Development Organization (New Delhi, India)
DSE	Regeneration of direct organogenesis from explants
EC	European Community
ECPGR	European Cooperative Program for Crop Genetic Resources Networks
EMBL	European Molecular Biology Laboratory
EMS	Ethylmethane sulfonate
EPSPS	5-Enol acetone, acyl Shikimate-3-phosphate synthase
ESM1	Epithiospecifier modifier 1 gene
ESP	Epithiospecifier protein gene
EST	Expressed sequence tag
ETS	External transcribed sequence
EURISCO	European Network of ex situ National Inventories
F_1	First filial (generation)
F_2	Second filial (generation)
FA	Fatty acid
FAE	Fatty acid elongation
FISH	Fluorescence in situ hybridization
FLC	*Flowering Locus C gene*
FMF2	*Embryonic flower 2* gene
FRI	*FRIGIDA* gene

FSE	Farm scale evaluation
GA_3	Gibberellic acid
*gap*C	Glyceraldehyde-3-phosphate dehydrogenase gene
gdc	Glycine decarboxylase gene
GDC	Glycine decarboxylase
*gdc*P	Glycine decarboxylase P gene
GFP	Green fluorescent protein gene
GISH	Genomic in situ hybridization
GLS	Glucosinolate compound
GLS-AOP	Glucosinolate 2-oxoglutarate-dependant dioxygenase gene
GLS-elong	Glucosinolate methylthioalkylmalate synthase gene
GLS-OH	Glucosinolate 2-oxoacid-dependant dioxgenase gene
GLS-OX	Glucosinolate flavin-monooxygenase gene
Glu	Glutamic acid
Gly	Glycine
GM	Genetic modification/ genetically modified
GMO	Genetically modified organism
GMU	Germplasm management unit
GRIN	Genetic Resources Information Network
GSL-ALK	QTL for production of alkenyl glucosinolate
HDL	High-density lipoprotein
HEA	High erucic acid
HEADE	High Erucic Acid Development Effort
His	Histidine
IBPGR	International Board for Plant Genetic Resources
Ile	Isoleucine
IO	Regeneration of explants through organogenesis from callus
IPGRI	International Plant Genetic Resources Institute
IPK	Institut fur Pflanzengenetik und Kulturpflanzenforschung
ISE	Regeneration of explants through somatic embryogenesis from callus
ISSR	Inter-simple sequence repeat
ITC	Isothiocyanate
ITS	Internal transcribed spacer
IUCN	International Union for Conservation of Nature
KAS	Ketoacyl-ACPsynthase
LDL	Low-density lipoprotein
Ler	Landsberg erecta ecotype
Leu	Leucine
LFY	Leafy gene
LG	Linkage group
Lys	Lysine
MAM1	Methylthioalkylmalate synthase 1 gene
MAS	Marker-assisted selection
MatK	Maturase K
*mat*K	Megakaryocyte-associated tyrosine kinase gene
MCT	Medium chain fatty acid
Met	Methionine

Mg	Magnesium
MISA	MIcroSAtelitte
MLO	Mycoplasma-like Organism
Mn	Manganese
MPSS	Massively parallel signature sequence
MS	Malate synthase gene
MS	Murashige and Skoog medium
Mt	Mountain
MTI	Mustard trypsin inhibitor
MtN21	*Medicago truncatula*-like nodulin 21 gene
MUFA	Mono-unsaturated fatty acid
MYA	Million years ago
N	Content of non-essential amino acid
NAA	Naphthaleneacetic acid
NAD(P)H	Nicotinamide adenine dinucleotide (phosphate) dehydrogenase
nad4	NADH4 gene
NADH	Nicotinamide adenine dinucleotide dehydrogenase
NBPGR	National Bureau of Plant Genetic Resources
NCBI	National Centre for Biotechnology Information
*ndh*F	NAD(P)H F gene
NeighborNet	A planar phylogenetic network construction program
NGRP	National Genetic Resources Program
NIL	Near-isogeneic lines
NMS	Nuclear male sterility
NPGS	National Plant Germplasm System
Nr	Nuclear ribosomal
Nt	Nucleotide
OIB	Outer involucral bract
Orf	Open reading frame
PAUP	Phylogenetic analysis using parsimony program
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PEP	Phosphoenolpyruvate
PGI	Phosphoglucose isomerase
Phe	Phenylalanine
PHYLIP	Phylogeny inference package
PhyML	Phylogenies by maximum likelihood program
PI	Pistillata gene
PMC	Pollen mother cell
PPT	Phosphinothricin
PRK	Phosphopyruvate kinase gene
Pro	Proline
PVC	Polyvinyl chloride
PVX	Potato virus X
PVY	Potato virus Y
QE II	Queen Elizabeth II
QTL	Quantitative trait loci
R&D	Research and Development

RAPD	Random amplified polymorphic DNA
*rbc*L	Rubisco (Ribulose bisphosphate carboxlyase/oxygenase) large subunit gene
RCA	*Ricinus communis* agglutinin
RCP	Reverse chromosome painting
rDNA	Ribosomal DNA
RFLP	Restriction fragment length polymorphism
RIL	Recombined inbreed line
rRNA	Ribosomal RNA
S	Selfing generation
SAAPLpNA	Succynil-ala-ala-pro-leu-*p*-nitroanilide
SACPD	Stearoyl acyl carrier protein desaturase
SAGE	Serial analysis of gene expression
SAMDC	*S*-Adenosylmethionine decarboxylase activity
Sar	Genome of *Sinapis arvensis*
SC	Self-compatible
SCAR	Sequence-characterized amplified region
SCRI	Scottish Crop Research Institute
Ser	Serine
S-GT	*S*-Glucosyltransferase
SHP 1	Shatteringprof 1
SHP 2	Shatteringprof 2
SINE	Short interspersed nuclear elements
SLR	*S*-Locus related
SNP	Single nucleotide polymorphism
SRAP	Sequence-related amplified polymorphism
SSR	Simple sequence repeat
SSRIT	SSR identification tool
STS	Sequence tagged site
T-DNA	Transfer-DNA
TFL 1	Terminal flower
Thr	Threonine
TIGR	The Institute Genome Rresearch
TOC1	True ossilator component
*trn*L-*trn*F	Intergenic space between introns tRNALeucine (UAA) and tRNA-Phenylalanine (GAA)
Trp	Tryptophane
TuMV	Turnip mosaic virus
Tyr	Tyrosine
UPM	Universidad Politecnica de Madrid
USDA	United States Department of Agriculture
UV	Ultraviolet
Val	Valine
VAM	Vesicular–arbuscular mycorrhizae
VLCFA	Very long chained fatty acid
WinClada	A data management analysis and tree editing program
WRPIS	Western Regional Plant Introduction Station

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Chapter 1
Arabidopsis

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1.1 Introduction

Arabidopsis belongs to the family Brassicaceae, which consists of several vegetable and oilseed crops including *Brassica oleracea, Brassica napus, Brassica nigra*, *Brassica rapa* (syn. *campestris*), and *B. juncea* among others. *Arabidopsis* genus consists of several species, among which *Arabidopsis thaliana* (L.) is used extensively for research in plant biology, although it has no potential economic value as a food or fuel crop. Although it cannot be used as oilseed crop, it has been used extensively for the genetic and genomic analysis of Brassicaceae crops (Panjabi et al. 2008). Since *Brassica* species genomes are many times greater than *Arabidopsis*, it is much convenient to work in *Arabidopsis* to use the information in the *Brassica* species for crop improvement efforts. *Arabidopsis* was used extensively as a model plant species in the 1950s for mutagenesis studies (Redei 1992). *A. thaliana* is a diploid species with $n = x = 5$ chromosomes, and a total genome size of approximately 125 Mb, one of the smallest known genomes among higher plants. Its small genome size is another feature that has led to its popularity as a model species. The genome sequence of *A. thaliana* var. Columbia was completed in 2000, and now most of the genes have been annotated (The Arabidopsis Genome initiative 2000).

Besides small genome size, it has less repetitive DNA, only 28,523 genes (of which 26,772 have been sequenced and the remaining thought to be pseudogenes) and about 50,000 proteins (Katam et al. 2010). Furthermore, microarrays were developed and are currently available for global gene profiling experiments, and data mining such as from *A. thaliana* GenExpress transcriptome database is possible. Comparison of diversion of genes between *Arabidopsis* and *Brassica* species may uncover the evolution of unique and species-specific genes that will unravel the evolution of each species in the Brassicaceace family under the presence of selective pressure (Hall et al. 2002).

Among other members of Brassicaceae, *Cardamine amara* has been estimated to have the smallest genome size of only 50 Mb (Bennett and Smith 1991). The variation in genome size among plant species, which can extend up to 120,000 Mb, have been resulted from duplication, reassembly, or by partial elimination of genes in the presence of selective pressure during plant evolution. Evolutionary trends suggest that the extant species resulted from many rounds of gene duplication, deletion, homologous recombination, unequal crossing, rearrangement, and polyploidization from their putative progenitors (Vision et al. 2000). Currently, 40% of Brassicaceae species are reported to be polyploids (Masterson 1994).

1.2 Species of *Arabidopsis*

Advances in molecular methods have enhanced our ability to better understand the function and evolution of genes that regulate during high temperature, drought, pathogens, insects, and other environmental challenges. Although no single model system can address the diverse range of ecological and evolutionary questions of interest, *A. thaliana* and its wild relatives

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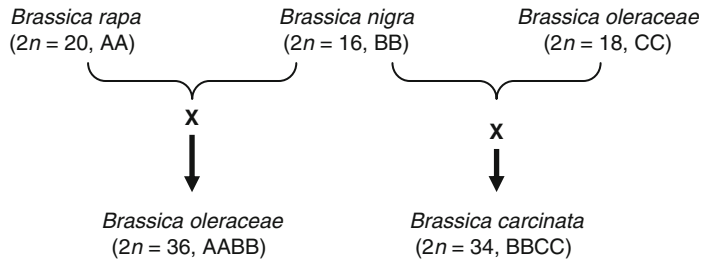

Fig. 1.1 Evolution of important oilseed crop plants in Brassicaceace family (modified from Snowdon 2007)

provide a model system that has a vast array of molecular tools, genetic resources, and biological information that can be used to address fundamental questions in ecology and evolution. Over the past 40 million years, approximately 3,000 crucifer species have radiated to many habitats worldwide (Koch et al. 2001). Recent molecular and morphological studies have outlined systematic relationships across Brassicaceae (Fig. 1.1) and across the closest relatives of *Arabidopsis* (Galloway et al. 1998).

The putative progenitors of *Arabidopsis* were likely polyploid, and this species may have evolved through extensive loss of genes (Blanc et al. 2000). There are nine closely related species in the genus *Arabidopsis* (Table 1.1). The closest relatives are *A. lyrata* and *A. halleri* ($2n = 8$), which are not cross-compatible with *A. thaliana*. However, *A. thaliana* (diploid) is cross-compatible with *A. arenosa* (tetraploid), resulting in a third bridge species, *A. suecica*, which is allotetraploid. The crosses involving these two species result in a few fertile seeds in F_1 generation. In F_7 generation, the progeny are quite stable, contemplating the involvement of micro-RNAs in such a phenomenon. There have been reports of successful crossing between *A. lyrata* × *A. hallari*, *A. lyrata* × *A. arenosa*, and *A. lyrata* × *A. arenosa* (Koch and Matschinger 2007), showing gene flow. Gene silencing in the bridge species involving various species of *Arabidopsis* at certain loci has led to speculation that these species evolved under the pressure of reproductive isolation. The genera *Arabidopsis*, *Brassica*, *Arabis*, and *Cardaminopsis* seem to have diverged from a common ancestor 40–50 million years ago (Mya), though *Arabis* and *Cardaminopsis* are often classified as *Arabidopsis* (Pigliucci and Schmitt 2004). There are 21 syntenic regions shared by *B. napus* and *A. thaliana* genome that show up to 90% homology that seem conserved for the last 20 million years

Table 1.1 *Arabidopsis* and their wild relatives (Koch .et al. 2008)

Species	Haploid chromosome	Ploidy (x)
Arabidopsis thaliana	5	Diploid
A. suecica	14	Tetraploid
A. pedemontana	9	Diploid
A. cebennensis	9	Triploid
A. kamchatica	16	Tetraploid
A. lyrata	4	Diploid
A. hallari	4	Diploid
A. arenosa	4	Diploid

(Parkin et al. 2005). Macrosynteny found between *B. oleracea* and *A. thaliana* also confirms large amount of chromosomal rearrangement to occur during the evolutionary process (Snowdon 2007). The knowledge of synteny is vital for quantitative trait loci (QTL) mapping in the other crop species, once QTL in *Brassica* is compared with the position in *Arabidopsis* chromosome. Nucleotide variation in eight effectively unlinked genes was surveyed in species-wide samples of the closely related outbreeding species *Arabidopsis halleri* and *A. lyrata* ssp. *petraea* and in three of these genes in *A. lyrata* ssp. *lyrata* and *A. thaliana* (Ramos-Onsins et al. 2004). Significant genetic differentiation was observed more frequently in *A. lyrata* ssp. *petraea* than in *A. halleri*. Average estimates of nucleotide variation were highest in *A. lyrata* ssp. *petraea* and lowest in *A. lyrata* ssp. *lyrata*, reflecting differences among species in effective population size. Average among-population variation in *A. halleri* and *A. lyrata* ssp. *petraea* was, respectively, 1.5- and threefold higher than that in the inbreeder *A. thaliana*. The detected reduction of variation in *A. thaliana* was less than that expected from differences in mating system alone, and therefore from selective processes related to differences in the effective recombination rate, but could be explained by differences in population structure.

1.2.1 Origin of New Species Through Polyploidy

Although comparative genomics can detect more ancient events than classical cytology, all events may not be detected. It has long been recognized that polyploidy has played an important role in speciation, especially in plants (Stebbins 1971). The new species will, therefore, evolve independently of its parental species, and if the polyploidization occurs only once, there will be no additional gene-flow into the species once it is formed. Even if it occurs several times, different polyploid lineages may well be isolated because of the rapid chromosome rearrangements (Pontes et al. 2004) and changes in gene expression (Osborn et al. 2003) that may accompany polyploidization. If polyploid species become diploidized over time, all plant species will have polyploid ancestors (Ku et al. 2000). *A. suecica* (Fr.) was first described as an allotetraploid ($2n = 26$), which has *A. thaliana* and *Arabidopsis* (*Cardaminopsis*) *arenosa* as its parental species, a suggestion supported by molecular phylogenetics (O'Kane and Al-shehbaz 1997). It is diploid ($2n = 10$), although tetraploid *A. thaliana* individuals are occasionally found. *A. arenosa* is a European species, which is tetraploid ($2n = 4x = 32$) in most of its range and reportedly diploid ($2n = 2x = 16$) in a small area in eastern Europe (Mesicek 1970). cpDNA sequencing confirmed that *A. thaliana* is the maternal parent of *A. suecica* (Säll et al. 2003). Furthermore, when producing artificial *A. suecica* through crosses, viable offsprings were produced from *A. thaliana* maternal parent (Comai et al. 2000). An important aspect of the formation of a polyploid species is the number of independent origins. Many allopolyploid species are believed to have multiple origins (Soltis and Soltis 1993). If all the polyploids form a single branch within the tree, there is evidence of a single origin, whereas if the allopolyploids appear at different positions relative to the parent, there is evidence of multiple origins. This method was used to analyze cpDNA from *A. suecica*, and the results indicated a single origin.

Speciation by polyploidization is instantaneous, which is the result of a well defined, discrete event (s). The new polyploid species is expected to be isolated from its parental species because crosses between a polyploid and its parental species are most likely infertile. However, repeated polyploidization events may result in a polyploid species with multiple origins. More than one polyploidization event of genetically very closely related parental individuals results in polyploidy species. The observation of monophyly at one or a small number of loci with respect to the parental species does not imply a single origin. Alleles in the polyploid species could be monophyletic with respect to the parental species since they coalesce more recently than the origins of the species (Rosenberg and Nordborg 2002).

1.2.2 Genome Differentiation in Closely Related Species

The phenomenon of heterosis or hybrid vigor is poorly understood despite its perceived importance in evolution and its practical significance in breeding programs that aim to increase yield of crop plants. The identification of positively selected chromosomal segments that increase the fitness of backcross progeny (Rieseberg et al. 1999) would provide a basis for the study of heterosis. Several models have been proposed to explain the genetic basis of heterosis, including the masking of deleterious alleles, complementation of allelic variants, and epistasis (Rieseberg et al. 2000). The interspecific hybrid system has a potential to facilitate progress to analyze biological processes that do not exist such as self-incompatibility and perennial growth habit and cannot be studied in *A. thaliana*. The interspecific hybrid system facilitates evaluation of the genetic basis of heterosis and understanding of genome evolution, specifically as a complement to the commonly used strategy of comparative mapping of the differentiated genomes of related species. However, the few molecular marker studies that have addressed this issue led to conflicting conclusions regarding the primary cause of heterosis possibly because a combination of causes might produce heterotic effects (Monforte and Tanksley 2000). Backcross populations of *A. thaliana*–*A. lyrata* hybrids may be used to investigate the extent of chromosome differentiation between *A. thaliana* and *A. lyrata* and the degree to which it might interfere with chromosome pairing and gene flow between the two species. It is critical to determine whether the backcross plants incur differential inheritance of different

chromosome blocks. These studies lead to the identification of loci that contribute to genetic isolation between the two species, because such loci are expected to be introgressed at a slower rate than neutral loci. The parental imprinting most likely is the dominant mechanism for the postzygotic hybridization barrier between *A. thaliana* and *A. arenosa* (Bushell et al. 2003). The ability to introduce a hybridization barrier may prove useful in the containment of genetically modified crops. Genomic strength could be modified to ensure that they would be unable to produce viable seeds by hybridization with wild relatives.

1.2.3 Arabidopsis *Hybrids*

In *A. thaliana*, considerable intraspecific genetic variation occurs among different geographical isolates, and this variation, which is largely quantitative in nature, is being studied by using methods developed for the analysis of QTL in crop plants (Alonso-Blanco and Koornneef 2000). Generation and analysis of interspecific hybrids between *A. thaliana* and related species provide an additional and unique resource for the functional analysis of the *Arabidopsis* genome.

Wide crosses and interspecific hybridizations have been used to investigate the genetic basis of complex traits that differentiate varieties within a species and related species in several plant families (Bernacchi and Tanksley 1997). The feasibility of generating interspecific hybrids of *Arabidopsis* and closely related species is suggested by the occurrence of *A. suecica,* an allotetraploid thought to be derived from *A. thaliana* and *C. arenosa* (O'kane et al. 1996) by crossing autotetraploid *A. thaliana* (generated by colchicine treatment) and tetraploid *C. arenosa*. Interspecific hybridizations were performed in an attempt to clarify the taxonomic relationships of *A. thaliana* to related species (Redei 1974). Hybridizations of *A. thaliana* with complex traits are amenable to analysis in *A. thaliana–A. lyrata* hybrids. Species within the immediate taxonomic vicinity of *Arabidopsis* show a range of interesting traits such as apomixis in *Arabis holboellii* (Roy 1995). Therefore, it is conceivable that the range of traits that may be investigated by an interspecific hybridization approach will be further expanded in the future, should it prove possible to hybridize *A. thaliana* with these other related species. By increasing the genetic variability available for study, the *A. thaliana–A. lyrata* hybrid populations should be useful in the analysis of a number of different plant processes.

1.3 Evolutionary Genomics

Progressive loss of individual genes from duplicated segments has been a fundamental feature of genome evolution. Plant genomes are shaped by a dynamic balance between rates of gene duplication and gene loss. Several studies have examined gene families and segmental duplications in the *Arabidopsis* genome. Extensive duplicated regions suggest that a polyploidization event occurred ~100 Mya. The half-life of duplicated genes in *Arabidopsis* is estimated at approximately 3.2 million years (Lynch and Conery 2000). During population differentiation, loss of gene function at alternative, duplicated loci provides a plausible speciation mechanism consistent with existing models. Several large genomic regions have been sequenced from relatives of *Arabidopsis* (Quiros et al. 2001). In comparison to *Arabidopsis*, functional genes are highly conserved, whereas intergenic regions display insertions, deletions, and higher levels of nucleotide substitution. Such sequence comparisons will be useful for genetic analysis of crop plants, and for annotation and regulatory studies of *Arabidopsis* genes. Furthermore, evolutionary analysis of comparative sequence data can provide insights into the evolution of gene families, the molecular basis of adaptation, and the rate of deleterious mutations.

Genetic and phylogenetic relatedness, which enables crosses between $x = 5$ and $x = 8$ taxa, provides a huge potential to move from the study of *A. thaliana* into its wild relatives to answer fundamental evolutionary questions, which may not be possible in *A. thaliana* because of inbreeding system and unspectacular distribution range. Evolutionary studies are restricted to single species or groups of populations.

The evolutionary split between *A. thaliana* ($x = 5$) and *Arabidopsis* taxa ($x = 8$) occurred 5 Mya (Kuittinen and Aguadé 2000) and initiated the evolution of *A. thaliana* with its unique characters compared with the $x = 8$ lineage and also changes on the chromosome level resulting in its derived genome structure (Yogeeswaran et al. 2005). On the contrary, there is

much more variation in the $x = 8$ lineage, resulting in the recognition of several species and subspecies. Three major lineages can be recognized, namely *A. lyrata, A. halleri*, and *A. arenosa*, and most species or subspecies can be treated within these three lineages. In addition, three species have been described that are not closely related to one of these three species groups: *A. croatica* (Croatia), *A. cebennensis* (France), and *A. pedemontana* (Italy). It can be expected that below the species level, the number of taxa will increase further as is the case for *A. halleri*, which segregates with five subspecies. The same will happen to *A. arenosa* segregates because actual taxonomic treatments are unconvincingly based on comparative cytological, morphological, or genetic analysis, and we are still lacking any comparative morphometric analysis.

1.4 Diversity in *A. thaliana* and Genetic Variation Among Wild Species of *Arabidopsis*

Plants are ideal for molecular ecological genetics because they are sessile and are confronted by measurable environmental challenges, such as abiotic (extreme temperatures, drought, salinity, etc.) and biotic (pathogens and insects, etc.) stresses. Many known genes function in response to these environmental challenges, so these loci could be influenced by natural selection. Analysis of DNA sequence variation within and between species can compare observed patterns of variation with predictions of neutral equilibrium models. Genetic variation was observed in recent population expansion in *A. thaliana* (Innan and Stephan 2000). A comprehensive study of nucleotide polymorphism at 400 loci found genome-wide evidence for population expansion, which corresponds to the large population size of *A. thaliana*. Such studies can determine whether QTL alleles are young, deleterious, and spatially restricted or whether they are old widespread polymorphisms contributing to local adaptation.

A great extent of natural variation has been found at phenotypic and molecular levels in *A. thaliana*. Studies at molecular levels have revealed the high level of interplay and complexity of genes determining the plant phenotype. Breeding system influences experimental tractability in *A. thaliana* and its relatives. High levels of self-pollination in *A. thaliana* and *A. drummondii* facilitate QTL mapping and progeny testing in advanced generation crosses, which can be hindered by inbreeding depression in outcrossing species (Karkkainen et al. 1999). However, many population genetic models assume random mating, which is approximated more closely in populations of outcrossing species such as *A. lyrata* and *A. halleri*. Outcrossing species are predicted to maintain higher levels of genetic variation within populations (Charlesworth and Charlesworth 1995a). This pattern has been observed among *Leavenworthia* species at PGI locus (Liu et al. 1999) but is less clear when the *Adh* gene is compared between *A. lyrata* and *A. thaliana* (Savolainen et al. 2000). Breeding system also influences transposon abundance and mobility (Charlesworth and Charlesworth 1995b) that can affect genetic variation for quantitative traits.

1.5 Comprehensive Phylogenetic Framework for *Arabidopsis*

Genetic variation of all evolutionary lineages of *A. thaliana* relatives was studied (Koch and Matschinger 2007) based on a representative geographic sampling by studying maternally inherited chloroplast DNA (cpDNA) haplotype variation and sequence diversity of the internal transcribed spacer region (ITS) of ribosomal RNA. The plastid data were compared with the nuclear data, and significant differences among the various evolutionary lineages are highlighted contributing to the systematic status of some taxonomical nomenclatural combinations such as *A. kamchatica* and *A. arenicola* (Shimizu et al. 2005). Using comparative DNA sequence analysis of a plastid and a nuclear locus across the whole genus *Arabidopsis*, a phylogenetic framework for all known closest relatives of *A. thaliana* was introduced.

1.6 Genetic Analysis of *Brassica* Crops Using *Arabidopsis* Genomics

Brassica lineage *nigra* ($n = 8$) and *rapa* ($n = 10$) are closely related to *Arabidopsis,* which diverged about 8 Mya (Lysak et al. 2005) and are diploids, although

they are probably paleopolyploids having several progenitor species and share similar evolutionary history to *A. thaliana* (Qiu et al. 2009).

The evolution of *Brassica* seems to be through extreme gene duplication and extensive rearrangement (Lagercrantz 2008). Polyploidy is responsible for evolution of diversity among plant species and observed frequently in *Brassica* members. Comparative genomic studies with the genome regions of *B. rapa*, *B. napus*, *B. oleracea* to *A. thaliana* proved consistency with the hypothesis of triplicated nature of *Brassica* (2*n*) genome (Park et al. 2005). Source for *Brassica* genome data primarily comes from genomic sequencing, bacterial artificial chromosome (BAC), and expressed sequential tag (EST) sequences. These ESTs were utilized to develop over 900 molecular markers and were mapped to 15 genetic maps (Lim et al. 2007). These markers are being used for marker-assisted selection (MAS), fine-mapping, QTL mapping, and map-based or synteny-based positional cloning for agronomic traits in *Brassica*. The evolutionary synteny among Brassicaceae family was investigated by performing chromosome painting with a 9 Mb BAC from *A. thaliana* chromosome 4 that found homologous regions in 21 members in *Brassica* and convincing pattern that may be due to evolution of a species from its progenitors, which had similar genome size as that of *Arabidopsis* (Lysak et al. 2005).

Although several segments of the genomes were found to be conserved across *Brassica* and *Arabidopsis*, there is distinct evidence of gene loss, which could have simply resulted from gene rearrangements from other parts of the genome, or through fragmentation and dispersal phenomena (Town et al. 2006). Comparative approach for mining similarity between *B. napus* (*B. olaracea* × *B. rapa*) has evidenced for several loci, which mapped to similar position in the *A. thaliana* genome. Since the *Arabidopsis* genome has been annotated, investigation of functional and comparative genomics is possible to find similar genes or orthologs in different species. Many genes controlling physiological processes in plants are highly conserved across plant species throughout the period of evolution and show various degrees of synteny.

Koch et al. (2000) analyzed sequence variation for chalcone synthase (*Chs*) and alcohol dehydrogenase (*Adh*) loci in 28 species in the genera *Arabidopsis* and *Arabis* and related taxa from the tribe Arabideae. *Chs* was single-copy in nearly all taxa examined, while *Adh* duplications were found in several species. Phylogenies constructed from both loci confirmed that the closest relatives of *A. thaliana* include *A. lyrata, A. petraea,* and *A. haller*. The genus *Arabis* is polyphyletic – some unrelated species appear within this taxonomic classification, which has little phylogenetic significance. *A. thaliana* diverged from its nearest relatives about 5 Mya and from *Brassica* roughly 24 Mya.

1.7 Introgression and Utilization of *Arabidopsis* for the Improvement of *Brassica*

It is critical to know about wild relatives in order to understand the evolution and transgression of genes of importance in *Arabidopsis* from its wild type progenitors and their basis of selection and differentiation from each wild type species. This results in a lot of complication about the evolution of individual characters. Furthermore, molecular markers such as simple sequence repeat (SSR) and amplified length fragment polymorphism (ALFP) reveal a detailed characterization of individual species. There are a large numbers of single nucleotide polymorphisms (SNPs), 249,052 reported by Clark et al. (2007) found from the *Arabidopsis* genome sequence projects, thus demonstrating that a large amount of variation in amino acids and proteins can be found in nature in response to different physiological conditions.

Introgression studies are critical for crop improvement, particularly at a time when a large amount of resources has been spent on gene sequencing and understanding the global profiling of proteins in plants. Introgression into the important *Brassica* species from their relatives *Arabidopsis* species could facilitate our understanding of gene introgression into the related species. Extensive similarity exists between *B. napus* and *A. thaliana* advocating the use of *Arabidopsis* genomic resources for *Brassica* crop improvement (Cavell et al. 1998). These two species have diverged about 20 Mya, and though *Brassica* species have acquired many more genes distributed in the additional chromosomes, the synteny of genes overshadow the significance of the evolutionary process in these two species (Hall et al. 2002). So the study and evolution of *Arabidopsis* and its synteny

with members of the Brassicaceae members (canola, oilseed mustards, and some vegetable crops like broccoli and Brussels sprouts) will shed light on the origin and development of key genes regulating yield and quality parameters.

Comparison of *A. thaliana* and *B. nigra* indicated that the *Brassica* genome has evolved through intensive genome replication and rearrangement of the genes. Many of the genes of importance to crop productivity traits must be present in *Arabidopsis*, and the availability of complete genome sequence indicates that data mining is possible in short time to identify the gene(s) of interest with agronomic significance.

1.8 *Arabidopsis* and *Brassica* Comparative Genomics

Comparative genomics among economically important crop species and their closely related model plant species can be useful in the identification of genetic markers and candidate genes for positional gene cloning (Qiu et al. 2009). The genome changes, which took place in these two plant species over millions of years will be possible and then comparing with *A. thaliana* is significant in the perspective of evolution from the genome sequence projects of *B. rapa* and *B. oleracea* (Yang et al. 2006). Among the cultivated *Brassica* species, *B. oleracea* has been estimated to have the largest genome, which is due to frequent insertions of transposons and mostly intron duplication (Alix and Heslop-Harrison 2004). To understand the evolution of genes associated with agronomic traits in these species, the ultimate aim is to decipher the control mechanism behind temporal and spatial expression of proteins and their complex interactions, which forms the basis of multitude of physiological functions in plants. This could in turn answer many other physiological problems related to crop (*Brassica* family) yield and tolerance to insect pests and diseases (Altmann et al. 2004).

1.8.1 *Comparison of* B. oleracea *with* Arabidopsis

Significant conservation was observed between the different *B. oleracea* and *A. thaliana* maps. Although conserved blocks were not found between *A. thaliana* Ch1 and linkage groups O1 and O2 of *B. oleracea*, a 2 Mb region was predicted to be syntenic to O3 in the A12XGD-206 (Fig. 1.2). Furthermore, a 4 Mb conserved block, from the lower half of *A. thaliana* Ch1, was predicted to be syntenic to O4 from A12XGD-206, A12XGD-210, whereas only 1 Mb of this region was syntenic with BolAG_1996_A O4. Conserved blocks between *A. thaliana* Ch2 and linkage groups O1, O5, O6, O8, and O9 of *B. oleracea* were not observed. However, a 9 Mb conserved block in the middle of *A. thaliana* Ch2 was predicted to be syntenic to BolAG_1996_A O7, and a smaller region of A12XGD-206 O7 was also syntenic with two smaller blocks. Similar to chromosome 2 of *A. thaliana*, no conserved blocks were identified between *A. thaliana* Ch3 and linkage groups O1, O2, O6, and O9 in their study. The 2 Mb conserved block at the top of *A. thaliana* Ch3 and 3 Mb inverted blocks at the lower half of the chromosome were predicted to be syntenic to A12XGD-206 and BolAG_1996_A. The predicted synteny between *A. thaliana* Ch4 and BolAG_1999_A was an inverted conserved block on O1, which was also present in A12XGD-210 O1. A 16 Mb conserved block at the lower half of *A. thaliana* Ch4 was also predicted to be syntenic to BolAG_1996_A O3. Only O4 from A12XGD-206 was predicted to be syntenic with *A. thaliana* Ch4. A 6 Mb conserved block in the middle of *A. thaliana* Ch4 was predicted to be syntenic to A12XGD-206 and BolAG_1999_A O7, whereas only 0.5 Mb syntenic block was detected on O7. The 6 Mb conserved block in the middle of *A. thaliana* Ch4 was predicted to be syntenic to O8 in all the populations except BolAG_1999_A, whereas 2 Mb conserved block in the middle of *A. thaliana* Ch4 was predicted to be syntenic to A12XGD-210 O9. While there were no other conserved blocks identified between *A. thaliana* Ch5 and linkage groups O4, O5, and O8 of *B. oleracea*, two conserved block at the top and bottom half of A12XGD-206 map O2 were predicted to be syntenic to *A. thaliana* Ch5. Furthermore, one 2 Mb block was found in the lower top of BolAG_1996_A O3, and a 0.5 Mb conserved block in O6 was predicted to have a syntenic region *A. thaliana* Ch5.

1.8.2 *Comparison of* B. napus *with* Arabidopsis

Comparative linkage study between *B. napus* and *A. thaliana* revealed that there were five conserved

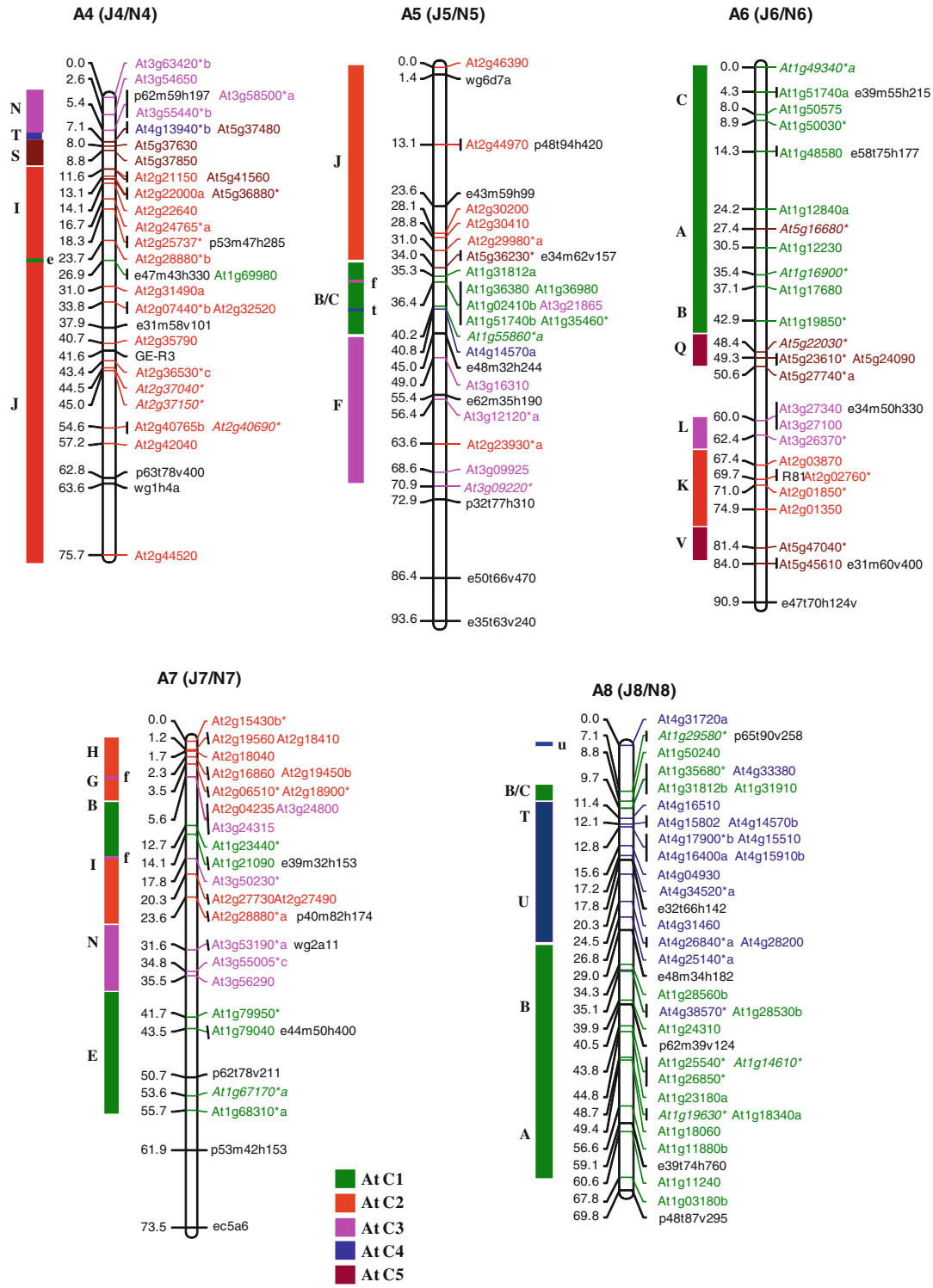

Fig. 1.2 Comparison of the *B. oleracea* maps GD206 (A12XGD-206), GD210 (A12XGD-210), Bol1996 (BolAG_1996_A), Bol1999 (BolAG_1999_A) to *Arabidopsis* chromosomes 1–5. *Source*: Lim et al. 2007

blocks between *A. thaliana* Ch1 and linkage groups N18, N13, N19, N15, and N16 (Fig. 1.3). The largest of these linkage groups, N15, spanned nearly the whole of *A. thaliana* Ch1, whereas the N18 region was found to be inverted. An earlier research identified syntenic regions between *A. thaliana* Ch1 and most linkage groups of *B. napus* (Udall et al. 2005). Several conserved regions between *A. thaliana* Ch2 and *B. napus* genome have been reported for N3, N5, N13, N14, and N18 with blocks larger than 3 Mb detected in N19, N4, and N12. Several conserved regions were found on *A. thaliana* Ch3 in another study in N2, N4, N5, N10, N12, N13, N14, N15, N18, and N19. Inverted blocks in N11 and N15 were

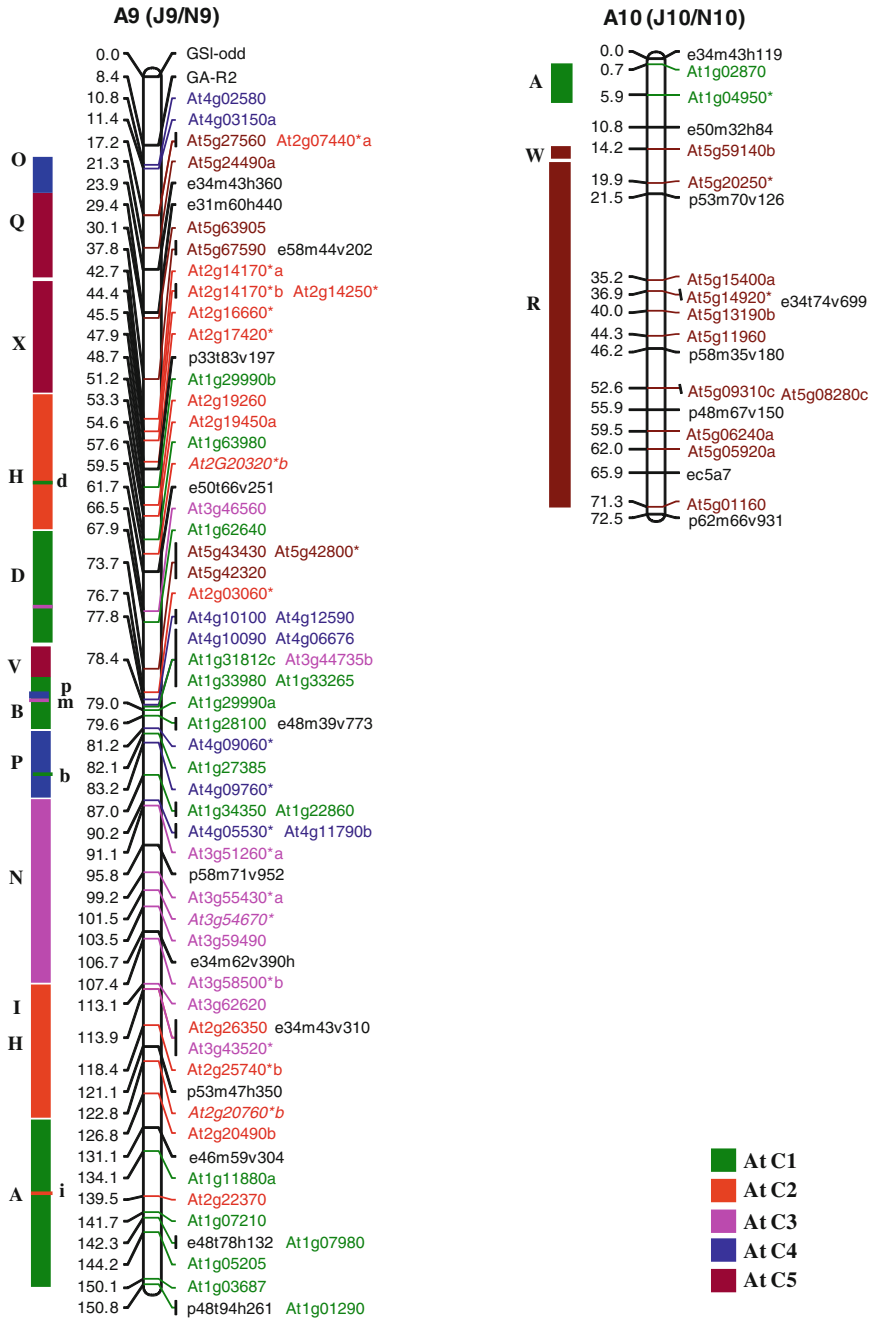

Fig. 1.3 Comparison of the *B. napus* maps to *Arabidopsis* chromosomes 1–5. *Source*: Lim et al. 2007

identified on the basis of another map (Parkin et al. 2005). Further conserved blocks for N11, N17, and N19 were found in the middle of *A. thaliana* Ch5. A 6 Mb inverted block was found for N6 in the middle of *A. thaliana* Ch5, and blocks larger than 4 Mb were found for N12, N13, N15, with a smaller block for N19 at the top of the chromosome. Furthermore, small blocks were found for N2, N6, N12, and N16 at the top of *A. thaliana* Ch5 (Mayerhofer et al. 2005).

1.8.3 *Comparison of* B. rapa *with* Arabidopsis

Under the ongoing effort of international genome sequencing and comparative mapping of *B. rapa* with respect to *A. thaliana,* Yang et al. (2005) sequenced Ch1 of *B. rapa*. Comparative sequence analysis revealed 82% similarity between *A. thaliana* and *B. rapa*. Homologous syntenic blocks over 200 with 72% of the sequenced and anchored *B. rapa* sequences were assigned to synteny blocks in *A. thaliana* (Mun et al. 2009), when multiple blocks overlapping the same region were considered (Fig. 1.4). According to them, the *B. rapa* and *A. thaliana* genomes share a minimum of 20 large-scale synteny blocks with substantial microsynteny blocks, which extend the length of whole chromosome arms. However, *A. thaliana* 1L (long arm) and *A. thaliana* 3 have only one or two synteny blocks in the *B. rapa* genome. Moreover, some genome regions of *A. thaliana*, including a smaller section of *A. thaliana* 2S and *A. thaliana* 4S, did not show significant synteny with *B. rapa* counterparts, indicating chromosome-level deletion of triplicated segments. Interestingly, *B. rapa* was found to show synteny with a major single chromosome along almost the entire length or fragments of multiple *A. thaliana* chromosomes in a complicated mosaic pattern, indicating frequent recombination of the *B. rapa* chromosomes.

1.8.4 *Comparison of* B. juncea *with* Arabidopsis

B. juncea genome has been mapped comparatively with *A. thaliana* to obtain a comparative picture of the genetic mapping. Molecular markers and relative position of molecular markers in the genome and usefulness of the same markers in both the species was investigated (Panjabi et al. 2008). Primers for PCR amplification were designed from exon sequences, which showed strong nucleotide conservation between *A. thaliana* and the corresponding EST sequences described for *Brassica* species. Out of 1,180 primer pairs, 383 (32%) showed polymorphism between the *B. juncea* lines. Genotyping using the 383 polymorphic primer pairs generated 486 loci in *B. juncea* of which 67% were scored as codominant markers and the remaining 33% were scored as dominant markers. A linkage map of *B. juncea* consisting of 533 *A. thaliana* loci is shown in Fig. 1.5. An uneven distribution of *A. thaliana* loci originating from each *Arabidopsis* chromosome was observed in the genome of *B. juncea*. Among the 10 LGs of the A-genome (A1–A10), for example, all the linkage groups except A2, A6, A7, A8, and A10 contained *A. thaliana* loci from each of the five *A. thaliana* chromosomes. Furthermore, the linkage group A8 was composed of markers from *A. thaliana* Ch1 and *A. thaliana* Ch4, while A10 was composed of markers from *A. thaliana* Ch1 and *A. thaliana* Ch5. The organization of the *B. juncea* linkage map with respect to the *A. thaliana* genome was also studied on the basis of the distribution of 24 genomic blocks described for a hypothetical ancestor of the *A. thaliana* and *Brassica* (Schranz et al. 2006). A conserved block was defined as a region that contained at least two *A. thaliana* loci from the same block region. Using this criteria, a total of 67 genomic blocks were identified in the A-genome of *B. juncea* with an average of 2.8 paralogous blocks for each block recognized in the hypothetical ancestral species.

1.9 Comparative Mapping of *Brassica* and *Arabidopsis*

Significant proportion of homology between members of the family is expected between *Arabidopsis* and *Brassica* since they are the members of the same family. Comparative studies with *Arabidopsis* with its members are described below for better understanding of the relatedness of these species with reference to the gene flow across the species and the syntenic relationship.

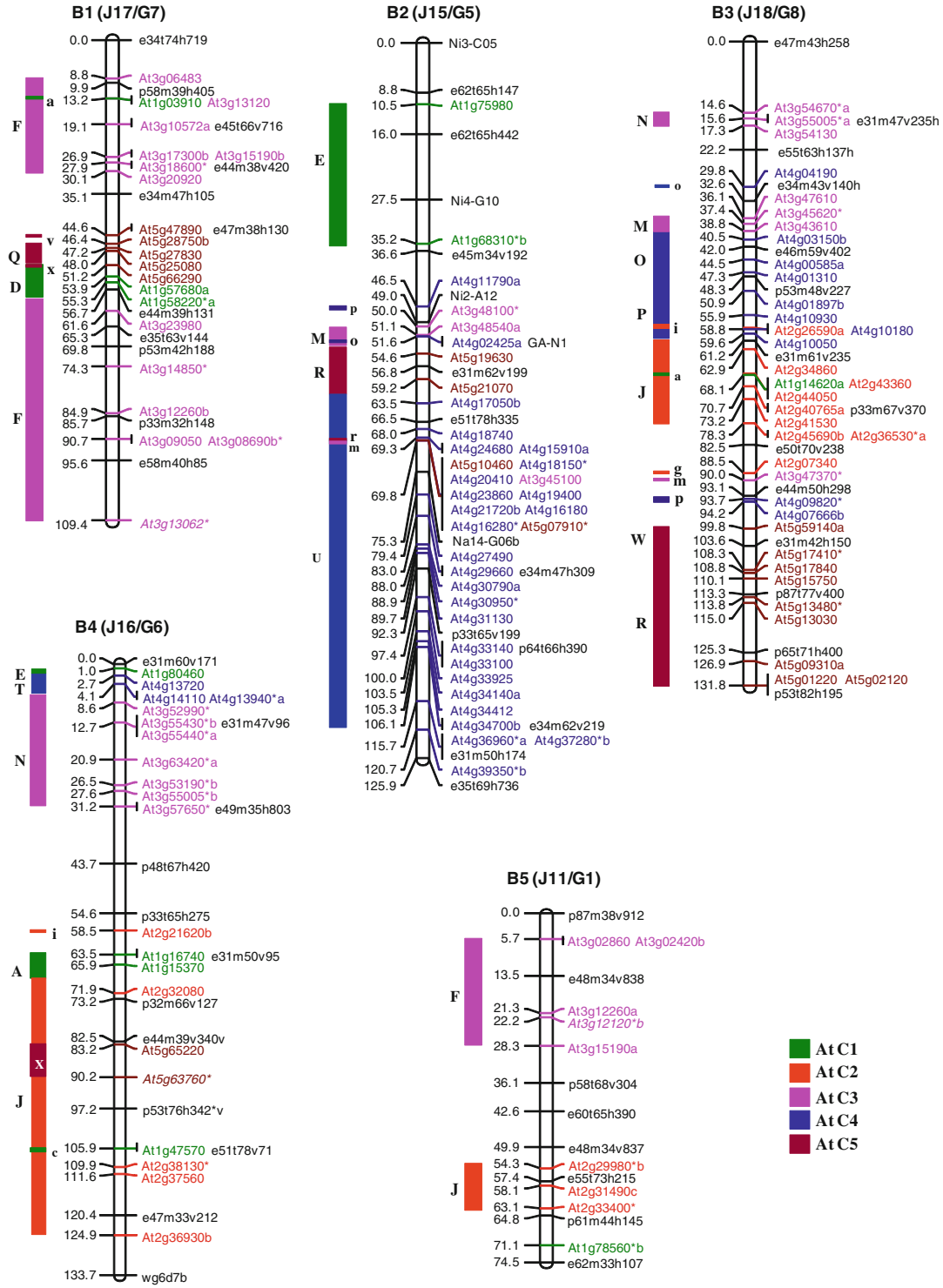

Fig. 1.4 Comparison of the *B. napus* maps to *Arabidopsis* genome. Synteny between the *B. rapa* and *A. thaliana* genomes. **(a)** Percent coverage of individual chromosomes showing synteny between *B. rapa* and *A. thaliana*. **(b)** Chromosome correspondence between *B. rapa* and *A. thaliana* represented by a dot-plot. *Source*: Mun et al. 2009

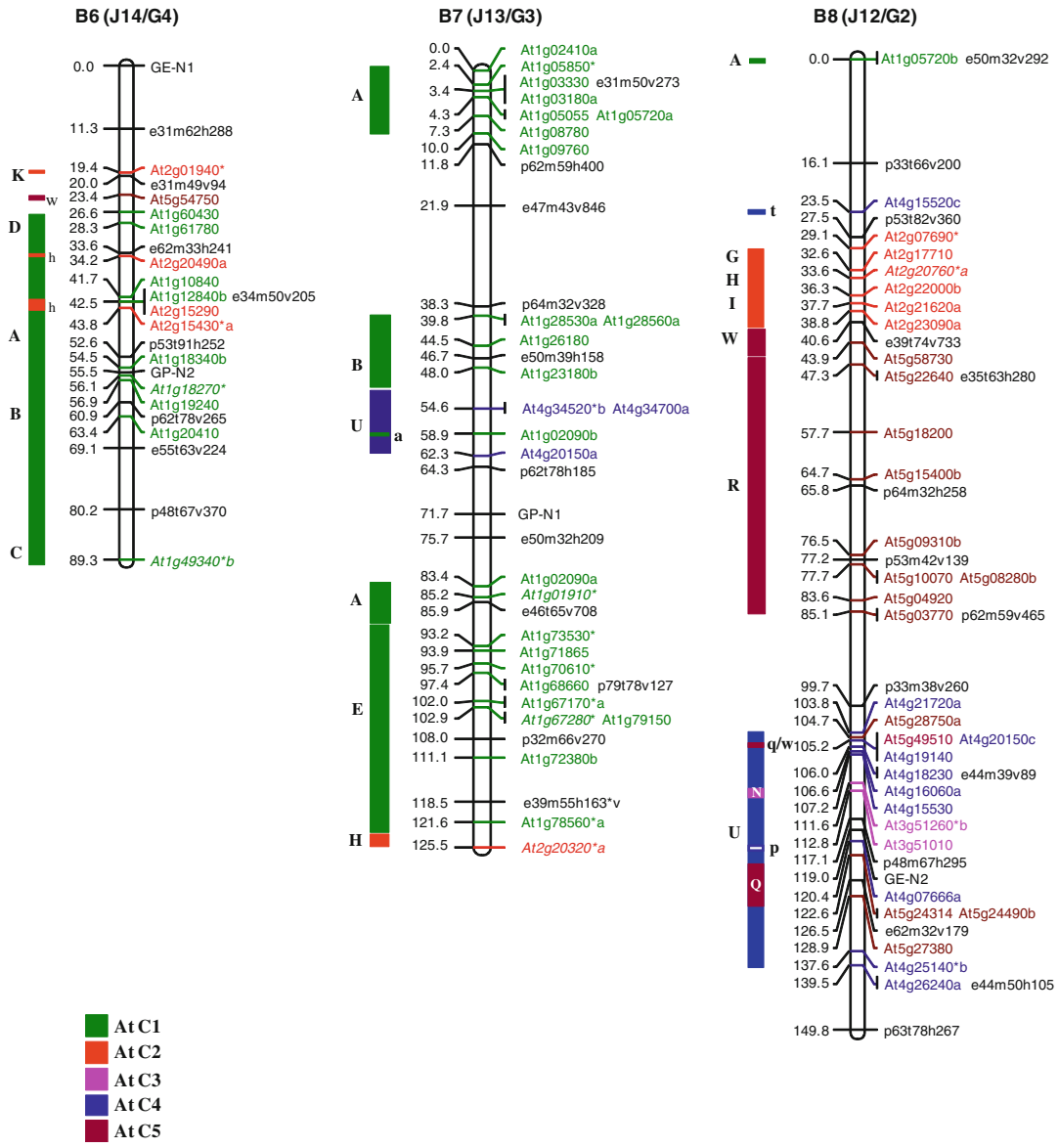

Fig. 1.5 Genetic map of *B. juncea* showing three linkage groups of the A-genome (A1, A2, and A3). The corresponding nomenclature followed earlier in *B. juncea* (J1–J3) and *B. napus* (N1–N3) is given in parentheses. Each genetic locus bears the name of the At (*A. thaliana*) gene and the color code of the *At* chromosome from which it is derived. *Source*: Panjabi et al. 2008

1.9.1 *Comparative Physical Mapping of* Arabidopsis *and* Brassica *Species*

Several comparative genetic maps between *Arabidopsis* and *Brassica* species have been constructed, which has significant role in applied plant breeding (Kaczmarek et al. 2009). Most of these markers (93.3%) gave successful amplification of target microsatellite motifs, which was confirmed by sequencing. Microsatellites markers were utilized to construct the comparative physical map between *B. napus, B. rapa*, and *Arabidopsis* to map serine and Gibberellic acid (Parida et al. 2010). The Brassica markers were physically anchored on the *A. thaliana* genome, which were useful in studying the evolutionary history of *A. thaliana* genomic duplications in relation to *Brassica* species. Comparative

physical mapping identified 85% of *Brassica* unigenes as single copy and gave clues for the presence of conserved primordial gene order. Complex chromosomal rearrangements such as inversions, tandem and segmental duplications, and insertions/deletions were evident between *A. thaliana* and *B. rapa* genomes. Similar map of *B. oleracea* and *A. thaliana* was also constructed using EST-based markers (Lan et al. 2000). Significant level of homology between *Brassica* and *Arabidopsis* genome was observed, indicating the possibility that genomic information obtained in *Arabidopsis* may be utilized in improving *Brassicas*. Such comparative mapping has been found to be useful in map-based cloning of genes, which can eventually be introduced into the crop plants to improve the traits of interest (Zhang et al. 2009).

1.9.2 *QTL Analysis in* Arabidopsis *and* Brassica *Species*

Small-scale sequencing from BAC clones for a particular plant species may provide useful information for undertaking comparative genomics as whole-genome sequencing of every other species is not feasible. Other genomic resources available include recombinant inbred lines (RILs), near isogenic lines (NILs), T-DNA insertion mutants in different genetic backgrounds, which can be used in fine-mapping of QTLs. The database information could also aid in MAS in crop plants, whose genomes has not been sequenced. Continuous breeding for improvement of crop productivity requires the study of quantitative genetics in plants and their effect on genetic variability.

A strong correlation between the genomes of *Arabidopsis* and *Brassica* was found from the restriction fragment length polymorphism (RFLP) analysis, which could be of potential interest for crop improvement in identifying certain genes (Qiu et al. 2004; Qiu et al. 2006). Panjabi et al. (2008) suggested the viability of using polymorphisms in introns for establishing genetic map using suitable markers. Lukens et al. (2003) were able to collate 34 regions in *A. thaliana* to at least 28% regions of *B. oleracea*. Advanced molecular and bioinformatics tools, by using MySQL database, MarkerQTL, EnsEMBL, and BioPerl language, are employed to analyze data on genetic markers and the physiological and agronomic traits. These will expedite QTL analysis for agricultural traits of importance.

As the *B. rapa* genome sequencing project (The Multinational Brassica Genome Project) is nearing completion, the sequence information will be used to further test hypotheses of gene conservation, duplication, or loss during the process of evolution, which can be extended to other oilseed crop relatives. Today, we have *B. napus* BAC clones representing most of the genes, extensive *Brassica* microsatellite markers and *Arabidopsis* serial analysis of gene expression (SAGE) data, and massively parallel signature sequence (MPSS) tags to expedite gene discovery (Hene et al. 2007). The comparative genomics approach has led to our understanding of the synteny between A, B, C sets of *Brassica* genome, and relating this information to *Arabidopsis* genome information will lead to facilitate precision breeding and positional cloning of genes of interest controlling agronomic traits (Panjabi et al. 2008). *Brassica* chromosomes showed extensive rearrangement as compared to *Arabidopsis* (Lagercrantz and Lydiate 1996). The presence of at least 74 rearrangements (38 in A and 36 in C-genomes) occurred during the divergence from *Arabidopsis* (Parkin et al. 2005). These studies enrich our knowledge about recombination and polyploidization events during *Brassica* evolution.

1.10 Conclusion

The world food production must match the predicted increase in human population amidst climate change in the coming years. Food production is a complex process and involves understanding of the multiple pathways, signaling components, and interaction with biotic and abiotic factors. *Arabidopsis* represents Brassicaceae – an economically important family; therefore, information from this species can be used for crop improvement. The presence of mobile DNA or transposons and polyploidy may have led to genome expansion in the *Brassica* species. As most genes remain conserved in nature since the time of evolution, showing greater degree of synteny among them, it is worth pulling our limited resources and investing on a model organism, since it has been proved that many genes with known function in

Arabidopsis show similar effect across species. Now, as many more species like rice, poplar, grapes, soybean, and sorghum have been sequenced, we can compare these genomes for certain conserved trait across taxa. As more genomic data become available, extensive genome survey sequences and putative physical maps can be used to dissect the crop genomes and relate them to the *Arabidopsis* model. Moreover, in the coming years, as the *Brassica* genome sequencing information becomes available, comparative genomics work will gain momentum by analyzing *Arabidopsis* and *Brassica* data. This will facilitate crop breeding for increasing productivity, oilseed crop in particular. More components, which are important for deciding productivity, can be more thoroughly analyzed and better strategies can be developed for genetic manipulation.

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Chapter 2
Brassica

Ferdinando Branca and Elena Cartea

2.1 Taxonomy of the Genus

Brassica species belong to the Brassicaceae (= Cruciferae) family and some of them are widely used in human diet mainly as an important source of vegetables, condiments, and edible oils. The use of the related crops is cited in some ancient civilized regions such as in the Mediterranean and in Asia. *Brassica* taxonomic studies started since 1700 by Tournefort and were continued by Linnaeus (1753), De Candolle (1821), Hooker (1862), Baillon (1871), Prantl (1891), Schulz (1919, 1936), and Beilstein et al. (2006). The genus *Brassica* is the most economically important genus within the Brassicaceae family and belongs to the subtribe Brassicinae, one of the nine subtribes of the Brassiceae tribe that shares with other 18 tribes a wide gene pool, which over time has been utilized directly or indirectly to improve several crops. Different species of the subtribes Raphaninae and Moricandiinae seem to be closely related to Brassicinae as confirmed by a long series of investigations on the chloroplast-DNA (cp-DNA) and restriction sites (Warwick and Black 1991; Pradhan et al. 1992; Warwick et al. 1992; Warwick and Sauder 2005). These authors distinguished vertically among these three tribes of two lineages represented by Rapa/Oleracea and Nigra as suggested earlier by Erickson et al. (1983), Yanagino et al. (1987), Palmer et al. (1983), and Song et al. (1988a, b, 1990).

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Since the last century, several cytogenetic investigations were carried out to determine chromosome numbers and chromosome pairing in interspecific *Brassica* hybrids. The small sizes and absence of evident distinguishing marks on the chromosomes did not permit to clarify *Brassica* pachytene, which was recognized for 36 species (Schulz 1919, 1936). The cytogenetic relationship of the main species of the *Brassica* genus was depicted in the U triangle (U 1935; Fig. 2.1) in which *Brassica nigra* (L.) Koch ($n = 8$), *Brassica oleracea* L. ($n = 9$), and *Brassica rapa* L. ($n = 10$) represent the three diploid species in the vertices, and they developed by intercrossing to the three amphidiploid species, *Brassica carinata* A. Braun ($n = 17$), *Brassica juncea* (L.) Czern. ($n = 18$), and *Brassica napus* L. ($n = 19$). The genome A was attributed to *B. rapa L.* (= *B. campestris* in the past), the genome-B to *B. nigra* and genome-C to *B. oleracea*. The genome-A is carried by Chinese cabbage, sarson turnip, turnip greens, turnip, and turnip rape crops, which on a morphological basis are assigned, respectively, to the leafy, rapifera and oleifera types. The Chinese cabbage is economically important in Asia as salad; sarson turnip is a minor crop in Europe and in New Zealand where it is utilized for food purposes; turnip greens and turnip tops are highly used in Portugal and northern Spain for culinary uses (Padilla et al. 2005); and turnip rape is widespread in the North America for oilseed production (McNaughton 1995a). *B. campestris* has been renamed as *B. rapa* according to the International Code of Botanical Nomenclature. Oost et al. (1987) used the name since this variant has been largely adopted. The genome-B is possessed by black mustard, which is nowadays diffused in Europe as weed but was well known in the Middle Ages in Europe as condiment. The genome-C is represented by *B. oleracea,*

C. Kole (ed.), *Wild Crop Relatives: Genomic and Breeding Resources, Oilseeds*,
DOI 10.1007/978-3-642-14871-2_2,

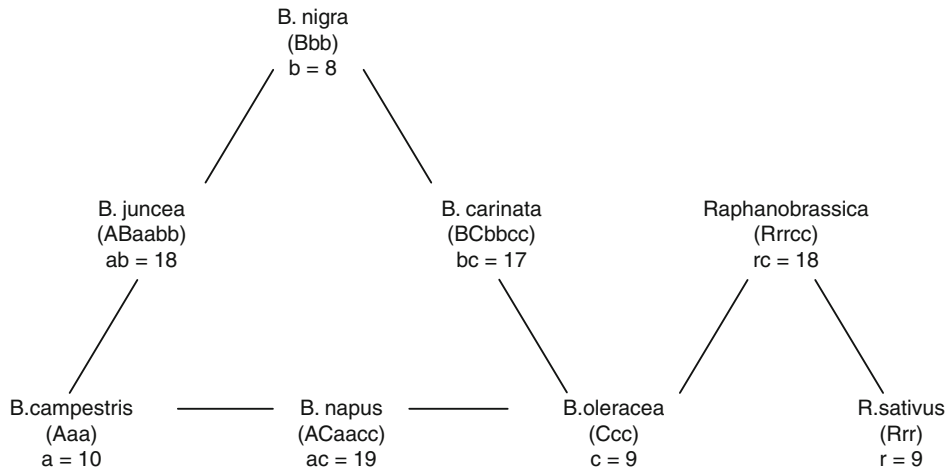

Fig. 2.1 U Triangle. In: Science, New Series, vol. 232, N. 4756, (Jun. 13, 1986), pp 1385–1389 published by American Association for the Advancement of Science

which diversified itself into several botanical varieties and related crops by domestication processes, such as var. *acephala,* var. *botrytis*, var. *capitata*, var. *gemmifera*, var. *gongylodes*, var. *italica*, and var. *sabauda*, which are represented, respectively, by kale, cauliflower, cabbage, Brussel's sprout, kohlrabi, broccoli and Savoy cabbage crops (Linnaeus 1753; Lamarck 1784; De Candolle 1821).

Among the amphidiploid species, *B. carinata* is represented by Ethiopian mustard diffused in Abyssinian Plateau derived from the union of the BB and CC-genomes; *B. juncea* by Indian mustard cultivated in Asia derived from the union of AA and BB-genomes; and *B. napus* mainly by oilseed rape grown in Asia, Europe, and North America derived from the union of the AA and CC-genomes (McNaughton 1995b). The genetic resources available for the breeding of *Brassica* crops are regulated by the genetic boundaries of their primary, secondary, and tertiary gene pools (Harlan 1975). *B. oleracea* represents the primary gene pool by itself, but several studies have been carried out to investigate the other gene pools and their potential utilization. The secondary gene pool was investigated by studies on the pachytene chromosome morphology, which permitted to identify the basic genomes of *Brassica* crops: AA ($2n = 20$) for *B. rapa*, BB ($2n = 16$) for *B. nigra*, and CC ($2n = 18$) for *B. oleracea*. Investigations on genomic libraries of *B. napus* and *B. oleracea* showed shared fragments among A, B, and C-genomes, suggesting their partial homology and the origin of the amphidiploid species *B. napus, B. carinata*, and *B. juncea* from the parental diploid ones (Hosaka et al. 1990; Slocum et al. 1990). The phylogenetic studies explain the evolution of *Brassica* and allied genera from a common ancestor with $n = 6$ through increase in the number of chromosomes and partial homology of A, B, and C-genomes (Prakash and Hinata 1980; Song et al. 1990).

Finally, the tertiary gene pool includes species and genera related to Brassica crops in 36 cytodemes capable of genetic flux, such as *Diplotaxis, Enarthrocarpus, Eruca, Erucastrum, Hirschfeldia, Rhynchosinapis, Sinapis, Sinapodendron,* and *Trachystoma* genera (Harberd 1976). These gene pools can confer favorable alleles and useful characteristics by using special methodologies. Tissue culture techniques, ovary and embryo rescue, and protoplast culture facilitated introgression of useful genes overcoming genetic boundaries.

In the last decades, a significant knowledge on pachytene studies was obtained through extensive use of molecular markers. In fact, molecular studies in *Brassica* species started with the determination of female parents of allopolyploid species using chloroplast-DNA (Palmer et al. 1983; Fukui et al. 1998) using genomic in situ hybridization (GISH) and fluorescence in situ hybridization methodology (FISH), in combination with ribosomal DNA markers (Schelfhout et al. 2004; Maluszynska and Hasterok 2005; Wang et al. 2005; Snowdon 2007). These methodologies

helped investigation on phylogenetic relationship within *Brassica* species and also with other related genera.

These studies have started to point out the potential germplasm of interest for Brassica genetic improvement that overcomes the biological boundaries. In addition, genomic investigations on *Arabidopsis thaliana* have facilitated understanding of evolution in the Brassicaceae family, especially for *Brassica* genus (*Arabidopsis* Genome Initiative 2000). These recent studies have indicated the constitution of the Brassica coenospecies formed by *Brassica* and allied taxa prefigured by Harbered (1972).

Even though the Himalayan region seems to be the primary center of diversity for Brassicaceae, from where dispersion extends to from North African and European Atlantic coasts to Saharo-Sindian phytochoreas, the southwest Mediterranean area seems to represent the secondary one, if not the primary, in the light of the recent evolutionary evidence recorded (Hedge 1976; Prakash et al. 2009).

With regard to morphological characters, high variation is evident among Brassiceae coenospecies with respect to cotyledon, adult leaf, and fruit shape (Gómez-Campo and Tortosa 1974; Prakash et al. 2009). The cotyledons are from small, slightly longer for *Diplotaxis* to wider with deeper notch for *Brassica, Raphanus, Coincya*, and *Sinapis*. Adult leaf typologies are (1) simple, entire to shallowly lobed; (2) lobed to pinnatifid; (3) pinnatisect with sinuses reaching the midnerve; (4) pinnatisect with reduced number of lateral segments (Prakash et al. 2009). The siliqua shows big variation for heteroarthocarpy, size, rib, rugosety, and wing. The evolutionary progress in *Brassica* species seems to be represented by the presence of seeds within the stylar cavity (Gómez-Campo 1999b). Half of the genera of this tribe present several types of seeded beaks showing heteroarthrocarpy. On the latter character, Gómez-Campo (1999a) formulated the "isthmus concept" of Brassiceae evolution and individuated in *Diplotaxis* genus the "bridge." Heteroarthropic siliqua with different beak size and shape is observed in *Erucastrum, Hirschfeldia, Sinapis, Coincya, Eurcaria, Trachystoma, Raphanus, Enarthrocarpus*, and *Brassica* genera. Although heteroarthrocarpy seems to represent an evolutionary crossroad, it does not support a monophylogenic evolution as showed by the chloroplast lineages distributed on both side of the "isthmus" (Prakash et al. 2009).

2.2 Conservation Initiatives

In the past decades, an important loss of natural genetic diversity of many crops has been observed due to many factors such as the introduction of new F_1 hybrids, droughts, changes in food habits and agricultural practices, and human activities such as deforestation and migration from rural to urban areas. This process is known as genetic drift. The loss of genetic variability represents not only the loss of wild germplasm but also the loss of evolved landraces resulting from the interaction of environmental selection with the genes present in both wild and cultivated populations. The *Brassica* genus has not been an exception and, in particular, conservation of wild *B. oleracea* species has been a high priority. During the 1970s, wild germplasm of *Brassica* was extensively collected and cytogenetic studies were started. Intensive efforts were made in the last decades to search and collect this material that, otherwise, would be irreversibly lost (Gómez-Campo et al. 2006, 2007). After 1970s, the introduction of the concept of biodiversity was a strong support for many improvements in ex situ and in situ conservation strategies.

Seed banks were created to maintain the genetic diversity of many crops, to minimize genetic erosion, and to supply seed material of landraces and of wild crop relatives for research. Most of the *Brassica* collections are conserved by means of seeds and, in general, they are conserved under long-term storage conditions to maintain seed viability for many years. The only exception within *Brassica* crops is a perennial kale (*B. oleracea* L. var. *ramose* DC) that can only be vegetatively propagated due to the loss of its ability to flower (Gómez-Campo 1999a).

Ex situ conservation of plant genetic resources in gene banks involves collecting traditional varieties and landraces from around the world and, in particular, from centers of genetic diversity of specific crops. The ex situ conservation also involves the selection of accessions to be conserved and the maintenance of these accessions for current and future users by regeneration. Decisions concerning both these aspects require knowledge about the distribution of genetic diversity within and between accessions sampled from the gene pool. However, they also require knowledge about changes in the variation of these samples as a result of regeneration activities.

One of the largest collections of wild *Brassica* species and allied cruciferous genera is kept by the Universidad Politécnica of Madrid (UPM), Spain. This seed bank was created in 1966 and its aim was the long-term ex situ conservation of wild taxa, thus making the accessions available for being used by researchers and breeders. The Plant Germplasm Bank from the UPM includes 600 crucifer accessions and rare and endangered species widespread in the western Mediterranean area and it is available at *http://www.etsia.upm.es/ANTIGUA/DEPARTAMENTOS/biologia/documentos/GC-2000-Int.htm*. Since 1982, several expeditions have been carried out by Professor Gómez-Campo from the UPM and his collaborators in order to rescue and collect Mediterranean populations of wild *Brassica* species. These missions were supported by the International Board for Plant Genetic Resources (IBPGR), later International Plant Genetic Research Institute (IPGRI), and now Bioversity International and were performed in the Mediterranean coast of Spain, Italy, Greece, and Tunisia and along the Atlantic coast of northern Spain, France, and the UK. As a result, different wild *B. oleracea* species with a chromosome number of $n = 9$ (including Atlantic *B. oleracea*) were collected. Four wild *B. oleracea*-related species were found in Sicily (*B. rupestris*, *B. incana*, *B. villosa*, and *B. macrocarpa*). Gómez-Campo and Gustafsson (1991) described the accessions collected in detail and the new locations found. According to the IPGRI policy, each sample was split into three parts, which were stored at the UPM (Spain), the University of Tohoku (Sendai, Japan) and also at seed banks of those countries, where the collection was done (Izmir, Turkey; Thessaloniki, Greece, Bari, Italy; Porquerolles, France; Kew, UK). Recently, two new expeditions have been carried out by the UPM team. The first one targeted the northern coast of Spain (Gómez-Campo et al. 2005) and the second one was focused on the northeastern coast of Spain in search of new localities and seeds of *B. montana* (Gómez-Campo et al. 2007).

In 1983, that collection was designated as the Global Base Collection for Wild Crucifers by the IBPGR, and in 1994, it was honored with the National Award for Environment by the Government of Spain. Recently, it has been included in the Global Biodiversity Information Facility database (*http://www.gbif.es*). The International Treaty on Plant Genetic Resources for Food and Agriculture that has recently been approved established a Multilateral System for having a facilitated access to the germplasm of a number of crops. This includes vegetables such as the Brassica complex with possible implications on the use of the diversity of these crops in the near future.

In Europe, and under the aegis of the European Cooperative Program for Crop Genetic Resources Networks (ECP/GR), a working group on Brassicas was established since 1991. One of the main efforts of this group has been to set up a European Brassica database (Bras-EDB), which was developed by the Center for Genetic Resources, Netherlands (Boukema and van Hintum 1998; *http://documents.plant.wur.nl/cgn/pgr/brasedb/*). This database includes cultivated materials as well as wild ones and contains 36 collections from 22 countries and more than 19,600 accessions. A list of wild *B. oleracea* species included in the European *Brassica* database is shown in Table 2.1. Major updates of Bras-EDB were done in 2001 and 2005, supported financially by the European Commission by means of the project RESGEN CT99 109-112: "*Brassica* collections for broadening agricultural use, including characterizing and utilizing genetic variation in *B. carinata* for its exploitation as an oilseed crop." The major aim was to create a core collection of the four *Brassica* species included in the project (*B. oleracea*, *B. rapa*, *B. napus*, and *B. carinata*). This project was an important attempt to unify efforts on Brassica germplasm within the EU and it was complementary to the activities of the ECPGR Working Group on *Brassica*.

Although wild species are included in ex situ collections, most of them are very difficult to regenerate ex situ to make them readily available to users. In this case, germplasm is conserved in its natural habitat (nature reserves) by specific in situ conservation activities. A strategy for in situ conservation of wild species related to *B. oleracea* has been elaborated by Maggioni et al. (1997). The implementation of a strategy for in situ conservation of wild species of the *B. oleracea* cytodeme has been recently suggested by the ECPGR Working Group on Brassica as a complementary way of preserving the diversity of these Mediterranean relatives of cultivated *Brassica* species with $n = 9$. Priority was assigned to the Sicilian center of diversity, where the level of variability is very high and the populations of *B. incana, B. macrocarpa*, *B. rupestris*, and *B. villosa* are often threatened by

Table 2.1 List of wild $n = 9$ *Brassica* species included in the European *Brassica* database and from the U.P.M. Crucifer Seed Bank

Species	Subspecies	Number accessions (Bras-EDB)[a]	Number accessions (UPM-seed collection)[b]
Brassica albogabra		62	1
Brassica bourgeaui		5	2
Brassica cretica		34	2
Brassica cretica	*aegaea*	25	5
Brassica cretica	*cretica*	38	
Brassica cretica	*laconica*	12	2
Brassica drepanensis		5	
Brassica hilarionis		4	1
Brassica incana		39	10
Brassica insularis		25	5
Brassica macrocarpa		9	2
Brassica montana		46	8
Brassica oleracea			9
Brassica oxyrrhina		1	1
Brassica rupestris		25	6
Brassica villosa		25	8

[a]Available from *http://documents.plant.wur.nl/cgn/pgr/brasedb/*
[b]Available from *http://www.etsia.upm.es/ANTIGUA/DEPARTAMENTOS/biologia/documentos/GC-2000-Int.htm*

human activities (Figs. 2.2 and 2.3). The objective will be the evaluation of these wild species under a different point of view (DNA analysis, morphological traits, and quality aspects focused on oils and nutraceutical compounds). The role of the Working Group is seen as a contribution to highlight the usefulness of the wild germplasm for breeding purposes and to select the most appropriate accessions of the future European Genebank Integrated System (Astley et al. 2007).

Since 2007, AEGRO GENRES project founded by the European Union deals with a basic research for an Integrated European in situ management work plan to implement Genetic reserves and "on farm" concept. The case of study is related to *Avena*, *Beta*, *Brassica*, and *Prunus* with a view to develop in situ management work plans for conservation of crop wild relatives (CWR) and landraces. For case study on Brassica, the attention has been paid on a Sicilian wild *Brassica* with $n = 9$ widespread in the Island. These studies have to contribute to the development of a CWR in situ conservation strategy for Brassica in Sicily, which will form part of the European integrated work plan for management of CWR (*http://aegro.bafz.de*).

Brassica diversity conservation has been stimulated by setting up a specific core collection named Diversity Foundation Sets (DFSs), designed to represent "an informative set of genetically fixed lines representing a structured sampling of diversity across a genepool," which is under development at the Warwick HRI. These collections are based on founder accessions sourced from ex situ genetic resource collections (see *http://www.Brassica.info/diversity/diversity_sets.htm*). They are designed to represent the diversity within the *B. oleracea* crop gene pool (BolDFS) whilst the *Brassica* C-genome Diversity Fixed Foundation Set (BCgDFS) aims to fix the diversity of *B. oleracea,* which represent the C-genome with the wild *Brassica* species ($n = 9$) that could be its wild relatives.

One of the main problems that germplasm curators must face is to maintain collections in active banks in good conditions of viability to minimize the need for regeneration (Gómez-Campo et al. 2006). Regeneration of *Brassica* is very costly. Therefore, good storage conditions are essential in order to maintain the seed viability. Gómez-Campo (2002) evaluated 40 different types of containers according to their ability to exclude water vapor, by using silica gel with a cobalt indicator. Only sealed brass cans, "Kilner" jars with rubber seals, laboratory bottles normally used for liquid chemicals, or flame-sealed glass ampoules were considered to be safe for use in long-term preservation. The 36 remaining containers allowed moisture to enter within 2 or 3 years or less. Currently, Brassica seeds at the UPM are kept in flame-sealed glass vials,

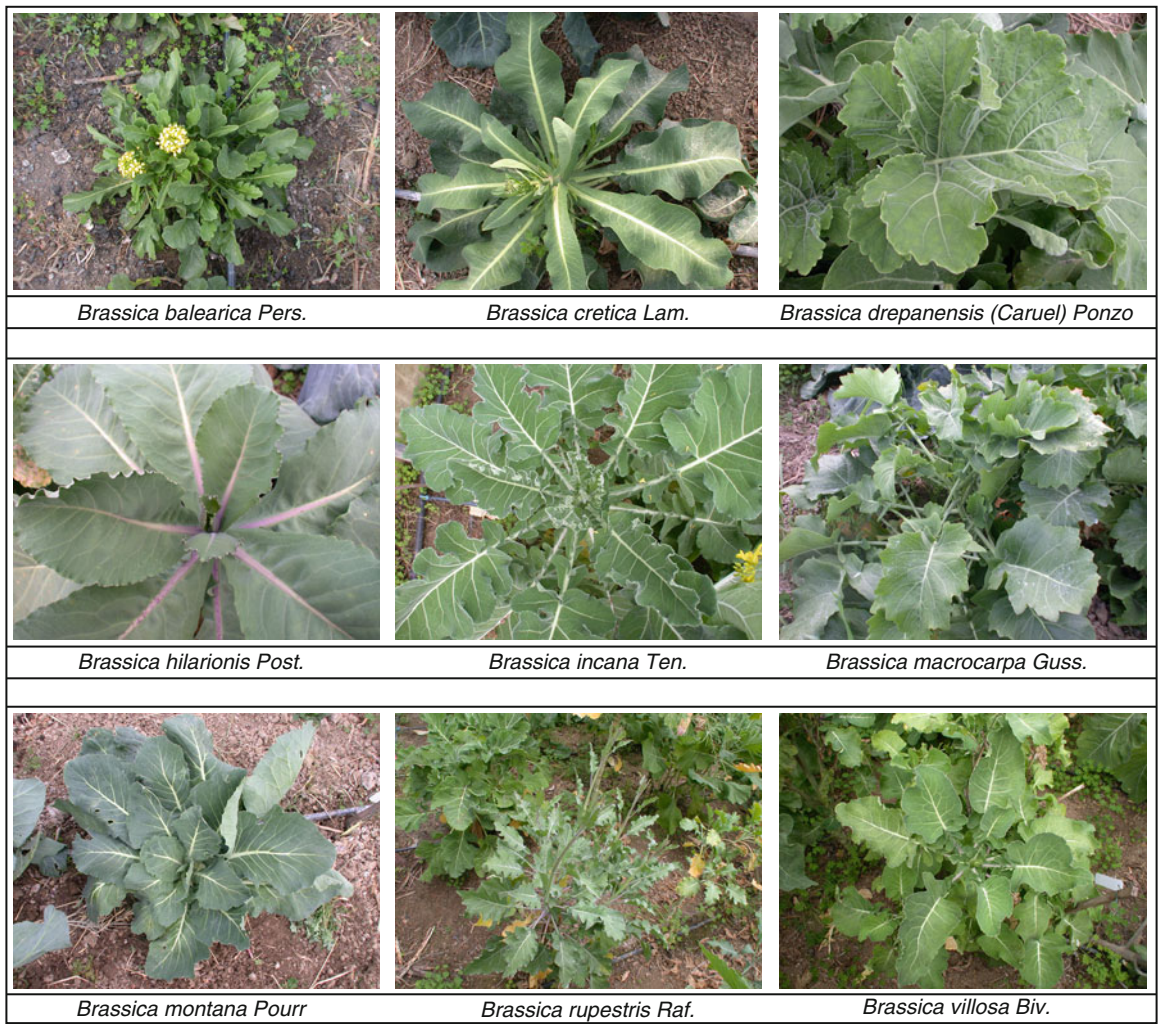

Fig. 2.2 Plant morphological diversity of Brassica wild species

having silica gel to ensure low moisture content, because this method is very convenient for small-sized seeds as those of Brassicas. Other possibilities to adapt this method to crop species have been explored (see *www.seedcontainers.net*). More recently, Pérez-García et al. (2007, 2008) concluded that the seed preservation method based on silica gel and low temperature (−5°C and −10°C) have proved to be highly efficient for Brassicaceae and other plant families and proposed the possibility of using ultra-dry methods for medium and long-term storage of orthodox seeds.

Another method to preserve seed germplasm is cryopreservation. It consists of storing the material at temperatures near that of liquid nitrogen (−196°C). Under these conditions, all enzymatic processes are practically halted, and it is thought that any type of biological plant material (meristems, embryos, pollen, seeds, somatic tissues, etc.) can thus be preserved for an infinite period of time. For an efficient cryopreservation, it is fundamental to avoid the intracellular formation of ice crystals, which are highly damaging for the cell internal structures.

Pérez-García et al. (1996) evaluated the effect of cryopreservation on seeds of seven wild and cultivated *Brassica* taxa. They concluded that *Brassica* seed cryopreservation is a suitable procedure for the long-term maintenance of seed accessions of this genus (wild and cultivated species) in seed banks. Low

Fig. 2.3 Plant morphological diversity of Brassica wild species

temperature preservation is very effective not only for maintaining germplasm but also for the storage of mature pollen, vegetative stems, cell and protoplast suspensions, and microspores of many species. Microspore cryopreservation is a potentially powerful method for long-term storage of germplasm destined to in vitro embryo production in plant species. Charne et al. (1988) described the method of cryopreservation of isolated microspores of rapeseed in liquid nitrogen without loss of embryogenic capacity and proposed this approach as a useful method to increase the efficiency of the rapeseed haploid system. On the other hand, Chen and Beversdorf (1992) found that isolated microspores of *B. napus* could be stored stably for an extended period of time by using cryopreservation and proposed that this storage system can be used in the

rapeseed breeding program to produce doubled haploid lines.

In the US, the database for Germplasm Resources Information Network (GRIN) maintain at the Beltsville Agricultural Research Center the accessions held by the Regional Plant Introduction Stations. This collection is duplicated and stored for a long term at the National Seed Storage Laboratory at Fort Collins (ARS-GRIN 1997). In China, the Chinese Genetic Resources Information System – CGRIS – supports the national network of regional gene banks coordinated by the Institute of Crop Germplasm Resources, which has the responsibility for the long-term conservation of genetic resources.

2.3 Origin and Evolution of Allied Crops

The relationship among the different *Brassica* species started to be explained by Morinaga (1934) and U (1935) with the already-cited U-triangle, and according to them, the diploid species *B. rapa* (AA-genome), *B. nigra* (BB-genome), and *B. oleracea* (CC-genome) originated along the same time as the allotretrapoid species *B. juncea* (AABB), *B. napus* (AACC), and *B. carinata* (BBCC). During domestication process of each species, divergent selection enriched the diversity of the correspondent cultivars and crops. Natural hybridization events have been the basis of genome evolution of *Brassica* and interspecific crosses enabling gene exchange contributed significantly to the differentiation of the genus by generating new types or species, allowing gene exchange across boundary species. Of course, genome similarity is required to ensure chromosome pairing and genetic recombination (Leflon et al. 2006). Several studies have permitted to study the possible phylogeny of the species also by the creation of new species since 1920s when Karpechenko developed the synthetic genus *Raphanobrassica* by crossing of *Raphanus sativus* with *B. oleracea* var. *capitata* to combine their desirable traits.

Archeological evidences of the main diploid species suggest *B. rapa* (turnip rape) and *B. nigra* (black mustard) to be the first domesticated species similarly as the amphidiploid *B. juncea* (Indian mustard) that originated from crosses among the two former species. Cytogenetic evidences suggest that the evolution of the diploid species started with *B. nigra* and was followed by *B. rapa* and *B. oleracea*. Molecular studies discard the monophyletic origin and suggest *B. oleracea* and *B. rapa* to have a common origin and the same progenitor, whereas *B. nigra* evolved from another one (Namai 1976; Prakash and Hinata 1980; Song et al. 1988a; Pradhan et al. 1992; Palmer et al. 1983). This is confirmed by the two lineages "nigra" and "rapa/oleracea" of subgen. *Brassica* as suggested by several authors (Song et al. 1990; Warwick and Black 1991; Pradhan et al. 1992). The former lineage show higher affinities with the genera *Hirschfeldia* and *Sinapis* and with some species of *Diplotaxis* and *Erucastrum*, whereas the latter with all the species belonging to Sect. *Brassica,* Sect. *Rapa* and Sect. *Brassicoide,* and with the species *B. barrelieri* and *B. oxyrrhina* of the Sect. *Sinapistrum* (Gómez-Campo 1999a).

Alloploid species were originated by unidirectional natural interspecific hybridizations, whereas *B. nigra* and *B. rapa* were the cytoplasmic donors of *B. carinata* and *B. juncea,* and *B. oleracea* was the cytoplasmic donor of *B. napus* (Erickson et al. 1983; Palmer et al. 1983; Warwick and Black 1991; Pradhan et al. 1992). The evolution of the species was identified by the mitochondria and chloroplast genomes, which are co-inherited and thereby could evidence for the more recent origin of the allopolyploid species. Among them, *B. juncea* seems to be originated earlier in comparison to *B. carinata* and *B. napus*. The analysis of the cytoplasmatic genomes offered by maternal parents indicated stable genome for *B. juncea* and *B. carinata,* whereas *B. napus* seems to have a polyphyletic origin where *B. oleracea* seems to play an important role (Song and Osborn 1992; Parkin and Lodiate 1997). This fits with the hypothesis that *B. oleracea* was originated later than other *Brassica* diploid species (Quirós et al. 1985).

B. nigra (L.) Koch. is well known since the Greek civilization for its medicinal proprieties (Hippocrates 480 BC). This species is widespread in the Mediterranean basin and in some central Asian and Middle East areas and is cited as "mustard" in the New Testament for its fast growing habit. During that time, attention had been paid to similar uses of *B. juncea* and *B. carinata* in spite of *B. nigra,* which contributed as parent to the origin of both the former species. The proposed ancestor of *B. nigra* is *Sinapis arvensis,* which show high homology in terms of nuclear

DNA, cp-DNA, and protein fraction with *B. nigra* (Song et al. 1988a; Warwick and Black 1991; Pradhan et al. 1992; Poulsen et al. 1994). In addition, high degrees of pairing and similar genetic sequences were detected for the interspecific hybrids of *B. nigra* × *S. arvensis* (Mizushima 1950; Cheng and Heneen 1995). Other candidate ancestors of *nigra* lineage seem to be *Hirshfeldia incana* ($n = 7$) with some *Erucastrum* species, of which fruits are less specialized in comparison to the species, and those belonging to the Sect. *Rhynchocarpon* of *Diplotaxis* and to *Sinapidendron* genera, the latter showing seedless beak Gómez-Campo (1999a). High degree of similarity for nuclear DNA and cp-DNA has been ascertained also for *B. fruticulosa* (Takahata and Hinata 1983; Song et al. 1990; Warwick and Black 1991; Pradhan et al. 1992).

Turnip is widespread in natural habitat from Mediterranean to central Asia as a weed and probably was the first Brassica domesticated because it is very rustic, invasive, and easy to grow. In addition, it has several uses, and for that, it has been considered along the time as a multifunctional crop. Since some millennia ago, *B. rapa* (syn. *B. campestris*) was domesticated to use its roots, young flowering shoots, and seeds by several civilizations. Turnip was recovered from the Neolithic sites and was proposed in cultivation around 2500–2000 BC (De Candolle 1886; Hyams 1971). Plinius (23–79 AD) distinguished domesticated forms having flat and round roots from wild ones with big roots, whereas Columella (ca. 60 AD) mentioned types called as "Long Roman," "Round of Spain," "Syrian," "White," and "Egyptian."

Leafy vegetable forms were differentiated in western Asia from oilseed forms of *B. rapa* introduced through central Asia, and among them, pak-choi (subsp. *chinensis*) was the first to be utilized in China (Li 1982). The subsp. *pekinensis,* well known as Chinese cabbage, originated around the tenth century from the hybridization between pak-choi and turnip and that has been confirmed by restriction fragment length polymorphism (RFLP) analysis (Song et al. 1988b). The European oleiferous *B. rapa* forms were developed in the Mediterranean basin whereas the Asian forms in the central Asia and Northwest India. In the latter area are distributed the brown sarson, toria, and yellow sarson. The oldest form seems to belong to the brown sarson, which is different from toria by plant morphology and size, and growing period, whereas the yellow sarson is distinguished by yellow colored seed and self-compatibility.

Yellow sarson is cited in the ancient Asian literature around 1500 BC (Prakash 1961; Watt 1989) and *B. rapa* seeds were identified in the stomach of a Tollund man who lived in Denmark in the fourth century BC (Renfrow 1973). Several authors agree with the comparative morphological studies of Sun (1946), which proposed two evolutionary lines of *B. rapa*, one western, in Europe and central Asia, where turnip and oilseed forms were domesticated, and the another eastern, in East Asia, in the areas of diversification of several vegetable forms. These two independent centers of origin of *B. rapa* are supported by morphological, geographical, and molecular evidences (Denford and Vaughan 1977; Song et al. 1988b).

B. oleracea, for example, is represented by several varieties, which originated several crops very different for growth habits and organ morphology. Among these, var. *acephala,* which is represented by several and diversified landraces of kale in the Mediterranean European countries, seems to be the evolutionary bridge for the other varieties of *B. oleracea*. In fact, the great variability exhibited by a core collection of European kale landraces studied by the Universities of Alnarp and Catania seems to support this hypothesis. In any case, kale landraces are often widely present in Europe in areas where the wild *Brassica* species related to *B. oleracea* are diffused and all of them are perennials. Furthermore, some wild *Brassica* species sprouts until now and are gathered and utilized in some Sicilian villages when young inflorescences start to flower to utilize the fleshy leaves and the tender shoots. Among all the varieties of *B. oleracea*, only var. *acephala* shows this characteristic, and its variants are cited in ancient Greek and Latin literature (Maggioni et al. 2010). Several authors indicate the Mediterranean basin as the center of diversity of *B. oleracea*, at least for broccoli and cauliflower, where several wild *Brassica* species ($n = 9$) are widespread and show great diversity (Gray 1982; Smith and King 2000). Recently, linguistic and literary considerations on the origin and domestication of *B. oleracea* crops suggest that its domestication process started in ancient Greek-speaking areas of the central and East Mediterranean areas (Maggioni et al. 2010). In any case, the *B. oleracea* gene pool is very rich in Sicily where *B. drepanensis, B. incana, B. macrocarpa, B. rupestris*, and *B. villosa* are

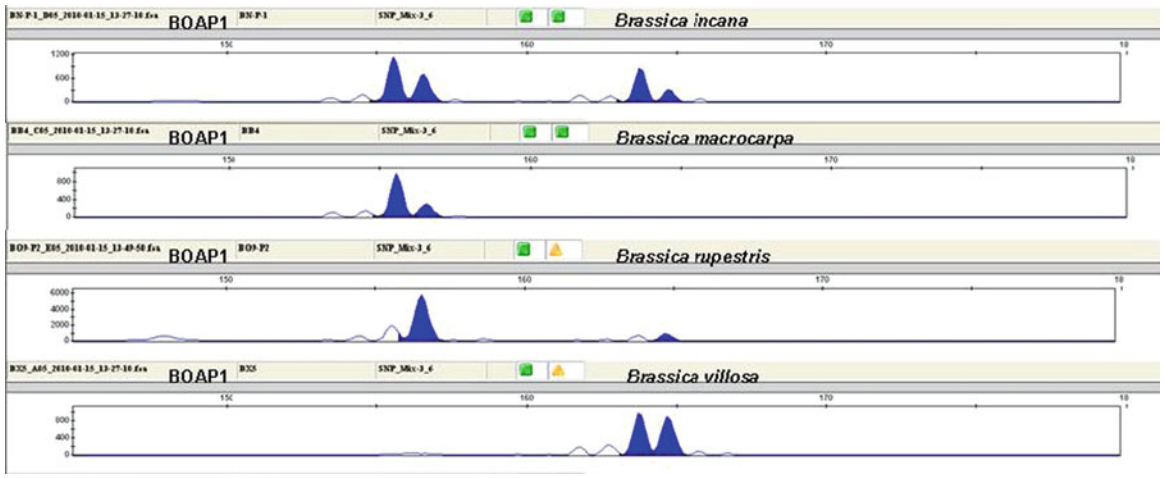

Fig. 2.4 Tetrasomy ascertained for BoAP1 SSR primer for some genotypes of crop wild relatives of Brassica widespread in Sicily

widespread, and in some areas, they are strictly associated among themselves and with *B. oleracea* crops (Branca 2008). Snogerup (1980) suggested multiple origins of several *B. oleracea* crops, which are the Atlantic coast for cabbage and the Mediterranean basin for kale, broccoli, and cauliflower. Glucosinolate profile of these wild species is also diversified in Sicily and, in some cases, is specific for each species (Song et al. 1990; Horn and Vaughan 1983; Mithen et al. 1987; Velasco and Becker 2000; Branca et al. 2002). In any case, F_1 hybrids between *B. oleracea* and wild *Brassica* are often fully fertile (Mithen et al. 1987; von Bothmer et al. 1995; Faulkner et al. 1998; Gómez-Campo 1999a).

Recent DNA analysis with molecular techniques support high similarity between Sicilian wild *Brassica* species ($n = 9$) and *B. oleracea* (Song et al. 1990; Lanner et al. 1996; Lázaro and Aguinagalde 1996; Lanner 1998; Tatout et al. 1999; Geraci et al. 2001). Recently, more similarity was observed between *B. oleracea* and the Mediterranean wild *Brassica* species than the UK *B. oleracea* wild types (Allender et al. 2007). Utilizing the simple sequence repeat (SSR) marker BoAP1, higher number of alleles were found in the wild *Brassica* species than in cabbage and cauliflower.

BoAp1-a locus located in a single genomic region on linkage group O6 chromosome of *B. oleracea* with the other ones (BoCAL, BoLFY, BoAP1-c, BoREM1, BoFULL, etc.) is related to MADs-box genes involved in flower development and evolution (Duclos and Björkman 2005). This O6 chromosomal region is strictly related to self-incompatibility controlled by the *S*-locus. This genomic region seems to be the key for selection while evolution of cauliflower that had been a subject of heavy pressure for diversification of *B. oleracea* originally utilized for its vegetative organs. Recently, characterization of Sicilian wild *Brassica* species carried out in the frame of the EU GENRES-AEGRO project, based on biomorphological, biochemical, and molecular descriptors, has started to provide important information on the genetic diversity of the *BoAP1-a* alleles of several wild populations in comparison to landraces of broccoli, cauliflower, and kale. Single nucleotide polymorphism (SNP) DNA sequence related to the BoAP1 SSR marker showed diversity of alleles for this locus. In addition, in some wild populations near villages, where *B. oleracea* crops are usually grown in several home gardens, have individuated trisonomy and tetrasomy, the signs of interspecific hybridizations with landraces (Fig. 2.4). Several studies are in progress to gain comprehensive knowledge on the genetic diversity in Sicily with a view to individuate adequate methodologies for on-farm and in situ conservation and, in the latter case, to establish genetic reserve for *Brassica*.

2.4 Role in Crop Improvement Through Traditional and Advanced Tools

Considerable progress has been accomplished in the cellular and molecular biology of *Brassica* species in the past years. The use of molecular markers in marker-assisted selection and breeding, genetic

transformation technology for the introduction of desirable traits and a comparative analysis of these traits are important components of the current research on this genus. Research priority in the *Brassica* genus was initially focused on polyploidy breeding. This original aim was later modified to the exploitation of wild allies for introgression of nuclear genes for desirable traits, cytoplasmic substitutions, and construction of chromosome maps. In the long history of the variety development of Brassica crops, genetic introgression from wild donor plants was a major approach for the introduction of valuable genes and traits.

Crosses between and among *B. oleracea* and C-genome relatives are known to produce fertile or semi-fertile offspring (Kianian and Quiros 1992; Gómez-Campo 1999a). Thus, transfer of desirable genes governing qualitative and quantitative characters from wild allies into cultivated forms can be achieved both by conventional crosses and biotechnology, depending on the relatedness and crossability of the donor wild species with Brassica crops. The new technologies have facilitated breeding programs, increased the efficiency of locating desirable traits, and have opened up new opportunities for using genes that were previously inaccessible. The level of success to transfer useful genetic variation from wild sources through crosses depends on many factors such as the extent of diversity that can be accessed to introduce useful variation, the risk to introduce deleterious traits, the possibility to use a particular valuable allele in different genetic backgrounds, and the efficiency with which useful alleles can be transferred. Moreover, most of the wild species are difficult to exploit in research programs mainly because of their extended vegetative phase or due to the difficulty to obtain homozygous material (doubled haploid lines) by in vitro culture.

As the wild germplasm belongs to second and tertiary gene pools, several kinds of hybridization operate. Consequently, many of the wild species are sexually incompatible with the crop species, thus making the genes present in wild forms inaccessible. Sexual incompatibility has been overcome by advances in cellular and molecular biology, which facilitate transfer of desirable genes into plants and cloning and manipulation of genes. Several forms of manipulations have been carried out to obtain sexual hybrids as bud pollinations, grafting or mixed pollinations, and subsequent ovule or embryo rescue techniques (Inomata 1985). In addition to traditional breeding methods, interspecific and intergeneric crosses have been facilitated with various approaches, such as somatic cell genetics and recombinant DNA techniques. Interspecific and intergeneric hybrids in Brassica crops produced by sexual and asexual hybridization, embryo rescue, and genetic manipulations have been described in several reviews (Glimelius 1999; Christhey 2004).

In the recent past, the development of in vitro techniques, such as ovary and embryo culture and somatic hybridization, has increased greatly. The embryo culture technique allows overcoming the post-fertilization barriers between distant related species, while the somatic approach becomes the best method of choice to realize hybridization where pre-fertilization barriers exist. Somatic hybridization has been extensively used in the Brassicaceae with the additional merit of inducing cytoplasmic variability and recombination of cytoplasmic and nucleic genomes, which is not possible through conventional methods of sexual hybridization (reviewed by Glimelius 1999; Christhey 2004; Liu et al. 2005).

The introgression of valuable traits by interspecific hybridizations from wild Brassicas can be traced back in literature to 1950 for sexual cross and to 1979 for somatic hybridization (Prakash et al. 2009). Despite the advantages, somatic hybridization also has some drawbacks. The technique needs to be improved since only a limited number of hybrids are produced in many experiments, thus reducing the possibility of selecting usable plants among hybrids. A detailed review of the intrageneric, intergeneric, and intertribal somatic hybrids along with the traits of interest incorporated has been published by Glimelius (1999).

Regarding *B. oleracea* wild relatives, hybrids between them and their cultivated forms resulted to be at least partially fertile. Hybrids like *B. napus* plants derived from in vitro culture of embryos resulting from crosses between *B. cretica* and *B. rapa* were obtained by Inomata (1985) and hybrids obtained from this cross may be valuable in broadening the narrow genetic base of oilseed rape. Other interspecific hybrids by embryo rescue between *B. cretica, B. montana*, and *B. bourgeaui* with *B. napus* and *B. rapa* were obtained by the same author (Inomata 1986, 1987, 1993, 2002). On the other hand, Prakash and Chopra (1990) and Mithen and Herron (1991)

obtained hybrids by sexual hybridization between *B. oxyrrhina* (as female) and *B. rapa* (as male) and between *B. atlantica* (as male) and *B. rapa* (as female).

Wild *Brassica* species possess a number of useful agronomic traits. They have been widely used in plant breeding programs to broaden the genetic base in most Brassica crop species. They have also been used as sources of donor elite alleles, controlling economically important quantitative traits including crop production, disease and pest resistance, tolerance to abiotic stresses (cold, salt and drought conditions), and specialty components of quality attributes (seed oil or glucosinolates) (Ramsey and Ellis 1994). Moreover, wild relatives could be incorporated into breeding programs, including cytoplasmic and nuclear male sterility for the development of hybrid seed production. Warwick et al. (2000) published a guide to wild *Brassica* germplasm that provides information on their growth form, chromosome number, geographical distribution, and quality and agronomic traits of interest. Examples of wild relatives of *B. oleracea* as potential sources of desirable traits are shown in Table 2.2.

Regarding biotic stresses, it has been found that wild *B. oleracea* species can carry important resistance traits related to biotic stresses, and a number of potential sources of resistance are available among wild allies against various pathogens. Gene introgression from wild relatives by using different approaches (sexual hybridization, embryo rescue, protoplast fusion, and genetic transformations) can be found in the literature. For instance, it has been found that wild *B. oleracea* populations from Sicily are resistant against the flea beetle disease (Palaniswamy and Bodnaryk 1994); *B. incana* has been proved to be the best source of resistance against *Verticillium wilt* among different cultivated and wild forms of *B. oleracea* species including *B. cretica*, *B. incana*, *B. insularis*, and *B. villosa* (Happstadius et al. 2003). Because sources of resistance to this fungal disease are not found in the oilseed rape germplasm, finding of these results are of great interest. Regarding pest resistance, Ellis et al. (2000) found germplasm resistant to *Brevicoryne brassicae* within wild *B. oleracea* including *B. villosa* and *B. incana* and also identified sources of resistance to *Delia radicum* in wild species (Ellis et al. 1999). In general, satisfactory genetic control of pathogens and virus diseases has not been achieved by using wild *Brassica* species. This is primarily due to the absence of sources of resistance for the most severe pathogens of Brassica crops. Thus, there is an urgent need to develop methods for identifying resistance genes in the wild species. Informative reviews have been published on pest resistance (Earle et al. 2004) and disease resistance (Tewari and Mithen 1999).

Regarding the quality attributes related to the seed fatty acid composition, the use of *B. villosa, B. incana,* and *B. rupestris* as sources of high erucic acid, which is highly appreciated for different industrial uses, merits a special mention (Velasco et al. 1998). The potential use of several wild *B. oleracea* species, mainly *B. villosa* as a donor of beneficial glucosinolates, such as glucoiberin or glucoraphanin that are closely related to human health due to anticarcinogenic properties has also been reported (Mithen et al. 1987; Faulkner et al. 1998). Among other wild *B. oleracea* species from the $n = 9$ complex that are useful as donors of valuable genes, special attention should be paid to *B. oxyrrhima* (Prakash and Chopra 1990), which has been used as a donor species for the development of new cytoplasmic male sterile (CMS) lines and *B. hilarionis and B. macrocarpa,* which have also been identified as potential donors of resistance to pod shattering (Mithen and Herron 1991; Table 2.2).

2.5 Genomics Resources Developed

Genetic studies in Brassicaceae can be traced back to the first half of the twentieth century. However, most progress in comparative mapping was made since the beginning of the 1990s, and this was coincided with a period of rapid progress in molecular marker technologies. Molecular markers have been intensively used in *Brassica* species, and preliminary maps were constructed by employing restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and DNA fingerprinting (Quirós 2001; Quirós and Paterson 2004). The development of genetic maps in *Brassica* has been used for two purposes: first, to utilize them in genetics and breeding, and second, to analyze the genetic relationships among Brassica crops and wild related species. More precisely, wild forms of *Brassica* (including some $n = 9$ Sicilian populations) have been studied by using RAPDs (Lanner et al. 1996; Lázaro and Aguinagalde 1996, 1998), RFLPs (Song et al. 1990),

Table 2.2 Wild *Brassica oleracea* species as sources of desirable traits

Trait	Species	Reference
Agronomic traits		
Resistance to pod shattering	*Brassica macrocarpa*	Mithen and Herron (1991)
	Brassica hilarionis	Mithen and Herron (1991)
Quality traits		
Glucosinolates	Wild *Brassica oleracea* complex	Mithen et al. (1987a)
High glucoraphanin	*Brassica villosa*	Faulkner et al. (1998), Mithen et al. (2003), Sarikamis et al. (2006)
High erucic acid (> 45–50%)	*Brassica cretica*	Yaniv et al. (1991)
	Brassica villosa	Velasco et al. (1998)
	Brassica incana	Velasco et al. (1998)
	Brassica rupestris	Velasco et al. (1998)
Breeding systems		
Cytoplasmic male sterility	*Brassica oxyrrhina*	Prakash and Chopra (1990)
Biotic stress		
Disease resistance		
Blackleg – *Leptosphaeria maculans* (*Phoma lingam*)	*Brassica insularis*	Mithen et al. (1987b), Mithen and Herron (1991), Mithen and Magrath (1992)
	Brassica atlantica	Mithen et al. (1987b), Mithen and Herron (1991), Mithen and Magrath (1992)
	Brassica macrocarpa	Mithen et al. (1987b), Mithen and Herron (1991), Mithen and Magrath (1992)
Verticillium wilt	*B. incana*	Happstadius et al. (2003)
Downy mildew – *Peronospora parasitica*	*Brassica oleracea,* wild accessions	Greenhalgh and Mitchell (1976)
Insect resistance		
Cabbage aphid (*Brevicoryne brassicae*)	*B. incana*	Ellis et al. (2000)
	B. villosa	Ellis et al. (2000)
Flea beetle (*Phyllotreta cruciferae*) and *P. striolata*	*Brassica incana*	Bodnaryk (1992)
Cabbage white fly (*Aleyrodes proletella*)	*Brassica villosa*	Bodnaryk (1992)
	Brassica cretica	Ramsey and Ellis (1994)
	Brassica incana	Ramsey and Ellis (1994)
	Brassica villosa	Ramsey and Ellis (1994)
	Brassica spinosa	Ramsey and Ellis (1994)
	Brassica. insularis,	Ramsey and Ellis (1994)
Cabbage root fly – *Delia radicum*	*Brassica incana*	Ellis et al. (1999)
	Brassica villosa	Ellis et al. (1999)
	Brassica macrocarpa	Ellis et al. (1999)

and by analyzing specific sequences of chloroplast DNA (cp-DNA) (Lanner 1998).

Nowadays, Brassica databases have been developed and are being made publicly available (*http://www.brassica.info/resources.php*), managing information related to *Brassica* genetics and genomics. A set of sequence accessions, genetic maps, and markers are accessible at *http://brassica.bbsrc.ac.uk/BrassicaDB/*. In addition, this database is currently the original source of information about the "BBSRC set" of Brassica SSR markers. Among genetic resources, bacterial *E. coli* clones are widely used to isolate and characterize subsets of DNA and RNA sequences and are especially useful for characterizing complex genomes such as of *Brassica*. Genomic bacterial artificial chromosome (BAC) libraries are now available for *B. rapa*, *B. oleracea*, and *B. napus*. These databases are complemented with expressed sequence tags (ESTs) together with reference to doubled haploid mapping populations, associated linkage maps, and

public domain molecular markers (*http://www.brassica.info/ssr/SSRinfo.htm*).

The major advances in comparative genetics and molecular cytogenetics in cultivated and wild species, as well as the potential of *Arabidopsis* genomic resources for comparative studies, have been the scope of recent reviews in the last 10–15 years (Qiu et al. 2009). Species of the *Brassica* genus are closely related to *A. thaliana*, which also belongs to the Brassicaceae family. This close relationship between the two genera, *Arabidopsis* and *Brassica*, is reflected by an average identity of exon sequences at the nucleotide level, which is estimated to be 87% (Cavell et al. 1998). The completion of the *Arabidopsis* genome sequence (The *Arabidopsis* Genome Initiative 2000) has provided a valuable resource for identifying genes involved in agronomic and nutritional aspects of *Brassica* species, including the genes responsible for head formation in cauliflower and broccoli (Lan and Paterson 2000) and the genes involved in glucosinolate biosynthesis (Li and Quirós 2003). As anticipated by Lan et al. (2000), the generation of ESTs in *A. thaliana* holds an enormous potential for the cross-genomic analysis of alleles conferring specific phenotypes to Brassica. For instance, ESTs from *Arabidopsis* have been used as RFLP markers in *B. oleracea*, for comparison of the genomes of both species (Babula et al. 2003). Batley et al. (2007) demonstrated the utility of EST-SSRs for the genetic analysis of wild *Brassica* populations and commercial *Brassica* germplasm, since these markers were polymorphic and showed a consistent amplification and genome specificity. A summary of EST clones in different *Brassica* species can be found at *http://www.brassica.info/resource/clones.php*.

The genetic diversity and relationships among C-genome species have been well studied based on ecogeographic, phenotypic, and genotypic information (see Gómez-Campo 1999a). However, it is difficult to make comparisons between molecular studies, as different genetic marker systems have been used on different populations and accessions of variable or loosely defined provenance. Both nuclear and organelle-based molecular markers have been used to generate genotypic datasets. Song et al. (1990) used RFLP analysis to compare *B. oleracea* and nine C-genome wild species with *B. rapa* and found that *B. oleracea* formed a paralogous clade with its wild relatives. However, studies using other marker systems suggest different evolutionary relationships. Dendrograms based on RAPD markers (Lázaro and Aguinagalde 1996; Geraci et al. 2001) and isozyme data (Lázaro and Aguinagalde 1998) indicated that *B. oleracea* clustered with species such as *B. montana* and *B. incana*. In contrast, Tatout et al. (1999) used short interspersed nuclear element (SINE) transposons as markers and found that *B. oleracea* and *B. incana* were more similar to species such as *B. hilarionis*. A detailed study of the relationships within the *B. oleracea* cytodemes was carried out by Lanner (1998) who used non-coding sequence from the chloroplast genome to examine diversity and relationships between *B. oleracea* and nine C-genome species. More recently, Allender et al. (2007) assessed the utility of chloroplast SSRs as markers for diversity and phylogeographic studies among the *Brassica* species ($n = 9$) and found that diversity revealed by chloroplast SSRs is present in the Mediterranean wild species and is apparently almost absent from the contemporary UK natural populations of *B. oleracea* itself. This finding has implications both for the conservation of natural genetic diversity and for the search for novel sources of alleles to be used in crop improvement programs.

2.6 Scope for Domestication and Commercialization

In the past, people depended exclusively on herbal remedies or traditional medicines and used some wild plants for cosmetic and perfumery purposes. Nevertheless, in the recent years, medicinal plants have represented a primary health care source for the pharmaceutical and perfumery industries. Global trend leading to increased demand of medicinal plants for pharmaceuticals, phytochemicals, nutraceuticals, cosmetics, and other products is an opportunity sector for wild and cultivated *Brassica* species. In that context, wild relatives of *B. oleracea* could have potential as sources of oil, condiments, and other products and, therefore, they can be used for food, medicinal purposes, (nutraceutical crops) and alternative uses as biocide crops.

Medicinal plants contain biologically active chemical substances such as coumarins, volatile oils, alkaloids, etc. In addition to these substances, plants may

contain other chemical compounds. In Brassicaceae, phytochemicals such as indole phytoalexins, phenolics, and glucosinolates are the most abundant. All of these phytochemicals contribute to the antioxidant, anticarcinogenic, and cardiovascular protective activities of Brassica vegetables (Podsedek 2007; Jahangir et al. 2009), which increase their value as therapeutic compounds to be used in medicine and as food supplements in the human diet and their value as biocides or pest deterrents in agriculture.

More specifically, and regarding glucosinolates, numerous studies have highlighted the idea that glucosinolates produced by plants may be useful as plant protection agents, as dietary supplements, and for obtaining pharmaceutical products for treating cancer, viral infections, or autoimmune diseases. After tissue damage, myrosinase (thioglucoside glucohydrolase) hydrolyzes the naturally occurring glucosinolates present, thus producing a number of end products including isothiocyanates, thiocyanate ions, nitriles, and epithionitriles according to the type of glucosinolates present and the exact hydrolysis conditions. Among these, isothiocyanates display different biological functions that allow their use as food, drugs, or fine chemicals.

Many wild members of the *B. oleracea* species complex have high levels of individual aliphatic glucosinolates (Mithen et al. 1987). The first studies about leaf and seed glucosinolates content in wild *B. oleracea* relatives can be found in the eighties. Mithen et al. (1987) analyzed leaf glucosinolates in 18 *B. oleracea* populations, including wild and cultivated crops, and found a great variability among species in glucosinolate content and profile. The major glucosinolate found in *B. montana, B. incana*, and *B. cretica* subsp. *cretica* was gluconapin, whereas *B. rupestris* does not contain gluconapin but contains glucoiberin; *B. drepanensis* contains glucoiberin and glucoiberverin, whereas *B. macrocarpa*, *B. insularis*, and *B. cretica* subsp. *laconica* showed a very high content of sinigrin. In the same study, wild populations showed a higher total glucosinolate content than the cultivars. Mithen (2001) provided further details about the range of glucosinolate contents found within wild and cultivated *Brassica* species, and environmental factors that influence glucosinolate expression. Afterwards, Branca et al. (2002) evaluated the content and profile of glucosinolates in the Sicilian *B. oleracea* germplasm including wild species related to it. They also found a great level of variability in glucosinolate content in most crops and wild species, and wild types showed low contents of glucoiberin and progoitrin.

Horn and Vaughan (1983) evaluated seed glucosinolates of 14 wild *Brassica* species including *B. insularis*, *B. incana, and B. oxyrrhina*. Neither *B. insularis* nor *B. incana* showed sinigrin in their seeds, while *B. incana* gave a high level of gluconapin. *B. insularis* showed an unusual pattern of glucosinolates, having low levels of progoitrin and high levels of gluconasturtiin and some benzyl glucosinolates. More recently, Velasco and Becker (2000) evaluated a germplasm collection of 20 *Brassica* species (including accessions of *B. incana, B. montana, B. oxyrrhina*, *B. rupestris*, and *B. villosa*). In that work, *B. montana* had the highest glucosinolate content, and authors concluded that the detected variability in this species might be useful for the development of *Brassica* crops containing high glucosinolate content and specific glucosinolate profiles.

Studies on the glucosinolate genetics in these taxa have been essential for elucidating the genetic pathway for glucosinolate biosynthesis. It is evident that certain species in this group could be valuable in *Brassica* breeding programs designed to specifically enhance glucoraphanin and/or glucoiberin and, by so doing, to enhance the anticarcinogenic potential of the plant. Several recent research programs indicate that isothiocyanates derived from the hydrolysis of glucoiberin and glucoraphanin glucosinolates may be important for human diet in preventing the development of cancer. Glucoiberin shows to have phase 2 enzyme induction activity, and glucoraphanin is the precursor of the anticarcinogenic isothiocyanate sulforaphane (Mithen 2001). The possible chemoprotective effect of these compounds has led to the interest in the dietary intake of glucosinolates and isothiocyanates in broccoli and other cruciferous vegetables. In an effort to obtain higher levels of these glucosinolates, formulated foods containing designed glucosinolates are being developed. The idea is to provide an elevated level of certain isothiocyanates to the consumer, particularly those that have been found to have health benefits.

The major finding from studies about glucosinolates in a wild *B. oleracea* complex was that they are members of the *B. villosa-rupestris* complex from Sicily, which possesses a nonfunctional *GSL-ALK* allele that turns these populations into useful donors of beneficial glucosinolates and into wild progenitors

of cultivated broccoli. Faulkner et al. (1998) described the use of *B. villosa* and other members of the *B. oleracea* complex as progenitors of cultivated broccoli and showed that F_1 hybrids, which had high levels of glucoraphanin and enhanced ability to induce quinone reductase in cell cultures. Hybrids between commercial broccoli cultivars and three wild members of the *B. oleracea* complex, *B. drepanensis*, *B. villosa*, and *B. atlantica,* resulted to be fully fertile and backcrossed populations were developed. In F_1 hybrids with *B. drepanensis* and *B. villosa*, the major glucosinolates were glucoiberin and glucoraphanin. This is similar to the profile found in broccoli, whereas in hybrids with *B. atlantica*, the major glucosinolates were sinigrin, gluconapin, and progoitrin (Faulkner et al. 1998). The different glucosinolate profiles in these hybrids resulted from the interaction of the genes in their respective parents.

Following with the interest in developing broccoli that can deliver high levels of sulforaphane, Mithen et al. (2003) described the use of these hybrids to develop broccoli breeding lines and experimental F_1 hybrids having enhanced levels of glucoiberin and glucoraphanin. Experimental hybrids were obtained through conventional breeding by the introgression of small segments of the *B. villosa* genome that express high glucoraphanin levels. Hence, it is feasible to develop broccoli lines with enhanced levels of glucoraphanin that may be valuable for experimental purposes in dietary intervention studies and for commercialization for specific purposes. For example, lines having a high glucoraphanin content for functional food development (cancer protection) and lines having a high sinigrin content for biological pest control (nematodes and fungal pathogens) may be produced. Sarikamis et al. (2006) described the development of ITC-enriched broccoli through the introgression of three small segments of the genome of *B. villosa*, each one containing a quantitative trait loci (QTL), into a commercial broccoli via marker-assisted selection and analysis of glucosinolates in the florets of backcross populations. An interesting feature to point out here is that the use of wild allied plants for improving quality in vegetable crops is a difficult approach, since commercial appearance is a major trait to ensure commercial success. Thus, it is important to define the minimum number of introgressed segments (from *B. villosa* to commercial broccoli) required to increase glucosinolate content sufficiently to achieve the health benefits.

As a conclusion, significant qualitative variations in the glucosinolate profiles of wild *B. oleracea* species suggest differences in the health-promoting properties among them. Leaves and seeds of Brassica may, therefore, be used as sources of glucosinolates and isothiocyanates in the diet, especially in formulated foods.

Other crucial metabolites because of their therapeutic value in Brassica crops are phenolic compounds, especially flavonoids. The main important biological effects derived from these compounds are the antioxidant activity, the capillary protective effect, and the inhibitory effects elicited in the various stages of a tumor. In many in vitro studies, phenolic compounds demonstrated to have a higher antioxidant activity than antioxidant vitamins and carotenoids (Podsedek 2007). Studies have been mainly focused on *B. oleracea* (Vallejo et al. 2004) and *B. rapa* (Fernandes et al. 2007) and very little studies have been focused on wild *B. oleracea* species. Only works of Aguinagalde et al. (1992) and Aguinagalde (1993) used these flavonoids as biochemical markers to study the interspecific variability among a set of wild *Brassica* populations and between wild and cultivated forms of *B. oleracea*. No differences were found between wild and cultivated *B. oleracea* accessions in that study; *B. bourgeaui* closely resembled the group formed by *B. oleracea* and *B. montana* (all lacking isorhamnetin) and a high diversity was found in *B. cretica*.

Among the alternative uses to which species of *B. oleracea* complex can be devoted, it is interesting to emphasize on biofumigation. This is an agronomic technique that is an alternative to chemical fumigants in order to manage soil-borne pests and diseases in an integrated way. Rotation with Brassica crops and incorporation of Brassica residues into soil have been reported to suppress a variety of pest and disease organisms, including fungi, nematodes, insects, bacteria, and weeds (Brown and Morra 1997). Plants from Brassicaceae have been recognized as having a potential use on biofumigation practices, based on production of active volatiles released after enzyme hydrolysis. The most common volatile compounds produced during the breakdown of Brassicas are isothiocyanates. In particular, the breakdown product of sinigrin seems to protect the plant against certain pests and possibly soil pathogenic fungi and nematodes. Numerous studies have been carried out with mustard

species (*B. juncea, B. nigra, B. carinata*) and with species within the *Sinapis* genus (*S. alba* and *S. arvensis*) with this aim. The major glucosinolate types in these species (allyl and *p*-hydroxybenzyl) have shown greater allelopathic effects compared to other glucosinolate types.

Regarding the wild *B. oleracea* complex, Branca (2004) evaluated the possible biofumigant activity of *B. macrocarpa* for the control of knot-root nematodes on cherry tomato crops. In a previous study carried out by the same author (Branca et al. 2002) with Sicilian wild *B. oleracea* species, *B. macrocarpa* resulted to have the highest glucosinolate content in leaves, of which about 90% are represented by sinigrin. As a conclusion, the insertion of *B. macrocarpa* dry biomass into the soil permitted to reduce the attack caused by soil nematodes. Nowadays, some chemical synthetic isothiocyanates are already utilized as nematocides for controlling nematodes in several commercial fumigant products.

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Chapter 3
Capsella

Pietro P.M. Iannetta

3.1 Basic Botany of the Genus

The genus name *Capsella* translates from Latin as "little box," owing to the fact that all the members of this genus are characterized by their fruits that are termed silicula. This means that they are flattened, and they are also "heart shaped" (hence species of *Capsella* are also referred to as "mother's heart"), with two loculi (seed chambers). This distinguishes them from the long and narrow "siliqua," with one seed per locule characterized by other *Brassicas* such as *B. napus* (canola or oilseed rape) and *Arabidopsis thaliana* L. Heynh. (thalecress).

Capsella is one of 338 genera that collectively account for the 3,710 species of the family Brassicaceae, and *Capsella* is most notable as its members may be found anywhere in the world (Stevens and Carson 2001), with the exception of the Antarctic polar region. Arguably, of the ca. 338 arable plant genera, *Capsella* may be the most prevalent, as it occurs at such high densities wherever it is found. Brassicaceous plants include many important crop species, many of which are grown over very large areas of farmed land throughout the world. For example, oilseed rape is cultivated for the vegetable oil that is extracted from its seeds, and is the greatest global contributor to edible seed oil yields. In addition, many others Brassicaceous species are also grown across the world at smaller scales. For example, ornamental forms of *Brassica oleracea* include kale (Acephala) and cabbage (Capitata). The Brassicaceae also includes more than 120 species of wild plants, of which the genus *Capsella* comprises only three species that are all capable of outcrossing, though to varying degrees. The genus *Arabidopsis* is the most well known within academia. Given the extremely high number of Brassicaceous species, and lack of phylogenetic characterization of genera, classification generally follows the artificial (i.e., non-comparative phylogenetic based) rules of Schulz (1936). Fruit characters, and to a lesser extent flower form, are usually still relied upon for identification of genera. The delimitation of genera in the Brassicaceae is also made more difficult due to the fact that there is frequent independent evolution among similar species.

3.2 Classifying Members of the Genus

While the various plant and flower parts could be described in exact botanical terms here, there are numerous excellent flora (cf. Clapham et al. 1962, 1987; Tutin et al. 1993) and internet-based resources that list full nomenclature for the Angiosperms, and the "Angiosperm Phylogeny Website" is highly recommended (Stevens 2001b; see also Broadley et al. 2004). In short, their vegetative form is (generally) a flat rosette that is laid low and flat to the ground, the leaves being subopposite, with different individuals of the group expressing a range of leaf phenotypes from simple (entire undivided) to a wide range of dissected leaf forms. Species of *Capsella* exhibit an annual growth habit, and can be found flowering from late spring to late autumn. *Capsella* may also exhibit a biannual habit, as it can develop winter frost tolerance, over-wintering in a vegetative form and flowering the following year. In particular, individuals within alpine

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populations (and probably those at higher latitudes) tend to most commonly demonstrate this shift from annual to biannual (Neuffer and Hurka 1986a) – an adaptation that may also be associated with delayed seed germination (Neuffer and Koch 1996; Baskin et al. 2004).

Capsella inflorescences are racemose (form bunches) or ebracteate (lack bracts); the flower sepals are erect and the petals usually white, and most significantly, the length of the petals can be used as a means by which one member of the genus, the obligate outbreeding diploid *Capsella grandiflora* (Fauche and Chamb; $2n = 2x = 16$), may be distinguished from its sister species. The genus encompasses only three real, that is reproductively active and isolated species, of which *C. grandiflora* is one. *C. grandiflora* flowers possess petals that are 4–5 mm longer than the sepals. In addition, and as the name suggests, this species can produce very large numbers of flowers, all of which possess large nectaries and a very attractive fragrance: traits often associated with an obligate outbreeding species that relies upon attracting insects to facilitate crosspollination (Fig. 3.1). This habit contrasts strongly with its two sister species, which are both highly self-fertilizing and possess small nectaries.

Distinguishing the two remaining breeding *Capsella* species is more difficult. These species are the diploid *Capsella rubella* ($2n = 2x = 16$), whose petals may be as long as, or scarcely extending beyond, the sepals, and the tetraploid *Capsella bursa-pastoris* L. Medic ($2n = 4x = 32$), commonly known as "shepherd's purse," whose flowers are 2–3 millimeters longer than the sepals. However, this is not a robust method by which these species may be discerned, and to further complicate the matter, *C. rubella* and *C. bursa-pastoris* are very similar phenotypically and at all life-history stages. It is also suggested that the former may be distinguished by the presence of bright red flower buds and leaf lobes (Clapham et al. 1962; hence "*rubella*," which is Latin and means "little red"). However, this feature is also not robust as a means to delimit these species, and unambiguous identification can be achieved only on the basis of ploidy analysis. The nomenclature described here is based upon Shull's systematic analysis (Shull 1909, 1914, 1918, 1929; see also Neuffer and Eschner 1995).

It should be noted that many presumed "species" of *Capsella* have been named, and this confusion is due to the fact that individual *Capsella* plants may appear very different. The genus is noted for the exceptionally high levels of diversity in leaf shape (Fig. 3.2). For example, and over the course of scientific history, the genus has been divided variously into as many as 200 different species types (Jordan 1864; Hopkirk 1869; Mott 1885; Almquist 1921, 1923). Such artificial divisions of the species are usually made on the basis of the wide variety of leaf shapes found among individuals of the genus. In addition to their arrangement as a rosette, and after environmental stresses such as winter vernalization, "bolting" (early flowering) may occur, and the rosette leaves may whorl around the flowering stem. Wherever the leaves occur, as a rosette or along the flowering stem, *Capsella* "leaf shape" character may only be reliably attributed using leaves from ninth node (or more) to assign specific leaf class (see Iannetta et al. 2007). However, the historic focus

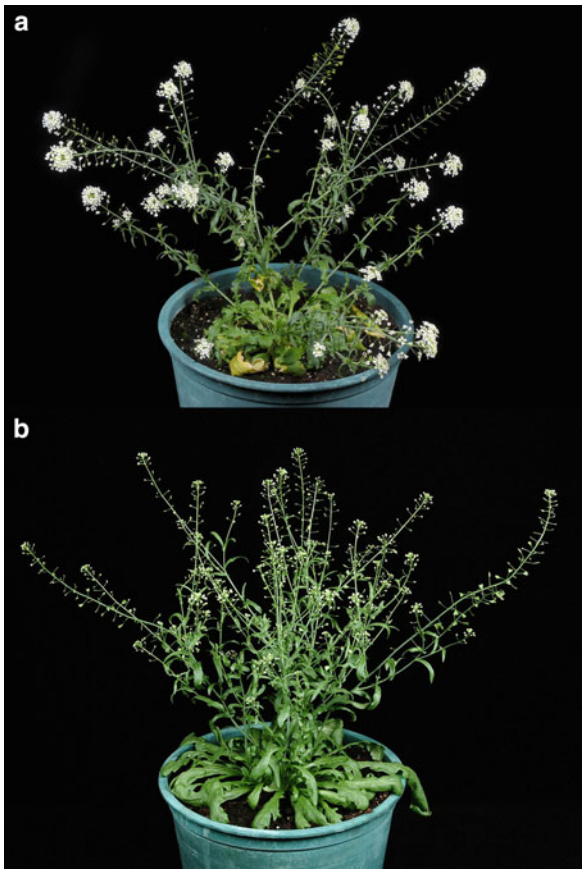

Fig. 3.1 This figure shows a comparison of the flowers and flowering stems of *C. grandiflora* (**a**) and shepherds purse (**b**) types. The large terminal inflorescences of *C. grandiflora* contrast with the more open and less prominent flowers of shepherd's purse

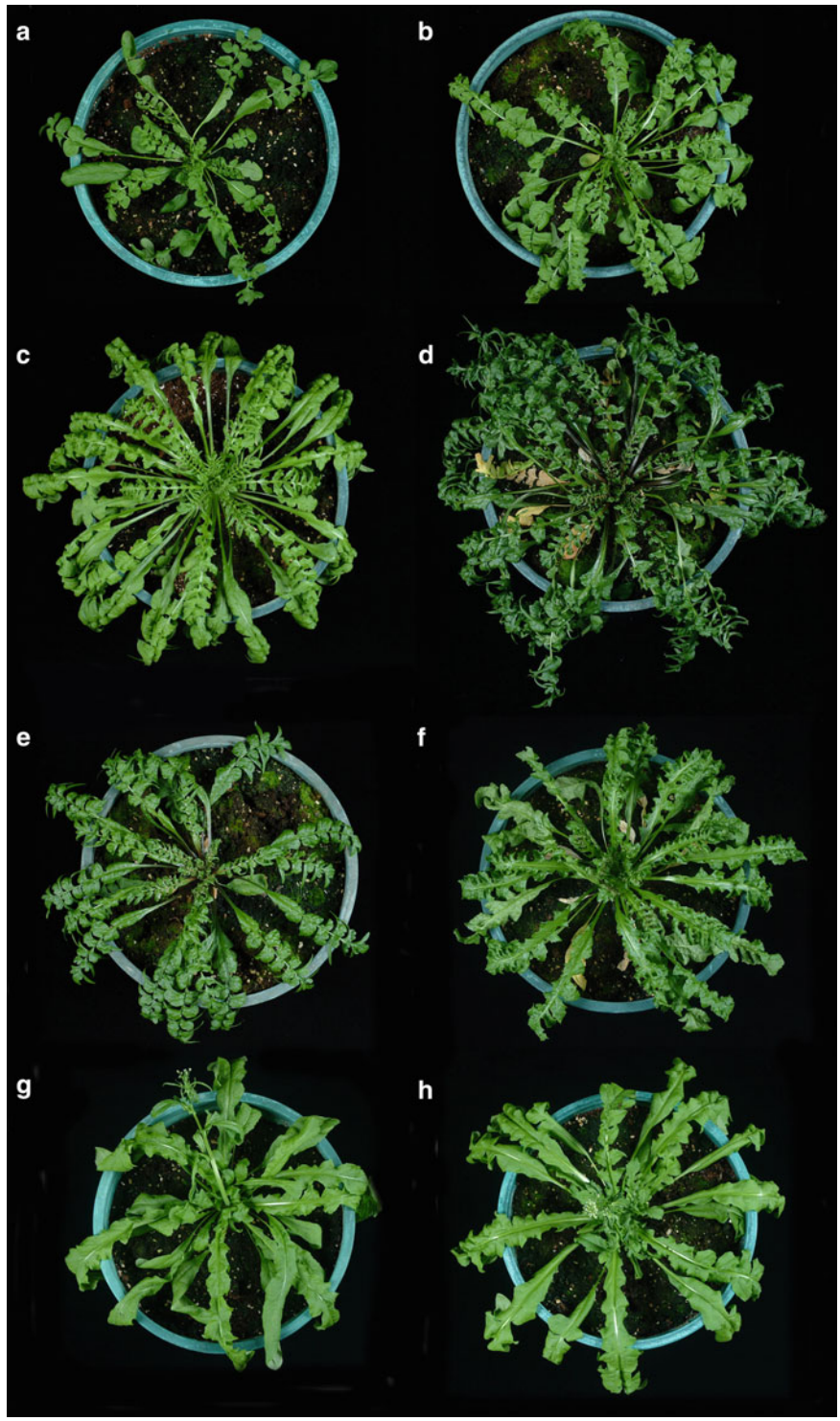

Fig. 3.2 Parts (**a**–**g**) demonstrates diversity among shepherd's purse leaf-shape types. For example, note the differences in the shade of green of the leaves (compare **d** and **c**), individuals breed true to their leaf shade and this does not appear to be a function of chlorophyll content, or leaf nitrogen (concentration or total amount). The depth of green shown by the leaves may reflect anthocyanin pigmentation (see Fig. 3.3), or perhaps leaf ultra structure: this nature of this difference has still to be discerned. Leaf number also varies predictably among types, with earlier time to flowering forms being more likely exhibit an entire or undivided leaf shapes (compare **a** and **d**). Other differences such as in leaf 3D structure, firmness or the degree to which their leaf edges may be serrated cannot be easily demonstrated here, though these are significant. In culturing these it is clear that those types with more divided leaves are more tolerant to water deficit. It is also worth noting that each of the pots shown in this collage are 30 cm in diameter, and the plants in then grown in unlimited conditions according to the principles of Gaudet and Keddy (1988)

Fig. 3.3 The center of the rosette of a late time to flowering ecotype of shepherd's purse. This highlights the deep purple pigmentation that is more commonly associated with later flowering types. Though this shows an extreme case, darker leaf types may not have this dark venation associated with their leaf petioles. One could speculate that the presence of such pigmentation will have some form of protective function

among leaf-shape differences among shepherd's purse types can distract scientific attention from other phenotypic differences: such as the presence (or absence) of anthocyanin pigmentation (SPAD, Iannetta et al. 2007), which and can lead to striking differences in color depth among the different types (Fig. 3.3). Also, the degree to which the leaves exhibit leaf hairs also varies dramatically between ecotypes (Fig. 3.4) and can affect plant–insect interactions (Karley et al. 2008). It should also be noted that such intraspecific variation does not seem to have appeared simply as a function of the polyploidization that led to the evolution of shepherd's purse. Ancestral species such as *C. grandiflora* also exhibit a diverse array of intraspecific phenotypic variation for leaf hairs (Fig. 3.4) and leaf shape (Fig. 3.5).

3.3 Species Distribution in Space and Time

Diploid members of the genus are predominantly localized in Mediterranean regions (Löve and Löve 1956; Davis 1965; Clapham et al. 1987; Aksoy 1996; Aksoy et al. 1998, 1999), *C. grandiflora* occurring over only a narrow geographical range that is confined mainly to the Greek Islands and the northwest coast regions of Albania. In contrast, "true" *C. rubella* may be considered a plant of central Europe and the Mediterranean coasts of France, Italy, and Yugoslavia (Meikle 1977). However, *C. bursa-pastoris* has a global distribution, and is excluded only from extremely dry tropical environments (Almquist 1929; Shull 1929; Svensson 1983; Neuffer and Eschner 1995; Hurka and Neuffer 1997). From molecular analyses, it would appear that the variable gene pool of shepherd's purse in Europe was introduced into the Americas, and from this, locally adapted types have arisen (Neuffer and Hurka 1986a, b, 1999). The wide geographic distribution of the tetraploid *Capsella* is likely to be a probable benefit of the evolutionary and adaptive advantages that are commonly associated with chromosome duplication. This species is especially abundant in disturbed ground of temperate regions, and most frequently found growing on soils of farmed fields under the frequent soil disturbance associated with arable cropping. Given its broader geographical range, shepherd's purse has received the most research attention to date.

C. rubella and *C. bursa-pastoris* do coexist in disturbed, semi-natural habitats, the former tending to show a later time-to-flowering and possessing leaves that are darker and more highly divided than the tetraploid form (Iannetta et al. 2007). Such differences are not a function of phenotypic plasticity but are genetically fixed attributes (Svensson 1983; Neuffer 1989; Iannetta et al. 2007). The tetraploid of the genus tends to flower earlier (within 50–90 days) than *C. rubella* (70–150 days). Earlier time-to-flowering forms tend to be highly fecund and produce more taller and slimmer flowering stems and with greater branching (Neuffer and Eschner 1995; Aksoy et al. 1999; Iannetta et al. 2007). Though both species may comprise individuals with a time from germination to flowering of up to 200 days, if subject to autumn cold (vernalization), late and early time-to-flowering ecotypes may flower and set seeds within a very short time frame of approximately 30 days. Ecotypes of the tetraploid and diploid forms, therefore, also reproduce within the same temporal space, especially in central European regions (Steinmayer et al. 1985; Neuffer 1989). These species may be considered reproductively isolated, as they are very self-fertile, and therefore, highly inbred (Shull 1929). Rare triploid interspecific hybrids may be found, which are known as *Capsella* × *gracilis*, which are sterile due to the abortion of their seed pods (Keble-Martin 1991). They are found most commonly where a large number of *C. rubella* and

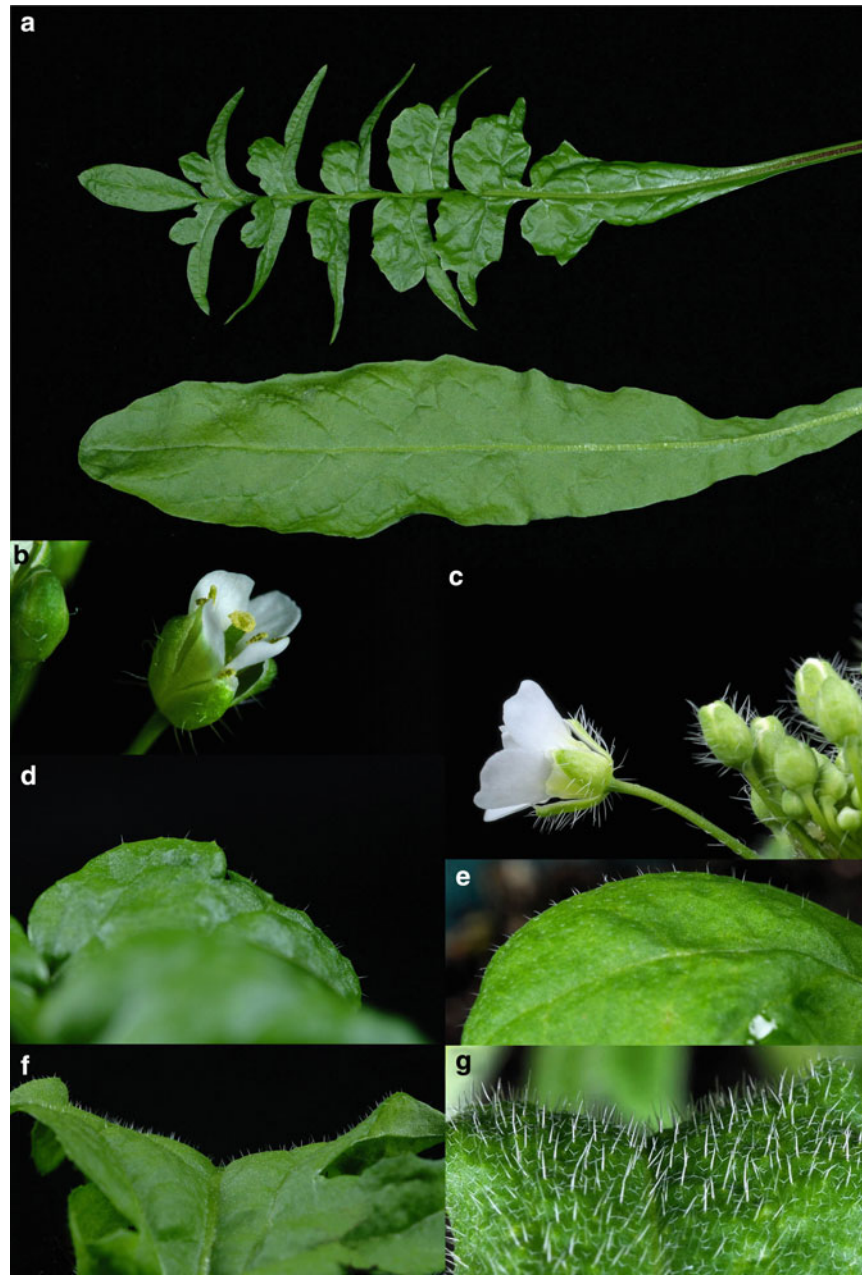

Fig. 3.4 *Capsella bursa-pastoris* demonstrates many different leaf shape types, and individuals breed true to their leaf shape form (and relative leaf number) (unpublished data). Leaf shape can only be reliably attributed using leaves from ninth node (or more) and care should be exercised to ensure that it is those leaves that are used to assign a specific leaf class. (**a**) shows two extreme leaf types within the tetraploid shepherd's purse. Such within species heterogeneity is thought to have evolved as a consequence of genome restructuring as a function of tetraploidy. However there is evidence that out breeding ancestral species also exhibit high intraspecific phenotypic diversity. (**b**) and (**c**) show the normal characteristic used to distinguish *Capsella grandiflora* (**c**), from other *Capsella species* (*rubella* and *bursa-pastoris* (**b**)), which is flower petal length. This difference aside, these species appear very similar, and individuals within each exhibit broad intraspecific differences. For example, *C. grandiflora* may also possess leaves that have; (**e**) only a low density of leaf hairs; or (**g**) a high density of leaf hairs. Similar leaf hair density differences are shown for shepherd's purse (**d** and **f**)

C. bursa-pastoris flower together within dense stands in localized in situ environments that are densely populated (e.g., arable fields), or artificial environments such as experimental glasshouses. While *C. rubella* has been recorded in the UK (Aksoy et al. 1998), a field survey found no definitive evidence of the coexistence of *C. rubella* and *C. bursa-pastoris* in arable fields of the British mainland (Iannetta et al. 2007).

3.4 Evolutionary Origins and Diversity

Until recently, there was doubt about the evolutionary origins of *C. bursa-pastoris*, as it shares biochemical (aspartate aminotransferase) isoenzymes (Hurka et al. 1989) and plastidic L-glutamate dehydrogenase (Hurka and During 1994) similarities with *C. grandiflora,* but is reproductively and phenotypically more similar to

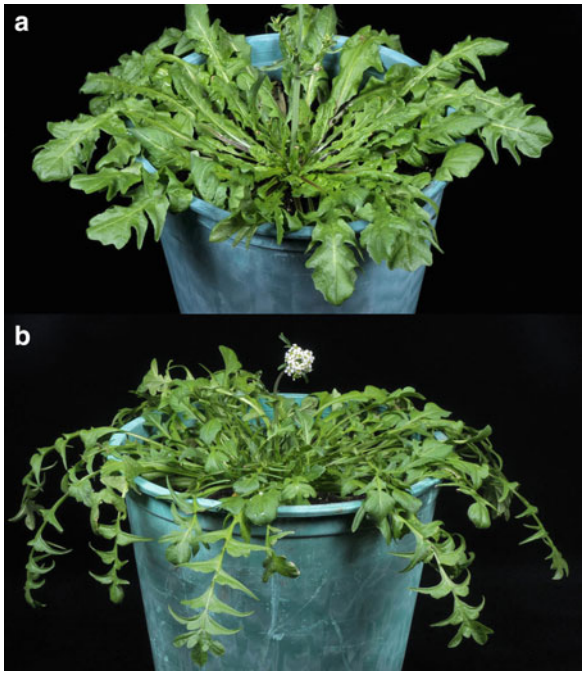

Fig. 3.5 Parts (**a**) and (**b**), show two *C.grandiflora* individuals which possess different leaf shapes. Again shown in 30 cm diameter pots, these types are as a large as shepherd's purse, and also exhibit different flowering times too. A characteristic attribute of *C. grandiflora*, is shown in (**b**), by the prominent tight ball-like arrangement of flowers on the terminal inflorescence (see Fig. 3.1)

C. rubella. However, and in contrast to the apparently high levels of intraspecific diversity, it has been suggested that the tetraploid shepherd's purse evolved only recently in plant evolutionary terms: after the last glaciations in Eurasia ca. 17,000 years ago (Slotte et al. 2006), as an introgressed hybrid of *C. rubella* (Slotte et al. 2007, 2008). The very high levels of intraspecific phenotypic and genetic diversity and colonization success for this species contrast with chloroplast-genome evidence suggesting that the species' molecular diversity is limited and the effective population size is small (<2,000; Nordborg and Innan 2002; Ceplitis et al. 2005). However, comparative genome mapping among members of the Cruciferae is a focus of intense research scrutiny, and estimating the age at which different lineages diverged is complex and may be inaccurate (Koch and Kiefer 2005). It is also acknowledged that the apparently limited genetic variability and theoretical predictions of small effective population size are many orders of magnitude smaller than the true numbers for this species (Ceplitis et al. 2005). It is, therefore, possible that *C. bursa-pastoris* may have a more ancient evolutionary origin. Studies of isolated *Capsella* seeds isolated from interglacial deposits from England suggest strongly that the seeds are from the tetraploid of the genus *C. bursa-pastoris* (Coope et al. 1961; West et al. 1964). Taken together, these facts imply that any definitions of *C. bursa-pastoris* as "recently evolved" or as a "poor competitor," or "ephemeral" may be misleading. In fact, the colonization success and the competitive ability of *Capsella* lie in its adaptive capacity to coexist and persist, not compete *per se* (e.g., Petry et al. 1993; Perera and Ayres 1992).

The high levels of occurrence and ease with which it may be gathered make shepherd's purse the most easily studied species of the genus. In a genetic context, this is especially true for key ecological traits of leaf shape and time to flowering. The occurrence of flowering time variants in shepherd's purse may be a consequence of environmental pressures, especially differences that are associated with latitude and altered day lengths (Slotte et al. 2007). This is suggestive of an adaptation to photoperiod, and the earlier and longer growing seasons that are commonly associated with southern latitudes encouraging the persistence of later time-to-flowering forms are likely to be significant. However, this assumption is contradictory to other publications (Iannetta et al. 2007) that report higher number of ecotypes with late time-to-flowering in the northern (as opposed to southern) UK. This pattern is suggested to be a consequence of factors associated with agricultural practices in that region. This feature is also plausible since the morphological and genetic diversity of shepherd's purse populations is heavily dependent upon the frequency of soil cultivation and appears adapted for coexistence with other (mainly crop) species. The functional and genotypic evidence also highlight that there is also order among the individuals on a local scale, which correlates time-to-flowering and reproductive duration with previous crop type and cropping intensity as well as soil physical and chemical characteristics (Iannetta et al. 2007). The findings are supported by Debeljak et al. (2007), who showed that the abundance and prevalence of *Capsella* (assessed at the level of species) in the arable seedbank differ greatly with geographical location and the intensity of field management. Note also, that genetic and functional

diversity among shepherd's purse individuals was far higher in plants from cultivated land than in those from ruderal localities (Beismann et al. 2004; see also Neuffer and MeyerWalf 1996), and intensive arable-farm practice may have encouraged the dominance of more recently evolved (earlier flowering) ecotypes (Iannetta et al. 2007). *FRIGIDA* (*FRI*) alleles determine natural variation in flowering time in *Arabidopsis*. Dominant alleles of *FRI* confer late flowering, which is reversed to earliness by vernalization. However, deletions in *FRI* disrupt the open reading frame, and "rapid cycling" or early flowering appears to have evolved independently at least twice from late flowering progenitors, at least in this model plant. Nevertheless, two major loci (*FRI* and *FLC*) appear as major determinants in the control of flowering time in *Arabidopsis* and the closely related *Brassica napus* and *B. rapa* (Osborn et al. 1997). However, Slotte et al. (2007) concluded that genes involved in regulation of the circadian clock (*CCA1* and *TOC1*) are adaptive flowering time variation in shephards purse, and this remains to be tested.

In this flowering time context, and as a very prevalent wild plant, *Capsella* has as its "corner stone" an ability to reproduce quickly and produce offspring, which do the same. *Capsella,* therefore, exhibits predictable traits that are geared towards attaining and maximizing their fecundity. Another very significant and important attribute of *Capsella* is its ability to produce large numbers of flowers, which do not rely upon specialist pollinators, are largely self-fertile, and employ only wind and generalist pollinators to support low levels of outbreeding. Such wild plants then produce many small seeds, despite possessing only a limited quantity of vegetative (leaf) material. The impact of such efficient reproduction is further enhanced by the fact that the resultant seeds are returned to the soil as a wild plant reservoir (the "*seedbank*"), where they may become dormant, retaining their viability for many years. When good growing conditions exist, the reproductive period may be extended and wild plant fecundity may be still greater. Collectively, this range of adaptations are evolved, as many wild plants have late time-to-flowering outcrossing ancestors (Roux et al. 2006), as evidenced for model grass and broad-leafed species, wheat, and *Arabidopsis* (Yan et al. 2003 and Komeda 2004, respectively). It should, therefore, be appreciated that the late time-to-flowering, obligate outbreeding habit is an archetypal condition, commonly associated with individuals that originate from those regions of the world that were arctic *refugia*.

The wild plant biota of central and northern Europe (including the UK) was established by the colonization of land exposed in the wake of a retreating ice cap and associated tundra, after the most recent ice age (Hewitt 1999). The wild plants of Europe are therefore the ancestors of those that survived in southern *refugia*, especially the peninsulas of Iberia, Italy, and the Balkans; and as the climate warmed, these species adapted and expanded rapidly northwards. In particular, specific plants families showed greater success than others in this respect, and members of the Cruciferae, Polygonaceae, Umbelliferae, Compositae, and Gramineae contribute usually large numbers of species to wild plant populations in temperate regions. As is common, the rapid wild plant recolonization was assisted by human activities. It has been generalized that the wild plant flora within areas of long agricultural histories (such as the *refugia* of southern Europe and North Africa) provided the successful wild plants for the newly exposed and disturbed regions (Gray 1897). The wild plants within agricultural areas of such *refugia*, having been exposed to many generations of selection by agricultural practices, developed traits that facilitate their coexistence with crops. Often, the lack of efficient seed cleaning methods would mean that wild plants would commonly be sown with crops, thereby aiding the development of wild plant adaptations. Selection for early time-to-flowering due to crop production and wild planting practices has been noted for wild oats (Imam and Allard 1965), *A. thaliana* (thalecress; Jones 1971), and shepherds purse (Iannetta et al. 2007).

Ecological theory dictates that the longer vegetative phase allowed to late flowering plants facilitates the accumulation of more resources and higher seed production. While this may be true for species inhabiting very different growing environments, within an arable ecosystem a disturbingly different scenario has been found. Detailed studies quantifying the fecundity of the common wild plant *Capsella* have shown that (for accessions gathered within arable fields) high seed production is an attribute of individuals with a shorter time to flowering: this success being achieved with less leaf biomass (Iannetta et al. 2007). Moreover, the late time-to-flowering forms comprised only a small percentage (less than 5%) of the accessions assessed. The behavioral characteristics of wild plants must therefore be

considered as having evolved in response to human arable farming activities.

Two key farming components that underpin *Capsella* diversity may, therefore, be highlighted. Firstly, frequent tillage of soil associated with arable farming appears an essential prerequisite for the simple rejuvenation of species and replenishment of the soil seedbank. Secondly, different farming styles and intensities provide essential and diverse selection pressures that facilitate the evolution of a wide array of functionally distinct intraspecies variants. As such, estimates of effective population size should therefore perhaps focus upon a representative range of individuals from diverse in-field arable locations and across a wide range of latitudes, longitudes, and farming landscapes (characterized by current management and management histories) to accurately encompass the breadth of genetic diversity that is available.

3.5 Diversity and Conservation

Of all the wild plant species that emerged from soil samples of arable farmed systems studied during the farm-scale evaluations (FSEs; *http://www.defra.gov.uk/environment/gm/fse/*), shepherd's purse turns out a "middle ranking" species. The FSE was an experimental release and impact assessment of the management associated with the commercial scale cultivation of various genetically modified (GM) crops (Firbank et al. 2003; Squire et al. 2003; Perry et al. 2003). However, from the above-ground diversity data, *Capsella* occurs so frequently that it has been identified as the second most prevalent above-ground common broad-leaved plant. In absolute number, wild plants, such as *Capsella,* are superseded greatly (a factor of 7–8 times more) by the most prevalent grass wild plant *Poa annua* L. (annual meadow grass; ca. 111,000). *Juncus bufonius* (toad rush; ca. 17,000) was the second most prevalent grass, occurring only a little more frequently than shepherd's burse (ca. 15,000). *Capsella* can be considered the most common above-ground, broad-leaved wild plant within (at least) UK arable fields from the FSE study. Though many wild plants appeared to occur with greater frequency (ca. 8,500 more counts per meter square) than *Capsella*, the estimate included a range of different *Matricaria* and *Tripleurospermum* species and was therefore a "genus x2" measure. With the exception of *Galium aparine* and *Fumaria officinalis*, grasses are the usual (primary) targets of herbicide applications, such as *Alopecurus myosuroides* (black grass) and *Bromus sterilis* (barren brome). This indicates that shepherd's purse, though prevalent, is not considered a noxious wild plant. Shepherd's purse is found with a frequency that is many orders of magnitude greater than *C. rubella*, which in turn is many orders of magnitude greater than *C. grandiflora*. Therefore, no coordinated strategy exists to conserve shepherd's purse; accessions of *C. rubella* are uncommon in their main niche (arable farmed habitats) and perhaps should be conserved where they are found, as it is possible that the occurrence of such late time-to-flowering types in field may be an indicator of sustainable farming practice (Iannetta et al. 2007). The conservation of *C. grandiflora* is even more justifiable on the grounds of its rarity and also because it is the only obligate outbreeder of the genus. This capacity is useful when there is a necessity to rely upon traditional plant breeding to improve a *Capsella* genus-specific trait, as the hybridization of extreme phenotypes could be accommodated more easily. In addition, when discerning the genetic basis of that specific trait, a diploid such as *C. grandiflora* has advantages that accommodate molecular discoveries in shorter time frames that than afforded by polyploids. The limitation with *C. grandiflora* is accessing a sufficiently broad range of functionally distinct types.

Many studies aim to understand the causative factors that drive ecosystem productivity and diversity (that is, natural selection and speciation). Such research is especially focused upon biodiverse tropical regions and research on animal and insect species due to their more obvious (at least to humans), behavioral and phenotypic shifts (Silvertown 2004). However, there is evidence that functional attributes of all important primary producers (wild plants) and within farmed systems do also segregate along environmental niche axes and give rise to assemblages with differing ecological properties (Bown et al. 2007; Pachepsky et al. 2007). In-field wild plants may be excellent indicators of system productivity and diversity (Marshall et al. 2003); and yet have been largely understudied (see Hawes et al. 2005). Therefore, while shepherd's purse should not be conserved *per se*, its utility as a diversity monitor on local scales (Paoletti et al. 1991; Hawes et al. 2005) demands

greater understanding through detailed characterization of large collections. In particular, in-field arable areas should be seen as sources of novel genetic and functional diversity for not only *Capsella* but also other wild (annual) plants. Recent evidence has established that in-field population structures for *Capsella* (at least within the UK) are heavily skewed toward the most fecund forms of the species (Iannetta et al. 2007). Many wild plant species that are not the primary targets of herbicides lack the capacity to adapt to this and other extreme selection pressures present in intensive agricultural practices (Marshall et al. 2003). However, *Capsella* does not appear to be one of these, and in this respect is similar to other commonplace crop-derived (and often Brassicaceous) volunteer and feral wild plants.

Assumptions that *Capsella*, and especially *C. bursa-pastoris,* is a "common wild plant" species accommodate the view that wild plant species are highly adaptive by virtue of their phenotypic plasticity. This perspective is misplaced and does not accurately reflect species structure. In a development of an original statement by Hurka and Neuffer (1997; cf. Steinmeyer et al. 1985), I highlight that, *the adaptive strategy of C. bursa-pastoris cannot be assigned to either ecotypic differentiation or phenotypic plasticity alone depends on the individual being studied*. This conclusion is supported by the classical genetic studies already carried out upon other model species, such as *Arabidopsis*. As such, there is no scientific basis upon which the "general purpose genotype" concept may be supported. The adaptive capability of a (wild plant) species must be seen as a product of those functionally distinct individuals that comprise that species. Ecologically important plant life history attributes such as leaf shape, number, rosette size, and flowering time differ greatly between accessions. As these traits do have a genetic basis, and are therefore heritable, the persistence of such broad traits will underpin species adaptation and resilience in the face of environmental (whether managed or climate) changes. However, as is common for almost all wild arable plants, there is a lack of information regarding even the most basic cytology and ecological significance (Hawes et al. 2005), an ignorance which is further compounded by scant detail regarding how these poorly characterized populations interact with the physical environment. While extensive habitat surveys of *Capsella* have been made in recent times, these do not extend to detailed analyses of in-field populations. Such studies are essential, though there appears a common non-recognition that the arable landscape is essential for food security (Rothstein 2007), and it is not yet accepted that common arable wild plants (such as *Capsella*) in this landscape are pivotal mediators of the essential ecosystem processes and services that underpin crop production. Therefore, when harvesting wild plant material with a view to its long-term study and conservation, other facts concerning the geophysical characteristics of the locality as well as its management history should also be obtained.

Collections of diverse intraspecific types from characterized in-field areas are, therefore, now being gathered. Ecotypes of *Capsella* (*bursa-pastoris*), which emerged as seedlings from soil seedbank samples, were collected from throughout the UK (130 accessions) and are held within a seedbank collection held at the Scottish Crop Research Institute (SCRI; to obtain seeds of the accession please contact the author). In addition, this collection is boosted by another *Capsella* collection, also derived from soil seedbank emergence studies. This second collection differs from previous collections, as these individuals were gathered from an exhaustive soil emergence sampling exercise in which all the individuals were isolated and grown on to provide seed for archiving. The soil samples were gathered from a wide range of farm types based along the arable East Coast regions of Scotland, and include fields under "intensive," "organic," or "integrated" crop management plans. This "East Coast of Scotland" collection includes around 1,300 *Capsella* individuals and a smaller number of *C. rubella* and *C. grandiflora* accessions. Despite the fact that *Capsella* has many suitable potentials, such as a tool for diversity monitoring or as a source of medicinal therapeutics (Defelice 2001), there appears to be no other equivalent extensive sampling (and plant-site) characterization strategy for *Capsella*. The author's seedbank collection also contains hundreds of other individuals from other (mainly) diploid self-fertilizing arable wild plant species collected using the same model approach. These other species include *Chenopodium album* (fat hen), *Atriplex patula* (Orach), *Polygonum aviculare* (knotgrass), *Persicaria maculosa* (redshank), *Persicaria lapathifolium* (pale persicaria), and *Lamium* (deadnettle) species. This collection is also joined by a third collection of "wild legume species" (from Scotland

and Sweden) that includes a bank of bacterial symbionts isolated from their nitrogen-fixing root nodules, which are also being used to develop their potential as cocontributors with other wild species to more sustainable arable farmed systems.

3.6 Comparative Functional Genomic Analyses

The genomic information data gathered from the genetic model plant *Arabidopsis* is duplicated in related dicotyledonous species, including crop plants (Rossberg et al. 2001; Horvarth et al. 2003), though the greatest correspondence is among other Cruciferous crop species, especially members of the *Capsella* complex (Kowalski et al. 1994; Lagercrantz and Lydiate 1996; Lagercrantz et al. 1996; Schmidt 1998; Acarkan et al. 2000, *Arabidopsis* Genome Initiative 2000; Schmidt 2002). Other genetically related wild (arable) relatives of *Arabidopsis* (and therefore *Capsella*) include *Sinapis arvensis* (wild mustard or charlock), feral *Brassica napus* (oilseed rape or canola) and *B. rapa* (wild turnip), and *Sysimbrium officinale* (hedge mustard). Arguably, *C. bursa-pastoris* because of it global prevalence, intraspecific functional diversity, and synteny with *Arabidopsis* represents a far more promising prospect for insightful studies of comparative genomics, adaptation, and evolution in situ. However, the diploid form (*C. rubella*) has received considerable attention as a genetic model, and its genome, along with other key and model *Brassica* species, is currently being sequenced (Schranz et al. 2006). In addition, and on the basis that the *C. rubella* genome sequence is available for comparative purposes with the genomes of close relative, a prioritized research agenda has been presented by Schranz et al. (2007) that encompassed a family-wide perspective of the Brassicaceae. Furthermore, and toward this end, a standardized gene nomenclature has been suggested by Lars Østergaard and King (2008) under the hospice of the Multinational *Brassica* Genome Project (MBGP; *http://www.brassica.info*), encompassing a *Brassica* gene registry, to manage gene names and encourage universal adoption.

Such comparative analysis and a functional genomic understanding of evolution and adaptation in model Brassicaceous species are welcome and essential to progress our academic understanding, and such knowledge is anticipated as being able to provide new perspectives on real-system processes. This assumption is driven by the extremely high levels of genetic synteny that exist between *C. rubella* and *Arabidopsis*. The small genome of *Arabidopsis* (0.2 pg of DNA representing 164 Mbp in five chromosomes per haploid genome, $2n = 2x = 10$; Schmidt 1998) is very much like that of *C. rubella* (170 Mbp on 8 chromosomes; Bennett and Smith 1976), with 90% identity for exon sequences (Acarkan et al. 2000). The high level of colinearity between the *Arabidopsis* and *Capsella* genomes exceeds that of *Arabidopsis* with the *Brassica* species, as discerned from genetic mapping of interspecific crosses of the two *Capsella* diploids (*C. grandiflora* and *C. rubella*; Boivin et al. 2004). However, though *Arabidopsis* is widely recognized as a model organism, its utility serves mainly academic priorities associated with plant molecular genetic analyses (Meyerowitz 1989), since research findings from this group may only be extrapolated to infer functional significance and ecological importance in situ. *Arabidopsis*, however, is only very rarely found at high population densities in natural situations (Roberts 1958), contributing negligible biomass toward the food web and productivity of higher trophic groups. Similar restrictions may apply to studies of *C. rubella*, given its restricted distribution too. It remains to be proven that *C. rubella* will bridge that gap in our understanding between academic model plants and other common wild species. In this context, I argue that shepherd's purse offers a more promising alternative.

Hybridizing functionally diverse ecotypes of *C. bursa-pastoris* from different geographical regions identified three quantitative trait loci (QTL) controlling flowering time. Flowering time is a trait that appears pleiotropic with rosette leaf number: the traits of later time flowering and more rosette leaves being associated (Linde et al. 2001). Similarly, Iannetta et al. (2007) also showed that rosette diameter (an indicator of resource capture potential) is negatively associated with time to flowering: i.e., late flowering plants possess markedly smaller rosettes (see also Neuffer and Bartelheim 1989; Neuffer and Eschner 1995; Slotte et al. 2006), and that flowering time did not appear to occur as a result of the same key genes identified as responsible in *Arabidopsis*. In fact, the genetic basis of life history traits, especially ecologically important ones, such as flowering time, will (probably) be different among intraspecific

variants. For example, flowering time variability in *Brassica nigra* is associated with the gene *Constans Like1* and not *FLC*/*FRI* (Kruskopf-Österberg et al. 2002). In addition, the late flowering habit in *Arabidopsis* and many other species is the archetypal form, early flowering and any associated (pleiotropic) life history trade-offs being a function of key gene modifications. However, unless a very deterministic biochemical pathway provides a phenotype, naturally selected variants may result from alterations in any one single gene from among a range of key genes that deliver that trait. This is best demonstrated for *Arabidopsis*, whose early flowering types have occurred via a variety of modifications and across numerous key flowering time genes (Komeda 2004). Similarly, the genetic control of other traits that are of pivotal ecological significance such as leaf shape is equally, or more, complex (Tsukaya 2006). Another example is that, in spite of their very high levels of genetic synteny, pivotal differences in key biochemicals can exist between *Arabidopsis* and *Capsella*, and among these are glucosinolates. These secondary metabolites are an important class of natural plant antifeedants that protect against leaf herbivory. Though glucosinolates occur in almost all species of the Brassicales including *Arabidopsis* and other closely related wild relatives, they are absent from the leaves of *Capsella* (Griffiths et al. 2001). While there is a question mark in relation to which antiherbivore defense mechanisms are used by *Capsella,* there is an even more important point to highlight, which is that very closely related species such as *Arabidopsis* and *Capsella* have very different mechanisms to protect from or deter herbivores. Therefore, if such divergent evolution can occur between these genetically highly syntenous species, it could, and indeed does, occur within other species also: emphasizing the importance of neutral selection, and perhaps the risks of extrapolating the findings of comparative genomics to inform crop breeding efforts (Broadley et al. 2008). After all, this key feature, diversity within species, was exploited to generate the crops we use today and their many varieties.

Nucleotide sequences from members of the genus *Capsella* can also be found on gene sequence databases. For example, main databases such as the National Center for Biotechnology Information (NCBI: *http://www.ncbi.nlm.nih.gov/*) currently hold around ca. 2,000 such sequences, though many of these are parts or duplicates. In addition, one may search the "phylogeny" database of this site to research the taxonomy of *Capsella* and any other genus: for example, from this source it may be discerned that other *Capsella* species have been named in addition to those three already named in the opening section here. From this data source, one would find other "provisional" species of *Capsella* listed including *Capsella heegeri* Solms, now extinct from its source of origin in western Germany, but maintained in botanical gardens (near Berlin). Also, *Capsella orientalis* (Klokov), isolated from Russia and western Ukraine, is noted at the NCBI site. In contrast, *Capsella pauciflora* is a synonym for *Hymenolobus pauciflorus*. However, it should be noted that among the three reproductive *Capsella*s and *Capsella* × *gracilis*, the only other potential *Capsella* is *C. procumbens* (L.) Fries, but this has been reclassified under the genus *Hymenolobus procumbens* (L.) (Yannitsaros 1973). While nucleotide databases offer essential insights for comparative genomic analyses between species, within-species variation may result from gross differences in DNA content that are less discrete than single nucleotide polymorphisms, such as the loss, or duplication, of chromosome parts. These differences are likely to have functional ramifications but can be difficult to detect. However, where different phenotypes present themselves, academic opportunities arise, such as the natural variant of *Capsella* termed "Spe," which exhibits stemenoid petals (the transformation of all the petals to stamens), which is being used to understand flower evolution (Hintz et al. 2006; Nutt et al. 2006). The array-based typing (Gupta et al. 2008) offers the possibility to identify the genetic regions, including smaller scale single nucleotide polymorphisms, which underlie such modified phenotypes.

The *Capsella* genome is being sequenced as part of a concerted research effort by the Community Sequencing Project (*http://www.jgi.doe.gov/CSP/*; US Department of Energy, and led by Prof. D. Weigel of the Max Planck Institute for Developmental Biology). In due course, *Capsella*-specific gene chips and associated technologies as well as databases are envisaged. While this approach claims to facilitate a "complete account" of the genetic basis of intraspecies polymorphisms for a species, I would argue that the understanding acquired will reflect genetic control at different scales: large – true for at least all the members of that genera, intermediate – true for only to species level, or small – that is, specific to those individuals that are assessed. Therefore, the accuracy

at each resolution can be verified only if sufficient sampling of individuals is achieved.

The interrelationship of *Capsella* with other (crop) species may also be inferred from expressed-gene relationships; and with this too, care must be exercised. The functional changes in many different genes also reflects the "neutral theory" of gene evolution, which dictates that many differences can also result from random events that are *not* under selection. For instance, from a study of expression levels among 18,000 gene transcripts from 14 taxa from the cabbage family, the differences in gene expression do not reflect functional adaptation: that is, genes expressed in the roots of a species are more similar to the genes expressed in the shoots of that species, rather than the roots of another and even very closely related species. There is, therefore, a necessity to use null models when interpreting gene expression data (Broadley et al. 2008), especially when using expression data to assess the interrelatedness of members of that group. These considerations should be borne in mind to identify comparative genomic research approaches that will have ecological (in situ) relevance.

Consequently, for ecological studies in situ, there is perhaps greater utility in being able to diagnose the relationship between and within the Brassicaceous genera using specific and robust molecular signature technologies (Sunnucks 2000), rather than attempting to discern the exact genetic basis of complex and interacting genetic components that underpin key life history traits. For example, simple sequence repeats (SSRs) designed to distinguish different functional types among one Brassicaceous species may be used on other similar species (Kresovich et al. 1995; Clauss et al. 2002; Iannetta et al. 2007). It is possible that existing genetic sequence information tools can be exploited to quickly facilitate molecular diagnostics for diverse *Capsella* species and ecotypes. Intersimple sequence repeats (ISSRs; Zietkiewicz et al. 1994; Wolfe et al. 1998; Bornet and Branchard 2001; Sica et al. 2005) present themselves as an especially useful tool since the method may be applied to small quantities of DNA in highly reproducible and high-throughput analyses with no prior DNA sequencing being necessary. These advantages are not accommodated by other dominant (and codominant) marker techniques (Wolfe and Liston 1998). Random fluorescently labeled ISSR primers have been used in automated laser-based genotyping approaches to assess the diversity of *Capsella* (Iannetta et al. 2007; Wishart et al. 2008) and "genetic individuality" in grassland systems (Fridley et al. 2007; Whitlock et al. 2007). The development of this technique to accommodate *very* rapid analyses across and within many different species depends upon the generation of data-handling methods and standard statistical procedures that are able of accurately assess dominant-marker data that are currently being investigated by Iannetta and coworkers at SCRI; that may also be extended to a unique form of genetic barcoding.

3.7 Scope for Domestication and Commercialization

New perspectives and developments using wild species such as *Capsella* are now being pursued to conflict with the "usual" (herbicide-biased) research attention that is focused upon such common wild species. That is to say, the efforts usually afforded to *Capsella* and other similar wild species by "developed" western cultures have been mainly geared towards developing methods that will facilitate their elimination. However, and as indicated by the term "Medic," which is associated with the full species name for shepherd's purse, *Capsella* has been recognized throughout history and across several diverse cultures as possessing powerful therapeutic qualities (Defelice 2001). While the fresh or dried roots may be used as a substitute for ginger (Duke 1992), *Capsella* leaves and flowering stems feature most commonly in the ethnobotanic literature. This is not surprising, since shepherd's purse is one of the earliest and most common wild greens to appear in the spring. The rosette leaves are best eaten before the flowering stems appear, when the leaves are tender and show no signs of senescence. The leaves of *Capsella* are used globally and are good served fresh in salads or cooked as greens, and have been described as having a pleasant and distinctive flavor that is similar to spinach, chard, asparagus, and even Brussels sprout. It is also interesting to note that, as a green vegetable, the later time-to-flowering forms of shepherd's purse possess more and larger leaves (Iannetta et al. 2007), which when grown in

good conditions can number up to 500 leaves and possess a rosette diameter of more than 60 cm (SCRI data). These ecotypes may be of greater commercial potential than the small rosettes of early flowering forms. However, also note that the converse will be true for fruit-bearing stems, commonly used for medicinal *Capsella* teas, with rapid cycling forms producing more flowering stem biomass. It is clear then that different ecotypic variants of *Capsella* may be suited to particular uses and, if development of commercial varieties is to succeed, their susceptibility harbor various microbial infections (see below) will need to be addressed.

3.8 As a Source of Medicine and as a Therapeutic

Shepherd's purse can be found as a prominent species in records from the ethnobotanic literature (Zhou 1998), especially from eastern and oriental cultures. In westernized societies today, *Capsella* tea may be commonly found in European markets, especially from sources in Hungary and Croatia, where is it is grown and marketed only at local scales. It is usually sold as chopped flowering stems (without seeds), being presented in a dried form for preparation as herbal tincture. *Caspella* root or leaf tea has been used to treat scour (prolonged diarrhea) in cattle (Vickery 1995). However, as scour has several different causes spanning viral, bacterial, protozoal, and insect-based forms, it would seem unlikely that *Capsella* would be effective against all sources.

The anticancer properties reported for extracts of *Capsella* were attributed to fumaric acid (Kuroda et al. 1976; Kuroda and Akao 1981), and ethanol extracts have been shown to cause vasodilatation and smooth-muscle contraction, including negative inotropic (muscle relaxing) and chronotropic (alteration to muscular rhythmic) action (Kuroda and Takagi 1969). The ability to decrease blood pressure and contract the small intestine and blood vessels was attributed to acetylcholine and choline, and tyramine, respectively (Kuroda and Kaku 1969). This may explain the use of *Capsella* a diuretic and febrifuge (antifever), and as a poultice for direct application to the surface of wounds. In terms of plant defense, novel phytoalexins have been isolated from *Capsella*, which were the subject of investigation due to their natural resistance to the crop pathogen *Alternatia brassicae* (Jimenez et al. 1997). In terms of human protection, and from 11 wild plants (isolated in Egypt) that were screened for their relative antibiotic potencies, Soxhlet-benzene extracts of *Capsella* were most effective; and this success was ascribed to the presence of alkaloids and flavenoids (El-Abyad et al. 1990). Novel antibiotic peptides from *Capsella* have also been noted (García-Olmedo et al. 2001). These peptides are localized mainly within the roots of *Capsella* and are called "shepherdins" and linear glycine/histidine-rich peptides (Park et al. 2000). These reports also lend scientific evidence to support the use of *Capsella* as a treatment for dysentery and eye infections. Other potential (human) health benefits have been highlighted, which include exploiting *Capsella* as a source of novel antioxidants (Kweon et al. 1996), anti-inflammatories (Raymond 2008), and a biomonitor of heavy-metal toxicity in soils (Aksoy et al. 1999).

As the potential of *Capsella's* commercial properties are realized, the question of how to improve the performance of the species in an agronomic context should also be considered. This includes enhancing the commercial trait for maximal benefits, perhaps using husbandry, breeding, or biotechnology, to enhance agronomic qualities such as disease resistance. Being of small genome size and diploid, species like *C. rubella* and *C. grandiflora* are sensible options for the generation of phenotypes in reverse genetic approaches, to understand the molecular basis of key traits, and to improve breeding germplasm. "Targeting induced local lesions in genomes" (TILLING; McCallum et al. 2000) is an excellent example of this approach. Originally developed using *Arabidopsis*, the method is being used to develop a wide range of crop species such as barley (Caldwell et al. 2004). While all these approaches offer possibilities, herbal-based medicines, whether improved or not using biotechnology, must be proven scientifically for use on humans. This demands robust clinical trials from the use of double-blind, placebo-controlled, and randomized experimental designs *as routine* (Ernst and Chrubasik 2000).

There are fewer restrictions for the development of *Capsella* over the shorter term as herbal therapies. These may be marketed readily, as their sale is faced with only

limited quality assurance legislation compared to products marketed as medicines. Consider Ocean Spray (cooperative of over 600 U.S. cranberry growers), which is a leading producer of drinks and whose turnover is $1.4 billion per year. The world market for herbal products is $60 billion, growing at 7% per year (Europe accounting for the largest portion, $23 billion), and projected to exceed $187 billion by 2010 (WHO). In China, the largest herbal tea producer (Wanglaoji) had revenues increase from $100 million (2004) to $375 million (2005). A fundamental and strategic knowledge base will secure a position for *Capsella* in this marketplace (Sucher and Carles 2008; Wishart et al. 2008). *Capsella* may also be exploited as stand-alone crop monoculture. This is also of large socioeconomic and environmental importance, as this approach adds to the essential diversity of agricultural production systems directly (Iannetta et al. 2007), developing the foundation for a commercial niche for arable wild plants like *Capsella* in nutraceutical-based cash crops (Meagher 1999). In addition, there are only around 8–10 main species of arable crop currently grown in Europe; any increase above this number is likely to diversify the economic base and land management strategies to enhance the resilience and sustainability of the industry and the environment (respectively).

There is also considerable potential for wild plants, such as *Capsella,* to be grown as companion plants among some crop species. Excellent examples of this can found in the peer-reviewed agricultural literature. Vieyra-Odilon and Vibrans (2001) note that Mexican families farming the Toluca Valley, on average, consume 4.5 kg of herbs per month. These are harvested from maize fields, and in fact, 55% of the net (crop maize) value was realized as green forage or human food. Wild plants can increase the useful biomass of a field and improve the nutrition of those choosing to use this directly as human food supplement.

The nutritional potential of volunteer wild crops, such as *Capsella*, cannot be addressed in detail here, as there are only a small number of peer-reviewed articles of this topic (see Guarrera 2003). Even many of those are not presented in English, and so there is scope to translate these potentially important scientific articles, particularly by Chinese (e.g., Zhou 1998) and Russian (e.g., Iurisson 1973, 1976) scientists. However, there are reports (non-peer-reviewed as far as I am aware) that *Capsella* leaves contain very high levels of B vitamins, especially thiamin (B1) and lower quantities of other vitamin B family members which include choline, inositol, and riboflavin (B2). Among the peer-reviewed literature, *Capsella* has also been noted as a source of ascorbic acid (vitamin C) and β-carotene (a vitamin A precursor), the levels being comparable to or greater that those vegetables (e.g., spinach) that are commonly marketed as best sources of these nutrients (Zennie and Ogzewalla 1977). *Capsella* is also noted for the high concentration of leaf vitamin K (K_1, phylloquinone), and it is curious that high levels of this vitamin are linked to the herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) resistance (Jansson 2006). In contrast, shepherd's purse could also present unappetizing flavors and they have also been noted to contain a wide range of different saponins (potential antifeedant glycosides) that can confer a bitter taste (Badia 1954; Garcia-Marquina et al. 1955). Whether or not *Capsella* nutrients or antifeedants are studied, there is little known about the levels of these bioactive chemical differing among ecotypes, throughout the plant life cycle or across the different plant parts, and in this respect, there is also scope for much more research.

Exploiting wild plants as "volunteer crops" need not adversely affect sown crop productivity. The coexistence of wild plants in maize plantations was not seen to reduce the yield of the main crop in Mexican field studies. In fact, the wild plant provided essential ecosystem services and not least among these was the control of soil erosion. The wild plants were removed only during the critical grain filling period (Vieyra-Odilon and Vibrans 2001). So while the use of wild plants is generally seen as a renaissance in western nations, in other ("less developed") regions of the globe it has been traditional to incorporate wild species as "volunteer crop-companion species" and essential elements of their production system. This approach is in contrast to the archeological findings from wild plant assemblages found throughout Europe, which indicate that intensive agricultural regimes based upon wild plant eradication were common among the early farmers in that locality (Jones 1992). I would claim that the legacy of this history is the mindset that has vilified in-field wild plants as nonessential: a perspective that must be adjusted using targeted suite of education and uptake strategies if we are to establish truly sustainable arable cropping practices.

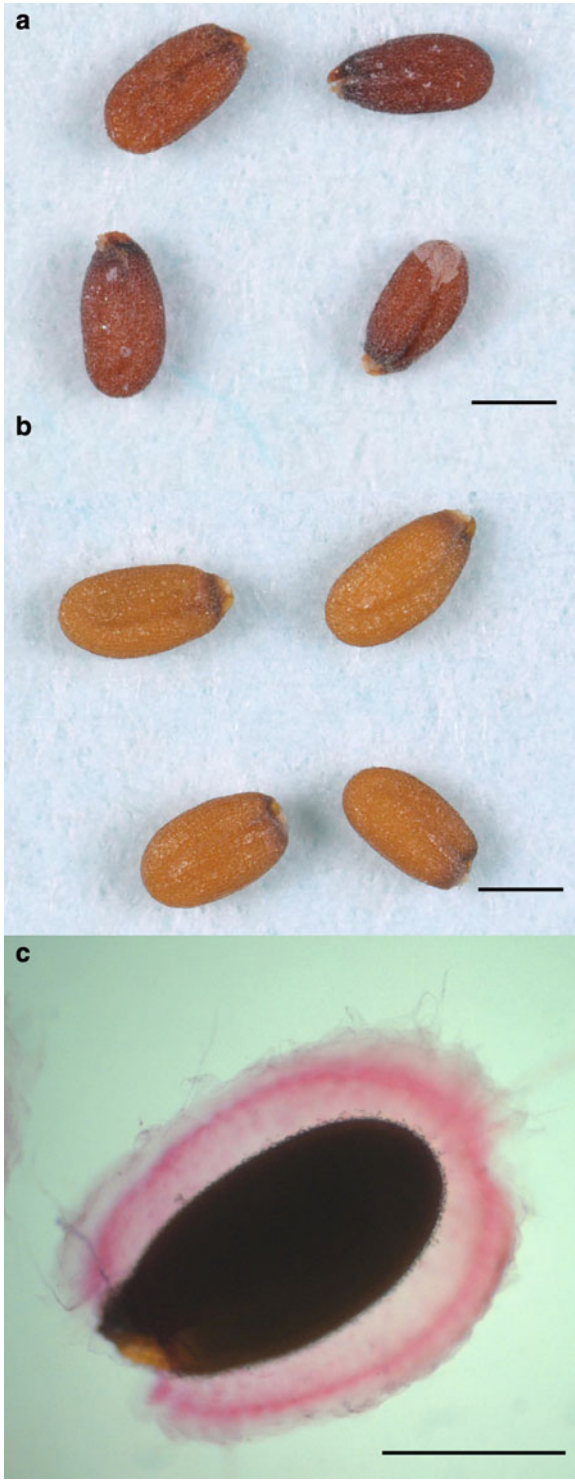

Fig. 3.6 Shepherd's purse produces seeds are heteromorphic. Smaller dark-brown (**a**), or larger light-brown seeds (**b**) are harvested from a single maternal source. The larger more mature seeds come from the lower most (oldest) siliques, and exhibit a fully myxospermous character. When hydrated the light-brown seeds exhibit a polysaccharide sheath around the seed. The ecological function of myxospermy has not been scientifically proven, though it is perceived as an adaptation to growth, and persistence, in water-limited environments. It is likely that seed dormancy, germination and longevity are also affected, though this too remains to be shown. The myxospermous sheath is comprised of pectinaceous polysaccharides as exemplified by the ruthenium red (pectin specific), stained light-brown shepherd's purse seed (**c**). Three layers may be discerned in the myxospermous coating: a pectin-only outer- and boundary-layers, and an inner layer in which a β-glucan (cellulose) fibrillar matrix is also localized. The glucan matrix strongly anchors the inner-most pectin layer to the seed coat, rendering this component very difficult to extract or remove. *Bars* = 0.5 mm

3.9 Commercial Potential Is Linked to Seed Conservation

Uses for seeds are also found, and harvested seeds of *Capsella* have been found at Catal Huyuk, one of Turkey's oldest cities (5950 BC) and in the stomach of Tollund Man (Scandinavia; 400 years BC; Mabberley 1997). Presumably, and like *Chenopodium album* (fat hen), these seeds were eaten in a form of porridge. Its potential in this respect is not being explored today.

More novel applications of the *Capsella* seeds extend to achieving greater understanding of its myxospermous seed-coat structures, whose ecological function has only recently been demonstrated (Fig. 3.6; see also Patel 2007; Toorop et al. 2008). Similar studies in *Arabidopsis* had only alluded to the potential functional role of myxospermy, and a survey of the literature shows that this trait has not been well characterized and the term (myxospermy) is used to describe a very wide range of different seed mucus structures. Beyond this academic and botanical perspective, the utility of the *Capsella* seeds as being able to attract and trap mosquito larvae has yet been tested in vivo (Mabberley 1997).

If agriculture is to be seen as the third axis of the neutraceutical industry (Meagher 1999), good scientific research will have to discern folklore from fact and build commercial opportunities for herbal remedies and neutraceuticals. This realization has already led academic interests, which normally focused on *Capsella* as a model for wild plant-control agents and management strategies, to compass an exploration of *Capsella*'s true potential. However, the example of the Chiang Mai Declaration should perhaps be

mirrored, though with modifications including a focus upon wild plants of temperate regions (Foster 1993). Using this model, health authorities, plant biologists, and environmental scientists could develop concerted strategies to cope with societal and environmental issues, focusing upon important ecosystems (Akerele 1993) such as the conservation of arable systems and associated plant species. This strategy would be most successful through application on a local scale that allows its tailoring to include more species and a wider range of habitats.

Despite the value of plant-derived medicine and food stuffs being $23 billion in Europe, agro-industrial technology is still not applied in a focused way towards the cultivation and processing of wild plants. Conservation of the *Capsella* genus as a diversity indicator species may, therefore, be extended by the growing interest in natural sources of medicines and other materials with commercial potential. Success in this respect will demand not only banking ecotypes but also concerted research action that encompasses phenotypic, biochemical, and genetic typing of the accessions. Note also, and ironically, that funding policies should embody serendipitous research approaches to accessing the potential of wild plants too because of the ca. 120 distinct plant-derived medicines used in primary health care (from 90 species of plant), 25% were not related to that traditional use for the species (Farnsworth et al. 1985; Farnsworth 1988).

3.10 A Model to Improve Arable Ecosystem Productivity and Sustainability

There is increasing awareness of global warming and concern over fuel and food security. This has driven a move toward lowered carbon footprints by increasing efficiency (on the farm) through reduced tillage and lowered application of agrochemicals. Within arable production systems, there is therefore a growing trend toward greater tolerance of wild plants within crops and the development of crops with environmental traits that include nutrient uptake and use efficiency, and especially for nutrients such as nitrogen and phosphorus. Water use efficiency is also a target environmental trait along with the development of crops with a natural wild plant suppression capability (Hoad et al. 2008). Beyond the direct cost saving that may be made, environmental benefits include increased wild plant abundance and greater nutrient capture and flow to natural food webs, which include natural predatory insects capable of controlling pests and supplying an important food source for birds. It is also interesting to note that *Capsella* has been the subject of other natural methods of wild plant control. For example, the allelopathic properties of buckwheat residues (*Polygonum convolvulus* L.) tested on *Capsella* showed good wild plant suppression (85% dry weight reduction), but this suppressive quality was overcome by the addition of nitrogen (Kumar et al. 2008). Hoad et al. (2008) also explored the interplay between natural inter- and intraspecific variation for cereals and their potential for non-herbicide-based control of wild plant populations. In this context of weed–crop coexistence and what levels are tolerable by the crop, *Capsella* has presented itself as a useful model again (see Freyman and Hall 1992). Research by Petry et al. (1993) revealed that a multispecies wild plant population (that included *Capsella*) was less competitive with a wheat crop than a wild plant population that comprised a single species. Also, it has been demonstrated that *Capsella* presented no threat to the yield of field grown radish (*Raphanus sitivus* L.) crops with which it coexisted (Perera and Ayres 1992) and at densities much greater than the usual 1% of in-field biomass.

3.11 Potential Disease Risk to Crops

The possibility that greater crop–wild plant coexistence will present increased risks to crop health may also be encountered. This is a particularly important consideration, with particular relevance to *Capsella* as a prevalent in-field species.

For example, just some of the fungal pathogens reported to infect shepherd's purse include *Alternaria brassicae*, *A. brassicicola*, *Leptosphaeria maculans* (blackleg), and *Verticillium dahliae* (Henriksson 1995; Petrie et al. 1995; Cooke et al. 1997). All of the aerial parts of *Capsella* may be susceptible to *Albugo candida* (white rust; Alexander and Burdon 1984). *A. candida* was originally described on shepherd's purse and as a host, and *Capsella* has been used to facilitate molecular characterization of this fungal

pathogen throughout the world (Choi et al. 2007). Shepherd's purse may also be host to other rusts *Puccinia trabutii* and *P. lagenophprae* (Paul and Ayres 1990). *Capsella* may also be infected by *Peronospera parasitica* (downy mildew; Alexander and Burdon 1984) and *Erysiphe communis* (powdery mildew). On a more positive fungal (symbiotic) note, the development of vesicular-arbuscular mycorrhizae (VAM) can be found in *Capsella* roots, though this genus (as is common for other members of the Brassicaceae) is characterized as non-mycorrhizal (Demars and Boerner 1994). In fact, VAM can colonize presumed "non-mycorrhizal" species such as *Capsella,* though this is dependent upon the root community matrix with which "non-mycorrhizal" plants exist.

Capsella seeds may also harbor viruses, and among those recorded are the beet yellowing viruses (Stevens et al. 1994), tomato spotted leaf virus (Bitterlich and McDonald 1993), potato leaf roll virus (Fox et al. 1993), and tobacco rattle virus (Wishart et al. 2007). Karley et al. (2008) established that late and early time-to-flowering forms of *Capsella* present a different resource (food) quality to aphids, which are also viral vectors. Aphids exhibit different survival and reproductive rates depending upon the *Capsella* ecotype. Cotton aphids (*Aphis gossypii*) may also infest *Capsella* (Hosoda et al. 1993; Belair and Benoit 1996). Other insects that predate upon *Capsella* include nematodes (*Meloidogyne hapla*), and arachnids (*Aceria drabae*) may cause galls on *Capsella*. *Capsella* is also very susceptible to grazing by all forms of mollusk (Duval 1971, 1973). Aksoy et al. (1998) highlighted that insects living on *C. bursa-pastoris* spanned many different insect orders including the families Lygaeidae (chinch or atypical seed) bugs and Aphididae that encompasses most plant viral vectors, such as *Myzus persicae* (the peach aphid; see also Karley et al. 2008). Both these families belong to the Hemiptera: that is, insects with sucking mouth parts. Also, members of the family Pieridea (order Lepidopdera) are known to encompass species of leaf feeders that are also common agricultural pests. In addition, among the order Coleoptera (beetles), there are other leaf mining beetles (Chrysomelidae), true weevil species (also known as snout beetles; Curculionidae), and "stout" and often "metallic bodied" beetles of the family Scarabaeidea, which are known to include *Caspella* root feeding species. Families within the Hymenoptera include species of sawflies (Tenthredinidae), gall-flies (Cecidomyiidae), and leaf-miners (Agromyzidae). It should also be noted that there is also one report in the peer-reviewed literature that presents indirect evidence of nitrite poisoning in pigs by shepherd's purse (Wiese and Joubert 2001).

It is likely that *Capsella* will harbor many more fungi, bacteria, viral pathogens, and insect species than reflected in the meager list above. It is also curious that despite the capacity for shepherd's purse to act as a harbor for these agents, individuals do not appear susceptible and avoid being affected deleteriously. Also, note that presence of a crop pathogen on a wild plant species (such as *Capsella*) is not proof of their culpability when severe disease outbreaks occur in crops, as severe diseased crop outbreaks are more likely to be a function of repeated cultivation over many years of the same susceptible host (crop) plants. Poor crop management is, therefore, the regulator of persistent reservoirs of crop infections. Good management might include "breaks" in the species or type of crop that is planted: for example, substitution of monocotyledonous plant for dicotyledonous plants – that is the use of crop that may not be hosts to pathogens. This "break" strategy should be a primary management option, as opposed to continued production of the host crop in conjunction with advocating wild plant extermination using indiscriminate herbicides.

3.12 Role in the Generation of New and Novel Crop Species

A potential role for *Capsella* in crop improvement is now being recognized, and there are reports in which shepherd's purse-specific traits, such as resistance to flea beetles (*Phyllotreta cruciferae* and *P. striolata*), cold tolerance, and rapid-cycling, have been introgressed into existing crop species. The protoplasts of shepherd's purse have been hybridized with inactivated protoplasts of *B. oleracea* to improve the potential of this commercial species (e.g., *Brassica oleracea*; Bonfils et al. 1992, Sigareva and Earle 1999). *Capsella*-specific genes conferring frost tolerance (and possibly salt and drought tolerance) have also been cloned (Sun et al. 2005; Wang et al. 2005; Fan and Wang 2006; Lin et al. 2007) for transformation

into other cold-intolerant species (Liu et al. 2004; Wang et al. 2004a, b). Bartholmes et al. (2008) also identified shepherd's purse as a very attractive system for studies concerned with understanding evolutionary and developmental biology that can be easily transformed using floral dip methods.

Recently, other research efforts have generated intertribal crosses between *C. bursa-pastoris* ($2n = 32$) as the pollen donor to *Brassica rapa* (also known as syn. *Brassica campestris*) ($2n = 20$) and *B. napus* ($2n = 38$). From each maternal source, two and three, respectively, F_1 hybrids have been characterized and had acquired agronomically important *Capsella*-based traits (Chen et al. 2007): namely, low erucic acid and glucosinolate content and high resistance to *Sclerotonia sclerotiorum* (water-soaked spot) that affects many crop species. Though such hybrids are generated in the absence of pollen competition, such studies do highlight that sexual isolation among members of the Brassicaceae is not absolute and, while such events in nature may be rare, they are possible. Shepherd's purse protoplasts have also been regenerated and used to generate intertribal somatic hybrids with rapid-cycling types of *B. oleracea* to confer resistance to *Alternaria brassicicola* (Sigareva and Earle 1999). Methods have also been described to rescue the embryos of intertribal hybrids from ovules of shepherd's purse (Monnier and Clippe 1992), as hybrids are at high risk of abortion. Though rarer than its sister species, *C. grandiflora* has also been subjected to some molecular dissection by academics, particularly with respect to the fact of its obligate outbreeding habit and evolution within the Brassicaceae (Paetsch et al. 2006). This has shown that, while there has been differing self-incompatibility along the two lineages (*Capsella*/*Arabidopsis* and *Brassica*), the diversification of allele regulating self-incompatibility can predate the phylogenetic separation of at least *Capsella*/*Arabidopsis* species.

These biotechnological opportunities must be managed carefully, especially given the global uptake of genetically modified (GM) technology among at least this wild plant family. Had it been my choice, GM safety and policing would not be left to any commercial companies; instead, a regulatory framework should be established and enforced by completely independent government sponsored research authority that would ascertain the suitability of the products for release. Commercial developers of GM plants would have to build the cost of this compliance into their product development plan.

3.13 *Capsella* as a Potential Invasive Plant

Shepherd's purse, as a plant that reproduces entirely using seeds, may be perceived as being of limited invasive potential. In addition, *Capsella's* wide global distribution excludes its potential as a true invasive species, which is defined as "a species whose introduction does, or is likely to adversely affect the wellbeing of economic productivity, the environment or health (human or animal)." While invasive species may spread using seeds, their capacity to dominate in new environments is attributed a lack of natural enemies and vigorous growth habit that includes a reliance upon vegetative, as opposed to sexual, reproduction, which supports their competitive exclusion of other plant species. However, sexual reproduction alone can, on occasions, be a successful strategy for an invasive species, especially self-fertile species such as *Capsella*. *Capsella*'s other life history characteristics, such as rapid growth, early maturation, high fecundity, and a wide dispersal capability, will also promote its invasion potential. In addition, the capacity to be self-fertile (like *Capsella*) may well be linked to a plant's ability to be a "colonizing" species (Price and Jain 1981). Its global distribution is testimony to the ability of this species to dominate and persist in many different landscapes, as long as they are occasionally disturbed. As such, *Capsella*'s persistence is not linked to its competitive ability or the displacement of other plant species, its capacity to adapt being facilitated through adaptations that encourage its coexistence with other most common crop species. The key in this respect is the potential number of seeds that can be produced by shepherd's purse, which has been recorded as 38,500 and 90,000 seeds per plant (Stevens 1932; Bosbach et al. 1982). In addition, and without the limitation of winter conditions, the underground resources of *Capsella* appear to be considerable, since plants that have even all their above-ground foliage removed are capable of producing new leaves and flowering stems several times. Aksoy et al. (1998) and Rutledge and McLendon (1996) limited this to two to three regeneration per

year. Our records have shown that the number of regenerations depends on the amount of resources accumulated during the vegetative stage, and may be repeated as many times as the growing season will facilitate. Regardless of the species, *Capsella* fruits contain elongated elliptical seeds, of which there are generally 12–24 and on occasions perhaps as many as 40 in the characteristic angustiseptate (heart-shaped) siliculae, each seed weighing only around 100 ± 20 μg. We have recorded seed numbers that range from approximately 80 to 300,000 per individual plant, which is 6 orders of magnitude greater than other published estimates of maximal fecundity. It is very interesting that the highest fecundities are achieved by plants with a good balance of shoot to root resources that are of intermediate (to short) flowering times. While ecological theory would dictate that slowly developing plants accumulate more resources to produce more or larger seeds (Aarssen and Taylor 1992; Aarssen and Jordan 2001), this is not the case for characterized types from in-field accessions of *Capsella*. In fact, the opposite is true, with the most fecundative individuals being the fast growing and of limited leaf area leading to the reiteration of the suggestion that agriculture has selected for a greater preponderance of resource-use-efficient and rapid cycling accessions, which were around 95% of all those tested (Iannetta et al. 2007).

It is often assumed that *Capsella* will not persist for more than 25 years in the soil seedbank unless the soil is repeatedly disturbed. However, the prevailing climatic conditions will affect any presumed in situ "shelf-life," and the potential to recover, or maintain, individuals from soil seedbanks is likely to be different in different regions of the world. It should be considered that *Capsella* seed developed on plants in temperate latitudes have lower mass but higher lipid content than seeds from colder regions. It was also indicated that this was due to the rigid genetic control rather than environmental variables (Mukherjee et al. 1984), perhaps indicative of local adaptation to cold. Estimates of in-soil longevity for *Capsella* seeds should also be treated with caution on the basis that we also have only limited knowledge of seed traits in field. Dormancy and germination characterization experiments have focused upon methods to maximize germination, rather than understand the dormancy among ecotypes, and this is especially true in relation to difficult to characterize traits such as secondary dormancy. Other important traits include a seeds ability to remain viable over time, which is perceived as a function of dehydration tolerance. Of those *C. bursa-pastoris* accessions characterized by Iannetta et al. (2007), germination tests established that, among all the accessions assessed and regardless of the flowering time type, virtually all (99%) were viable despite their maturity class. While heterodiaspory has been recognized in shepherd's purse (Teppner 2003), and the formation of seeds structurally characterized (Shamrov 2002), this had not been translated to life-history strategies and trade-offs. However, Toorop et al. (2008) reported two seed classes within seed batches from individual accessions. These were less mature, darker seeds that were produced from higher up the flowering stem closer to the terminal inflorescences, which lack the testa mucosa, and more mature light colored seeds that might be considered fully myxospermous. While the light and dark seed classes showed similar levels of viability (ca. 100%), their relative dormancies differed, and were related to the time-to-flower character of the mother plant. In addition, their relative sensitivity to light and nitrogen as well as longevity differed significantly depending upon maternal time-to-flowering type (Toorop et al. 2008). While it is useful for scientific discovery to artificially segregate the *Capsella* seeds into light and dark forms, this disguises reality. In fact, there is a gradation in seed maturity status and the associated seed traits, and therefore, the adaptive potential within a single batch of seeds may be more finely tuned than the extremes would predict. The important conclusion is that a single *Capsella* individual produces many seeds that are graded in their life history potentials for dormancy, germination, and persistence. Such diversity in seed life history capacity promotes "bet hedging" (Lawrence 2007) to present clear survival and persistence advantages for this species and is likely to be repeated in other plant species.

The capacity for longevity of viable seeds of wild species in the soil seedbank has also not been fully addressed. The persistence of *Capsella* seeds in the soil has been estimated at 2–5% seed viability after 10 years of burial (Conn and Deck 1995), suggesting a half-life of 5 years. However, Baskin et al. (2004) reported ca. 100% viability of *Capsella* seeds after 2½ years of burial. Perez-Garcia et al. (2007) reported ca. 95% viability of *Capsella* seed held in storage for 34 years. *Capsella* seeds return to the seedbank in very

large numbers, and it is possible that their persistence will extend beyond what is anticipated. If we assume, even using the 5-year half-life, that the seed decay is an exponential function, from 1,000,000 seeds set (which could easily be produced by only 50 plants), after 100 years one seed would persist, or 62,500 viable seeds after even 20 years; these are likely to be underestimates. This theoretical exercise demonstrates, albeit crudely, that the persistence of such seeds in the soil extends far beyond our expectations – thankfully this potential could accommodate the recovery of lost ecotypes, and even species lost generations ago.

Throughout the world, an increasing number of facilities are being built that catalog seeds and store them in "libraries" under optimal storage conditions that maximize seed longevity. This is occurring at a pivotal stage in human cultural evolution, as this is also a time when there is also increased global recognition of the ecological interdependence of crop and wild species for maximal system productivity. Yet, most seedbanks aim to preserve mainly crop species or crop-related germplasm (for example, the Svalbard Global Seed Vault; Fowler 2008). Future crops, the current wild species, are largely ignored. There are exceptions, of course, and some seedbank focus only on wild species, such as the Royal Botanic Garden Kew's Millennium Seedbank (*http://www.kew.org/msbp*). The responsibilities of seedbanks should be broadened to include (1) wild species; (2) molecular assessment to ensure a minimum breadth of intraspecific diversity; and (3) the long-term storage of seed in their natural soil bank. The storage of soil seedbank samples might also allow preservation of associated microbial fauna, which may also be ecologically important for optimal development of the seed in situ.

Most importantly, it should be acknowledged that wild plant traits are still evolving: adapting to the intense selection pressures that they face from modern agricultural practices: competing spatially (within crops) or temporally (in the time windows between plantings); and in response to the commonly used tilling approaches and chemical applications. The most dramatic example of this capability is the increasing prevalence of wild plants resistant to commonly used herbicides, such as glyphosate and other chemicals (Powles 2008; Holt and LeBaron 1990). Selection for such an obvious adaptation (herbicide tolerance) is no more ecologically significant than the enrichment of wild plant populations for other less obvious traits (e.g., time to flowering and fecundity; as described above) as a result of non-chemical interventions such as crop type or tillage practice (Hawes et al. 2005; Owen 2008). Diversity must be organized into all aspects of the cropping system if the selection pressures that drive wild plant adaptation are to be reduced and sustainable wild plant management achieved. Fundamental to our success in obtaining truly sustainable cropping is maximizing wild plant growth in-crop and parallel integrated pest management approaches (Kogan 1998).

It is fortuitous that wild plants possess such a rapid evolutionary capacity, given their importance in arable ecosystems: sustaining diversity through their passage of energy and essential nutrients to the wider environment, especially the soil and the food web. Our future challenge is, therefore, to increase our understanding of wild plant species, especially their ecological importance in different agricultural and landscape contexts and continue the manipulation that we have begun. Future management should not proceed as it has done, but should use informed decision making to balance the obvious environmental and commercial benefits of greater wild plant–crop coexistence with other obvious costs such a decreased yield (Costanza et al. 1997; Mader et al. 2002). In addition, we must quantify (where it is found to occur) whether there is any reality in the perceived increased risk posed to crops by their coexistence with wild plants that harbor pathogens.

3.14 Containment of Gene Flow

Considerations of *Capsella*'s long-term persistence is especially important in the context of transgene flow from GM species such as canola (*B. napus*), and other related species. The flow of transgenes from crop plants to wild *Capsella* has not been proven, though specific experiments to generate such hybrids have been attempted (Pu et al. 2005). Where such cross-species transgene movement is possible, GM components could persist in the soil seedbanks indefinitely. Gene flow from crops to wild or wild plant relatives is often cited as a potential risk in the commercialization of transgenic crops (Wolfenbarger and Phifer 2000), and it has been demonstrated that

some crop species have exchanged genes with wild plants for centuries and this is especially true for cerealcrops and wild grasses. Among broad-leaved species, spontaneous hybridization of rape with wild mustard and wild hoary mustard (*Hirschfeldia incana*) and wild radish (*Raphanus raphanistrum*) has been demonstrated to occur when using a male-sterile oilseed rape cultivar as the pollen recipient. Interspecific gene flow between GM oil seed rape and partially allogamous wild cruciferous relatives has been the focus of intense research investigation (cf. Jørgensen et al. 1996). These related species include gene flow to shepherd's purse and other wild species such as charlock, hoary mustard, wild radish, wild turnip, hedge mustard (*Sisymbrium officinale*), and white mustard (*Sinapis alba*). Gene flow was detected from oil seed rape to wild turnip at hybridization frequencies up to 50% (Norris and Sweet 2004), and some of these were backcrossed in the direction of both parents, and the high densities suggested persistence and gene flow can occur over a prolonged period. Gene flow to wild radish was also detected, though this was in the absence of pollen competition (Chèvre et al. 1996). Lefol et al. (1996a, b) showed that the reciprocal cross can occur in the field with hoary mustard. Interspecific hybrids in the field between (non-GM) oilseed rape and wild radish as the seed parent have also been reported (Eber et al. 1994; Darmency et al. 1998). In contrast, gene flow to *Capsella* has not been demonstrated and is highly unlikely. However, given the potential of *Capsella* and other wild *Brassica* relatives as a GM crop, this situation may change, especially if this it developed in parallel with the introduction of new *Capsella* × *Brassica* hybrids (Sigareva and Earle 1999; Monnier and Clippe 1992). This concern is heightened if we consider the possibility that the genetically engineered plants may be equipped with fitness benefits that could led to an exacerbated wild plant problem. For example, sunflowers modified to express a fungal-derived insect antifeedant, *Bacillus thuringiensis* (*Bt*) toxin, benefited from an increased seed yield (Snow and Moran-Palma 1997).

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Chapter 4
Carthamus

Deepmala Sehgal and Soom Nath Raina

4.1 Introduction

The genus *Carthamus* L. is a member of the tribe Cynareae, subfamily Tubuliflorae, and family Asteraceae. The eastern part of the Mediterranean region is regarded as the center of origin of the genus (Ashri and Knowles 1960; Weiss 1971). It includes ~25 species and subspecies (Hanelt 1963; Weiss 1971). Of these, *C. tinctorius,* commonly known as safflower, is the only species that is widely cultivated as an oilseed crop. All the remaining species grow wild from northwestern India westward to and around Mediterranean Sea (Knowles 1980). Some species have spread far beyond these regions; *C. lanatus* is widespread in the entire Mediterranean region, and together with *C. creticus* and *C. leucocaulos*, in all the Mediterranean-like climate regions of the world including Argentina, Australia, California, Chile, and South Africa (Ashri and Knowles 1960; Hanelt 1963; Estiali and Knowles 1978).

Safflower ranks eighth after soybean, groundnut, rapeseed, sunflower, sesame, linseed, and castor crops grown worldwide for oil. India, Mexico, United States, Ethiopia, Argentina, and Australia together account for 99% and 87% of the world safflower growing area and oil production, respectively (Damodaram and Hegde 2002).

The genus *Carthamus* has tribasic ($x = 10, 11, 12$) chromosome number. Most of the wild species of the genus including the cultivated *C. tinctorius* are diploid with $2n = 2x = 20$, $2n = 2x = 22$, and $2n = 2x = 24$. The cultivated species and its immediate close allies, *C. oxyacantha*, *C. palaestinus*, and *C. flavescens* have 24 somatic chromosomes. Two successful weedy species, *C. flavescens* (syn. *C. persicus*) and *C. oxyacantha*, grow widely in Turkey, Syria, and Lebanon, and Iran and Iraq to northwestern India, respectively. *C. palaestinus* grows in desert areas from Israel to western Iraq. *C. flavescens* is self-incompatible and *C. palaestinus* is self-compatible species, whereas *C. oxyacantha* is partly self-incompatible.

A large group of closely related species with $2n = 2x = 20$ occupies the Middle East from Libya to Iran. *C. divaricatus*, the only species with $2n = 2x = 22$, is self-incompatible and is found growing wild in coastal areas of Libya.

The three self-compatible species, *C. lanatus*, *C. turkestanicus*, and *C. baeticus,* are polyploid ($2n = 4x = 44$ and $2n = 6x = 64$) in constitution. *C. lanatus* ($2n = 44$) is distributed over almost the entire distribution range of the genus. It is presumed to be the result of a cross between species with $2n = 20$ and $2n = 24$ followed by doubling of chromosomes (Ashri and Knowles 1960; Khidir and Knowles 1970a, b). The remaining two polyploid species have $2n = 64$. *C. baeticus* (syn. *C. creticus*) extends from Turkey westward to Spain, while *C. turkestanicus* extends from Turkey to the easternmost range of the genus upto Ethiopia (Kumar 1991). The $2n = 64$ taxa are believed to have originated from crosses between *C. lanatus* ($x = 22$) with $2n = 20$ species. Polyploidy has extended the boundaries of the genus with *C. lanatus* and/or *C. baeticus* reported to occur in California, Chile, and Australia (Ashri and Knowles 1960; Hanelt 1963; Estiali and Knowles 1978).

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4.2 Economic Importance

Safflower has been grown for centuries in India for the orange-red dye (carthamin) extracted from its brilliantly colored flowers (Knowles 1958; Weiss 1971; Singh and Nimbkar 2007). It is still used for this purpose in parts of northern India. With the introduction of cheap synthetic dyes, its importance as a dye source had almost vanished since the middle of the last century. Breeding for high oil content and modified fatty acid composition (Knowles 1968, 1969a, 1972; Ladd and Knowles 1970; Hamdan et al. 2008) in the later half of last century gave rise to promising cultivars that established it as an important high-quality oil crop for cooking as well as industrial purposes (Ashri et al. 1975). Safflower cultivars yield two types of oil – a polyunsaturated type which is rich in linoleic acid and a monounsaturated type which is rich in oleic acid. The standard polyunsaturated type is used in soft margarines and for cooking, as well as in surface coatings because of its high linoleic acid content. The monounsaturated type is used as a high-quality frying oil and also has industrial uses. The monounsaturated fatty acid (oleic acid) is known to reduce low-density lipoproteins (LDLs; bad cholesterol) without affecting high-density lipoproteins (HDLs; good cholesterol) in blood (Smith 1996). Safflower oil is highly stable. Its consistency remains the same at low temperatures, thereby making it suitable for application in frozen/chilled foods (Weiss 1971). It is also better suited to hydrogenation for margarine production than soy or canola oils (Kleingarten 1993). Safflower oil is non-allergenic and therefore suitable in injectable medications (Smith 1996). Safflower is considered to be ideal for cosmetics and is used in "Macassar" hair oil and Bombay "Sweet oil" (Weiss 1971). Safflower oil is preferred for the paint and varnish industries owing to its specific properties such as the absence of linolenic acid, the presence of high linoleic acid, low color values, low free fatty acids, low unsaponifiables, and no wax, which makes the excellent quality paints, alkyd resins, and coatings (Smith 1996). Nutritionally, the safflower oil is similar to olive oil. The seeds contain ~30% oil, 20% protein, and 35% crude fiber. The seeds are also a rich source of minerals (Zn, Cu, Mn, and Fe), vitamins (thiamine and β-carotene) and the tocopherols (α, β, and γ) (Nagaraj 2001).

Safflower flowers are known to have many medicinal properties. They cure several chronic diseases. They are widely used in Chinese herbal preparations (Li and Mundel 1996). The tender leaves, shoots, and thinnings of safflower are used as pot herb and salad. They are rich in vitamin A, iron, phosphorus, and calcium. The young plants are commonly sold as a green vegetable in markets in India and some neighboring countries (Nimbkar 2002). Safflower can be grazed or stored as hay or silage. Safflower forage is palatable, and its feed value and yields are similar to or better than those of oat or alfalfa. Safflower cake in combination with all-purpose flour in 1:3 proportions was found to be highly suitable for manufacturing protein (~22%) enriched biscuits (Singh and Abidi 2005).

Another important and interesting use of safflower seed has recently emerged from genetic modification to produce high-value proteins for pharmaceuticals and industrial enzyme purposes (Singh and Nimbkar 2007). SemBioSys, a Calgary-based (Canada) company, transforms safflower tissue genetically in order to get the proteins of interest, which accumulate in the seeds of the transgenic plant (Mundel et al. 2004). The process of transformation of safflower tissues followed the patented Stratosom Biologics system, which facilitated the genetic attachment of target proteins of interest to oleosin, which is the primary protein coating the oil-containing vesicles (oil bodies) of the seed (Markley et al. 2006). The attachment of proteins to the oil bodies of safflower is expected to stabilize intracellular accumulation of foreign proteins, and also provide a useful attachment matrix and deliver benefits for end-use applications. Safflower has also been developed as a production platform for insulin (*http://www.sembiosys.com/*).

C. oxyacantha and *C. lanatus* are other species of economic importance. *C. oxyacantha* is considered a useful plant for desert areas of India. It is spineless when young and is therefore used as fodder and sometimes as a vegetable. The seeds are eaten in many parts of India, either parched alone or with wheat or sometimes ground and mixed with wheat flour for bread (Stewart 1869). The oil, called pohli oil, obtained from seeds of *C. oxyacantha*, is used for culinary and lighting purposes, and in the preparation of *roghan* used in the manufacture of Afridi wax-cloth (Deshpande 1952). The seeds of *C. lanatus* yield a pale-yellow oil, which dries to a hard, pale film, and is almost comparable in composition to safflower oil (Weiss 1971).

4.3 Domestication and Migration History of Safflower

It is believed that the cultivated species had its origin in the Near East in the area delimited by Turkistan, southern Turkey, western Iran, Iraq, Syria, Jordan, and Israel, probably from an ancestral type that gave rise to all closely related $x = 12$ diploid taxa (Weiss 1971, 2000). The oldest evidence of safflower as a cultivated crop plant comes from archeological records dating 1600 BC in Egypt, where its brilliantly colored florets were an important source of red-yellow and orange dyes for cotton and silk (Weiss 1971). Mummies found in Egyptian tombs had their bindings dyed with safflower's orange dye. The dye was also exported to Italy, France, and England, where it was used for coloring and preparation of cheese. Egyptians also used charred safflower plants for making "Kohl."

The evidence of safflower in Arabia comes from the work of Mesua (ca. 1000 AD), who was the author of a major work on contemporary Arab and Greek medicine. He noted that the plant with white seeds in both wild and cultivated state the most valuable. Another Arabian author, Abu Hanifa, also mentioned wild and cultivated safflower in Arabia (Weiss 2000). The great Arab traveler Ibn Batutah described safflower during his journeys in Africa and Asia between 1325 and 1354. Western expansion of the Arabs in creating the Muslim Empire of the fifth and sixth centuries expanded the cultivation of safflower along the North African coast and into Europe via the Iberian Peninsula. It is considered to have been introduced into East Africa also by the Arabs.

Safflower was introduced into Britain in 1551 from Egypt, where it was grown by monks mainly as a pot herb but was also used for coloring foods (Hanelt 1961). It has been used as a source of dye from ancient times by carpet weavers of the Irano-Afghanistan area from where it was probably introduced into southern Russia (Weiss 2000).

It was introduced into Asia Minor by Turks emigrating from Middle Asia, and has been extensively cultivated on a peasant scale since then in Turkey. Like sesame, it was apparently unused by the Jews in antiquity, and not until the second century AD was it mentioned in their writings (Weiss 1971).

The history of the crop in Ethiopia is obscure, although this area is considered to be a secondary center of origin of the species. It is well known and widespread in the country and has been cultivated for centuries, but documentary or other evidence of its early history is not available. Surprisingly, none of the early travelers to India mentioned the dye being used. In Afghanistan and India, it is used mainly as an edible oil. The florets are added to rice, bread, and pickles for an attractive orange color. In India and Myanmar, leaves and thinnings of the less spiny varieties were used as a vegetable. The tubular florets are still used as an adulterant for true saffron.

The date of safflower's introduction to China, where it was known as "hung-hua," has been widely stated to be the second century BC. It is believed to have come from Afghanistan along the historic "Silk Road" (Weiss 1971). Its main use was as a dye, but it had a prominent place as a cosmetic, and to a minor extent in medicine. Safflower was later grown extensively for its dye in many areas of China, particularly in Yangtze and Szechwan provinces. From China, it was introduced into Japan about the third century AD. The oil was sparingly used in cooking until the twentieth century.

It was grown for its flowers in Mexico and was probably introduced for that purpose by the Spaniards. Elsewhere in South America, its introduction coincided with the establishment of either Portuguese or Spanish rule. One of the early references of safflower in the United States is contained in a research report of the University of California at the beginning of twentieth century.

Cultivation of safflower in the New World began in 1899, but it was not commercially grown until the early 1950s (Knowles 1958, 1989). In the United States, *C. tinctorius* has been reported to have escaped cultivation in California (Munz 1968; Hickman 1993), Iowa (Rydberg 1971), Illinois (Henry 1992), Kansas (Gates 1971; Rydberg 1971), New Mexico (Martin and Hutchins 1998), Ohio (Vincent and Cusick 1998), and Utah (Shaw 1989).

4.4 Original and Derived Basic Chromosome Numbers

Three basic numbers ($x = 10$, 11, and 12) are reported in the genus *Carthamus*. There are conflicting arguments with regard to original basic number vis-à-vis

derived basic numbers. All the three basic numbers have been considered original basic numbers by various authors (Khidir 1969b; Estiali and Knowles 1976; Kumar 1991; Vilatersana et al. 2000b). Khidir (1969b) is of the view that $x = 12$ is the original basic number in the genus. According to Kumar (1991), $x = 10$ is the primitive basic number and the other two are derivatives of $x = 10$. Kumar (1991) believes that the presence of structural chromosome morphology in certain species with $x = 10$ chromosomes (Schank and Knowles 1964; Estiali and Knowles 1978) favors the increase in basic chromosome number from 10 to 12 through aneuploid addition as a result of reciprocal translocations. Estiali and Knowles (1976) proposed all the three possibilities of 10, 11, or 12 being the original basic number in the genus *Carthamus*.

Estiali and Knowles (1976) are of the opinion that the original basic number was $x = 11$. The basic number of 10 arose through the fusion of two telocentric chromosomes of *C. divaricatus* forming a large metacentric chromosome. However, geographic evidence does not favor such an evolutionary step since *C. divaricatus* is confined to Libya only, with the nearest wild species with $x = 12$ being *C. flavescens* in the continental regions of Turkey, Syria, and Lebanon or *C. palaestinus* in the desert areas of Israel. Alternatively, the two telocentric chromosomes of *C. divaricatus* (the only species with $x = 11$) could have derived from the large metacentric chromosome of $x = 10$ species. This is supported by the good homology between the two telocentric chromosomes of *C. divaricatus* and a large metacentric chromosome of *C. dentatus* (Estiali and Knowles 1976). *C. divaricatus* has characteristics of both the $x = 10$ and $x = 12$ species; the number of florets per head, yellow pollen and flower color, and reddish brown color of the terminal portion of the middle involucral bract. Descending dysploidy has been a well-known trend in many genera of Asteraceae such as *Crepis*, *Astranthium*, *Calycadenia*, and *Leontodon* (Tobgy 1943; Stebbins 1950, 1971; Sherman 1946; DeJong 1965; Baldwin 1993). It has been extensively documented in subtribe Centaureinae of family Asteraceae (Garcia-Jacas and Sussana 1992; Garcia-Jacas et al. 1996).

Recent DNA sequence analysis in *Carthamus* (Vilatersana et al. 2000b; Sasanuma et al. 2008) provided molecular evidence of $x = 12$ as the original basic number. Based on nucleotide sequence differences in chloroplast *trnL-F* intergenic region of the 13 *Carthamus* species, Sasanuma et al. (2008) identified three cytoplasm types: A, B, and C. The cytoplasm C was found only in *C. arborescens* ($x = 12$). It had intermediate sequence between types A and B. Upon sequence comparison with the outgroup species, *Cirsium japonicum*, it was clear that *C. arborescens* possessed the prototype of cytoplasm for the genus *Carthamus* (Table 4.1). Figure 4.1 represents schematic representation of direction of evolution of *Carthamus* based on *trn*L-F sequence data.

Table 4.1 Revised genomic formula, and plastome types of *Carthamus* species[a]

Taxon	Genomic formula	Substitution site in *trn*L-F					Plastome
		72	345	456–461	473	477	
C. tinctorius var. *tinctotius*	BB	G	G	–[b]	A	C	B
C. tinctorius var. *inermis*	BB	G	G	–	A	C	B
C. oxyacantha	BB	G	G	–	A	C	B
C. palaestinus	BB	G	G	–	A	C	B
C. glaucus	BB	G	G	–	A	C	B
C. lanatus	XXYY	G	A	TCAATT	G	C	A
C. lanatus ssp. *lanatus*	XXYY	G	A	TCAATT	G	C	A
C. lanatus ssp. *montanus*	XXYY	G	A	TCAATT	G	C	A
C. lanatus ssp. *turkestanicus*	AAYYXX	G	A	TCAATT	G	C	A
C. lanatus ssp. *creticus*	AAYYAA	G	A	TCAATT	G	C	A
C. glaucus ssp. *anatolicus*	AA	G	A	TCAATT	G	C	A
C. boisserii	AA	G	A	TCAATT	G	C	A
C. arborescens	CC	C	A	TCAATT	A	T	C
Cirsium japonicum		C	A	TCAATT	A	T	

[a]Sasanuma et al. (2008)
[b]Deletion of 6 bp

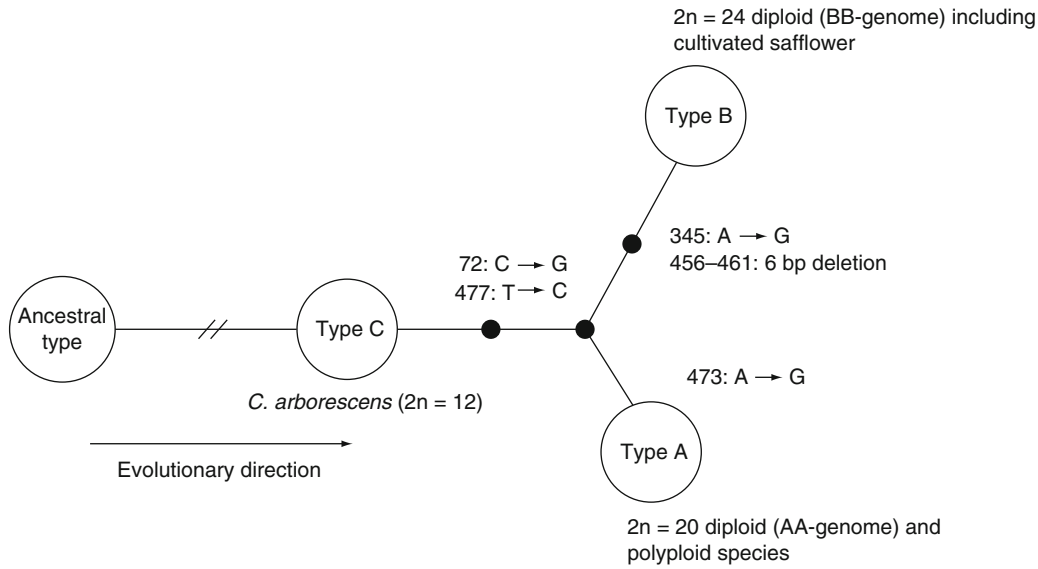

Fig. 4.1 A hypothetical model showing evolution of plastomes A and B from primitive plastome type C in *Carthamus* (Sasanuma et al. 2008)

4.5 Genome Size Variation

Genome size variation has been widely used for understanding the genome organization and evolution of the plant species (Price 1976; Raina and Rees 1983; Raina and Srivastav 1986; Raina and Bisht 1988; Parida et al. 1990; Lysak et al. 1999; Murray 2005).

The genome size in 17 *Carthamus* species and subspecies was estimated by flow cytometry (Garnatje et al. 2006). *Carthamus* taxa generally have low nuclear DNA amounts compared to the plant DNA C-values recorded to date (Plant DNA C-values Database, Bennett and Leitch 2004). The estimated 2C DNA content was 2.76 pg for the cultivated species *C. tinctorius*. For the wild species, the 2C values ranged from 2.26 pg for *C. leucocaulos* ($2n = 20$) to 7.46 pg for *C. turkestanicus* ($2n = 64$). Mean DNA values differed significantly between diploids and tetraploids as well as between diploids and hexaploids. According to Garnatje et al. (2006), allopolyploid species (*C. lanatus*, *C. lanatus* ssp. *montanus*, *C. turkestanicus*, *C. creticus*) had nuclear DNA content more or less equal to, or a little less than, the sum of the parental species.

4.6 Origin and Evolution of the Cultivated Species, *C. tinctorius* ($2n = 2x = 24$)

There have been many speculations on the origin of the diploid cultivated safflower *C. tinctorius* (Bamber 1916; Kupzow 1932; Deshpande 1952; Knowles 1958; Ashri and Knowles 1960; Hanelt 1963; Ashri and Efron 1964, 1965; Imrie and Knowles 1970). One of the two wild species, either *C. palaestinus* or *C. oxyacantha*, has been proposed as a possible progenitor of the cultivated species. The cytogenetic studies of interspecific hybrids involving the above species (Ashri 1957; Ashri and Knowles 1960; Ashri and Efron 1965) suggest that these species are closely related as evidenced by the average number of bivalents (11.94) in their interspecific hybrids (Ashri and Knowles 1960). Ashri and Efron (1964) analyzed the mode of inheritance of 10 morphological characters in the interspecific crosses involving the three species. They concluded that many of the morphological differences between the three species are solely due to single gene differences. Baker (1970) referred to the three species as one "biological species."

On the basis of morphological characters, Bamber (1916), Knowles (1958), Hanelt (1963), and Deshpande (1952) consider *C. oxyacantha* as the wild progenitor of *C. tinctorius*. Cultivated safflower, however, has some morphological characters that distinguish it from *C. oxyacantha*, the most distinctive of these being obpyramidal seeds with pappus. *C. oxyacantha,* on the other hand, has oval-shaped seeds that are without pappus. These seed characters, according to Ashri and Knowles (1960) and Ashri and Efron (1964), are quite distinctive in *C. oxyacantha*. These seed characteristics also mark *C. oxyacantha* as a divergent type in the whole genus. Bassiri (1970), on the basis of isozyme patters, suggested two possibilities: one being that *C. oxyacantha* is the wild progenitor of *C. tinctorius*, and the other being that both the species evolved from a common ancestor.

According to Imrie and Knowles (1970), both cultivated (*C. tinctorius*) and weedy species (*C. oxyacantha*) have evolved concurrently from the common progenitor *C. palaestinus* through adaptive radiation. *C. tinctorius* is the product of selection by humans in an agricultural environment, while *C. oxyacantha* is a weed of disturbed areas.

To address this issue, the genomes of the three species, *C. tinctorius*, *C. palaestinus,* and *C. oxyacantha*, have recently been screened with nuclear, mitochondrial, and chloroplast DNA markers as well as gene sequences (Chapman and Burke 2007; Sasanuma et al. 2008; Sehgal et al. 2008c). Chapman and Burke (2007) studied the diversity of seven nuclear genes, selected from a set of universal markers recently developed for Asteraceae (Chapman et al. 2007), in five *Carthamus* species, *C. tinctorius*, *C. palaestinus*, *C. oxyacantha*, *C. curdicus*, and *C. gypsicola*. The gene trees, both alone and in combination, revealed *C. palaestinus* as the most closely related species to safflower with maximum likelihood bootstrap and Bayesian posterior probabilities. *C. oxyacantha* was found to be most distantly related to *C. tinctorius*. Sasanuma et al. (2008) reached the same conclusion by studying the nuclear stearoyl acyl carrier protein desaturase (*SACPD*) gene and chloroplast *trnL-F* intergenic sequences.

The study by Sehgal et al. (2008c), utilizing nuclear, mitochondrial, and chloroplast markers and gene sequences, was the most informative in identifying the progenitor of *C. tinctorius*. Internal (ITS) and external (ETS) transcribed sequences of ribosomal DNA, *SACPD* nuclear gene sequence, and ITS-CAPS (cleaved amplified polymorphic sequences), cpDNA-CAPS, and mtDNA-CAPS markers were used in this study for assaying the genomes of two botanical varieties of *C. tinctorius, C. tinctorius* var. *tinctorius*, and *C. tinctorius* var. *innermis*, *C. oxyacantha* and *C. palaestinus* (Sehgal et al. 2008c). DNA marker analysis of the nuclear and mitochondrial genomes did not provide any clear evidence with regard to the ancestry of *C. tinctorius*. However, significant changes were revealed in the chloroplast genomes of these taxa. The data on cpDNA did not discriminate between *C. tinctorius* var. *tinctorius* and C. *oxyacantha*, and between *C. tinctorius* var. *innermis* and *C. palaestinus*, but the two taxa in each pair were distinguishable from each other by considerable differences in the mobility of several individual restriction fragments (Fig. 4.2). The existence of profound cpDNA variation between the two varieties of *C. tinctorius* was one of the most interesting results. It is an uncommon event of chloroplast genome evolution at intraspecific level. A comparison of *C. tinctorius* cpDNA with those of the two wild species revealed a startling information: the restriction patterns of *C. tinctorius* var. *tinctorius*

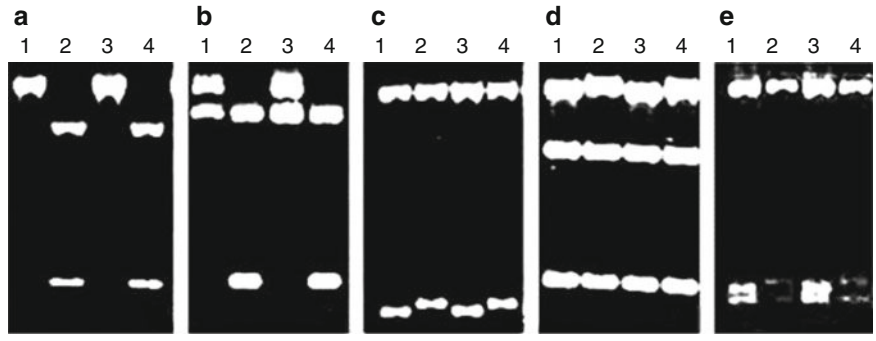

Fig. 4.2 Restriction fragment size patterns of the amplified chloroplast *psaA* gene digested with *Bgl*II (**a**) and *Taq*I (**b**) and the amplified *trnS–psbC* intergenic region digested with *Dra*I (**c**), *Hae*III (**d**) and *Pst*I (**e**) in *C. tinctorius* var. *tinctorius* (*lane 1*), *C. tinctorius* var. *innermis* (*lane 2*), *C. oxyacantha* (*lane 3*) and *C. palaestinus* (*lane 4*) (Sehgal et al. 2008c)

and *C. tinctorius* var. *inermis* exactly correspond to that of *C. oxyacantha* and *C. palaestinus*, respectively (Fig. 4.2). These results suggested that the two varieties of *C. tinctorius* had their origin from two different wild species. From practical application point of view, this information could be of importance for breeders and biotechnologists alike looking for the best possible sources of germplasm and for specific cytoplasm in their safflower genetic enhancement programs.

4.7 Center(s) of Origin/Similarity of *C. tinctorius*

Vavilov (1951) proposed three centers of origin for cultivated safflower (*C. tinctorius* L.). The one in India was based on variability and ancient culture of safflower production. A second center was proposed in Afghanistan, which was based on safflower diversity and proximity to wild species. A third center of origin, in Ethiopia, was primarily based upon the presence of the wild safflower species in the area. Kupzow (1932) had also proposed the same centers of safflower origin as proposed by Vavilov (1951). Ashri and Knowles (1960) and Hanelt (1961) indicated the center of origin to be in the Near East. This assumption was based on the similarity of cultivated safflower to two closely related wild species, *C. flavescens* reported from Turkey, Syria, and Lebanon, and *C. palaestinus* found in the desert areas of western Iraq and southern Israel. Knowles (1969) described the safflower centers of cultivation as the "centers of similarity," and not as the centers of origin or diversity, as there is a conspicuous similarity between the types existing in some or most of the centers. These centers are as follows:

1. Far East (Vavilov's center I – Chinese): China, Japan, and Korea
2. India–Pakistan (Vavilov's center II – India): India, Pakistan and Bangladesh
3. Middle East (Vavilov's centers III and IV – Central Asiatic and Near Eastern): Afghanistan to Turkey, and southern USSR to the Indian Ocean
4. Egypt (Vavilov's center V – Mediterranean): Bordering the Nile, north of Aswan.
5. Sudan (the southern reach of Vavilov's center V): Bordering the Nile in northern Sudan and southern Egypt
6. Ethiopia (Vavilov's center VI – Ethiopian)
7. Europe (western portion of Vavilov's center V): Spain, Portugal, France, Italy, Romania, Morocco, and Algeria.

Table 4.2 lists a few of the distinguishing characteristics of the safflower of each center of similarity. Ashri (1975) extended the number of centers to 10 and named them as "regional genepools." The Middle East center of Knowles (1969) was subdivided into three genepools; Iran–Afghanistan, Israel–Jordan–Iraq–Syria, and Turkey, with the Kenya genepool added to the list (Ashri 1975).

4.8 Origin and Nature of the Polyploid Species

It has been suggested that *C. lanatus* taxa with $2n = 44$ (*C. lanatus* ssp. *montanus* and *C. lanatus* ssp. *lanatus*) are allotetraploids built on $x = 10$ and $x = 12$ (Ashri 1957; Ashri and Knowles 1960; Kumar 1991). *C. lanatus* ssp. *turkestanicus* and *C. lanatus* ssp. *creticus* with $2n = 64$ are allohexaploids and believed to have resulted from crosses between taxa with $2n = 44$ and

Table 4.2 Characteristics of safflower's centers of similarity listed in order of decreasing frequency[a]

Center	Height	Branching	Spines	Head size	Flower color
Far East	Tall	Intermediate	Spiny, spineless	Intermediate	Red
India–Pakistan	Short	Many	Spiny	Small, intermediate	Orange, white, red
Middle East	Tall	Few	Spineless	Intermediate, large	Red, orange, yellow, white
Egypt	Intermediate	Few	Spiny, spineless	Large, intermediate	Orange, yellow, white, red
Sudan	Short, intermediate	Intermediate	Spiny	Small, intermediate	Yellow, orange
Ethiopia	Tall	Many	Spiny	Small	Red
Europe	Intermediate	Intermediate	Spiny, spineless	Intermediate	Orange, red, yellow, white

[a]Knowles (1969)

$x = 10$ (Ashri and Knowles 1960; Harvey and Knowles 1965; Khidir and Knowles 1970a, b; Kumar 1991).

Hybrids between *C. lanatus* and $x = 12$ species have been produced in all possible combinations (Knowles 1958; Ashri and Knowles 1960; Harvey 1964; Harvey and Knowles 1965). None of these hybrid combinations, however, exhibited the level of chromosome homology that would suggest one of them to be the parent. The number of bivalents ranged from 1 to 7 in crosses between *C. lanatus* and species with $x = 12$, which is much less than expected 12. On the basis of these results, it has been postulated that the present $x = 12$ species either has diverged from the ancestral $x = 12$ species or is now extinct.

On the other hand, a mean of 8.13 bivalents was obtained when *C. lanatus* was crossed with $x = 10$ species (Ashri and Knowles 1960). With regard to the $2n = 20$ taxa, Ashri (1957) and Ashri and Knowles (1960) suggested *C. leucocaulos* to be the $x = 10$ donor species on the basis of expected 10II in the hybrid with *C. lanatus*. Harvey and Knowles (1965) are of the view that even the $x = 10$ taxon donor of the allotetraploid *C. lanatus* seems to be extinct.

Further, from a morphological standpoint, the characteristics of *C. lanatus* are present in most of the diploid species investigated (Ashri 1957; Ashri and Knowles 1960; Harvey and Knowles 1965). *C. flavescens*, *C. palasetinus* (wild progenitor of *C. tinctorius*), *C. dentatus*, *C. glaucus* ssp. *glaucus*, and *C. leucocaulos*, all appeared to be good possibilities as parents of *C. lanatus*. In the synthetic alloploids, however, the expected combination of characters was never evident in the hybrid combinations of $n = 10 \times n = 12$ species. In other words, there was a consistent lack of morphological homology between natural *C. lanatus* and that of *C. lanatus* $\times$ $n = 10$ or *C. lanatus* $\times$ $n = 12$ species hybrids.

Based on the morphological and cytogenetic data, Khidir and Knowles (1970a, b) hypothesized that the allohexaploid *C. lanatus* ssp. *creticus* ($2n = 64$) originated from the hybridization between the tetraploid *C. lanatus* ($2n = 44$) and the diploid *C. leucocaulos* ($2n = 20$), and *C. lanatus* ssp. *turkestanicus* ($2n = 64$) originated from the hybridization between the *C. lanatus* and the diploid *C. glaucus* ssp. glaucus ($2n = 20$). The synthetic allohexaploids exhibited combined characters of their parents and showed intermediacy in habit, pubescence, capitulum size, degree of saccation of the florets, and color of the florets, anthers, and pollen (Khidir and Knowles 1970a, b) as in the natural allohexaploids. Crosses of *C. lanatus* ($n = 22$) with either *C. creticus* or *C. turkestanicus* ($n = 32$) produced hybrids with 22 bivalents and 10 univalents (Khidir and Knowles 1970a, b; Estiali and Knowles 1978). Based on these observations, Khidir and Knowles (1970b) assigned genomic formulas for *C. glaucus* ssp. *glaucus* and all other $2n = 20$ species, except *C. leucocaulos*; AA, *C. leucocaulos*; A_2A_2, *C. tinctorius* and other $2n = 24$ species with yellow flowers; BB, *C. lanatus*; $A_1A_1B_1B_1$, *C. creticus*; $A_1A_1B_1B_1$ A_2A_2 and *C. turkestanicus;* $A_1A_1B_1B_1$ AA.

The analysis of alcohol dehydrogenase allozymes by Efron et al. (1973) demonstrated that *C. lanatus*, *C. creticus* and *C. turkestanicus* share a unique allele for one subunit of this enzyme not found in other species of *Carthamus* (Table 4.4). The results of this study suggested that an ancestor of *C. lanatus* may have been one of the progenitors of the hexaploid ($n = 32$) species of *Carthamus*.

DNA markers such as repetitive sequences and low copy nuclear and chloroplast genes/intergenic sequences have been used to identify the origin of allopolyploid taxa in a number of plant species (Sang et al. 1997; Doyle et al. 1999a, b, 2000; Ford and Gottlieb 1999; Ge et al. 1999; Mason-Gamor 2001). The use of DNA markers to discern the origin of polyploid species has gained attention recently in *Carthamus*. Sequences of the plastid intergenic spacer *trnH–psbA* and the intron *trnK* and three introns of nuclear low-copy genes of the RNA polymerase family (RPD2 and the duplicated RPC2), as well as random amplified polymorphic DNA (RAPD) markers, were used to infer the progenitors of hexaploid species of *Carthamus* (Vilatersana et al. 2007). Phylogenetic analyses of the nuclear introns and additivity analysis of the RAPD markers support the hypothesis of Khidir and Knowles (1970a, b) that the two hexaploids are sharing a tetraploid progenitor lineage *C. lanatus* combined with different diploid progenitor lineages, consistent with the different geographic distributions of the hexaploids. Whereas *C. leucocaulos* from the southeastern Greek Islands represented the diploid progenitor lineage of the western *C. creticus*, the Irano-Turanian *C. glaucus* represents the diploid progenitor lineage of the eastern *C. turkestanicus*. The plastid data suggested that the diploid lineages served as the maternal progenitors of the hexaploids.

Sequences of nuclear *SACPD* gene and chloroplast *trnL-F* intergenic spacer (Sasanuma et al. 2008) have been used to identify the wild progenitors of tetraploid and hexaploid species of *Carthamus*. Two and three distinct types of sequences were identified at *SACPD* locus for tetraploids and hexaploids, respectively. This confirmed the allopolyploid nature of these taxa. The sequence data also revealed that the tetraploid taxa are devoid of both AA- and BB-genome species. It provided evidence of the existence of not-yet-identified diploid X-and Y-genomes. Based on the results, new genomic formulas for polyploid species (Table 4.1) were provided by Sasanuma et al. (2008).

Analysis of repetitive sequences (ITS and ETS; Sehgal et al. 2009) suggested that *C. glaucus* ssp. *anatolicus* ($2n = 20$) is likely one of the ancestral diploid species of *C. lanatus* ssp. *turkestanicus* ($2n = 64$) while *C. boisserii* ($2n = 20$) is one of the ancestral diploid species of *C. lanatus* ssp. *creticus* ($2n = 64$), *C. lanatus* ($2n = 44$), *C. lanatus* ssp. *lanatus* ($2n = 44$), and *C. lanatus* ssp. *montanus* ($2n = 44$).

There are conflicting accounts with respect to the taxonomy of various species. Based on morphological and cytogenetic evidences, *C. alexandrinus* and *C. tenuis,* and *C. creticus* and *C. turkestanicus* are treated either as subspecies of *C. glaucus* and *C. lanatus*, respectively, or recognized as distinct species (Ashri 1957; Ashri and Knowles, 1960; Hanelt 1961, 1963; Schank and Knowles 1964; Khidir 1969a; Khidir and Knowles 1970a, b; Estilai 1977). The controversy with respect to *lanatus* species complex, constituted by *C. lanatus* ssp. *creticus*, *C. lanatus* ssp. *turkestanicus*, *C. lanatus* ssp. *montanus*, and *C. lanatus* ssp. *lanatus*, needs to be highlighted. *C. lanatus* ssp. *creticus* and *C. lanatus* ssp. *turkestanicus* have been either given a rank of subspecies of *C. lanatus* or recognized as two (*C. turkestanicus* and *C. creticus*) distinct species (Ashri 1957; Ashri and Knowles 1960; Hanelt 1961, 1963; Khidir 1969a; Khidir and Knowles 1970a, b; Estilai 1977) in the same (Hanelt 1961, 1963; Vilatersana et al. 2005) or different taxonomic sections (Ashri and Knowles 1960; Estilai 1977).

On the basis of morphological similarities, Hanelt (1961, 1963) assigned the taxa with $2n = 44$ and $2n = 64$ to one species, *C. lanatus*. The key morphological difference between the taxa with $2n = 44$ and $2n = 64$ lies in the color of pollen grains and anthers. The former have yellow pollen and yellow anthers with no stripes, while the latter have white pollen and brown and purple stripes on light yellowish anthers. In the absence of anthers and/or pollen, the identification, therefore, becomes difficult. Several collections in the past, therefore, identified morphologically as *C. lanatus* with $2n = 44$ turned out to have $2n = 64$ and vice versa (Khidir 1969a; Khidir and Knowles 1970a; Weiss 1971; Estilai 1977; Vilatersana et al. 2000a).

Molecular evidence for the specific treatments for *C. alexandrinus*, *C. creticus*, *C. tenuis*, and *C. turkestanicus* was provided by Sehgal et al. (2009) and Vilatersana et al. (2005). The dendrogram based on RAPD and ITS-CAPS markers revealed species-specific groups for the four taxa.

4.9 Taxonomic Sections

4.9.1 Morphological, Biogeographical, Cytogenetic, and Biosystematic Evidences

The position of *Carthamus* in the tribe Cardueae is unclear (Cassini 1819; De Candolle 1819; Dittrich 1969; López González 1989); so is the circumscription of the genus (Knowles 1958; Ashri and Knowles 1960; Hanelt 1961, 1963; Schank and Knowles 1964; Estiali and Knowles 1976, 1978; Estilai 1977; Vilatersana et al. 2000a, 2005). Delimitation of *Carthamus* and a close ally, *Carduncellus*, has been difficult due to morphological similarities and convergent evolution of several variable characters utilized by taxonomists. These two genera constitute a large group termed the *Carduncellus–Carthamus* complex (Vilatersana et al. 2000a, b). Morphological and cytogenetic data are insufficient to delimit the species of the *Carduncellus–Carthamus* complex into discrete sections and genera (Hanelt 1963; Dittrich 1969; López-González 1989; Vilatersana et al. 2000a, b). Depending on the particular taxonomist and the morphological characters, several species in the complex have been moved in and out of *Carthamus* and *Carduncellus* (Table 4.3). The existing confusion was further compounded by the incorporation of a new genus, *Femeniasia* Susanna (Susanna et al. 1995; Susanna and Vilatersana 1996) into the complex.

Table 4.3 Sectional classifications proposed for the genus *Carthamus*[a]

De Candolle (1838), Cassini (1819)	Ashri (1957), Knowles (1958), Ashri and Knowles (1960)	Hanelt (1961,1963)	Estilai (1977)	Vilatersana et al. (2000b, 2005)
Genus *Carthamus* ($x = 12$)	Section I ($n = 12$)	Section Thamnacanthus	Section I ($n = 12$)	Genus *Phonus*
C. tinctorius	*C. tinctorius*	*C. arborscens*	*C. tinctorius*	*P. arborscens*
C. palaestinus	*C. palaestinus*	*C. rhiphaeus*	*C. palaestinus*	*p. rhiphaeus*
C. oxyacantha	*C. oxyacantha*	*C. martis*	*C. oxyacantha*	
C. flavescens			*C. flavescens*	
	Section II ($n = 10$)	Section Carthamus		Genus *Carthamus*
Genus *Kentrophyllum* ($x = 10$, 11 and 12)	*C. alexandrinus*	*C. persicus*	Section II ($n = 10$)	Section Carthamus[b]
Remaining species	*C. tenuis*	*C. palaestinus*	*C. alexandrinus*	*C. persicus*
	C. syriacus	*C. gypsicolus*	*C. tenuis*	*C. palaestinus*
	C. glaucus	*C. curdicus*	*C. syriacus*	*C. gypsicolus*
		C. oxyacantha	*C. glaucus*	*C. curdicus*
		C. tinctorius		*C. oxyacantha*
	Section III ($n = 22$)		Section III ($n = 22$)	*C. tinctorius*
	C. lanatus	Section Odontagnathius	*C. lanatus*	Section Atractylis[c]
		C. dentatus ssp. *dentatus*		*C. dentatus* ssp. *dentatus*
		C. dentatus ssp. *ruber*		*C. dentatus* ssp. *ruber*
				C. glaucus ssp. *glaucus*
	Section IV ($n = 32$)	Section Lepidopappus	Section IV ($n = 32$)	*C. glaucus* ssp. *glandulosus*
	C. baeticus	Ser. Lepidopappi	*C. baeticus*	*C. glaucus* ssp. *alexandrinus*
		C. glaucus ssp. *glaucus*	*C. turkestanicus*	*C. boisserii*
	Others	*C. glaucus* ssp. *glandulosus*		*C. tenuis* ssp. *tenuis*
	C. arborescens	*C. glaucus* ssp. *alexandrinus*	New section (n = 11)	*C. tenuis* ssp. *gracillimus*
	C. caeruleus	*C. boisserii*	*C. divaricatus*	*C. tenuis* ssp. *foliosus*
		C. tenuis ssp. *tenuis*		*C. leucocaulos*
		C. tenuis ssp. *gracillimus*	Others ($n = 12$)	*C. rechingeri*
		C. tenuis ssp. *foliosus*	*C. arborescens*	*C. nitidus*
				C. lanatus ssp. *lanatus*
		Ser. Leucocauli	*C. caeruleus*	*C. lanatus* ssp. *creticus* var. *divaricatus*
		C. leucocaulos		*C. lanatus* ssp. *montanus*
		C. rechingeri		*C. lanatus* ssp. *turkestanicus*
		C. nitidus		
		Section Atractylis		
		C. lanatus ssp. *lanatus*		
		C. lanatus ssp. *creticus* var. *divaricatus*		
		C. lanatus ssp. *montanus*		
		C. lanatus ssp. *turkestanicus*		

[a]Sehgal et al. (2009)
[b]Species of section Carthamus of Hanelt (1961)
[c]Species of sections Odontagnathius, Lepidopappus, and Atractylis of Hanelt (1961)

Several species from *Carthamus* have also been transferred to two other genera, *Lamottea* and *Phonus*, on the basis of detailed morphological studies (Ashri and Knowles 1960; Hanelt 1963; López-González 1990) and cytogenetic analysis of interspecific hybrids (Ashri and Knowles 1960; Estiali and Knowles 1976, 1978). Crosses between *C. caeruleus* and other species of *Carthamus* failed to produce seeds except for a single cross with *C. leucocaulos*. It produced a single sterile F_1 plant with low pollen viability (Ashri and Knowles 1960; Estiali and Knowles 1976, 1978), indicating thereby the distant relationship of *C. caeruleus* with other members of *Carthamus*. Similarly, crosses between *Carthamus arborescens* and other species, including *C. divaricatus* and *C. leucocaulos*, and several *Carthamus* species with $n = 12$, did not produce seeds. This indicated the divergence of *C. arborescens* from the rest of the genus taxa (Ashri and Knowles 1960; Estiali and Knowles 1976, 1978).

López González (1990) proposed a system of classification based on anatomical characteristics, biogeographical distribution, and biosystematic information. In this classification, *Carthamus* and *Cardencellus* are grouped along with two other genera, *Phonus* and *Lamottea*. Three potential criteria were listed for differentiating between *Carthamus* and *Cardencellus*: structures of the pappus and the pericarp, and morphology of the middle bracts of the capitulum. Two main types of pappus are found in the *Carduncellus–Carthamus* complex. The more common type is a double pappus, composed of two whorls, a very short convergent inner pappus, and an outer pluriseriate pappus with longer, unequal, linear, or paleaceous setae. This type of pappus can be persistent or deciduous and is present in *Carthamus* and in a group of *Cardencellus* species that has been segregated to a different genus (*Lamottea*) on the basis of this character. The second type of pappus is characterized by simple, more or less equal linear setae, deciduous as a single unit by means of a basal ring. This pappus is typical of *Cardencellus*. On the basis of pericarp structure primarily, some species were segregated from *Carduncellus–Carthamus* complex into a new genus, *Phonus*.

On the basis of some differences in vegetative characters and in the cypselas, Cassini (1819) and De Candolle (1838) included the cultivated species *C. tinctorius* and closely related wild species in the genus *Carthamus*, and all the remaining wild species in a separate genus *Kentrophyllum*. Chromosome numbers supported this separation. *C. tinctorius* and the closely related wild species *C. oxyacantha*, *C. palaestinus*, and *C. flavescens* have the basic chromosome number $x = 12$, whereas the remaining species transferred to genus *Kentrophyllum* show a complex dysploid series from $x = 12$ to $x = 10$.

Chromosome numbers have been used in the delimitation of the sections within the genus *Carthamus*. Knowles (1958) divided the genus into four taxonomic sections on the basis of chromosome numbers. Section I, II, III, and IV contained taxa with $2n = 24$, $2n = 20$, $2n = 44$, and $2n = 64$, respectively (Table 4.3). Three species were assigned to section I, viz. *C. tinctorius*, *C. oxyacantha*, and *C. palaestinus*. Two perennial species, *C. arborescens* and *C. caeruleus* with $2n = 24$, were not assigned to any section. Section II included *C. alexandrinus*, *C. glaucus*, *C. syriacus*, and *C. tenuis*. Later, *C. leucocaulos* was added to this group. Only *C. lanatus* was included in section III. One species, *C. baeticus*, was assigned to section IV. Later, Khidir (1966) added another species to section IV, viz. *C. turkestanicus*. Estilai (1977) added a new section in Knowles' (1958) classification to accommodate *C. divaricatus*, the only species with $2n = 22$. Hanelt (1961, 1963) delineated the genus into five sections on the basis of morphological characters and chromosome counts. Section *Thamnacanthus* ($n = 12$) included perennial and primitive taxa (*C. arborescens, C. rhiphaeus*, and *C. martis*) of the genus. Section *Carthamus* ($n = 12$) contained *C. tinctorius* and the wild taxa closely related to *C. tinctorius*. Section *Odontagnathius* ($n = 10$) contained only *C. dentatus*. Section *Lepidopappus* ($n = 10$) contained remaining species with $2n = 20$ and *C. nitidus* with $2n = 24$. Section *Atractylis* ($n = 11$, 22 and 32) included the species of polyploid origin.

The taxonomic status of *C. nitidus* and *C. leucocaulos* is unclear. Conflicting accounts have been reported for the relationship of *C. nitidus* with the other species of *Carthamus*. Crosses between *C. tinctorius* ($n = 12$) and *C. nitidus* ($n = 12$) produced sterile F_1 progeny (Knowles and Schank 1964; Knowles 1989). Crosses of *C. nitidus* with both *C. dentatus* and *C. glaucus* ($n = 10$) failed to produce hybrids (Knowles and Schank 1964). Based on these hybridization experiments, Knowles and Schank (1964) suggested that *C. nitidus* was more closely allied to section *Carthamus* ($n = 12$).

4.9.2 DNA Marker Assay Evidences

As is clear from above, the morphological, biosystematic, and cytogenetic approaches have not been able to resolve the taxonomic problems of the genus *Carthamus*. Divergent views have come to stay even after comprehensive utilization of the above approaches. Lately, DNA markers have been widely utilized for fast and unambiguous understanding of phylogenetic relationships of plant species of both diploid and polyploid origin (Raina and Ogihara 1994, 1995; Song et al. 1995; Wendel et al. 1995; Ge et al. 1999; Khan et al. 2000; Raina et al. 2001; Shiran and Raina 2001; Lee et al. 2002; Sharma and Jana 2002; Jung et al. 2003; Vander Stappen et al. 2003; Volkov et al. 2003; Dillon et al. 2004; Drossou et al. 2004; Xiang et al. 2004; Taketa et al. 2005).

Vilatersana et al. (2000a, b) analyzed DNA sequence from the internal transcribed spacers of nuclear ribosomal DNA (ITS1 and ITS2) in representatives of the *Carduncellus–Carthamus* complex. The analysis effectively differentiated between the annual eastern *Carthamus* and the perennial western genera, *Carduncellus, Phonus*, and *Femeniasia,* without intermediates. Vilatersana et al. (2000a, b) obtained strong bootstrap support for the removal of *C. caeruleus,* and *C. arborescens* and *C. rhiphaeus* from *Carthamus* and their placement in *Lamottea* and *Phonus*, respectively. Analysis of the ITS region within *Carthamus* (Vilatersana et al. 2000a, b) established two different well-supported clades. Section *Carthamus sensu* Hanelt emerged as a well-defined natural group. The remaining species of the genus formed another group, a conglomerate that was considered by some authors to be a different genus *Kentrophyllum* (De Candolle 1810; Cassini 1819).

Later, Vilatersana et al. (2005) (Table 4.3) carried out RAPD marker analysis on two populations each of *C. creticus*, *C. glaucus* ssp. *glaucus*, *C. lanatus*, and *C. turkestanicus*, and one population each of *C. alexandrinus*, *C. boissieri*, *C. dentatus* ssp. *ruber*, *C. leucocaulos*, and *C. tenuis,* and suggested two sections, *Carthamus* and *Atractylis,* for the genus. Section *Carthamus* contained the same taxa as in section *Carthamus* of Hanelt (1963). Section *Atractylis* was comprises sections *Atractylis*, *Odontagnathius*, and *Lepidopappus* of Hanelt (1963).

Lately, on the basis of comprehensive nuclear DNA assay by multiple DNA markers including RAPD, rDNA-restriction fragment length polymorphism (RFLP), and ITS-CAPS, as well as sequence data of ITS and ETS regions of ribosomal DNA, Sehgal et al. (2009) reported that the *Carthamus* taxa consistently resolved into three lineages. Lineage 1 in all the cladograms was constituted by *C. arborescens* ($2n = 24$) alone. Lineage 2 was comprised of the $2n = 24$ taxa of section I of Ashri and Knowles (1960) and Estilai (1977) or section *Carthamus* of Hanelt (1961, 1963) and Vilatersana et al. (2005). Lineage III comprises the $2n = 20$, $2n = 44$, and $2n = 64$ taxa included in the sections II, III, and IV by Ashri and Knowles (1960) and Estilai (1977), and sections Lepidopappus and Atractylis by Hanelt (1961, 1963), and section Atractylis by Vilatersana et al. (2005). Figure 4.3 represents strict consensus parsimonious tree based on ITS + ETS sequence data. Another strong feature to emerge from this study was the clear evidence for the extreme divergence between *C. arborescens* and the remaining *Carthamus* taxa. Even one DNA marker assay was sufficient to distinguish between the two. The taxonomic history of *C. arborescens* is contentious. Hanelt (1961, 1963) included this species in section Thamnacanthus of *Carthamus,* while Ashri and Knowles (1960) have not been able to classify it and thus incorporated it in "others." Vilatersana et al. (2000a) and López-González (1989) are of the view that *C. arborescens* should be considered as a species (*P. arborescens*) of a separate genus *Phonus*. The ITS (6.3%) and ETS (10.4%) divergence between *C. arborescens* and the remaining *Carthamus* taxa is much less than the intergeneric sequence divergence reported so far (Baldwin 1992; Kim and Jansen 1994; Baldwin and Markos 1998; Clevinger and Panero 2000; Vilatersana et al. 2000a; Kelch and Baldwin 2003), which suggests that it should be a species of *Carthamus*. According to Sehgal et al. (2009), the genus *Carthamus* should be divided into two subgenera, placing *C. arborescens* as the only species in one subgenus and all the other *Carthamus* taxa in two sections in another subgenus.

Five unnamed *Carthamus* species, provided by United States Department of Agriculture (USDA), were also analyzed by Sehgal et al. (2009). Four species had $2n = 6x = 64$. The remaining species had $2n = 2x = 24$. Four out of five species were close to the hexaploids. The remaining species was

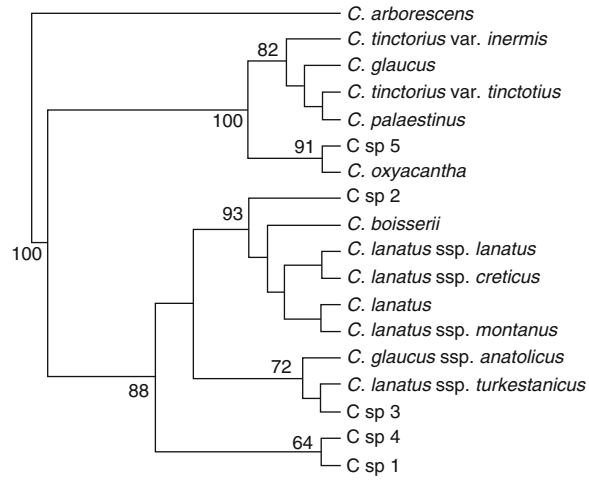

Fig. 4.3 Strict consensus tree of 12 most parsimonious trees based on ETS + ITS data of *Carthamus* species. Numbers at nodes represent bootstrap values. Csp1, Csp2, Csp3, Csp4, and Csp5 represent unidentified *Carthamus* species (Sehgal et al. 2009)

close to the diploid taxa with $2n = 24$. ITS restriction patterns produced by *Stu*I, *Hae*III, *Taq*I, *Hinf*I, and *Hap*II were the most informative in classifying the unverified species *C. turkestanicus* and the two species shared similar restriction pattern in ITS + *Hap*II combination. *C. creticus* and the other two species with $2n = 64$ shared similar restriction pattern in ITS + *Stu*I combination. The restriction patterns produced by the remaining three enzymes were similar between the diploid ($2n = 24$) taxa and the lone unverified species with $2n = 24$. The power of DNA markers, as in the case of *Carthamus*, to classify germplasm independently of other taxonomic information have been demonstrated in other plant taxa as well (van de Ven et al. 1993; Millan et al. 1996).

4.10 Genetic Diversity

The evaluation of genetic diversity of the germplasm resources of an important cultivated species and its close relatives in primary, secondary, and tertiary genepool is a prerequisite to genetic enhancement, effective management of crop genetic resources, designing breeding and conservation strategies, monitoring genetic erosion, development of "core collection," and detection of contaminants and redundancies (Sehgal and Raina 2008). There are 25,179 accessions of safflower germplasm conserved in 22 genebanks of 15 countries in the world (*http://www.ipgri.cgiar.org/regions/apo/safflower.html*) (Dwivedi et al. 2005). The USDA maintains a collection of safflower germplasm at the Western Regional Plant Introduction Station (WRPIS), Pullman, WA, which currently has more than 2,300 accessions (Johnson et al. 2007). The details of the accessions are available on Germplasm Resources Information Network (GRIN) (*http://www.ars-grin.gov/npgs/*). These accessions, representing germplasm from more than 50 countries, are available to scientists worldwide for research. Presently, India maintains about 7,316 accessions at the Germplasm Management Unit (GMU) of the Directorate of Oilseeds Research (DOR), Hyderabad (Anonymous 2002). The germplasm collection at Hyderabad consists of the accessions received from safflower centers of the All India Coordinated Research Projects on Oilseeds (AICORPO), local collections from traditional and nontraditional safflower growing areas, and exotic collections.

4.10.1 Genetic Diversity Based on Morpho-Agronomic Traits

Phenotypic diversity was assessed for quantitative and qualitative traits in a salt-tolerant subset of the safflower (*C. tinctorius* L.) germplasm collection originating from 11 countries in three regions (central Asia, Southwest Asia, and Africa) of the Middle East (Jaradat and Shahid 2006). Phenotypically, the

germplasm, among and within regions, was highly variable, especially for rosette and yield-related traits. Frequency of desirable variants of seven agronomically important traits ranged from 14% for long rosette period to 50% for no or few spines. The level of population differentiation was high for the number of capitula per plant (30%), whereas most traits partitioned their diversity (82–87%) within populations. A multivariate selection criterion for high biological and seed yield, long rosette period, and no or few spines identified five accessions from Southwest Asia that can be introduced into subsistence farming systems as a multipurpose crop under saline agriculture.

The evaluation of 721 accessions from the U.S. safflower collection in Mexico for *Alternaria* resistance and seed oil content identified 84 accessions possessing tolerance to *Alternaria* and 37 accessions having good agronomic characteristics. An oil content of >32.1% on an average was recorded, with maximum being 42.03%. This study emphasized that U.S. safflower collection can be used as a source for *Alternaria* resistance and high oil content for breeding purposes (Cervantes-Martinez et al. 2001).

Considerable diversity was found in 23 accessions of safflower in Spain for morphological, physiological, and chemical characters (Pascual-Villalobos and Alburquerque 1996).

A core collection is intended to contain, with minimum repetitiveness, the genetic diversity of a crop species and its wild relatives. Brown (1989) suggested that a core collection should contain 5–10% of the germplasm collection and that this size should retain over 75% of the variability in the whole collection. Such manageable collection with high diversity would provide the starting material for breeders in search of new and novel variation for genetic improvement and related biotechnological programs (Sehgal and Raina 2008).

A core collection of 207 accessions was identified in the U Safflower germplasm collection based on country of origin and morphological traits like branching pattern, flower color, flowering time, growth habit, head diameter, plant height, iodine number, lysine content, and oil content (Johnson et al. 1993). The core collection in effect was evaluated for seven quantitative characters, which indicated considerable diversity within the core collection. Correlation analysis showed the strongest association between plant height and flowering, followed by association between outer involucral bract (OIB) width and OIB length. The evaluation of accessions on the basis of the country of origin revealed that accessions from Southwest Asia were very different from those of other regions and there were significant differences among the regions for all traits evaluated except OIB length and yield per plant. Further studies revealed that this core subset captured a large fraction of the diversity in oil and meal characteristics (Johnson et al. 1999). Suresh and Balakrishnan (2001) and Balakrishnan and Suresh (2000, 2001a, b) proposed several strategies and sampling methods to develop core collection using geographical origins and 28 descriptors on 3,250 safflower accessions from 32 countries.

A core subset of 570 accessions was identified from 5,522 safflower accessions based on geographical distribution and 12 morphological descriptors having high heritability and are least influenced by genotype × environment interaction (Dwivedi et al. 2005). South Asia and Southeast Asia together accounted for 79.8% (4,406 accessions) of the accessions in the entire collection of 5,522 accessions and this predominance in number was also reflected in the core subset that contained 77.7% (443 accessions) of the accessions from these regions. About 7.2% (41) of the accessions in the core subset were from the Americas, 4% (23) from the Mediterranean, and 3% each from European (17) and West Asian (17) regions. Australia, the former USSR, and Africa represented 1.2%, 1.4%, and 1.8% accessions, respectively, in the core subset. Differences among means of the entire collection and core subset for the 12 morphological descriptors used in developing the core subset were not significant and the variance of the entire collection and core subset were homogeneous for all the traits except for growth habit ($P = 0.024$). The core subset captured 100% range variation for 11 morphological descriptors and 80% for shape of upper stem leaves.

4.10.2 Genetic Diversity Based on Molecular Markers

Molecular marker approach has advanced our understanding of the genetic resources more than any other approach. However, the use of molecular techniques for genetic variation analysis in safflower is a recent event. Like in most other crops, isozymes were the

first markers to be used for studying genetic variation in safflower germplasm resources. Using isozymes, Bassiri (1977) identified nine introduced and five local cultivars of *C. tinctorius,* and seven ecotypes of *C. oxyacantha*. Two isozymes, acid phosphatase and cathodal peroxidase, together identified all the 21 cultivars and ecotypes. Efron et al. (1973) studied alcohol dehydrogenase (ADH) allozyme pattern in the seeds of 1,553 accessions from the world collection of safflower (*C. tinctorius*) and 36 collections of 14 wild species. Two genes Adh_1 and Adh_2 were identified. Adh_1 and Adh_2 loci control the anodal and cathodal bands, respectively. The codominant alleles $Adh^s{}_1$ and $Adh^f{}_1$, specifying allozymes with different migration rates in the anodal zone, were found in cultivated safflower. The frequency of $Adh^f{}_1$ allele was very low. The safflower collection was found to be more or less uniform. A third allele $Adh^T{}_1$ was present only in the polyploids. The authors concluded, based on ADH genotypes (Table 4.4) of the diploids and polyploids, that *C. lanatus* is the common progenitor of both polyploid species. Isozyme markers were also used to identify hybrid individuals in safflower populations (Carapetian and Estilai 1997) and to study divergence in 89 accessions that originated from 17 countries (Zhang 2001). The latter study revealed that materials from East Asia had the maximum estimates for both mean allele frequency and mean gene diversity. The accessions of unknown origin showed resemblance to those from India, Turkey, and the Middle East (Zhang 2001).

RAPD markers were used by Yazdi-Samadi et al. (2001k.) to detect variation in 28 safflower accessions that included Iranian landraces and several wild and exotic genotypes as well. The clusters based on RAPD markers correlated fairly well with a classification scheme based on morphological traits.

RAPD, intersimple sequence repeat (ISSR), and amplified fragment length polymorphism (AFLP) markers were used to fingerprint safflower cultivars released by AICORPO (Sehgal and Raina 2005). These cultivars cover 90% of the total acreage of cultivation in various agro-climatic regions of India (Table 4.5). Out of the three marker systems employed, AFLP proved to be better than both RAPD and ISSR markers. Figure 4.4 represents RAPD and ISSR profiles of the safflower cultivars. Two AFLP primer combinations vis-à-vis 36 RAPD and 21 SSR primers could effectively fingerprint the 14 cultivars. The most important finding of this study was the identification of cultivar-specific markers. Eight RAPD (OPA08, OPA02, OPA01, OPK07, OPC08, OPI14, OPC04, and OPA17), four

Table 4.4 ADH genotypes of *Carthamus* species[a]

Species	ADH genotype
$n = 10$	
C. alexandrinus	Adh_1^s/ Adh_1^s
C. anatolicus	Adh_1^s/ Adh_1^s
C. dentatus	Adh_1^s/ Adh_1^s
C. glaucus	Adh_1^s/ Adh_1^s
C. leucocaulos	Adh_1^s/ Adh_1^s
C. tenuis	Adh_1^s/ Adh_1^s
$n = 11$	
C. divaricatus	Adh_1^s/ Adh_1^s
$n = 12$	
C. flavescens	Adh_1^s/ Adh_1^s
C. nitidus	Adh_1^s/ Adh_1^s
C. oxyacantha	Adh_1^s/ Adh_1^s
C. palaestinus	Adh_1^s/ Adh_1^s
C. tinctorius	Adh_1^s/ Adh_1^s
$n = 22$	
C. lanatus	Adh_1^s/ Adh_1^s; Adh_1^T/ Adh_1^T
$n = 32$	
C. baeticus	Adh_1^s/ Adh_1^s; Adh_1^s/ Adh_1^s; Adh_1^T/ Adh_1^T
C. turkestanicus	Adh_1^s/ Adh_1^s; Adh_1^s/ Adh_1^s; Adh_1^T/ Adh_1^T

[a]Efron et al. (1973)

Table 4.5 Safflower cultivars of Indian origin fingerprinted by DNA markers[a]

Cultivar	Pedigree	Research center[b]	Remarks
A-1	Hybridisation (Pedigree method) (A-482-1 × A-300)	Annigeri	Suitable under scanty and assured moisture regions
Bhima	Selection from A-300	Jalgaon	
Girna	Hybridization (Pedigree method) (A-1 × G 1254)	Jalgaon	Moderate wilt tolerant
JSF-1	Selection from IC 11839	Indore	Suitable for early and late sowings
Sharda	Selection from No. 168	Latur	
HUS-305	Selection from germplasm	Varanasi	Salt and wilt tolerant
S-144	Pure line selection from local variety	Raichur	Suitable for dry areas
Nira	Hybridization (Pedigree method) (NS 1572 × EC 32012)	Phaltan	For irrigated conditions
CO-1	Selection from PI 250528	Coimbatore	Non-spiny
APRR-3	Selection from EC 27250	Hyderabad	Rust resistant
Nari-6	Hybridization (Pedigree method) (CO-1 × JL-8)	Phaltan	Non-spiny
Manjira	Pure line selection from SF-65	Hyderabad	Medium statured
JSI-7	Selection from JSF 1909	Indore	Non-spiny
Nari-2	Selection from HUS-296-3	Phaltan	Non-spiny

[a]Sehgal and Raina (2005)
[b]The Research center where the cultivar was bred

SSR (UBC 880, UBC 842, UBC 895, and UBC 872) primers, and one AFLP primer combinations (EACG + MCAG) generated 14, 5, and 5 diagnostic markers, respectively, for 11 out of 14 cultivars. The maximum number of diagnostic markers was obtained for Nari-2 followed by HUS-305, Bhima, and JSI-7. These markers could be of potential use for detecting mixtures and duplicates in the germplasm.

RAPD, ISSR, and AFLP markers were also used to assess (1) the genetic diversity of 85 accessions (originating from 24 countries of five continents, viz. Asia, Europe, Africa, America, and Oceania) obtained from USDA and representing global germplasm variability of safflower, and (2) the interrelationships among safflower "centers of similarity" or "regional genepools" (Sehgal et al. 2008d). This study also revealed the relative superiority of the AFLP marker system vis-à-vis RAPD and ISSR markers in uncovering variation in safflower. Figure 4.5 represents AFLP profile of safflower accessions. AFLP markers could effectively demarcate most of the known morphological-trait-based centers of similarity/regional genepools (Ashri 1973, 1975; Ashri et al. 1974, 1975). The "regional genepools" that were convincingly supported by the AFLP data were India– Pakistan, Europe, Far East, Turkey–Israel–Iraq–Jordan (Middle East), Egypt, and Iran–Afghanistan (Fig. 4.6). The grouping of accessions from Iran and Afghanistan separately from accessions of other Middle East countries supported the views of Ashri (1973, 1975) and Ashri et al. (1974, 1975) that Iran–Afghanistan should be considered as a separate genepool. The trivial genetic differences obtained between accessions from Turkey, Israel, Iraq, and Jordan supported the views of Zhang (2001) that Turkey should be treated as part of the Middle East genepool. Ethiopia, Kenya, and Sudan were not supported in this study as separate genepools. The accessions from Ethiopia were part of the Middle East group, while accessions from Sudan and Kenya were distributed in the dendrogram. These results suggested that safflower germplasm should be reclassified into six genepools; India–Pakistan, Europe, Far East, Turkey–Israel–Iraq–Jordan, Egypt, and Iran–Afghanistan, which is more congruent with classification. The variation between and within the regional genepools was calculated by Shannon's index and analysis of molecular variance (AMOVA). Shannon's diversity for the species (H_{sp}) was 12.38 and the proportion of diversity within ($H_{w/n}$) and between ($H_{b/w}$) regional genepools was 0.45 and 0.55, respectively. Estimates of genetic diversity with Shannon's index (H_{pop}), proportion of polymorphic genes, and mean genetic similarity revealed that diversity was highest in Iran–Afghanistan, which was equivalent to the total diversity

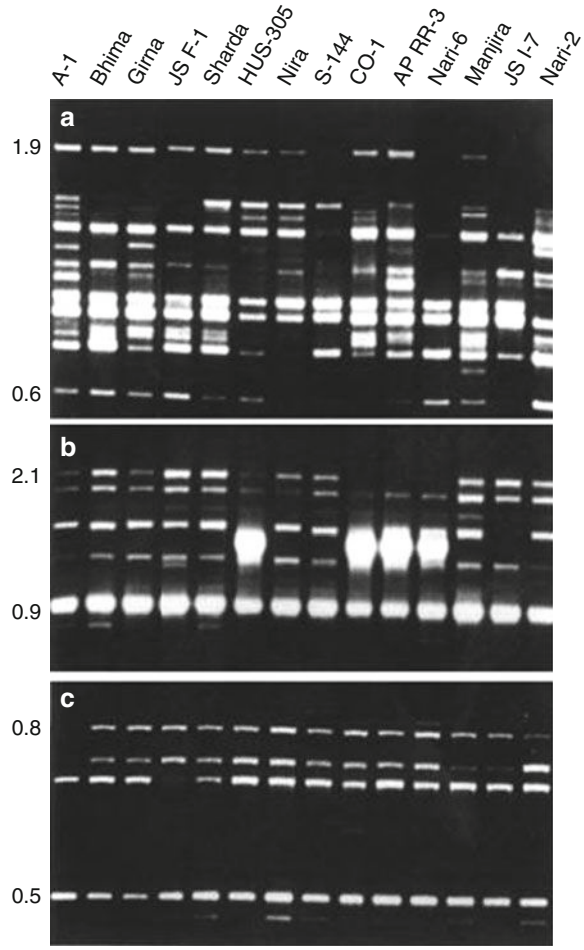

Fig. 4.4 Gel electrophoresis of amplification products obtained with RAPD primers OPC04 (**a**), OPI04 (**b**), and the ISSR primer UBC 881 (**c**) in safflower cultivars. The size of fragments in kilobases is indicated *on the left* (Sehgal and Raina 2005)

of the species. The least diversity was noticed in the Far East (Table 4.6) genepool. This information is important from practical applications standpoint.

Both RAPD and agro-morphological traits were used to assess the genetic diversity in safflower (Amini et al. 2008). Sixteen selected lines derived from landraces growing in various agro-climatic regions of Iran along with four exotic genotypes were evaluated for days to emergence, days to initial flowering, days to flowering, days to maturity, plant height, branches per plant, capitula per plant, seeds per capitulum, 1,000-seed weight, seed yield per plant, seed yield, and reaction to powdery mildew (*Leveillula taurica* Arnaud). The results indicated significant differences among genotypes for the agro-morphological traits. A relatively high range of genetic variation was observed for days to 50% emergence, seeds per capitulum, seed yield, and seed yield per plant. Number of branches and capitula per plant, seeds per capitulum, and 1,000-seed weight showed relatively high broad-sense heritabilities, which indicated that selection can be effectively employed for improvement of these yield components. Analysis of the RAPD markers revealed the highest genetic similarity between the cultivars "AC Sunset" and "AC Sterling" from Canada. The lowest relatedness was observed between a local breeding line "E2428" and genotype "GE62923" from Germany. Comparing the clusters based on agro-morphological traits with those from RAPD data showed little similarities.

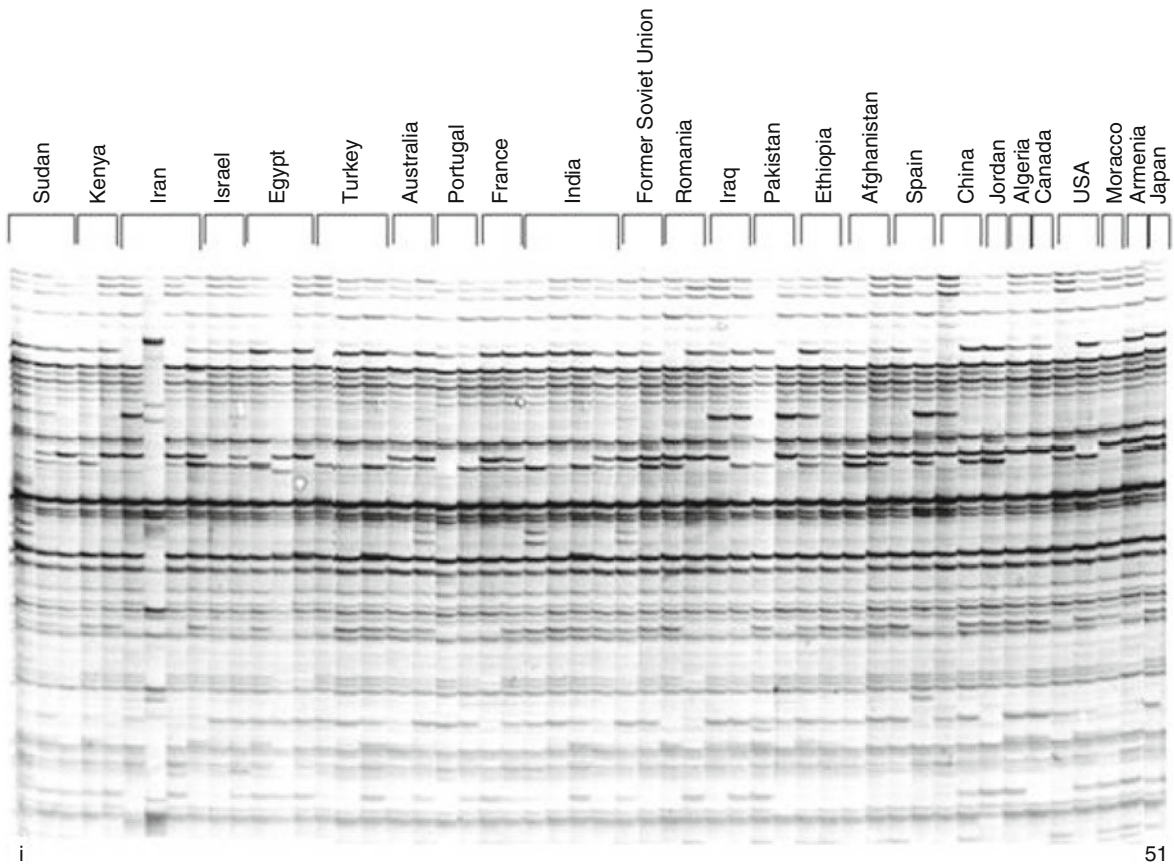

Fig. 4.5 AFLP profile of 51 *C. tinctorius* accessions (*lanes* 1–51) with primer combination E–CCA/M–CCA (Sehgal et al. 2008d)

Similarly, AFLP markers and phenotypic characters were used for assessment of genetic diversity in 96 safflower accessions representing seven regions (the Americas, China, East Africa, East Europe, the Mediterranean, South central Asia, and Southwest Asia) and to determine the association between phenotypic traits and AFLP marker data (Johnson et al. 2007). The within-region comparisons showed that the accessions from the Americas were more diverse than from other regions. This could be attributed to the wide range of genetic material used in the U.S. breeding programs and the selection of progeny with a wide range of agronomical traits including hull characteristics, disease resistance, meal attributes, and so on. The accessions in the other regions were predominantly the landraces used by local people without the introduction of exotic germplasm. The correlation of the distance matrices based on AFLP data with average Euclidian distance matrices based on phenotypic factors was the strongest within China, accounting for 39% of the variation. In most cases, the correlations within regions were either weak or not significant, which suggested that marker data in safflower should be balanced with phenotypic traits in order to obtain a complete picture of overall diversity.

In another study, AFLP markers proved useful for discrimination of 28 safflower populations in China (Zhang et al. 2006). ISSR markers used to study genetic variation in safflower accessions from 32 different countries unveiled a high level of polymorphism (Yang et al. 2007). This study revealed higher genetic variation in the accessions from India and the Middle East as compared to other regions. Genetic diversity of 23 populations of *C. tinctorius* and two populations of related wild species *C. lanatus* in China was investigated using sequence-related amplified

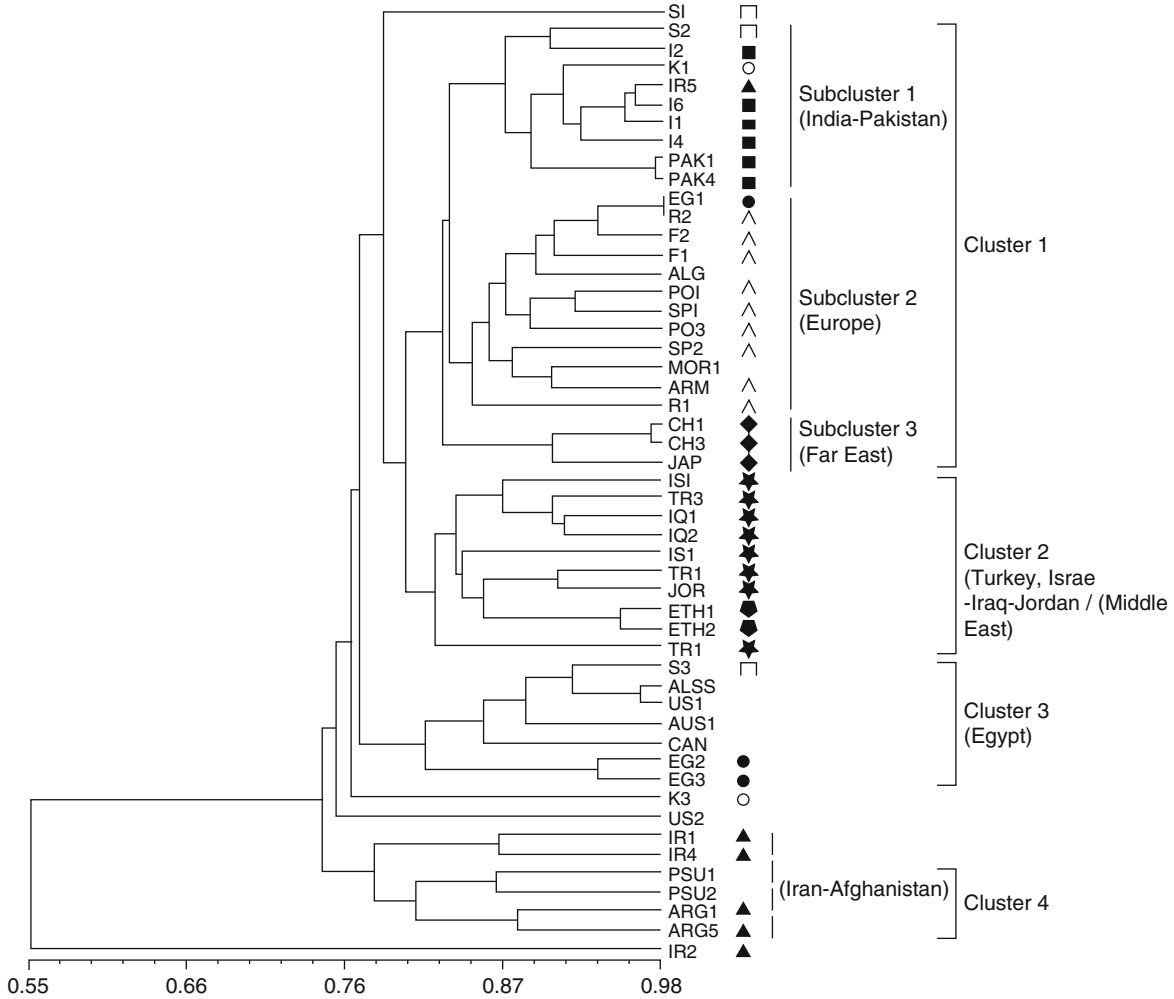

Fig. 4.6 UPGMA phenogram of *C. tinctorius* accessions based on AFLP marker data: (*open square*) Sudan; (*open triangle*) Europe; (*filled diamond*) Far East; (*filled square*) India–Pakistan; (*filled circle*) Egypt; (*open circle*) Kenya; (*filled triangle*) Iran–Afghanistan; (*filled star*) Turkey–Israel–Iraq–Jordan; (*dotted*) Ethiopia; *US* United States; *AUS* Australia; *MOR* Morocco; *FSU* Former Soviet Union; *CAN* Canada; *ALG* Algeria (Sehgal et al. 2008d)

polymorphism (SRAP) markers (Peng et al. 2008). This marker analysis distinguished populations of *C. tinctorius* from *C. lanatus*. Five species-specific bands were identified for *C. lanatus*.

ISSR markers revealed distinct genetic groups within the *C. lanatus* population from New South Wales (Ash et al. 2003). The analysis indicated that there are two distinct groups of *C. lanatus* that correlated with location (northern and southern regions). The Shannon's diversity index indicated higher variation in the southern group (0.33) than in the northern group (0.22).

4.11 Physical Mapping of Sequences

Knowledge of the physical localization of coding and repetitive DNA sequences on chromosome arms is becoming increasingly important because of the need to link genetic and physical maps (Raina and Mukai 1999; Raina et al. 2001; Sharma and Raina 2005), to transfer known chromosome segments carrying useful genes between species, and to follow and identify chromosomal regions consequent upon hybridization and backcrossing breeding program (Biagetti et al. 1999). Repetitive DNA sequence localization, in

Table 4.6 Proportion of polymorphic genes, Shannon's diversity (H_{pop}) and mean genetic similarities values for ten regional gene pools[a]

Regional gene pool	Proportion of polymorphic genes	H_{pop}	Mean genetic similarity
Far East	0.07	2.22	0.96
India–Pakistan	0.16	4.64	0.89
Iran–Afghanistan	0.46	11.76	0.69
Turkey	0.15	4.91	0.83
Israel–Iraq–Jordan	0.27	7.45	0.80
Egypt	0.18	5.67	0.81
Sudan	0.19	5.92	0.84
Ethiopia	0.10	3.37	0.92
Europe	0.23	6.05	0.86
Kenya	0.11	3.91	0.84

[a]Sehgal et al. (2008d)

particular, can support the unequivocal identification of plant chromosomes (Castilho and Heslop-Harrison 1995; Cuadrado and Jouve 1997; Cuadrado and Schwarzacher 1998; Raina and Mukai 1999; Raina et al. 2001), which otherwise cannot be identified by simple morphological features alone (Fuchs and Schubert 1998; Raina and Mukai 1999; Galasso et al. 2001). The tandemly arranged repetitive DNA elements also provide information on the large-scale organization and evolution of plant genomes (Pich et al. 1996; Vershinin et al. 2001; Badaeva et al. 2002; Sharma and Raina 2005).

Two repetitive DNA sequences, *pCtKpn1 and pCtKpn2*, were isolated from *C. tinctorius* and cloned (Raina et al. 2005). Both are tandemly repeated sequences. The *pCtKpnI-1 and pCtKpnI-2* clones constitute repeat units of 343–345 bp and 367 bp, respectively, with 63% sequence heterogeneity between the two. The physical location of these two repeated sequences was studied by fluorescence in situ hybridization (FISH). The *pCtKpnI-1* sequence was found to be exclusively localized at subtelomeric regions on most of the chromosomes. On the other hand, sequence of the pCt*Kpn*I-2 clone was distributed on two nucleolar and one non-nucleolar chromosome pairs (Fig. 4.7). The satellite and the intervening chromosome segments between the primary and secondary constrictions in the two nucleolar chromosome pairs were almost wholly constituted by pCt*Kpn*I-2 repeated sequence. The *pCtKpnI-2* repeated sequence, showing partial homology to intergenic spacer (IGS) of 18S–25S ribosomal RNA genes of an Asteraceae taxon (*Centaurea stoebe*), and the 18S–25S rRNA gene clusters were located at independent but juxtaposed sites in the nucleolar chromosomes (Fig. 4.7). Variability in the number, size, and location of the two repeated sequences has made possible the identification of most of the chromosomes in the otherwise not too distinctive homologues within the complement in *C. tinctorius*.

4.12 Breeding Objectives and Achievements

4.12.1 Disease Resistance

Patil et al. (1993) have reported that safflower is infested by 57 pathogens around the world, including 40 fungi, two bacteria, 14 viruses, and one mycoplasma. Of these, *Alternaria* leaf spot caused by *Alternaria carthami* and wilt caused by *Fusarium oxysporum* are the most devastating and can cause from 13 to 49% losses. The outbreak of *Alternaria* may sometimes wipe out the entire crop in the region under conditions conducive to its spread as happened in India in 1997–1998, when the entire crop of safflower in the major safflower growing states of Maharashtra and Karnataka was lost (Anon 1997–1998). Disease resistance, therefore, has been a major objective in safflower breeding programs. Safflower germplasm resources in USDA is a valuable source of genotypes resistant to rust, *Fusarium* wilt, *Verticillium* wilt, *Alternaria* leaf blight, and *Phytophthora* root rot (Urie and Knowles 1972; Da Via et al. 1981; Klisiewicz and Urie 1982). Ashri (1973) and Karve (1979) identified germplasm with tolerance or resistance to major diseases and insects in Israel and

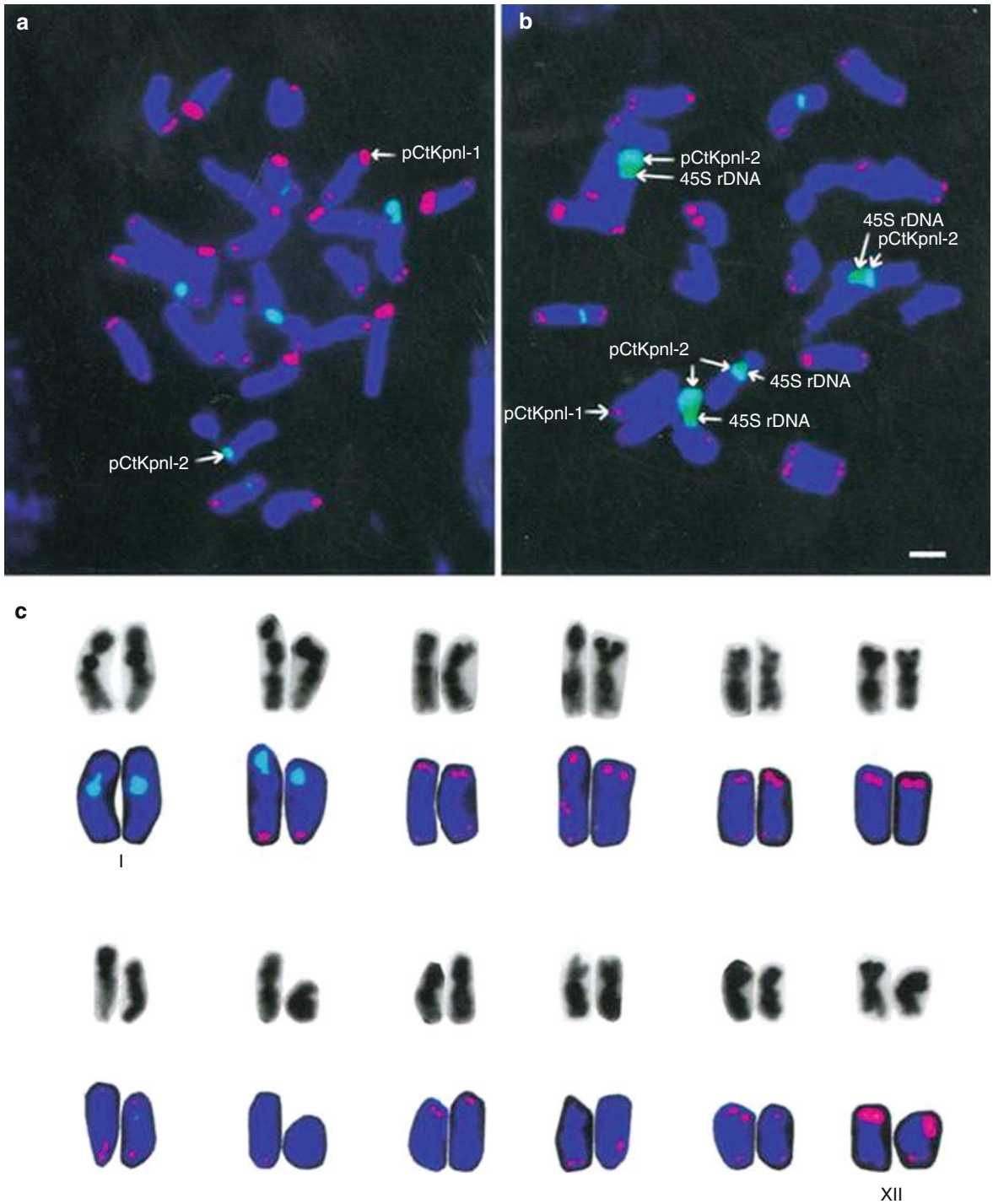

Fig. 4.7 Fluorescence in situ hybridization (FISH) of *C. tinctorius* ($2n = 24$) metaphase chromosomes with pCtKpnI-1 (*red fluorescence*) and pCtKpnI-2 (*green fluorescence*) probes (**a**). Metaphase chromosomes after FISH with pCtKpnI-1, pCtKpnI-2, and 18S–26S rDNA (*yellowish green fluorescence*) probes (**b**). The chromosomes were counterstained with DAPI (4′6-diamidino-2-phenylindole). Karyograms of metaphase chromosomes after DAPI staining (*upper row*) and FISH with pCtKpnI-1 and pCtKpnI-2 repeats (*lower row*) (**c**) (Raina et al. 2005)

India, respectively. Moreover, cultivars have been bred with resistance to wilt caused by both *Fusarium oxysporum, F. carthami* and *Verticillium albo-atrum* and to rust caused by *Puccinia carthami* (Singh et al. 2003a, b). Breeding for wilt resistance in safflower following backcross breeding resulted in the development of wilt-resistant genotypes giving an increase in seed yield by 31% (Singh et al. 2003a, b). Breeding safflower varieties for resistance to multiple diseases resulted in the development of germplasm line VFR-1. This line was derived from the breeding line Nebraska 4051, and it showed resistance to *Verticillium* wilt, *Fusarium* wilt and root rot, and *Rhizoctonia* root rot (Thomas 1971). The Australian safflower cultivar Sironaria, showing resistance to *Alternaria* blight and moderate resistance to *Phytophthora* root rot, has been developed by backcross breeding (Harrigan 1987, 1989). Safflower cultivars resistant to *Alternaria* blight (Sidwill, Hartman, Oker, Girard, and Finch) have been successfully developed in the United States (Bergman and Riveland 1983; Bergman et al. 1985, 1987, 1989a, b).

Wild species closely related to crop plants have played an important role in raising the economic value of cultivated crop species by providing genes for various biotic and abiotic stresses, yield and yield components, as well as physiological attributes (Jena and Khush 1990; Jauhar 2006). Among the wild species, the taxon that shares common ancestry with the cultivated species is a preferable choice, as it will facilitate genetic exchange through homoeologous pairing, and thus could serve as a useful germplasm for successful breeding of improved varieties.

Several wild species of *Carthamus* have successfully been used as a source of disease and pest resistance in safflower breeding programs (Table 4.7). Among these, *C. oxyacantha*, *C. palaestinus*, and *C. flavescens* are most promising. *C. oxyacantha* has useful genes for profuse branching, a large number of capitula, and resistance to many diseases and moisture stress (Table 4.7; Deshpande 1952; Ashri 1973; Karve 1976; Kumar et al. 1990). Introgression of desirable genes from *C. oxyacantha* into *C. tinctorius* showed an increased seed yield ranging from 2.3 to 44% in cultivar HUS 305 with an improved level of tolerance to *Alternaria* leaf blight and moisture stress (Singh 1994). Heaton and Klisiewicz (1981) synthesized a disease-resistant alloploid through chromosome doubling of *C. tinctorius* ($2n = 24$) × *C. lanatus* ($2n = 44$). The alloploid was highly resistant to *Alternaria carthami*, *Pseudomonas syringae*, and *F. oxysporum* f. sp. *carthami*.

To make safflower much more competitive than sunflower and soybean in cooking oil production, there is still a need to develop cultivars with greater resistance to foliar diseases caused by *Alternaria* spp., *Puccinia*, *Botrytis cinerea*, and *P. syringae*. This would also facilitate establishment of the crop in more humid areas.

4.12.2 Oil Content and Quality

The primary objective of safflower breeding program has been the development of cultivars with high oil content. Cultivars, HUS-305, NARI-6, and the non-spiny hybrid NARI-NH-1 released for commercial production in India contain ~35% oil (Singh and Nimbkar 2007) vis-à-vis 28–35% in other cultivars. Significant improvement in oil content of safflower seed has been achieved in the cultivars developed in the United States (Bergman et al. 1985; Rubis 2001). Safflower cultivar Oker contains 45% oil (Bergman et al. 1985). A safflower line having oil content as high as 55% has been reported by Rubis (2001).

Vegetable oils with high concentration of saturated fatty acids have important applications in the food industry, especially for the production of shortenings, margarines, and spreads. Conventional safflower oil contains 60–80 g kg^{-1} palmitic acid, 20–30 g kg^{-1}

Table 4.7 *Carthamus* species with resistance to various diseases and pests

Species	Resistance	
	Fungi	Insects
C. oxyacantha	Leaf blight, rust, Ramularia leaf spot, powdery mildew	–
C. flavescens	Ramularia leaf spot	Safflower fly
C. palaestinus	–	Safflower fly
C. lanatus	Leaf blight, rust, Ramularia leaf spot, powdery mildew	*Euribia* fly
C. baeticus	Rust, powdery mildew, Ramularia leaf spot	*Euribia* fly

stearic acid, 160–200 g kg^{-1} oleic acid, and 710–750 g kg^{-1} linoleic acid (Knowles 1989). Breeding for modified fatty acid composition of the safflower oil by a few major genes has been a major breakthrough. The allele (*ol*) has been bred into cultivars in United States, which led to two types of cultivars: high linoleic (polyunsaturated, 71–75% linoleic acid, 16–20% oleic acid) and high oleic (monounsaturated, 14–18% linoleic acid, 75–80% oleic acid) types (Knowles 1989). Ladd and Knowles (1970) increased stearic acid levels (50–120 g kg^{-1}) in several germplasm accessions. Velasco and Fernandez-Martinez (2001) reported the development of lines with modified fatty acid composition having high palmitic acid content (10.3% of the total fatty acids), medium or high stearic acid content (3.9 and 6.2%), and high or very high oleic acid content (>78 and 86%), together with reduced levels of the saturated fatty acids palmitic and stearic acid (<5%), and very high linoleic acid content (>86%). Recently, lines with increased palmitic acid (CR-50; 98.2 ± 7.9 g kg^{-1} vs. 64.0 ± 3.4 g kg^{-1} in the check) and stearic acid (CR-13; 92.8 ± 9.2 g kg^{-1} vs. 22.2 ± 3.4 g kg^{-1}) have been developed (Hamdan et al. 2008).

4.12.3 Spineless Cultivars

In areas where hand harvesting of the capitula containing seeds is practiced, the spine on the plant is a major bottleneck. Spineless cultivars CO-1 and JSI-7 have been developed in India but their poor yield vis-à-vis the spiny cultivars hinders their wide cultivation. Recently, the non-spiny cultivar NARI-6 and non-spiny hybrid NARI-NH-1 (Singh et al. 2003a) have been released. Their yield is comparable to the spiny cultivars, and in addition, they have better tolerance to foliar and wilt diseases than the spiny ones.

4.13 Construction of Genetic Linkage Maps

Genetic linkage maps for *C. tinctorius* and its wild relative *C. oxyacantha* have recently been constructed using SSR and RFLP markers (Mayerhofer et al. 2010). An F_2 population of 138 progenies derived from a cross of the safflower cultivars Centennial × NP-12 and a backcross (BC1) population of 120 progeny derived from *C. oxyacanthus* × Centennial were used to generate the two maps, respectively. The segregation of a total of 116 and 166 loci was scored in the *C. tinctorius* F_2 and *C. oxyacanthus* BC_1 populations, respectively. Using a limit of detection (LOD) threshold of 3.0 and a maximum recombination frequency of 0.3, the loci coalesced into 13 linkage groups for each of the *C. tinctorius* and *C. oxyacanthus* maps. However, some of the linkage groups consisted of only two linked markers, which might be due to the paucity of polymorphism and mappable loci in the two populations. The linkage groups ranged in genetic length from 1.3 to 170 cM and comprised 2 to 27 loci. The length of linkage maps in *C. tinctorius* and *C. oxyacanthus* was 954 and 580 cM, respectively.

The two maps showed significant colinearity of markers in several regions and an apparent translocation or inversion event on one linkage group (T5 and O5; Fig. 4.8). No apparent clustering was observed in *C. tinctorius* map as compared to *C. oxyacanthus* map where dense clusters were present on O4b and O7, with 12 and 7 markers cosegregating, respectively.

Another interspecific (*C. tinctorius* × *C. palaestinus*) linkage map is developed (Ravikumar et al. 2008) in safflower utilizing SSR markers from chickpea, sorghum, and sunflower. However, only nine SSR markers have been mapped on three linkage groups that spanned a total length of 124.6 cM.

4.14 Scope of Transcript Genetic Maps or Functional Maps

A large amount of expressed sequence tag (EST) data has been generated in safflower, and 40,996 sequences are currently available in the public domain (*http://plantta.jcvi.org/cgi-bin/plantta_release.pl*; accessed July 2007). From these, 18,067 EST singletons have been identified. The utilization of these loci into genetic maps would generate a transcript map/gene map and/or functional map. To achieve this, ESTs can be converted into an effective marker assay. These could be in the form of RFLP, STS, CAPS, SSR, or single nucleotide polymorphism (SNP). For instance, a given EST can be amplified from genomic DNA and the polymerase chain reaction (PCR) product obtained

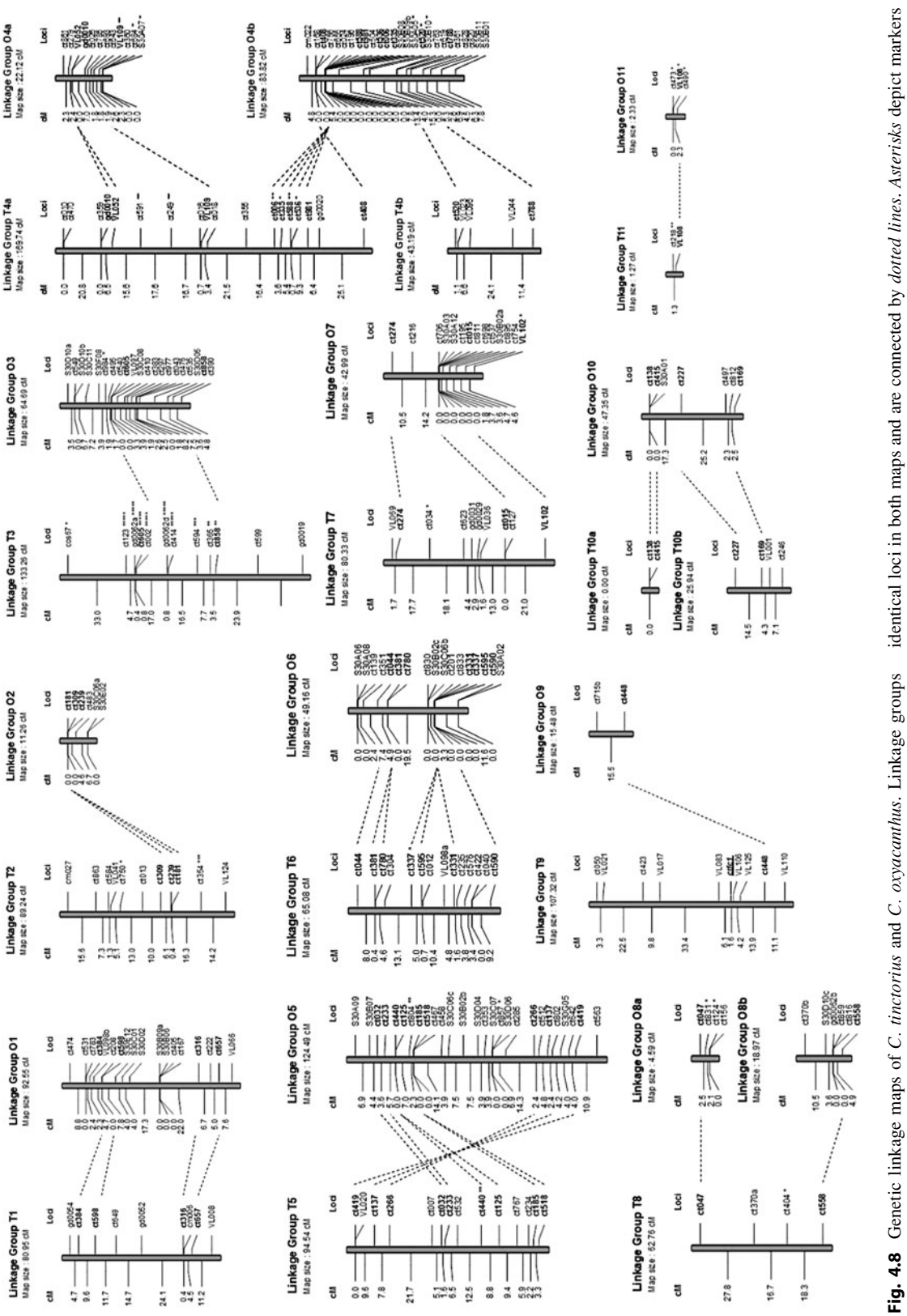

Fig. 4.8 Genetic linkage maps of *C. tinctorius* and *C. oxyacanthus*. Linkage groups T1-T11 and O1-O11 are derived from *C. tinctorius* and *C. oxyacanthus*, respectively. Genetic distances are given centiMorgans (cM) on the *left*. Markers in *bold* share identical loci in both maps and are connected by *dotted lines*. *Asterisks* depict markers with different degrees of segregation distortion (Mayerhofer et al. 2010)

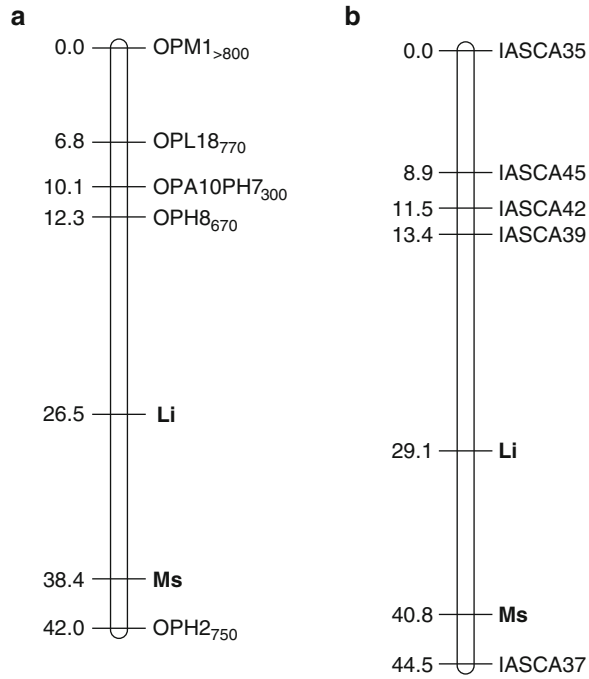

Fig. 4.9 Linkage maps containing the *Li* gene for very high linoleic acid content and the *Ms* gene for nuclear male sterility. Linkage map for RAPD marker loci, *Li* and *Ms* (**a**). Linkage map for SCAR marker loci, *Li* and *Ms* (**b**). The cumulative distances in centiMorgans (Kosambi) are shown at the *left of the map* (Hamdan et al. 2008)

can be used as an RFLP probe in a Southern hybridization or it can be tested directly for length or sequence polymorphism between the parents of a mapping population. Sequence variation between homologous PCR products can be detected directly by sequencing (EST-SNP), indirectly by digestion with restriction enzymes (CAPS). ESTs can also be searched for SSRs utilizing free available software such as SSRIT (SSR Identification tool; *http://www.gramene.org/db/searches/ssrtool*), MISA (MIcroSAtellite; *http://pgrc.ipk-gatersleben.de/misa*) and primers can be designed to amplify EST-SSRs. An important feature of EST-SSR markers is their applicability across species (Varshney et al. 2005; Asp et al. 2007; Heesacker et al. 2008; Sehgal et al. 2008a), which makes them valuable for comparative mapping.

4.15 Molecular Markers for Marker-Assisted Selection

Very little work on marker-assisted selection (MAS) has been carried out in safflower. Sequence-characterized amplified region (SCAR) markers have been developed for the closely linked genes *Li*, controlling very high linoleic acid content, and *Ms*, controlling nuclear male sterility (NMS) (Hamdan et al. 2008). For developing these markers, a mapping population of 162 individuals was developed from the nuclear male sterile line (NMS) CL1 (64–79% linoleic acid) and the line CR-142 (84–90%), and phenotyped in the F_2 and F_3 generations. A linkage map including the five SCAR markers and the *Li* and *Ms* genes was constructed (Fig. 4.9). SCAR markers flanked both the loci at minimum distances of 15.7 cM from the *Li* locus and 3.7 cM from the *Ms* locus. These are is the first molecular markers developed for trait selection in safflower, and it will be useful for MAS programs aimed at introgressing *Li* and *Ms* alleles into elite safflower cultivars.

4.16 Concluding Remarks

Insights into the origin of crop plants and knowledge of the identities of their progenitors are of great value in both basic and applied research programs. For instance, the comparative analysis of crop plants and

their wild progenitors can shed light on the genetic mechanisms underlying organismal evolution (Doebley and Stec 1991; Matsuoka 2005). Comparative analyses of this sort can also be a powerful tool for identifying genes underlying agronomically important traits (Doebley et al. 1997; Frary et al. 2000; Li et al. 2006; Jauhar 2006). The explicit identification (Sehgal et al. 2008c) of *C. palaestinus* and *C. oxyacantha* as maternal progenitors of safflower's botanical varieties *C. tinctorius* var. *innermis* and *C. tinctorius* var. *tinctorius*, respectively, has opened the door for improvement for safflower.

The precise cataloging of world germplasm resources of safflower (Johnson et al. 2007; Sehgal et al. 2008d) including cultivars (Sehgal and Raina 2005) by molecular DNA markers have opened up new vistas for marker-assisted improvement and related genomic analysis of this important crop plant. With the structuring of genetic diversity using AFLP fingerprints, it is now possible to clarify the genetic framework of morphological traits based "centers of similarity" or "regional genepools" reported in safflower. AFLP markers and morphological descriptors can now be used as coherent tools for the development of core collection(s) in safflower.

Further, comparison of DNA sequences including RAPD, ISSR, AFLP, ITS and ETS sequences, chloroplast and single copy nuclear gene sequences, and repetitive element families have and will continue to provide a better understanding of genomic relationships within and between, the cultivated species *C. tinctorius* and the wild species (Sehgal et al. 2008a, b; Sehgal and Raina 2005 ; Raina et al. 2005; Vilatersana et al. 2005, 2007; Chapman and Burke 2007; Sasanuma et al. 2008). In addition, the identification of novel repeated DNA sequences in safflower (Raina et al. 2005) will be of particular utility in the study of phylogenetic and evolutionary pathways not only in *Carthamus* taxa but also in the family Asteraceae. From a practical application standpoint, such tools and information facilitate identifying and manipulating homologous genes or alleles in related wild species or homoeologous loci within polyploid taxa for trait improvement.

Breeding efforts over the past five decades have contributed tremendously to the genetic improvement of safflower in terms of releasing disease-resistant and high oil yielding and spineless cultivars. However, traditional approaches to crop improvement have several limitations. To enhance productivity and quality and to develop resistance to the constraints such as drought, diseases, and insect pests, much more needs to be done. Molecular tools like linkage mapping, identification, characterization, and expression of genes as well as genetic engineering are needed to address limitations of classical breeding efforts. It will accelerate identification and incorporation of useful genes into cultivars, facilitate positional cloning of genes, and provide new opportunities for assessing and expanding the genepool in safflower through comparative mapping of related and unrelated taxa, and contribute to the understanding of the biological basis of complex traits and phenomena important to crop improvement and in the development of transgenics. Gene identification and mapping deserves special attention in the genus because several of the valued traits are unique to safflower. For instance, safflower is unique among the oil crops to have the highest amount linoleic acid (710–750 g kg^{-1}) (Hamdan et al. 2009).

ESTs are currently the most widely sequenced nucleotide commodity from plant genomes in terms of the number of sequences and the total nucleotide count. ESTs provide a robust sequence resource that can be exploited for gene discovery, genome annotation, and comparative genomics (Rudd 2003). A large number of ESTs (40, 996) are available in safflower. Safflower researchers, therefore, can use EST resources developed in safflower to design markers or identify gene–trait relationships for molecular applications in safflower. Also, genes can be searched in the safflower's EST database and perhaps DNA chips could be developed to conduct MAS. The identification of genes underlying economically important traits will enable the identification of "functional markers" such as SNPs within the key genes enabling efficient, high-throughput selection at the genic rather than the phenotypic level, or eventually even the design and substitution of improved alleles for these genes by some form of gene-specific transformation.

Development of genetic linkage maps in safflower is still in its infancy. Nevertheless, one intraspecific and one interspecific map are currently under development (Mayerhofer et al. 2010), and it will soon be available to safflower researchers for carrying out molecular breeding. Currently, some of the LGs (linkage groups) have significant numbers of markers available (LG1) while others (LG7 to LG12) do not. More markers will be required to saturate the linkage map

to make it more reliable and practically feasible for molecular breeding. In this regard, efforts are underway to design markers from the genomic DNA libraries (both small-insert SSR libraries and random genomic libraries) to saturate the linkage groups (Mayerhofer et al. 2010). The resulting markers can then be used to study the genetic basis of a number of key agronomic traits including flowering time, seed yield, and other traits of interest to safflower researchers.

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Chapter 5
Crambe

Eicke Rudloff and Youping Wang

5.1 Basic Botany

5.1.1 Geographical Distribution and Morphology

The genus *Crambe* L. is one of the species-richest in the tribe Brassiceae, involving 34 or 35 species (Schulz 1919; Leppik and White 1974; Gomez-Campo 2000; Warwick et al. 2000; Warwick and Al Shehbaz 2006). Due to a revision of section *Dendrocrambe* DC. recently conducted by Prina and Martinéz-Laborde (2008), the number of species and subspecies increased to 44 (Table 5.1). The geographic distribution area ranges in north–south direction from the Arctic Polar Circle to the equator and in west–east direction from Macaronesian archipelago to western China and northern India; some species were introduced to Australia, China, and America (Schulz 1919; Wang et al. 2000; Appel and Al-Shebaz 2003; Prina and Martinéz-Laborde 2008). The vertical distribution ranges from sea-level till 3,800 m in the Himalayas; the climatic conditions involved range from moderate maritime climates to semi-arid and arid climates. The three sections, in which the genus is divided, are geographically separated. The species of section *Dendrocrambe* DC. are the most west located and endemic to the Macaronesian archipelago (Canary Islands and Madeira). The adjacent geographic region involving the northern part of central Europe as well as the Mediterranean and the African area is the distribution region of section *Leptocrambe* DC., and the section *Sarcocrambe* DC. is native to the Eurasian–Asian area till the West Himalayas.

The species are annual or perennial, taprooted, often glaucous, glabrous, or pubescent herbs or subshrubs. The cauline leaves are 3-pinnatisect to entire or rarely pinnate. The sepals are ascending, the lateral pair is subsaccate, petals are white or rarely sulfureous, ovate to oblong and shortly clawed. The anthers are oblong to ovate; the median filaments are wing-like flattened and mostly appendaged. The lateral nectaries are more or less semi-annular, intrastamial, median large, globose, and distinct. The style is obsolete or lacking, the stigma is capitate and entire. There are two ovules; the globose, ellipsoid, or ovoid fruits (silicles) are heteroarthrocarpous and ascending to divaricate. The distal segment is one-seeded and terete to quadrangular, usually corky and often longitudinally ribbed and rugose or reticulate. The proximal segment is obsolete and seedless, but with one rudimentary ovule. Thus, the silicles bear only one seed, which is orthoplocal with conduplicate cotyledons. At maturity, the silicles do not dehisce and are of grayish-brown color. The fruit articulation is dedicious or non-dedicious. The seed is 1–3 mm in diameter and tan to black colored (Prantl 1891; Schulz 1919; Appel and Al-Shebaz 2003) (Fig. 5.1).

5.1.2 Taxonomy and Chromosomal Status

The taxonomic position of the genus *Crambe* L. is as follows:

order Capparales
family Brassicaceae
tribe Brassiceae
subtribe Raphaninae

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Table 5.1 Compilation of the three sections and the respective species of the genus *Crambe* (according to Schulz 1919; Francisco-Ortega et al. 1999; Prina 2000; Warwick et al. 2006; Prina and Martinéz-Laborde 2008)

1. Section *Sarcocrambe* DC.

19 species; gynaecium without pistil, stigma sessile, perennials, distribution area Eurasian–Asian region to the West Himalayas:

- *Crambe aculeolata* (N. Busch) Czerniak.
- *Crambe amabilis* Butkov & Majlun
- *Crambe armena* N. Busch
- *Crambe aspera* M. Bieb.
- *Crambe cordifolia* Steven
- *Crambe edentula* Fisch. & C.A. Mey. ex Korsh.
- *Crambe gordjaginii* Sprygin & Popov
- *Crambe grandiflora* DC.
- *Crambe grossheimii* I. Khalilov
- *Crambe hedgei* I. Khalilov
- *Crambe juncea* M. Bieb.
- *Crambe koktebelica* (Junge) N. Busch
- *Crambe kotschyana* Boiss.
- *Crambe maritima* L.
- *Crambe orientalis* L.
- *Crambe persica* Boiss.
- *Crambe schugnana* Korsh.
- *Crambe steveniana* Rupr.
- *Crambe tataria* Sebeók

2. Section *Leptocrambe* DC.

7 species and subspecies; annuals to short perennials with pronounced leafy main stem; distribution area Mediterranean region and East Africa:

- *Crambe filiformis* Jacq.
- *Crambe hispanica* subsp. *hispanica* L.
- *Crambe hispanica* subsp. *abyssinica* Hochst. ex R.E.Fr.[a]
- *Crambe hispanica* subsp. *glabrata* (DC.) Cout.
- *Crambe kralikii* subsp. *garamas* (Maire) Podlech
- *Crambe kralikii* subsp. *kralikii* Coss.
- *Crambe sinuato-dentata* Hochst. ex F. Petri

3. Section *Dendrocrambe* DC.

18 species and subspecies; subshrubs (3–4 m), profusely branched, endemic in the Macaronesian archipelago (13 in the Canary Islands, 2 in Madeira):

- *Crambe arborea* Webb ex H. Christ[b]
- *Crambe feuilleei* A. Santos[b]
- *Crambe fruticosa* subsp. *fruticosa* L.f.[b]
- *Crambe fruticosa* subsp. *pinnatifida* (Lowe) Prina & Martin.-Laborde[b]
- *Crambe gigantea* (Ceballos and Ortuño) Bramwell
- *Crambe glaberrima* (Bornm.) Mouterde ex Greuter & Burdet
- *Crambe gomerae* subsp. *gomerae* Webb ex Christ[b]
- *Crambe gomerae* subsp. *hirsuta* Prina[b]
- *Crambe laevigata* DC. ex H. Christ
- *Crambe microcarpa* A. Santos
- *Crambe pritzelii* Bolle[b]
- *Crambe santosii* Bramwell[b]
- *Crambe scaberrima* Webb ex Bramwell[b]
- *Crambe scoparia* Svent.
- *Crambe strigosa* L'Hér.
- *Crambe sventenii* B. Pett. ex Bramwell & Sundell
- *Crambe tamadabensis* A. Prina & A.Marrero
- *Crambe wildpretii* A. Prina & D. Bramwell

[a]In the further text indicated as *C. abyssinica*

[b]Species and subspecies that were introduced as distinct by Prina and Martinéz-Laborde (2008) but not indicated as "accepted" in Warwick et al. (2006)

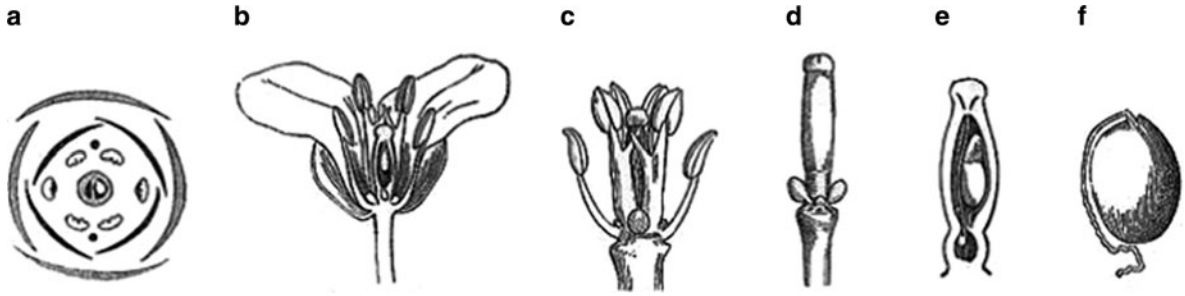

Fig. 5.1 *Crambe* L. (**a**) flower diagram; (**b**) flower cut vertically; (**c**) androeceum; (**d**) pistil and nectaries; (**e**) silicule; (**f**) seed (modified from Watson and Dallwitz 1992)

The genus is monophyletic (see Sect. 5.2.1) and it is according to morphological characters and to the geographic distribution divided into several sections. The original division, made by de Candolle (1778–1841) according to morphological characters and geographical distribution, considered the three sections *Leptocrambe* DC., *Dendrocrambe* DC., and *Sarcocrambe* DC. It was confirmed by Prantl (1891) and Schulz (1919). Khalilov (1991 cited from Francisco-Ortega et al. 1999; Prina and Martinéz-Laborde 2008) defined seven sections: section *Dendrocrambe* DC. (Schulz 1919) was divided into two new sections *Dendrocrambe* and *Rhipocrambe*, the section *Leptocrambe* DC. remained largely unaffected, and the species distributed over Eurasia, which belonged to the section *Sarcocrambe* DC., according to Schulz (1919), were divided into four new sections *Crambe*, *Astrocrambe*, *Flavocrambe* and *Orientecrambe*. Due to the application of molecular techniques to phylogenetic investigations, Warwick and Black (1997) as well as Francisco-Ortega et al. (1999) revealed results, which coincided rather with the original classification (Schulz 1919) into three sections (see also Sect. 5.3). The results were supported by the chemotaxonomic analyses of Aguinagalde and Gomez-Campo (1984), who detected distinct flavonoid patterns for the three sections. After detailed morphological studies also Prina (2000, cited from Prina and Martinéz-Laborde 2008) confirmed the classification made by de Candolle and applied by Schulz (1919). Thus, according to their morphological characterization and distribution area, the three sections displayed in Table 5.1 are to discern (Prantl 1891; Schulz 1919; Prina 2000; Prina and Martinéz-Laborde 2008). The names of the species in Table 5.1 are in accordance with the species check list of Warwick et al. (2006), which comprises the accepted botanical names. The section *Leptocrambe* DC. was completed with the species additionally mentioned by Warwick et al. (2006). In this section, the species *C. abyssinica* and *C. glabra* were reassigned as subspecies to *C. hispanica* according to the new findings from molecular phylogenetic studies (Prina 2000). The former distinct species *C. kilimandscharica* was not accepted in Warwick et al. (2006) but integrated into *C. hispanica* subsp. *abyssinica*. Similarly, the hitherto distinct species *C. garamas* had been reassigned as subspecies to *C. kralikii*, which was also accepted by Warwick et al. (2006). The section *Dendrocrambe* DC., which is endemic in the Macaronesian archipelago, was revised by Prina and Martinéz-Laborde (2008). Since their paper was published later than the species check list of Warwick et al. (2006), their new classification was integrated into Table 5.1 but marked with two asterisks.

The genus *Crambe* has a unique basic chromosome number of $x = 15$ and is exclusively polyploid (Marhold and Lihova 2006; Warwick and Al Shehbaz 2006; Lysak et al. 2007). The available data about chromosome number and genome size are summarized in Table 5.2. In former papers, the species names differed from those of Warwick and Al Shehbaz (2006) or Prina and Martinéz-Laborde (2008). In these cases, the name from the original paper was used but prefixed with an "as" and attached to the accepted name (e.g., second row in Table 5.2). The diploid chromosome number ($2n$) varies from 30 to 150 resembling a polyploid series of 30, 45, 60, 90, 120, and 150 chromosomes. Lysak et al. (2007) revealed 6, 12 copies of the analyzed block in the *C. maritime* ($2n = 60$), *C. cordifolia* ($2n = 120$),

Table 5.2 Chromosome number and nuclear DNA content in the genus *Crambe* L.

Species	Chromosome no.[a]		2C-DNA content (pg)	Reference
	n	2*n*		
Crambe aculeolata	n.a			
Crambe arborea		30	1.86[b]	Suda et al. (2003)
as *C. astrigosa* var. *arborea*	15			Warwick and Black (1997)
Crambe armena		60		Warwick and Al Shehbaz (2006)
Crambe aspera		30		Warwick and Al Shehbaz (2006)
Crambe cordifolia	ca. 60	120		Warwick and Al Shehbaz (2006)
C. cordifolia	60			Warwick and Black (1997)
C. cordifolia		120	9.468 [b]	Lysak et al. (2007)
C. cordifolia		ca. 120		Manton (1932)
Crambe edentula	n.a			
Crambe feuilleei	n.a			
Crambe filiformis	15			Warwick and Al Shehbaz (2006); Warwick and Black (1997)
C. filiformis		30		Manton (1932)
Crambe fruticosa	15	30		Warwick and Al Shehbaz (2006)
C. fruticosa		30; 60		Manton (1932)
Crambe gigantea	n.a			
Crambe glaberrima	n.a			
Crambe gomerae	15			*http://www.mobot.org/*
C. gomerae	n.a			
Crambe gordjaginii		150		Warwick and Al Shehbaz (2006)
Crambe grandiflora		120		Warwick and Al Shehbaz (2006)
C. grandiflora		ca. 120		Manton (1932)
Crambe grossheimii	n.a			
Crambe hedgei	n.a			
Crambe hispanica subsp. *hispanica*	30	60		Warwick and Al Shehbaz (2006)
as C. hispanica	30			Warwick and Black (1997)
C. hispanica subsp. *hispanica*		60	4.05[c]	Mulder and Mastebroek (1996)
as *C. hispanica*		60		Manton (1932)
C. hispanica subsp. *abyssinica*	45	90		Warwick and Al Shehbaz (2006)
C. hispanica subsp. *abyssinica*			7.04[c]	Mulder and Mastebroek (1996)
as *C. abyssinica*		90		Wang et al. (1995); Manton (1932)
Crambe hispanica subsp. *glabrata*	15	30		Warwick and Al Shehbaz (2006)
C. hispanica subsp. *glabrata*			3.9[c]	Mulder and Mastebroek (1996)
Crambe juncea		60; ca. 120		Warwick and Al Shehbaz (2006)
as C. orientalis var. *juncea*		ca. 120		Manton (1932)
Crambe koktebelica		30		Warwick and Al Shehbaz (2006)
C. koktebelica	15			Warwick and Black (1997)
as *C. orientalis* var. *koktebelica*		30		Manton (1932)
Crambe kotschyana	15	30		Warwick and Al Shehbaz (2006)
C. kotschyana	15			Warwick and Black (1997)

(*continued*)

Table 5.2 (continued)

Species	Chromosome no.[a]		2C-DNA content (pg)	Reference
	n	2*n*		
Crambe kralikii	15; 30; 45	30		Warwick and Al Shehbaz (2006)
C. kralikii	15; 30; 45			White and Solt (1978)
Crambe laevigata	15			Warwick and Al Shehbaz (2006)
C. laevigata		30	1.90[b]	Suda et al. (2003)
C. laevigata	15			Warwick and Black (1997)
Crambe maritima	15; 30	30; 60		Warwick and Al Shehbaz (2006)
C. maritima		60	4.837[b]	Lysak et al. (2007)
C. maritima	15; 30			Warwick and Black (1997)
C. maritima		60		Manton (1932)
Crambe microcarpa	n.a			
Crambe orientalis	15	30		Warwick and Al Shehbaz (2006)
C. orientalis	15; 30			Warwick and Black (1997)
Crambe persica	n.a			
Crambe pritzelii	n.a			
Crambe santosii	n.a			
Crambe scaberrima		30		www.mobot.org
C. scaberrima		30	1.85[b]	Suda et al. (2003)
C. scaberrima	15			Warwick and Black (1997)
Crambe schugnana		30		Warwick and Al Shehbaz (2006)
Crambe scoparia	15			Warwick and Al Shehbaz (2006)
Crambe sinuato-dentata	n.a			
Crambe steveniana	n.a			
Crambe strigosa	15			Warwick and Al Shehbaz (2006)
C. strigosa		30	1.98[b]	Suda et al. (2003)
as C. strigosa var. *strigosa*	15			Warwick and Black (1997)
Crambe sventenii		30		Warwick and Al Shehbaz (2006)
C. sventenii	15			Warwick and Black (1997)
Crambe tamadabensis	n.a			
Crambe tataria		30; 60; ca. 120		Warwick and Al Shehbaz (2006)
C. tataria	15; 30; 60			Warwick and Black (1997)
C. tataria		ca. 120		Manton (1932)
as *C. tataria* var. *pinnatifida*		60; 120		Manton (1932)
Crambe wildpretii	n.a			

[a]n.a.: data not available
[b]2.0 C level as reference value (for details see reference)
[c]0 C level as reference value (for details see reference)

respectively, by chromosome painting using an approximately 11.5-Mb chromosome segment of the upper arm of *Arabidopsis* chromosome At3. Data about the DNA content of the nucleus were rare.

5.1.3 Utilization and Agricultural Status of Crambe Species

There are three kinds of utilization for *Crambe* species, i.e., as vegetables (culinary herbs or roots), for phytoremediation of contaminated soils, and as an oil crop. The different kinds of utilization concern diverse features of the plants.

5.1.3.1 Vegetables

The distribution area of the genus *Crambe* involves a variety of landscapes and environmental conditions (see Sect. 5.1.1). That requires a huge adaptability, which in turn needs a sufficient degree of genetic variability. The species are not dominant for, but a well adapted part of, the respective habitat. Thus, kinds of tribal uses had already evolved in ancient times, mainly as vegetables or food.

Crambe maritima was probably already known in ancient Rome. The species grows commonly on the European seaside of the Atlantic Ocean as well as of the English Channel and the Baltic Sea. Since the seventeenth century, it was used as vegetable in England, France, and the North American Colonies (Hedrick 1919; Hahnelt 1997). The plants were covered up with sand in their natural habitat and the leaves blanched while growing. After boiling, they were appreciated as a delicacy. For the nineteenth century, it was described as very popular in England and France (Hedrick 1919; Renfrew and Sanderson 2005). In the 1990s, some attempts were made in France to introduce it again as a vegetable (Péron 1990; Quinsac et al. 1994; Briard et al. 2002). In 1992, a breeding program was initiated in France, which involved a systematic search for wild populations alongside the French coast of the English Channel (see Sect. 5.3.2). Investigations of glucosinolate content in fresh and boiled etiolated sprouts were reported by Quinsac et al. (1994). Glucosinolates are sulfur-containing secondary metabolites, which are characteristically found in tissues and seeds of cruciferous plants. In damaged cells, a glycoprotein enzyme myrosinase, which is located in the idioblast, comes into contact with the glucosinolates, stored in the same cell. That induces the hydrolysis of the glucosinolate, thus producing breakdown products, which are antinutritious compounds (Agnihotri et al. 2007). The total glucosinolate content in fresh sprouts was between 5.4 and 7.3 μmol/g fresh matter, which constitutes to 80% of epiprogoitrin. Freezing resulted in nearly complete decomposition of the glucosinolates, whereas blanching (cooking for 4 min) reduced it by about 30%. *C. orientalis* is domestic in Asia Minor and Persia. The shape and the taste of its roots resemble that of horse radish and they were used in an analogous manner. The root and foliage of *C. cordifolia*, which is growing in Persia and from Caucasus to Tibet and the Himalayas, is edible. In the Garhwal Himalayas (India), several parts of the plant are consumed during famine (Duhoon and Koppar 1998). *C. tatarica* is domestic in the steppes of eastern Europe (rivers Danube, Dnepr, and Don) where its fleshy, sweet roots were eaten raw as well as cooked (Hedrick 1919). Ul'chenko et al. (2001) reported that in central Asia, all aerial parts of *C. kotschyana* are used as feed for all types of livestock, whereas the roots are edible and give starch and alcohol. An extract is used for treatment of congestion of respiratory tract.

5.1.3.2 Phytoremediation

C. abyssinica is reported to be able to tolerate and accumulate unusually high amount of arsenic (As). Artus (2006) compared the As uptake of *B. juncea* with *C. abyssinica*. In a hydroponic system, the As accumulation in the leaves of *C. abyssinica* was fourfold higher than in *B. juncea* (140 vs. 34 mg/kg, dry weight base). She recommends considering the crop for use in phytoremediation research. Paulose et al. (2007) described the search for As-induced genes in *C. abyssinica* to elucidate the mechanisms of arsenic tolerance in plants.

5.1.3.3 Oil Crop for Industrial Use

The history of agricultural utilization is very young and dates back to the 1930s, when *C. abyssinica* was

recovered as agricultural valuable in Russia. Its oil bearing seeds, yield capacity, and adaptation on the regional conditions made it attractive as an alternative oil crop for Eurasian regions. After World War II, similar attempts were made in central Europe. The success was low till the unique seed oil composition of the species was recorded, which made it highly attractive for industrial purposes. This will be described in detail in Sect. 5.3.2.

5.1.4 Conservation Activities

Several species of the genus *Crambe* are considered as a part of the threatened biological diversity and get legal protection on a national or international level. The 1977 IUCN Red List of Endangered Species 132classified the threat level of seven species to be intermediate (*C. steveniana*), rare (*C. fruticosa*, *C. scaberrima*), vulnerable (*C. gomerae*, *C. laevigata*), and endangered (*C. arborea*, *C. sventenii*). Three additional species (*C. koktebelica*, *C. scoparia*, and *C. tataria*) were classified under legal protection (Ozinga and Schaminée 2005). Several *Crambe* species are as crop wild relatives (CWR) of additional interest in terms of utilization for crop improvement.

One of the main instruments to protect the biological diversity is germplasm collection and the ex situ conservation in gene banks. Therefore, for long-term storage, the seed is air-dried and stored at −15 to −20°C. EURISCO counts 251 ex situ accessions conserved in European gene banks (CWR 2009). The European gene banks with the most accessions are located in Austria (AGES, eight accessions of *C. abyssinica*), Bulgaria (Institute for Plant Genetic Resources "K. Malkov," 36 accessions of three species), Germany (IPK gene bank, 71 accessions of eight species), and Hungary (Institute for Agrobotany, 13 accessions of *C. abyssinica*) (EURISCO 2009).

An ex situ Crucifer germplasm collection established by César Gómez-Campo at Universidad Politécnica, Madrid, Spain, contains 20 accessions of 20 species (Gomez-Campo 2000). A large germplasm collection in US is held by the National Genetic Resources Program (NGRP) of the USDA-ARS. It includes 165 accessions from ten species (GRIN 2009).

The main institutions for the in situ conservation of such wild species are botanical gardens. Botanical Gardens Conservation International (BGCI), an international association of botanical gardens, lists 193 accessions of 23 *Crambe* species worldwide (BGCI 2009).

5.2 Phylogenetic Relationships, Development of Molecular Markers, and Cytogenetic Stocks

5.2.1 Phylogenetic Relationships of the Genus Crambe

A few reports have used internal transcribed spacers (ITS), cpDNA, random amplified polymorphic DNA (RAPD), and similar techniques to analyze phylogenetic relationships between different members of the *Crambe* genus (Warwick and Black 1997; Francisco-Ortega et al. 1999; 2002; Warwick and Gugel 2003). A phylogenetic analysis of nucleotide sequences of the ITS of nuclear ribosomal repeat was conducted with 27 species of *Crambe* (Francisco-Ortega et al. 1999). Cladistic analyses using weighted and unweighted parsimony support *Crambe* as a monophyletic genus with three major lineages. The first comprises those taxa endemic to the Macaronesian archipelagos. Taxa with a predominant Mediterranean distribution form the second assemblage, and a disjunction between East Africa (*C. abyssinica*) and the Mediterranean (*C. hispanica*) occur in this clade. The third lineage includes all Euro Siberian–Asian taxa and *C. kilimandscharica*, a species from the highlands of East Africa. A basal biogeographic split between East Africa and Eurasia is present in the third clade. The patterns of relationships in the ITS tree are concordant with known climatic events in North Africa and southwestern Asia since the middle Miocene. The ITS trees are congruent with the current sectional classification except for a few members of sections *Crambe*, *Leptocrambe*, and *Orientecrambe* (*C. cordifolia*, *C. endentula*, *C. kilimandscharica*, and *C. kotschyana*). Warwick and Gugel (2003) compared genetic relationships among *C. abyssinica*, *C. hispanica*, and *C. glabrata* and a taxonomic separation of them using traditional morphological traits, agronomic and seed quality data,

chromosome number, and various molecular data sets including nuclear-DNA-based random amplified polymorphic DNA (RAPD) data, chloroplast (cpDNA) restriction site data, and ITS sequence data for the ITS region of the nuclear ribosomal DNA. The three species can be distinguished most reliably by chromosome number. Accessions could generally, but not always, be distinguished morphologically by plant branching pattern, fruit articulation and color, leaf pubescence, and leaf shape. cpDNA restriction site data and ITS sequence data, two relatively conserved DNA data sets, supported the recognition of *C. glabrata* as a distinct species separate from the *C. hispanica*/*C. abyssinica* accessions. Within the latter group, both RAPD data and field evaluation data revealed greater amounts of genetic variation in *C. hispanica* compared to accessions of *C. abyssinica*, with the latter included as a subset of *C. hispanica*. *C. glabrata* was genetically distinct for all data sets and warrants separate species status. Phylogenetic studies have indicated that *C. filiformis* Jacq. ($n = 15$), an endemic of southern Spain, Algeria, and Morocco, is the most closely related species to *C. abyssinica* and *C. hispanica*, and that the $n = 15$ taxon, *C. kralikii*, an endemic of Morocco and Algeria, formed a sister group to the clade containing these three taxa (Warwick and Black 1997; Francisco-Ortega et al. 1999).

Glabrous leaf was used as a dominant marker in measuring outcrossing percent between *C. abyssinica* and *C. hispanica*. Pubescence appears to follow a normal diploid segregation controlled by one gene with glabrous leaf dominant to pubescent leaf (Beck et al. 1975). Three separate sets of molecular data are consistent with the inclusion of *C. abyssinica* and *C. hispanica* in the same species and the recognition of *C. glabrata* as a separate species. This conclusion is supported by hybridization and flavonoid data from previous studies. Mulder and Mastebroek (1996) reported successful crosses between *C. abyssinica* and *C. hispanica*, and a lack of success in crossing *C. hispanica* and *C. glabrata*. Meier and Lessman (1973a, b) also reported successful reciprocal crosses between *C. abyssinica* and *C. hispanica*; they also suggested that the parents used in their study should be classed as a single species based on similar chromosome numbers and raised the possibility that the plants that were thought to be *C. hispanica* may not have been that species. The *C. hispanica* parent was later determined to be an ecotype of *C. abyssinica* (Lessman 1975). Flavonoid studies by Aguinagalde and Gomez-Campo (1984) indicated identical patterns for *C. abyssinica* and *C. hispanica* and the presence of two unique flavone glycosides in the *Crambe* taxa surveyed, whereas *C. glabrata* was distinctly different, having a much more complex range of flavonoids similar to that found in other *Crambe* species.

5.2.2 Molecular Markers

Somers and Demmon (2002) used direct amplification of minisatellite DNA (DAMD) by PCR to clone fragments of DNA from *C. abyssinica*, *C. hispanica* var. *hispanica*, and *C. hispanica* var. *glabrata*. The DAMD PCR fragments were used as probes to hybridize the genome, and the *Crambe* genome-specific probes (pCa17.3, 420 bp) can be used to distinguish other crucifer species. In 1992, a systematic search for wild populations of *C. maritima* was undertaken in France, from Quiberon (South Brittany) to Dunkerque (North France near Belgium). Morphological descriptors and molecular markers (RAPD) were used to study the phenotypic variability of the collected plants. A great variability for leaf and leaf-stalk color, limb, flowers, and silique sizes was observed. Among the wild collected plants, molecular similarity varied from 25 to 85%. The mean distance from all the wild genotypes to the breeding material already in collection was large (50%) (Briard et al. 2002).

5.2.3 Addition Lines

Two *B. napus*–*C. abyssinica* monosomic addition lines ($2n = 39$, AACC plus a single chromosome from *C. abyssinica*) were obtained from the F_2 progeny of the asymmetric somatic hybrid between *B. napus* and *C. abyssinica* (Wang et al. 2006). The progeny plants with 39 chromosomes exhibited minor morphological differences from the control plant (*B. napus*), for example, in the presence of numerous trichomes on the stems in the young plant or very dark green leaves; both of these characteristics are typical for the chromosome donor (*C. abyssinica*). Genomic in situ hybridization (GISH) analysis of these two plants indicated that they each contained

39 chromosomes comprising the complete diploid genome of *B. napus* ($2n = 38$) and a monosomic addition chromosome from *C. abyssinca*. After colchicine doubling of 13 haploids derived from microspore culture, two plants were confirmed to possess 40 chromosomes, representing 38 *B. napus* chromosomes and two homologous *C. abyssinica* chromosomes (disomic addition line). The meiosis of these plants showed normal 20 bivalents (II) at the diakinesis. These doubled haploids had a low seed set, from 2.4 to 6.9, and compared to the control (*B. napus*), they contained a higher level of erucic acid (51.9%). The intergeneric hybrid between *Brassica chinensis* and *C. abyssinica* with 55 chromosomes was obtained. After several generations of in vitro propagation by tissue culture, the chromosomes of the hybrid were remarkably reduced, ranging from 25 to 28. The reduction of chromosomes and the high numbers of bivalents in the hybrid were possibly due to the *Crambe* chromosome elimination and the *Brassica* genome doubling in the cells (Tang et al. 2006).

5.3 Role in Crop Improvement

5.3.1 Natural Variability for Desired Traits

5.3.1.1 Agronomic Characters

Agronomically important characters related to plant development and growth depend to a great extent on environmental and cultivation conditions. Therefore, they are not reviewed in detail. Mulder and Mastebroek (1996) and Warwick and Gugel (2003) compared the genetic variation of *C. abyssinica*, *C. hispanica*, and *C. glabrata* regarding their agricultural applicability in Netherlands (Wageningen) and Canada (Saskatoon), respectively. At both the places, the variability in *C. hispanica* (29 and 29 accessions) was greater than that of *C. abyssinica* (4 and 58 accessions) for agronomic traits. There were significant differences between *C. abyssinica* and *C. hispanica* in days to anthesis (61.3 vs. 57.8 days in Wageningen and 54 vs. 49 days in Saskatoon) and plant height (97.7 vs. 121 cm in Wageningen and 93 vs. 114 cm in Saskatoon). The range for days to anthesis in Wageningen was 60.4–62.6 days and 45.9–85.5 days in *C. abyssinica* and *C. hispanica*, respectively, and in Saskatoon, 45–58 days and 42–66 days, respectively. Similar species differences occurred in plant height and other agronomic characters. Compared to *C. abyssinica, C. glabrata* displayed significant differences in both trials with regard to days to anthesis but not plant height. Obviously, the growth is delayed under Chinese conditions; Wang et al. (2000) recorded 212–224 days to maturity for several selections from cv. "Meyer" compared with 90–100 days for similar cultivars in Italy (Lazzeri et al. 1994).

5.3.1.2 Fruit and Seed Characters and Seed Compounds

The harvest product of a *Crambe* cultivar is the seed bearing fruit, because the pod in contrast to other Brassica oil crops, e.g., oilseed rape (*B. napus*) or sarson (*B. juncea*), remains closed at maturity. This is a disadvantage because it increases volume to be transported, complicates oil extraction, and decreases the proportion of oil and protein. In *C. abyssinica*, the husk or pericarp proportion was 21.2% in c.v. "Meyer" (Reuber et al. 2001) and between 25 and 40% in samples of 75 field trials in 17 US states (Earle et al. 1966). Downey (1971) reported that pericarp proportion in Canada differed from these results, displaying 14–20%. In trials in Italy, cv. "BelEnzian" and "BelAnn" displayed a hull content of 27.4% and 28.8%, respectively (Lazzeri et al. 1994). The only available study with other species was that from Comlekcioglu et al. (2008), whose data for Turkish wild populations of *C. orientalis* and *C. tataria* indicated a pericarp content of 45% and 25%, respectively.

The formation of more than one seed in the fruit of *Crambe* species occurs occasionally, but Duhoon and Koppar (1998) collected in altitudinal ranges from 500 to 3,800 m at the Garhwal Himalayas (India) a *C. cordifolia* with 3–5 seeds per siliqua. Introgression of such trait into the *C. abyssinica* crop would be of great advantage for the *Crambe* business.

Table 5.3 summarizes results of several seed characters (content of oil, protein and glucosinolates, and 1,000-fruit weight). As mentioned above, the siliques do not dehisce at maturity, but the seed remains covered with the pericarp. For the processing of *C. abyssinica,* mechanical peeling of the seeds is recommended to get hulled seeds. This is possible

Table 5.3 Variation of several seed characters in the genus *Crambe* L.

Species	1,000 fruit weight (g)	Seed protein (%)		Seed glucosinolate (μmol/g or %)		Oil content (%)		Reference[b]
	Whole seed	Whole seed	Hulled	Whole seed	Hulled	Whole seed	Hulled	
Crambe abyssinica						38.7[a]		Dolya et al. (1977) (1)
C. abyssinica	6.9–8.7					25.7–29.0		Mulder and Mastebroek (1996) (4)
C. abyssinica	6.0–7.8	22.7–32.2		81.6–118.7		31.3–38.5		Warwick and Gugel (2003) (58)
C. abyssinica	5.5–6.5	24.2	31.2	71.5–82.2	116.1	34.48	44.47	Wang et al. (2000) (cv. Meyer)
C. abyssinica	6.54					32.2		McKillican (1966) (1)
C. abyssinica	7.0/6.8			65.4/64.2		30.7/34.4		Mastebroek et al. (1994) (22)[c]
C. abyssinica						33.6		Mandal et al. (2002) (1)
C. abyssinica						35.6–42.8		Castleman et al. (1999) (25)
C. abyssinica				10.4–26.3%			38.8–45.5	Lessman (1975) (162)
C. abyssinica		26.5/26.7		83.1/92.0		35.2/36.3	–/44.8	Lazzeri et al. (1994) (BelEnzian/BelAnn)
C. abyssinica			22–37		4–10%	24–37	36–54	Earle et al. (1966) (75)
C. abyssinica			25.8		8–10%			Erickson and Bassin (1990) (?)
C. abyssinica	4,2/5,3	32/25				36/34		Mikolajczak et al. (1961) (2)
C. abyssinica	5.7–7.9					27.8–35.3		Oplinger et al. (1991) (8 cv.)[d]
Crambe amabilis						18.41(1)		Umarov and Kisapova (1973)
Crambe cordifolia	17.5	45					26	Miller et al. (1965) (1)
Crambe fruticosa						6.9		Kumar and Tsunoda (1978) (1)
Crambe glabrata	9.9–14.2					24.6–27.5		Mulder and Mastebroek (1996) (7)
C. glabrata	8.0–10.5	21.9–26.5		59.9–87.0		22.9–30.6		Warwick and Gugel (2003) (7)
Crambe hispanica	5.7–13.2					22.9–29.5		Mulder and Mastebroek (1996) (29)
C. hispanica	5.1–9.5	19.1–35.5		83.4–120.5		17.2–35.3		Warwick and Gugel (2003) (29)
C. hispanica	4.7	29					45	Miller et al. (1965) (1)
Crambe kotschyana						23.0[a]		Ul'chenko et al. (2001) (1)
Crambe. kralikii						19.0		Kumar and Tsunoda (1978) (1)
Crambe maritima as *C. pontica*						41.7		Dolya et al. (1973) (1)
Crambe orientalis	10.0–29.0					11.0	26.0	Comlekcioglu et al. (2008) (1; 20 plants)
C. orientalis	6.7	31					43	Miller et al. (1965)
Crambe scaberrima						11.0		Kumar and Tsunoda (1978) (1)
Crambe schugnana						18.58[a]		Umarov et al. (1972) (1)
Crambe tataria	9.0–18.0					15.0	25.0	Comlekcioglu et al. (2008) (1; 20 plants)
C. tataria	15.5	40					33	Miller et al. (1965) (1)

[a]It is not clear if the oil content is related to the fruit (whole seed) or the hulled seed
[b]In parentheses: no of accessions studied or name of cv
[c]Means of different years (1991/1992)
[d]Different cultivars in different years

with special devices (Reuber et al. 2001), but most studies were done without peeling, i.e., with the whole seed. Therefore, in Table 5.3, it is considered if hulled or whole seeds were analyzed.

The 1,000-seed weight displays a considerable intra- and interspecific variability and ranges in *C. abyssinica* from 4.2 to 8.7 g; and from 8.0 to 14.2 g and 4.7 to 13.2 g in *C. glabrata* and *C hispanica*, respectively. The heaviest seeds were observed in *C. orientalis* (average 17.5 g). The respective weight of hulled seed was 9.7 g (Comlekcioglu et al. 2008).

The oil content of hulled seed attained in *C. abyssinica* was more than 44%, and it is expected from several data in the "whole seeds" column that it would be outreached by several accessions. Similar values were displayed from *C. hispanica* (35.3%) (Warwick and Gugel 2003). Some other species display remarkable high oil content, too. That applies to *C. pontica* with 41.7% (Dolya et al. 1973) and *C. orientalis* with 43% (Miller et al. 1965).

The protein content is of particular value for the oil crop *C. abyssinica*, since the seed meal after the extraction of oil provides a protein-rich and nutritious feeding stuff. The protein content of hulled seeds ranged in *C. abyssinica* from 22% to 31.2%, which resembled oilseed rape (*B. napus*). The protein content of defatted seed meal was similar in *Crambe* and oilseed rape as well. It was 45–47% in rapeseed (Downey 1971) and 44–46% in *C. abyssinica* cv. "Meyer" (Reuber et al. 2001), respectively. The utilization of the seed meal of Brassica crops is limited by the glucosinolates in the seed. The whole seed contains about 60–110 μmol/g seed meal, which consists mainly of epiprogoitrin (90%). The breakdown products (isothiocyanates, thiocyanates) due to the myrosinase-caused hydrolysis in damaged tissues affect the iodine uptake by the thyroid gland in non-ruminant animals and reduced the palatability of the feed and its efficiency (Agnihotri et al. 2007). The glucosinolate level shown in Table 5.3 is similar to conventional cultivars of oilseed rape (non-Canola quality) and wild species of Brassicaceae. A source for low glucosinolate content is not known so far in the genus *Crambe*. Besides its importance as a deleterious compound in food and feed, glucosinolates have biological functions, which could open up new fields of application. An important function is to defend the plant against herbivores (insects, molluscs, and nematodes) as well as pathogenic microbes and viruses (Kliebenstein et al. 2005; Wink 2007). Lazzeri et al. (1993) and Mari et al. (1993) reported on the in vitro activity of the breakdown products (isothiocyanates) against nematodes and post-harvest fruit pathogens, respectively. Breakdown products of glucosinolates have been indicated as inhibitors of chemically induced carcinogenesis. Attempts to produce enantiomerically pure fine chemicals from the glucosinolates of *Crambe* meal, which is attractive due to its high amount of progoitrin (>90 μmol/g) as described by Daubos et al. (1998), will support those applications. The available methods of detoxification of *Crambe* meal (Shahidi and Naczk 1990) are able to attain both an improved residual meal and a good quantity of epiprogoitrin as a by-product (Lazzeri et al. 1994).

5.3.1.3 Seed Oil Composition

The most important product of the oil crop *C. abyssinica* is the seed oil, especially the erucic acid (see Sect. 5.2). Therefore, one of the main reasons for the utilization of the wild species of the genus *Crambe* is expected to be the variability of the seed oil composition. The available data on seed oil composition in several *Crambe* species are compiled in Table 5.4. As with other characters, there was no systematic evaluation of seed oil composition so far. Of the 43 species and subspecies mentioned in Table 5.1, data only for 15 species on this matter are available. The extent differs between the species, being highest in *C. abyssinica* and *C. hispanica*, as expected. With *C. abyssinica*, both wild populations and cultivars were involved in the review. Obviously, there was no substantial difference in erucic acid content between cultivars (Lazzeri et al. 1994; Mulder and Mastebroek 1996; Wang et al. 2000) and wild populations (Mikolajczak et al. 1961; Earle et al. 1966; Dolya et al. 1977; Mastebroek et al. 1994). Erucic acid varied from 48.5% to 62.5%. In two other members of the section *Leptocrambe* DC., viz. *C. hispanica* and *C. glabrata*, a comparable amount of erucic acid was found (50.0–60.1%, and 45.4–59.9%, respectively), whereas in one accession of *C. kralikii* (Kumar and Tsunoda 1978), a lower content (45.5%) was estimated. Considerably high amounts of erucic acid (55.1% and 50.4%, respectively) were observed in *C. scaberrima* and *C. fruticosa* (Kumar and Tsunoda 1978) as well, which belong to the section *Dendrocrambe* DC.

Table 5.4 Seed oil fatty acid composition in *Crambe* species (when several accessions were analyzed, the respective range is given)

Species	Fatty acid (%)[a]							Reference[b]
	$C_{16:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:1}$	$C_{22:1}$	$C_{24:0}$	
Crambe abyssinica	1.53	15.24	9.46	5.22	2.80	57.07	1.62	Dolya et al. (1977) (1)
C. abyssinica	n.a.	n.a.	n.a.	6.8/6.8	9.9/8.8	54.0/55.9	n.a.	Mastebroek et al. (1994) (22)[c]
C. abyssinica	2.8	16.3	11.0	8.7	3.5	54.9	n.a.	McKillican (1966) (1)
C. abyssinica	n.a.	13.30–15.60	8.45–9.40	6.00–8.00	1.20–1.55	55.5–57.3	1.80–2.25	Mulder and Mastebroek (1996) (4)
C. abyssinica	n.a.	17.0	9.0	6.0	5.0	55.0	n.a.	Princen (1983)
C. abyssinica	2.18	16.49	9.34	4.80	4.69	62.50	n.a.	Wang et al. (2000) (5)
C. abyssinica	0.2–0.3	16.0–22.8	6.9–8.5	4.3–6.0	3.7–5.6	48.5–57.9	1.3–1.6	Warwick and Gugel (2003) (58)
C. abyssinica	1.9/1.8	17.2/17.6	8.7/8.5	5.2/5.4	3.4/3.7	56.2/56.3	1.6/1.5	Lazzeri et al. (1994)(cv. BelEnzian/cv. BelAnn)
C. abyssinica	1.6	17.9	6.9	6.7	2.5	58.6 (51–60)	0.3	Earle et al. (1966) (75)
C. abyssinica	2.0/2.0	18.0/18.0	11.0/9.0	4.0/6.0	2.0/3.0	59.0/59.0	0/0.4	Mikolajczak et al. (1961) (2)
C. abyssinica	1.7	16.7	7.8	6.9	2.1	55.7	n.a	Downey (1971) (?)
Crambe amabilis	2.50	19.35	12.94	8.05	19.92	34.82	–	Dolya et al. (1977) (1)
C. amabilis	3.47	22.83	12.97	24.99	n.a.	30.78	n.a.	Umarov and Kisapova (1973) (1)
Crambe cordifolia	2.31	27.11	14.44	7.59	17.28	28.04	1.35	Dolya et al. (1977) (1)
C. cordifolia	4.0	22.0	14.0	6.0	12.0	36.0	0.3	Miller et al. (1965) (1)
Crambe fruticosa	6.0	17.7	43.4	9.5	1.8	50.4	n.a.	Kumar and Tsunoda (1978) (1)
Crambe glabrata	n.a.	20.30–24.40	2.95–5.90	3.20–5.45	1.60–4.15	53.6–59.9	1.60–2.45	Mulder and Mastebroek (1996) (7)
C. glabrata	0.3–0.5	18.0–23.0	5.1–7.3	3.9–6.1	4.8–8.5	45.4–54.8	1.0–1.6	Warwick and Gugel (2003) (7)
Crambe hispanica	0.3	17.0	9.0	7.0	4.0	55.0	0.8	Miller et al. (1965) (1)
C. hispanica	n.a.	14.5–23.8	5.25–10.60	2.70–7.25	1.25–4.30	53.1–60.1	1.20–2.60	Mulder and Mastebroek (1996) (29)
C. hispanica	0.2–0.4	17.0–20.6	5.4–8.4	4.0–7.4	2.6–6.6	50.0–58.6	1.0–108	Warwick and Gugel (2003) (29)
C. hispanica	3.7	20.1	10.7	5.5	4.6	52.4	n.a.	Downey (1971) (?)
Crambe koktebelica	1.32	31.14	19.11	4.68	17.47	24.71	–	Dolya et al. (1977) (1)
Crambe kotschyana	2.36	29.29	16.57	7.66	17.10	25.66	–	Dolya et al. (1977) (1)
C. kotschyana	0.9	21.5	8.3	24.5	n.a.	42.1	n.a.	Ul'chenko et al. (2001) (1)
Crambe. kralikii	4.0	22.2	8.4	7.5	11.2	45.5	–	Kumar and Tsunoda (1978) (1)
Crambe maritima	4.1	26.7	25.3	4.8	14.5	21.6	0.4	Goffman et al. (1999) (1)
as *C. pontica*	1.38	22.25	20.30	8.27	17.49	28.41	–	Dolya et al. (1973) (1)
Crambe orientalis	2.09	18.14	13.07	9.07	19.39	34.69	0.99	Dolya et al. (1977) (1)
C. orientalis	3.27	1.61	12.42	21.21	11.34	39.39	0.99	Comlekcioglu et al. (2008)
C. orientalis	2.0	18.0	11.0	10.0	20.0	36.0	0.7	Miller et al. (1965) (1)
Crambe scaberrima	3.2	14.1	12.2	13.0	1.5	55.1	–	Kumar and Tsunoda (1978) (1)
Crambe schugnana	2.45	21.64	12.93	26.29	n.a.	34.86	n.a.	Umarov et al. (1972) (1)
Crambe steveniana	1.26	19.95	23.37	8.54	18.47	24.49	–	Dolya et al. (1977) (1)
Crambe tataria	1.72	28.69	22.17	7.81	16.46	20.72	–	Dolya et al. (1977) (1)
C. tataria	2.30	1.41	9.00	15.01	7.70	29.87	0.68	Comlekcioglu et al. (2008) (1)
C. tataria	2.0	21.0	15.0	11.0	21.0	27.0	–	Miller et al. (1965) (1)
as *C. pinnatifida*	1.99	17.95	11.66	9.09	3.62	47.37	3.20	Dolya et al. (1977) (1)

[a] *n.a.* not analyzed; – not detected. $C_{16:0}$ palmitic; $C_{18:1}$ oleic; $C_{18:2}$ linolic; $C_{18:3}$ linolenic; $C_{20:1}$ eicosenic; $C_{22:1}$ erucic; $C_{20:0}$ nervonic acid

[b] In parentheses: number of included accessions, and name of cultivars, respectively; (?): several, no. unknown

[c] Results of 2 years (1991/1992)

The oleic acid content in *C. orientalis* and *C. tataria* as given by Comlekcioglu et al. (2008) seems to be very low (1.61% and 1.41%, respectively). The results from Dolya et al. (1977) and Miller et al. (1965) are rather expected. A considerable variation in linolenic acid seems to exist in some species of the section *Sarcocrambe* DC. It concerns *C. amabilis* (8.05% and 24.99%) and *C. kotschyana* (7.66% and 24.5%). Another species, *C. schugnana*, of which only one accession was analyzed, contains 24.5% linolenic acid (Ul'chenko et al. 2001).

5.3.1.4 Abiotic and Biotic Stresses

The different environmental influences, which are acting in the distinct geographic areas of distribution, present a great diversity in abiotic and biotic stresses, which the plants have to combat. Results on variability in tolerance to abiotic stresses, such as for instance drought, temperature, and soil conditions, are not available. But general characterization was made for several species. Thus, *C. maritima* was considered to be salt-tolerant, but no data were available. Cultivars and released germplasms of *C. abyssinica* responded with decreased seed yield on increasing soil salinity (Francois and Kleiman 1990). *C. abyssinica* generally is considered as a low-input crop but with drought tolerance (Erickson and Bassin 1990). Of course the fact that several species are also distinguished due to their adaptation to highly different natural habitats indicates a great variability in their tolerance to abiotic stresses.

C. abyssinica is infested with similar pests and diseases, which also occur in other species of the Brassicaceae family. For Germany, Amelung (1995) listed the pathogenic fungi *Plasmodiophora brassicae, Albugo candida, Peronospora parasitica*, *P. crambes, Erysiphe polygoni, Sclerotinia sclerotiorum, Puccinia trabutii* [*Aecidium crambes*], *Alternaria alternata*, *A. brassicae*, *A. brassicicola, Botrytis cinerea, Chromelosporium fulvum, Fusarium acuminatum*, *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. redolens*, *F. solani*, *F. sulphureum, Verticillium dahliae, Ascochyta crambes*, *Asteromella brassicae*, *Mycosphaerella brassicicola* and the pests *Meligetes aeneus, Meligetes* spp., *Phyllotreta atra*, *P. nigripes*, *P. undulata*, *P. nemorum, Gastroidea polygoni*. The pathogens *Sclerotinia sclerotiorum*, *Alternaria alternata*, *A. brassicae*, *A. brassicicola*, and *Botrytis cinerea* were often observed to cause considerable damages to the crop.

With cultivation in greenhouse or growth chamber, Scholze and Hammer (1997) evaluated the disease resistance of 900 accessions of Brassicaceae including six accessions each of *C. abyssinica* and *C. hispanica* after inoculation with *Plasmodiophora brassicae*, *Alternaria brassicicola*, *A. brassicae*, and *Phoma lingam*. All *Crambe* accessions exhibited resistance against *A. brassicae* and *P. lingam* and were susceptible for *A. brassicicola* and *P. brassicae*. In field trials, which compared 58 accessions of *C. abyssinica*, 29 accessions of *C. hispanica*, and seven accessions of *C. glabrata,* Warwick and Gugel (2003) observed a low blackleg (*P. lingam*) severity with mean values (scale 0–6) of 1.3, 0.6, and 0.8, respectively.

Anderson et al. (1992) compared the feeding preference of the flea beetle (*Phyllotreta cruciferae*) for oilseed rape and *C. abyssinica* and classified the latter as resistant against this pest. This agreed with the observations reported by Carlson et al. (1996) in the field crop in North Dakota. The results on resistance against cabbage seedpod weevil (*Ceutorhynchus obstrictus*) are contradictory. The results of Kalischuk and Dosdall (2004), who estimated a high resistance in *C. abyssinica,* were not confirmed by Cárcamo et al. (2007), who compared a few species of Brassicaceae including *C. abyssinica*, *C. glabrata*, and *C. hispanica*. Due to the lack of synchrony between pod development of the plants and reproduction of the weevil, definite results were not obtained.

5.3.2 Traditional Breeding Efforts

C. abyssinica is the only species with applicability in agricultural use. There are three reasons, which once made and still make *Crambe* a unique oil crop:

- Its oil contains the highest amount of erucic acid among Crucifer oil crops, which is of great interest for industrial use
- *C. abyssinica* and C. *hispanica* display a high-yielding ability, comparable to rapeseed, but need lower cultivation efforts
- This species does not naturally hybridize with other Brassica oil crops, especially rapeseed (Wang and Luo 1998), which allows to grow the crop in close neighborhood to them (Carlsson et al. 2007)

The first attempts of breeding *C. abyssinica* were reported from the USSR around 1932 with the aim to use the low demands of the species to climate and soil conditions for oil crop breeding (Zimmermann 1963; Mastebroek et al. 1994; Carlsson et al. 2007). These attempts were continued in Germany and Poland in the 1950s and 1960s (Zimmermann 1963). In the middle of the last century, there was a growing interest in domestic vegetable oils as a renewable source of industrially useful products. One important compound of vegetable oil was the erucic acid ($C_{22:1}$). This very long chained (VLCFA) and monounsaturated fatty acid (MUFA) is a high valuable industrial feedstock for the manufacturing of plastics, lubricants, coatings, and surfactants. The most important oil crop of the northern latitude was rapeseed (*B. napus* and *B. rapa*) with oils containing about 50% erucic acid. Ten years later, plant breeding created rapeseed with only traces of erucic acid in the seed oil for food application (Downey 1971). But rapeseed was not a crop in the US. In search for candidate crops for industrial use, USDA evaluated numerous cruciferous and other species regarding their oil content and fatty acid composition. Thus, the cruciferous species *C. abyssinica* became of growing interest because of its high content of erucic acid in the seed oil (Earle et al. 1959; Miller et al. 1965).

Since the 1960s, there were a couple of studies about the utilization of high erucic *Crambe* (*C. abyssinica*) seed oil as an industrial feedstock (Bruun and Matchett 1963; Miwa and Wolff 1963; Mustakas et al. 1965; Nieschlag and Wolff 1971; Princen and Rothfus 1984; Lazzeri et al. 1994). The first studies concerning agronomy and breeding were initiated by Lessman at the Purdue State University involving 11 introduced lines of *C. abyssinica* and resulted in the release of three cultivars "Prophet," "Indy," and "Meyer" (Carlson et al. 1996). Very soon, Lessman noted that the variability regarding agronomic characters as well as oil content was too low to achieve further breeding success (Lessman and Meier 1972; Meier and Lessman 1973b). Continued breeding efforts including the introgression of wild accessions into cv. "Indy" resulted in the release of additional cultivars "BelAnn" and "BelEnzian" and breeding lines "C-22," "C-29," and "C-37" (Campbell et al. 1986a, b). In 1991, HEADE (High Erucic Acid Development Effort) was initiated in North Dakota. It was a cooperation of USDA institutions and several universities throughout the US with the aim to force the commercialization of *Crambe* oil. The evaluation of breeding lines in North Dakota indicated that many of them outperform the adapted cultivars, but no one possessed all the desired traits (Knights 2002).

Vegetable oils as industrial feedstock became of interest also in Europe (Röbbelen 1984). In the funding programs for renewable resources and sustainable agriculture, respectively, which were initiated by the European Union (EU) in the 1980s and 1990s, *C. abyssinica* was one of the new crops with particular consideration (Mangan 1995). In two specific *Crambe* programs from 1995 to 1998 and 1999 to 2002, respectively, agronomic and breeding prospects of *C. abyssinica* were studied under the conditions of different sites in Europe (for details see AIR 1998; FAIR 2003).

Breeding in Europe resumed in the beginning of the 1990s at the Plant Research International (PRI), Netherlands. The breeding target was aimed at improving seed and oil yield, enhancing the erucic acid content in the oil, improving the disease resistance and decreasing the glucosinolates content in the seed (Mastebroek et al. 1994; Mastebroek and Lange 1997). The results clearly demonstrated that *C. hispanica* had more variation than *C. abyssinica* for most of the traits measured. This variability may be valuable for *Crambe* breeding programs since *C. abyssinica* and *C. hispanica* can be successfully crossed (Mulder and Mastebroek 1996). Genotypic and phenotypic variations in *Crambe* are rather limited; thus only incremental progress has been made in cultivar improvement. Large numbers of selections are being evaluated followed by hybridization of superior lines. These crosses should expand the genetic diversity of *C. abyssinica* and allow improvements in yield, oil quality, and agronomic traits such as maturity, height, and seed retention. Also, major efforts will be directed to identify low glucosinolate materials, which, if found and incorporated into superior cultivars, would enhance meal quality. The variable ploidy levels of the related species could prove useful for creating a wide range of variability for long-term crop improvement. An interesting option is *C. kralikii* with its three ploidy levels (White and Solt 1978; see also Table 5.2). Perhaps greater cold and drought tolerance could be transferred into *C. abyssinica* from *C. kralikii*. Since only one accession of *C. kralikii* has a gametic number of 45, additional germplasm from Algeria should be collected and tested for ploidy level.

Another potentially interesting objective would be to try to genetically fix two- or three-seeded fruits, which might make combine hulling feasible. These multi-seeded fruits occur naturally in very low percentages in seedlots (White et al. 1994). Another possibility could be the introduction of a respective trait from the *C. cordifolia* accession described by Duhoon and Koppar (1998, see also Sect. 5.1.2), but there are differences in chromosome number (see Table 5.2).

Mastebroek et al. (1994) evaluated 42 accessions of crambe agronomic characteristics in 1990 and 1992. Large variation was found for earliness, length of the top branch, thousand seed weight, and fatty acid composition. These characteristics showed a high correlation between the years tested. Earliness had a large impact on crop development and seed quality. Seed yield and oil content were considerably affected by environmental conditions. Little variation was found for the content of erucic acid and glucosinolates in the seeds. Three accessions, two early flowering American breeding lines and a late flowering European land race, were used in a crossing program (Mastebroek and Lange 1997). Five generations were raised. From the F_3 generation onward, selection was performed for agronomical characteristics. Estimation of broad-sense heritability values on the basis of variation between F_3 lines revealed high values for thousand seed weight, moderate for plant height, earliness and seed oil content and low for seed yield and number of seeds produced per square meter. In comparison to the reference populations, only limited progress in seed yield was obtained by selection. Improvement of seed yield performance was also observed in the reference populations after growth of the subsequent generations. For the Dutch climatic conditions, earliness appeared desirable and selection for earliness in crossing populations might be an effective tool for indirect selection for higher yields of seed oil. Three selection cycles resulted in improved breeding lines, which exceeded the standard variety "BelAnn" in seed oil content and seed oil yield.

Although *C. abyssinica* has potential as an oilseed crop, it lacks genetic variation for important agronomic traits, including hardiness, maturity time, and pest resistance (Papathanasiou et al. 1966; Lessman and Meier 1972; Leppik and White 1974; Mastebroek et al. 1994). Improvements in the genetic variability of *C. abyssinica* may be possible by mutagens. Chu et al. (2001) treated crambe seeds with different concentrations and doses of ethylmethane sulfonate (EMS) or ^{60}Co, and found that low level of them could stimulate the respiration and raise the activities of ascorbic acid oxidase and peroxidase, which were associated with higher rate of seed germination and growth of seedling. High level of them had inhibiting effects on respiration and the activities of related enzymes, the germination potential of seed and the rate of germination, and the vigor of root system decreased.

5.3.3 Gene Introgression Due To Distant Hybridization

5.3.3.1 Intrageneric Crosses

As mentioned in Sect. 5.3.2, the genetic variability for desired traits is limited in *C. abyssinica*. But as is indicated in Sect. 5.3.1, there seems to be variability in desired traits in several other *Crambe* species. Therefore, intrageneric crosses should be a suited means for increasing genetic variability and selection of novel trait combinations. Whereas crosses between *C. abyssinica* and *C. hispanica* have already been made, there seems to be a lack of knowledge on intrageneric hybridization in *Crambe*. Also, a detailed evaluation of *Crambe* species other than *C. hispanica* is necessary for targeted intrageneric crosses for the transfer of desired traits from one *Crambe* species to the other.

5.3.3.2 Intergeneric Crosses

Crambe species may also serve as a useful source of genes for *Brassica* crop improvement, particularly for flea beetle resistance (Anderson et al. 1992) and tolerance to heat stress (Johnson et al. 1995). Artificial crosses between *C. abyssinica* and *Brassica* via use of embryo rescue (Wang and Luo 1998; Tang et al. 2006) could facilitate the introduction of such traits into *Brassica*. In crosses of *B. napus* × *C. abyssinica* and *B. rapa* × *C. abyssinica*, pre-fertilization incompatibility was observed. In crosses of *B. juncea* × *C. abyssinica*, pollen grains of *C. abyssinica* were compatible to the stigmas of the *B. juncea* pistils, the pollen tube growth was normal, and the pollen tube reached the micropyle in 3–4 days after pollination. Through comparative studies on embryo development

in generic crosses of *B. juncea* × *C. abyssinica* and self-cross of *B. juncea*, the causes of the hybrid embryo abortion were found. For the earlier aborted embryo sac, the hybrid embryos were in starvation state from 2-cell stage, with disintegration of nucellar parenchyma and degeneration of endothelium. For late-aborted embryo sac, the development of hybrid embryo was lagged in contrast with that of self-cross. During 32–64 cell or multicellular globular embryo stage, the development of embryo proper was slowed down and cellular endosperm was formed 4 days earlier than that of self-cross. An intergeneric hybrid between *B. juncea* × *C. abyssinica* was obtained through conventional crossing method combined with ovary culture when *C. abyssinca* was used as a paternal parent (Wang and Luo 1998). Tang et al. (2006) obtained intergeneric hybrid between *B. chinensis* and *C. abyssinica* via sexual cross and embryo rescue.

5.3.3.3 Somatic Hybridization

The regeneration system of *C. abyssinica* from single cell culture was developed by Gao et al. (1998). PEG-induced asymmetric somatic hybridization between *B. napus* and *C. abyssinica* was carried out by Wang et al. (2003). Prior to fusion, the protoplasts of *C. abyssinica* were exposed to different doses of UV, ranging from 0.05 to 0.30 J/cm^2. UV-irradiated mesophyll protoplasts of *C. abyssinica* cv. "Carmen" and cv. "Galactica" were fused with hypocotyl protoplasts of different genotypes of *B. napus* cv. "Maplus" and breeding line "11502." Twenty asymmetric somatic hybrids were obtained and verified by nuclear DNA content and AFLP analysis. Cytological analysis of these hybrids showed that 9 out of 20 asymmetric hybrids had 38 chromosomes, and the others contained 40–78 chromosomes, having additional chromosomes between 2 and 40 beyond the 38 expected for *B. napus*. Sexual progenies of asymmetric somatic hybrids between *B. napus* and *C. abyssinica* were analyzed with respect to the chromosomal behavior, *fae1*-gene introgression, fertility, and fatty-acid composition of the seed (Wang et al. 2004). Among 24 progeny plants investigated, eleven plants had 38 chromosomes and were characterized by the occurrence of normal meiosis with 19 bivalents. Thirteen plants had more than 38 chromosomes, constituting a complete chromosomal set from *B. napus* plus different numbers of additional chromosomes from *C. abyssinica*. Analysis of cleaved amplified polymorphic sequence (CAPS) markers derived from the *fae1* gene showed novel patterns different from the *B. napus* recipient in some hybrid offspring. Most of the progeny plants had a high pollen fertility and seed set, and some contained significantly greater amounts of seed erucic acid than the *B. napus* parent.

5.3.4 Isolation and Use of Genes

Till now, more than 200 nucleotide sequences cloned from the genus *Crambe* are available in the website (*http://www.ncbi.nlm.nih.gov*): *C. abyssinica* (30), *C. hispanica* (26), *C. hispanica* subsp. *abyssinica* (13), *C. strigosa* (12), *C. scaberrima* (10), *C. microcarpa* (8), *C. filiformis* (7), *C. hispanica* subsp. *hispanica* (6), *C. fruticosa* (6), *C. pritzelii* (6), *C. feuillei* (6), *C. kralikii* (5), *C. maritima* (5), *C. gomerae* (4), *C. arborea* (4), *C. cordifolia* (3), *C. kotschyana* (3), etc. Liang et al. (1998) isolated and characterized gene promoter from *C. abyssinica*. The existence of a large number of novel and highly variable thionin variants in *C. abyssinica* has been deduced from cDNA sequences that were amplified by the PCR from RNA of seeds, leaves, and cotyledons (Schrader-Fischer and Apel 1994). Wang et al. (2004) demonstrated the successful transfer of new allelic variants of the *FAE1* gene controlling erucic acid biosynthesis from the distantly related, triploid oilseed plant *C. abyssinica* ($n = 3x = 45$) into *B. napus* by somatic hybridization. Overexpression of the *C. abyssinica FAE1* gene in *B. carinata* resulted in a substantial increase in the proportion of erucic acid in seeds compared to the wild type control (Mietkiewska et al. 2007). The increased pool of oleic acid in seeds of the transgenic plants was subsequently elongated with assistance from the heterologously expressed *C. abyssinica FAE1*, which resulted in a net increase of the proportion of erucic acid in T_1 segregating seeds of up to 16% (Mietkiewska et al. 2008). PCR primers were designed according to the cDNA sequence of *S*-glucosyltransferase (*S-GT*) gene in *B. napus*, and full length of the *S-GT* genes in *C. abyssinica* was obtained by using the genomic DNA as PCR templates. Sequence alignment revealed that *CaSGT* gene had an intron of

74 bp and coded a protein of 465 amino acids and shared a similarity of 93.4% at the DNA sequence level and a similarity of 95.06% at the amino acid level with the *B. napus* (Liu and Qiao 2008).

5.3.5 Transformation

Recently, attempts have been made in cooperation between European Commission (EC) and United States Department of Agriculture (USDA) to evaluate the possibilities of genetic transformation of *C. abyssinica* to get special seed oil composition suited for industrial utilization, especially wax ester production. Crucial motivations to choose this crop were the sexual incompatibility with other Brassica oil crops (oilseed rape) as an oil crop, and that the crop has never been a food (Carlsson 2006; Carlsson et al. 2007).

A transformation protocol is critical in the process of providing *Crambe* with the necessary genes for novel industrial oil qualities. An *Agrobacterium*-based protocol for *Crambe* transformation was established (Karin Sonntag personal communication). Cotyledons from 1 to 2 mm petioles were cut from 6-day seedlings after germination and placed for 20 min in *A. tumefaciens* suspension containing MS medium with 20 g/l sucrose, 5 mg/l BAP (benzylaminopurine), 0.5 mg/l NAA (naphthaleneacetic acid), and 2 mg/l $AgNO_3$. Then the explants were transferred to cocultivation medium containing the same elements as the infection medium but modified by the addition of 10 mg/l acetosyringone and 8 g/l phytagar. After cocultivation, explants were placed on shoot induction medium. The experimental results revealed a great difference between three genotypes ("BelAnn," "Carmen," and "Galactica") regarding their regeneration response after the infection with *A. tumefaciens*. The highest transformation frequency was 1.7%. After transfer to the glasshouse, the plants grew normally and developed seeds.

5.4 Conclusions

The genus *Crambe* is one of important germplasms for vegetable production and industrial uses of its oil. It is a low-input oil crop compared to many others. In order to develop crambe into a nonfood oil crop platform for production of special industrial oils, the following programs should be focused: (1) Breeding for high seed yield by mutagenesis or assisted molecular markers, (2) Developing transformation protocol to produce genetically modified (GM) plants for designing novel characters, (3) Breeding for increased disease resistance, (4) Breeding for winter varieties, etc.

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Chapter 6
Cuphea

Jan Olejniczak

6.1 Introduction

Cuphea is a New World genus and the largest of the 32 genera of Lythraceae (Graham et al. 1981), with about 600 species of herbaceous perennials and small shrubs. Many of these species have seed oil that is rich in medium-chain fatty acids (MCFAs), as well as lauric (C-12:0) and myristic acid (C-14:0). These fatty acids are important to the chemical industry for the produce of detergents, surfactant, lubricants and other products. *Cuphea* also has the potential to replace coconut (*Cocos nucifera* L.) and palm kernel (*Elaeis guinaaesis* Jacq.) important in the world.

Modern studies on systematics in *Cuphea* plants rely upon many sorts of comparative data, such as morphological, cytological, biochemical and molecular relationships. Recently, attention has been focused on the domestication of the species within the genus *Cuphea*. *Cuphea* generally exhibits characteristics that are typical of undomesticated plants, such as indeterminate pattern of flowering and growth, seed shattering from ripening fruit, seed dormancy, viscid and glandular hairs on stems, leaves and flowers. These traits are major constraints to agricultural use. Several breeding methods (e.g., mutation, interspecific crossing), *culture* in vitro, and modern biotechnology have been attempting to remove these limitations to domestication and production of *Cuphea*. Recently, molecular markers have entered the scene of genetic improvement of alternative oil crops, including *Cuphea*. Among the major traits targeted for domestication in oil breeding programs are dormancy, shattering, indeterminate growing and flowering.

For new industrial crops, which will be commercialized, many technical, ecological and economic constraints must be overcome. However, little is known about the best agricultural management practices for their production. Several agronomical studies have been conducted to determine the adapted areas: seeding rate, sowing date, row spacing, weed control, water requirements, and harvest procedure. Production and research of *Cuphea* will also require a growth staining system for proper crop management regarding timing for herbicide control, insecticide and fungicide treatment of applications.

Development of a crop such as *Cuphea*, which can be integrated into sustainable agricultural production systems, supports the achievements of national rural development and environmental quality objectives. Gene flow between *Cuphea* and their wild relative *Lythrum* is an important process that has strong implications for both conservation of genetic diversity and for plant breeding.

Understanding how to manage and use the new oil crops, including *Cuphea,* is a complex task, and it is important to minimize the economic and environmental risks, and to maximize the benefits to farmers and consumers.

6.2 Systematic Botany

The Lythraceae are a moderate-sized family (*Myrtales*), a family of 32 primarily subtropical and tropical genera. Only *Lythrum* is of warm to cool temperature in range. The species of *Cuphea* in the Antilles have extensive ranges and distributions elsewhere in the

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New World tropics and subtropics. Numerous collections are known from Australia and the islands in the Pacific. The genus of *Lythrum* extensive range extensively in the New World, Eurasia, and in areas of Europe. The biogeographic patterns of the Lythraceae have been established following multiple changes, and long-distance dispersal events from multiple directions (Graham et al. 1993).

6.2.1 Morphological Phylogenetic Analysis

Cuphea is a New World genus and the largest of 32 genera of Lythraceae (Graham et al. 1993), with about 600 species of herbaceous perennials, and small shrubs. As traditionally circumscribed (Koehne 1903), the family comprises 28 genera and is easily recognized by a suite of characters: opposite entire leaves; a persistent perigynous campanulate to tubular floral tube with crinkled petals inserted at the rim; two whorls of stamens inserted in the tube; and a many seeded capsular fruit. The genera are clearly delimited and are regarded as monophyletic. The genus is recognized by the ribbed floral tube, which terminates in six dilated calyx lobes, and especially by a unique seed dispersal mechanism. *Cuphea* seeds are exposed for dispersion of a placenta that becomes exerted through matching longitudinal slits in the adaxial (dorsal wall of the capsule and floral tube). The current taxonomic classification of the Lythraceae (Koehne 1903) divided the narrowly circumscribed family into two tribes, each with two subtribes (Table 6.1). The classification is highly ambiguous, with the tribes and subtribes delimited by few non-exclusive characters. Further, Tobe et al. (1998) found the defining features of the tribes, the presence or absence of complete septal wall in the ovary, to be erroneous. Anatomical section indicated incomplete septal wall at the apex of the placenta in all genera, negating the sole basis for the primary division of the family. A morphological cladistic analysis of the Lythraceae did not recover clades equivalent to the tribal or subtribal groupings, nor did it produce well-supported alternative relationships (Graham et al. 1993).

The evolution of several characters of taxonomic importance in the Lythraceae has been exceptionally labile and complex. The presence of an inferior to semi-inferior ovary (the floral tubes epigynous or hemi-epigynous) was influential in recognition of

Table 6.1 Classification of the Lythraceae based on Koehne (1903) excluding unnamed series (Graham 1989)

Classification and genera	Defining characters
Subfamily Sonneratioideae:	
Sonneratia	
Duabanga	
Subfamily Punicoideae:	
Punica	
Subfamily Lythroideae (*Lythracea sensu stricte*):	Septum of the ovary interrupted or split above the placenta: the placenta is not with the style
Tribe Lythreae:	Seed without a marginal or, margined, the flowers always zygomorphic
Subtribe Lythrineae:	Seed dorsally compressed and by a wing: placenta of the mature fruit strongly depressed basal: flowers always actinomorphic
Rotala, Ammanniia (*Hionanthera*), *Peplis, Didiplis*	Septrum of the ovary complete, the placenta thus continuous with the style; flower always actinomorphic
Lythrum, Woodfordia, Cuphea, Pleurophora	Seed coat neither thickened nor winger
Subtribe Diplusodontinae:	Seed coat either extended as a wing or the apex truly spongy
Galpinia, Pemphis,(*capuronia*), *Diplusodon*	
Prysocalymma, Lafoensia	
Tribe Nesaeeae:	
Subtribe Nesaeinae:	
Crencea, Nesaea, Heimia, Decodon, Pebria, Adenaria, (*Koelnteria*), (*Laurtella*), *Tetrataxis, Ginoria,* (*Haitia*)	
Subtribe Lagerstroemiinae:	
Lagersoemia, Lawsonia	

Genera in parentheses were described after 1903

subfamilies of the Lythraceae. Cytological analyses indicate that the superior ovary (perigynous floral tube) is plesiomorphotic for the Lythraceae, having evolved on the branch leading to the family from an ancestor with an inferior ovary in an epigynous flower. Most genera of the Lythraceae have a superior ovary, with the staminal filaments emerging near the base of the ovary (Graham and Cavalcanti 2001).

Pollen of Lythraceae is the most diverse of any family of the Myrtales. Patel et al. (1984) proposed a harmomegtic function for pseudocopoli suggestion, that evolution of pseudocopoli was radiation of lineages into wetter or arid habitat. However, in the Lythraceae, there is no distinction in genera habitat between genera with pseudocolpate versus non-pseudocolpate pollen. For systematics of *Cuphea*, pollen grains, seed coat and leaves have been investigated intensively so far and the first were described by Koehne (1881). In leaves, cuticular waxes and trichomes have been studied most frequently. Genus *Cuphea* exhibits a wide variety of floral morphologies, pollinations, and seed coats in approximately one of all the genera of the Lythraceae. Lythraceae are unique among all angiosperms in having inverted exotestal trichomes, formed by protrusion of a membranous into the cell lumens of an outer most seed coat cell layer (Graham 1995).

The genus *Cuphea* have been grown for a long time, but even 40 years later new species continue to be discovered (Cavalcanti and Graham 2005). As the genus has become better known through sectional revisions, the classification has become more difficult to apply, and it is increasingly apparent that relationships among the taxa implied by the classification are unsupportable. Pollen diversity, in section *Melvilla* for example, strongly suggests that this large section is a collection of species based on convergent floral morphology related to pollinator specialization. Species that share similar elongate, thick-bodied, intensely-colored floral tubes, a floral syndrome under selection to attract bird and large bee pollinators, fall into at least four very different pollen categories, each known from characteristics of other sections. Other relationships implied by the taxonomy are likely to be inaccurate, due to convergent floral morphology related to changes in the breeding system from outcrossing to self-fertilization. Section *Brachyandra* comprises species having pale green, very small flowers (3–8 mm long), with deeply included anthers and stigma characters, suggestive of self-fertilization. Species of the section have been confirmed as facultatively self-fertilizing, and a morphological phylogenetic analysis indicated that at least four distinct lineage groups constitute this section. Each lineage was defined by a unique combination of pollen and seed morphology, but shared the same homoplastic floral features (Graham et al. 1993). The questions of species relationships raised by morphological data, the degree to which the classifications reflect natural lineages, and hypotheses about the historical biogeography of the genus have yet to be examined using a date source other than morphology (Table 6.2).

Modern studies in plant systematics rely upon many kinds of comparative data such as cytology, cytogenetics, metabolities and molecular studies, which are important source of information to depict evolution.

Table 6.2 Current and distribution of the genus *Cuphea* (Graham 1989)

Subgenus *Cuphea* (*LythroCuphea*)
Sect. *ArchoCuphea* – 3 ssp., NE Brazil, 1 widespread
Sect. *Cuphea* (*EnantiocCuphea*) – 15 spp., Central America to Argentina, Antilles
Subgenus *Bracteolotae* (*EuCuphea*)
Sect. *Heteranthus* – 10 ssp., NW South America to South Mexico
Sect. *Melicyathium* – 1 ssp., SE Brazil
Sect. *Brachyandra* – 22 ssp., South America and widespread weeds
Sect. *Euandra* – 74 ssp., primarily Brazil to Argentina
Sect. *Amazonia* – 17ssp., Guianan and Amazonian region
Sect. –*Trispermum* – 16 ssp., N. South America, including Amazon
Sect. *Pseudocircaea* – 5 ssp., Brazil to Argentina and Bolivia
Sect. *Heteredon* – 28 ssp., W. Mexico to Costa Rica
Sect. *Melvilla* – 39 ssp., W. Mexico to South America
Sect. *Leptocalucx* – 7 ssp., Mexico to Panama
Sect. *Diploptychia* – 20 ssp., Mexico to Nicaragua
Sect. *OrnithoCuphea* – 3ssp., W. Mexico

6.2.2 *Chromosomal* Phylogenetics *Studies*

Chromosome number is a well known source of information to clarify relationships at several taxonomic levels in plants. Chromosome numbers are generally stable, however, variations are witnessed during the evolutionary processes leading to speciation.

Chromosomes of *Cuphea* are generally small, 1–3 μm in length, metacentric or submetacentric, and the meiotic metaphase I chromosomes are often globose. Meiosis is regular in diploids, but frequently irregular in polyploids. Clumping at metaphase I, presence of dark-staining and similarity in size of the chromosomes in some species make their counting difficult. In the family Lythraceae, a moderate sized patropical family of genera and about 600 species, chromosome number data have led to the determination of an original basic number of the family (Graham and Cavalcanti 2001). The basic chromosome number is an important factor to be accounted for in hypothesizing regarding phylogenetic relationship and evolutionary pathway among the genera, and also in considering the families of the order of *Myrtales*. Among the 78 species for which number is known, chromosome number ranged from 6 to ca. 56 (Graham and Cavalcanti 2001). The basic number for *Cuphea* is considered to be $x = 8$. Tobe et al. (1998) have suggested two base numbers of *Cuphea* ranging from 4 to 6, and another yet older basic number of 5. If this were true, species with $x = 8$ would actually be ancient tetraploid. In this case, the ancestral diploid state would appear to have become extinct because no species are known to have $x = 4$, and only two species, the annual *C. lanceolata* and *C. viscosissima* of eastern Mexico and eastern United States, have $x = 6$. Chromosome number has been surveyed across the genus *Cuphea*. Supernumerary chromosomes are present in several species of *Cuphea*, which also have 22 species with more than basic ploidy level. These genus appear to be in the middle of rapid evolutionary radiation, employing more than one mechanism of change. Specification in the genus *Cuphea,* based on clues from chromosome number including autopolyploidy, hybridization and alloploidy, disploidy, and caryological and cytogenetic analyses, is needed to be verified for further interpretations. Domestication of *Cuphea* may depend on combining of genetic material from different species through introgressive hybridization, and for this purpose, chromosome number data are a starting point for planning interspecific hybrid programs.

The herbaceous genera *Cuphea* and *Lythrum* are chromosomally the most diverse genera in the family Lythraceae. The pattern of distribution of diploid versus polyploid numbers in *Lythrum* indicated the generic center of origin to be Circum-Mediterranean: all species with $x = 5$ occur there. Knowledge of the origin of this genus and distribution of chromosome numbers among the species does not clarify the evolutionary history of heterostylous breeding system in *Lythrum*. Both homostylous and tristylous species exhibit the basic chromosome number of this genus. Among the 19 species, which are known chromosomally, seven are homostylous with haploid numbers of 5, 10, 15, and 20. From Europe and the Mediterranean, two *Lythrum* species have different numbers as well as *Lythrium virgatum* ($n = 15$) and *Lythrium salicaria* ($n = 15, 25, 30$), which are widespread in Europe and taxonomically not easy to be separated (Graham and Cavalcanti 2001). Further understanding of chromosomal change and evolution in the family Lythraceae, will depend on phylogenetic relationship, as well as cytological investigations, as different chromosome numbers occur. The meiotic analyses are valuable for the study of domestication of the genus and for clarification of the evolutionary relationships among taxa. The data on meiotic pairing included those on hybrids between different species, including triploid, tetraploid and other configurations of chromosomes (Gathman and Ray 1987). Meiotic analysis of two *Cuphea* hybrids showed different pairing of chromosomes: univalents (I), bivalents (II), trivalents (III) and tetravalents (IV) (Figs. 6.1 and 6.2) (Olejniczak 1996).

6.2.3 *Biochemical Phylogenetics Studies*

Plant secondary metabolites have been widely used as taxonomic characters for comparisons in different genera of plants. Flavonoids have been more frequently used for comparisons at lower hierarchic levels such as genera and species. In spite of the widespread use of secondary metabolites in taxonomy, they have never achieved the same status as characters

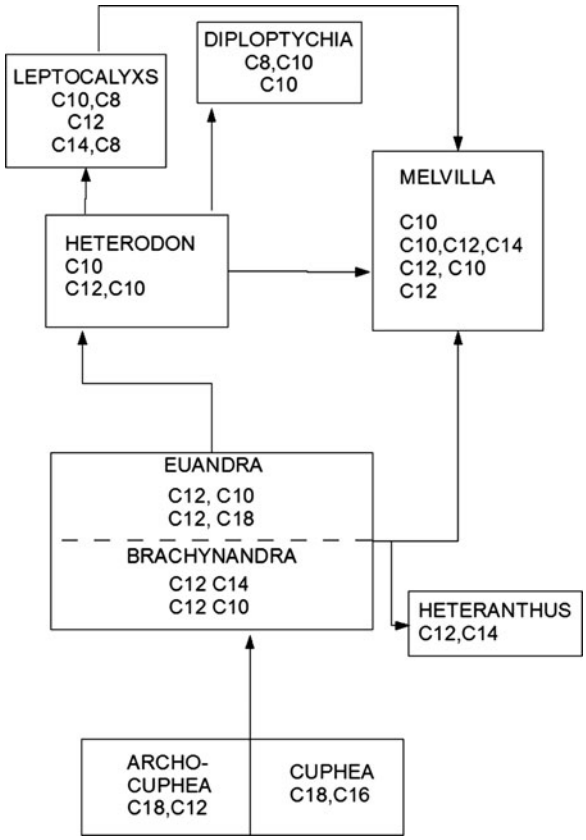


Fig. 6.1 Taxonomic relationships in the genus *Cuphea* and the mains fatty acids. Characteristics of fatty acid for each section is in large type: Boxes are proportional to the size of the section (Graham et al. 1981)

for the establishment of phyletic relationships among plant taxa as macromolecules, such as proteins, fatty acids, sequence of nucleic acid, which have recently received much more attention in molecular systematics (Hillis et al. 1996). Relatively little is known about flavonoids' distribution in the family *Lythraceae*. Graham et al. (1980) reported the flavonoids of *Ammania coccinea*; Blatt et al. (1994) on the flavonoids of 27 species of *Diplusodon*; Santos et al. (1995) on 16 species of *Cuphea* and Santos et al. (2000) on three species of *Lafoensia*. Assuming *Cuphea* as more advanced than *Diplusodon*, the following attributes that could be regarded as evolutionary advances of the flavonoid chemistry of the Lythraceae include (a) absence of glucuronic acid; (b) rare occurrence of glucuronic acid; (c) presence of methoxylated flavonoids, such as rhamnetin and isorhamnetin, and (d) more frequent occurrence of galactose glycosides.

Fig. 6.2 Initial form *C. tolucana*, line 629 (C-5) and dwarf mutant (Olejniczak 1996)

However, the present knowledge of the chemistry of the Lythraceae is too sketchy to accept these assumptions as definite proof of either primitive or chemical advance in the family, because the differences observed between two genera might not be reproduced in other taxa with a similar distance of relative advancement. An intriguing aspect emerges, if one attempts to include the flavonoid pattern of *Lafoensia* into the cladogram, of bearing in mind the flavonoid of *Cuphea* and *Diplusodon* already commented upon. The flavonoid patterns of *Lafoensia* and *Cuphea* are substantially different, and up to the present, myricetin, *O*-methylated flavonols, and flavonoids with glucuronic acid are constituent flavonoids of *Diplusodon* and *Cuphea*. It becomes difficult tounderstand that such a simple flavonoid profile as that of *Lafoensia* in the simply topology *Lafoensia* should have some characteristics common to the other two genera. The flavonoid chemistry suggests that *Lafoensia* belongs to an evolutionary line different from *Cuphea*, or it is a more advanced group. However, interpretation from this flavonoid profiles is not compatible with the distribution of fatty acids in *Lafoensia*.

Many species within the genus *Cuphea* (Lythraceae) produce seed with high levels of MCFAs. Composition of

Fig. 6.3 Initial form, *C. tolucana*, line 629 (C-5) and compact mutant (9M) (Olejniczak 1996)

these fatty acids depends on taxonomic section (Fig. 6.3). The *Cuphea* pattern of seed fatty acids is regarded as a derived condition (apomorphy). Similar profiles have not been found in other genera of *Lythraceae* (Graham and Kleimann 1987). For the first attempt to construct a phylogenetic framework for *Cuphea*, data from nuclear internal transcribed sequence (ITS) sequence for 54 species and four outgroup taxa were analyzed by Graham et al. (2006). Independent results employing morphological and molecular data sets confirmed *Cuphea* as monophyletic with *Pleurophora* as sister. The ITS parsimony and maximum likelihood phylogenies indicated South America as the initial center of diversification, and identified a deep trichotomy, one branch of which was equivalent to subgenus *Cuphea*. The ITS analyses also recognized seven well supported clades, each composed of members from two to four taxonomic sections. The ITS analyses provided the initial phylogenetic hypotheses for the genus that clarify relationships previously obscured by the highly homoplastic nature of the morphological taxonomic characters.

Molecular and morphological data have significantly widened the understanding of evolution and development of the family Lythraceae. The crown clades recovered by using parsimony and likelihood approaches on sequences from chloroplast and nuclear genomes, have substantially improved the general hypotheses.

6.3 Domestication

Domestication is a selection process conducted by humans to adapt plants to the needs of humans whether as farmers or consumers. *Cuphea* domestication traits include increased seed or fruit size, more determinate growth and flowering; suppression of natural dispersal through shattering of seed; dormancy of seed; and self-incompatibility. These traits are major constrains to agricultural use. Several breeding methods (mutation, interspecific crossing), in vitro culture and biotechnology have been attempting to remove these limitations to domestication and production of *Cuphea*. Olejniczak (1996) selected different mutants and hybrids with change of MCFA spectrum (Table 6.3).

Tissue culture techniques can also be used to micropropagate, as well as produce, high-yielding, somaclonal variants with extended genetic variability in *Cuphea* (Przybecki et al. 2001a, b). In vitro culture could also be adapted for both genetic transformation, and somatic hybridization studies to establish a basis for the hybridization of *Cuphea*, aimed at a long-term program for creating material of experimental and commercial interest.

The total system of new crops, as well as *Cuphea* research and development, is composed of four major components of activities. The steps proceed in an essentially sequential fashion, but they are not discrete, and varying amounts of overlap may occur among them (Fig. 6.4). The first phases involve three major activities of the germplasm system – collection, evaluation, and enhancement and development. These three major activities involve the utilization of germplasm in cultivar development, which is intimately involved and development with agronomical evolution and the development of appreciate cultural and management systems. The last, but not least, involves an array of activities associated with full-scale commercialization. Types of activities and input of the various scientific disciplines and industrial components are indicated within each of the four areas of the total system, so that they are adaptable to agriculture use.

Table 6.3 Composition of medium chain fatty acids (MCFAs) in mutant M_5 and hybrids F_5 generation in seeds (Olejniczak 1996)

Genotypes		C10:0 (%)	C12:0 (%)	C14:0 (%)
C. tolucana	Line 629	25.4	60.1	3.9
Mutants	9	23.6	61.4	4.1
	19	25.0	62.9	3.7
	20	21.9	60.5	4.6
	21	26.1	63.9	4.1
	27	21.5	71.1	4.5
	41	15.5	71.1	4.5
	42	17.2	68.1	4.6
	43	21.3	64.4	3.9
	44	18.7	66.7	4.9
	45	15.6	70.5	4.8
	46	23.6	61.9	4.2
	47	22.7	62.5	4.0
	48	22.0	63.0	4.4
	49	24.8	61.9	3.9
	50	19.4	66.8	4.8
	51	18.0	67.5	4.9
	52	22.7	63.0	4.2
X ± s		21.5 ± 3.2	64.7 ± 3.2	4.3 ± 0.4
C. wrigthii	Line 651	30	56.4	3
Hybrid line 629 x	23	24.6	56.4	3.9
line 651	24	19.3	63.8	4.2
	25	24.9	58.6	4.4
	26	25.2	58	4.2
	28	26.2	58.5	4.3
	29	28	58.3	4.1
	32	29.9	57.9	2.5
	33	21	64.5	4.2
	35	24.7	59.8	4.3
	36	23.9	60.5	4
	37	24.3	60.6	3.9
X ± s		24.7 ± 2.0	60.0 ± 2.3	3.7 ± 0.3

6.3.1 Shattering

Seed shattering and dormancy usually determine the difference between undomesticated and domesticated forms and species. The wild progenitors of several crops, including wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), oats (*Avena sativa* L.), and barley (*Hordeum vulgare* L.) are characterized by seed shattering (Ladizinsky 1985). Non-shattering phenotypes are simply inherited in these species, and were presumably fortuitously discovered, selected, and fixed in populations sometime within the last 10,000 years. Seed shattering system is a taxonomic trait of all existing *Cuphea* species. Wild *Cuphea* species disperse seeds through a dorsal abscission layer along the corona tube. The fully exposed seeds mature and dehisce after the placenta separates from the corolla tube. Following Vavilov's idea of parallel variation of shattering, it is very difficult to select full non-shattering traits, although after crossing among different species, Knapp (1993) selected forms with partially improved shattering.

6.3.2 Dormancy

Seed dormancy could be considered simply as a block to the completion of germination of an intact viable seed under favorable conditions, but it is one of the least understood phenomena in the field of seed

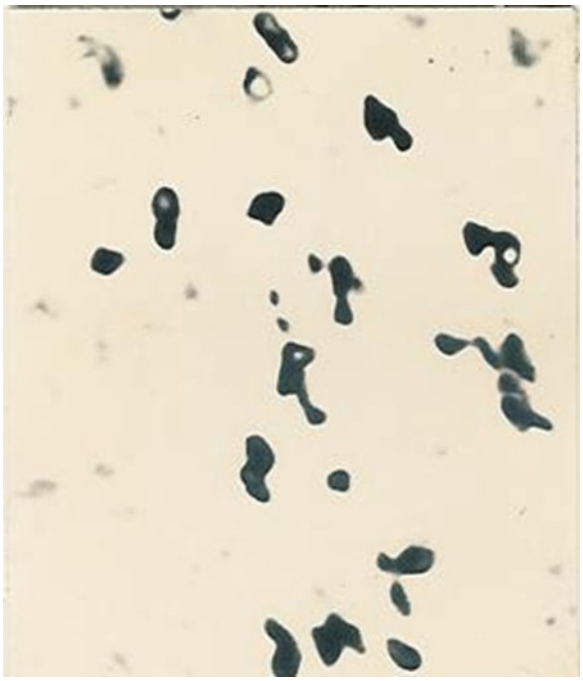


Fig. 6.4 Metaphase I meiosis of hybrids F5 *(C. tolucana 629 x C. wrighii 651:I-6, II-8, III-2, IV-1)* (Olejniczak 1996)

biology. Seed dormancy is known to be a trait strongly influenced by environmental factors, and determined by seed coat embryo and endosperm factors. Olejniczak (1996) showed (Table 6.4) that mature seeds of *Cuphea* (i.e., with brown and black seed coat) germinate successfully after a few months' of storage. However, it is still unclear, how seed dormancy is associated with coat color (Torada and Amano 2002). Dormancy decreases after ripening time but the rate varies between and within species and population. Genetic variation in other species (*C. tolucana* and *C. wigthii*) has been also reported by Olejniczak (1996). Use of mass selection in Cuphea greatly reduced seed dormancy. Treatment of immature seeds of *Cuphea* (green coat) with chemical mutagens noticeable stimulated germination and also induced mutation (Olejniczak 1996).

6.3.3 Morphological Traits

Most wild plant species of *Cuphea* have a sticky coating on their stems, leaves and flowers, making harvesting of seed more difficult. Morphological studies have been carried out (Hirsinger and Knowles 1984; Olejniczak 1996) showing that the growth potential of *Cuphea* species is sufficient, and that using induced mutation and crossing, major wild plant characteristics, such as indeterminate growth and flowering, and seed setting and seed weight can be overcome. Koehne (1903) first described five *Cuphea* interspecific natural hybrids in *Cuphea*. Röbbelen and Hirsinger (1982) observed spontaneous outcrossing among species in their germplasm collection. Many different hybrids were obtained by Olejniczak (1996). Different genotypes were selected with improved morphological traits (Figs. 6.5 and 6.6). Lorey and Röbbelen (1984) attempted interspecific hybridization among 18 species, with five sections of the genus *Cuphea,* as well as among biogenetic groups characterized by different predominant fatty acids in seed oil. Reciprocal crosses were made between different species to assess them for *Cuphea* breeding programs. Mutation breeding and crossing in *Cuphea* has been suggested as a means of increasing genetic variability and selection, to overcome wild characteristics.

6.3.4 Molecular Perspective

Arabidopsis and *Cuphea* disperse their seeds, but the pod shattering mechanism is known as fruit dehiscence. Recently, molecular genetic studies in *Arabidopsis* have identified the major genes that control fruit development, leading to a model for the regulatory interaction between genes that pattern on shattering. Valve margin of functionality in *Arabidopsis* required the activities of the functionally redundant *SHATTERRPPOOF1* (*SHP1*), and *SHATTEROOF2* (*SHP2*), protein member of the extended family of *MADS* (Ostergaard et al. 2006). This group showed also that ectopic expression of the *Arabidopsis FRUITFUL* gene in *Brassica juncea,* is sufficient to produce pod shattering-resistant fruit, and that the genetic pathway leading to valve margin specification is conserved between *Arabidopsis* and *Brassica*. Genetic strategy for the control of seed dispersal will be applied to diverse *Cuphea* species to reduce seed loss. Konishi et al. (2006) reported the qSH1, a major quantitative traits loci controlling seed shattering in rice, demonstrated, that a single-nucleotide polymorphism (SNP) in the 5′ regulatory region of the qSH1 gene caused loss of seed shattering, owing

Table 6.4 Germination rate (%) of *Cuphea wrigthii* line 651 seeds depending on color of coat and storage periods (Olejniczak 1996)

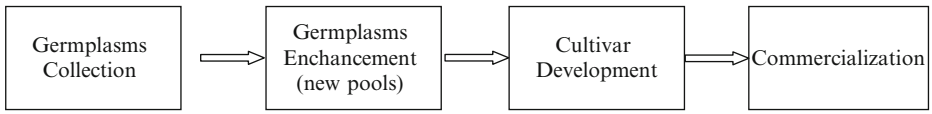
	Period of storage (months)						
	0	2	4	6	8	10	12
Light-green	0	0	0.7	39.3**	48.0**	69.3**	70
Dark-green	0	3	23.3**	83.3**	100.0**	90.7	90
Light-brown	0	2	28.0**	80.0**	98.0**	99	98
Middle-brown	0	4.7	55.3**	77.3**	99.3**	99.3	98.7
Dark-brown	0	6.7*	74.7**	79.3	99.7**	98.7	99.7
Black	0	8.7**	85.7**	84.7	98.7**	98.7	98.7

Significant $P_{0.05} = 6.60$*; $P_{0.01} = 8.23$**. Analyses was done in classes of coat color and between time of storage

Fig. 6.5 Metaphase I meiosis F4 (Mutant 1/186x *C. procumbens): I-12, II-12, III-1, IV-1* (Olejniczak 1996)

to the absence of abscission layer formation. Use of *FUL* gene to control fruit opening and seed dispersal can be directly transferred from *Arabidopsis* into *Cuphea,* suggesting that biotechnology should be broadly applicable for controlling pod-shattering in *Cuphea*.

The *TERMINAL FLOWER* 1 (TFL1) gene, and the two *EMBRYONIC FLOWER* (FMF) genes, *EMF1* and *EMF2*, are involved in delaying vegetative to reproductive transition and floral initiation in *Arabidopsis*. Loss of function mutations in *TFL1* gene shortens both rosette and inflorescence development (Bradley et al. 1997). Similar loss of function in *EMF* mutants display more dramatic phase-reduction phenotypes; there is no rosette shoot development, only a reduced inflorescence with several flowers lacking petal is produced. Mutants of the *TFL1* and *EMF1* genes display another similar phenotype, the conversion of the inflorescence apex from indeterminate to determinate by production of a terminal flower (Chen et al. 1997). Flowering is controlled by a number of endogens and environmental factors. The environmental factors include day-length, and stress conditions, while the endogenous factors include age, circadian rhythm, hormone, sugar content, etc. The factors or signals are perceived and transmitted to the nucleus to cause a change in gene expression that would lead to flower development. In *Arabidopsis*, more than 50 genes have been identified that are involved in the signaling pathway of flowering development (Blasquez 2000). Shoot architecture and flowering time in angiosperms depend on the balanced expression of a large number of flowering time and flower meristem genes. Loss of function mutants in the *Arabidopsis* EMF gene causes elimination of rosette shoot growth, and transformation of the apical meristem from indeterminate to determinate by producing a single terminal flower on all nodes (Aubert et al. 2001). The early flowering transgenic plants with a determinate inflorescence and normal leaves demonstrated the role played by the *EMF1* gene. The inflorescence development might be regulated by the level of *EMF1* activity, as showed by various levels of RNA in the antisense transgenic plants with terminal flowers or determinate inflorescences (Aubert et al. 2001). Molecular characterization of these two genes, *EMF1* and *EMF2*, has provided information on the mechanism of floral repression during vegetative development. It might be possible to engineer *Cuphea* plants with determinate flowering. Attempt to develop novel transgenic c*Cuphea* will probably be done in the future.



*taxonomic systematic *analyses of variability *selection of useful species *storage and collection	*increase variability - mutation -crossing -biotechnology *selection of improved lines	*plant breeding and genetics -selection of lines with improved yield *agronomic system *yield trials *preliminary evolution by industry	*ecological risk *economic assessments -farmers -industry -consumers

Fig. 6.6 A generalized system for reasearch and development of *Cuphea* plants (Olejniczak unpublished)

Seed dormancy provides a mechanism for plants to delay germination until conditions are optimal for the survival of the next generation. Dormancy release is regulated by a combination of environmental endogenous signals, with both synergistic and competing effects. Molecular studies of dormancy have correlated changes in transcriptomes, proteomes, and hormone levels with dormancy states ranging from deep primary or secondary dormancy to varying degrees of release. However, a number of genes that influence the regulation of seed dormancy are known. Dormancy gene *DELAY OF GERMINATION* (*DOG-1*) is an alternatively spliced gene, which encodes a protein of unknown function. Mutant alleles of *DOG-1* cause complete non-dormancy indicating this gene to be absolutely required for seed dormancy induction (Schwab 2008).

During the last few years, molecular studies have entered into the scene of genetic improvement of alternative oil crops including *Cuphea*.

6.4 Biosynthesis of Medium Chain Fatty Acids

Gene sequences are rapidly accumulating for many commercially and scientifically important plants. These resources create the basis for developing sequence-based markers for mapping and tracking known (candidate) genes, thereby increasing the utility of gene maps. Members of most of the gene families underlying the synthesis of seed oil fatty acids have been cloned from the medium-chain oilseed, *Cuphea*. Genes controlling the production of MCFAs are interesting for understanding this major variation in plant fatty acid synthesis and for genetically engineering the desired chain length in heterologous species.

The effect of fatty acid composition, alternatively, may be more relative to the interaction of isoforms with enzymes involved in fatty acid biosynthesis, rather than with the fatty acid themselves. Although the critical role of ACP in fatty acid biosynthesis has been established, the role of the diverse number of isoforms has yet to be elucidated. *Arabidopsis* transgenic plants with a *Fat*B gene, cloned from California Bay tree (*Umbellularia californicia* H.A.; Voelker et al. 1992), were able to accumulate up to 70% C-10:O and C-12:0 in the seed. Thioesterases cloned from different *Cuphea* species to transform oilseed plants have produced reduced MCFA. The conversion of *Arabidopsis* and canola to MCFA-producing plants, by transformation with thioesterase-encoding sequences, indicates that foreign enzymes can interact productively with heterologous fatty acid synthase systems. The spectrum of fatty acids present in the seed oil of transgenic plants, however, has not been strictly comparable to these of the MCFA-producing plants from which thioesterases were obtained even when the activity was massively over-production. For example, California bay accumulates 30% C-10:0 and

60% C-12:0 in the seed but rapeseed plants engineered with the bay thioesterase produce up to 56% C-12:0: and no C-10:0 (Voelker et al. 1996). Similarly, the seeds of *C. hookeriana* contain 50% C-8:0 and 25% C-10:0, but transgenic rapeseed expressing the *C. hookeriana* fatty acyl-thioesterase gene produced seed oil with 12% C-8:0 and 27% C-10:0 (Dehes et al. 1996). The condensing enzymes that extend growing fatty acid chains, B-ketoacyl acyl carrier protein (ACP) syntheses (KAS) are the likely candidates to be codeterminants of chain length. A KAS with specificity for short and medium chains fatty acids might favor certain chain lengths by augmenting acyl-ACP pools that provide substrate for medium chain-specific thioesterases. Additionally, competition between thioesterases and KAS for a given acyl-ACP substrate might influence the rate of chain termination for that fatty acid. The increased C:10/C:12 ratio produced in *Cuphea wrightii* seed plastid incubations with cerulenin, a inhibitor of certain condensing enzymes, provides indirect support for the hypothesis that fatty acid phenotype is influenced by KAS activity. Genetic changes induced by domestication are generally not comparable with those currently brought about by genetic engineering (GE) as since they represent a loss of function, in contrast to the gain of function associated with genes introduced by GE. In addition, GE is not a faster and more precise alternative to traditional plant breeding. It is principally a powerful and useful way of generating additional genetic diversity that could be exploited later for crop improvement by the proven methods and techniques of plant breeding. The potential benefits of GE should be investigated on a case-by-case basis, taking into account the advantages or disadvantages of alternative technologies. Further advances will increase this technology on relevance of consumer and environmental friendliness. Potential solutions to some of these problems are being developed.

6.5 Impact of Genetically Modified (GM) Plants to Non-GM Plants

The use of genetic modification techniques have increased the number of potential applications for alternative oil crops. However, it is important to address potential environmental concerns during the development of such crops although many potentially useful developments in oil crops have been made. Genetic modification has numerous potential applications in the improvement of crops for non-food uses. It has contributed to the production of new oil profiles with valuable industrial properties, by the introduction of single genes from wild species, for example, from *Cuphea* plants into oilseed rape, which produced MCFAs, reducing the use of petrochemically-derived oil. Environmental assessments are further complicated when considering the implication of GM plants, compared to naturally evolved systems. A particular response to non-food industrial crops on the ecosystem should also be considered for both GM and non-GM plants. This risk of gene transfer from *Cuphea* crops to related weedy species, as well as *Lythrum* depends very much on the plant itself. *Cuphea* has compatible indigenous related wild species in Europe, for example, *Lythrum* that could receive transgenes via pollen flow.

The extent of outcrossing depends on climatic conditions, agricultural practices, viability of pollen, and viability of outcrossing partners. Many domesticated plants can hybridize with relatives when they are grown in proximity, and whatever genes the cultivated plant had can then be passed on to the hybrid. This applies equally to transgenic plants and conventionally bred plants, as in either case, there are advantageous genes that might have negative consequences to an ecosystem upon release. This is normally not a significant concern, despite fears over "mutant superweeds" overgrowing local wildlife: although hybrid plants are far from uncommon, in most cases these hybrids are not fertile due to polyploidy, and will not multiply or persist long after the original domestic plant is removed from the environment. In some cases, the pollen from domestic plants may travel many miles in the wind before fertilizing another plant. This can make it difficult to assess the potential harm of cross-breeding, as many of the relevant hybrids are far away from the test site. Among the solutions under study for this concern are systems designed to prevent transfer of transgenes, such as terminator technology, and the genetic transformation of the chloroplast only, so that the seed of transcendence plant would bear the transgene. With regard to the former there is some controversy that the technologies may be inequitable, and might force dependence upon producers for valid seed

in the case of poor farmers, whereas the latter has no such concern, but has technical constraints that still need to be overcome.

Outcrossing of transgenic plants not only possesses potential environmental risk, but it may also trouble farmers and food producers. Many countries have different legislations for transgenic and environmental plants, as well as the derived food and feed, and the consumers demand the freedom of choice to buy GM-derived or conventional products. Therefore, farmers and producers must separate both production chains. This requires coexistent measures on the field level, as well as traceability measures throughout the whole food and feed processing chain. It can be interesting to investigate how the farmers can avoid outcrossing and mixing of transgenic and non-transgenic crops, and how processors can ensure and verify the separation of both the production chains.

6.6 Evolution of Domesticated Plants

Plants evolutionary theory has been greatly enriched by the studies on crop species. Over the last century, important information has been generated on many aspects of population biology, specification, mutation, hybridization, polyploid and molecular genetics. Plant breeders regularly employ quantitative trait loci (QTL) analyses to tag the key genes regulating many agronomic traits, and evolutionary biologists have found the techniques to be valuable in identifying the genetics of important adaptive traits and reconstructing the speciation process. A considerable amount of work has been conducted to identify those QTLs associated with the process of crop domestication.

One of the most striking findings in these studies is that a few major genes often influence a large amount of the genetic variability, even though a large number of genes may be affected by artificial selection during domestication (Wright et al. 2005).

The process of domestication dramatically changed the performance and genetic architecture of ancestral species, through the process of hybridization and selection as originally described by Darwin (1859). Not all genetic variation is created similarly. Darwin first introduced the concept of evolution, and challenged the prevailing view that species were fixed entities with a single invariable genetic identify. The concept of natural selection pre-supposed that species were comprised of genetically variable individuals such that selection could act on them. The genetic variants differ in the alleles (version of genes) they carry. Alleles that are deleterious in terms of survival and reproduction of the organism will eventually be eliminated, while the alleles that are favorable or neutral will be perpetuated in the population. Recombination in natural population allows alleles that may be deleterious in one genetic background to be reassessed in a different genetic context. Over time, the alleles that are transmitted at high frequency across generations represent those with a substantial likelihood of contributing positively to organisms' long-term viability in a viable environment.

N. I. Vavilow, a Russian geneticist and biologist, was one of the first to explore and actively collect wild relatives and early landrace varieties as sources of genetic variation for the future of agriculture. His botanical collection expeditions amassed thousands of rare and valuable specimens. Vavilow's concepts in evolutionary genetics, such as the law of homologous series in variation and the theory of centers of origin of cultivated plants, were major contributions to the understanding of the distribution around the world.

Natural evolution of different species of *Cuphea* was established by natural crossing between cross-pollinated species (Koehne 1903). Spontaneous mutations also have a great role, particularly in self-pollinated *Cuphea* species. The potential for adaptive evolution in wild species is provided by standing genetic variation, compared to that from new mutation. The four types of evolutionary changes that might promote rapid evolution in introduced ranges are bottlenecks, hybridization, polyploidy, and stress-induced modification of genome. Domestication syndrome is associated with relative simplicity and involves comparatively few quantitative and qualitative genes with major phenotypic effects. For example, in a survey of different cereals, Ladizinsky (1985) found that seed dispersal was controlled by only one or a maximum of up to three major genes in several crops. He has also showed that the genes controlling domestication-related characteristics are frequently clustered or linked within the genome.

It has been proposed that linkage of domestication traits would only have been advantages for the adaptation of outcrossing crops, as those crops are more

likely to have suffered from the introduction of unadapted genes from crosses with the wild ancestors (Koinange et al. 1996).

Triangle of U (UN 1935) is a theory about the evolution and relationships between members of the genus *Brassica*. This triangle shows three ancestral genomes, AA-, BB-, or CC-. These three species exist as separate species. Because such genetic and morphological variation has occurred as a result of natural interspecific hybridization, the remarkable diversity of *Brassica* is an excellent example for understanding how genetic and morphological variations have developed during the evolution of plant genomes.

Natural evolution of the *Cuphea* kingdom has been going on since a long time, but it is only in the last three decades that the geneticist and breeders have been using different tools, and trying to domesticate these species all over the World.

6.7 Invasive *Lythrum,* a Close Relative to *Cuphea*

The genera *Cuphea* and *Lythrum* are in a close relationship within the family Lythraceae based upon cytological and polygenetical pattern. Purple loosestrife (*L. salicaria*) is a European plant species that is now widespread also in the wetland areas of North America (Graham et al. 1993). Reportedly, it was introduced in the early1800s via ships, and is now widely cultivated for its ornamental and pharmacological values. There are many examples of invasive species causing damage to population of wild and domestication plants, and significant alteration of ecosystems. The destruction wrought by invasives occurs through predication, introduction of disease, competition for food and other resources, hybridization and habit degradation. These problems are likely to become graver with increasing global trade, global change and changing of land use pattern. These are especially relevant to wetlands, where much damage has been caused in the temperate areas. The actual impact of climatic change on plant distribution, whether the plants are native or exotic species, will depend on the biology of the species. The success and geographic range of plant populations may be influenced much by the changes in the distribution of precipitation during the year, as in the variation in the average amount of precipitation. Changes in temperature means extremes could also affect plant population. Climate change is expected to substantially alter biodiversity, causing changes in phenology, genetic composition and species ranges, and affecting species interactions and ecosystem processes (Myers et al. 2004).

Most treatments of species' response focus on native species, or on those for whom preservation is the primary concern. Invasive species will also responds to climate change, and their response will have ecological and economic implications. Many different wild species, for example *Cuphea*, have characteristics that are different from non-invasive species. Invasive plant species also often have characteristics that facilitate rapid range shift, such as dormancy, shattering of seed, and short time of maturing. Invasive species have been introduced in different ways; some non-native species, for example, *Lythrum* and *Heracleum*, were intentionally introduced for beneficial purposes, and later they turned out to be invasive as superweeds. The Giant Hogweeds (*Heracleum*), a perennial plant native to the Caucasus Mountains (Sobisz 2007), was introduced as an agricultural crop into Europe in the 1940s, where its large biomass was ensilaged to provide fodder for livestock. Besides the ecological problems, (superweed) tall invasive hogweed species also represent a serious health hazard for humans. The plant exudes a clear water sap, which contains several photosensitizing furanocoumarin. When they come in contact with the human skin and in combination with ultraviolet radiation, these compounds cause burning of the skin. Invasive plant species inflict tremendous economic and ecological cost on agriculture and ecosystems. Environmental weed invasion is a widespread phenomena in the plant kingdom.

Exotic plants introduced to a new region may or may not be adapted to a new environment they encounter. Exotic plants that become a permanent component of the flora of the new regions are referred to as naturalized plants. A small percentage of naturalized exotic plants earn the term of invasive plants. Invasive plants typically spread rapidly, and replace native vegetation in their new habitat. Giant foxtail, multiflora rose velvetleaf, hogweed, garlic mustard and possibly *Cuphea* are examples of exotics that have earned invasive status. Many invasive species behave differently in the region they colonize as compared to their native land. Thus, an introduced species

that is aggressive and displaces native species in a new region may be much less competitive in its native range. These plants act as good neighbors in their native range, and do not dominate plant communities. The traditional explanation for this new-found aggressiveness is that in new territories the plant is released from the suppressive effect of natural enemies (insects, diseases). The foundation of classical biological control of native weeds range and introduce them as the natural enemies to the new territory of the plants. The focus of much of the research on invasive weeds has been species that are adapted to less disturbed habitats than agricultural fields. This would include prairies, rangeland, woodland, and wetland. Invasive plants reduce the value of these areas in many ways including (1) replacing native species and reducing diversity (garlic mustard); (2) interfering with recreation multiflora rose, and (3) eliminating food and nesting areas for wildlife (purple loosestrife).

Invasion by wild species has been identified as a threat to biodiversity, second only to habitat loss. In addition, introduction of the species is perhaps the most permanent protection to native biodiversity and ecosystems processes.

6.8 Gene Flow Between Domesticated Crops and Wild Relative Species

Along with other evolutionary forces including genetic drift, mutation, recombination and selection, the process of migration is affecting the variability in gene frequency in the natural population of plants. Difference between populations can be created by genetic drift because of complete or partial isolation, or by heterogeneous selection among different genes. Migration of individuals (e.g., seed) or gametes (pollen) between subpopulations counteracts this divergence between population, causing reduction in the genetic diversity between subpopulation and increasing the level of genetic diversity within subpopulations. Gene flow between wild and domesticated populations is likely to have been important following domestication, and would have partially restored the genetic diversity of domesticated populations (Badr et al. 2008). From the family Lythraceae, genus *Cuphea* contains, about 600 wild species native to North, Central and South America, but *Lythrum* species have a close relationship based on phylogenic analyses (Graham et al. 2006). Genus *Lythrum* is naturally distributed in Eurasia and Europe. Both of these wild species may cross in natural environmental conditions. This flow of gene from domesticated *Cuphea* crops may cause reduction in the genetic diversity of the wild population.

Crops and their wild progenitors show marked phenotypic difference that have been referred to as the domestication syndrome. These differences result from natural or human selection pressures that are imposed at various stages of the plant under cultivation.

In some cases, crossing between wild and domesticated populations can promote the development of weedy populations, which can be found in areas of cultivation in abandoned fields or in other distributed areas, e.g., roadsides, railsides, and these will be present as intermediate characters between the wild and domesticated crops. Gene flow between wild and domesticated populations is also limited by their phenology. Nevertheless, the reproductive and the propagation system of domesticated crops represent crucial factors that affect the rate of gene flow between the mentioned populations. Clearly, in an allogamous species, higher outcrossing results in much higher frequency of domesticated-to-wild hybridization as compared to autogamous species.

Gene flow from wild to domesticated plant species has an important role in relation to the evolution of crop plants. After domestication, gene flow in the center of origin can partially resolve the low genetic diversity included in the first domesticated population. For instance, the difference between domesticated barley from the Himalayas and India, compared to the Near East germplasm, is probably due to the introgression from Asian populations of wild barley (*Hordeum vulgare* ssp. *spontaneus*) after domestication (Salamini et al. 2002). Gene flow still plays an important role as a source of new alleles for domesticated crops, where traditional farming continues (Jarvis and Hodgkin 1999). Gene flow from domesticated to wild populations is also an important issue relative to the release of transgenic varieties because of the potential effect on the genetic diversity of wild relatives, and possibly the production of new aggressive weeds (superweeds). Several examples of intergeneric gene transfer between species of wild and

domesticated plants from the family Brassicaceae were observed (Ford et al. 2006). However, in some cases, domesticated crops and their progenitors belong to different biological species, in particular when domestication has involved polyploidization and/or interspecific hybridization. Useful traits from crops to domesticated wild plants from different taxa are still a big problem. However, modern biotechnological techniques, which do not depend on sexual transfer that occur in nature, overcome this sexual barrier by making gene transfer possible between unrelated species.

6.9 Utilization of Other Plant Genetic Resources

The conservation, management and utilization of the plant genetic resources, also known as germplasm, form the basis for harnessing genetic diversity to create and sustain agricultural production system. Germplasm is both the genetic material (genes, group of genes, chromosomes), that controls heredity, and the development of tissues, organs and organisms that express the useful variation contained in that genetic material.

Lauric and capric acids (medium-chain fatty acids) have several industrial, medical, and nutritional uses. Development of plants, such as *Cuphea*, that can be integrated into sustainable agricultural production systems, supports the achievement of national rural development and environmental quality objectives. Several others, especially palm, are mentioned in "economic botany" literature as a potential source of lauric oils although few of them seem to have attracted agronomists enough to collect information on yield. "*Manicaria sacciera*" (Gaert and Ynesia) Cilenda (O.S. Cook), whose fruit contains 35% kernel; and *Schelea macrocarpia* (Karsts,) *Maxmiliana spp*. and *Boctris gaipaes* (H.B.K.,) all of which have oil monocarps as well as kernels (Arkcoll and Aguiar 1984) have been included. Data on the yield of *B*. *gaispaes* are now being collected because the pulp oil appears to be worth developing. Unfortunately, this plant generally has a small kernel, with low oil (20.4%) and low lauric acid content (33.3%) (Hammond et al. 1982). However, the fruits are very variable, and some with large kernels have been seen. A number of dicotyledons from Lauriaceae and Miristicacea are also rich sources of lauric acid. *Virola* ssp. fruit consists of 82% kernel that contains from 60 to 74% oil, which typically has 73% myristic and 15–20% lauric acids. Several other trees are mentioned in the literature (Princen 1984), but like most of the above, they suffer from the logistical problems of collection from the wild, and slow growth and low yield, compared to the common plantation crops, such as coconut and oil palm. Synthetic fats and oil can of course be made from petrochemical feedstock. However, this is too expensive to be an attractive source, and the toxicological and nutritional consequences of the branched chain, and add-numbered carbon acid may contaminate the products. The properties of *Cuphea* oil make it ideal for overcoming the challenges of the existing biodiesel products, for the utilization of used vegetable oil for biodiesel is provenly efficient (Geller et al. 1999). Actually, biodiesel is now being made available and is sold commercially in some parts of the globe. The use of these as substitutes for fossil fuels can reduce the toxic air pollution in the environment. Biodiesel is also biodegradable in a short period. Up till now, biodiesel is generally more expensive to purchase than petroleum diesel, but can be made at home for a much cheaper price. This differential may diminish due to economies of scale of the rising cost of petroleum, and government subsidization favoring the use of biodiesel. Biodiesel is free from such substances as sulfur and aromatics, which are found in traditional petroleum. This fuel, like all other renewable energy sources, was developed specifically for enhancing the sustainability of our use of green energy resources (Olejniczak et al. 2006).

6.10 Conservation of Plant Genetic Resources

Interest in the conservation of wild relatives of cultivated plants has increased considerably in the recent periods, and is recognized as one of the priority activities all over the world. Wild plants, for example, have to provide resources for the improvement of a lot of crop plants, for example, oil seed crops, and particularly for improvement of resistance to biotic and abiotic stresses. Genetic diversity provides plant breeders with the option to develop, through selection and

breeding, new and more productive crops that are resistant to virulent pests and diseases, and adapted to changing environments. The concept of germplasm conservation demands that collection methods initially capture maximum variation, and subsequently, conservation and regeneration techniques minimize losses through time. Collection involves gathering samples of a species from populations in the field, or natural habitat, for conservation and subsequent use. The unit of collection may be botanically small seed, for example, *Cuphea*. In vitro collecting methods were also developed for *Cuphea* (Przybecki et al. 2001a, b). Among the various ex situ conservation methods are seed storage, field genebank and botanical gardens. Among the various ex situ conservation methods, seed storage is the most convenient for long-term conservation of plant genetic resources. This involves desiccation of seed to low moisture content and storage at low temperature, −5°C for *Cuphea*. Cryopreservation involves storage of plant material at ultra-low temperature, cell division and metabolic activities remain suspended, and the material can be stored without change for a long period of time.

6.11 Development for Commercialization

The introduction of a new and improved agricultural plant, germplasm has been an ongoing process since the beginning of civilization. Although in early times, improvements were made through a simple selection based on individual observation, in our modern era such improvements are often the results of close cooperation among different disciplines, and among different government, industrial, and academic institutions. For new industrial crops and the use of traditional crops to be commercialized, many technical and economic constraints must be overcome, and the involvement of the public and private sectors is needed. The extent of this involvement and the speed at which new technologies will be developed and commercialized, will be influenced by many factors. Efficient data management is the key to any crop research or its promotion. Projects to develop novel crops are no exception. However, new crop development requires especially robust management, due to often insecure resourcing, the potentially narrow genetic base, uncertainty over intellectual property right issues, the need to document performance, and the risks for grower, promoter and regulatory agencies. Perception of a new crop's potential may fluctuate with large external factors, such as progress with alternative sources of target products (Finlay 2004). Research support is highly sensitive to external circumstances, such as budgets, periodicity of crisis, such as the recent increase in oil price, or poor markets for crops currently in production, causing the grower to look for new crops or market solutions. Such projects, for example, novel oil crop *Cuphea*, may be suspended or downsized for extended periods. Given the uncertain prospect for new crops, robust data management can protect valuable knowledge over periods of inaction, and ideally, this should detail records of crosses, selection and evaluations.

The characteristics of new crops are that, while intellectual property rights are problematic for established crops (Kimpel 1999), careful documentation of the crop development process will provide stakeholders' prosperity, economically and ethically. As new crops typically have a short and limited history, information on expected performance is especially valuable. The foremost interest concerns yield and market value. The producers also need comprehensive information on management, including assessments of risk factors, such as from pests or drought. Buyers or processors may seek additional information on interaction of quality with growing conditions and management, and the regulators may require product safety or other topics. Efficient management of information can help ensure that stokeholders' concerns are answered reliably and quickly, including performance attributes, and final yield.

Agricultural research requires managing large sets of data (Olejniczak 1996; Olejniczak and Adamska 1999). Plant breeding requires tracking the performance of large numbers of genetically distinct plants or population. Agronomic studies often involve experiments conducted over multiple years and locations, with recorded data including management practices, weather conditions, nutrient levels in the plant and soil (Olejniczak and Adamska 2000), as well as the crop's development and performance attributes, including final yield. An integrated information system differs from a database in the scope of data to be managed, and the emphasis on tools to facilitate the use of data for objectives, rather than the span of

multiple disciplines. Thus, integration involves joining together diverse types of data (Fig. 6.4) and integrating those data with analytical tools.

Agricultural research often seeks to understand crop response in terms of genotypes, environments and management systems' components. Ecophysiological models, which integrate knowledge on physiology, genetics, soil chemistry, and climatology can predict both crop performance and effects of crop on the environment, such as water and nutrient requirements and soil organic matter.

Model applications are of potential interest to new oil crop management decisions, especially in relation to climatic risk. Information on agronomic management traditionally comes from field experiments at a limited number of localization; but geospatial tools increase options for analyzing crops' response across environments (Olejniczak 1996).

6.12 Agricultural Production

All of our existing major crop species have been through a continual process of domestication and production since the beginning of agriculture, more than 10,000 years ago (Gepts and Papa 2003). The surplus in the production of conventional cereals and oilseed in the world has stimulated interest in the development and production of crops for specific non-food use.

Experimenting with an alternative crop, *Cuphea*, involves both risk and opportunities from the production and marketing standpoint. Information regarding alternative crops are limited especially when compared to that of prevailing crops, such as corn, wheat and soybean. *Cuphea* ssp. largely thrives in the tropical climates, but many of them are also well adapted to regions that are moist and temperate. Indeed, the small taproot characteristic of the species most likely limits their adaptation to wetter environment, and is perhaps a morphological trait, which induces wilting in the absence of water. A few *Cuphea* species have been found in more arid environments. Thick leaves or large taproots allow these species to cope with drought stress. Growing staging systems in crop are used for determination of application timings for post emergence herbicide, insecticide and fungicide, desiccation, swathing or direct harvest and for yield-loss chart for crop insurance. Berti and Johnson (2008) created a simple and descriptive growth staging system useful for individuals involved in *Cuphea* production. Only a few field experiments are known to identify the best agricultural conditions for *Cuphea* production. Gesch et al. (2006) assessed the impact of sowing date and row spacing on growth and seed yield of *Cuphea* in the northern Corn Belt of the United States. The researchers also found that *Cuphea* sown in wide rows compensated for seed yield by producing more branches and seed pod than when sown in narrow rows. The other known experiment examined the impact of depth and rate of sowing on seedling emergence and seed yield of *Cuphea* in Iowa (Roath 1998). However, Roath (1998) suggested that intensive rainfall events besides sowing and seed shattering late in the season can cause poor seed yield. Sowing early allows plants to transcend theoretical stages of development before the occurrence of late-season water stress (Brenton and Gesch 2004). For future large-scale production, need exists to identify additional broad leaf weed control option and there is need to study *Cuphea*'s soil nutrient requirements. Improved harvest management to reduce moisture content of *Cuphea* seed can be reduced to about 20% following swathing and drying in the field for approximately 14 days. It is required to develop lines with higher shattering resistance and determinate growth habit in *Cuphea*. Besides, equipment and techniques for harvesting are required to be designed for combating shattering problems. *Cuphea* is very sensitive to competition from summer-growing weeds, and no research has been conducted previously on the tolerance of this broad leaf plant to herbicides. For this reason, Forcella et al. (2005) conducted a greenhouse study on a large number of preplant incorporated, pre-emergence and post-emergence herbicides for tolerance of *Cuphea*. The promising herbicides were thereafter tested in the field for 2 years. Only a few herbicides appeared suitable for *Cuphea* production. There were two suitable preplant incorporated herbicides (ethalfuralin and trifluralin), and one suitable pre-emergence herbicide (isoxaflutde). Knowledge that these herbicides can be used in *Cuphea* production will aid domestication and acceptance of *Cuphea* by policy makers, researchers and the oil companies. Field study was initiated in Minnesota (USA) to determine the impact of sowing date, row spacing on soil and water use, and rooting characteristics of Cuphea (Sharratt and Gesch 2004). This study indicated that

biomass, seed yield, and water use a tendency for these plants to utilize water efficiently in seed production early in the spring. Significant effect of different irrigation levels of water in greenhouse conditions on agronomic traits and length of root systems was observed by Olejniczak and Adamska (2000).

An alternative crop may make a contribution by increasing the diversity of the farm income base, spreading out risk, reducing weaknesses in the farm system or broadening the base of operations. Alternative crops such as *Cuphea* provide additional markets or greater profitability as compared to standard crops. They can also be included in crop rotation to break up insects, pests, weeds and disease cycles, to scavenge nutrition for other crops, to improve soil, tilt and fertility, or to clean up weedy fields.

Most of the plants have already been adapted to European climatic conditions, with higher yield and well established systems for production, harvest and storage, and plant architecture using the different new technologies. *Cuphea* has become a viable crop in the United States and Europe, and an economic and reliable source of MCFAs from agriculture.

6.13 Conclusion

The genus *Cuphea* (Lythraceae) contains about 600 species native to North, Central and South America. Modern studies in *Cuphea* plant systematics rely upon many kinds of comparative data, such as on morphology, cytology, and biochemistry. A little over 100 crop species are now grown intensively around the world. Every crop plant cultivated today is related to a wild species occurring naturally in its center of origin, and progenitors of many of our crops are still found in the wild environment. Similar situations may also occur after the commercial production of Cuphea in America and Europe. Many of *Cuphea* species have the ability to synthesize and store MCFAs in their seeds. Some of these MCFAs, such as capric, lauric and myristic acids, are important in feedstock manufacturing, offering potential in the production of toothpastes, shampoos and detergents. Recent development has also proven that MCFAs are viable replacements for petroleum in uses such as motor oil and diesel fuel. *Cuphea* generally exhibits wild characteristics that are typical of undomesticated plants, such as indeterminate pattern of growth and flowering, seed shattering, seed dormancy, viscid and glandular hairs on stems, leaves, and flowers. Many breeding tools, such as mutagenesis and crossing, in vitro culture and biotechnology have been attempting to remove these limitations to domestication and production. Research and production of *Cuphea* will require a growth staging system for proper management regarding pest control, and application of herbicide and fungicide. Researchers in recent times have focused on non-shattering and non-dormancy development. Modern biotechnological tools have entered into the scene of improvement of oil crops including *Cuphea*, to solve the problems of dormancy, shattering and indeterminate growth and flowering. Introduction of new and improved agricultural germplasms has been an on-going process since the beginning of civilization. Although in the early ages, improvements were made through a simple selection, in our modern era such improvements are often the results of close cooperation among scientists of many emerging disciplines, and among many sectors, including government, industrial and academic institutions.

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Chapter 7
Diplotaxis

Domenico Pignone and Juan B. Martínez-Laborde

7.1 Basic Botany of the Species

The genus *Diplotaxis* DC. (family Brassicaceae, tribe Brassiceae) comprises 32 (Warwick et al. 2006) to 34 species (listed in Table 7.1) plus several additional intraspecific taxa, native to Europe, the Mediterranean region, Southwest Asia (up to the Himalayas), and Macaronesia, with the highest diversity in the Iberian Peninsula, Northwest Africa, and Cape Verde archipelago. A few species have become naturalized elsewhere (North and South America, Australia, etc.). The genus displays a considerable degree of heterogeneity in morphology, molecular marker profiles, chromosome numbers, and geographical amplitude.

7.1.1 Morphology

Diplotaxis species are mostly herbaceous annuals, with a group of perennials or subshrubby taxa with a more or less woody base. The perennials are *D. ibicensis*, *D. tenuifolia* and the *D. harra* aggregate that includes the closely related species from Cabo Verde – *D. antoniensis*, *D. glauca*, *D. gorgadensis*, *D. gracilis*, *D. hirta*, *D. sundingii*, *D. varia*, and *D. vogelli*, as well as *D. kohlaanensis* from Yemen and *D. nepalensis* from Nepal; *D. ibicensis* and *D. tenuifolia*. Only in *D. tenuifolia* the roots produce adventitious buds from which new shoots arise (Caso 1972). Trichomes are always simple, patent or retrorse, abundant or scarce on stems, leaves, pedicels, and sepals. The leaves tend to have a pinnatifid or pinnatisect blade, though in a few cases they are subentire to shallowly lobed. Upper cauline leaves are sometimes different from the basal or median ones; they are remarkably sessile, with a broad (in *D. erucoides*) or even clasping (in *D. assurgens* and *D. tenuisiliqua*) base.

The flowers form terminal racemes with fruiting racemes rather long. Bracts are rarely present, and always confined to one to a few basal flowers of the raceme. Pedicels are rather thin and erect-patent to patent, but remarkably thick and appressed in *D. assurgens*. Lateral sepals are somewhat saccate at base, while the median ones are moderately to markedly cucculate at apex.

Petals may be white (*D. erucoides* subsp. *erucoides*), violet (subgen. *Hesperidium*), or yellow (in most species, including 2–3 taxa with pale yellow petals). The petal limb may contract rather abruptly into a claw with parallel margins (in sections *Rhynchocarpum* and *Heterocarpum*), or taper gradually towards its base, forming a poorly defined claw with more or less converging margins (in section *Heteropetalum* and subgen. *Diplotaxis*). Petal venation was found to vary in a meaningful way within the tribe Brassiceae by Muñoz and Bermejo (1980), who adapted the terms employed by Hickey (1973) for types of leaf venation to petals. In *Diplotaxis*, most species show one of two well-defined patterns (Martínez-Laborde 1992): petals are brochidodromous (i.e., all secondary veins anastomose in one or more series of prominent loops or arches, forming a rather dense pattern) in most taxa of subgenera *Diplotaxis* and *Hesperidium*, as well as in section *Heteropetalum*, and cladodromous to eucamptodromous (i.e., secondary veins ramify towards the margin, sometimes curving up, never or very rarely anastomosing to form

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Table 7.1 Species and subspecies of *Diplotaxis* arranged according to the subgenera (SG) and sections (S) proposed in Gómez-Campo and Martínez-Laborde (1998), their known diploid chromosome numbers ($2n$) and geographical areas

SG[a]	S[b]	Species (and subspecies)	$2n$[c]	Geographical area[d]
D		*D. tenuifolia* (L.) DC.	22 (Harberd 1972)	Europe, Middle East*
		D. cretacea Kotov	22 (Harberd 1972)	Ukraine
		D. muralis (L.) DC.		
		subsp. *muralis*	42 (Harberd 1972)	Europe*
		subsp. *ceratophylla* (Batt.) Mart.-Laborde	–	N Algeria
		D. scaposa DC.		Island of Lampedusa
		D. simplex (Viv.) Spr.	22 (Harberd 1976)[e]	N Africa
		D. viminea (L.) DC.	20 (Harberd 1972)	Europe, N Africa, Middle East*
		D. harra (Forssk.) Boiss.		
		subsp. *harra*	26 (Gómez-Campo 1980)	N Africa, Middle East
		subsp *crassifolia* (Raf.) Maire	26 (Harberd 1972)	Sicily
		subsp. *lagascana* (DC.) O. Bolòs & Vigo	26 (Harberd 1972)	SE Spain
		D. kohlaanensis A. G. Miller & J. Nyberg	–	Yemen
		D. villosa Boulos & Jall.	–	Jordan
		D. pitardiana Maire	–	NW Africa
		D. nepalensis Hara	–	Nepal
		D. antoniensis Rustan	–	Cape Verde
		D. glauca (J.A. Schmidt) O.E. Schulz	26 (Harberd 1972)	Cape Verde
		D. gorgadensis Rustan		
		subsp. *gorgadensis*	–	Cape Verde
		subsp. *brochmannii* Rustan	26 (fide Rustan 1996)	Cape Verde
		D. gracilis (Webb) O.E. Schulz	26 (Rustan 1996)	Cape Verde
		D. hirta (A. Chev.) Rustan & Borgen	26 (Rustan 1996)	Cape Verde
		D. sundingii Rustan	26 (Rustan 1996)	Cape Verde
		D. varia Rustan	–	Cape Verde
		D. vogelli (Webb) Cout.	–	Cape Verde
H		*D. acris* (Forssk.) Boiss.	22 (Amin 1972)	N Africa, Middle East (to Iraq)
		D. griffithii (Hook.f. & W. Thomps.) Boiss.	–	Afghanistan, Pakistan
R	Rh	*D. assurgens* (Delile) Grenier	18 (Harberd 1972)	Morocco
		D. berthautii Braun-Blanq. & Maire	18 (Takahata and Hinata 1978)	S Morocco
		D. brachycarpa Godron	18 (Martínez-Laborde 1988a)	N Algeria
		D. catholica (L.) DC.	18 (Harberd 1972)	Iberian Peninsula, N Morocco
		D. ollivieri Maire	–	S Morocco
		D. siifolia Kunze		
		subsp. *siifolia*	20 (Harberd 1972)	Iberian Peninsula, NW Africa
		subsp. *bipinnatifida* (Coss.) Mart.-Laborde	–	S Morocco
		subsp. *vicentina* (Sampaio) Mart.-Laborde	20 (Fernandes and Queirós 1970–1971)	SW Portugal
		D. tenuisiliqua Delile		
		subsp. *tenuisiliqua*	18 (Harberd 1972)	N Morocco, NW Algeria
		subsp. *rupestris* (J. Ball) Mart.-Laborde	–	S Morocco
		D. virgata (Cav.) DC.	18 (Harberd 1972)	Iberian Peninsula, Morocco
	Hc	*D. ibicensis* (Pau) Gómez-Campo	16 (Gómez-Campo 1980)	E Spain coast, Balearic Islands
		D. brevisiliqua (Coss.) Mart.-Laborde	16 (Martínez-Laborde 1991b)	NE Morocco, NW Algeria
		D. ilorcitana (Sennen) Aedo, Mart.-Laborde & Muñoz Garm.	16 (Martínez-Laborde 1991b)[f]	E Spain
		D. siettiana Maire	16 (Takahata and Hinata 1978)	Island of Alboran
	Hp	*D. erucoides* (L.) DC.		
		subsp. *erucoides*	14 (Harberd 1972)	Europe, N Africa, Middle East*
		subsp. *longisiliqua* (Coss.) Gómez-Campo	14 (Gómez-Campo 1980)[g]	N Algeria

[a]D: subgen. *Diplotaxis*; H: subgen. *Hesperidium* (O. E. Schulz) Nègre; R: subgen. *Rhynchocarpum* (Prantl) Mart.-Laborde
[b]Rh: section *Rhynchocarpum*; Hc: section *Heterocarpum* Mart.-Laborde; Hp: section *Heteropetalum* Mart.-Laborde
[c]Only one reference is given. Some chromosome numbers were reported under a different name or synonym, but only those not based on the same basionym are further clarified in footnotes e–g
[d]Some taxa, indicated with (*) have, in addition, become naturalized in other continents
[e]sub. *D. pitardiana* (see Martínez-Laborde 1990)
[f]sub *D. gomez-campoi* Mart.-Laborde
[g]sub *D. virgata* subsp. *cossoniana* (Reut.) Maire & Weiller

arches and yielding a more or less loose pattern), in sections *Rhynchocarpum* and *Heterocarpum*.

There are always six stamens (two shorter and lateral plus four longer and median); lateral stamens of *D. viminea* have notably small and probably sterile anthers. There are four nectaries: two are more or less ovoid, located between each pair of median stamens, and the other two are cushion-like, flat at the top, located on the inner side of each lateral stamen. The pistil is linear to more or less elliptical, slightly to shortly stipitate at base, with a bilobate or decurrent stigma.

The fruit is always a silique, with considerable variation regarding position, length, and width, and proportional size of its parts. Siliques are generally erect-patent (sometimes tending to patent); erect to appressed siliques are characteristic of *D. assurgens*, whereas in *D. harra*, as well as in some of the closely related taxa from Cape Verde and at least partly in *D. kohlaanensis*, are patent to hanging. There is always a carpophore at the base, which is rather inconspicuous (less than 0.5 mm long, or even subnull) in most species but can achieve 1–5 mm in *D. tenuifolia*, *D. harra* aggregate, *D. acris*, and *D. griffithii*. The valvular portion, corresponding to the development of the ovary after fertilization, is generally linear to narrowly elliptical to lanceolate in outline, though in some species this shape is slightly (*D. tenuisiliqua*) to markedly (*D. brachycarpa*, *D. berthautii*) oblanceolate to obovate. It is always dehiscent and consists of two locules (separated by a false septum or dissepiment), which allocate all or most of the seeds. Seeds in this segment show the typical arrangement of two rows of seeds in each locule, explicitly alluded to in the ethymology of the generic name *Diplotaxis* (gr. *diplóos*, *diploûs* = double; gr. *táxis*, *-eos* = arrangement). However, the number of seed rows in the locule seems to depend on seed size and varies among species. Thus, fruits of *Diplotaxis siifolia* may have one row per locule, whereas those of *D. harra*, *D. acris*, or *D. siettiana* generally show 3(–4) rows of seeds per locule. The valves can be more or less flat or rather convex, keel-shaped, and vary from almost smooth to more or less torulose.

The distal, stylar segment, usually called the beak, is a key organ in the morphological characterization and variation of the tribe Brassiceae, since most genera of the tribe have heteroarthrocarpous fruits: the stylar segment is hollow and holds one to several seeds (Appel and Al-Shehbaz 2003), and in some genera (*Crambe*, *Raphanus*) can even be the only seed-containing part of the fruit. The adaptive and evolutionary meaning of such trait is a matter of debate (Hernández Bermejo and Clemente Muñoz 1977; Appel 1999; Gómez-Campo 1999; Gómez-Campo and Prakash 1999). In *Diplotaxis* (as in *Brassica*), both heteroarthrocarpous and non-heteroarthrocarpous fruits coexist: all species in subgenera *Diplotaxis* and *Hesperidium* have fruits with aspermous (seedless) beak, whereas in subgenus *Rhynchocarpum* species of sections *Rhynchocarpum* and *Heteropetalum* have 1–2 seeds in the (oligospermous) beak and those of section *Heterocarpum* may show either type of fruit.

The seeds are always rather small (0.6–1.3 mm long), and ovoid to ellipsoid in shape, with the exception of *D. siifolia*, which has spherical seeds, more similar to those of *Brassica* or *Sinapis*.

7.1.2 Geography

There is a considerable degree of endemicity in the genus, combined with several widely distributed species (Table 7.1). All the eight species from Cape Verde Islands are narrow endemics only found in one or two islands (Rustan 1996). In the *D. harra* aggregate, other endemics are *D. kohlaanensis* (Yemen), *D. villosa* (Jordan) and *D. nepalensis* (Nepal), whereas *D. harra* extends from Morocco to Pakistan (Jafri 1973), with two local endemic subspecies (subsp. *crassifolia* endemic to Sicily and subsp. *lagascana* endemic to SE Spain; Martínez-Laborde 1991a).

Both *D. acris* and its close relative *D. griffithii* have rather wide geographical areas: *D. acris* ranges from South Algeria to the Middle East and Iraq, while *D. griffithii* extends across Afghanistan and Pakistan (Jafri 1973). While *D. simplex* extends across North Africa, and *D. tenuifolia*, *D. viminea*, and *D. muralis* subsp. *muralis* are widely found in most central and South Europe and the Near East, *D. cretacea* is only known from Ukraine, *D. pitardiana* extends regionally in South Algeria, South Morocco, and Mauritania, and *D. muralis* subsp. *ceratophylla* is endemic to Northeast Algeria (*D. scaposa*, of uncertain status, has only been reported from the island of Lampedusa). In the

subgenus *Rhynchocarpum*, all the four species of section *Heterocarpum* show remarkably narrow geographical ranges: *D. ilorcitana* on SE Spain, *D. brevisiliqua* on Northeast Morocco and Northwest Algeria, *D. ibicensis* on a few Balearic Islands and nearby Iberian coast, and the most strikingly narrow endemic in the genus *D. siettiana*, only known to grow in the very small island of Alboran (Martínez-Laborde 1991b, 1998). In section *Heteropetalum*, *D. erucoides* subsp. *longisiliqua* is limited to Northeast Algeria, while *D. erucoides* subsp. *erucoides* is widely circum-Mediterranean (southwards reaching Ethiopia). In the section *Rhynchocarpum*, most species have a medium range, spreading across extensive areas of the Iberian Peninsula (*Diplotaxis catholica*), Morocco (*D. assurgens*, *D. berthautii, D. tenuisiliqua*), or both (*D. virgata*, *D. siifolia*); *D. ollivieri* and *D. brachycarpa* have, however, narrow areas, in South Morocco and North Algeria, respectively.

A few *Diplotaxis* species have widely transgressed their original boundaries and became naturalized in North Europe but also in remote continents. The two related taxa *D. tenuifolia* and *D. muralis* subsp. *muralis* are found in North America (Rollins 1993), South America (Brako and Al-Shehbaz 1993; Martínez-Laborde 1999), and Australia (Hewson 1982), and the latter also in South Africa (Marais 1970), India (Henry and Janarthanam 1993) and China (Taiyan et al. 2001), whereas *D. erucoides* subsp. *erucoides* has been reported from North and South America (Rollins 1993; Martínez-Laborde and Méndez 2001).

All *Diplotaxis* species are, more or less, ruderal and commonly found along roads or on periurban or otherwise altered locations, as well as on cultivated fields. Both *D. muralis* subsp. *muralis* and *D. tenuifolia* are successful colonizers (Eschmann-Grupe et al. 2004), and the latter is considered as an annoying weedy invader in Argentina (Caso 1972), whereas *D. erucoides* subsp. *erucoides* is rather a common weed in vineyards and other crops (Farré and Sebastiá 1992; Sans and Bonet 1993; Sans and Masalles 1994). Varying degrees of seed dormancy – rather easily overcome by gibberellic acid (GA_3) applications – were found in different populations of *D. virgata* and *D. erucoides* subsp. *erucoides* (Pérez-García et al. 1995; Pita Villamil et al. 2002; Martínez-Laborde et al. 2007).

7.1.3 Cytology, Cytodemes, and Reproductive Biology

Chromosome numbers are known for most taxa, as recorded in Table 7.1, and indicate that practically the whole genus is diploid. Polyploidy is practically absent within *Diplotaxis*, but instead there is a remarkable degree of disploidy. The series of gametic numbers ranges from $n = 7$ in *D. erucoides*, through $n = 8$, 9, 10 and 11, to $n = 13$ in the *D. harra* aggregate, whereas *D. muralis* has $n = 21$, the only species in the genus with a higher ploidy level. According to Harberd and McArthur (1972), *D. muralis* would be an amphidiploid probably arisen from *D. tenuifolia* ($n = 11$) and *D. viminea* ($n = 10$), a hypothesis strongly supported by isozyme (Eschmann-Grupe et al. 2003; Sánchez-Yélamo and Martínez-Laborde 1991; Warwick and Anderson 1997), seed protein (Sánchez-Yélamo et al. 1992), and flavonoid (Sánchez-Yélamo 1994) analyses, as well as by inter-simple DNA sequence repeat markers (Martín and Sánchez-Yélamo 2000). On the other hand, isoelectric focusing (IEF) pattern of Rubisco (Mummenhoff et al. 1993), as well as random amplified polymorphic DNA (RAPD) analysis (Eschmann-Grupe et al. 2003) confirm *D. viminea* as the maternal parent and allow for either *D. tenuifolia* or *D. simplex* (also $n = 11$) as the parental one.

Little is known about the reproductive biology in the genus. The floral syndrome (showy petals, nectaries) suggests that they are, in general, at least partly cross-pollinated. The Cape Verde species are self-incompatible (Rustan 1996) and *D. tenuifolia* is an outbreeder (Eschmann-Grupe et al. 2004). Some degree of self-pollination has been found in *D. erucoides* subsp. *erucoides* (Farré and Sebastiá 1992; Sans and Bonet 1993). On the other hand, *D. viminea* is a clearly autogamous species, as indicated by its much smaller flowers, apparently sterile lateral anthers, and high degree of ovule fertilization and seed formation, and *D. muralis* is predominantly self-pollinated (Eschmann-Grupe et al. 2004), probably due to maternal inheritance.

Crossing experiments have revealed that *Diplotaxis* species are reproductively rather isolated (Martínez-Laborde 1988b) and belong to at least 13 different

cytodemes (Harberd 1972; Takahata and Hinata 1983; Prakash et al. 1999). Apart from the case of *D. tenuifolia*, *D. cretacea*, and *D. simplex*, which constitute one cytodeme (with $n = 11$), and all Cape Verde species, which belong to the same cytodeme (with $n = 13$) as *D. harra* (Sobrino Vesperinas 1993; Rustan 1996), all other investigated species (*D. assurgens*, *D. berthautii*, *D. catholica*, *D. erucoides*, *D. muralis*, *D. siettiana*, *D. siifolia*, *D. viminea* and *D. virgata*) are each in its own cytodeme.

7.1.4 Molecular Markers

The molecular evidence accumulated along the past 25 years indicates that *Diplotaxis* species constitute a remarkably heterogeneous group. Phylogenetic analyses of chloroplast-DNA restriction site variation carried out first on a group of *Brassica* species (Palmer et al. 1983) and then on several genera belonging to tribe Brassiceae (Warwick and Black 1991, 1993; Warwick et al. 1992) consistently showed a clear separation into two clades, designated as the Rapa-Oleracea and Nigra lineages by Warwick and Black (1991) or the *Brassica* and *Sinapis* lineages according to Pradhan et al. (1992). Polyphyly was evident for *Diplotaxis* (Warwick et al. 1992; Warwick and Black 1993), with its species distributed in both lineages. All studied taxa belonging to subgen. *Diplotaxis*, plus both subspecies of *D. erucoides* (subgen. *Rhynchocarpum*, section *Heteropetalum*) were in the Rapa/Oleracea lineage, whereas all remaining taxa of subgen. *Rhynchocarpum* were placed in the Nigra lineage. Groups or clades within each lineage were rather consistent with known chromosome numbers and cytodemes as recognized by Harberd (1976) and Takahata and Hinata (1983). In the Rapa/Oleracea lineage, one clade included two sister groups, one with of *D. harra* and the other with *D. tenuifolia* and related taxa ($n = 11$), while *D. muralis* and *D. viminea* appeared in a third clade; a fourth clade corresponded to subgen. *Rhynchocarpum*, section *Heteropetalum*. In the Nigra lineage, one clade contained most species of section *Rhynchocarpum*; *D. brachycarpa*, of the same section, appeared in a second clade, while a third clade corresponded exactly with section *Heterocarpum*. Martín and Sánchez-Yélamo (2000) studied nuclear DNA markers (microsatellites) in 10 *Diplotaxis* species, and obtained a dendrogram showing two main branches, approximately corresponding to the two mentioned lineages detected with chloroplast DNA. One branch included five species, all of them corresponding to the Rapa/Oleracea lineage and to subgen. *Diplotaxis*. However, in the other branch appeared *D. harra* and *D. erucoides*, of the Rapa/Oleracea lineage, together three species corresponding to the Nigra lineage and to subgen. *Rhynchocarpum*.

7.2 Conservation Initiatives

In the early 1990s, under the aegis of IPGRI's (International Plant Genetic Resources Institute, now Bioversity International) program Underutilized and Neglected Crops, financed by the Italian Government and led by Dr. Stefano Padulosi, a specific network was started to foster initiatives of conservation and valuation of *Eruca* and *Diplotaxis* genetic resources. The idea came forth from the observation that the wild populations of *Diplotaxis* in many Mediterranean regions, and in South Italy in particular, were endangered by over-collecting from the wild, due to the growing market request for rocket. The Rocket Genetic Resources Network, or simply Rocket Network, had a first meeting in 1994 in Lisbon, Portugal (Padulosi 1995). Twelve lectures were presented at that meeting regarding conservation and evaluation activities (Pignone and Api Ngu 1995), genetic characterization, and utilization of these crops. During the meeting, it was decided to establish a survey of the collections available all over the world (Table 7.2) and to start a descriptor list in order to facilitate characterization. This event represented the first organized activity aimed at establishing a coordinated activity of conservation and evaluation of these neglected species.

A second congress was organized within the frame of the Rocket Network in Padua, Italy, in 1995 (Padulosi and Pignone 1997). One of the most relevant achievements of that meeting was to bring to the attention of the scientific community the studies carried out during the previous couple of years tending to establish cultural practices to cultivate *Diplotaxis*. Improved agricultural and marketing techniques were developed thereafter, such as mechanized hydroponic cultivation (Bianco and Boari 1997; Pimpini and Enzo 1997;

Table 7.2 Accessions of major collections of the genus *Diplotaxis* stored in gene banks

Gene bank	No. of *Diplotaxis* accessions	No. of *D. erucoides* accessions
IPK, Gatersleben (D)	18	2
GRIN-NPGS (USA)	30	1
UPM, Madrid (E)[a]	24	1

[a]One accession per each of 24 taxa (species and subspecies) of *Diplotaxis* are conserved in long-term conditions at the Plant Genebank of the Universidad Politécnica de Madrid (BGV-UPM, *http://www.etsia.upm.es/banco_germoplasma/inicio_bgv.htm*). Additionally, for a limited number of taxa there are extra accessions used as working collection to assess intraspecific variation

Santamaria et al. 2002) and bagging of harvested leaves under controlled atmosphere for increased shelf-life (Martínez-Sanchez et al. 2006; Wagstaff 2007), which have made available a clean and ready-to-eat product, easy to transport also very far from the production area. The result of this booming activity is that the anthropic pressure over natural population has practically been reduced to a negligible level.

Rocket most definitely can no longer be considered a neglected crop.

7.3 Role in Elucidation of Origin and Evolution of Allied Crop Plants

Diplotaxis species differ in their mating systems, their life forms, and in their evolutionary history, but some of them are unbeaten colonizers. In fact, it is not difficult to observe fields in which *D. erucoides*, or *D. tenuifolia*, or *D. muralis* are the most, or among the most, abundant species. The diploid perennial *D. tenuifolia* is an outbreeder, and the allotetraploid annual to biennial *D. muralis* is predominantly self-pollinated. Eschmann-Grupe et al. (2004) studied the genetic structure of populations of these two species in order to estimate the relative influence of the breeding system and genetic drift effects on the extent and structure of genetic variation. A first result was that no evident correlation between population divergence and geographic distance could be revealed. *D. tenuifolia* populations were found to be in complete Hardy–Weinberg equilibrium, thus inferring complete and obligate outbreeding behavior. However, a significant allelic differentiation across populations was observed together with deviation of genotypic frequencies from panmictic expectations. On this basis, the authors inferred low or absent gene flow between populations. *D. muralis*, on the contrary, was nearly devoid of genetic variation due to the recent phylogenetic history of this species, and its success appeared to be in relation to polyploidy (Eschmann-Grupe et al. 2004). The low variation at the molecular level in *D. muralis*, however, does not correspond to a low level of phenotypic variation. This is not a unique case; nevertheless, this discrepancy needs to be explored.

Different and apparently contrasting results were reported by Hurka et al. (2003). *Capsella bursa-pastoris* (L.) Medik., a tetraploid species, showed the greatest colonizing ability among the species considered in their very interesting review. They inferred the success to be associated with polyploidy, predominant self-pollination, high genetic diversity, and to the introduction of pre-adapted genotypes. The observed difference with *D. muralis* might be attributed to the fact that apparently *C. bursa-pastoris* is not of recent origin as one might predict from its prevalence for manmade habitats. Many genetic data indicate that the observed genetic variation in this species does not reflect adaptation to local environments but, more probably, population history and colonization events, and archeological data support the long evolutionary history of this species. The authors, therefore, warn that genetic diversity at the molecular level may not be informative of the adaptation status but rather reflect the ancestry of populations and their history.

The authors argue that it appears that natural interspecific hybridization is not infrequent in the wild Brassicaceae and the consequent introgression is one of the most important mechanism for the establishment of high levels of genetic variation in diploid species or of successful colonizing polyploids, especially in manmade habitats. This mechanism might have a strong impact on Brassica crops' evolution or domestication. In fact, it appears that natural hybridization of crop Brassicas with wild species (*Sinapis, Raphanus*, etc.) is not a rare event (Warwick and

Small 1999). Hurka et al. (2003) report also on some examples of *Cardamine* and *Nasturtium* to demonstrate the evolutionary significance of hybridization in rapidly changing environments, and infer that an increase in interspecific gene transfer due to habitat modification is to be expected.

The success of polyploidy in the Brassicaceae might have a genetic base: that is, might be due to the presence of specific meiotic mutants facilitating the production of non-reduced gametes at meiosis. Malallah and Attia (2003) reported the observation of 1.5% cytomixis and 7.8% aneuploidy in pollen mother cells of a Kuwaiti diploid ($2n = 26$) population of *D. harra*. In addition to a genetic control of cytomixis, stress factors such as high temperature or drought during definite periods of the growing season may contribute to the occurrence of cytomixis. Non-reductional meiosis, besides favoring the establishment of polyploids, may also be one of the factors involved in gene flow among species at different ploidy level, as observed in hybrids involving *Triticum* and *Aegilops* (Pignone 1993). Recently, Marhold and Lihovà (2006) have reviewed studies on polyploidization and hybridization events in the Brassicaceae. They have recognized these phenomena as important evolutionary forces in the family. They describe the reticulated pattern of evolution in Brassicaceae, which derives from allopolyploid and homoploid hybrid speciation.

7.4 Role in Classical and Molecular Genetic Studies and in Crop Improvement

7.4.1 Trypsin Inhibitors

Many evidences indicate that trypsin inhibitors can confer to plants resistance to the attacks of phytophagous insects. From *Sinapis*, the MTI-2 (mustard trypsin inhibitor) inhibitor was isolated and found to be encoded by the gene *mti-2* (Ceci et al. 1995; Volpicella et al. 2001; De Leo et al. 2002) belonging to a novel family specific of the Brassicaceae. In order to identify other inhibitors with higher or qualitatively different activity with respect to MTI-2, a search of new variants of *mti-2* was undertaken, within the Brassicaceae, based on the nucleotide sequence homology (Pignone et al. 2001). New interesting variants of the *mti-2* gene were found in *D. tenuifolia* and *D. muralis*.

In order to characterize the activity of the newly identified genes, they were in vitro expressed using the yeast vector *Pichia pastoris*. The recombinant proteins so produced were tested for their ability to inhibit commercial (trypsin, chymotrypsin) and *Helicoverpa zea* larvae gut proteinases, using specific chromogenic substrates (BApNA, SAAPLpNA), in order to estimate the K_i against the respective proteinase. The results indicated that the activity against insect proteases was much higher than against commercial enzymes, indicating that the usually adopted procedure of reporting activity of plant protease inhibitors against bovine trypsin may lead to wrong estimation of their effect on insect proteases (Volpicella et al. 2009).

These results open new perspectives to horticultural crop breeding, since they offer the possibility to identify new sources of useful genes to be used in specific programs of introgressive hybridization.

7.4.2 Glucosinolates

The genus *Diplotaxis*, as other Brassicaceae, is a major producer of glucosinolates. Glucosinolates are a group of sulfur-rich thioglucoside natural products common in the Brassicaceae and related plant families. Isothiocyanates are the volatile products of (enzymatic) hydrolysis of glucosinolates, and they are responsible for the characteristic aroma of many crucifers (Macleod 1976; Rodman 1991). According to a revision by Fahey et al. (2001), as many as 16 compounds have been reported in the nine species of *Diplotaxis* so far investigated (Table 7.3). It is of interest that two compounds found in *D. tenuifolia* [4-(Methylsulfinyl) butyl and 4-(Methylthio)butyl] are the same glucosinolates found in *Eruca vesicaria*, which may account for taste similarities, flavor, and culinary uses between these two widely used species.

The role of these compounds on plant protection (Rosa et al. 1997; Fahey et al. 2001) and their influence on human and animal health have been largely described (Srinibas et al. 2000). The glucosinolate content and profile were studied on 44 accessions of different origins, belonging to 17 different *Diplotaxis*

Table 7.3 Glucosinolates found in species of *Diplotaxis*, grouped into classes according to their chemical structure (as reported by Fahey et al. 2001)

Class[a]	Glucosinolates	Species of *Diplotaxis*[b]								
		Dca	Der	Dgr	Dha	Dmu	Dsi	Dtf	Dvm	Dvr
A	4-(Methylsulfinyl)butyl			+				+		
	4-(Methylthio)butyl		+	+				+	+	
	3-(Methylthio)pentyl		+							
C	1-Methylethyl								+	+
	1-Methylpropyl								+	+
D	3-Butenyl	+	+				+			+
	2-Hydroxy-3-butenyl									+
	2-Propenyl		+			+			+	
	4-Pentenyl						+			
G	Benzyl		+							+
	4-Hydroxybenzyl	+	+	+	+		+		+	
	2-Phenylethyl		+							+
I	4-Hydroxyindol-3-ylmethyl		+							
	Indol-3-ylmethyl		+							
	1-Methoxyindol-3-ylmethyl		+							
	4- Methoxyindol-3-ylmethyl		+							

[a]Chemical classes as follows: (A) sulfur containing side-chains, (C) aliphatic, branches chain, (D) straight and branched chain, (G) aromatic, (I) indole.
[b]Species acronyms as follows: Dca (*D. catholica*), Der (*D. erucoides*), Dgr (*D. griffithii*), Dha (*D. harra*), Dmu (*D. muralis*), Dsi (*D. siifolia*), Dvm (*D. viminea*), Dvr (*D. virgata*)

taxa (D'Antuono et al. 2006). Total glucosinolate content ranged from 0.25 to more than 70 g/kg of dry mass. Sinigrin, sinalbin, glucobrassicin, and gluconasturtin represented the main components of the glucosinolate rich accessions, accounting for 50–100% of the total glucosinolate content. The glucosinolate content of *D. tenuifolia* reached a maximum of 4.6 g/kg of dry mass. As regards the presence of glucosinolates with recognized cancer protective effects, glucoraphanin was widespread in *D. tenuifolia*, glucobrassicin was very abundant (6.7 g/kg) in one accession of *D. siifolia*, and gluconasturtin was relevant in *D. brachycarpa* (2.8 g/kg) (D'Antuono et al. 2006).

It is also worth noting that these compounds fall within a broad category of compounds called allelochemicals, and are exclusive of food that influences growth, health, or behavior of other organisms (Brown and Morra 2005). The interest in allelochemicals is in relation to their potential for use as alternative pest management agents. In fact, the use of plant-produced allelochemicals in agricultural and horticultural practices could possibly reduce the use of synthetic pesticides to a minimum. The plant products, in fact, do not require energy from fossil sources, which help in reducing the CO_2 balance of the environment, leading therefore to a more sustainable agricultural system.

7.4.3 Wide Hybridization

The genus *Diplotaxis* is a somewhat close relative of the genus *Brassica*, one of the most commercially important genera in the world. Therefore, much effort has been spent in producing hybrids and amphyploids of *Diplotaxis* with Brassicas in order to transfer the useful genes present in the wild gene pool to the crops. Attempts have been made to obtain intergeneric hybrids between *D. siifolia* and the species *Brassica campestris*, *B. juncea*, and *B. napus* (Batra et al. 1990). Due to unilateral incompatibility, hybrids were obtained only using the *Diplotaxis* parent as a female. Through ovary culture it was possible to establish hybrids showing no fertility and therefore maintained using axillary bud propagation. The amphiploids derived from these hybrids were partially self-fertile and could be used for establishing backcross derivatives.

Wide hybridization has also been used to establish cytoplasmic male sterile (CMS) lines via alloplasmy. For instance, CMS lines were developed in *Brassica juncea* using the bridgecross hybrids (*D. erucoides* × *B. campestris*) × *B. juncea* and (*D. berthautii* × *B. campestris*) × *B. juncea*. Both lines resembled the *B. juncea* parent in morphology and were as

much female fertile as the *Brassica* parent. Differences could be observed in the floral morphology and in the smaller anther with empty and sterile pollen (Malik et al. 1999).

Pathania et al. (2003) reported on CMS based on alloplasmic *B. juncea* carrying *D. catholica* cytoplasm. In these plants, using genetic segregation data, they could demonstrate that a single, dominant nuclear gene governs fertility restoration. The male sterility appeared to be based on interaction between the *Brassica* nuclear and the *Diplotaxis* mitochondrial (mt) genomes. However, in Northern analysis, out of eight mt genes studied, an altered transcript pattern was recorded only for *atpA*. In fertility-restored plants, the *atpA* transcript appeared shorter and it was associated with the CMS trait.

Recently, Bhat et al. (2008) reported on the development of improved CMS lines of *B. juncea* carrying cytoplasm of *D. berthautii*, overcoming some of the problems of the lines described by Malik et al. (1999), whose female fertility was comparable to that of the euplasmic lines. Moreover, the fertility restorers of *Moricandia arvensis* or *D. catholica*-based alloplasmic CMS lines appeared to be capable of restoring male fertility also to this newly described CMS line. Also in this case, the fertility restoration appeared to be monogenic and gametophytic. As in Pathania et al.'s (2003) report, Northern analysis using eight mitochondrial gene probes revealed an altered expression of the mt gene *atpA* associated with male sterility. The paper by Bhat et al. (2008) contains an extensive, up-to-date, and illuminating literature for those who are interested in this topic. Wide hybridization and the production of alloplasmic CMS lines appears at present the most promising system to improve *Brassica* crops.

7.4.4 Genomics Resources Developed

A search of the EMBL database (*http://srs.ebi.ac.uk*) using *Diplotaxis* as a keyword for organism name returned 261 entries. Most of them refer to plastidial or nuclear (rDNA) spacers sequences. These sequences are mostly used for phylogenetic reconstruction studies, since they are considered to be neutral markers of evolutionary events.

Just for the sake of comparison, a search for *Arabidopsis* or *Brassica* returned over 2 and 1.6 billon entries, respectively. The Brassicaceae sequences present in the EMBL database include over 4.2 billion entries. This means that the genomes of *Diplotaxis* species have not even been looked at!

The abundance of information present in sequence databases, comprising nuclear, plastidial, and expressed sequence tag (EST) sequences, is a powerful resource to explore the genomes of this genus, which is waiting to be exploited in order to analyze the genetics of some traits, like perenniality, which cannot be studied in model organisms like *Arabidopsis* or in annual crops.

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Chapter 8
Eruca

Domenico Pignone and César Gómez-Campo

8.1 Basic Botany of *Eruca* spp.

Rocket or arugula is an edible plant that grows wild in the Mediterranean basin and eastwards to Iran. It belongs to the botanical genus *Eruca*, a member of the tribe Brassiceae within the family Brassicaceae (erstwhile Cruciferae). The taxonomic limits of the genus have been moved with time, but today, we can safely agree that *Eruca* contains a single species *Eruca vesicaria* (L.) Cav., which, in turn, includes three infraspecific taxa. All of these can be found in the wild state, but only *E. vesicaria* subsp. *sativa* (Miller) Thell. (syn. *Eruca sativa* Miller) has been domesticated and occupies a wider geographical area in the world. The second (type) subspecies, *vesicaria*, as well as the third subspecies, *pinnatifida* (Desf.) Emberger & Maire, is only local in the West Mediterranean area. Although another subspecies, *longirostra* (Uechtr.) Maire, from the West Mediterranean area has also been described, a detailed morphometric analysis based on fruit dimensions does not confirm its distinct status (Gómez-Campo 2003a).

The accepted botanical name for the cultivated rockets is, therefore, *E. vesicaria* subsp. *sativa* (Miller) Thellung, though it is still frequently referred to by the simplified synonym *E. sativa* Mill. Other ancient synonyms are *Brassica eruca* L. and *Raphanus eruca* (L.) Crantz.

The plants of *Eruca* are annual, up to 1 m high, either glabrescent or more or less roughly hispid, with simple hairs, mostly in the stem. The basal leaves occur in a rosette and are lobed to pinnatifid (sinuses not reaching the midnerve), with the lobes usually acute. Caulinar leaves are often simplified. Floral racemes have many flowers with no bracts at their base. Sepals are erect and more or less hairy; the lateral ones are swollen at their base and slightly hooded at their apex (the median ones also sometimes slightly so). The petals are 1–2 cm long, whitish, creamy or somewhat yellowish, and unguiculate, with very conspicuous violet brochydodrome venation. Median nectaries are ovoid. Stigma is small and bilobed. Siliques are short (10–35 mm), usually erectopatent or sometimes patent, erect or adpressed, with their valves oblong and subcylindrical, showing a well-defined and visible straight median vein. The stylar portion is flat, ensiform, or spadiciform (swordlike), and always asperm (seedless). Seeds are small, 1–3 (4) mm, somewhat flattened, and biseriate or subbiseriate in each locule. Cotyledons are conduplicate (embracing the radicule in the embryo) (Tutin 1993; Gómez-Campo 1993).

Eruca has a pan-Mediterranean distribution and is also diffused into the Near East. As a cultivated form, it is also present and popular in Asia (India, Pakistan) and, more recently, in the Americas. All the above-mentioned subspecies can be found in the southwest Mediterranean region, while only one of them (subsp. *sativa*) has spread to other parts of the world. Their distribution suggests that the Southwest Mediterranean region is the center of diversification for this species. Throughout the area of subsp. *sativa*, plants that have escaped from cultivation show larger and more robust fruit and seeds , a trend that can be used to distinguish them from the real wild ones. Consequently, toward the east, Iran contains both escaped and wild phenotypes, while further to the east (Pakistan and India), only the escaped forms can be found. The same obviously occurs in North and South America. On the contrary,

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subsp. *vesicaria* (the originally described type subspecies) is only present in gypsaceous soils of the central, eastern, and southeastern regions of the Iberian Peninsula and also in some areas of Northwest Algeria, around Tlemcem. Subspecies *pinnatifida* grows in subdesertic areas of Morocco and Algeria. Subspecies *vesicaria* is not cultivated (Gómez-Campo and Prakash 1999), while subsp. *pinnatifida* is sometimes cultivated as a leaf vegetable or grown as a fresh forage in oases in the Sahara (Specht and Diederichsen 2001).

The exact identification of the three subspecies is difficult in herbarium material as this often contains too young inflorescences. Also, some distinctive characters of the inflorescence, for instance, anther shape or sepal cucullation (hooding), are not as constant as described in the books. Others are altered by the pressure used during the drying process. For this reason, Gómez-Campo (2003a) recommended the use of fresh material and built a table after side by side cultivation of samples (Table 8.1) collected during various expeditions. To better describe the subspecies *vesicaria* and *sativa*, the same author cultivated more than 100 accessions, recording a number of characters, and conducted a morphometrical analysis where, in some cases, subsp. *pinnatifida* were included. Some of the characters were found to be distinctive, but most of them could not be considered definitive due to some difficulties in recording them. In fact, differential characters appeared to be copious, but some were dependent on environmental conditions, while some others were easily observed in cultivation but were difficult to score in nature, and others were erratic or restricted only to limited geographical areas. Similar difficulties were also reported for the correct identification of the subsp. *pinnatifida*. The ecogeographic distribution of the samples is also of much help because susbsp. *vesicaria* or subsp. *pinnatifida* are practically impossible to find outside their areas of distribution.

The possibility of studying living plants growing side by side under identical conditions (Fig. 8.1) was considered a way to avoid environmental bias with respect to the expression of some distinctive characters, thus facilitating the gaining of new insights into the infraspecific levels of variation of this species (Fig. 8.2). Table 8.1 gives a critical account of the characters, which have been used to distinguish the three subspecies. However, the following key might be more useful in the practice. It is only based in a short number of characters, those that were judged the most practical to distinguish all the three subspecies:

A key for *Eruca* subspecies:

1. Seeds not larger than 1 (1.1) mm	subsp. *pinnatifida*
1. Seeds from 1.0 to 2.5 mm	
2. Calyx persistent to maturity.	
Lower fruit pedicels 8–15 mm	subsp. *vesicaria*
2. Calyx deciduous shortly after fertilization.	
All fruit pedicels ≈6 mm	subsp. *sativa*

The chromosome number of *Eruca* is $2n = 22$ ($n = 11$), and it does not change among the different subspecies of this species (Harberd 1972). This basic number is rarely present in other related genera of the Brassiceae (*Guenthera sensu lato*, *Diplotaxis* Sect. *Diplotaxis*), and it is noteworthy that it occurs

Table 8.1 Some possible characters for distinguishing between subsp. *vesicaria* and subsp. *sativa* and their limitations (after Gómez-Campo 2003a)

Subsp. *vesicaria*	Subsp. *sativa*	Observations
Plant height 80–140 (150) cm	Plant height 60–100 (120) cm	Of variable expression under natural conditions
Plant contor hemispherical	Plant contor obconical	Of difficult observation under natural conditions
Plant very hispid	Plant less hispid to glabrous	High interindividual and interpopulation variation
Lower leaves pinnatilobed	Lower leaves pinnatifid	Pinnatilobed leaves are often present in subsp. *vesicaria*
Racemes with 12–15 (20) flowers	Racemes with 20–25 (30) flowers	Of difficult observation in poorly developed subsp. *sativa*
Calyx swollen at the base	Calyx not swollen	Not fully applicable in all the subsp. *vesicaria* area
Calyx persistent	Calyx deciduous	Of difficult evaluation in late fruiting stages or in dry years
Sepals all cucullate	Sepals only two cucullate (hooded)	Only applicable to the first 2–3 flowers of each raceme
Corola cream, yellowish	Corola cream, whitish	Only applicable within the Iberian Peninsula
Anthers subacute	Anthers obtuse	Difficult to asses
Lower fruit pedicels 9–12 (15) mm	Lower fruit pedicels <6 mm	Only applicable to basal siliques

Fig. 8.1 (*Left*) inflorescence of *Eruca vesicaria* subsp. *sativa*; (*Right*) *Eruca vesicaria* subsp. *vesicaria*. In the latter taxon, the calyx persists for a long time, while in *Eruca vesicaria* subsp *sativa*, the calyx is short-lived

in taxa showing a seedless stylar portion, as *Eruca* does.

A collection of names for wild forms of this species includes roquette, roquette sauvage (French), rocket, wild rocket (English), grote zandkool (Dutch), oruga, rúcula, roqueta silvestre (Spanish), Doppelsame, Schmalblättriger, Doppelrauke, Ölrauke, Rauke, Ruke (German), rucola, rucola americana, and ruquascine (Italian). For cultivated forms, they are gargir, girgir (Arabic), Salatsennep (Danish), roquette, roquette cultivée (French), arugula, rocket, garden rocket (English), rokietta siewna (Polish), mindau (Russian), Senapskål (Swedish), Garten Senfrauke, (German), Roka (Turkish), and Rucola, Rughetta (Italian) (List produced by the Chamber of Commerce of Savona, Italy). In many European languages, therefore, the common name for *Eruca* contains a root that might derive from the archaic "*roc,*" which in early Latin meant "harsh, rough, scratchy," possibly in relation to the pungent taste of its leaves and from which the classical Latin name "*eruca*" derived. Nevertheless, the etymology is not fully disclosed yet, and the name might derive from the Latin name *eruca* ("caterpillar") or from the verb "*urere*" (to burn, to be spicy) due to the bitter, sour taste of *Eruca* leaves.

Ethnobotanical knowledge regarding *Eruca* is vast. It is accepted that the "*gargir*" mentioned in the Talmud (fifth to seventh centuries) is rocket, and that rocket is the Biblical "*oroth*." In the Book of Kings

Fig. 8.2 Infrutescences of *Eruca vesicaria* subsp *sativa* (*left*: cultivated, and, *center*: wild forms and *right*: subsp. *vesicaria*)

(II Kings 4:39–40) of the Bible, it is written: "one of them went out into the field to gather oroth" (Yaniv 1997). The plant was sacred to Priapus, god of the fertility, identified in Italy with the god Mutinus Tutinus, an ancient Roman divinity. According to Pliny, the Roman naturalist of the first century, a decoction of rocket seeds was used to eliminate intestinal worms. The Romans paid a lot of importance to *eruca*, which they considered a powerful remedy against impotence.

In one of the epigrams of the famous Roman poet Martial, we can read that a certain Lupercus was so deadly impotent that not even *eruca* seeds could be of any benefit. The Hispano-Romans also compared the aphrodisiac power of rocket precisely with the anaphrodisiac power of lettuce. In Hispano-Visigoth culture, Isidoro de Sevilla supports the use and knowledge of this plant's powers, and Hispano-Arab agronomists, for instance, Ibn Hayyay (eleventh century), Ibn Wafid (eleventh and twelfth century), and, of course, Ibn al-Awwam (twelfth century) (Nuez and Hernández-Bermejo 1994), also mention its cultivation.

The Italian physician Matthioli, in a treaty printed in 1557, cites Dioscorides and states that eating abundant raw rocket "*desta Venere. Il che fa parimente il suo seme: commodo anchora à provocar l'orina,*" that is: "This being eaten raw in any great quantitie doth provoke Venery, and the seed of it also doth work ye like effect, being vreticall and digestiue, and good for ye belly. Wakes the sexual desire up" (Goodyear, in Gunther 1933).

8.2 Agricultural Status and Uses

Rocket is a fast-growing, salt-tolerant, cool season crop and bolts under long days and high temperature. Under Mediterranean conditions, the wild forms flower from February to June and the cultivated form right into mid-summer. It can be harvested after 20–27 days and then sequentially harvested from regrowth (Morales and Janick 2002). Until some 15 years ago, this species was only traditionally consumed either from collections or cultivated on a small scale, mostly for the local markets (Padulosi and Pignone 1997). A number of scientific and technical initiatives have radically changed the processing and marketing of this crop and have increased its production. One of these initiatives, the "Rocket Network," an R&D international network promoted by the former IPGRI (now Biodiversity International), under the frame of the Underutilized Mediterranean Species program, put together scientists and stakeholders in a very concerted manner and produced as an outcome the diffusion of modern practices for cultivation and post-harvest technology, which resulted in a strong diffusion of *Eruca* among the European consumers (Padulosi and Pignone 1997; Pignone 2004). Now-a-days, this species is quite widely diffused in European markets and is highly appreciated by the consumers.

Rocket is considered to be an excellent stomachic, stimulant, and aphrodisiac, and is also used as a diuretic and antiscorbutic. The leaves have a bitter flavor, which is made milder by cooking or frying. The seeds are hot, although rather less so than mustard seeds. It contains glucosides, such as allyl sulphocyanate, mineral salts, and vitamin C. The oil of the seed contains erucic acid. The oil is used for human nutrition, as lamp oil, as a lubricant, and for medicinal and cosmetic purposes (Yaniv et al. 1998). In Asia, the seed cake and the entire plant are also used as fodder for domestic animals. The seed oil content of subsp. *sativa* ranges from 22 to 41% and is rich in erucic acid (Al-Shehbaz 1985; Yadava et al. 1998; Yaniv et al. 1998), which makes this species a potential future source of industrial oil. The seed also contains significant amounts of 4-methylthiobutyl glucosinolate (glucoerucin), which has both direct and indirect antioxidant activity (Barillari et al. 2005).

This plant's pungent qualities are much appreciated in Italy, and it is widely consumed either alone as a green or as part of a salad mix. In countries such as Spain, France, and Great Britain, cultivation is rarer. In Turkey and Egypt, non-pungent varieties are also appreciated in common salads as a cheaper substitute for lettuce. In India and Pakistan, both uses coexist and, additionally, the seeds are also used to extract oil. It is also popular in the Sahel, especially in Sudan (Ibn Oaf 2004). In Europe, *Eruca* oil is also appreciated in industrial processes due to its erucic acid content. In fact, selection for edible plants has created a demand for high erucic acid material. However, its use in the kitchen is not limited to salads. *Eruca* leaves can be cooked to prepare dishes of pasta and to prepare a "pesto di rucola," a sort of cream that includes garlic and cheese, to dress pasta or bread toasts; it is one of the main ingredients of the Italian "breasola," a dish prepared with slices of dry meat, olive oil, parmesan scales, and rocket leaves. The Food Down Under database (*http://www.fooddownunder.com*) lists 102 recipes using rocket as an ingredient. This testifies to the great importance *Eruca* has gained with the consumers nowadays , as stated above.

8.3 Conservation Initiatives

The already described the Rocket Network was established in the early 1990s in order to foster research and development activities aimed at combating the genetic erosion consequent to the increased harvesting from the wild of *Eruca* and *Diplotaxis* to meet the growing market needs (Padulosi 1995). At that time, a survey of the available genetic resources of *Eruca* stored in genebanks was carried out. The results of that survey demonstrated that at least 150 different samples were already stored in different genebanks all over the world. Many of these accessions, some two-thirds of the total, were cultivated accessions of Asian origin, while the remaining accessions (wild and cultivated) had been collected around the Mediterranean.

This pattern provides evidence of the uses of *Eruca* in different cultures, at least anciently. In Asia, and particularly in the Indian subcontinent, *Eruca* is mostly cultivated for producing oil from its seeds. This oil, known as Jamba oil or Taramira oil, is rich in erucic acid (C22:1-*cis*-13-docosenoic acid), and therefore not counseled for human consumption (Uphof 1963). *Eruca* seed oil has mainly industrial uses as a lubricant, for soap making, and as an illuminating agent; it is also used in oils for massaging, and in the production of medicines. It is sometimes used for adulterating other oils. The cake can be used as an ingredient of cattle feed and as manure.

After the end of the Rocket Network project, no special initiative was taken to preserve *Eruca* germplasm. At present, the most important ex situ collections of genetic resources of this genus are those held at Universidad Politécnica de Madrid (Spain) holding some 130 entries, the National Plant Germplasm System of the USDA (USA) holding 246 entries, and the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben (Germany) holding 142 accessions. The level of duplication among these accessions has not been explored, but it is highly probable that it is quite high having been originated by exchange of germplasm among the institutions. GRIN and IPK focus mainly on cultivated accessions. The UPM seed bank of Madrid is distinguished in as much as it stores mainly directly collected wild material with a good representation of subsp. *vesicaria*. Also a part of its material is preserved with ultra-dry methods that ensure a highly efficient long term seed preservation (>96% germination after 40 years) (Pérez-García et al. 2007).

Regarding wild variability, a present matter of concern should be the fate of some wild populations of subsp. *sativa* in certain areas of the Mediterranean basin. In Italy, for instance, it is very common to find the same subspecies to have escaped from cultivation, and these supposedly displace and/or interbreed with the original wild populations. Though they might be difficult to distinguish in the first instance, escaped populations show larger siliques and larger seeds than the original wild populations.

8.4 Role in Elucidation of Origin and Evolution of Allied Crop Plants

The tribe Brassiceae has recently been the object of considerable studies because it includes economically important crops such as *Brassica oleracea, B. rapa, B. napus, Sinapis alba, Raphanus sativus,* etc. (see for a recent review, Al-Shehbaz et al. 2006). These and other studies point out that the Brassiceae is perhaps a monophyletic tribe and consists of 46 genera and some 230 species that share some common characters, such as conduplicate cotyledons, segmented fruits (partially), and simple or no trichomes. The vast part of the genera within this tribe are easily recognized by their fruit morphology, while vegetative characters, such as leaf or flower ones, are often valid but, in general, less adequate to this end. Moreover, the recent work of Warwick and Sauder (2005), based on cpDNA analysis on this tribe, clearly demonstrates that the traditionally recognized generic boundaries need to be revised. This work confirms previous observations (Yanagino et al. 1987; Song et al. 1990; Warwick and Black 1991) that within this tribe, two main clades are present, weakly defined at the morphological level but not at the molecular one. For this reason, Al-Shehbaz et al. (2006) suggest that the larger and most commonly known genera of the tribe (e.g., *Brassica*, *Diplotaxis*, *Sinapis*, etc.) should be reshaped. Nevertheless, these genera include economically important crops and weeds, so this proposal might have slow initial acceptance. Morphologically, Gómez-Campo (1999) has insisted in the evolutionary importance of the presence of seeds in the stylar portion, a

trend that can only be found in approximately one half of the genera. On this basis, the genus *Guenthera,* with a seedless stylar portion, was split from *Brassica* (Gómez-Campo 2003b). It shows a clear relation to *Eruca*, but it needs to be further defined because it contains three molecular clades. *Eruca* is not affected itself with this readjustment although its position within an ampler group of tentative relatives depends from further studies.

Within the tribe Brassiceae, *Eruca* is a relatively isolated genus. One could perhaps aim to find which other genera are more or less related to it (*Diplotaxis*, *Brassica*, etc.), but it would be difficult to identify the nearest one.

Based on restriction fragment length polymorphism (RFLP; Song et al. 1990) and cpDNA data (Warwick and Black 1991; Pradhan et al. 1992), it has been suggested that *Raphanus sativus* ($n = 9$) and *E. sativa* ($n = 11$) are genetically close. They possess some morphological similarities such as large sized flowers with prominent veins in the petal, but the dissimilarities are deeper and more abundant. For instance, fruit structure is diametrically different.

Of particular interest is the reference to its closeness to *Brassica*. If we consider the group of taxa disclosed from *Brassica* under *Guenthera* (Gómez-Campo 2003b), these might be much closer to *Eruca,* at least morphologically (seedless stylar portion, leaves never lyrate-pinnatisect, shallower cotyledon notch than in more advanced *Brassica* species, etc.) and also by their chromosome number. Some rare material of this group from the Rif and Thell mountains in North Africa have even been described as *Eruca* (*Eruca setulosa* Boissier & Reuter, *Eruca loncholoma* (Pomel) O. E. Schulz) based upon similarity in the fruits. *E. vesicaria* might have been a "progressive" annual taxon derived from such perennial material, which extended around as a weed in the Mediterranean basin. If we go to real *Brassica* species where the fruits show a seeded beak and the leaves are lyrate-pinnatisect, the morphological relation is less clear. The possibility of transferring useful characters between *E. vesicaria* and *B. oleracea* has often been suggested (Bansal et al. 1997), and crosses between both species are possible but problematic. In a recent ITS-based molecular dendrogram, *E. vesicaria* (two subspecies) form a neat group with *Diplotaxis tenuifolia* and *Brassicaria repanda* (of the *Guenthera* group) (Warwick and Sauder 2005). Similar relations had been suggested by Pradhan et al. (1992) using both cp-DNA and mt-DNA. In short, if we go to the treatment of Gómez-Campo (1999), *Eruca* may be much more related – morphologically and molecularly – to *Brassica* subgen. *Brassicaria* than to *Brassica* subgen. *Brassica*.

8.5 Classical and Molecular Genetic Studies

Based on chloroplast DNA restriction experiments using 20 different endonucleases, Warwick and Black (1991) attempted a molecular taxonomy of *Brassica* and allies, analyzing 33 diploid taxa of the subtribe Brassicinae. They observed a total of 419 mutations, 221 (53%) of which phylogenetically useful. In their analysis, Warwick and Black (1991) concluded that the genus *Eruca* pertained to a clade they defined the "Rapa/Oleracea lineage," which also included *Brassica*, some *Diplotaxis*, and *Raphanus*, other taxa possessing pungent leaves and therefore used in salads, as distinguished from the "Nigra lineage," which also included *Sinapis*. Though correlation with morphology is not high, the existence of these lineages is useful to understand molecular relationships among genera and groups of genera.

As for *Eruca* itself, a dendrogram by Warwick et al. (2007), based on 47 accessions and a total of 234 polymorphic amplified fragment length polymorphism (AFLP) fragments, neatly separates all the three subspecies and includes a separate consistent branch with Asiatic *sativa* accessions, the latter of probable cultivated origin.

Hybridization among both subspecies has been conducted by Sobrino-Vesperinas (1995), who found fertility only in one direction – subsp. *sativa* (female) × subsp. *vesicaria* – and high sterility in the opposite way – subsp. *vesicaria* (female) × subsp. *sativa*. He suggests that both subspecies should be given the rank of species. So far, we ignore of the existence of hybridizations involving subsp. *pinnatifida*.

Hybridization with other supposedly related taxa is also rare because while chromosome number $2n = 22$ is present in some other related genera in the Brassiceae, these are often poorly known, and germplasm is often not available. With reference to the described

cytodemes with $n = 11$ (Harberd 1972; Warwick and Al-Shehbaz 2006; Prakash et al. 2009), the five that have been found are *Diplotaxis acris*/*Diplotaxis tenuifolia*/*Brassica souliei*/*Brassica elongata*/*Eruca vesicaria*.

As far as we know, the four other members of these cytodemes have never been successfully crossed with *E. vesicaria,* probably because it has never been intended. Both *Brassica* species mentioned belong to the *Guenthera* group (formerly *Brassica* subgen. *Brassicaria* (Godr.) Gómez-Campo 1999). *Diplotaxis* with seedless stylar portion, especially *D. tenuifolia*, constantly appear close to *E. vesicaria* in molecular dendrograms.

Somatic hybrids of *E. vesicaria* subsp. *sativa* × *Brassica juncea* were reported by Sikdar et al. (1990), as well as of *E. vesicaria* subsp. *sativa* × *Brassica napus* (Fahleson et al. 1988). In both cases, chromosome pairing was very poor. Several other interesting achievements by plant breeders are described below. The status of wide hybridization in the Brassicinae was reviewed in Warwick (1995) and more recently by Marhold and Lihová (2006).

Genomic in situ hybridization has effectively been used to determine the alien chromosome status at mitosis in some of the somatic hybrids and their progeny, e.g., in *E. sativa* × *B. napus* (Fahleson et al. 1988). Also, several cytoplasmic male sterility (CMS) systems in *B. napus* and *B. juncea* based on *E. vesicaria* subsp. *sativa* have been obtained following protoplast fusion.

No autopolyploids have ever been induced or detected.

8.6 Role in Crop Improvement Through Traditional and Advanced Tools

Although known and appreciated for centuries, *Eruca* has been given little attention by geneticists and breeders until now. Different from *Diplotaxis* species, both are often called by the same common name and used in the same way. Only *Eruca* shows a certain level of domestication. Gómez-Campo (2003a) suggested that some collections from the wild are, in fact, in feral forms: plants that have established natural stands after escaping from cultivation. True wild forms (estimated by the smaller fruits and seeds and by the internal variability of the populations) can only be found in Spain and Iran. Still, it seems that the use of this species eastward to India dates back to very ancient times. At present, nothing is known for sure regarding the time and place of domestication of this species, but one could imagine that *Eruca* represents one of the many experiments men conducted through pre-domestication cultivation of wild forms and empiric selection of the forms most adapted to their needs (Hammer 1988; Balter 2007; Pignone 2009). The domestication of *Eruca* is in any case at a very initial state and presently, as it happens to other extensively cultivated vegetables of the Brassicaceae family, only a few open-pollinated cultivars have been registered, while the majority of seeds present in the market have no registration. *Eruca* is a cross-pollinated species, which is mostly self-incompatible, and may suffer from severe inbreeding depression. For this reason, breeding of this species is mostly based on mass selection for the desired traits (Morales and Janick 2002). The hybrid nature of the varieties has the advantage of maintaining a high level of heterosis but at the same time reduces the uniformity of the crop. A complex system of self-incompatibility, mainly gametophytic, is present, but with some alleles acting sporophytically. The existence of genic male sterility has been verified.

To overcome such a limitation and to obtain the most benefit from the heterosis in hybrid populations, the availability of highly homozygotic lines is essential. To overcome the problems of self-incompatibility and inbreeding depression, one strategy might be that of the production of doubled haploids via anther or microspore culture, a technique commonly used for haploid production in the Brassicaceae. Recently, Leskovšek et al. (2008) reported a successful protocol for the obtainment via microspore culture of *Eruca* embryos able to develop into plantlets. These authors could induce the formation of embryos from the microspore cultures, which turned to be in most cases diploid. In fact, out of some 500 embryos analyzed, about two-thirds were diploid, while less than one-third was haploid and higher ploidy levels represented only some 5% of the regenerated plantlets. The genetic nature of diploid plantlets was not ascertained, so it is unknown whether they are doubled haploids or the result of fusion of haploid cells. Besides the question being still open, this technique evidences for new perspectives for *Eruca* breeding.

As stated earlier, *Eruca* is molecularly included in the Rapa/Oleracea lineage (Warwick and Black 1991), and with the restrictions described above, it is an undoubted molecular relative of *Brassica*. For this reason, it has been suggested as a possible donor of genes for *Brassica* improvement. Magrath and Mithen (1997) reported on a sexually obtained amphiploid *E. sativa* × *B. rapa*, which was self-incompatible, but could be crossed with the *B. rapa* parent to attempt gene transfer. In fact, in the Brassiceae, the meiotic chromosome pairing regulation is not too strict and alien chromosome pairing is not unexpected. *Eruca* could be a source of genes for some fungal diseases that affect *Brassica* species (Tewari et al. 1996) and for the fatty acids' pathway, particularly for the production of erucic acid. Moreover, amphiploids could help elucidate the genetics of the different fatty acids' production and the different genes involved in saturation and length of the acid chain.

A different approach to the obtainment of amphiploids between distant species is somatic hybridization. Using this approach, Fahleson et al. (1997) could study the problems and the fate of alien chromosomes introduced in a different background after the hybrid is backcrossed to one of the parents. Using genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH) techniques, it was possible to distinguish the chromosomes belonging to *Brassica* and to *Eruca* in each examined cell. Moreover, GISH and FISH allowed the identification of recombinant chromosomes. These results are of great importance as molecular cytogenetic techniques could be easily used to select the appropriate plants in marker-assisted selection programs.

Cytogenetic studies in *Eruca* are scarce. Besides the chromosome number, there are a few reports on more detailed cytogenetical studies. Blangiforti and Venora (1995) used an automated image analysis system to investigate karyotype morphology in two taxa of *Eruca*, viz. *E. vesicaria* subsp. *sativa* and subsp. *pinnatifida*, and their relative *D. tenuifolia*. All the plants analyzed showed a karyotype with $2n = 22$ chromosomes. The use of image analysis allowed a detailed description of chromosomal morphology. It is interesting to observe that the karyotypes of these three taxa do not differ strikingly from each other, suggesting little karyotype evolution, a finding somewhat confirming the vision of Warwick and Sauder (2005), who hypothesized a need of reconsidering the generic delimitation within the Brassicinae. Recently, Lysak and Lexer (2006) have analyzed different taxa of the Brassiceae using molecular cytogenetic tools, including chromosome FISH, using chromosome-specific bacterial artificial chromosomes (BACs) from *Arabidopsis thaliana*. Although *Eruca* was not investigated in that study, the results demonstrate that a vast level of chromosome repatterning has occurred during the evolution of the Brassicaeae. These results do not easily comply with the observation of Blangiforti and Venora (1995); a possible explanation could be that this latter study regarded only two genera of the Brassicinae, viz. *Eruca* and *Diplotaxis*, while that of Lysak and Lexer (2006) only considered *Diplotaxis erucoides* ($2n = 14$), not much related to *D. tenuifolia*, as a representative of this ranking level. Moreover, both studies did not include *Brassica* for a possible comparison.

These and similar studies using molecular cytogenetic tools, including FISH, on extended DNA fibers have shown that it is possible to use currently available DNA libraries, such as those developed in *A. thaliana* or in *Brassica* species, to achieve a better understanding on the genome organization in *Brassica* and allied species and to obtain better insights into the mechanisms involved in the evolution of these complexes (Heslop-Harrison 2004; Alix et al 2005; Lysak et al. 2005; Yang et al. 2006; Howell et al. 2009).

Zhang and Wessler (2005) have described a new family of short interspersed elements (SINEs), referred to as BoS family, widespread in Brassicaceae and present at some 2,000 copies in *B. oleracea*. SINEs are a family of retrotransposons diffused in the eukaryotic genomes. This new family shares structure and target site preference with previously described SINEs, and in addition, it possesses several unusual features. This study has demonstrated that BoS is the most abundant SINE family in Brassicaceae that is clearly differentiated from similar elements described in other taxonomic groups. The BoS family is further subdivided in a number of subfamilies sharing common features but clearly differentiated from each other. For this reason, Zhang and Wessler (2005) proposed the BoS retrotransposons as a valuable phylogenetic marker for resolving the still open questions in the evolution of species and genera in the Brassicaceae family.

It appears that the molecular approaches described above can shed substantial light on many still unresolved taxonomical problems in the Brassicinae and could provide new powerful tools for breeding new

varieties of different crops. This problem is not only related to food applications, but also to oil quality, as many Brassicinae are used for oil production. The growing demand for new oil qualities to fit the needs of the green chemistry or the production of renewable fuels (Cardone et al. 2003; Lazzeri et al. 2004) makes this an argument of extreme interest for geneticists and breeders.

8.7 Scope for Future Domestication and Commercialization

In addition to food quality of *Eruca* and the need to meet the growing demands of an expanding market for this crop, and to the potential use of the genetic resources of this genus for addressing the scientific and technological problems, *Eruca* possesses pharmacological and biological activities. The organs of the plants are rich in glucosinolates and their derivatives, isothiocyanates. These compounds are the major organoleptic and bioactive elements of the Brassicaceae and a few other related families. Endogenous degradation of the glycosides liberates volatile, pungent products, which are responsible not only for flavor and smell of these plants, but also for their biological properties. The major degradation products are isothiocyanates, and to a minor extent, nitriles, thiocyanates, and thionocarbonates.

Glucosinolates and/or their derivative products are known to possess fungicidal, bactericidal, and nematocidal activity (Zasada and Ferris 2004). Recently, research on these compounds has been stimulated by their cancer chemoprotective attributes (Higdon et al. 2007). As a counterpart, these compounds are also attributed negative characteristics such as antinutritional or goitrogenic (they can negatively affect thyroid functionality) properties. A review (Fahey et al. 2001) analyzes the complex family of these biologically active and chemically diverse compounds, which are so abundant in *Brassica* and in the Brassiceae. More recently, Bennett et al. (2006) have analyzed the content of secondary compounds, including glucosinolates in *Diplotaxis* and *Eruca* taxa. In addition, a similar work was done also by D'Antuono et al. (2008) but mostly with a phylogenetic scope. This study has evidenced that some *Eruca* accessions are relatively rich in glucosinolates and particularly in one compound, namely glucoraphanin. This is an important compound, since, under the action of the enzyme myrosinase, present in the leaves and released after chewing, and present also in the intestines of humans, it is converted to sulforaphanin, rapidly absorbed, transported systemically in the blood, metabolized, and excreted in the urine. In the process of uptake and elimination from the cells, sulforaphanin induces the formation of a variety of detoxifying and antioxidant peptides and proteins. Moreover, it exerts a direct antibiotic effect on *Helicobacter pylori*, the etiological agent of ulcers and gastritis, and a major risk factor for gastric cancer (Fahey et al. 2002).

E. vesicaria has also been used as a model for glucosinolate biosynthesis investigations (Graser et al. 2000; Falk et al. 2004).

8.8 Some Dark Sides and Their Addressing: Recommendations for Future Actions

Gene flow from wild or weedy *Eruca* to crop species is improbable because, as commented above, *E. vesicaria* is never too close to its nearest relatives. Recent studies of possible flow from *Raphanus raphanistrum* to *B. rapa* (Chèvre et al. 2004) might exemplify studies on this type of subject. Only interactions and competence between wild and cultivated or escaped *Eruca* might be a matter of concern. Still, *Eruca* is a weed and might be a host for several fungi and viruses that attack other cruciferous crops (Al-Shehbaz 1985).

Regarding germplasm collection, Algeria still holds a high variability of wild susbsp. *sativa* in the northcentral of the country, as well as of subsp. *vesicaria* in the northwestern end. However, very few accessions of this area, if any, are now available in seed banks.

As stated before, rocket is gaining a growing importance on the European market. This event is the result of several different events: the increase of research and development on this species, the diffusion of off-soil growing techniques, the increased mechanization in leaf production, the development of new promising varieties, and, last but not least, the diffusion of the fourth generation packaging

technology to rocket (Padulosi and Pignone 1997; Morales and Janick 2002; Pignone 2004). Nevertheless, this new packaging technology also has a dark side that needs to be addressed. One problem comes from the growth of microorganisms in the package atmosphere. To better understand the microbial development, see Nielsen et al. (2008). To this end, cut-leaves were decontaminated and then inoculated with selected microorganisms. The package was stored in different atmospheres and temperatures, and the accumulation of off-odors was monitored. Several compounds were detected, and sulfides were identified as the substances causing the unpleasant smell. Pseudomonadaceae and xanthamonadaceae were particularly effective in producing sulfides, and the level of oxygen and temperature were found to have a strong effect (Nielsen et al. 2008).

Another problem that affects the shelf-life of rocket is yellowing, which is naturally induced by ethylene, even when the cut-leaves are stored at low temperatures. Koukounaras et al. (2006) have demonstrated that a treatment with 1-methylcyclopropene could prevent the action of ethylene in inducing yellowing on cut *Eruca* leaves and extending the shelf-life of some 20%. In another study, the same authors (Koukounaras et al. 2007) analyzed the influence of leaf age and post-harvest conservation temperature on the product quality and shelf-life. They concluded that lower temperature, the best close to 0°C, reduces the emission of endogenous ethylene, leaf respiration, and overall metabolic activity, resulting in a reduced chlorophyll degradation, hence yellowing, and extension of shelf-life. The storage temperature appears to be a limiting factor especially when transportation of processed vegetable is considered, since the authors demonstrate that the usual temperatures employed in transportation are not adequate to fully develop the shelf-life potential of this crop.

In addition to post-harvest problems, also some agronomic issues related to plant growth need to be explored. Pita Villamil et al. (2002), for instance, analyzed the influence of seed storage parameters on seed germination and plantlet development. This parameters are of great importance when off-soil growing techniques are applied, e.g., in floating beads over nutrient solution (D'Anna et al. 2003; Nicola et al. 2004), due to the mechanization process that these techniques imply. Soilless systems, in fact, are being used for increasing yield and quality of vegetables. The floating system is easy and cheap and offers the possibility to increase the nutritional value of the crops by controlling the nutrient solution.

The diffusion in recent years of studies regarding the advanced cultivation techniques, e.g., soilless, and post-harvest treatment of *Eruca*, once more demonstrates the growing importance of this emerging crop in the European economy.

There is an increasing world demand for the amide of erucic acid (erucamide). Hydrogenation of erucic acid with subsequent fractionation yields behenyl alcohol, which is used in cosmetics and was used in large quantities as foam control in machine detergents. Esters and amides of behenic acid are used in lubricants for PVC and antiblocking agents for polyolefin films. Ozonolysis of erucic acid yields brassylic acid used in the manufacture of polyamides (nylon 6/13 and 13/13) and esters, which are used as low temperature plasticizers and as lubricant components. Current world production of erucic acid is 25,000 million t/annum. Consumption of high erucic acid (HEA) in the EU was 40,000 t/annum in 2000 and has increased to 55,000 t/annum by 2005.

The largest current applications for HEA oil are in polymer additives and detergents. As crude oil is biodegradable, it provides an alternative to mineral oil in many industrial applications. It has wide application in the steel casting industry and as a lubricant for chainsaws.

Eruca is a nutritious vegetable, and although not everybody appreciates its characteristic pungent flavor, more attention should be given to its production and improvement. Sufficient genetic variation exists to be able to modify its taste. It also deserves further testing as an oil (edible or industrial) seed crop for drought-prone regions, since it tolerates high salinity and low rainfall conditions.

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Chapter 9
Helianthus

Felicity Vear

9.1 Introduction

The genus *Helianthus* originated in North America and the wild species now found in other continents are almost certainly introductions. Wild forms of *Helianthus annuus,* the species now largely cultivated, are extremely common weeds in many parts of the US. Domestication of this species is generally thought to have been in the US, although there are some suggestions that it could have occurred in Mexico. The other *Helianthus* species have varying distributions in the US, Canada, and Mexico. It should be noted that the first real development of sunflower as an oilseed crop occurred in Russia during the nineteenth and early twentieth century, and the renewed interest in wild *Helianthus*, *H. annuus*, or other species, as a source of variability for breeding cultivated sunflowers is quite recent (the 1970s) .

9.2 Taxonomy (After Seiler and Gulya 2004)

The identification of sunflower species has long been problematic. Heiser et al. (1969) felt that the greatest contribution of his sustained efforts to understand sunflower taxonomy was not providing an easy way to identify sunflowers, but rather an explanation for why they are so difficult. The taxonomic complexity of the genus *Helianthus* stems from many different factors. Natural hybridization and introgression occur between many of the species, often resulting in morphological intergradation between otherwise distinct forms. Polyploidy in the perennial species also contributes to the complexity of species classification in *Helianthus*. This has led to various taxonomic treatments of the genus. There are still specimens, variously of hybrid origin or growing in unusual conditions or incompletely collected, which defy certain placement into a single species. Since many of the species are wide ranging geographically, they exhibit extensive phenotypic variation, which appears to include both heritable and non-heritable (environmental) components. Many species are also genetically quite variable, making rigorous identification and classification difficult.

The genus *Helianthus* has been considered to comprise only ten species to more than 200. Linnaeus described nine species in 1753. Asa Gray (1889) recognized 42 species in North America. In the early twentieth century, Watson (1929) accepted 108 species, 15 of them from South America. Heiser et al. (1969) recognized 14 annual species and 36 perennial species from North America in three sections and seven series, as well as 17 species from South America. Subsequently, Robinson (1979) transferred 20 perennial species of South American *Helianthus* to the genus *Helianthopsis*. The taxonomic classification of *Helianthus* by Anashchenko (1974, 1979) was a radical departure from all previous schemes. He recognized only one annual species, *H. annuus* (with three subspecies and six varieties), and only nine perennial species with 13 subspecies. Schilling and Heiser (1981) proposed an infrageneric classification

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This article is based on reviews and articles by specialists in this field, particularly G. Seiler and C. C. Jan (USDA, Fargo, ND, USA), E. Schilling, Tennessee University, Knoxville, TE, USA, H. Serieys and A. Bervillé (INRA, Montpellier, France), D. Skoric (IFVC, Novi-Sad, Serbia), and M. Christov (DAI, General Toshevo, Bulgaria).

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of *Helianthus*, using phenetic, cladistic, and biosystematic procedures, which place 49 species of *Helianthus* in four sections and six series.

The classification of Schilling and Heiser (1981) is presented here with the following six modifications. First, the sectional name Atrorubens used by Anashchenko (1974) has taxonomic priority; thus, the section Divaricati of Schilling and Heiser is replaced by section Atrorubens Anashchenko. Second, *Helianthus exilis* is recognized as a species as opposed to an ecotype of *H. bolanderi* (Oliveri and Jain 1977). Third, the species name *H. pauciflorus* has priority over *H. rigidus* and is treated accordingly. Fourth, *Viguiera porteri* has been transferred to *Helianthus porteri* (Pruski 1998). Fifth, *Helianthus verticillatus* has been rediscovered and redescribed and is now recognized as a species (Matthews et al. 2002).

Sixth, *Helianthus niveus* ssp. *canescens* has been transferred to *Helianthus petiolaris* ssp. *canescens*. This brings species number to 51, with 14 annual and 37 perennial (Table 9.1).

9.3 Conservation Initiatives (After Seiler and Gulya 2004)

Preservation of wild sunflower species populations is critical because there are insufficient resources to preserve all wild species and locally adapted sunflower populations in seed banks. Furthermore, a significant proportion of wild diversity would likely be lost while regenerating banked germplasm accessions. Unfortunately, the long-term outlook for survival of a number of sunflower species is not promising; some species are already rare and endangered, and *H. nuttallii* T. and G. ssp. *parishii* (A. Gray) Heiser has probably become extinct. Efforts to conserve the wild sunflower species will have to be a combination of preserving some of the species as populations in nature and also collecting of seeds for gene bank preservation.

The collection efforts have resulted in the assemblage of the USDA-ARS wild species collection that is the most complete collection in the world. It is presently located at the National Plant Germplasm System, Plant Introduction Station at Ames, IA. Currently, the wild *Helianthus* collection contains 2,163 accessions, about two-thirds of which are annual species (Brothers and Seiler 2002). The germplasm collection contains seeds or rootstocks from populations of all but one species, *H. laciniatus*, and one subspecies, *H. niveus* ssp. *niveus*, but lacks sufficient populations of many species to be completely representative of the genetic variability in nature. The wild species collection maintained at INRA, Montpellier, France, has more than 600 accessions of 45 wild sunflower species (Serieys 1992). Notable also are the collections at the Institute of Field and Vegetable Crops, Novi Sad, Serbia (39 wild species, Cuk and Seiler 1985), and at the Dobroudja Agricultural Institute (DAI) at General Toshevo, Bulgaria, where 428 accessions represent 37 species of *Helianthus* (Christov et al. 2001).

While progress has been made in the collection and preservation of the wild sunflower species, the present germplasm collection contains only a portion of the available genetic variability in *Helianthus*. Additional populations of several species should be collected; particularly those species that are endangered, threatened, or indigenous to habitats where development is threatening.

9.4 Use of Molecular Markers to Improve Taxonomy (After Schilling 2000 and Sossey-Alaoui et al. 1999)

9.4.1 *Definition of the Genus* Helianthus

Schilling (2000) considered that the systematics of *Helianthus* and its relatives has been largely influenced by the influx of molecular phylogenetic data. An initial molecular data set (Schilling and Jansen 1989; Schilling 1997) was based on restriction site analysis of chloroplast DNA (cpDNA), which was one of the first approaches to have widespread use in plant phylogenetic studies. The resulting tree thus represents a maternal gene tree, because the chloroplast genome is mostly inherited maternally in flowering plants and is rarely if at all subject to recombination. A data set from sequencing of the nuclear ribosomal internal transcribed spacer region (ITS) has also been obtained (Schilling and Panero 1996; Schilling et al. 1998), which provides a second, independent gene tree. The results from cpDNA and ITS analyses are mostly but not entirely congruent.

The availability of molecular data has made it possible to refine with confidence the circumscription of *Helianthus*. As a result, several species or groups of

Table 9.1 Classification of *Helianthus* species (after Seiler and Gulya 2004)

Section-series	Species	(*n*)	Section-series	Species	(*n*)
Helianthus			*Atrorubens Corona-solis*		
	H. annuus L.	17		*H. californicus* DC.	51
	H. anomalus Blake	17		*H. decapetalus* L.	17, 34
	H. argophyllus T.& G.	17		*H. divaricatus* L.	17
	H. bolanderi A. Gray	17		*H. eggertii*	51
	H. debilis			*H. giganteus* L.	17
	ssp. *debilis* Nutt.	17		*H. grosseserratus* Martins	17
	ssp. *cucumerifolius* (T & G) Heiser	17		*H. hirsutus* Raf	34
	ssp. *silvestris* Heiser	17		*H. maximiliani* Schrader	17
	ssp. *tardiflorus* Heiser	17		*H. mollis* Lam.	17
	ssp. *vestitus* (Watson) Heiser	17		*H. nuttallii*	
	H. deserticola Heiser	17		ssp. *nuttallii* T.and G.	17
	H. exilis A. Gray	17		ssp. *rydbergii* (Brit.)Long	17
	H. neglectus Heiser	17		*H. resinosus* Small	51
	H. niveus			*H. salicifolius* Dietr.	17
	ssp. *niveus* (Benth.) Brandegee	17		*H. schweinitzii* T.and G.	51
	ssp. *tephrodes* (Gray) Heiser	17		*H. strumosus* L.	43, 51
	H. paradoxus Heiser	17		*H. tuberosus* L.	51
	H. petiolaris		*Atrorubens Microcephali*		
	ssp. *canescens* (AGray)Schilling	17		*H. glaucophyllus* Smith	
	ssp. *fallax* Heiser	17		*H. laevigatus* T.and G.	34
	ssp. *Petiolaris*	17		*H. microcephalus* T.and G.	17
	H. praecox			*H. smithii* Heiser	17, 34
	ssp. *hirtus* Heiser	17	*Atrorubens Atrorubentes*		
	ssp. *praecox* Englm & AGray	17		*H. atrorubens* L.	17
	ssp. *runyonii* Heiser	17		*H. occidentalis*	
Agrestes				ssp. *occidentalis* Riddell	17
	H. agrestis Pollard	17		ssp. *plantagineus* (T & G)Heis.	17
Porteri				*H. pauciflorus*	
	H. porteri (A. Gray) Pruski (Schilling and Heiser 1981)	17		ssp. *pauciflorus* Stiff	51
Ciliares Pumili				ssp. *Subrhomboides* (Rd) Spring	51
	H. arizonensis R.	17		*H. silphioides* Nutt.	17
	H. ciliaris DC.	34, 51	*Atrorubens Angustifolii*		
	H. laciniatus A.Gray	17		*H. angustifolius* L.	17
Ciliares Pumili				*H. carnosus*	17
	H. cusickii A.Gray	17		*H. floridanus* Gray Chapman	17
	H. gracilentus A.Gray	17		*H. heterophyllus* Nutt.	17
	H. pumilus Nutt.	17		*H. longifolius* Pursh	17
	H. radula (Pursh)T.and G.	17		*H. simulans* E.E.Wats.	17
	H. verticillatus Small (Schilling and Heiser 1981)	17			

species can certainly be excluded from it, and two species of questionable affinities have been included. As now circumscribed, *Helianthus* is geographically coherent, being restricted to North America, but it is not easily diagnosed morphologically. The key feature that classically identifies *Helianthus* within subtribe Helianthinae is the caducous pappus of two awns, with few or no intervening scales. *Helianthus* is also characterized by the pales that are trilobed at the apex. Both these features are homoplasious in subtribe Helianthinae and are found in other groups. Furthermore, they are both absent in one species, now included in *Helianthus, H. porteri*. A third feature that is found throughout *Helianthus* is the well-developed terminal style appendage, but a similar structure is also observed in a few other members of the subtribe.

Molecular data allow confirmation of the placement in *Helianthus* of two species whose affinities were not certain. The placement of one, *H. agrestis*, had been questioned because it has several distinctive traits for *Helianthus*, including tuberculate cypselas and self-compatibility. The other species, *H. porteri*, has been placed in *Viguiera* (or in *Heliomeris* by those who recognize it as distinct) by most botanists. The molecular data, however, agree with chromosome number, crossing behavior, and biogeographic evidence in indicating that *H. porteri* is accurately placed in *Helianthus*. The inclusion of *H. porteri* in *Helianthus* creates a problem in the morphological circumscription of the genus because it lacks two of the three apparent synapomorphies of the genus. The pales of *H. porteri* are not trilobed at the apex, and it entirely lacks a pappus. It does, however, have the prominent terminal style appendage that characterizes *Helianthus*. There are several other members of subtribe Helianthinae that exhibit a similar terminal style appendage, so there is not a single absolute synapomorphy to allow morphological diagnosis of *Helianthus*.

9.4.2 *Divergence Within* Helianthus

The most striking feature of molecular phylogenetic studies of *Helianthus* to date is that there is strikingly little variation within the genus for either ITS or cpDNA. The lack of divergence in particular has limited the conclusions that can be drawn regarding relationships within *Helianthus*. This observation was extended by our recent survey of variation for the *trn*T–*trn*F spacer/intron region. Comparison of *H. porteri*, *H. angustifolius*, and *H. giganteus* revealed only two base pair changes and six insertion/deletion changes from a total of 1,417 bases that were sequenced for this region of the cpDNA genome. By contrast, studies of this gene region in other plant families have revealed extensive variation, sometimes even within a single species. The incongruence between results from cpDNA and ITS data sets for relationships within *Helianthus* is not readily explainable, although it may reflect past hybridization between different lineages. The cpDNA results would suggest a relatively straightforward division of the genus into four main lineages, with *Phoebanthus* as part of the perennial group. The ITS data suggest a more complex scenario in which the perennial species are paraphyletic to the rest of the genus, based on placement of *H. heterophyllus* and *H. carnosus* as basally branching clades. In the ITS tree, *Phoebanthus* is placed as the sister group to *Helianthus* rather than as part of the perennials.

Neither the tree from cpDNA nor the one based on ITS is in accord with the current infrageneric classification of *Helianthus* of Schilling and Heiser (1981). Section *Helianthus*, which contains the bulk of the annual species including *H. annuus*, is shown to be monophyletic in both trees. Both trees also place the peculiar annual species, *H. agrestis* and *H. porteri*, as distinctive lineages. In neither tree is *H.* sect. *ciliares* shown to be monophyletic. For the most part, however, there are few differences among the perennial species from either data set. The lack of variation leaves unresolved the relationships of the polyploid species of *Helianthus*, all of which are perennial. It has not been possible even to identify the maternal genome of any of the polyploids. The only exception to this involves the sterile triploid, *H.* × *multiflorus*, in which it has been possible to show that the cpDNA genome is from a polyploid species and that it contains rDNA genomes from both *H. annuus* and a perennial species. Thus, further work is needed to identify molecular markers that show the appropriate, high level of variation that will be needed to resolve relationships of the polyploid species of *Helianthus*.

It is potentially notable that the basally diverging clades of *Helianthus* in the ITS tree all are species of the extreme southeastern US with relatively narrow geographic distributions. This suggests the hypothesis that after migration to North America from a probably Mexican origin, the ancestral lineage to *Helianthus* may have been limited during glaciation events to areas near the southeastern coastline. Thus, *Phoebanthus, H. carnosus*, *H. heterophyllus*, *H. agrestis*, and *H. porteri* may represent relictual species from one or more early rounds of divergence in *Helianthus*. The subsequent migration from the southeastern US across much of North America and differentiation to form the current suite of species may have been relatively recent.

Using nuclear DNA markers, annual and perennial species have been found to differ in their genomic constitution. Sossey-Alaoui et al. (1999) reported studies of random amplified polymorphic DNA (RAPD) on 36 *Helianthus* species. Of the 118 RAPD

fragments analyzed, 33 were common to all *Helianthus* species, 29 were unique to section Helianthus, and 56 to perennial species, of which 24 were only found in the section Atrorubentes. None were specific to the Ciliares section. Phenetic trees constructed using Jaccard distances and Sokal and Michener similarity were very similar to the current taxonomy. Each set of common or specific fragments was assumed to belong to a genome (1) the C-genome carrying the fragments common to all species of the three sections, (2) the H-genome unique to sect. Helianthus, (3) the P-genome common to perennial species (sects. Atrorubentes and Ciliares), and (4) the A-genome unique to sect. Atrorubentes. The genomic structure was therefore HC for sect. Helianthus, CPA for sect. Atrorubentes, and CP for sect. Ciliares. Molecular hybridizations with amplification products revealed homologies between *Helianthus* genomes and several other genera in the Helianthinae subtribe.

9.5 Hybridization (After Christov et al. 1996; Faure et al. 2002; Jan et al. 2008)

Christov et al. (1996) reported that the progenies of interspecific hybrids could be characterized by extremely wide formative processes, which could give rise to new characters in sunflower. With annual species, the F_1 hybrids may be very vigorous (an example is shown in Fig. 9.1) and may give sufficient seed set, and this is often true also when further generations are backcrossed to cultivated sunflower, sibcrossed, or even selfed. In contrast, there are difficulties with producing the second generation of hybrids obtained from perennial species because of partial or total sterility of F_1 plants. It is useless to self-pollinate them, and most will not give seed even when open-pollinated. These authors found that pollination with cultivated sunflower pollen is the most successful procedure to overcome this barrier. Pollen may be collected from several different lines or varieties and this is mixed with pollen from the interspecific F_1 plants, which are pollinated with this mixture. This operation can be repeated over several generations. The disadvantage is that the genotype of the pollen that gives seed is unknown.

The poor crossability and frequent F_1 sterility in interspecific hybrids limit the usefulness of many wild *Helianthus* species. Abortion of the hybrid embryo is one mechanism that prevents interspecific hybridization. An embryo rescue technique has facilitated the success of several interspecific crosses (Chandler and Beard 1983).

Sterility associated with meiotic abnormalities of F_1s can be decreased by doubling chromosomes using colchicine (Jan and Chandler 1985). Colchicine treatment of interspecific F_1 hybrids resulted in high frequencies of chromosome doubling and the production of amphiploids (Jan and Fernández-Martínez 2002). The tetraploid amphiploids produced included crosses of sunflower × *H. bolanderi*, *H. gracilentus* × sunflower, *H. grosseserratus* × sunflower, and *H. cusickii* × sunflower. These amphiploids had restored fertility and provided easily available genetic diversity for the improvement of cultivated sunflower. The first hexaploid amphiploids in sunflower were produced from crosses of *H. hirsutus* × sunflower and *H. strumosus* × sunflower. These interspecific amphiploids should enable the establishment of a number of chromosome addition lines for genetic studies of specific chromosomes of both cultivated and wild *Helianthus* species.

The low fertility associated with some interspecific crosses presents a serious impediment to hybridization, but such infertility sometimes can be overcome by using different populations of species for crossing. Some populations of a species have variable pollen staining indicating differences in pollen fertility (Rieseberg et al. 1995). Another problem observed

Fig. 9.1 *Helianthus argophyllus* (*on the left*) and hybrids between this species and cultivated sunflower (*on the right*) (photo INRA Clermont-Ferrand)

in interspecific crosses is achene dormancy. A very useful technique using achene scarification and a low level of growth hormone (GA3) has increased germination to 70% in interspecific crosses as well as in wild species populations, especially perennial species (Chandler and Jan 1985).

Crosses between the cultivated sunflower and annual species belonging to the section *Helianthus* have been produced without difficulty, but the resulting progenies are more or less male-sterile due to translocations (Quillet et al. 1995). In such crosses – mainly performed for breeding – sunflower is usually used as the female parent, unless the objective of the study is to transfer the cytoplasm of the wild form (Serieys 1999). Crosses between sunflower and perennial species belonging to section Atrorubentes are more difficult to perform except when the male is polyploid, such as Jerusalem artichoke (*H. tuberosus*). Crosses with the diploid species *H. occidentalis*, *H. maximiliani*, and *H. mollis* are difficult to obtain. As an example, when Faure et al. (2002) crossed cultivated sunflower with *H. mollis*, 62 plants were obtained naturally from mature seeds, 22 others following embryo rescue procedures, and only 22 from the reciprocal cross following natural seed development. All of the resulting plants were found to be diploid $2n = 34$ but reciprocal crosses led to different progenies, with phenotypes that were predominantly similar to those of the female parent, sunflower or *H. mollis*, especially after natural seed development. The embryo rescue procedure enhanced the level of partial hybridization, determined using RAPD or restriction fragment length polymorphism (RFLP) markers but with a maximum of 8.5 markers from the male parent where normal hybridization should have given 25. Figure 9.2 shows an F_4 plant selected from this cross with sessile leaves characteristic of *H. mollis*.

9.6 Economic Value of Wild *Helianthus* Species (After Seiler and Gulya 2004; Seiler 2002; Skoric 1992)

Wild species have contributed many agronomically important traits to cultivated sunflower. The estimated economic value of the contribution of the wild species to cultivated sunflower is $384 million per year (Prescott-Allen and Prescott-Allen 1986). Another estimate is $269.5 million per year (Philips and Meilleur 1998). The greatest value is derived from the PET1 cytoplasmic male sterile (CMS) cytoplasm from *H. petiolaris* (Fig. 9.3). The main characters introduced into cultivated sunflower from wild *Helianthus* are the following:

9.6.1 Cytoplasmic Male Sterility

Cytoplasmic male sterility (CMS) is a maternally inherited trait preventing plants from producing normal pollen. It is used to generate F_1 hybrids in sunflower, which is now the second largest hybrid crop in the world. Almost all the hybrids in the world use a single cytoplasm derived from wild *H. petiolaris* (Leclercq 1969). Several research programs generating alloplasmic combinations have identified 62 new

Fig. 9.2 F_4 plant from a cross between sunflower and *Helianthus mollis* (photo INRA, Montpellier)

Fig. 9.3 *Helianthus petiolaris* (photo Clarence A. Rechenthin @ USDA-NRCS PLANTS Database)

sources of male sterile cytoplasms, 40 annual and 12 perennial (Serieys 1999), and in most cases, the necessary fertility restoration genes have been obtained from the same species. However, all these cytoplasms are mostly used only in experimental crosses or are kept in reserve, in case of a problem with the PET1 cytoplasm.

9.6.2 Disease Resistance

Wild sunflower species have been one of the few sources of resistance genes for many of the common pathogens of cultivated sunflower. *H. annuus, H. petiolaris*, and *H. praecox* are the major sources of genes for Verticillium wilt (*Verticillium dahliae*) resistance (Hoes et al. 1973). These species plus *H. argophyllus* are also the major sources of resistance genes for downy mildew (*Plasmopara halstedii*) and rust (*Puccinia helianthi*) in cultivated sunflower (Quresh et al. 1993). Resistance genes for these pathogens occur frequently in the wild annual species (Tan et al. 1992). Resistance to broomrape (*Orobanche cernua*) has been observed in most of the wild perennial species (Jan and Fernandez-Martinez 2002) and annual *H. anomalus*. Phoma black stem (*Phoma macdonaldii*) resistance has been reported in several perennial species including *H. decapetalus, H. resinosus*, and *H. tuberosus* (Skoric 1985). Phomopsis stem canker (*Diaporthe helianthi*) resistance has also been found in perennials such as *H. maximiliani, H. pauciflorus,* and *H. hirsutus* (Skoric 1985). Alternaria leaf spot (*Alternaria helianthi*) resistance was observed in perennials *H. hirsutus, H. pauciflorus*, and *H. tuberosus* (Morris et al. 1983). Sclerotinia (*Sclerotinia sclerotiorum*) head rot tolerance was observed in many perennials such as *H. resinosus* and *H. pauciflorus* (Mondolot-Cosson and Andary 1994; Ronicke et al. 2004). Sclerotinia root and mid-stalk rot tolerance was observed in perennials including *H. resinosus* and *H. tuberosus* (Skoric 1987; Köhler and Friedt 1999).

9.6.3 Insect Resistance

Wild sunflowers are native to North America where their associated insect herbivores and entomophages coevolved in natural communities, making it logical to search for insect resistance genes in the diverse wild species. Sunflower moth (*Homoeosoma electellum*) tolerance was observed in annual *H. petiolaris* and perennials such as *H. maximiliani* and *H. ciliaris* (Rogers et al. 1984). Stem weevil (*Cylindrocopturus adspersus*) tolerance was found in perennials including *H. grosseserratus*, *H. hirsutus*, and *H. salicifolius* (Rogers and Seiler 1985). Sunflower beetle (*Zygogramma exclamationis*) tolerance was observed both in annuals *H. agrestis* and *H. praecox* and in many perennial species (Rogers and Thompson 1980).

9.6.4 Oil Quality

Reduced concentrations of saturated palmitic and stearic fatty acids have been observed in a population of wild *H. annuus*, which had a combined palmitic and stearic acid concentration of 58 g kg^{-1} (Seiler 1998), this is 50% lower than in oil of cultivated sunflower. A combined palmitic and stearic acid concentration of 65 g kg^{-1} was observed in a wild perennial species, *H. giganteus* (Seiler and Brothers 1999).

9.6.5 Salt and Drought Tolerance

Several species of *Helianthus* are native to salt-impacted habitats. Interspecific germplasm derived from *H. paradoxus* has been identified with high salt tolerance withstanding salt concentrations up to EC 24.7 dSm^{-1}. It appears that one major gene controls salt tolerance, although a modifier gene may also be present, possibly recessive in control. Evaluation of 19 perennial and one annual wild sunflower species indicated that perennial species had higher diffusive resistance, transpiration, and stomatal densities than annual species (Seiler 1983). In all perennial species, stomatal densities were higher on the bottom surface than the top surface, similar to cultivated sunflower, while it was opposite in the annual species. Blanchet and Gelfi (1980) evaluated stomatal resistance, leaf-water potential, photosynthetic activity, leaf structure, and number of stomata. They concluded that *H. argophyllus* is the most likely source of drought tolerance because its pubescent leaves reflect sunlight, reduce water loss, and exhibit low transpiration rates. Serieys

(1991) selected interspecific pools with *H. argophyllus* (Fig. 9.3) for increased and reduced transpiration.

9.6.6 Herbicide Tolerance

A population of wild *H. annuus* from a soybean field in Kansas that had been repeatedly treated with imazethapyr developed resistance to the imidazolinone and sulfonylurea herbicides (Al-Khatib et al. 1998), which may also control broomrape in areas of the world where this parasitic weed attacks sunflower. This resistance has been introduced into cultivated sunflower (Al-Khatib and Miller 2000). Table 9.2 presents a summary by Christov (2008) of the species, which could be of use in breeding sunflowers for many characters.

9.7 Problems with Wild *Helianthus* Species (After Muller et al. 2008)

In North America, where weedy sunflowers occur quite widely, they can decrease substantially yields of corn and soybean (Kane and Rieseberg 2008). Moreover, the potential intercrossing between cultivated and weedy sunflower raises the question of the transfer of advantageous crop traits to the weeds (Mercer et al. 2007), which can contribute to the evolution of more aggressive weeds. Weedy forms morphologically close to American wild *H. annuus* have been observed in France and other European countries in recent years. Weedy sunflowers affected around 15% of sunflower fields in the area studied and caused yield losses that reached 50% in strongly infested patches. Until now, the only available method to control these weeds is mechanical weeding at the very beginning of the infestation of a field.

These wild *H. annuus* weeds probably came from pollution of basic and hybrid seed production in North America, but it is very important that institutes maintaining wild *Helianthus* collections around the world take all the necessary precautions such that there are no escapes or pollution of cultivated sunflower by wild sunflowers, which is difficult to control in these crops.

9.8 Conclusions

Seiler and Gulya (2004) concluded that wild *Helianthus* species have been and continue to be an invaluable source of new genes for the improvement

Table 9.2 Sources of new characters transferred into cultivated sunflower (from Christov 2008)

Character	Species
Resistance/tolerance to:	
Plasmopara helianthi	*H. annuus* (w.f.), *H. argophyllus*, *H. bolanderi*, *H. debilis*, *H. exilis*, *H. neglectus*, *H. paradoxus*, *H. petiolaris*, *H. praecox*, *H. divaricatus*, *H. doronicoides*, *H. giganteus*, *H. glaucophyllus*, *H. grosseserratus*, *H. mollis*, *H. maximiliani*, *H. microcephallus*, *H. multiflorus*, *H. nuttallii*, *H. occidentalis*, *H. orgialis*, *H. pumilus*, *H. salicifolius*, *H. smithii*, *H. decapetalus*, *H. hirsutus*, *H. laevigatus*, *H. scaberimus*, *H. tomentosus*, *H. eggertii*, *H. californicus*, *H. ciliaris*, *H. pauciflorus*, *H. resinosus*, *H. strumosus*, *H. tuberosus*, *H.* × *laetiflorus*
Phomopsis helianthi	*H. annuus* (w.f.), *H. argophyllus*, *H. debilis*, *H. eggertii*, *H. pauciflorus*, *H. glaucophyllus*, *H. laevigatus*
Erysiphe cichoracearum	*H. decapetalus*, *H. laevigatus*, *H. glaucophyllus*, *H. ciliaris*
Orobanche cumana	*H. tuberosus*, *H. eggertii*, *H. smithii*, *H. argophyllus*, *H. pauciflorus*, *H. strumosus*
Phoma helianthi	*H. argophyllus*, *H. laevigatus*, *H. eggertii*, *H. debilis*
Sclerotinia sclerotiorum	*H. praecox*, *H. argophyllus*, *H. annuus* (w.f.), *H. petiolaris*, *H. eggertii*, *H. pauciflorus*, *H. smithii*
Earliness	*H. praecox*, *H. scaberimus*, *H. glaucophyllus*, *H. giganteus*, *H.rigidus*, *H. nuttallii*, *H. ciliaris*, *H. annuus* (w.f.)
Seed size	*H. annuus* (w.f.), *H. argophyllus*, *H. tuberosus*, *H. strumosus*
High oil content	*H. annuus* (w.f.), *H. debilis*, *H. petiolaris*, *H. praecox*, *H. pauciflorus*, *H.* × *laetiflorus*
Genes controlling CMS	*H. annuus* (w.f.), *H. argophyllus*, *H. debilis*, *H. petiolaris*, *H. praecox*, *H. pauciflorus*, *H. strumosus*
Rf genes	*H. annuus* (w.f.), *H. argophyllus*, *H. bolanderi*, *H. debilis*, *H. exilis*, *H. neglectus*, *H. paradoxus*, *H. petiolaris*, *H. praecox*, *H. divaricatus*, *H. doronicoides*, *H. glaucophyllus*, *H. giganteus*, *H. grosseserratus*, *H. maximiliani*, *H. microcephallus*, *H. mollis*, *H. multiflorus*, *H. nuttallii*, *H. occidentalis*, *H. orgialis*, *H. pumilus*, *H. salicifolius*, *H. smithii*, *H. decapetalus*, *H. hirsutus*, *H. laevigatus*, *H. scaberimus*, *H. tomentosus*, *H. eggertii*, *H. ciliaris*, *H. resinosus*, *H. pauciflorus*, *H. strumosus*, *H. tuberosus*, *H. californicus* and *H.* × *laetiflorus*

of cultivated sunflower. The majority of gene transfers carried out so far have been from the annual *Helianthus* species, which comprise only a third of the species of the genus. Exploiting the genetic diversity of the wild perennial *Helianthus* species will present more of a challenge than the annual species for a number of reasons, the most important being the different polyploidy levels, which complicate the production of fertile seed in crosses with diploid cultivated sunflower.

Compared with many cereal crops, breeding of sunflowers is quite a recent technology. There was a "bottle neck" due to the limited variability of the sunflowers which reached Russia and which were the basis of the present day oil producing crop. However, eastern Europe became a secondary area of diversification of cultivated sunflowers. As soon as there was a renewal of breeding with the development of hybrids, it was realized that the existence of a large number of species within the *Helianthus* genus, present in North America, close to and/or easily available to many breeders and research institutes was an extremely valuable resource. The research effort compared with that on maize, rice, or wheat is small, but the existence today of about 50 *Helianthus* species should facilitate improvement in the sunflower crop compared with these species where close relatives either no longer exist, are difficult to collect, or have already been largely exploited. Sunflower breeding is still relatively young, but the *Helianthus* genus should assure its success for a long period in the future.

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Chapter 10
Hirschfeldia

Johannes Siemens

10.1 Basic Botany of the Species

Hirschfeldia Moench is a small genus taxonomically close to *Erucastrum* C. Presl and consists of *Hirschfeldia incana* (L.) Lagréze-Fossat and two very localized species in North Africa and Socotra (Gómez Campo 1993; Gómez-Campo and Martinez Laborde 1998). The genus is under constant discussion and some former species of the genus are already allocated to other genera and renamed. Snogerup and Snogerup (2002) recently proposed to dispose the name *Hirschfeldia* due to synonymy with *Brassica geniculata* as the correct name for *H. incana*. In an overview about the phylogeny of the Brassicaceae, Al-Shehbaz et al. (2006) also stated serious problems relating to generic boundaries in the tribe Brassiceae and recommended a change in nomenclature regarding several genera among *Brassica*, *Sinapis*, and *Hirschfeldia*. Until now, such a nomenclatural change has not generally been accepted and it will not be followed in this deliberation. Most specimens are collected and classified under the name *H. incana* (L.) Lagréze-Fossat, and in most of the recent literature, this name has been used.

H. incana is a thermophilous and nitrophilous annual to biennial crucifer up to 1.5 m tall, usually divaricately branched with the potential to turn to a tumble-weed with high individual seed production. The plant shows certain variability in hairiness and size of siliqua and beak. *H. incana* is a widespread annual occupant of waste habitats in the Mediterranean region extending to Southwest Asia. Beyond these regions, it has spread as an often casual adventive to warm–temperate regions, e.g., it grows as an introduced weed in southern Australia and New Zealand.

H. incana belongs to the tribe Brassiceae and has a somatic chromosome number of 14 ($n = 7$). Hoary mustard is self-incompatible due to protogyny (Al-Shehbaz 1977) and *S*-locus-related (SLR) genes control late pollen adhesion (Darmency and Fleury 2000; Luu et al. 2001), although some plants have some ability to self-fertilize (Lee et al. 2004). Furthermore, cytoplasmic male sterility occurs regularly in wild populations of this annual crucifer. In these plants, numbers of seed per fruit and total seed weights per plant are slightly higher than in the hermaphrodites (Horovitz and Beiles 1980).

Now, *H. incana* is regarded mostly as a weed or as a plant with a potential use in phytoremediation. In ancient times, it had probably been used as a wild herb. Dioscorides mentions a wild pot herb called *lapsane* by the Greeks and *napicium* by the Romans and says both leaves and stalks were eaten. Pliny refers to *lapsana* as a sort of wild cabbage. It seems probable that the *lapsana* of the ancients is the hoary mustard, *H. incana*, a common wild plant found around the Mediterranean Sea, where, even today, the young sprouts are gathered in spring, boiled in water, and seasoned with olive oil and lemon juice to make a tasty dish (Andrews 1942). However, since the times of Dioscorides, *H. incana* certainly remained as a wild plant of no economic importance, belonging to the category of wild herbs used by country people as supplementary food.

10.2 Conservation Initiatives

Regarded as a weed, there are no comprehensive attempts of in situ and ex situ conservation or large collections of genetic material of *H. incana*. All

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germplasm banks have some specimens, and several specimens are also stored in the botanic gardens, especially in the Mediterranean region.

10.3 Role in Elucidation of Origin and Evolution of Allied Crop Plants

Sikka and Sharma (1979) performed chromosome studies on 28 species and varieties belonging to 18 genera including *Brassica* and *Hirschfeldia* of the tribe Brassiceae, which is characterized by a graded karyotype with medium to short chromosomes and constrictions mainly median to submedian in position.

These studies were improved by analysis of meiotic chromosome pairing in interspecific hybrids of the genus *Brassica* with each of the following genera: *Diplotaxis*, *Eruca*, *Erucastrum*, *Hirschfeldia*, *Hutera*, *Sinapis*, and *Sinapidendron* (Harberd and McArthur 1980). Introgression studies over several generations with *B. napus* and five related species (*B. oleracea, H. incana, Raphanus raphanistrum, B. nigra*, and *S. arvensis*) obtained by embryo rescue provided further information about *Hirschfeldia* chromosomes (Chevre et al. 1991). Ali et al. (2005) has studied rRNA-gene localization on chromosomes as a structural marker on chromosomes of 45 species in the tribe Brassiceae including *H. incana*.

The in situ hybridization studies and the introgression studies provided tools to analyze the karyotype of *Hirschfeldia*; the latter additionally proved the potential of *H. incana* as a gene donor for the crop plants in the genera *Brassica, Raphanus*, and *Sinapis*.

However, the phylogenetic analysis was highly enhanced by the analysis of chloroplast genomes in the family Brassicaceae (Warwick and Black 1991, 1993; Warwick and Sauder 2005; Koch et al. 2007).

The diversity in the chloroplast genomes analyzed by restriction fragments indicated a clear division of the subtribe into two ancient evolutionary lineages, namely the "Nigra" lineage including *H. incana*, and the "Rapa/Oleracea" lineage. The genera *Coincya*, *Hirschfeldia*, and *Sinapidendron* were monophyletic within the "Nigra" lineage. A high level of congruence was observed between recognized cytodems or crossing groups in the subtribe and the clusters defined by the cpDNA data. Levels of genetic divergence suggested by the cpDNA provided evidence for inconsistencies in the current generic delimitations based on morphology (Warwick and Black 1991, 1993). For example, the chloroplast DNA data also indicated the close genetic relationship of *Sinapis pubescens* to *H. incana*. On the basis of their data, morphological similarities, and prior taxonomic treatments, Warwick and Black (1993) proposed renaming *H. incana* as a subspecies in the genus *Sinapis*. In contrast, Koch et al. (2007) differentiated *Hirschfeldia* and *Sinapis* species by chloroplast genome analysis based on variation of *trn*F pseudogenes and of the *trn*L-F intergenic spacer regions. The phylogenetic distribution of microstructural changes for the *trn*L-F region supports ancient patterns of divergence in crucifer evolution for some but not all gene loci. Current phylogeny of tribe Brassiceae (Brassicaceae) based on sequences data is summarized by Warwick and Sauder (2005).

10.4 Role in Development of Cytogenetic Stocks and Their Utility

As already mentioned, *H. incana* has been introduced in programs for interspecific crossing with the genus *Brassica* (Harberd and McArthur 1980; Chevre et al. 1991). But instead of focusing on introgression as a breeding tool, most of the subsequent studies of interspecific hybrids with *H. incana* attempted to quantify the agricultural and ecological risks of transgenic crops in European *B. napus* production areas, focusing on the possible spread of transgenic crops and the spread of the target transgene through hybridization (Lefol et al. 1996; Chevre et al. 1997, 1998, 1999, 2001, 2003; Chadoeuf et al. 1998; Wei and Darmency 2008). Concomitantly, these studies pinpoint the bottleneck in the hybridization approach for breeding. Genetic stability of the resistance traits in the genetic background of the crop plant and within the subsequent backcross generations is the crucial characteristic for an introgression of new genes in the genepool of crop plants. Furthermore, as a side effect of these studies, quite a large collection of hybrids and recurrent backcross generations with the potential of addition and substitution lines have been established and analyzed in the Institut Scientifique de Recherche

Agronomique (INRA, Rennes, France). However, the potential of these lines for introgression of useful traits from *H. incana* into crop plants remains to be published in detail.

10.5 Role in Classical and Molecular Genetic Studies

H. incana is used as parent in interspecific (Harberd and McArthur 1972) and intergeneric crosses (Chevre et al. 1991; Plümper 1995), but data regarding classical and molecular genetic linkage maps are not published in detail. Most studies characterized physiological traits like resistance to heavy metals (Poschenrieder et al. 2001; Gisbert et al. 2006, 2008) or host–parasite interactions (Roland 1952; Minz 1954; Salisbury 1987; Fernandez Garcia 1988; Plümper 1995; Paz et al. 1997; Scholze and Hammer 1998; Wheeler and Hoebeke 1999; Fletcher 2001; Shapiro 2006; Kavak et al. 2007) under natural ecological conditions collecting plants in areas with abiotic or biotic stress conditions. A classical genetic analysis or a molecular genetic linkage map correlated to these traits is not published. Physiological traits like sterol composition (Tzakou et al. 1993) or glycosinolates composition (Sang and Salisbury 1987; Belkhiri and Lockwood 1994) are characterized in a small number of plants and no variation within the species has been described. However, phytosociological studies indicate variation within the species (Chronopoulos et al. 2005).

10.6 Role in Crop Improvement Through Traditional and Advanced Tools

Plümper (1995) has screened a large collection of wild relatives of *B. napus* for resistance to the important fungal pathogens *Leptosphaeria maculans*, *Alternaria brassicicola, A. brassicae,* and *A. raphani*. A similar screening of a large collection of wild relatives of *B. napus* has been extended to the protist *Plasmodiophora brassicae* (Scholze and Hammer 1998). In both studies, *H. incana* lines revealed to be resistant to *L. maculans*. Successful hybridizations with *B. napus* have been achieved using *H. incana*. The hybrids showed resistance to *L. maculans* in a cotyledon test and have been successfully backcrossed to *B. napus* (Plümper 1995). However, the resistance was not characterized genetically, and further studies were stopped due to the development of more promising resistance phenotypes against blackleg (*L. maculans*) and blackspot diseases (*Alternaria brassicae* and *A. brassicicola*) in hybrids of *Brassica elongata, Sinapis alba, Diplotaxis tenuifolia*, and *Diplotaxis erucoides* with *B. napus* (Klewer 2005). Resistance to *A. brassicae* and *A. brassicicola* of *D. erucoides* has been characterized by interspecies cross and is inherited as monogenic dominant trait (Klewer 2005).

However, these intergeneric hybrids point to the possibility of introgression from *H. incana* into crop plants. They clearly stress the value of introgression from wild relatives (Siemens 2002). Other studies regarding resistance to abiotic or biotic stress might be reflected under this aspect.

H. incana is described as a host for the cucumber mosaic virus (Fletcher 2001), potato Y potyvirus (PVY) (Paz et al. 1997), and cabbage black ring spot virus (Roland 1952). The latter virus infection causes only very weak symptoms in *H. incana* (Roland 1952). Hoary mustard has been described as spring host of *Pieris rapae* (Fernandez Garcia 1988) and as an oviposition substrate for *Phulia nymphula* (Shapiro 2006). The scentless plant bug *Rhopalus tigrinus* (Wheeler and Hoebeke 1999) and the root-knot nematode *Meloidogyne* sp. (Minz 1954) have been found on *H. incana*. Kavak et al. (2007) described a heavy infection with *Albugo candida* on hoary mustard. In all mentioned studies, no variation of *H. incana* has been described. In contrast, resistance to blackleg disease caused by *Leptosphaeria maculans* has been described by several authors in different material (Salisbury 1987; Plümper 1995; Scholze and Hammer 1998), indicating that a screen for variation in this species can be precious.

In regard to abiotic stress, *H. incana* is described as pseudometallophytes due to the ability of the species to grow under extreme Cu-toxicity conditions and on highly contaminated soils (Poschenrieder et al. 2001; Gisbert et al. 2006, 2008). The potential usefulness of this trait for phytoremediation but not for introgression into crop plants has been discussed. There is great interest in the identification of autochthonous plant species capable of accumulating

elevated amounts of heavy metals in their tissues, with the aim of employing them for phytoremediation of contaminated soils. *H. incana* is the dominant species in many metal-contaminated sites in Spain and is one possible candidate. The species is able to support the extreme Cu-toxicity conditions on soils with 5–16.8 mg g^{-1} extractable Cu and shows high shoot/root Cu ratio. Furthermore, *H. incana* can grow in soils with concentrations of heavy metals such as Pb, Zn, Cu, Cd, and arsenic (As). Rio-Celestino et al. (2006) analyzed the phytoextraction efficiency of plant species to estimate their suitability for remediation of Pb and Zn polluted soils. *H. incana* was highly effective in removing Pb and Zn from the contaminated soil, when the biomass values were taken into account. Together with *Bassia scoparia* (Chenopodiaceae), *Inula viscosa* (Asteraceae), and *Solanum nigrum* (Solanaceae), *H. incana* (Brassicaceae) also had the highest values for arsenic accumulation (Gisbert et al. 2008). Furthermore, *H. incana* showed thallium accumulation in the shoots and especially in the flowers, which might be due to high concentrations of nitrogen and also high values of P, S, and K in the flowers (Madejon et al. 2005, 2007).

None of these physiological traits of *H. incana* have been genetically analyzed; none have been used for introgression either. In fact, these traits are discussed as phytoaccumulation risks for crop species in those soils. Thus, a risk would arise if these soils, or those nearby with similar plant-available heavy metal levels, were cultivated or used for livestock grazing.

H. incana showed a high accumulation of metals in shoots, which could make it interesting for phytoremediation of contaminated soils. The results also suggest that *H. incana* may be useful as an indicator of soil heavy metal contamination, as the metal concentrations in the aerial part reflect the soil concentrations (Gisbert et al. 2008).

10.7 Genomics Resources Developed

The genus *Hirschfeldia* is clearly underrepresented in databases. Molecular studies are rare, and consequently, sequence data are also limited. Genetic variation is described (Lee et al. 2004) but not collected intentionally. Therefore, the specimens in the germplasm banks will probably not show very high genetic diversity.

10.8 Scope for Domestication and Commercialization

Since the times of Dioscorides, hoary mustard was a side dish in traditional food gathering. However, *H. incana* was included in a study to estimate alternative crops (Branca 1995). Yields were high and quality of the edible parts was satisfactory, particularly with regard to mineral and ascorbic acid contents. Wild Brassicaceae species could offer a significant contribution to vegetable diversification.

10.9 Some Dark Sides and Their Addressing

H. incana is integrated into many studies regarding outcrossing of transgenes as consequences of commercial release of transgenic crops, especially oilseed rape (*B. napus*). *B. napus* is partially allogamous and presents numerous wild relatives growing nearby in cultivated areas and having an overlapping of the flowering period. In principle, transgenes can escape via seeds and volunteer rape, and seeds of interspecific hybrids between rape and wild relatives can survive and germinate after several years, ensuring genetic and spatial spread of transgenes (Chadoeuf et al. 1998; Devos et al. 2009). Spontaneous backcrossing with hoary mustard has been found. The performance of the hybrids indicated that hybrids and hoary mustard would not be better competitors against oilseed rape, but hybrids might be more competitive in a hoary mustard population (Lefol et al. 1995). Field experiments have been carried out under natural conditions to develop and validate gene flow models. Experiments under normal agronomic conditions have revealed a very low frequency of hybrids (Chevre et al. 1997, 1999, 2001, 2003). In summary, under European agricultural conditions, *H. incana* do not exhibit a supposable potential for superweed due to gene flow from transgenic crops.

10.10 Recommendations for Future Actions

As a non-crop plant, the genus *Hirschfeldia* is not associated with farmers' rights and IPR-related issues. Like many other plants, hoary mustard is a neglected plant and has been displaced by more high-yielding crop plants. Besides all other suggestions for neglected plants, it might be a good attempt to encourage people to collect wild species as side dishes like it is a fashion in autumn seasons with fungi. Introducing an attitude as a pleasant and tasteful fashion for several thousand people might be more effective to stress the importance of natural diversity and creating awareness than negotiations about in situ conservation and collection of germplasm. However, in populated areas, *H. incana* might be found in rural spots, presumably with heavily contaminated soils and, therefore, it cannot be regarded as healthy natural food. Therefore, hoary mustard can only be a minor exponent for this movement of care and preservation of ancient food.

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Chapter 11
Linum

Christopher Cullis

11.1 The Basic Botany of the Genus

The family Linaceae is comprised of 22 genera of which genus *Linum* is the most well known. The genus *Linum* is widely distributed mostly throughout the temperate regions of the world and a few species extend into subtropical areas. Most species are found in the Northern Hemisphere, although there are 14 mainly shrubby species with small flowers that are found in the Cape region of South Africa and one similar species in New Zealand (Strange and Rix 2007). Their habitat is rather open on rocks or well-drained calcareous or sandy soils. Flax is fastidious about the nutrients in the soil and is sensitive to the lack of boron. Flax thrives on medium and light weakly podzolized loamy soils with pH of 5–6. *Linum* probably originated in an area in Northwest India, from where it spread northward, westwards and southwards to Europe and Russia and Ethiopia. Currently, the main centers of diversity of the genus are the Mediterranean area, the Fertile Crescent, Ethiopia, and India. Various estimates of the number of species in this genus vary from 150 to more than 200 (Flora Europaea 1968; Strange and Rix 2007). The species present in the genus *Linum* are divided in five subsections (McDill 2009); *Linum*, *Dasylinum*, *Syllinum*, *Cathartolinum*, and *Linastrum*. The taxonomic characters used to define these sections include morphological characters such as flower color, the presence/absence of stipular glands, trichome distribution, presence/absence of staminodia, and leaf arrangement. Some of the relationships can be reexamined using new molecular data. Preliminary data indicate the separation between the blue-flowered species and the yellow-flowered species (McDill 2009).

In Europe, *Linum* contains 36 species, covering the five sections. The commonest is the small annual, *Linum catharticum* L. which has white flowers and opposite leaves. It is the only species in section *Cathartolinum* in Europe, but most of the eight species from eastern North America belong to this section. The species from western North America are in the section *Hesperolinon*. The section *Linum* includes the blue-flowered species *L. usitatissimum* L. (the cultivated common flax) and the cultivated ornamental perennials *L. narbonense* L. and *L. perenne* L. This section also includes about 12 species in Europe. The section *Dasylinum* (Planchon) Juz. contains four European species, including the pink-flowered *L. viscosum* L. and *L. pubescens* Banks & Solander; this section is characterized by its pubescent leaves. The section *Linastrum* (Planchon) Bentham, which contains yellow-, pink- and white-flowered species, mostly annuals like the yellow-flowered *L. maritimum* L is less significant as far as horticulture is concerned.

11.1.1 Morphology

The general characteristics of the Linaceae are:

- Leaves are alternate (rarely opposite) and simple
- Stipules are small or absent
- Flowers are bisexual and regular
- Sepals are either free or united at the base, imbricated, persistent, and usually number 5 (but on rare occasions 4)
- Petals are of the same number as sepals with which they alternate, imbricated, and convoluted

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- Stamens are of the same number and alternate with petals. The filaments are monadelphous and often have a glandular base
- Ovary has 2–5 carpels but appear to be 4–10-celled because of the intrusion of false septa, with 1–2 ovules
- Style number the same as ovule number

The *Linum* species are annual or perennial herbs. They are usually erect growing with tough cortex. The leaves are alternate, sessile, narrow, and usually entire with or without stipular glands. The flowers that can be blue, red, yellow or white are borne on terminal or axillary racemes. They first open soon after dawn in the sunshine. The flowers have five petals with five- or ten-celled ovaries (depending on the absence or presence of false septa), with two ovules per cell. The five styles can be free or united to the center and the capsules can be either dehiscent or indehiscent (Flora Europaea 1968).

11.1.2 Karyotype

Karyotypic analysis of *Linum* L. species started more than half century ago and allowed several flax species to be recognized (Muravenko et al. 2003). It has been observed that chromosome number ranges from 12 to 72 in the genus, and that chromosomes are small (1–4 μm) and morphologically similar. Although monochrome staining reveals only general karyotypic differences, the results obtained with this method have made it possible to construct a putative phylogenetic tree both for the Old World and for New World species of the genus *Linum* (Muravenko et al. 2003). Table 11.1 contains a listing of some of the *Linum* species chromosome numbers.

11.1.3 Taxonomy

Flax is an annual of the family Linaceae. The overall place of flax in the plant classification is:

Division: Magnoliophyta
Order: Malpighiales
Family: Linaceae
Genus: *Linum*
Species: *Linum usitatissimum*

Table 11.1 Chromosome numbers of *Linum* species. Chromosome numbers taken from Harris (1968) and Ray (1944)

Species	Chromosome number (*n*)	Reference
L. africanum	16	Ray (1944)
L. alatum	15	Harris (1968)
L. alpinum Jacq	18	Ray (1944)
L. alpinum	9	Ray (1944)
L. altaicum	9	Ray (1944)
L. angustifolium	15	Ray (1944)
L. arboretum	14	Ray (1944)
L. arenicola	18	Harris (1968)
L. aristatum	15	Harris (1968)
L. aristale	15	Harris (1968)
L. austriacum	9	Ray (1944)
L. bahamense	18	Harris (1968)
L. campanulatum	14	Ray (1944)
L. capitatum	14	Ray (1944)
L. carteri small	30	Harris (1968)
L. catharticum	8	Ray (1944)
L. collinum	9	Ray (1944)
L. compactum	15	Ray (1944)
L. corymbytherium	15	Ray (1944)
L. extra-axillare	9	Ray (1944)
L. flavum	15	Ray (1944)
L. floridanum	18	Harris (1968)
L. gallicum	10	Ray (1944)
L. grandiforum Desf.	8	Ray (1944)
L. hirsutum	8	Ray (1944)
L. hologynum	9	Ray (1944)
L. hudsonioides	15	Harris (1968)
Li. Imbticatum	15	Harris (1968)
L. intereusum	18	Harris (1968)
L. julicum	9	Ray (1944)
L. lewisii	9	Ray (1944)
L. loreyi Jord	9	Ray (1944)
L. maritinum	10	Ray (1944)
L. medium	15	Ray (1944)
L. mexicanum	18	Harris (1968)
L. monogynum Forst	43	Ray (1944)
L. mulleri	9	Ray (1944)
L. narbonense	9	Ray (1944)
L. nelsonii	31	Harris (1968)
L. neo-mexicanum Greene	13	Harris (1968)
L. nervosum	15	Ray (1944)
L. perenne	9	Ray (1944)
L. pratense	9	Harris (1968)
L. pronglri	26	Harris (1968)
L. puburelum	15	Harris (1968)
L. punctatum	9	Ray (1944)
L. rigidum	15	Ray (1944)
L. rupestre	18	Harris (1968)
L. salsoides	9	Ray (1944)
L. scabrellum	36	Harris (1968)
L. schiedeanum	18	Harris (1968)

(*continued*)

Table 11.1 (continued)

Species	Chromosome number (*n*)	Reference
L. striatum	18	Harris (1968)
L. strictum	9	Ray (1944)
L. sunteres	15	Harris (1968)
L. sulcatum	15	Ray (1944)
L. tenuifolium	9	Ray (1944)
L. tommasinii	9	Ray (1944)
L. usitatissimum	15	Ray (1944)
L. vernale	15	Harris (1968)
L. virginianum	18	Harris (1968)
L. viscosum	8	Ray (1944)
L. westii	18	Harris (1968)

The Malpighiales are a large order of flowering plants, included in the group named eurosids I in the recent Angiosperm Phylogeny Group classification. Its internal systematics are still uncertain. This diverse order includes violets and willows, passion-fruit and mangrove, poinsettia, and flax.

11.1.4 Genome Size

This family only has a single member for which the genome size has been determined that is flax (*L. usitatissimum*). The value from the RBG Kew DNA C-values database is 1C = 0.70 pg (1C = 686 Mbp). However, there appears to be significant variation in the genome sizes even within flax varieties. Thus, genome size determinations on nine different flax varieties gave sizes ranging from 381 to 575 Mbp (Michael personal communication), a variation of 194 Mbp, which is greater than the complete *Arabidopsis* genome size. It will be important to determine the genome sizes across the *Linum* germplasm and determine the function, if any that this range of genome sizes has on phenotypic characteristics. One of the interesting characteristics of the flax genome is that it appears to contain few retrotransposable elements but does have a large portion of the genome in repetitive sequences arrayed in tandem (Cullis 1981). However, the kinetic complexity of the single copy sequences (which make up 44% of the total genome) is only 3.25×10^8 nucleotide pairs. This kinetic complexity corresponds to a weight of DNA of 0.38 pg/1C nucleus, which is very closer to half the estimate from Feulgen staining (0.31 pg/1C nucleus). Therefore, flax has one of the smaller genomes in plants as far as the complexity is concerned. This is true because of the fact that much of the genome (35%) comprises highly repetitive tandemly arrayed sequences. These are likely to be the sites of the heterochromatic regions of the genome. A light satellite region making up about 15% of the total genome is also present. It will be instructive to determine the distribution of the genome organization in the genus as a whole and determine if flax is typical or atypical.

11.1.5 Status as an Agricultural and Model System

Within the genus, only flax (*L. usitatissimum*) is used as an agricultural crop. Other species such as *L. flavum*, *L. grandiflorum* and *L. komarovii* are used as ornamental plants.

Flax is valued as a crop for its stem fiber and seed oil (as well as medicinal secondary metabolites), and there are both shared and unique aspects of each of these processes that are scientifically and economically interesting (Fig. 11.1). For example, although the composition of flax xylem is similar to the stems of poplar, flax phloem fibers (i.e., linen) have a composition that is much more similar to tension wood. Similarly, flax makes many of the same fatty acids as soybean, and additionally makes a higher proportion of unsaturated and longer-chain fatty acids valued for industrial and nutritional applications. Thus, studies of processes such as the development of plant architecture, stems, cell walls, and lipids may be conducted more efficiently in flax than in other systems, and the results have the potential to be informative in other species already sequenced, as well as contributing to more general questions of gene and genome evolution, including further clarifying phylogenetic relationships within the Malpighiales and higher clades. For example, as a member of the Malpighiales, flax is closely related to other potential biofuel species, such as poplar and willow. However, flax is more suitable to forward genetics and other types of techniques, as it has all of the practical advantages of *Arabidopsis* as an experimental organism, including a short generation time, self-pollination, efficient transformation systems,

Fig. 11.1 Flax plant

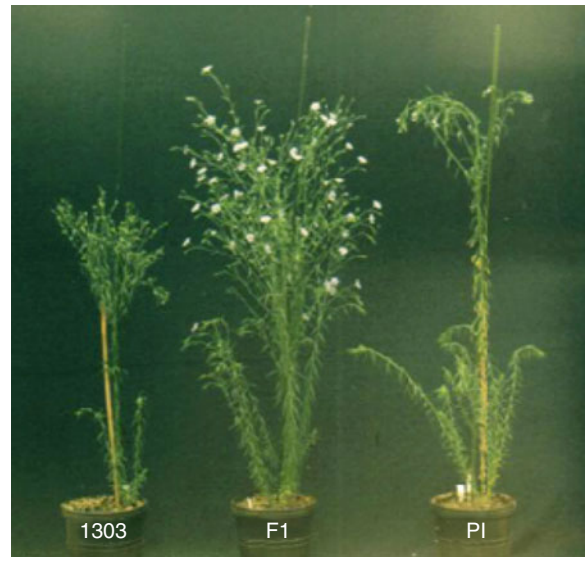

Fig. 11.2 Inheritance of height and branching. The cross between the lines CI1303 and Stormont cirrus (Pl) with an F_1 plant

and can be grown in laboratory or field settings at high density.

One of the interesting questions that could be addressed within the cultivated flax with genomic sequences from both a fiber and an oil variety is how the genome organization and control has allowed the development of a bifunctional crop – namely, one that can produce both high-quality fiber as well as high oilseed yield. The importance of identifying the control of the two phenotypes and the system integration of the development will be greatly facilitated by complete genome sequences of both types. In crosses between tall and short varieties, height is dominant but the branching patterns are synergistic so that the F_1 is much more extensively branched than either of the parents. These phenotypes then segregate into various classes in the next generation. The description of the phenotypes in the segregating families along with the ability to identify the segments of the genome arising from each of the parents will allow the combinations of chromosomal regions responsible for each of the quantitative trait loci (QTL) controlling these characters (Fig. 11.2).

Flax is an excellent subject for studying both the development of fiber-rich stems, and the biosynthesis of a range of lipids. Therefore, flax is informative with regard to two potential sources of bioenergy: cellulose and biodiesel. Moreover, flax is already an established crop grown on over 3.5 million ha. worldwide, and significant potential exists for developing this into a multi-use crop including the production of environmentally friendly sources of high-quality fiber for textiles and composites. Currently, more than one million tons annually of flaxseed straw, as a byproduct of the linseed industry, constitutes a major environmental disposal problem. Understanding and manipulating this waste product to either make it useful as a fiber source or fermentable as a biofuel source will reduce the problem and possibly increase the production of flax as a crop within the northern USA.

Apart from the traditional uses of flax as a fiber and oil crop, there has been an increasing interest in the medicinal and nutraceutical value of flax. The initial interest was in the omega-3 fatty acid, α-linolenic acid, but the use has widened to other biologically active molecules such as the flax lignans, which are being considered because of their effect on several medical conditions. These medicinal uses are of ancient origin such as when Charlemagne prescribed flaxseed production for all the subjects in the Roman Empire for its health properties (Muir and Westcott 2003).

11.2 Conservation Initiatives

Flax is one of the founding crops of the Near East. It has been subjected to disruptive selection resulting in a dual use crop – the seed flax (linseed) for oil and the fiber flax for fiber. This is one of the few domesticated plants that have been selected for two totally different crop uses. Therefore, the diversity of collections is an important underpinning for the continued improvement of these crops as well as for the development of a truly bifunctional crop where the same planting can be used for both oil and fiber harvest.

An international flax database has been managed by the AGRITEC Company Ltd., Sumperk in the Czech Republic since 1994. Accessions in this database are described using 22 passport descriptors as well as 24 special descriptors. Included in the latter group are 14 morphological traits, four biological, and six yield characters. As noted by the name, this database is mostly populated by cultivars. These data are mainly from the Czech, Russian, Romanian, and Dutch seed collections (Pavalek 1998). Currently, the FDB includes data from 8,387 accessions of flax and linseed, stored in 13 contributing genebanks from 11 countries. This is estimated at 33% of the total number of flax accessions (possibly around 25,000) conserved in Europe. The types of accessions are 38% advanced cultivars, 27% genetic resources, 20% breeding material, and 14% landraces, primitive cultivars, and wild forms. Passport data are included in the database for 82% of the accessions, while 16% are described by specific characterization descriptors (*http://www.ecpgr.cgiar.org/databases/Crops/flax.htm*). One of the current objectives is to develop and use molecular markers to identify duplicate accessions to reduce the seed storage needs while still maintaining the diversity available for this crop. An extensive overview of ex situ collections of cultivated flax (*L. usitatissimum* L.) and 53 other species of the genus *Linum* L. is available (Diederichsen 2007) (Table 11.2). Included in the data are 33 genebanks in 23 countries that are preserving *Linum* germplasm. These world genebanks are engaged in the ex situ preservation of about 48,000 accessions of cultivated flax (*L. usitatissimum*). However, it is possible that only 10,000 accessions of these are unique. The number of species of the genus *Linum* is not generally agreed upon but there are about 200 identified. Fifty-three species (excluding *L. usitatissimum*) covering about 900 accessions are reported to be represented in these ex situ collections. A large number of these accessions are of the wild progenitor of cultivated flax, *Linum bienne* Mill (279 accessions). However, *L. bienne* from the region of origin of cultivated flax is rarely represented in genebank collections, possibly as the differentiation between the progenitor of the cultivated species and escapes from earlier episodes of domestication are difficult to discern. There is still need for the clarification of taxonomic species delimitation within the genus *Linum* and the continued development of molecular markers, in particular DNA markers, will greatly aid these efforts. Outside of the need for collections and descriptions of the agronomically useful germplasm, more detailed studies of the variation available in *Linum* species for ornamental use or use in breeding is needed. An example of these efforts is the National Russian Flax Collections of the All Russia Flax Research Institute (VNIIL). During the period 1991–1997, 2,219 samples of the genus *Linum* were collected and these included 2,077 samples of the species *L. usitatissimum* and 142 samples of 28 wild species of the genus *Linum*. As of 1998, the Institutes collection totaled more than 6,130 samples, which included 142 wild species in addition to the nearly 6,000 flax and linseed samples (Rozhmina and Zhuchenko 1998).

Table 11.2 Flax ex situ collections (Diederichsen and Fu 2008)

Site	Number of accessions
VNIL Torzhok, Russia	6,100
VIR St. Petersburg, Russia	5,700
RITC China	4,000
PGRC Canada	3,500
DSV Lippstadt, Germany	3,500
Adis-Abeba, Genbank Ethiopia	3,100
GRIN, USA	2,800
RICIC Romania	2,700
Other collections (81)	22,300

The diverse germplasm stored in genebanks will be essential for the long-term sustainability of flax production since the diversity maintained through agriculture has mostly disappeared. This variation is a critical need in order to increase the potential of the crop in its many modern uses. An extended evaluation of genebank germplasm is necessary to identify

those accessions, which have genetic variation for useful traits that are not found in current cultivars. This characterization is important to occur for both the molecular and phenotypic characteristics, since the phenotypic expression of characters can be affected both by the original seed production environment and the evaluation environment. As mentioned earlier, the possible duplication within and among genebank collections needs to be determined and cooperation among the major flax genebanks in this endeavor will ensure the maximum genetic diversity preservation with the minimum of effort. The aim would be to preserve diversity without loosing unique material. Associated with these efforts should be a closer focus on the wild progenitor of flax (*L. bienne*) and other wild interfertile species as sources of useful characteristics in flax breeding. *L. bienne* from the region of origin of cultivated flax, the Fertile Crescent, is presently very rare in genebanks and these collections should be extended (Diederichsen 2007).

The survey and management of the *Linum* ex situ collections will be greatly enhanced for both accuracy and ease with the deployment of molecular markers. These are being developed for flax and the first large set of simple sequence repeats (SSRs) have been isolated and characterized (Cloutier et al. 2009). Full genome shotgun sequencing of flax is also underway and these molecular resources will greatly enhance diversity analysis and identification. However, the screening of the world collection of *Linum* is a major undertaking that will need considerable funding to achieve.

11.3 The Origin and Evolution of Allied Crop Plants

The Linaceae is an outlier in the phylogenetic tree. The group is not closely related to other crop plants, the closest being poplar for which there are extensive molecular data. Clearly, the development of molecular data for flax will enable the taxonomic relationships to be more clearly defined. Most of the molecular taxonomic data have used the ITS regions of the ribosomal RNA genes. The most used molecular markers in *Linum* are random amplified polymorphic DNA (RAPD) and intersimple sequence repeat (ISSR). These markers show considerable variation. In addition, the chloroplast rDNA region has been used for interspecific comparisons.

The basic morphological markers that were initially employed in the taxonomic evaluation of each of the sections (Flora Europaea 1968) and the characteristics of the different sections are described as below.

Section Syllinum has stems with narrow wings decurrent from leaf bases. The leaves are alternate with a pair of glands at the base. The sepals are sometimes glandular-ciliate. The petals are slightly joined at the base of the claw and are usually yellow. The stigmas are usually linear or oblong-linear. They can be homostylous or heterostylus.

Section Linum has alternate, glabrous leaves without basal glands. The sepals are eglandular. The petals are free and can be blue, purple, or pink. The stigmas are capitate, clavate, or linear. The flowers can be homostylous or heterostylus.

Section Dasylinum has leaves that are alternate, pubescent, and lacking basal glands. The sepals are glandular and ciliate while the petals are free and colored blue or pink. The stigmas are linear and the flowers are homostylous.

Section Linastrum has leaves that are mostly glabrous and without basal glands. The sepals are glandular and ciliate while the petals are free and colored yellow, pink, or white. The stigmas are linear or capitate and only rarely clavate. The flowers can be homostylous or heterostylus.

Section Cathartolinum has leaves that are opposite without basal glands while the petals are small, free, and white. The stigmas are capitate. The flowers are homostylous.

The data on the chemical constituents of members of the family are relatively limited. Owing to its usefulness, most of the emphasis has been on the constituents of linseed oil. However, an increasing focus on other constituents of the seed that may have medicinal properties has occurred.

The components of the seed that are of interest include the lipids, proteins, polysaccharides, and the phytochemicals. The phytochemical components include cyanogenic glycosides, lignans, phenolic acids, isoprenoids, and flavanoids. There are considerable variations in these components within flax varieties and between *Linum* species. No systematic use of these chemicals for taxonomic studies has been reported. Additional interest in these components in both flax and its relatives is due to their potential use in preventative medicine.

Seed storage protein analysis was carried out on 12 Iranian species of *Linum* (*L. strictum*, *L. tenuifolium*, *L. corymbulosum*, *L. album*, *L. nodiflorum*, *L. austriacum*, *L. glaucum*, *L. usitatissimum*, *L. bienne*, *L. catharticum*, *L. bungei*, and *L. nervosum*) to study species interrelationships and evaluate the taxonomic treatments proposed for the genus (Sharifnia and Assadi 2003). The cluster analysis of protein data and resulting placement of species in different sections was in agreement with previous phenetic morphology-based studies. Thus, the sodium dodecyl sulfite–polyacrylamide gel electrophoresis (SDS-PAGE) profile of seed proteins can be another useful molecular marker in the biosystematics of genus *Linum* at subgeneric level.

11.4 Cytogenetic Stocks and Their Utility

Most of the flax improvement has been carried out using traditional breeding methods. These traditional breeding programs have been successful in improving grain yield and agronomic fitness of linseed in western Canada (Rowland and Wilen 1998) but these efforts are time-consuming. One possible mechanism for reducing the time for variety development would be through the development of doubled haploid plants through anther or microspore culture. These techniques would be very useful where interspecific crosses are concerned for the immediate production of homozygous lines for the evaluation of diverse germplasm. The doubled haploid technology for linseed is not yet in wide use.

The so-called twinning cultivars are known in flax, where the second embryo is formed from one of the antipodal cells, and it has the haploid chromosome number (Green and Salisbury 1983). However, this phenomenon is insufficiently frequent to produce haploids for breeding purposes. The first source of haploid plants reported in *Linum* was obtained via polyembryony (Kappert 1933), but anther culture is currently the most successful method of producing dihaploid (DH) lines in flax (Preťová et al. 2006). In spite of the improvements in the technology, DH production is still not suitable for practical breeding. Thus, flax is considered recalcitrant to anther culture-based regeneration of DH plants.

One of the major side effects of tissue culture of higher plants is the occurrence of somaclonal variation. The somaclonal variation observed in anther culture-derived flax plants included changes in petal color, other morphological changes including branching patterns and growth rate, and variation in resistance to Fusarium wilt (Rutkowska-Krause and Mankowska 2002).

11.5 Classical Mapping

Flax has been the subject of inheritance studies for nearly a century and included the characterization of the inheritance of petal, anther, and seed color along with other characteristics. Flor's work on disease resistance genes laid the foundations for a substantial part of modern disease resistance breeding (Beard 1962). The early mapping work identified genes involved in flower and petal color (Tammes 1922, 1928), anther color, filament color, stigma color, or seed color (Shaw et al. 1931). White flower color was controlled by a single gene (Myers 1936; McGregor 1937). Seed color is used in Canada to differentiate between the two main market types. The traditional, high linolenic acid flax must have brown seed, while solin or zero linolenic acid flax must be yellow-seeded. The four loci governing seed color were essentially inherited independently (Mittapalli and Rowland 2003).

The flax–flax rust interaction, used first by Flor to demonstrate the gene-for-gene relationship between host plant resistance genes and corresponding pathogen avirulence genes (Flor 1956), has been the subject of extensive classical genetic analysis. The rust resistance genes were placed at five loci (*K*, *L*, *M*, *N*, and *P*) (Flor 1956). Additional disease resistance loci have been identified including Fusarium wilt resistance that was conditioned by multiple loci (Knowles and Houston 1955).

The genetic mapping in flax has until very recently been relatively limited. Comstock (1963) investigated the linkage relationships between the color determinants rust resistance loci, and six chlorophyll mutants (*Yg 1–4*, *Y1*, and *St1*). The identification of a large number of polymorphic SSR loci (Cloutier et al. 2009) will substantially enhance the mapping and breeding efforts in *Linum*, and supercede those maps based on RAPDs and amplified fragment length polymorphisms (AFLPs) because of their ease of utility across diverse germplasm.

A small number of genes for economically important traits, such as those controlling the oil profile of the seeds and seed color have been used in breeding populations but without the development of an overall genetic map. The small size of the chromosomes and the only recent identification of these through banding patterns have also hindered the development of genetic maps with linkage groups assigned to identified chromosomes. A series of isozymes including those for diaphorase, leucine amino-peptidase, 6-phosphogluconate dehydrogenase, acid phosphatase, phosphoglucomutase, and peroxidase have been mapped (Gorman et al. 1992). Gene discovery through ethyl methane sulfonate (EMS) mutation screens have identified genes controlling the oil profile of the seeds and these have been incorporated into commercial varieties (Green 1986; Rowland et al. 1995; Saeidi and Rowland 1999). The fatty acid (FA) biosynthetic genes β-ketoacyl CoA synthase, FA elongase, stearoyl-ACP desaturase, and FA desaturase (Fofana et al. 2004) and a pectin methylesterase isoform (Al-Qsous et al. 2004) have been cloned.

Mutation breeding in flax (*L. usitatissimum* L.) has been used to develop a new type of edible flaxseed oil that has nearly eliminated linolenic acid and quadrupled the level of palmitic acid. A variegated seed coat color mutant was used as a phenotypic marker to distinguish varieties with this particular fatty acid profile from those of linseed (high linolenic acid) or solin (low linolenic acid) varieties (Saeidi and Rowland 1997).

All these efforts have been directly utilizing the *L. usitatissimum* and virtually no input from wild relatives (Diederichsen 2007). Linum bienne would be the most likely source of new genetic variation but the variation in this wild germplasm itself has not yet been characterized. The identification through molecular markers indicates that the diversity within the cultivated crop is low, so this will certainly spur on efforts to make more diverse combinations. Again, the availability of molecular markers to rapidly introgress useful genes from wild relatives will also make this process manageable.

11.6 Crop Improvement

Substantial breeding efforts to improve yield and quality factors are underway in both the fiber and the oil crops. These classical efforts are being supplemented by additional techniques including mutation breeding, somaclonal variation, and transgenic technologies.

Flax generates variants in tissue culture (somaclonal variation) (McHughen and Swartz 1984; Rowland et al. 1988; Rakousky et al. 1999). Anther cultures were less effective in plant regeneration than somatic cell cultures, but the regenerants derived from anther cells showed valuable breeding features, including increased resistance to fungal wilt.

11.6.1 EMS and Radiation Mutation

EMS and radiation mutation induction has been successfully applied in flax to generate new mutants, especially those with altered oil quality (Rowland et al. 1995; Bhatia et al. 1999). Additional phenotypes that have been selected include curly stem (Tejklová 2002) and mutations that affect cell walls (Chen et al. 1998). Oil modifications have been induced in three cultivars, Gleneig (Green 1986), Rauloinus (Nichterlein et al. 1988), and McGregor (Rowland and Bhatty 1990; Rowland 1991; Ntiamoah et al. 1995; Ntiamoah and Rowland 1997) and used in breeding programs to generate new varieties.

11.6.2 Transgenic Flax

Transgenic flax is already a reality since flax has been transformed both by *Agrobacterium tumefaciens* and by particle bombardment (Jordan and McHughen 1988; Dong and McHughen 1993). Flax-specific promoters are available and will become better characterized as a result of the molecular initiatives currently underway. Therefore, the specific tailoring of tissue-specific transgene expression is likely to be more easily achieved (Jain et al. 1999). A new herbicide-resistant flax line, Triffid, subsequently withdrawn, was created by *Agrobacterium*-mediated transformation in which the transfer-DNA (T-DNA) contained the *ALS* gene from a chlorsulfuron-tolerant line of *A. thaliana* (McSheffrey et al. 1992). An alternative to modified flax as a conventional crop, would be to produce novel chemicals. An example is the synthesis of polyhydroxybutyrate in transgenic flax.

The synthesis of both the fibers and the polyhydroxybutyrate for composites in a single plant has been reported (Wróbel et al. 2004).

11.7 Genomic Resources

11.7.1 Genome Characteristics

The known characteristics of the flax genome have been identified using whole leaf DNA, and therefore included the chloroplast and mitochondrial components. The presence of two cesium chloride satellite fractions that comprise about 20% of the genome has been demonstrated (Fig. 11.3).

The heavier satellite is mainly composed of the large and 5S ribosomal RNA gene clusters that occur in long tandem arrays as demonstrated by hybridization cross the gradient. The large ribosomal RNA gene repeat unit (8.6 kb) has been cloned and sequenced, and the sequence deposited in Genbank. A series of 5S rRNA gene families have also been sequenced.

Renaturation kinetics (Cullis 1981) indicated that about 50% of the genome is low-copy-number sequences, a small fraction of intermediately repetitive sequences (the normal component of transposons) and about 35–40% highly repetitive sequences, with all these sequences organized in a long-period interspersion pattern. The kinetic complexity calculated was only half that expected from the DNA estimation. Since this genome is certainly derived from a tetraploid, it is possible that the renaturation kinetic experiments were not stringent enough to differentiate between the two ancestral genomes. The sequencing will be able to do so since many protein-coding regions are polymorphic and appear as duplicate genes. Therefore, the sequencing is likely to be able to distinguish among the progenitor genomes and indicate whether or not *L. usitatissimum* is an ancient allo- or auto-polyploid.

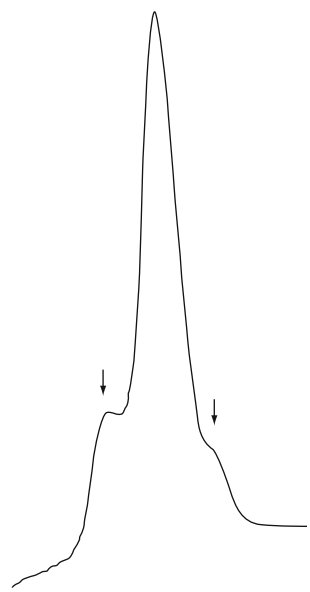

Fig. 11.3 Equilibrium cesium chloride gradient centrifugation using a Beckman Model E ultracentrifuge. The light and heavy satellite fractions are *arrowed*

The picture of the genome organization of flax being in a long-period interspersion pattern has been confirmed from a small 454 shotgun sequencing run that yielded 1.6 MB of sequence data and the sequencing of a selected bacterial artificial chromosome (BAC) clone. The Genbank nonredundant database was then searched with the flax 454 sequence using both Blastx and Blastn (Table 11.3). The Blastn results mainly identified similarities to chloroplast DNAs, mitochondrial DNAs, the ribosomal RNA genes, and some of the known tandemly repeated families that had homology to certain *Linum* expressed sequence tags (ESTs). The distribution of the sequences was as expected from the renaturation kinetics. Thus, the proportion of the reads in each of these categories is given in Table 11.3.

The data from the Blastx searches was very instructive and confirmed that the flax genome appears to be very much gene rich and relatively free from retrotransposons. The initial cut-off value for significance was $e < -5$ although all similarities were also interrogated. The data showed that many more reads were similar to known genes than to retrotransposons.

Table 11.3 Percentage of sequence types in preliminary 454 sequencing. The fraction of the reads in the chloroplast and ribosomal gene fractions were in agreement with earlier work estimating the representation of these sequences in such a DNA preparation

Sequence type	%
Large ribosomal RNA genes	2.9
5S similarities	1.1
Chloroplast similarities	8.7
Mitochondrial similarities	0.65

Hits to transposable elements ($e < -5$) – 1.4%. These hits were to the type proteins for both LTR and non-LTR retrotransposons. They included copia-type polyprotein, retrotransposon protein – putative Ty1-copia subclass, reverse transcriptase, gag-pol polyprotein, integrase, putative TNP2-like transposon protein [*Oryza sativa* (japonica cultivar-group)], retrotransposon protein, putative, Ty3-gypsy subclass (*O. sativa*), non-LTR retroelement reverse transcriptase-like (*Arabidopsis thaliana*), RNA-dependent RNA polymerase family protein (*A. thaliana*), retrovirus-related Pol polyprotein from transposon TNT 1-94, and a putative transposase (*A. thaliana*).

Hits to known proteins ($e < -5$) – 4.6% of all the reads representing 221 different proteins. Of particular interest is that the reads only represent 0.25% of the total flax genome. Therefore if, as appears likely, these data are representative of the genome as a whole, then the flax genome is very much gene rich and the assembly from shotgun reads should result in long contigs of gene-rich sequence information.

Most of the repetitive families have been shown to be arranged in tandem arrays and also comprised about 20% of the total reads. The reads were categorized with respect to their A + T content. As expected, about 14% of the reads were of an A + T content of >70%. Included in this set were three families that were organized in tandem arrays. One of these tandemly arrayed, high A + T families, also has similarity with a *Linum* EST (*L. usitatissimum* clone LU0014H04 mRNA sequence). Therefore, all of the sample sequencing to date has confirmed the earlier characterization of the flax genome, particularly the presence of most of the repetitive sequences in tandem arrays and the low representation of known retrotransposons.

11.7.2 Available Genomic Resources

The Canadian flax biotechnology program has sequenced more than 250,000 ESTs. Additional data that these projects will provide includes:

A physical map based on ~400,000 BAC-end sequences.

A 50× shotgun sequence of the genome of the linseed cultivar Bethune.

Approximately 10,000 single nucleotide polymorphisms (SNPs) mapped to existing populations.

Obviously, all this data are directly derived from the cultivated *L. usitatissimum*. Once collected, it will provide an extensive basis for then developing the appropriate information concerning the wild relatives. Early indications are that the genome of this genus has evolved very rapidly and so can be useful as a model to determine the mechanisms and influences that affect genome evolution.

11.8 Flax as a Functional Food

The National Cancer Institute has identified flax seeds as a food product that deserves further study and attention because of its potential cancer-fighting properties and many health benefits. According to the US Department of Agriculture, flax seeds contain 27 identifiable cancer-preventive compounds. Medical sources that have published clinical results concluding that golden flax seed can have a positive impact on the overall health abound. Many potential health benefits of flax seeds that have been reported include lowering of blood cholesterol levels and blood pressure, reduced threat of blood clots and as a natural laxative.

The components that make flax a useful dietary component clearly also are present in the many relatives. In particular, *L. carthicum* derives its name from its effect on the digestive tract. However, since there are a few studies on the relative abundances on the important lipid, protein, and phytochemical constituents of *Linum* species (Muir and Westcott 2003) more information about the useful ranges and additional compounds needs to be acquired.

11.9 Impediments for Introducing Transgenic Flax

Flax is being evaluated as a crop platform for the production of bio-industrial and nutraceutical products. Since it is already capable of being transformed, novel traits can be introduced through genetic

engineering. The current climate related to the use of genetically modified organisms (GMOs) is moving towards greater control so that the requirements for the release of GMOs are becoming more stringent. One of the most important considerations when GMOs containing any novel trait are proposed for release is the potential for gene flow to wild or weedy relatives and the impact it may have on their populations. Interspecific hybridization and cytogenetic studies between flax and congeneric species have demonstrated that cultivated flax has the ability to hybridize and form viable F_1 plants with at least nine species of *Linum* (*L. africanum, L. angustifolium, L. corymbiferum, L. decumbens, L. floccosum, L. hirsutum, L. nervosum, L. pallescens*, and *L. tenue*) (Jhala et al. 2008). Although the hybridization of flax with many other wild relatives has yet to be reported, the data already to hand indicates that the potential for gene flow from transgenic flax to wild or weedy relatives is possible. Therefore, the release of transgenic flax with novel combinations of genes will need to be monitored to determine the extent of gene flow and its likely environmental consequences. Since all the components are in place for novel flax products and new genetic material to be integrated into the commercial germplasm, public acceptability is likely to limit their adoption rather than any technical difficulties in getting them developed. The status of Triffid, a transgenic flax that has contaminated some Canadian flaxseed exports adds to the difficulties in introducing transgenic flax.

11.10 The Future

Flax has some unique properties that make it suitable to be used as a model system. It has clearly been important in the elucidation of the structure of disease resistance genes. It is also the best-studied example of genomic responses to the environment. In both of these cases, the understanding of the molecular events would have been facilitated by all the additional genomic resources that will soon be available.

In many ways, flax is ideally positioned to become a useful model system. It is a multifunctional crop and can be effectively used to unravel both fiber and oil biosynthesis. The small size of the genome and its organization also make it relatively easy to isolate genes and ideal for genomic sequencing using the next generation sequencing methodologies.

Essentially, the future for flax depends on the developing markets. If the importance and use for food (both health foods/nutraceuticals for humans and animal feed) and additional use for the fiber increase, then there will be a greater importance in understanding and manipulating flax. A detailed molecular marker map, as well as sharing of molecular resources, is a first priority. The development of the molecular map will also facilitate the screening of the germplasm and the detection of the extent of genetic variation present therein. The ease of transformation and regeneration is an additional asset since constructs can be tested in vivo and putative gene functions directly tested. As the crop becomes more important commercially, efforts to identify useful wild germplasm and the availability of such germplasm will become more important. One of the early steps in the use of molecular markers will be to determine the real diversity present in the germplasm collections and identify needs for additional collections in centers of wild species diversity.

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Chapter 12
Moricandia

Muhammad Tahir and Roger Watts

12.1 Introduction

Moricandia has been used as an ornamental garden flowering plant commonly called Purple Mistress (Harvill et al. 1977) and Violet Cabbage (Harvey 1975). However, the genus *Moricandia* represents a diverse group of hardy herbaceous plants found in North Africa, Mediterranean basin, West Asia, and Southeast Asia. The genus is considered as one of the tribes of Brassiceae based on the original establishment of boundaries of Brassiceae (Schulz 1936), later identification of 38–63 cytodemes of the coenospecies of *Brassica* (Harberd 1972; Gomez-Campo 1999), identification of *Moricandia*, *Pseuderucaria*, and *Rytidocarpus* as *Brassica* coenospecies and part of a subtribe Moricandiinae (Warwick and Black 1997), and morphological or cytogenetic similarities with the *Brassica* coenospecies (Gomez-Campo 1999). Based on these literary resources, the genus *Moricandia* and its species can be classified as given in Table 12.1.

Some research efforts have been made to find the relationship of *Moricandia* with species of *Brassica*. Early reports (Takahata and Takeda 1990) indicated that interspecific crosses of *Moricandia* with *Brassica* A- and B-genome show genetic similarity with A- and B-genome indicating partial chromosome homology. Example of successful interspecific hybridization using ovary culture to produce hybrid lines between *Moricandia* and *Brassica napus* and *B. juncea* were then reported (Takahata et al. 1993). Pradhan et al. (1992) also identified *M. arvensis* to share similarity to the *Brassica* lineage as compared to *Sinapis*, *Diplotaxis*, and *Erucastrum* lineages. Later, chloroplast restriction site analysis revealed that *Moricandia* belonged to the lineage of *B. rapa* and *B. oleracea* (Warwick and Black 1997), supporting the classification suggested by Gomez-Campo of *Brassica* subtribes and confirms that *Moricandia* is a coenospecies of *Brassica* (Warwick 2005). In more recent studies (Inaba and Nishio 2002), physiological analysis of *S*-locus shows a distant relationship between *B. rapa* and *B. olereaca*, as it is grouped separately from the rest of *Brassica* group. This physiological analysis shows a distant relationship between the current *Brassica* cultivated lines and *Moricandia*.

The species of *Moricandia* are differentiated from each other based on the morphological characteristics, growth habit, and habitats. Some prominent features of the species in terms of their growth and geographical distribution are summarized in the Table 12.2.

The genome size of *Moricandia* has not been studied to date; however, *Moricandia* species are polyploid containing 14 chromosomes ($2n = 28$) based on the smallest chromosome number of 7 observed in the *Brassica* genera (Harberd 1972; Warwick et al. 2009). The original diploid homolog for *Moricandia* sp. is still unknown. *Moricandia* has species that exceed tetraploidy containing cytodemes of $6x$ and $8x$ (Sobrino-Vesperinas 1980). For example, *M. suffruticosa* is a tetraploid with $n = 28$ (Sobrino-Vesperinas 1980), and *M. spinosa* is a hexaploid with $n = 42$ (Sobrino-Vesperinas 1980).

Currently, there are eight recognized species of *Moricandia*. Preservation of *Moricandia* sp. is concentrated in Spain but accessions are preserved in multiple institutions within Europe (Table 12.3).

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Table 12.1 Scientific classification of Moricandia sp.

Kingdom:	Plantae	(Plants)
Subkingdom:	Tracheobionta	(Vascular plants)
Superdivision:	Spermatophyta	(Seed plants)
Division:	Magnoliophyta	(Flowering plants)
Class:	Magnoliopsida	(Dicotyledons)
Subclass:	Dilleniidae	
Order:	Capparales	
Family:	Brassicaceae	(Mustard family)
Genus:	*Moricandia*	(Moricandia)
Species:	*Moricandia arvensis*	(Purple Mistress)
	Moricandia foetida	
	Moricandia foleyii	
	Moricandia oricandioides	(Violet cabbage)
	Moricandia nitens	
	Moricandia sinaica	
	Moricandia spinosa	
	Moricandia suffruticosa	

References: Schulz (1936); Harberd (1972); Harvey (1975); Harvill et al. (1977); Warwick and Black (1997); Gomez-Campo (1999)

Table 12.2 Geographical distribution and growth habits of identified *Moricandia* sp. (adapted from Warwick et al. 2009)

Species	Growth habit[a]	Habitat	Distribution	Origin
Moricandia arvensis (L.) DC.	A, B, P	Coastlines; Hilly regions	S. Europe; N. Africa	Mediterranean
Moricandia foetida Bourg. ex Coss.	B	Coastal hills	S. Europe	Mediterranean
Moricandia foleyii Batt	A	Desert regions	N. Africa	Mediterranean
Moricandia moricandioides (Boiss.) Heywood	P	Semi-arid regions	S. Europe	Mediterranean
Moricandia nitens (Viv.) E.A. Durand & Barratte	P	Coastal deserts	N. Africa; W. Asia; Middle East	Saharo-Sindian
Moricandia sinaica (Boiss.) Boiss.	A, B, P	Desert regions	W. Asia; Middle East	Saharo-Sindian
Moricandia spinosa Pomel	P	Desert regions	N. Africa	Saharo-Sindian
Moricandia suffruticosa (Desf.) Coss. & Durieu	P	Desert regions	N. Africa	Saharo-Sindian

[a]*A* Annual, *P* Perennial, *B* Biennial

Table 12.3 Accession line of *Moricandia* species maintained in Europe

Location	Species	Source
Comunidad de Madrid: Madrid, Spain	*M. arvensis, M. spinosa, M. suffruticosa, M. moricandioides, M. sinaica, M. nitens, M. foleyii*	Algeria, Italy, Spain, Tunisia, Morocco
Junta de Andalucia: Almeria, Spain	*M. moricandioides, M. arvensis*	Spain
Genebank: Leibniz, Germany	*M. arvensis*	Italy, Tunisia
Millenium Seed Bank Project: West Sussex, UK	*M. suffruticosa, M. nitens, M. arvensis*	Morocco, Egypt, Tunisia

Source: Eurisco 2010

Protocols for preservation of Brassiceae tribes have been developed by storing the seeds in a sealed glass vial containing silica gel and the removal of oxygen. This produces an anhydrous and anaerobic environment for long-term storage under cold conditions such as −5 to −10°C (Gomez-Campo 1972). This method of preservation has been shown to maintain high viability in the seed for up to 40 years (Perez-Garcia et al. 2007).

Cryopreservation of *M. arvensis* cell suspensions has also been used as a method of preserving *Moricandia* germplasm (Craig et al. 1997a).

Interspecific hybridization of *Moricandia* with other *Brassica* species has been used for the study of phylology, physiology, and trait introgression. The interspecific crosses using *Moricandia* sp. has been used to study C_3–C_4 intermediate characteristics of *M. arvensis* and to introduce cytoplasmic male sterility (CMS) and restorer genes into *B. juncea*. A number of techniques have been practiced to develop wide hybridization between *Moricandia* and it distant *Brassica* relatives (Table 12.4).

Somatic hybridization using protoplast fusion has been used to introduce the *Moricandia* genome into cultivated species. Early somatic hybridization was completed using *B. oleracea* and *B. juncea* for introducing *Moricandia* nuclear and plasmid DNA (Toriyama et al. 1987; Kirti et al. 1992). Somatic hybrids of *M. arvensis* and *B. juncea* were used to develop CMS and restorer lines (Prakash et al. 1998a; Bhat et al. 2006). Protoplast fusion has also been used to study the heritability of C_3–C_4 intermediate photosynthesis by developing hybrids with *B. napus*, *B. rapa*, and *B. oleracea* (O'Neill et al. 1996; Meng et al. 1999; Yan et al. 1999).

Table 12.4 Interspecific and intergenic crosses involving *Moricandia* sp. (adapted from Warwick et al. 2009)

Cross	Crossing Method	References
Moricandia arvensis + *Brassica juncea*	Protoplast fusion	Kirti et al. (1992)
M. arvensis × *B. juncea*	Ovary culture, reciprocal unsuccessful	Takahata et al. (1993)
(*M. arvensis* + *B. juncea*) × *B. juncea*	Backcross	Prakash et al. (1998)
(*M. arvensis* + *B. juncea*) × *B. juncea*	Sexual hybridization/backcross	Bhat et al. (2006)
B. napus × *M. arvensis*	Ovule culture, reciprocal unsuccessful	Zenkteler (1990)
B. napus + *M. arvensis*	Protoplast fusion	O'Neill et al. (1996)
B. napus + *M. nitens*	Protoplast fusion	Meng et al. (1999)
M. arvensis × *B. napus*	Ovary culture	Meng et al. (1997)
M. arvensis × *B. napus*	Ovary culture	Meng (1998)
M. arvensis × *B. napus*	Ovary culture, reciprocal unsuccessful	Takahata et al. (1993)
M. nitens × *B. napus*	Ovule culture	Rawsthorne et al. (1998)
M. nitens + *B. napus*	Protoplast fusion	Meng et al. (1999)
(*M. nitens* × *B. oleracea*) × *B. napus*	Ovary/ovule culture	Beschorner et al. (1999)
M. arvensis × *B. nigra*	Ovary culture	Takahata and Takeda (1990)
B. oleracea × *M. nitens*	Ovary/Ovule culture, reciprocal successful	Beschorner et al. (1999)
B. oleracea + *M. nitens*	Protoplast fusion	Meng et al. (1999)
B. oleracea + *M. nitens*	Protoplast fusion	Yan et al. (1999)
B. alboglabra × *M. arvensis*	Sexual hybridization	Apel et al. (1984)
M. arvensis × *B. oleracea*	Ovary culture, reciprocal unsuccessful	Takahata and Takeda (1990)
M. arvensis + *B. oleracea*	Protoplast fusion	Toriyama et al. (1987)
M. nitens × *B. oleracea*	Ovary/ovule rescue, reciprocal successful	Beschorner et al. (1999)
M. nitens + *B. oleracea*	Protoplast fusion	Meng et al. (1999)
B. rapa × *M. arvensis*	Ovary culture, reciprocal successful	Takahata and Takeda (1990)
B. rapa × *M. arvensis*	Embryo culture	Matsuzawa et al. (1998)
M. arvensis × *B. rapa*	Ovary culture, reciprocal successful	Takahata and Takeda (1990)
M. nitens + *B. rapa*	Protoplast culture	Meng et al. (1999)
D. muralis × *M. arvensis*	Sexual hybridization	Razmjoo et al. (1996)
D. tenuifolia × *M. arvensis*	Ovule culture	Zenkteler (1990)
R. sativus × *M. arvensis*	Embryo culture, reciprocal successful	Bang et al. (1997)
M. arvensis × *R. sativus*	Ovary culture, reciprocal unsuccessful	Bang et al. (1995)
M. arvensis × *R. sativus*	Sexual hybridization, reciprocal unsuccessful	Bang et al. (1996)
M. arvensis × *R. sativus*	Ovary/embryo culture	Bang et al. (1996)
M. arvensis × *R. sativus*	Embryo culture, reciprocal successful	Bang et al. (1997)
M. arvensis × *B. oleracea*	Protoplast fusion	Ishikawa et al. (2003)

Wide hybridization of *Moricandia* sp. with other species of the Brassicaceae family using ovary and ovule culture has successfully developed interspecific hybrids between *Raphanus*, *Brassica*, and *Diplotaxis* species (Takahata and Takeda 1990; Zenkteler 1990; Takahata et al. 1993; Bang et al. 1996; Beschorner et al. 1999). Ovary culture has been used to attempt to introduce C_3–C_4 intermediate photosynthesis in to *B. oleracea* (Takahata 1990) and *B. napus* (O'Neill et al. 1996).

Other techniques that have been used to produce interspecific hybrids of *Moricandia* include embryo rescue and sexual hybridization. The use of embryo rescue has been completed successfully in *B. napus*, *B. oleracea*, *B. rapa*, and *Raphanus sativus* (Meng et al. 1997; Matsuzawa et al. 1998; Meng 1998). Embryo rescue was used to develop C3–C4–C3 intermediate hybrids between *B. napus* and *M. nitens* (Rawsthorne et al. 1998). Sexual hybridization has been less commonly used in *Brassica alboglabra* and *Diplotaxis muralis* (Apel et al. 1984; Razmjoo et al. 1996). The development of these wide hybrids have allowed the introduction of novel traits from *Moricandia* into cultivated Brassicaceae genera.

Moricandia sp. has been studied extensively due to C_3–C_4 intermediate photosynthesis observed in the species. Early characterization of *Moricandia* identified enzymatic and morphological characteristics that produced the C_3–C_4 intermediate characteristics (Holaday et al. 1981, 1982). The C_3–C_4 intermediate phenotype was determined to have a concentration of chloroplast in the bundle sheath surrounding the vascular bundles in a Kranz-like structure. This is complemented by a naturally occurring mutation that allows the recapture of carbon lost by photorespiration by having glycine decarboxylase (GDC) function only in bundle sheath cells due to the lack of the glycine decarboxylase P protein in mesophyll cells (Rylott et al. 1996). This produces a lower CO_2 compensation concentration, effectively reducing the rate of loss due to photorespiration by allowing for the capture of lost carbon due to the changes in GDC and chloroplast and mitochondrial clustering in the bundle sheath cells (Hylton et al. 1988; Rawsthorne et al. 1988a, b). This unique morphology was identified as the *Moricandia* syndrome, which is observed in *M. arvensis*, *M. nitens*, and *M. suffruticosa*. Other related species in Brassicaceae, *Diplotaxis* and other *Moricandia* species, have variations of C_3–C_4 intermediate photosynthesis (Apel et al. 1997).

The C_3–C_4 intermediate phenotype in *Moricandia* was first identified by a concentration of photosynthetic and photorespiratory components in the mesophyll and bundle sheath cells of leaf tissue (Winter et al. 1982; Apel et al. 1996). A detailed anatomy of the *Moricandia* leaf structure showed that, in the leaf tissue, there is an increased lateral contact between mesophyll cells into the vascular system. The leaf morphology shows a curvature of the palisade and spongy parenchyma cells towards the bundle sheath cells to increase contact. Additionally, there are a large amount of minor veins within the leaf to increase the contact of mesophyll and bundle sheath cells (Beebe and Evert 1990). This leaf architecture shows an increase in the efficiency of the movement of photosynthates in the vascular bundles to decrease losses due to photorespiration. These characteristic have allowed *Moricandia* to be successful in drier regions due to an increase in water use efficiency (McVetty et al. 1989; Apel 1994b).

The *Moricandia* syndrome, which defines the C_3–C_4 intermediate photosynthesis observed in several *Moricandia* sp., is thought to be a clue in the evolutionary development of C_4 photosynthesis (Apel 1994a). The anatomy of this C_3–C_4 intermediate is unique, as it does not incorporate phosphoenolpyruvate (PEP) carboxylase to recover carbon lost to photorespiration as observed in C_4 species (Brown 1997). Studies have shown that photorespiration in *M. arvensis* was not impacted by PEP carboxylase, which is observed in another C_3–C_4 intermediate *Flaveria floridana* (Leegood and Voncaemmerer 1994). This may suggest that the physiological characteristics observed in *Moricandia* C_3–C_4 intermediates represent an early transition of C_3 to C_4.

The C_3–C_4 intermediate characteristics of *Moricandia* are of interest to plant breeders, as it could be used as a source for improving the drought tolerance of cultivated *Brassica* sp. Interspecific crosses have been developed to study the inheritance of the C_3–C_4 intermediate in attempts to introduce it into breeding lines. Early sexual crosses with *B. alboglabra* and *M. arvensis* produced an intermediate phenotype between C_3 and C_3–C_4 photosynthesis (Apel et al. 1984). An interspecific cross between related species *M. moricandioides* and *M. arvensis*, which have C_3 and C_3–C_4 phenotypes, respectively, produced an

intermediate carbon compensation level (Rawsthorne and Hylton 1991). Additional crosses using protoplast fusion and ovary/ovule culture have produced similar intermediate phenotypes (O'Neill et al. 1996). Backcrosses to C_3 parental lines have produce reduced carbon compensation, which suggests that the C_3–C_4 intermediate phenotype is controlled by nuclear genes (Ueno et al. 2007). This was further supported by the development of monosomic addition lines, which showed that the glycine decarboxylase and bundle sheath adaptations were present on one chromosome of *M. arvensis* (Bang et al. 2009).

GDC has been studied with respect to the differential expression of the GDC P protein in the bundle sheath cell and mesophyll cell. The expression of GDC P has been linked to light intensity (Hunt et al. 1987) and serine hydrooxymethyltransferase in the bundle sheath cell (Morgan et al. 1993). The increase in GDC P expression suggests a role in the localization of glycine metabolism in bundle sheath cells to recapture lost carbon due to photorespiration (Morgan et al. 1993). This compartmentalization has been used to reduce the carbon loss by inhibiting the metabolism of glycine in the mesophyll cell, allowing it to be recovered in the bundle sheath cells. A dominant marker gdcP was developed from the *gdc* gene for C_3–C_4 intermediate phenotype in the integration of into *B. napus* using a *M. nitens* × *B. olereaca* somatic hybrid (Zhang et al. 2004).

Moricandia sp. has been identified as a CMS source in *B. juncea*. CMS makes the development of hybrid cultivars in open-pollinated crops by inhibiting the production of pollen or anther development. *M. arvensis* was identified as a source of CMS in *B. juncea* by inhibiting pollen production (Kirti et al. 1998). The developed lines were chlorotic and required backcrossing and selection to produce viable female lines. Characterization of CMS systems of *B. juncea* shows that *Moricandia* CMS has unaltered floral organs. The only identifying characteristic is its non-viable pollen making it similar to *B. oxyrrhina* CMS in *B. juncea* (Singh and Srivastava 2006).

Analysis of the source of CMS in *B. juncea* using *Moricandia* cytoplasm has identified *atpα* as a candidate for the mitochondria source gene for CMS (Gaikwad et al. 2006). Analysis of transcription of this gene produced a 2,800-bp RNA fragment in the male sterile line, which was found to be different from the 1,900-bp transcription factor observed in the restored lines. It is, therefore, suspected that altered transcription or RNA editing is responsible for the male sterility (Gaikwad et al. 2006). In CMS *B. juncea* using *Moricandia* cytoplasm, an open-reading frame gene *orf108* was identified as being cotranscribed *atpα* in male sterile flowers. The function of this gene has not been identified but it is suggested that it may produce a soluble protein, and it is unclear whether the protein is toxic in the cell or inhibits translation of *atpα* to cause the sterility to occur (Ashutosh et al. 2008).

CMS hybrid production also requires a restorer gene to allow for the hybrid lines to be viable. These genes are generally nuclear-inherited and *M. arvensis* was identified as a source for restorer genes in *Moricandia* CMS in *B. juncea* (Prakash et al. 1998a). Other Brassicaceae species *Diplotaxis berthautii*, *D. eruciodes*, *D. catholica*, and *Brassica oxyrrhina* were identified as sources of CMS in *B. juncea*. *Moricandia* restorer lines were used to restore male fertility in these CMS, making it a valuable source of restorer lines to multiple *B. juncea* CMS lines (Bhat et al. 2005, 2006, 2008; Singh and Srivastava 2006).

Tissue culture is an important tool in the genetic study of plant species and for the development of transgenic plants. Multiple techniques have been used in *Moricandia* sp. to allow the generation tissue culture derived plants. Plant regeneration has been completed using a number of tissues including hypocotyl, cotyledon, and stem explants (Khehra and Mathias 1992; Craig et al. 1997b). Plant regeneration has also been successfully completed using mesophyll protoplasts to produce plants in *M. arvensis* and *M. nitens* (Murata and Mathias 1992; Tian and Meng 1998). *Agrobacterium tumefaciens*-mediated transformation to produce transgenic plants in *M. arvensis* has been competed using leaf-disk-derived explants with good success (Rashid et al. 1996).

Moricandia has been shown to have potential health benefits unique to this species. The leaves of the *Moricandia* plant have been used in traditional cooking in Tunisia (Skandrani et al. 2009) and have been used in the treatment of syphilis (Le Floch 1983). Recently, *Moricandia* leaf extracts has been shown to have a number of potential health benefits. The leaf extract has been shown to have high antioxidant and antigenotoxic effects (Skandrani et al. 2010b). It has been shown to have free-radical scavenging properties, effectively reducing the reactive oxygen species (ROS) in *E. coli* (Skandrani et al. 2007).

Moricandia extract has been shown to reduce the proliferation of human cancer cells (Skandrani et al. 2010a). The four novel phenolic glycosides identified in *M. arvensis* were found to have antioxidant characteristics (Braham et al. 2005). These compounds could be used in nutraceutical research by introducing them into other field crops or the development of a cultivated *M. arvensis* cultivar.

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Chapter 13
Orychophragmus

Li Rong Zhou, Jun Wu, and Shenghua Wang

13.1 Introduction

The genus *Orychophragmus* comprises two species, *O. violaceus* and *O. limprichtianus*, known as members of the tribe Brassiceae in the family Cruciferae and endemic to China and Korea (Al-Shehbaz and Yang 2000). One of them, *O. violaceus*, has received a lot of attention. On one hand, it is cultivated as an ornamental plant in the gardens or at the roadside in China due to its beautiful purple flower; on the other hand, it is used in studies on genetics and breeding for oil quality improvement of oilseed crops because of its excellent nature of seedoil since 1980s. In this chapter, we introduce and review the research and utility of *O. violaceus* as a wild-plant resource for improvements of edible oilseed crops.

13.2 Basic Botany

13.2.1 Morphology of O. violaceus

The genus *Orychophragmus* shows atypical morphological characters compared to the remainder of the Brassiceae species (reviewed by Anderson and Warwick 1999; Warwick and Sauder 2005). *O. violaceus* is an annual or biennial herbaceous plant with a height of 10–50 cm, erect stem, single branch, or base partial branch. The leaves vary greatly in size and form, lowermost leaves and the lower leaves of the stem pinnately divided, lyrate-shaped, with the terminal lobe large and rounded or ovate and the lower lobes much smaller, and the brim has blunt toothed. The upper leaves of stem are sessile, oval, or slightly oval, the top acuta and base auricular and amplexicaul, and the brim has irregular teeth. Its flowers bloom in January to June, fruiting occurs from May through July. The pollination study revealed that *O. violaceus* is mainly an often cross-pollinated plant (Luo et al. 1991). Its flower is raceme terminal, petals 4, deep purple, lavender, or white, broadly obovate. The fruits are silique, terete, and somewhat 4-angled. Seeds are oblong, with black and brown color, and have the ability of self-sowing (Fig. 13.1).

13.2.2 Taxonomic Position

Orychophragmus Bunge is a small genus with only two species, *O. violaceus* and *O. limprichtianus* as below according to Flora of China (Al-Shehbaz and Yang 2000; Wu 2001a). *Note*: The data of the following classification are from Wu (2001b):

1a. Cauline leaves auriculate; sepals linear, erect, (6–) 8–13(–16) mm, base of lateral pair strongly saccate; petals deep purple, lavender, or rarely white, (12–) 16–25(–32) mm, apex rounded, claw well differentiated and as long as sepals; anthers linear, (3–) 4–6(–8) mm; style (0.3–) 0.7–3(–5.5) cm ..... 1. *O. violaceus*

2a. Cauline leaves not auriculate; sepals oblong, ascending, 2–3.5 mm, base of lateral pair slightly saccate; petals white, (6–) 7–9 mm, apex shallowly emarginate, claw obscurely differentiated and shorter than sepals; anthers oblong,

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DOI 10.1007/978-3-642-14871-2_13,

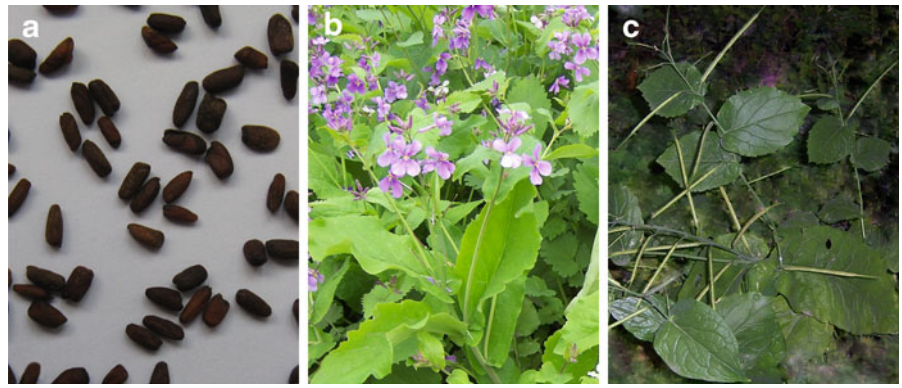

Fig. 13.1 The morphology of *O. violaceus*: (**a**) seeds; (**b**) florescence; and (**c**) fruits

Table 13.1 The difference in morphology among *O. hupehensis*, *O. taibaiensis, O. diffusus*, and *O. violaceus*

Species	Shape of leaf	Amplexicaul leaves	Branch	Stem
O. violaceus	Narrowly ovate or oblongate leaves without lobe	Amplexicaul leaves at the top	A few branches	Erect, upward
O. diffusus	Cordate or reniform and the tip is blunt	Amplexicaul leaves at the base	Many branches	Diffuse, Underdeveloped
O. taibaiensis	Cordate or ovoid triangles	Not amplexicaul	Few branches	Erect, upward
O. hupehensis	Inverse-triangle and apex acute to acuminate.	Not amplexicaul	Many branches	Erect, underdeveloped

1–1.5 mm; style 0.1–0.3 cm .... 2. *O. limprichtianus*

Because *O. violaceus* displays a high variability in morphology, its taxonomy has long been a controversial subject, with its variety identification and species status frequently revised. Schulz (1903) recognized one species with five varieties: *O. violaceus* var. *homaeophyllus* (Hance) O. E. Schulz; *O. violaceus* var. *hupehensis* (Pampanini) O. E. Schulz; *O. violaceus* var. *intermedius* (Pampanini) O. E. Schulz; *O. violaceus* var. *lasiocarpus* Migo, and *O. violaceus* var. *subintegrifolius* (Pampanini) O. E. Schulz, while Tan et al. (1998) identified three additional species: *O. taibaiensis* Tan & Zhao, *O. diffusus* Tan & Xu, and *O. hupehensis* (Pampanini) Tan & Zhang. Among them, *O. hupehensis* was ever defined as a variety of *O. violaceus* in Flora of China (Zhou 1987). Later, Al-Shehbaz and Yang (2000) believed that the variation of *O. violaceus* did not seem to follow any geographical distribution pattern. Hence they re-amalgamated these (including three additional species proposed by Tan et al. (1998)) into the original species *O. violaceus* as a highly variable species without any infraspecific taxa.

In fact, *O. hupehensis*, *O. taibaiensis*, and *O. diffusus* are different significantly from *O. violaceus* not only in morphology (especially in leaf morphology, see Table 13.1), but also in genomes (Table 13.2). Even in *O. violaceus* as the representative species of the *Orychophragmus* genus, there exists chromosome differentiation. This implies cryptic speciation in *O. violaceus* (Zhou et al. 2009a). Furthermore, the molecular phylogenetic results also supported that the groups of *O. hupehensis* and *O. taibaiensis* should not be allocated in *O. violaceus* (Zhou et al. 2009b).

13.2.3 Distribution and Geographical Locations of Genetic Diversity

Species of *Orychophragmus,* as a good ground cover plant, are narrowly distributed in China and Korea, and the main species of this genus *O. violaceus,* endemic to China, is native to northeastern and north of China with strong cold resistance. Now it widely spreads in the provinces of Anhui, Gansu, Hebei, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Liaoning, Nei Mongol, Shaanxi, Shandong, Shanxi, Sichuan, and Zhejiang, and naturalized in Japan (Al-Shehbaz

Table 13.2 Somatic chromosome number ($2n$), base number (n), ploidy level (x), karyotype formula, mean chromosome length (L), and mean length of heterochromatic bands (Het.cr.) of the populations of *Orychophragmus* investigated

Taxa and accessions	$2n$	$n(x)$	Formula[a]	Karyotype	Het.cr. (%)	L (m)
O. violaceus JJ	24	12(2*x*)	16m+2msat+4sm+2smsat	2A	0.508	1.7–2.9
O. violaceus HS	24	12(2*x*)	16m+2msat+4sm+2smsat	2A	0.489	1.3–2.7
O. violaceus SNJ	24	12(2*x*)	16m+2msat+6sm+2smsat	2A	0.503	1.8–3.1
O. violaceus TS	24	12(2*x*)	16m+2msat+4sm+2smsat	2B	0.482	1.9–3.7
O. violaceus BJ	24	12(2*x*)	16m+2msat+4sm+2smsat	2B	0.496	1.6–3.2
O. violaceus CD	24	12(2*x*)	16m+2msat+4sm+2smsat	2B	0.51	1.6–3.0
O. violaceus CS	24	12(2*x*)	14m+2msat+6sm+2smsat	2B	0.484	1.3–2.7
O. violaceus NJ	24	12(2*x*)	14m+2msat+6sm+2smsat	2A	0.481	1.4–3.1
O. violaceus XH	24	12(2*x*)	14m+2msat+6sm+2smsat	2A	0.49	1.4–2.8
O. taibaiensis JK	24	12(2*x*)	14m+2msat+6sm+2smsat	2B	0.517	1.2–3.2
O. taibaiensis HP	48	12(4*x*)	28m+4msat+12sm+4smsat	2B	0.544	0.9–2.7
O. hupehensis WD	22	11(2*x*)	12m+2msat+6sm+2smsat	2A	0.457	1.6–2.7
O. hupehensis SY	22	11(2*x*)	12m+2msat+6sm+2smsat	2B	0.455	1.5–2.8
O. hupehensis DJ	22	11(2*X*)	12m+2msat+8sm	2A	0.447	1.3–2.3
O. diffusus SH	20	10(2*X*)	10m+2msat+6sm+2smsat	2B	0.417	1.5–3.1

[a]*m*, Metacentric; *sm*, Submetacentric; and *SAT*, Satellites

and Yang 2000). *O. violaceus* often grows by roadsides, in gardens, forests, fields, thickets, valleys, hillsides, and near sea level to 1,300 m.

The locations of *O. violaceus, O. taibaiensis*, and *O. hupehensis* are not overlapping but neighboring in their distribution (Fig. 13.2). The present phylogenetic reconstruction indicated that the independence of *O. hupehensis* and *O. taibaiensis* from *O. violaceus* has been karyologically paralleled by a differentiation in chromosome number and karyotype asymmetry (Zhou et al. 2009a). Hence they are supposed as additional species (Zhou et al. 2009a) with the characters of approximate triangle, petiolate, and not amplexicaul upmost stem leaves.

Wide distribution of *O. violaceus* brings about high genetic diversity. Morphology, especially leaf morphology, of *O. hupehensis*, *O. taibaiensis*, and *O. diffusus* differ significantly from *O. violaceus*. *O. violaceus* and *O. diffusus* are biennial herbs mostly with ovate leaves. *O. violaceus* features narrowly ovate or oblongate leaves without lobe and erect stems growing upward, a few branches at the base, and some amplexicaul leaves at the top (Luo et al. 1995a), whereas *O. diffusus* grows with diffuse caules and many branches at base. All of its leaves are lyrata with serrate edge; the terminal leaf is cordate or reniform and the leaf tip is blunt (Tan et al. 1998). *O. taibaiensis* and *O. hupehensis* are annuals and characterized by the uppermost stem leaves having approximate triangle, being petiolate and not amplexicaul. *O. taibaiensis* is different from *O. hupehensis* in that its uppermost stem leaves have slightly pinnate cleavage, cordate, or ovoid triangles with apiculate or obtuse ends, but for *O. hupehensis* its stem is underdeveloped, with all leaves largely pinnated cleavage, inverse-triangle, and apex acute to acuminate.

Similarly, populations of *Orychophragmus* were habitated in various geographical environments that unfolded its genetic diversity. *O. taibaiensis*, growing in Mexican region of altitude of 1,150–1,470 m (Mt. Taibai, Shaanxi), possesses the base number $2n = 4x = 48$ and $2n = 2x = 24$, *O. hupehensis* ($2n = 2x = 22$) limited in regions of elevation of 450–1,000 m (Danjiankou to Shiyan of Hubei); the population of $x = 10$ (*O. diffusus*) located in shanghai, where altitude is 80 m and climate is much warmer.

In the region from the upper and middle Yangtze River to Qin Mountain, the diversity of *O. violaceus* complex is the richest, including populations with various base numbers, such as $x = 11$, 12, and tetraploids and becomes less outward (Fig. 13.2). Therefore, this region can be considered as the center of recent differentiation of the *O. violaceus* complex.

13.2.4 Cytology and Karyotype

According to the scanty literature available, *Orychophragmus* is characterized by the base number $x = 12$

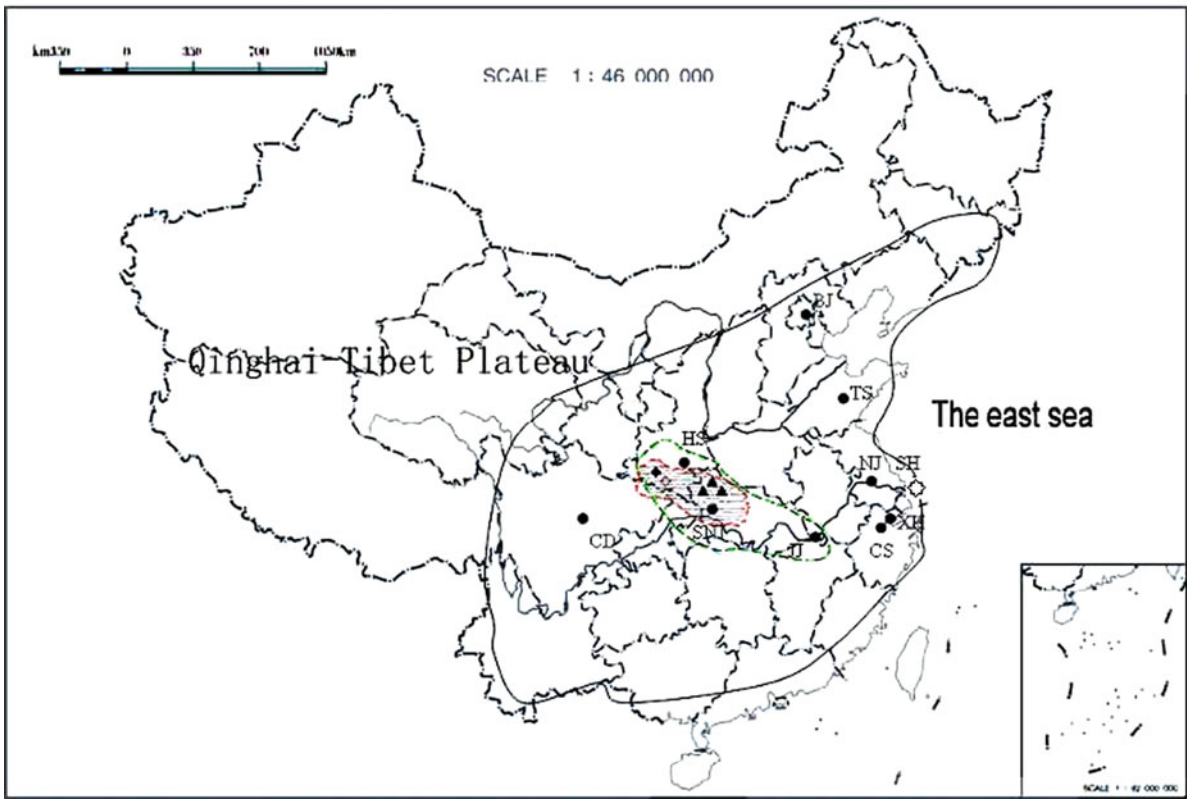

Fig. 13.2 Map of the locations of 15 populations of *O. violaceus* complex and the distribution ranges of different groups. The *red dashed line* indicates the range of *O. hupehensis* and *O. taibaiensis*, the *black line* indicates the range of *O. diffusus* and *O. violaceus*, and the *green dashed line* indicates the region of the presumed center of recent differentiation (Zhou et al. 2009a, b). *Filled dot* represents populations of *O. violaceus*, filled triangle represents populations of *O. hupehensis; Filled diamond* represents populations of *O. taibaiensis,* hollow polygon represents populations of *O. diffusus*

with *O. violaceus* being diploid of $2n = 24$ in the somatic cells and $n = 12$ in the gametic cells (Maton 1932; Li et al. 1994b; Lan et al. 1995). The whole set of chromosomes are divided into nine metacentric chromosomes (one SAT chromosome) and three submetacentric chromosomes (one SAT chromosome). The karyotype formula is: $2n = 24 = 18$ m (2 SAT) + 6 sm (2 SAT) (Li et al. 1994a, b; Luo et al. 1995a, b) and showed a gradual decease in the relative length of the chromosomes, the gaps among them being very small (Li et al. 1994a, b). Obviously, the karyotype of *O. violaceus* belongs to symmetry type 1A–2A (Stebbins 1971).

Otherwise, high frequency of B-chromosomes in population of translocation heterozygote was found in root tip cells, derived from A-chromosomes generated by reciprocal translocation (Li et al. 1994a, b).

However, Zhou et al. (2009a) indicated that besides $x = 12$, *Oruchophragmus* genus also has other base numbers of genomes $x = 10$ and 11 and tetraploids (Table 13.2). Correspondingly, the groups with different karyotypes have different morphological characters. The karyotypes of all populations (or species) investigated have symmetry karyotypes with mainly metacentric chromosomes. *O. violaceus* is comparatively the original type in Brassicaceae, according to the standard of Stebbins (1971).

Lan et al. (1995) observed that chromosomes of *O. violaceus*, from the pachytene to early diakinesis during miosis, often congregate into two chromosome clumps, one with five bivalents (5II) and another with seven bivalents (7II) and believed that *O. violaceus* may be allotetraploid with two different base numbers of chromosomes, which might originate from a

wide-cross hybrid with $x = 7$ and $x = 5$. But Li and colleagues (Li et al. 1995, 1997c; Li and Liu 1995) believed that *O. violaceus* may be tetraploid with $x = 6$ based on chromosome behaviors and configurations in pollen mother cells. This hypothesis was supported by observations based on mitosis of somatic cell and plantlet cell of anther culture (Wu and Luo 1996; Wu et al. 1996b). Recently, the opinion on *O. violaceus* being tetraploid taxa was powerfully supported by CCP data (Lysak et al. 2007).

Additionally, various chromosome configurations, bar-shaped, ring-shaped and cross-shaped bivalents and 8-shaped, ring-shaped, and chain-shaped quadruples appeared in diakinesis caused by chromosome translocations (Lan et al. 1995). Li and Liu (1995) also observed other multivalents in pollen mother cells of *O. violaceus*, such as hexavalents, octovalents, and decavalents. These showed high homology in the genome of *O. violaceus*.

13.2.5 Genome Size

DNA content was measured by Lysak et al. (2007). 2C value of *O. violaceus* is 2.944, which is larger than the genome sizes of Brassiceae species with $2n = 14–36$ (varying from 1.206 to 1.781). However, no significant correlation between base composition and genome size was found (tested non-parametrically by Spearman's rho).

13.2.6 Ecological Characters

O. violaceus has strong ecological adaptability, for example, cold tolerance, leaves dark green in winter, good shade-endurance with normal growth, blooming, and seed set under some diffused light, so that it widely distributes from north to south, from east to west in China. Moreover, in terms of altitude above sea level, its habitat is also wide from tens of meters to more than 2,000 m altitude. It may grow well in half-shade or sunny environments. It often grows in plain, mountains, and roadside or in forest. It is not strict on soil type and can grow in acidic or alkaline soils. However, in loose, fertile, and deep soil, its root system is well developed and can grow well with high output.

13.2.7 Agronomic Characters

O. violaceus has good agronomic characters as found by Luo et al. (1991). It has many branches with the number of primary branches between 13.4 and 14.9, more siliques, more seeds per silique (39.0–39.7), more 1,000-seed weight (3.8–3.98 g); more theoretical yield of seeds reaching up to 2,047.5–2,085 kg/ha. It has virtues of resistance to viral disease and aphid. *O. violaceus* flowers from January to June and is mainly an often cross-pollinated plant species (Luo et al. 1991), fruiting during May through July, with high seed-setting rate of even 100% by natural pollination (Luo et al. 1995a).

Seed oil of *O. violaceus* is high-quality edible oil with high percentage of oleic acids, linoleic acids, and palmic acids and lower percentage of linolenic acids and erucic acids.

13.2.8 Agricultural Status

Since *O. violaceus* not only is green in winter and purple in spring, but also can continue by self-fan and do not need too much maintenance, it is used by a lot of green project, parks, tourist attractions, etc. At present, it is widely planted as flowering ornamental in garden, forest, parks, residential community and highway roadside, etc., and in many cities in north and south of China and has excellent effect of greening and beautification.

Furthermore, it was shown that *O. violaceus* is a potential edible oil crop and a new germplasm source for genetic improvement of *Brassica* crops, since it has a high seed yield potential and its seed oil has a desirable fatty acid composition, especially nearly zero erucic acid and low linolenic acid contents (Luo et al. 1994a, b). The intergeneric hybridizations between the six cultivated *Brassica* species in the U-triangle (Nagahara 1935) and *O. violaceus* were made in order to improve the fatty acid component of the *Brassica* crops, and the cytogenetic studies have been documented (Li et al. 1995, 1998a, b).

13.3 Role in Elucidation of Origin and Evolution of Allied Crop Plants

The tribe Brassiceae with the distinct characters of pungent or acrid juice, cruciate corolla, tetradynamous stamens, and silique or silicle is one of the few natural entities (Al-Shehbaz 1984) in the Brassicaceae family, and it includes about 240 species under 49–54 genera (Gómez-Campo 1999; Warwick et al. 2000). Of them, some species are economically important crops (Table 13.3), for example, *Brassica napus* is one of the world's fourth major oilseed crops and provides 10% of cooking oils, and *Raphanus sativus* and *Brassica chinensis* are widely cultivated as vegetables.

13.3.1 Morphological Traits and Taxonomy

Orychophragmus Bunge, as a member of the Brassicaceae family, according to Zhou (1987), was assigned to the tribe Brassiceae that in China contains eight genera including *Brassica, Sinapis, Diplotaxis, Eruca, Raphanus, Crambe, Orychophragmus,* and *Conringia*. However, it was excluded by Gomez-Campo (1980), Li et al. (1996), and Khosravi et al. (2009) but included again in the tribe Brassiceae by Anderson and Warwick (1999), and Warwick and Sauder (2005). This controversy over taxonomic status of genera and species existed continuously in the Brassiceae family due to the common existence in convergent evolution and reticulate evolution of morphological characters used in systematic classification of plants and in unsynchronized evolution at genus or tribe level of different characters.

Obviously, elucidation of the taxonomic status of *Orychophragmus* is an important basis for developing and utilization of the genus itself as a useful wild plant resource (see Sects. 13.1 and 13.8) and depicting its phylogenetic relationship with the alien crop species. For this, it needs to include more botanical characters to the traditional systematics and taxonomy, including leaf venation, micromorphology, such as indumentum, gland (nectary; Deng and Hu 1995), stomata, and even anatomical characters. For example, leaf venation pattern is nearly the same in plants of the same species and exhibits stability (Nam et al. 2008). Leaf venation character can be used as an evidence for taxonomy of interspecific, intergeneric, and interfamilial classification (Zhang and Xia 2007). By observing the leaf venation characteristics of nine species of six genera of Brassiceae, Liu et al. (2006) suggested that *Brassica* have a more close relationship to *Eruca* and *Crambe*

Table 13.3 *Orychophragmus* and its some major allied crop plants in tribe Brassiceae

Genus	Species	2*n*/1C DNA (pg)	Genome	Economic utility
Brassica	*B. nigra*	16/0.65	BB	Oilseed, vegetable and condiment
	B. oleracea	18/0.71	CC	Vegetable
	B. rapa syn. campestris	20/0.54	AA	Oilseed and vegetable
	B. carinata	34/1.31	BBCC	Oilseed and vegetable
	B. juncea	36/1.09	AABB	Vegetable
	B. napus	38/1.15	AACC	Oilseed
	B. bourgeaui	18	?	
	B. napobrassica	38		Vegetable
	B. alboglabra	18	CC	Vegetable
	B. chinensis	20	AA	Vegetable
	B. parachinensis	20	AA	Vegetable
	B. pekinensis	20	AA	Vegetable
	B. tournefortii	20/0.60	TT	Condiment
Orychophragmus	*O. violaceus*	24/2.5	OO	Ornamental, medicinal
Raphanus	*R. salivus*	18/0.53	RR	Vegetable
Sinapis	*S. alba*	24/0.57	SS	Condiment, medicinal
Crambe	*C. kotschyana*	30		Oilseed
	C. maritima	60		Vegetable
Eruca	*Eruca sativa*	22/0.67	EE	Vegetable

Note: Data of 1C DNA amount (pg) are from Lysak et al. (2009)

than to *Conringia*, *Orychophragmus*, and *Raphanus* and *B. oleracea* var. *acephala* might be the comparatively primitive species in *Brassica*. Recently, Nam et al. (2008) proposed a scheme for similarity-based leaf image retrieval not only to save time and energy but also to increase the efficiency.

13.3.2 Biochemical Techniques and Systematics

A morphological trait, after all, is the last comprehensive appearances of metabolic activities controlled by a variety of genes. Morphotaxonomy is more sensitive and less accurate than biochemical and molecular systematics to elucidate the origin and evolution in the Brassicaceae family.

Biochemical markers might constitute a feasible approach for studying the genetic relationship among species, and this approach has benefited more in cases of animals than plants. Anderson and Warwick (1999) claimed, based on the variation of isozyme patterns in the tribe Brassiceae, that *Orychophragmus* should be included in the tribe Brassiceae and *Calepina* and *Conringia* should be excluded. However, biochemical systematics would be more successful for taxonomy of Brassicaceae family if the biochemical markers are extended, which in the current uses were the isozymes mainly involved in carbohydrate metabolism, such as development of protein production of supergene families related to growth and development of plant. Selection of protein productions of family genes linking 2D-electrophoresis and immunoprecipitation techniques perhaps plays some beneficial effects.

Chemotaxonomy. Although there are some research works on chemotaxonomy of *Orychophragmus* and *Brassica*, respectively (Tsukamoto et al. 1993; Tang et al. 1996), little research was done on the chemotaxonomic comparison of *Orychophragmus* and its allied crops.

13.3.3 Molecular Biology and Evolution

Molecular biological methods are widely believed as the best effective strategy for plant taxonomy and relationship among species. For all that, appropriate selection and discovery of molecular straits are very important. The molecular straits here are defined as the independent and indicable fragments of DNA or genome (Table 13.4). The molecular marker techniques, such as restriction fragment length polymorphism (RFLPs), simple sequence repeat (SSR), intersimple sequence repeat (ISSR), microsatellites,

Table 13.4 The cp DNA fragments in molecular systematics

Gene or DNA fragments	Main application scope	References
cpDNA fragment		
*Rbc*L	Genera, family	Xiang et al. (1993); Sehwarzbach and Rieklefs (2000); Sosa and Chass (2003)
*Mat*K	Intergeneric and interspecific relationship in family	Plunkett et al. (1997); Hu et al. (2000)
*Trn*L, intron, and *trn*L-F intergenic region	Interrelated family, subfamily stribe or genus	Clegg et al. (1994); Downie et al. (2000b)
*Ndh*F	In family or interfamily	Scotland et al. (1995); Clark et al. (1995); Smith et al. (1997)
*Atp*B	Genera, family	Soltis et al. (1999)
Mitochondria		
*Cox*I	Taxa above family	Malek et al. (1996); Bowe et al. (2000)
*Nad*5		Wang et al. (2000)
*Mat*R	Taxa above genus	Qiu et al. (1999); Meng et al. (2002)
*Atp*A	Taxa above family	Davis et al. (1998, 2004)
Nuclear genome		
18s rRNA	Family and above family	Soltis et al. (1997)
ITS (18S rDNA – 5.8S rDNA – 26S rDNA)	Phylogeny and taxonomy of family, subfamily, stribe, genus, and group	Baldwin (1993); Baun et al. (1994); Downie et al. (2000a); Álvarez and Wendef (2003)

amplified fragment length polymorphisms (AFLP), and random amplified polymorphic DNA (RAPD), are widely used for analysis of phylogenetic relationship among species.

Wang et al. (2005) believed that through analyzing the phylogenetic relationship among 11 species of six genera in Cuciferae, *CYP*86*M F* gene, a member of cytochrome P450 supergene family, is applicable to genus taxon because of its lower differences of nucleotide and amino acid sequences between species than genera. Ding et al. (2007) presented that *S*-adenosylmethionine decarboxylase activity (SAMDC), a key enzyme involved in biosynthesis of the polyamines, viz. spermidine and spermine, is also feasible marker for elucidating the relationship among crucifer species.

More recently, the ITS region and *trn*L intron are prevailing in phylogenetic analyses of the Brassicaceae (Warwick et al. 2002, 2004; Koch et al. 2003). The base for inclusion of *Orychophragmus* in tribe Brassiceae by Warwick and Sauder (2005) was that it shared monophyletic origin with Brassiceae revealed by comparison analysis of the cpDNA and ITS and ITS/*trn*L sequence data. Based on molecular phylogenetic analysis of ITS/5.8S and matK sequences, Zhou et al. (2009a) indicated the exclusion of *O. hupehensis* and *O. tabaiensis* from *O. violaceus* but inclusion of *O. diffusus* in *Orychophragmus*.

Fluorescence in situ hybridization (FISH) provides alternative measure with intuitively detecting and localizing interested genes or sequences in chromosome, analysis of chromosome structure variation (Nath and Johnson 2000). Many new techniques have been currently derived from it, such as multicolor-FISH, comparative genomic hybridization (CGH), genomic in situ hybridization (GISH), chromosomal in situ suppression (CISS), bacterial artificial chromosome-fluorescent in situ hybridization (BAC-FISH), chromosome painting (CP), comparative chromosome painting (CCP), and reverse chromosome painting (RCP). GISH has been widely used for the comparison of genetic relationship between species and detection of introgressed chromosome fragment and the origin and evolution of species (Schwarzacher et al. 1992; Hua and Li 2006). CCP began to be used to the origin and evolution of Brassiceae (Lysak et al. 2005, 2007). Lysak et al. (2007) believed that *O. violaceus* is a tetraploid taxon sharing the same ancestor with Brassiceae and represents a bridge between the diploid *Calepina*/*Conringia* lineage and the hexaploid Brassiceae.

Brassica is a polyploid complex comprising diploidy and tetraploidy, which covers many economically important crops. The evolution and relationships between members of the genus *Brassica* can be elucidated with the U-triangle (Nagahara 1935). The hybridization results between *Brassica* species and *O. violaceus* and behaviors of their hybrids indicated that *O. violaceus* has not undergone the Brassiceae-specific triplication event (Lysak et al. 2007).

13.4 Role in the Development of Cytogenetic Stocks and Their Utility

Until now, the intergeneric crossing of *O. violaceus* with alien plants in Brassiceae tribe performed mainly in oilseed crops aims at improving seed oil quality. Distant genetic relationship of *O. violaceus* and *Brassica* species has often led to unsuccessful hybridization between them because of the reproductive barrier. But the development of hybrid progenies is effectively stimulated by ovary culture and embryo rescue (Li et al. 1995, 2000; Wang et al. 2008). These hybrid progenies are extremely unstable (Comai 2000) and produce a wide separation profile, for instance, various *Brassica* aneuploids, including alien additions, substitutions and hypoploids, and create novel species, which makes possible to stimulate basic research on genome structures and phylogenetic relationship of *Brassica* and to the improvement of crop output and quality. Hence, distant hybridization between *O. violaceus* and alien cultivated crops in crucifer family is an effective approach to construct various alien crop stocks for genetic analysis.

13.4.1 *Mixoploid Hybrids from Distant crosses of* O. violaceus

The F_1 hybrids of *O. violaceus* and its cultivated alien *Brassica* crop species are almost mixoploid (Li and Ge 2007; Zhao et al. 2008).

The U-triangle *Brassica* species are derived from the different combinations of three different genomes (A- B- and C-genome; Nagahara 1935). The difference

of these genomes controlling chromosomes behaviors results in the diversity of chromosome composition in hybrid cells of *O. violaceus* with *Brassica* species (Li and Ge 2007), some cells possess both parental chromosome complements, such as the hybrids of *B. oleracea* ($2n = 18$, CC) with *O. violaceus* ($2n = 24$, OO) containing 21 chromosomes; some cells possess chromosomes from only one parent and other cells possess complete and incomplete genome from one parent and some additional chromosomes from another parent (Li et al. 1998a, b; Li and Heneen 1999).

13.4.2 Developments of Aneuploids from O. violaceus with Alien Crops

Apart from the types with similarity to crop chromosomal structures, the mixoploid hybrids of *O. violaceus* with alien oilseed crops will separate many types of offsprings with different chromosomal constitutions in the following generations, leading to the production of various genetic stocks, including hypoploids, alien substitution and addition lines, trisomics, double trisomics and double tetrasomics through different assembling strategies of crosses, such as seilfing or backcrossing, and selections for several generations.

13.4.2.1 Alien Chromosome Addition and Substitution Plants

Alien substitution and addition lines, i.e., intergeneric aneuploids, were separated from the mixoploid hybrids of cultivated *Brassica* species. The chromosomes of *O. violaceus* were easy to be identified due to the characteristics of its chromosome sizes and morphology (Li and Ge 2007), such as more darker and condensed chromosomes (Xu et al. 2007a, b). Researches reported that the mixoploid hybrid ($2n =$ 30–42) of *B. juncea* ($2n = 36$) with *O. violaceus* produced some substitution lines with 1–4 pairs of additional chromosomes of *O. violaceus*, and some lines with additional chromosomes of *O. violaceus* (Li et al. 2003). Some substitution plants with 1–3 pairs of chromosomes from *O. violaceus* were also produced from mixoploid hybrids ($2n =$ 29–34) of *B. carinata* ($2n = 34$) with *O. violaceus* (Li et al. 2003). Liu and Li (2007) reported again that they had obtained *Brassica juncea–Orychophragmus violaceus* addition and substitution lines.

The same hybridizations performed at different times might obtain different mixoploid profiles, for example, research group of Li at the Huazhong Agricultural University reported in 1995 that mixoploid hybrids ($2n =$ 12, 19, 24, 31, and 38) with fertility derived from *B. napus* with *O. violaceus* produced two kinds of progenies: a high percentage of parental *B. napus* (84–95%) and a low percentage of mixoploid offsprings (5–16%), while Cheng et al. (2002) found that the F_1 plants from the same hybridization might be classified into three groups: one had $2n = 29$ chromosomes with 9.2–11.7% *O. violaceus*-specific RAPD fragments, two had $2n =$ 35, 36, and 37 chromosomes, and the 3rd group had $2n =$ 19, 37, 38, and 39 chromosomes. Both 2nd and 3rd group had no *O. violaceus*-specific RAPD fragments. This phenomenon observed by Cheng et al. (2002) was substantiated by Li's group (Hua and Li 2006).

13.4.2.2 Hypoploids

Naturally, hypoploids (nullisomics, monosomics, and hyperploids as trisomics, tetrasomics) are obtained from the mixoploid plants with $2n$ less one parental chromosome number, too.

The case of *Brassica* species hypoploids was from the hybrids of *O. violaceus* with *B. napus*. From one F_4 hybrid ($2n =$ 50, AACCO) population, Wu et al. (2004d) identified three monosomic plants ($2n = 37$) with different morphology and fertility. One of these three monosomics grew vigorous and the unpaired small chromosome had no negative effects on plant development. Accordingly, it can produce nullisomic lines through selfing. Besides that, they also found four types of hypoploid plants with 29–32 chromosomes from the progenies of P3 (aneuploids) with 41–44 chromosomes.

13.4.2.3 Other Aneuploids from Mixoploid Hybrids of *O. violaceus* with *Brassica* Alien Crops

Hua et al. (2006b) reported that the new materials with $2n = 39$, 40, and 42 were obtained after one offspring plant with $2n = 42$ and no *O. violaceus* chromosome

was backcrossed to *B. napus*. This offspring without *O. violaceus* was derived from in vitro microspore culture of mixoploid hybrid ($2n$ = 19–37) between *B. napus* and *O. violaceus*. Obviously, these new materials are trisomics, double trisomics, and double tetrasomics of *B. napus*, because chromosome pairing patterns in pollen mother cells (PMCs) were 19II + 1I, 19II + 2I, and 19II + 2II at diakinesis.

13.4.3 Approaches to Produce Brassica Aneuploids

Several routes are used to construct aneuploids for genetic analysis.

The first one is conventional sexual cross. Aneuploids are produced by cross-synthesizing alloploids and then selfing or backcrossing accompanied by oriental selection of generations. This is the most commonly used approach for effective construction of cytogenetic stocks. For instance, Xu et al. (2007a) reported that many novel lines with $2n$ = 36–40 and without intact *O. violaceus* chromosomes detected by GISH were established from the mixoploid hybrids ($2n$ = 23–42) with partial fertility from the cross between *B. rapa* ($2n$ = 20, AA) and *O. violaceus* ($2n$ = 24, OO) through successive selection for fertility and viability. These lines showed high productivity, a wide phenotypic spectrum, and obvious variations of fatty acid profiles in seed oil and glucosinolate contents in seed meal.

The second is somatic hybridization. Somatic hybridization between untreated and X- or γ-irradiated protoplasts is helpful to the transfer of partial genomes (Gupta et al. 1984; Bates et al. 1987). Successful cases in this asymmetric somatic cell fusion as well as symmetric cell fusion have been provided by (Hu et al. 2002; Mei et al. 2003; and Zhao et al. 2008).

Additionally, gametosomatic hybridization may be an alternative approach of cell fusions for development of alloploid cytogenetic stocks of *Brassica*. Li et al. (1994a, b) had successfully documented pollen-somatic protoplast fusion in *Brassica*. *O. violaceus* has a strong ability for in vitro plantlet regeneration (see Sect. 13.5). Wiegand et al. (1995) had ever attempted in the *Brassiceae* to produce alien addition and substitution lines of *B. napus* by gametosomatic hybridization.

The hybrid progenies will recover the fertility through successive selection for fertility and viability (Xu et al. 2007a).

13.4.4 Theoretical Basis of Aneuploids

In hybrids of *O. violaceus* as paternal parent and *Brassica* species as maternal parent (very difficult to success in reciprocal crosses), cytological events, including genome doubling, complete or partial separation of genome, elimination of chromosomes, DNA fragment introgression, changes, and recombination, often occur in cell cycles (Li et al. 1995, 1998a, b; Li and Heneen 1999; Hua et al. 2006a, b; Ma et al. 2006; Li and Ge 2007). These events can appear in mitosis and meiosis from zygote development to reproductive process (Li and Liu 2001). If parental genome doubling and separation of chromosomes accompanied by partial elimination of *O. violaceus* chromosomes occur, substitute lines and hyperploids including addition lines can be established. On the contrary, hypopids, monosomic, double monosomic and nullisomic can be established if parental genome doubling and separation of chromosomes accompanied by complete elimination of *O. violaceus* chromosomes occur (Li and Ge 2007).

Hua et al. (2006a) reported that some mixoploid F_1 ($2n$ = 34) only contains *B. carinata* maternal chromosome complement, implying *B. carinata* genome doubling and elimination of the entire *O. violaceus* chromosome complement. However, for all that, some *O. violaceus*-specific AFLP bands and new bands were detected in the leaves of F_1 plants; some F_2 plants that were predominantly like *B. carinata* showed some *O. violaceus*'s traits, brown seed coat, and the purple color. Obviously, some introgression of *O. violaceus* genetic substances took place in hybrid offsprings that provides the theoretical basis for minor improvement of good oil crop variety.

Hence, the cytological events at the whole chromosomal level in hybrid cells are also accompanied by DNA fragment recombination and introgression, producing *Brassica* type plants with modified genetic constitutions and phenotypes (Liu and Li 2007).

Wu and Luo (1996) also reported that plantlets regenerated from cultured ahther of *O. violaceus* were also mixoploid. This type of mixoploid comprises only cells with different numbers of *O. violaceus* chromosome,

suggesting *O. violaceus*'s polyploidy property and the possibility of aneuploids of *O. violaceus*.

13.4.5 Transfer of Other Genomes to Brassica Crops

Until now, in the *Brassica* genetics and breeding researches, the attention is mainly focused on the behaviors of nuclear genomes from hybridization parents, while the behaviors and effects of other two genomes of parents, mitochondrial genome and chloroplast genome, on hybrid progenies, are also worth studying. The inheritance and role of organelle genomes from *Orychophragmus* can be explored by asymmetric fusion of *Orychophragmus* protoplast X-ray or γ-ray irradiation.

13.5 Role in Classical and Molecular Genetic Studies

13.5.1 Chromosome Behavior of O. violaceus and Their Role

The meiosis process of pollen mother cell of *O. violaceus* is similar to *B. napus* and other allied crops, but its chromosomal behavior also has its own traits. The bivalents in diakinesis present circle and club-shaped, etc. Moreover, chromosomal configurations vary in this period. For example, some appear as shapes of "8," some as circles, and some as catenulate quadruples. These configuration series may reflect chromosomal reciprocal translocation. Additionally, polymorphism of the number and shape of nucleolus also appeared (Luo et al. 1995a, b).

Due to the peculiar behaviors of *O. violaceus* chromosomes and its distant relationship to Brassiceae species and because of some possible hybridization compatibility factors unknown, hybridization between *O. violaceus* and allied species, including somatic hybridization, only can get a few seeds. These hybrid seeds with rich genetic base often show the abnormal phenomena: univalents, precocious chromosome migration to the poles, laggard and micronucleus at anaphase/telophase II, tetrads with some microcytes and I, extra division of tetrad or microspore, and parental genome separation and thus produce diversity of genetic structures and more available information or new materials for theoretical genetic research.

Intergeneric hybrids derived from *O. violaceus* as parent often consist of mixoploidy cells (mixture of diploid and polyploid cell; Table 13.5). For example, somatic tissue of F_1 plants from the cross *B. napus* L. (AACC, $2n = 38$) × *O. violaceus* were mixoploid, consisting of haploid ($2n = 2x = 19$) and diploid cells ($2n = 4x = 38$) of *B. napus* as well as hybrid cells ($2n = 31$; Li et al. 1995).

Genome doubling and chromosome elimination by separate, complete, and partial elimination of parental chromosomes change ploidy level of some genomes in the offsprings of distant hybridization, such as the intergeneric hybrids of *B. napus* × *O. violaceus* (Cheng et al. 2002). Even though there is no change in chromosome number, ploidy level of some genomic fragments can also change due to exchange and translocation. Ge and Li (2006) and Sakhno et al. (2007) found no chromosome replication and nuclear fusions

Table 13.5 Wide crosses of *O. violaceus* (OO, $2n = 24$) as male parent

Female parent	Hybrids	References
B. napus ($2n = 38$, AACC)	F1: $2n = 19$, 35, 36, 37, 38, 39	Luo et al. (1994b); Li et al. (1995, 1996); Wu et al. (1997a, b, c); Cheng et al. (2002); Hu et al. (2002); Mei et al. (2003)
B. alboglabra ($2n = 18$, CC)	F1: $2n = 21$	Yin et al. (1998); Luo et al. (2000b)
B. juncea ($2n = 36$, AABB)	F1: $2n = 12$–43	Li et al. (1997a, b, c, 1998a, 2002, 2003); Lin et al. (2005a); Xu et al. (2007a, b)
B. carinata ($2n = 34$, BBCC)	F1 = 12–35	Li et al. (1998b, 2002); Hua et al. (2006)
B. oleracea ($2n = 18$, CC)	F1: $2n = 21$	Li et al. (1998b)
B. nigra ($2n = 16$, BB)	F1: $2n = 17$–26, 11–17 and 14–17	Li and Heneen (1999)
B. campestris ($2n = 20$, AA)	F1: $2n = 23$–42	Li and Heneen (1999)
B. chinensis ($2n = 20$, AA)	F1: $2n = 22$	Wu et al. (1996a, b, c)
B. rapa ($2n = 20$, AA)	F1: $2n = 23$–42	Li and Ge (2007)

in the nuclei of tetrads produced by wide hybrids between an artificially synthesized *Brassica* allohexaploid ($2n = 54$, AABBCC) and *O. violaceus* (OO). They reported that they have obtained phosphinothricin-resistant intergeneric hybrids through somatic hybridization between *B. napus* and *O. violaceus* transformed by the *Bar* gene. Hence, wide cross of *O. violaceus* and other allied species can provide more novel materials for genetic studies.

Because of its high-quality seed oil, *O. violaceus* was used to improve the fatty acid profile of *Brassica* crops through intergeneric hybridization (see Sect. 13.6). During the process of practicing for transfer of the favorable traits of *Orychophragmus* to oilseed crops, discovery of the arcanum of controlling these favorable traits is a necessary work through genetics and molecular means.

13.5.2 In Vitro *Culture and Transformation of* O. violaceus

The plants of *Orychophragmus* have strong regeneration capacity. Since Pan and Huang (1986) first reported successful plantlets regeneration from *O. violaceus* flower buds, nearly every part of *O. violaceus* plant could be as explant for in vitro culture (Table 13.6). Its advantages showed the following four aspects.

13.5.2.1 Strong Plantlet Regeneration In Vitro

Explants from various parts of *O. violaceus* can propagate and regenerate plantlets with over 78% success (Luo et al. 1996) under cultured conditions (Table 13.6).

Furthermore, the explants stimulated by different combinations of phytohormones may give rise to intact plants through several pathways including direct organogenesis, indirect organogenesis, somatic embryogenesis, and indirect somatic embryogenesis. Of them somatic embryogenesis was originated from single-cell, similar to zygotic embryogenesis (Zhang and Luo 1995).

13.5.2.2 An Ideal Material for Protoplast Culture

The protoplasts from petiole and mesophyll or hypocotyl and cotyledon of *O. violaceus* had high capacity for plant regeneration in culture with over 40% division frequency and 100% regeneration frequency

Table 13.6 Tissue cultures from different explants of *O. violaceus*

Explants		Regeneration pathways[a]	References
Organ culture	Leaves	IO, DSE	Xu and Xu (1987); Zhang and Luo (1995); Wu et al. (2004b, c)
	Petiole	ISE, DSE	Xu and Xu (1987); Zhang and Luo (1995); Wu et al. (2004b, c)
	Hypocotyl	DO, IO, DSE	Zhang and Luo (1995); Chen et al. (2001)
	Cotyledon	DO, DSE	Zhang and Luo (1995); Luo et al. (1996); Zhou et al. (1996a)
	Flower bud, flower stalk	IO	Pan and Huang (1986)
	Rachis	DO	Ye and Lan (1998)
	Petal, sepal and filament	IO	Ye and Lan (1998)
	Anthers	DSE	Luo et al. (1996); Wu and Luo (1996); Wu et al. (2000)
	Receptacle	DO, ISE	Zhang and Luo (1994); Luo et al. (1996); Ye and Lan (1998)
	Pollen	ISE	Jia et al. (1999)
	Embryo	ISE	Wu et al. (1996a, b, c)
Protoplast culture	Mesophyll	C-SE?	Xu and Xu (1988); Hu et al. (1999)
	Hypocotyl, cotyledon	IO or ISE	Luo et al. (1996); Zhou et al. (1996a, b)
	Petiole	IO	Luo and Luo (1992)
Flower induction in vitro	Seedling		Luo et al. (1998a, b, c, 2000)
	Receptacle, pedicel		Zhang et al. (1999)
In vitro maturation of microspores			Zhao et al. (2007b)

[a]*IO*, Regeneration of explants through organogenesis from callus; *DO*, Regeneration of direct organogenesis from explants; *ISE*, Regeneration of explants through somatic embryogenesis from callus; and *DSE*, Regeneration of direct organogenesis from explants

(Xu and Xu 1988; Luo and Luo 1992; Luo et al. 1996; Zhou et al. 1996a; Hu et al. 1999).

13.5.2.3 In Vitro Flower Induction

Zhang et al. (1999) reported that flower was directly induced from receptacle and pedicel and this potency continued to be stable under artificial conditions. This means that some molecules controlling flower development exist in receptacle and pedicel that are remembered and transferred in one mode or the other. However, this memory is wiped out if the cultural conditions changes, receptacle and pedicel dedifferentiate into callus and then re-differentiate into plantlets (Zhang and Luo 1994; Luo et al. 1996; Ye and Lan 1998). Besides, germinating seeds treated by low temperature 5–7°C for 7 days and cultured with the medium containing GA3 (gibberellic acid 3) do not undergo vegetative growth but undergo reproductive growth, giving rise to flowers (Luo et al. 1998c).

13.5.2.4 In Vitro Maturation of Microspores

Microspores isolated from *O. violaceus* developed into mature pollen with bio-function under controlled conditions (Zhao et al. 2007a, b). This initiated a novel possibility for the development of male gamete and male gametophyte and deciphering their gene regulation under artificial conditions and suggesting possibility of gene transfer through the male gametophyte.

Hence, *O. violaceus* may be an excellent model plant in Cruciferace for plant cell and tissue culture. This characteristic is very helpful to basic and applied research including genetics and breeding.

13.5.3 *Genetic Transformation*

Based on the established regeneration system, *Agrobacterium*-mediated transformation protocols for *O. violaceus* cotyledons and hypocotyls were developed, with the transformation frequency over 5% (Zhou et al. 1996a, 1997). Moreover, the PEG-mediated transformation using hypocotyl protoplasts of *O. violacues* (Zhou et al. 1996c) was also documented. Thus, it provides a new possibility for genetic improvement of *O. violaceus* and carries forward research on molecular mechanism related to lipid metabolism and agronomic traits. Sakhno et al. (2007) identified hybrids resistant to phosphinothricin (PPT) from transformed *O. violaceus* through somatic hybridization with *B. napus*.

13.5.4 *Other Characters of* O. violaceus

O. violaceaus has also strong resistance against aphid and Downy mildew. But *O. violaceaus* still has some disadvantages, such as weak resistance to Sclerotium disease and it also can be used to study the biological characteristics and pathogenesis of pathogens. Additionally, *O. violaceus* is a host plant for cauliflower mosaic virus (CaMV). Its protoplast is often used to study the interaction between virus promoter sequence and translation initiation of mRNA in planta (Gordon et al. 1992; Fütterer et al. 1996; Pooggin et al. 2000).

13.6 Role in Crop Improvement Through Traditional and Advanced Tools

B. napus (AACC, $2n = 38$) is one of the most widely cultivated rapeseed crop species in the Crucifer family (Brassicaceae) over the world, providing 10% of edible oil for human beings. However, long-term and large-scale cultivation of this single species resulted in a depleted gene pool of oilseed crops, which is not desired for breeding for edible oil as it targets for high oleic acid, low saturated fatty acids and linolenic acid and <30 mmol glucosinolate/g in the seed oil; high oil yield including seed yield and oil content of seed; and strong tolerances to lodging, disease, herbicides, and pod shattering. Therefore, introduction of new germplasm from natural resources including wild plants is an effective strategy to widen gene pool and improve rapeseed.

13.6.1 O. violaceus*: A germplasm of Superior Quality for Rapeseed Breeding*

O. violaceus has been found to have good oil quality with high palmitic and oleic acids, and low linolenic and erucic acids in seed oil (Table 13.7). Moreover,

Table 13.7 Seed oil profile of *O. violaceus* and *B. napus* cv. Oro (after Luo et al. 1991)

Species	Palmitic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Eicosenoic acid (%)	Erucic acid (%)	Glucosinolate (μmol/g oil free meal)
O. violaceus	14.31	20.32	53.17	4.76	4.49	0.94	
B. napus cv. Oro	2.92	14.56	12.94	9.00	9.42	51.15	64.9[a]

[a]Data of *B. napus* cv. Oro are from Ma and Li (2007)

Table 13.8 Summary of crosses between *Brassica* species and *O. violaceus*

Crosses	Characters of hybrids	References
B. napus ($2n = 38$, AACC) × *O. violaceus* ($2n = 24$, OO)	Morphologically intermediate between parents, but could produce a lot of seeds when selfed, $2n = 31$	Li et al. (1994a, b, 1995, 1996); Wu et al. (1997b, 2002); Cheng et al. (2002); Hu et al. (2002b)
B. juncea ($2n = 36$, AABB) × *O. violaceus* ($2n = 24$, OO)	An intermediate morphology except for petal color and partially fertile, mixoploid ($2n = 12$–42), and cells mostly with 24, 30 or 36 chromosomes	Li et al. (1997a, b, c, 1998a, b, 2003); Xu et al. (2007a, b)
B. carinata ($2n = 34$, BBCC) × *O. violaceus* ($2n = 24$, OO)	A matroclinous morphology and nearly fertile, mixoploids ($2n = 12$–34), and cells most frequent with $2n = 34$	Li et al. (1998a, b)
B. alboglabra ($2n = 18$, CC) × *O. Violaceus* ($2n = 24$, OO)	Morphologically intermediate between parents, $2n = 21$, totally sterile	Yin et al. (1998); Luo et al. (2000)
B. campestris ($2n = 20$, AA) × *O. violaceus* ($2n$=24, OO)	Morphologically intermediate, except for partial fertility and yellow petals, mixoploid ($2n = 23$–42)	Li and Heneen (1999)
B. oleracea ($2n = 18$, CC) × *O. Violaceus* ($2n = 24$, OO)	An intermediate morphology, but petals purple, $2n = 21$, sterile	Li and Heneen (1999)
B. nigra ($2n = 16$, BB) × *O. violaceus* ($2n = 24$, OO)	Majority of the F1 plants of maternal type ($2n = 16$), a small fraction with *B. nigra* morphology but mixoploids, $2n = 16$–18	Li and Heneen (1999)
B. napus with OguCMS × *O. violaceus* ($2n = 24$, OO)	Morphological characters intermediate between parents, high male sterility and low female fertility, $2n = 31$	Wu et al. (2004a)

O. violaceus possesses a high seed yield potential, with the clustering of stems, high number of pods per plant, profuse seeds per pod, and large seed size (Luo et al. 1991; Table 13.14). Consequently, *O. violaceus* is endowed with a valuable wild plant spermplasm resource for oilseed crop improvement (Luo et al. 1994a).

13.6.2 Strategy for Improvement of Alien Crops Using O. violaceus

13.6.2.1 Sexual Wide Crossing

The desired traits can be introduced into *Brassica* species by appropriate approaches from wild relative species. In fact, Ma and Li (2007) have obtained many novel *B. napus* inbred lines with significant improvement of oil quality, for example, oleic acid was 10% higher than that of parent *B. napus*, linoleic acids 40% higher than that of parent *B. napus*, and less than 30 μmol/g of glucosinolate in oil free meal, by pedigree selection through F12 generation in the progenies of one *B. napus* cv. Oro × *O. violaceus* F5 hybrid plant ($2n = 31$), which had slight yellow petals.

A wide sexual cross is a traditionally effective means for transfer of these traits from *O. violaceus* into rapeseed crops (Li et al. 1995, 1998a, b; Qu et al. 1996; Séguin-Swartz et al. 2000). Luo and colleagues in the Sichuan University, Li and colleagues in the Huazhong Agricultural University, and other researchers have conducted a lot of research works in this direction (Table 13.8). The current information show that in the hybridizations between the cultivated *Brassica* species and *O. violaceus*, all these F1 hybrids turn out to be non-classical, i.e., composed of cells with divergent chromosome numbers, except for the hybrids with *B. oleracea* (Li and Ge 2007). It is noted that ovary culture and embryo rescue techniques

should be effective in the intergeneric hybridization between *Brassica* and *Orychophragmus* (Luo et al. 1994a, Li et al. 1995; Wu et al. 1996a, b, c).

13.6.2.2 Somatic Hybridization

Somatic hybridization, an asexual wide hybridization, which may overcome the reproductive barriers of sexual incompatibility, often happened during sexual wide hybridization, is another effective means for transfer of desired traits from related wild plant resources into crop species. Somatic hybridization based on protoplast fusion may be performed by symmetric fusion, asymmetric fusion, and microfusion of protoplasts. *O. violaceus* is a plant material with high in vitro regeneration capability including protoplast culture as mentioned above (see Sect. 13.5). Therefore, it is technically feasible to perform intergeneric somatic hybridization between *Brassica* and *Orychophragmus*.

Recently, several successful cases of protoplast fusion between *Brassica* species and *O. violaceus* have been reported (Hu et al. 2002; Mei et al. 2003; Zhao et al. 2008). Zhao et al. (2008) reported that somatic hybrids between *B. napus* and *O. violaceus* through asymmetric fusions of mesophyll protoplasts were mixoploids containing different numbers of *O. violaceus* chromosomes and gave rise to *B. napus–O. violaceous* chromosomal addition lines through further backcrossing and embryo rescue. On the other hand, some sterile materials have been obtained from somatic hybrids between *O. violaceus* and *B. napus* by Hu et al. (2002). Among the progenies of asymmetric fusions of the donor parent, *O. violaceus,* protoplasts irradiated prior to fusion, Hu et al. (2002) reported the possibility of somatic hybridization between *Brassica* and *Orychophragms* for improvement of important agronomic traits of the rapeseed crop.

13.6.2.3 Heterosis Breeding

A male sterile material involving cytoplasmic male sterility, genic male sterility, self-incompatibility, and male gametocide property is an important base for utilization of heterosis in crops. Male sterile materials in rapeseed can be acquired following two strategies: discovery of natural sterile plant and innovation of male sterile materials. In the crosses of *Brassica* species and *O. violaceus*, the hybrids obtained are generally sterile, especially male sterile for the great difference in their inheritance background. Thus, this provides the possibility for utilization of heterosis of rapeseed genic male sterile materials and cytoplasmic male sterile plants produced during the hybridization of *Brassica* and *Orychophragmus* (Mei et al. 2003; Lin et al. 2005), for example, the nuclear male sterile lines, G585, G587, G590, and G591 from inbred crossing of the intergeneric hybrids of *B. napus* or *B. juncea* and *O. violaceus*, cytoplasmic male sterile lines 405A, 407A, and 409A from the backcrossing of the intergeneric hybrids between *B. napus* and *O. violaceus* (Lin et al. 2005).

Additionally, Hu et al. (2002) reported that 131 sterile plants with no pollen grains were successfully obtained from symmetric fusions between *O. violaceus* and *B. napus* and were characterized as alloplasmic male sterile controlled by one pair of recessive nuclear genes (Hu et al. 2002; Mei et al. 2003) and would be of great potential for the development of a new cytoplasmic male sterility (CMS) system.

13.6.3 Chromosome Behaviors in Intergeneric Hybrids

From intergeneric hybridization between *Brassica* species and *O. violaceus*, all the hybrids between *O. violaceus* as pollen parent and six *Brassica* species were obtained, while reciprocal crosses were always unsuccessful; and all the hybrids were mixoploids, except for *B. oleracea* × *O. violaceus* (Li and Heneen 1999). These hybrids derived from distant crossing between *Brassica* and *O. violaceus* were genetically unstable. Due to lack of coordination between two parental chromosome behaviors in hybrid cells, genomes from one parent separated completely or partially, which produced mixture of cells with different chromosomes, or haploid or diploid complements of two parents and or hyperploid and hypoploid complements resulting in substitution and addition lines (Li and Ge 2007).

Additionally, introgressive transfer of genetic substances occurs accompanying separation or elimination

of parental chromosomes in distant crossing as reported by Ma and Li (2007).

13.6.4 Molecular Breeding

Traditional hybridization is very important for improvement of crops, as enormously documented. But in practice, the favorable characters are often transferred not independently but with undesirable genes into the receipt plant. So molecular breeding provides an alternative strategy for effectively improving rapeseed oil quality and production. However, the basic research on the important traits of *O. violaceus*, especially related to fatty acid metabolism in seeds, is almost limited. Strengthening the research on molecular genetics of *O. violaceus*, undoubtedly, is very important for improving the oil quality and production of rapeseed.

Effective plant regeneration in vitro has been developed in *O. violaceus* (see Sect. 13.2) that will provide several possibilities for genetic transformation of *O. vioaceus*'s allied crops. One is transfer of the gene of interest into recipient crops from *O. violaceus*, i.e., targeted gene transfer into *O. violaceus* and then into target crops from the gene-transformed *O. violaceus*; another is through *O. violaceus* pollen as gene media (Zhao et al. 2007a, b).

13.6.5 Other Desirable Traits

Through comparison and analysis of the difference of inorganic nutrition of *O. violaceus* and *B. napus*, *B. juncea*, and *B. campestris* at lime soil, Wu et al. (1997a) found that *O. violaceus* had powerful tolerance to low concentration levels of mineral elements P, B, K, Zn, and Mn and powerful refusal to absorb Na and Cu.

13.7 Genomics Resources Developed

Although *O. violaceus* was considered as available plant resource of high-quality edible oil in Brassiceae, the works are still fragmentary on gene mining from it. Until now, only a few gene databases, such as chalcone synthase – the key enzyme of flavonoid biosynthesis pathway (Jiang and Cao 2008), a critical enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate biosynthesis pathway (Gong et al. 2006), a peroxidase gene related to salt stress (Du et al. 2003), ITS (internal transcribed spacer) and *mat*K (maturase K) sequences (Warwick and Sauder 2005; Zhou et al. 2009a, b) mainly used in plant taxonomy and systematics, and Delta-12 desaturase, have been deposited in genbank (Table 13.9).

Table 13.9 Genes and EST of *O. violaceus* deposited in genbank

Gene or EST	Accession No.	Functions	References
SLR1	AB009872	Self-incompatibility	Sakamoto et al. (1998)
Beta-ketoacy-CoA synthase (FAE1)	AY888042	Lipid metabolism	Lu et al. (2005)
Delta-12 desaturase	DQ518316	Lipid metabolism	Zhang et al. (2006)
Protein kinase Rci	AY428037		Du et al. (2003)
OvRCI	AY428037	Peroxidase	Du et al. (2003)
Toc33	AF517946, AF517947	Chloroplast protein import machinery	Liao et al. (2003)
EPSPs (5-enolpyruvylshikimate-3-phosphate synthas)	AF440389	Shikimate biosynthesis pathway	Liu et al. (2002); Gong et al. (2006)
OvCYP86MF		Similar to cytochrome P450	Wang et al. (2005)
Chalcone synthase	EF408918	Flavonoid biosynthesis pathway	Jiang and Cao (2008)
ITS	EU306540–EU306554, AY722506		Zhou et al. (2009a, b); Warwick and Sauder (2005)
*mat*K	EU306555–EU306557, EU543181	Maturase of trnK	Zhou et al. (2009a, b)

Toc Translocon at the outer envelope membrane of the chloroplast

Besides, the gene related to drought tolerance, *REB2B*, was cloned 20031 (Lin et al. 2005b), and its role in improving plant drought and freeze resistance identified.

Hence, many available genes related to agronomic straits including lipid metabolism and stress tolerance are cloned and their functions have been systematically identified.

13.8 Good Plant Resource for Multiple Utility

O. violaceus, besides being an excellent wild plant genetic resource for oil quality improvement of rapeseed crops and an ornamental plant for landscaping in gardens, is also being developed as a vegetable and new oil seed crop.

13.8.1 Potential as an Edible Vegetable

O. vioaceus, as an edible wild vegetable, has a long history in the folks in China, and even today, picking tender leaves and stems as vegetable is still a usual practice in Jiangsu and Anhui province.

Zhugecai, the Chinese name of *O. violaceus*, is from a story that happened in ancient China 1800 years ago. In the Three Kingdoms period, Zhuge Liang, the prime minister of Shu Han, asked his army to sow seeds of "Manqing" wherever they camp when he had learnt how to cultivate it. As the tender shoots of this wild plant is edible and the plant is extremely robust, fast-growing, and easy to store and transport, by this measure the army solved the limited provisions for the army on their plates. Later on, this plant "Manqing" was also known as "Zhugecai" or the "Zhuge-veggie."

13.8.1.1 Protein Content and Nutritional Value

O. violaceus is highly rich in nutrients. First of all, it is rich in protein. Current research reports presented that the tender stems and leaves contain 4.29% protein in the fresh sample (Luo et al. 1998a, b, c) and flower stalks contain 36.29% protein in the dry sample (Wu et al. 1997a, b, c), and therefore it is obviously superior to other vegetable crops in Cruciferae (Luo et al. 1995a, b). In terms of amino acid composition, 18 amino acids and all of the eight essential amino acids (lysine, phenylalanine, methionine, threonine, isoleucine, leucine, and valine) for human body have been found in *O. violaceus* (Wu et al. 1997a, b, c; Weng and Huang 2001). Moreover, assessing proteins in vegetables by using egg protein as standard protein and using WHO/FAO reference model of essential amino acid as an appraisal criterion, the nutrient value of *O. violaceus* is significantly higher than many common vegetables including *Glycine max* sprout and *Medicogo sativa* (Tables 13.10 and 13.11).

Secondly, *O. violaceus* is rich in vitamins and mineral elements, especially in vitamin C and β-carotene, microelements Fe and Zn that are necessary for human body (Tables 13.12 and 13.13). The content of glutamic acid is above 2% in *O. violaceus*, which can relieve ammonia poison produced during metabolic process in tissue through formation of glutamine without toxicity to cells after combining with blood ammonia, participate in metabolism of brain tissue and make its function active. Therefore, *O. violaceus*, especially its flower stalk, has good health care properties. Besides, *O. violaceus* contains γ-aminobutyric acid (Li et al. 1997a, b, c) that is a chief inhibitory neurotransmitter in the mammalian central nervous system, helpful for functional recovery of brain cell, lowering blood pressure, and treatment of hepatic coma. This enhances the health care potential of edible *O. violaceus*.

As the dish vegetable on the table, with special flavor, Zhugecai smells lightly fragrant, tastes fresh, and is delicious due to its richness delicious amino acids, glutamic acid, and aspartic acid over 3% in leaves and stems.

Edible method of O. violaceus: The picked tender leaves and stems are boiled for a few seconds in boiling water and then used in cold mixed vegetables, fried dish, soup and as filling, fried pork, or stew. But note that the boiling time must not be too long for many nutrient elements as β-carotene and vitamins could be affected.

Hence, according to the nutritional chemistry as above, *O. violaceus* is worthy of developing into a novel vegetable crop, characterized by high protein, β-carotene, Ca and Fe, and low sugar and fiber.

Table 13.10 Close degree of the appraised food protein and the standard protein[a]

Appraised food	Protein content (%)	Close degree to standard protein	Appraised food	Protein content (%)	Close degree to standard protein
O. violaceus	3.01	0.8851	*Glycine max* sprout	3.9	0.7986
P. sativum	3.1	0.9114	*Vicia amoena* fisch	2.0	0.7888
Capsella bursa-pastoris	2.7	0.8800	*Phaseolus radiatus* sprout	1.8	0.7513
Osnunda japonica	3.1	0. 8611	*Chenipodium serotinum*	2.8	0.7332
Medicogo sativa	4.0	0.8323	*Rorippa indico*	2.5	0.7243
Houttuymia cordata	2.2	0.8230	*Kochia scoparia*	2.3	0.7172
Toona sinensis	8.1	0. 8207			

[a]*Note* 1. The standard protein is egg protein. The appraised samples of *O. violaceus* are its shoots

Note 2. The closed degree to standard protein of appraised protein is calculated with the formula: $\mu(a, ui) = 1 - 0.09 \sum_{k=1}^{8} \frac{|ak-uik|}{ak+uik}$

Here, $\mu(a, ui)$ presents the closed degree to standard protein of appraised protein; the better is the nutrient value of the protein if the μ value is more closed to 1. ak ($k = 1, 2, \ldots 8$) is the eight essential amino acid contents in standard protein a, uik is the content of the No. k essential amino acid in appraised protein

Note 3. The data are from Weng and Huang (2001)

Table 13.11 Contents of crude protein and amino acids in vegetables (mg/100 g)

	Legume (average of 8 vegetables)	Rhizome (average of 13 vegetables)	Tender stem, leaf, flower (average of 25 vegetables)	Melon-fruit (average of 11 vegetables)	Solanaceous (average of 11 vegetables)	Egg	*O. violaceus*[a]
Water (g)	72.79	86.25	90.17	94.19	93.52	75.3%	87.05
Protein (%)	5.64	1.8	2.15	0.69	1.045	12.4%	3.13
Ile[b] (mg)	236.25	45.62	65.67	21	33.72	664	4,314.5
Leu[b]	433.25	75.46	108.25	32.09	47.5	1,153	8,165
Lys[b]	314.38	63.77	92.08	29.27	48.45	991	6,024
Met[b]	267	24.67	23.75	8	18	334	661
Phe[b]	214.38	40.91	70.25	20.18	36.09	692	3,943.5
Val[b]	269.25	67.46	194.18	24.45	39.18	813	6,347.5
Trp[b]	94	26.9	25.6	8.5	10.875	221	1,293
Thr[b]	205.5	53.31	68.58	18.11	33.64	504	4,338.5
Tyr	161	40.91	50.7	15.11	26.9	601	282
Cys	88.29	21.0	32.5	9.25	20.75	194	684
Arg	424.71	106.11	148.8	39.5	45.27	803	4,296
His	137.75	28.92	33.91	15.18	17.73	303	2,019
Ala	265.13	61.67	92.10	29.8	39.82	645	6,153
Asp	765.63	272.75	210.1	54.11	117.82	1,081	8,077.5
Glu	865.5	194.91	333.55	115.7	169.2	1,589	11,514
Gly	217.25	49.083	78.957	21.818	36.818	410	5,204
Pro	204.43	48.11	66.5	21.875	36.56	428	10,346
Ser	262.38	67.31	76.67	25.36	41.73	845	4,260
T	2034.01 + 3392.07	398.1 + 890.773	648.36 + 1123.787	161.6 + 332.593	267.455 + 552.598	5,372	+ 6,899 35,087 + 52,835.5
Delicious amino acid (%)	1631.13/30.06	467.66/36.28	543.65/30.68	169.81/34.36	287.02/35.00	2,670/ 21.76	19,591.5/ 22.82
E/N	0.5996	0.4469	0.5769	0.4859	0.4840	0.7787	0.6641
E/T	0.375	0.309	0.366	0.327	0.326	0.438	0.399

Egg: The data are derived from Modern Food Composition and Dietary Nutrition (Ma and Duan 1998)

[a]*O. violaceus*: The data are derived from Weng and Huang (2001)

[b]*E* means content of essential amino acids; *T* means content of total amino acids, and *N* means content of non-essential amino acid

Content of delicious amino acid = Asp + Glu

Table 13.12 Nutrient composition in vegetables (g/100 g)

	Water (g)	Energy (kcal)	Protein (g)	Fat (g)	Fiber (g)	Carbohydrate (g)	Ash (g)	β-carotene (μg)	Retinoic acid (μg)	Thiamine (mg)
Legume (average)	86.7	43.4	4.6	0.90	2.7	4.5	0.8	228	38	0.09
Rhizome (average)	78.1	74.5	2.01	0.5	2.35	15.6	1.8	818	100	0.05
Tender stem, leaf, flower	76.02	33.07	2.52	0.39	2.44	7.01	1.03	487.65	81.35	0.08
Melon fruit	99.79	29.33	1	0.25	2.00	5.8	0.58	133.33	22.22	0.03
Solanaceous vegetables	86.39	37.73	2.37	1.23	4.85	4.25	0.9	314.44	52.44	0.12
O. violaceus	89.18	–	4.29	0.58	0.67	1.375	1.19	1,620	–	0.104

Note: 1. The data in Tables 13.12 and 13.13 are derived from Modern Food Composition and Dietary Nutrition (Ma and Duan 1998)
Legume vegetables: hycacinth bean string bean, white string bean, soybean sprout, green Asparagus bean, Purple yard long bean, mung bean sprout, green soy bean, and dwarf bean
Rhizome and bulb vegetables: water chestnut, Arrowhead, sweet potato, pachyrhizus, sweet potato (Shanyu), sweet potato meal, carrot, ginger, dried ginger, radish, turnip, red radish, red-core radish, green skin radish, potato, lotus root, *kohlrabi,* Chinese yam, Chinese yam bean, sugarbeet, Jerusalem artichoke, Spring bamboo sprout, and bamboo shoots
Tender stem, leaf, flower vegetables: spinach, cauliflower, Chinese cabbage, welsh onion (produced in plains), welsh onion (produced in Hills), white garlic, day lily, fennel, shepherd's purse, cane shoots, leek, Bitter Vegetables, radish leaf, radish leaf (radish), celery, Chinese chives, garlic sprout, celtuces, tender leaves of Chinese toon, shallot or chopped green onion, potherb mustard, rape/cole, bolt of rape, fruits of elm, cabbage, garlic, and caraway/coriander
Melon-fruit vegetables: muskmelon, snake melon, Fang melon (Square melon), dipper melon, gourd strip (dry), early cucumber, winter melon, bitter gourd, pumpkin, sponge gourd loofah, winter squash, watermelon, and summer squash
Solanaceous vegetables: lantern pepper, big capsicum, tomato, tomato (pink), hot pepper, dry red apical hot pepper, green pepper, round eggplant, long eggplant, green eggplant, and purple eggplant
2. The data about *O. violaecus* in Tables 3 and 4 are derived from Luo et al. (1995a)

13.8.1.2 Productivity of *O. violaceus* as a Vegetable Crop

As a resourceful wild grass distributed widely, *O. violaceus* has strong vigorous vitality and adaptability, such as strong drought and flood tolerance, cold resistance and antifreezing, and fast growth, no serious diseases and insect–pest incidence. It has no specific soil requirement for soil and can grow in acid soil or alkaline soil or in even poor gravel soil. It has anti-weed ability and strong regeneration ability, such as producing stump sprouts from cut-stump following a harvest. Thus, *O. violaceus* as an ornamental vegetable can be planted in park, by forest edges, urban streets, in residential districts, flower beds, highways, under viaduct, hillside, under-wood plantings, or both sides of railways, that is, without any competition for agricultural field and labor force. However, *O. violaceus* grows better in the porous, deep, and fertile land and with developed root system growing well and gives high yield. Naturally, if *O. violaceus* is planted in barren lands, strengthening of water and fertilizer management is necessary for high yield.

When *O. violaceus* is cultured as a vegetable crop, the sowing of the seeds is performed from March (spring sowing) to October (autumn sowing) every year. The seed rate is about 22.5–30 kg/ha. As the seedling grows up to 15–18 cm height, harvesting of the tender stem and leaves could be started followed by fertilizing and watering in time. It is better to apply a small amount of water and fertilizer at regular intervals. In *O. violaceus*, first flowering begins in early spring (February) and full-bloom stage is from March to April every year. The whole flowering period is up to 2 months or more. Flower colors are bright and blue, purple, or faint red. If planted in a large-scale, *O. violaceus* will make a beautiful scene composed with green leaves and flower.

Hence, with long growth and development period, *O. violaceus* can provide a benefit to local vegetable market in autumn, winter, and early spring. The production of tender shoots and leaves is high, up to 30,000–45,000 kg/ha/year (Luo et al. 1995a). Otherwise, as ground cover plant with good-looking flowers, *O. violaceus* makes beautiful environment with flowering in early spring and greens during the winter.

Table 13.13 Contents of vitamins and mineral elements in vegetables (DW)

	Riboflavin (mg)	Niacin (mg)	Vitamin C (mg)	Vitamin E (mg)	K (mg)	Na (mg)	Ca (mg)	Mg (mg)	Fe (mg)	Mn (mg)	Zn (mg)	Cu (mg)	P (mg)	Se (mg)
Legume	0.07	0.8	15.3	1.62	329.3	7.5	73	37	1.6	0.58	0.78	0.26	67	0.90
Rhizome	0.02	0.6	15.3	0.58	244	62	73	27	1.9	0.90	0.51	0.24	38	0.75
Tender stem, leaf, flower	0.07	1.03	55.11	1.03	252.68	102.78	111	33.46	2.48	0.56	76.01	0.11	51.58	0.67
Melon-fruit	0.02	0.39	13.42	0.539	149.17	19.97	29.86	17.93	1.006	0.22	0.43	0.02	32.57	0.52
Solanaceous	0.038	0.75	29.89	2.41	222.35	6.84	18.18	21.33	0.89	1.41	1.03	0.22	25.36	0.36
O. violaceus	0.05	–	27.1	–	–	–	168.07	–	4.655	–	–	–	35	–

13.8.2 Potential as a Source of Herbal Drugs

The stem and leaves have a high content of flavonoid (with total flavone of 0.568%) including 0.329% of quercetin, 0.228% of kaempfero, and 0.011% of isorhamnetin (Weng et al. 2000), which are useful to reduce inflammation, suppress tumor, protect liver, and prevent cardiovascular diseases. Several patents in China (patent No. 200510136472 and 200510136474) reported that *O. violaceus* could be made into healthy tea for antihypertensive, reducing blood lipid, lowering cholesterol, and hypoglycemic benefits. The anticancer bioactive glucoraphanin can be isolated from *O. violaceus* seeds (Patent No. CN01129168).

13.8.3 Potential as a New Oilseed Crop

O. violaceus can be developed as a potential oil crop. Besides its high oil quality in seeds as described above, *O. violaceus* possesses many excellent agronomic traits (Table 13.14), including dwarf plant, high number of branches and pods per plant, moderate-long pod, 25–45 seeds per pod, larger grain, long seed dormancy, high disease and insect resistance, and high yield potential per plant (1,500 kg/ha of seed output, i.e., 750 kg/ha of oil) (Luo et al. 1994a, b). However, the agricultural properties are to be improved through oriented cultivation, such as disease resistance to Sclerotinia disease, increase of total pods, seed number per pod and seed weight, etc. Therefore, *O. violaceus* has a great potential to emerge as a good oilseed crop.

13.9 Utilization of *Orychophragmus* and Ecological Safety

Although the intergeneric hybrid incompatibility in the tribe Brassiceae is high, only a few hybrid seeds were produced in the distant hybrid between the *Brassica* genus and the *Orychophragmus* genus. But different modes of crossing produced different results. The cross of *Brassica* (♀) × *Orychophragmus* (♂) may produce 0.1–0.7% hybrid seeds; on the contrary, the

Table 13.14 Some major agronomical characters of *O. violaceus*

Years	Planting density	Growing period	Plant height	Plant types	Branch position	First effective branch number	Pod number per plant	Seed number per pod	1,000 GW	Yield per plant
1987–1988	90,000/ha	214	70	Tower, Broom	6	13.4		39.0	3.8	60.9
1988–1989	135,000/ha	231	67.9	Tower, Broom	6	14.9	246.4	39.7	3.0	23.9

Notes: Data in table are from Luo et al. (1991)

hybrid combination of *Orychophragmus* (♀) × *Brassica* (♂) may result in only a few offsprings (Wang et al. 2006b). This situation may be explained as due to a pre-fertilization barrier, that is, the sperm cells cannot be transported into the embryo sac and thus cannot fertilize the egg cell and central cell, because of failure of pollen grain adhesion, failure of pollen germination, slowness and/or arrest or abnormal of pollen tube growth, pollen tube abnormality, and strong callose deposition in papillose cells of stigma (Wu et al. 1999; Wang et al. 2006a).

On the other hand, *O. violaceus* pollinated with pollen grains of transgenic oilseed rapes cannot germinate because of very low sexual compatibility between the tribe Brassiceae and *O. violaceus* (Song and Qiang 2003; Pu et al. 2005; Zhao et al. 2005, 2006, 2007a, b). Therefore, it is inferred that there is very low possibility of *O. violaceus* becoming super-weeds, which has been propounded by Du et al. (2009).

O. violaceus has strong disease and insect resistance. However, some reports claim that *Orychophragmus* is easily infected by Rape Sclerotiniose (Huang et al. 2000) and turnip mosaic virus (TuMV) (Chen and Li 1999) and Potato virus X (PVX), which is widespread in potato, tobacco, and tomato in China and shows mosaic and distortion (Li et al. 2008). They indicated that *O. violaceus* could serve as the virus reservoir in winter, when the Brassica crops are harvested (Li et al. 2008).

13.10 Recommendations for Future Actions

The understanding on *Orychophragmus* endemic to China and Korea came mainly from the research on the species *O. violaceus* and is still primitive. However, and as an important valuable wild plant resource for the Cruciferae family, it may play an important role in many ways, such as genetic analyses and genetic improvement of allied Brassica crops; elucidation of many principles related to physiology and biochemistry; and development of new oilseed and vegetable crops. Therefore, it is necessary to enhance protection of *Orychophragmus* resources.

13.10.1 *Investigation on* O. violaceus *Resources*

Till now, the classification of *Orychophragmus* is controversial. A comprehensive survey for *Orychophragmus* resources is required to be performed over the countries of China and Korea, especially for the natural distributions of the genus: kinds, quantity, and distribution of various different morphological phenotypes and their distribution patterns including *O. violaceus*, *O. limprichtianus*, and two forming species, *O. hupehensis* and *O. taibaiensis*.

13.10.2 *Research and Evaluation of Germplasm Resources of* Orychophragmus

Ideal samples of every kind of phenotypes in a genus are collected and identified and classified morphologically. And then further cytological and genetic studies, such as biological characteristics, genetic characters, including in multi-ingredients, molecular marker and DNA-fingerprints, genetic diversity, phylogenetic analysis, genetic mapping, gene location and as well as physiological and biochemical researches undergo at the same time.

On the basis of the above researches, the corresponding protection and preservation site or region is determined, either in situ protection site or resite reserve according to the features of germplasm resource to effectively preserve existing germplasm resources is done. The appropriate protection and management schemes are designed and realized to effectively protect *Orychophragmus* resource and provide for crop breeding and for molecular and biotechnological applications.

13.10.3 Information System for O. violaceus Resource

Based on the research results mentioned above, the information system of *O. violaceus* resource is constructed. This system includes basic database, geographic information system, model library, and expert system. The databases in the information system are dynamic, changing with deepening of survey, research, and utilization.

Of course, propaganda and education on the importance of germplasm protection of *Orychophragmus* is very important. Only when the strengthening of public consciousness on environment, protecting environment of *Orychophragmus* as well as other plants, avoidance of destructive development during the utilization process of *Orychophragmus* resources are powerfully carried out, it can just effectively protect *Orychophragmus* resources and realize full utilization of their favorable traits for improvement of allied crops.

13.11 Conclusion

Orychophragmus, endemic to China, comprises *O. violaceus*, *O. limprichtianus*, and two forming species, *O. hupehensis* and *O. taibaiensis*. *O. violaceus* is a great valuable wild plant material in the tribe Brassiceae with healthy oil qualities and high capability of plantlet regeneration. It is sexually crossable with allied oilseed crops despite very small number of seed setting. Its protoplast fusion ability with allied crop species makes it a new genetic and breeding stock for theoretical research and also for effectively improving the Brassica crops' quality and yield. *O. violaceus* possesses rich nutritive profile, many good agronomic traits and high ecological adaptability, and can be developed into new vegetable crop. The beautiful purple flowers and green leaves make *O. violaceus* a good ground cover ornamental plant. Hence, *O. violaceus* is a valuable multipurpose wild plant species in Cruciferae.

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Chapter 14
Pachycladon

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14.1 Introduction

The islands of New Zealand are located on the ring of fire in the South Pacific and are the product of intense geological activity including tectonic faulting and volcanic eruptions. The Pliocene epoch, occurring 2.5–5.2 million years ago (Mya), was a time of great geological change in the South Island of New Zealand predominated by the uplift of the southern Alps and Kaikoura Mountain Range (Batt et al. 2000). The southern Alps resulted from trans-current movement of the Alpine Fault that runs along almost the entire length of the South Island, and was formed by the colliding of the Indo-Australian and Pacific tectonic plates. The Pleistocene (0.01–2.5 Mya) was a significant epoch for vascular plants with numerous glacial–interglacial cycles that have shaped both the landscape and distribution of contemporary plants in New Zealand and around the world. The geological and glacial activity of the last 5.2 million years has resulted in a variety of parent-rock substrates, an array of diverse environmental variables, and potential new microhabitats for plants. It is not surprising that this rich and recent geological activity was paralleled by many Late Tertiary/Early Quaternary species radiations to give rise to the incredible diversity of plants found in New Zealand (McGlone et al. 2001).

The genus *Pachycladon* belongs to the Brassicaceae (Cruciferae) family and its member species are found exclusively in New Zealand and Australia (Tasmania). These plants are perennial herbs that grow on rocky substrates and exhibit extensive morphological diversity and a range of habitat preferences. This genus has had a recent allopolyploid origin (<2 Mya) and has undergone rapid speciation and radiation in the last million years. Its unique history, natural diversity, and close relationship to the model plant *Arabidopsis thaliana* have resulted in its development as an emerging model system to study the molecular genetic basis of plant speciation and radiation, and these studies are yielding molecular resources that can be used to study other traits of interest in the Brassicaceae.

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14.2 Basic Botany of the Species

14.2.1 Distribution

There are nine species of *Pachycladon* that are endemic to New Zealand and an additional species, *Pachycladon radicatum* (Hook.f.) Heenan & Mitchell, endemic to Tasmania. The New Zealand species of *Pachycladon* are restricted to the South Island, found predominantly in sparse isolated populations and form three distinct groups based on morphological characters and habitat preference (Heenan and Mitchell 2003). The distribution of New Zealand *Pachycladon* species is illustrated in Fig. 14.1 and their substrate and altitudinal preferences are summarized in Table 14.1.

The first group includes two species. *Pachycladon cheesemanii* Heenan & Mitchell is the most widely distributed species occurring in eastern South Island, having a wide latitudinal and altitudinal range and occurring on a number of different rock types. Its altitudinal range is from near sea level (10 m) to mountaintops (1,600 m), and it is a geological generalist occurring on graywacke, Haast schist, and basaltic

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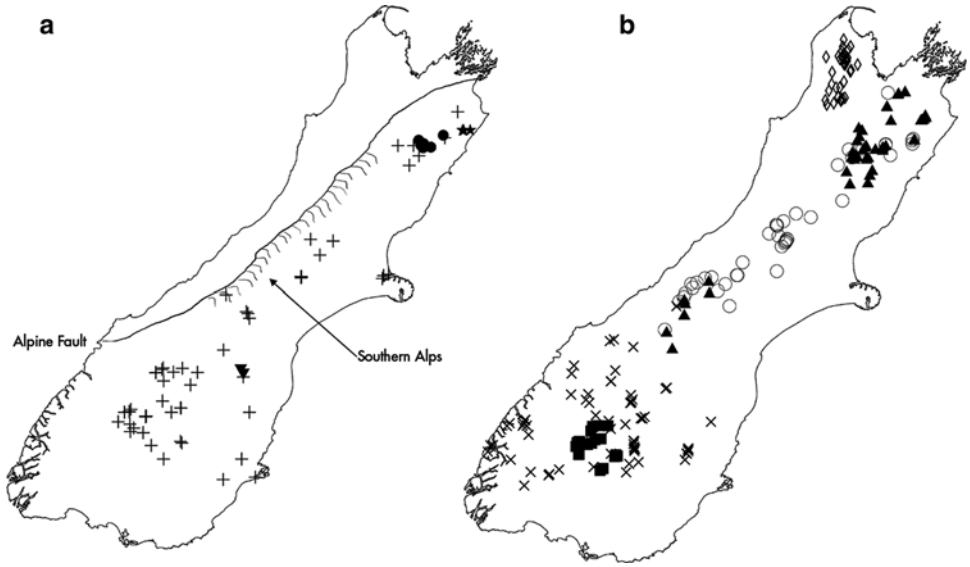

Fig. 14.1 Distribution of *Pachycladon* species. (**a**) *P. cheesemanii* (*plus sign*), *P. exile* (*solid inverted triangle*), *P. stellatum* (*star*), and *P. fasciarium* (*solid circle*) (**b**) *P. enysii* (*open circle*), *P. fastigiatum* (*solid triangle*), *P. latisiliquum* (*open diamond*), *P. novae-zelandiae* (*cross sign*), and *P. wallii* (*solid square*)

and andesitic volcanic rocks (see Fig. 14.1a). The closely related *Pachycladon exile* (Heenan) Heenan & Mitchell has a very restricted distribution in northern Otago and it is known with certainty from only three low-altitude (<500 m) sites comprising calcareous substrates, including limestone and volcanic rock (see Fig. 14.1a).

The remaining species of *Pachycladon* are generally more specialized in their distributional and habitat requirements. The second group is comprised of *Pachycladon* species located in southern South Island that predominantly occur on Haast schist. *Pachycladon novae-zelandiae* (Hook.f.) Hook.f. occurs on rocky outcrops and fell field between 1,080 and 2,031 m (see Fig. 14.1b). *Pachycladon crenatus* Philipson[1] is a western Southland taxon found in the Fiordland area growing on gneiss. *Pachycladon wallii* (Carse) Heenan & Mitchell is found on rocky outcrops and bluffs between 1,100 and 1,875 m (see Fig. 14.1b).

The last group comprises of four alpine species that grow on shaded rocky bluffs and cliffs. Of these, *Pachycladon latisiliquum* (Cheeseman) Heenan & Mitchell, is a Northwest Nelson endemic growing in a narrow latitudinal range and at an altitude of 1,036–1,768 m on a wide variety of rock types including granite, sandstone, marble, limestone, and volcanics (see Fig. 14.1b). The other three species are restricted to greywacke. *Pachycladon enysii* (Cheeseman) Heenan & Mitchell occurs throughout the southern Alps from 975 to 2,492 m above sea level, reaching the highest altitude of all *Pachycladon* species and one of the highest of any vascular plant species in New Zealand (see Fig. 14.1b). Heenan and Mitchell (2003) suggested that *P. enysii* probably survived on nunataks during glacial periods, especially during the last glaciation (~14,000–18,000 years ago). In contrast, *Pachycladon fastigiatum* (Hook.f.) Heenan & Mitchell has a lower altitudinal range (914–2,031 m) and disjunct populations in the northern and southern parts of the southern Alps (see Fig. 14.1b). It is absent from the high mountains of the central southern Alps, and it was suggested that this distribution reflects its extirpation from this area during the last glaciation (Heenan and Mitchell 2003). *Pachycladon stellatum* (Allan) Heenan & Mitchell is restricted to a small geographic area in

[1]The taxonomic standing of this species is questionable as *P. crenatus* appears to grade into *P. novae-zelandiae*, with whom it shares many features. For the purpose of this chapter, therefore, a broad taxonomic concept of *P. novae-zelandiae* will be adopted that includes *P. crenatus*.

Table 14.1 Morphological and distributional characteristics of *Pachycladon* species

Species	Growth habit	Leaves	Inflorescence	Siliques	Seed wings	Altitudinal range (mean ± SD) m	Geological parent material of substrate
P. cheesemanii	Polycarpic, woody caudex, subshrub habit	Leaf heterophylly, ovate to broadly elliptic, lobed to serrate; lamina and petiole with branched hairs	Slender and terminal; narrow petals	Terete; seeds uniseriate	Absent	10–1,600 (811 ± 49)	Greywacke, semi-schist, schist, plutonics
P. enysii	Monocarpic, caudex stout and soft	Ovate to ovate-lanceolate, serrate; lamina and petiole with branched hairs	Terminal and very stout; broad petals	Laterally compressed; seeds biseriate	Present	975–2,492 (1,885 ± 42)	Greywacke
P. exile	Polycarpic, caudex woody	Leaf heterophylly, ovate to broadly elliptic, lobed to serrate; lamina and petiole with branched hairs	Slender and terminal; narrow petals	Terete; seeds uniseriate	Absent	25–500 (276 ± 137)	Limestone, calcareous tuff and basaltic breccia, and alluvium
P. fasciarium	Monocarpic, caudex stout and soft	Narrowly linear to narrowly linear-lanceolate; glabrous	Terminal and very stout; broad petals	Laterally compressed; seeds biseriate	Present	884–1,058 (1,021 ± 59)	Limestone
P. fastigiatum	Monocarpic, caudex stout and soft	Narrowly elliptic to lanceolate, serrate; lamina usually glabrous (rarely with simple hairs), or with simple hairs only on the petiole and leaf margin	Terminal and very stout; broad petals	Laterally compressed; seeds biseriate	Present	914–2,031 (1,485 ± 33)	Greywacke, semi-schist
P. latisiliquum	Monocarpic, caudex stout and soft	Narrowly elliptic to lanceolate, serrate; lamina glabrous, usually with simple hairs on the petiole and leaf margin	Terminal and very stout; broad petals	Laterally compressed; seeds biseriate	Present	1,036–1,768 (1,441 ± 22)	Sandstone, marble, limestone, plutonics, argillite, granite
P. novae-zelandiae	Polycarpic, caudex semi-woody	Oblong to broadly elliptic, usually lobed or crenate; lamina and petiole glabrous or hairy, hairs simple or branched	Lateral, more-or-less slender; narrow petals	Laterally compressed; seeds biseriate	Absent	1,080–2,031 (1,587 ± 26)	Semi-schist, schist, plutonics
P. radicatum	Polycarpic, caudex semi-woody	Obovate to broadly elliptic, serrate; glabrous	Lateral, more-or-less slender; broad petals	Laterally compressed; seeds biseriate	Present	No data available	Dolerite and siliceous rock
P. stellatum	Monocarpic, caudex stout and soft	Narrowly elliptic to lanceolate, serrate; lamina and petiole with branched hairs	Terminal and very stout; broad petals	Laterally compressed; seeds biseriate	Present	900–1,371 (1,021 ± 34)	Greywacke
P. wallii	Polycarpic, caudex semi-woody	Oblong to oval, usually lobed or crenate; glabrous	Lateral, more-or-less slender; broad petals	Laterally compressed; seeds biseriate	Present	1,100–1,875 (1,403 ± 37)	Sandstone, semi-schist, schist

southern Marlborough, where it occurs on greywacke bluffs at a relatively low altitude (900–1,371 m) (see Fig. 14.1a). Finally, a recently identified species, allied to *P. fastigiatum* and named *P. fasciarium* Heenan (see Fig. 14.1a), is found restricted to limestone in eastern Marlborough (Heenan 2009).

14.2.2 Morphology

Pachycladon species are short-lived perennial, rosette-forming herbaceous plants with leaves that are glabrous or bear simple or branched trichomes, terminal or lateral inflorescences, white flowers, and narrow siliques. The growth habit and extent of morphological variation of several species of *Pachycladon* can be seen in Fig. 14.2. Differences in their morphology are further summarized in Table 14.1.

A phylogenetic analysis of morphological characters identified four species groups in *Pachycladon* (Heenan and Mitchell 2003); three of these groups are New Zealand endemics and the fourth represents the single Tasmanian species *P. radicatum*. *P. radicatum* is polycarpic, with decumbent branches, semi-woody caudex, ovate leaves, more or less slender, lateral inflorescences, short, laterally compressed siliques, and seeds that are winged.

Of the New Zealand species, *P. exile* and *P. cheesemanii* (Fig. 14.2a) are polycarpic and have woody caudices, short aerial branches, exhibit heterophylly, have slender, terminal inflorescences, terete siliques with uniseriate seeds, and seeds without wings. These two species are most similar, morphologically, to the closest relatives of *Pachycladon* in the Brassicaceae, viz., *Transberingia* and *Crucihimalaya* (Heenan et al. 2002; Joly et al. 2009). It has been suggested that the ancestral *Pachycladon* may have been the most similar to modern-day *P. cheesemanii* because of this morphological similarity and its generalist nature (Heenan and Mitchell 2003).

A second group includes *Pachycladon novae-zelandiae* (Fig. 14.2d) and *P. wallii* (Fig. 14.2f), which are also polycarpic but have lobed leaves, lateral inflorescences, short and laterally compressed siliques with biseriate seeds, and seeds with or without wings. The siliques of *P. novae-zelandiae* have a unique lanceolate shape.

A final group consists of *P. enysii* (Fig. 14.2b), *P. fastigiatum* (Fig. 14.2c), *P. latisiliquum*, and *P. stellatum* (Fig. 14.2e), and these are monocarpic, have a stout and soft caudex, serrate leaves, stout terminal inflorescences, long and laterally compressed siliques with biseriate seeds, and seeds with wings. *P. fasciarium* is closely related to *P. fastigiatum*, but differs with leaves that are shorter and narrower, linear, glabrous, and having fewer, smaller teeth on the margin.

14.2.3 Reproduction

Pachycladon plants are monocarpic or polycarpic and flower from early to mid-summer between September and January. The monocarpic species develop inflorescences at the end of summer (February to March) and these overwinter (April to August) prior to flowering the next summer. The flowers are usually bisexual and have four white petals, six stamens, and one pistil. *Pachycladon* species are self-compatible (SC) (Heenan unpublished data) and thus are able to self-fertilize. While this mode of reproduction can ensure survival in the absence of other plants to cross-fertilize with, and is therefore a feature that is essential for the success of neopolyploid species (see Sect. 14.4.1), it can also lead to inbreeding depression through fixation of deleterious alleles. Hence, for an established species, outcrossing is a more favorable reproductive strategy.

Mitchell and Heenan (2002) found low pollen:ovule ratios in *P. cheesemanii* (76:1), *P. exile* (74:1) and *P. novae-zelandiae* (181:1) and selfing floral morphology, suggesting an autogamous breeding system for these species. Higher pollen:ovule ratios were determined for *P. fastigiatum* (339:1), *P. stellatum* (525:1), and *P. latisiliquum* (786:1), and examination of their floral morphology suggest that these SC plants are encouraged to outcross. Gender dimorphism (gynodioecy) has also been reported in *P. wallii*, *P. stellatum*, *P. fastigiatum*, and *P. latisiliquum* (Garnock-Jones 1991; Heenan and Garnock-Jones 1999). These findings, and estimates of the percentage of polymorphic loci and among-population genetic diversity (G_{ST}) from AFLP analyses, are consistent with *Pachycladon* species exhibiting predominantly selfing to mixed mating and endemic to regional distribution.

Fig. 14.2 Growth habit and morphological variation of *Pachycladon* species. (**a**) *P. cheesemanii*, (**b**) *P. enysii*, (**c**) *P. fastigiatum*, (**d**) *P. novae-zelandiae*, (**e**) *P. stellatum*, (**f**) *P. wallii*

Pachycladon species also remain interfertile. This was confirmed by the successful production of artificial F_1 hybrids from interspecific crosses under laboratory conditions and the recent discovery of naturally occurring hybrids of greywacke species *P. enysii* × *P. fastigiatum* and *P. fastigiatum* × *P. stellatum* as well as hybrids of *P. novae-zelandiae* × *P. fastigiatum* (Heenan 1999; Bicknell et al. 2009; Heenan unpublished data). Figure 14.4b summarizes the parent plants of both the artificial interspecific hybrids generated, and the naturally occurring hybrids discovered to date.

14.2.4 Cytology and Embryology

Pachycladon species have a somatic chromosomal complement of $2n = 20$ as determined by DAPI staining and chromosomal counting of cells from floral or

root tip tissue (Dawson 1995; Dawson 2000; Lysak et al. 2009). The C-values (1C) of seven species of *Pachycladon* have been estimated recently including *P. exile*[§], *P. cheesemanii**, *P. novae-zelandiae*[§,*], *P. enysii**, *P. fastigiatum*[§,*], *P. wallii**, and *P. stellatum** and were found to range from 0.44 to 0.56 pg ([§]Lysak et al. 2009; *Bicknell and Heenan unpublished data), corresponding to genome sizes of ~430 Mbp for *P. exile*, ~450 Mbp for *P. cheesemanii*, ~460* Mbp for *P. novae-zelandiae*, ~489 Mbp for *P. enysii*, ~499[§]–509* Mbp for *P. fastigiatum*, ~518 Mbp for *P. wallii*, and ~548 Mbp for *P. stellatum* (1 pg = 978 Mbp; Dolezel et al. 2003). This suggests that the genomes of *Pachycladon* species are about three times bigger than the *A. thaliana* genome (1C = 0.16 pg, ~157 Mbp) but smaller than the family average (1C = 0.63 pg; ~616 Mbp; Lysak et al. 2009). They are comparable in size to the genomes of diploid crop species like *Brassica rapa* and *Raphanus sativus* but about half the size of the genomes of polyploid crop species like *Brassica juncea* and *B. napus*. Further, despite recent speciation (see Sect. 14.4.1), significant variation in genome size exists between *Pachycladon* species. To date, there are no published studies that characterize or compare karyotypes of *Pachycladon* species. However, a chromosomal painting study is currently underway to map the genomes of *P. cheesemanii*, *P. enysii*, *P. exile*, and *P. novae-zelandiae* (Mandáková, Lysak and Heenan unpublished data).

Embryological studies of *P. cheesemanii* and *P. exile* (Luo et al. 2003) reveal that their megasporogenesis and embyogenesis are similar to their relative and model plant *A. thaliana* (Mansfield and Briarty 1991). Meiosis was found to occur at Stage 10 and anthesis at Stage 13 (day 9). However, *Pachycladon* showed significant delays in the first cell divisions of the endosperm, occurring at Stage 16 (7 days post-anthesis) and the initiation of embryogenesis, which occurs at Stage 17 (~9–10 days after anthesis) in comparison with *A. thaliana*, where these processes are initiated just hours after fertilization (Mansfield and Briarty 1991). Interestingly, the fruit experienced its most rapid growth during the delay to endosperm formation and embryogenesis, with a rapid decline in growth rate after day 15. Silique dehiscence was observed in *P. exile* 7 days prior to *P. cheesemanii* (day 29 and 36, respectively). It is unknown if this delayed embryogenesis is restricted to these two *Pachycladon* species or if it occurs in all species.

14.2.5 Taxonomy

14.2.5.1 Morphotaxonomy

Pachycladon has a befuddled taxonomic history and this reflects issues of generic circumscription that are common in the Brassicaceae (e.g., Al-Shehbaz et al. 1999). When the species now assigned to *Pachycladon* were first named and described in the late 1800s and early 1900s, the majority were placed in the well-known European and North American genera *Arabis* L., *Braya* Sternb. & Hoppe, *Cardamine* L., *Nasturtium* R.Br., and *Sisymbrium* L., as they appeared morphologically similar to species from these genera. The genus *Pachycladon* was described by Hooker (1867) to accommodate *P. novae-zelandiae*, an alpine species from southern South Island that had been previously placed in *Braya*. Later, a major study of Brassicaceae genera by Schulz (1924, 1936) resulted in the erection of the two new endemic New Zealand genera *Cheesemania* O.E. Schulz (seven taxa) and *Ischnocarpus* O.E.Schulz (two taxa).

In the past, *Cheesemania*, *Ischnocarpus*, and *Pachycladon* have not been considered to have a particularly close relationship as they have different growth habits (monocarpic or polycarpic), fruit (biseriate or uniseriate), and seed types (accumbent or incumbent; winged or not winged). *Cheesemania* was placed in the tribe Arabideae and *Ischnocarpus* and *Pachycladon* in different subtribes of the tribe Sisymbrieae (Schulz 1924, 1936). Much of this confusion has arisen because the Brassicaceae emphasis has been placed on morphological characters that show considerable convergence in the family and therefore are not reliable indicators of true taxonomic relationships (e.g., Mummenhoff et al. 1997; Koch et al. 1999, 2001; Al-Shehbaz et al. 2006).

14.2.5.2 Molecular Taxonomy

Molecular phylogenetic analyses of the nrDNA internal transcribed spacer (ITS) region using PAUP vers. 3.1.1 (Swofford 1993) and the Maximum Likelihood program DNAML, from PHYLIP vers. 3.5c (Felsenstein 1993) showed that species of *Pachycladon*, *Cheesemania*, and *Ischnocarpus* formed a monophyletic clade (Mitchell and Heenan 2000). When species from these genera

were topologically constrained to the tribes they were classified under, the resulting trees were far less parsimonious (requiring up to 75 additional steps) than the original tree, therefore rejecting these traditional taxonomic placements (Mitchell and Heenan 2000).

In order to more accurately infer the taxonomic position of *Pachycladon* within the Brassicaceae, several taxa including those from two studies (Koch et al. 1999; Mitchell and Heenan 2000) were subjected to a more comprehensive molecular phylogenetic analysis of nrDNA ITS data using PAUP vers. 4.0b6 (Swofford 2000; Heenan et al. 2002). The recent origin and close relationship between *Pachycladon*, *Cheesemania*, and *Ischnocarpus* was reflected in their grouping together with high bootstrap support (98%), within a monophyletic clade that lacked well-resolved topology, and showed low sequence divergence (2.6%). Based on the results from these two studies, a reassessment of morphological characters, and the generation of fecund interspecific hybrids between species from each of the three genera (Heenan 1999, Heenan unpublished data), *Pachycladon* was recircumscribed to include species placed in *Cheesemania* and *Ischnocarpus*, and *I. novae-zelandiae* was renamed as *P. cheesemanii* (Heenan et al. 2002).

The *Pachycladon* complex was found to belong to the Arabidopsoid clade of the Brassicaceae family and forms a distinct and relatively young New Zealand lineage estimated to be ~1.0–3.5 million years old based on molecular clock estimates for the nrDNA ITS region (Heenan et al. 2002). It is sister to *Crucihimalaya* from Asia and *Transberingia bursifolia* found in eastern Russia and North America. The ITS region of *Pachycladon* species and the model plant *A. thaliana* were estimated to have diverged ~9–11 Mya, indicating that they are closely related (Heenan et al. 2002).

Since these efforts were made to understand the phylogenetic relationships of *Pachycladon* species and their closest relatives, several *Pachycladon* species have been included in more recent and comprehensive studies of the taxonomy and phylogeny of the Brassicaceae family. Al-Shehbaz et al. (2006) critically reviewed the taxonomy of the Brassicaceae family in terms of morphological traits, generic circumscription, and major clades and proposed a new tribal alignment of 25 tribes to the family, placing *Pachycladon* along with other members of the Arabidopsoid clade in the tribe Camelineae. Bailey et al. (2006), with the aim of determining a global phylogeny for the family, performed a parsimony ratchet analysis in WinClada (Nixon 1999) of the nrITS region for 461 species representing 24 of the 25 Brassicaceae tribes and a super-matrix parsimony ratchet analysis in WinClada of five nuclear genes (nrITS, *PI*, *CHS*, *Adh*, *LFY*) and five chloroplast genes (*rbc*L, *mat*K, *atpb*, *ndh*F, *trn*L-*trn*F). Even though only nrITS and *rbc*L sequences were available for the five species of *Pachycladon* included in these analyses, the phylogenetic position of *Pachycladon* within the Camelineae was confirmed with high bootstrap support (100%). The sister clade of *Pachycladon* consisted of the genera *Transberingia* and *Crucihimalaya*, though in this super-tree, there was only poor bootstrap support for this relationship (37%).

14.2.5.3 Interspecific Relationships

Molecular support for interspecific relationships hypothesized based on habitat preference, morphology, and amplified fragment length polymorphism (AFLP) data (Mitchell and Heenan 2002; see Sect. 14.2.2) was sought using DNA sequence data from three nuclear markers (ITS, *FRIGIDA*, and *gap*C) and one chloroplast marker (*trn*L-*trn*F) that had parsimony informative nucleotides (McBreen and Heenan 2006). Marker sequence was recoverable from most species and subjected to NeighborNet analyses (Huson and Bryant 2006). Overall, the splits graphs generated are characterized by star-like patterns of radiation with little structure in the internal branches suggesting that the onset of diversification occurred very rapidly or simultaneously. In this multigene study, some interspecific relationships within the *Pachycladon* genus were well supported. *P. exile* and *P. cheesemanii* were the most frequently retrieved *Pachycladon* subgroup, occurring in the *FRIGIDA*, ITS, and *trn*L-*trn*F networks. The monocarpic species *P. enysii*, *P. fastigiatum*, *P. latisiliquum*, and *P. stellatum* were only partially retrieved in the *trn*L-*trn*F, ITS, *FRIGIDA*, and *gap*C-copy B networks. Interestingly, the species *P. enysii*, *P. fastigiatum*, and *P. stellatum* clustered together for all genes investigated to date (McBreen and Heenan 2006; Joly, Heenan and Lockhart unpublished data). Finally, the third morphological group consisting of *P. novae-zelandiae* and *P. wallii* was not retrieved in any of the networks.

14.2.5.4 Chemotaxonomy

Current research work reveals that *P. fastigiatum* and *P. enysii* demonstrate variable profiles of major secondary metabolites like glucosinolate compounds (GLS), glucosinolate breakdown products, and flavonoids (Voelckel et al. 2008; Voelckel and Reichelt unpublished data). Both *Pachycladon* species appear to have GLS chemotypes that reflect their innate glucosinolate composition. Instead of two species-specific GLS profiles, five GLS profiles (= chemotypes) were found, two specific to *P. fastigiatum* (chemotypes two and five) and three specific to *P. enysii* (chemotypes one, three, and four). In terms of major GLS compounds, chemotype one produced mainly 4-methylsulfinylbutyl GLS (4MSOB) and 3-butenyl GLS, chemotype two produced mostly 3-methylsulfinylpropyl GLS (3MSOP), chemotype three produced predominantly allyl GLS and 3-butenyl GLS, and chemotypes four and five produced mostly 3MSOP and allyl GLS. Chemotype four and five differed in the minor compound 3-methylthiopropyl GLS (3MTP), which was only produced in chemotype four. All chemotypes produced 7-methylsulfinylheptyl GLS (7MSOH) as a major compound, albeit to different degrees. Interestingly, these chemotypes were not randomly distributed across populations. Instead, individuals of one chemotype were found to dominate each site (except for one *P. fastigiatum* site). It remains to be determined if this is due to local adaptation or neutral processes such as drift and inbreeding. The five *Pachycladon* chemotypes can be matched with three *A. thaliana* ecotypes for which the allelic configuration at two major glucosinolate biosynthetic loci is known (Kliebenstein et al. 2001). Therefore, it is predicted that the chemotypes observed in *P. enysii* and *P. fastigiatum* can be explained by segregation of the *GLS-elong* locus and the *GLS-AOP* locus. These chemotypic differences may be useful in distinguishing, at the interspecific and intraspecific levels, populations that should be targeted for conservation efforts. Glucosinolate profiles are currently being obtained for additional *Pachycladon* species (see Sect. 14.5.2.2).

14.2.6 Agricultural Status and Research Use

Pachycladon species are considered to be important endemic New Zealand plants, some of which are considered nationally threatened and therefore subject to preservation efforts by the New Zealand Department of Conservation to protect them from invasive species. There are no documented uses of these plants by the Maori (indigenous people of New Zealand). The very recent origin and radiation of *Pachycladon* make it an excellent candidate for development as a model system to determine the genetic and molecular basis underlying plant speciation, adaptive radiation, and evolution.

Studies of the model plant *A. thaliana* and its close relatives are leading to a much greater understanding of the genetic processes involved in plant development and evolution. It is now accepted that major changes in plant form can be accomplished by changes in a few key genes (Doebley 1992; Doebley and Lukens 1998). However, there is still much to learn about the drivers and mechanisms most important to morphological and ecological diversification during plant species radiation (Mitchell-Olds 2001; Shepard and Purugganan 2002; Remington and Purugganan 2003). For example, is diversifying selection on a small number of key genes sufficient for plant radiation? Testing this hypothesis is becoming increasingly tractable due to our improved understanding of the genetic processes operating within model plants, and comparative studies with species that have undergone recent morphological and ecological diversification in the wild (Falconer and Mackay 1996; Schmidt 2000; Weinig et al. 2003).

The close relationship between *Pachycladon* and *Arabidopsis* enables the use of available genomic resources. By taking advantage of the vast amount of resources that are available for *Arabidopsis*, and the natural diversity within *Pachycladon*, important advances in understanding the genetic processes underlying plant speciation and adaptive radiation will be made. In particular, *Pachycladon* can be used to understand whether diversifying selection on a small number of key genes is sufficient for plant radiation, and in answering this question, identify some of the genes involved.

14.3 Domestication and Conservation

Currently, *Pachycladon* species are primarily used in research programs to elucidate speciation, adaptive radiation, and evolutionary processes. As they have

no direct agricultural potential per se, domestication in a traditional sense is not an issue being addressed. However, cultivated plants are an essential resource for experimental genetic, molecular, reproductive, and ecophysiological study and *Pachycladon* plants have been found to be amenable to growth and maintenance in growth chambers and greenhouses.

14.3.1 Growth and Maintenance of Pachycladon *Plants*

14.3.1.1 Cultivation and Propagation

Plants are generally readily grown from seed, and if the seed is only a few days old, rapid germination occurs. Fresh seed will often spontaneously germinate in damp conditions around potted plants. Seeds older than 2–3 weeks benefit from stratification at 4°C for 7 days. Germination occurs about 7–12 days after stratification. Young seedlings are most easily potted up when they are 10–15 mm in diameter, as smaller and younger plants are sensitive to disturbance and can be difficult to transplant.

Propagation can also be achieved by cuttings, and these will root in 3–5 weeks when placed in a mist unit with bottom heat. Plants are easily cultivated under greenhouse conditions, but do require overhead shading (prefer about 350 μmol s^{-1} m^{-2}), temperatures below 20–22°C, and good airflow. Cultivated plants respond to long day conditions and during the winter months can be induced to flower if subjected to 16 h light and 8 h dark. Monocarpic species need to be regularly replaced after flowering and fruiting, but the polycarpic species can be maintained for 3–4 years.

14.3.1.2 Pests and Diseases

The main pests and diseases of cultivated plants include root mealy bug (*Rhizoecus* species), white rust (*Albugo candida*) that can disfigure leaves and inflorescences in warm and humid conditions, gray cabbage aphids (*Brevicoryne brassicae*) that occur throughout the summer growing season, and white butterfly larvae (*Pieris rapae*). However, the most serious issue is the recent identification of turnip mosaic virus in *Pachycladon* (Fletcher et al. 2010).

14.3.2 Conservation Initiatives

Three species of *Pachycladon* are listed as nationally threatened according to the New Zealand threat classification system (de Lange et al. 2009). *P. exile* and *P. stellatum* are assessed as Nationally Critical. Today, *P. exile* is known from a single site on a limestone outcrop in northern Otago, although historically it was known from other sites in the area. It has undergone a significant decline, and at its single known location, the population is estimated to be about 50 individuals. Regular weeding of the naturalized *Hieracium pilosella*, *Dactylis glomerata*, and *Sedum acre* is undertaken to prevent smothering of *P. exile* and to provide an open habitat for its establishment. Fencing has also been required to prevent rabbits from accessing the site and damaging the plants by browsing. The site is under private ownership but has been preserved into perpetuity after being designated a National Queen Elizabeth II Covenant.

P. stellatum has a very restricted, naturally sparse distribution and specific, shaded, habitat requirements, and is known from a few scattered and very small populations in Marlborough. Competition from weeds, such as *Hieracium* spp. and *Echium vulgare*, which occupy similar habitats, are a threat to *P. stellatum*.

P. cheesemanii is considered to be Nationally Vulnerable. It is a widespread but sparsely distributed species that generally has small-sized populations (< 50 plants). A study of the impact of exotic weed competition on *P. cheesemanii* suggests that competition with invading weeds threatens current *P. cheesemanii* populations, seedling survival and plant establishment can be enhanced by weed removal, and considerable potential exists for artificially expanding populations by sowing seed into appropriate weed-free habitat (Miller and Duncan 2004).

P. fasciarium is found in a small population of less than 50 plants restricted to limestone cliffs in Marlborough, and has been the subject of a cryopreservation study, whereby successful plant regeneration was achieved from cryopreserved meristems (Hargreaves et al. 1997). These techniques should be applicable to all species of *Pachycladon*. In contrast, seed germplasm is likely to be of limited use in the conservation of species of *Pachycladon*. Hargreaves et al. (1997) reported that after 4 years of storage at 4°C, seed germination reduced from 81 to 43%. These data are

also supported by anecdotal observations that *Pachycladon* seed stored for over 5 years has little or no germination (Heenan unpublished data), which is in contrast to the ex situ longevity reported for seeds of many Brassicaceae species that were found to remain viable even after 20 years of storage in the Millennium Seed Bank (Probert et al. 2009).

14.4 Origin and Evolution of *Pachycladon* Species

14.4.1 Allopolyploid Origin

14.4.1.1 Evidence for Allopolyploid Origin

A study by McBreen and Heenan (2006) revealed a duplication at the glyceraldehyde 3-phosphate dehydrogenase (*gap*C) gene, a nuclear marker, present in all species of *Pachycladon* with greater sequence divergence between the copies (7.9%) than among species, suggesting a possible polyploid origin for *Pachycladon*. To confirm that *Pachycladon* had a polyploid origin, Joly et al. (2009) sequenced several *Pachycladon* and Brassicaceae species for five single-copy nuclear gene markers (*CHS*, *PRK*, *MS*, *CAD5*, and *MtN21*) that map to distinct regions of the *A. thaliana* genome and different blocks of the reconstructed ancestral Brassicaceae karyotype (Schranz et al. 2006). These sequences were subjected to maximum likelihood and Bayesian phylogenetic analyses using PhyML vers. 2.4.4 (Guidon and Gascuel 2003) and BEAST vers. 1.4.7 (Drummond and Rambaut 2007), respectively. *Pachycladon* species were found to have two copies of all five of these genes and gene copies for every gene were distantly related and had the same phylogenetic position within the Brassicaceae, thus lending strong support that this genus has an allopolyploid origin. A Bayesian analysis of the *CHS* gene, which has been sequenced for many Brassicaceae species, was reproduced here using denser taxon sampling in the deeper parts of the Brassicaceae phylogeny (see Fig. 14.3). This *CHS* phylogeny shows that one genome copy of *Pachycladon* (genome-A) is in a derived position in the Arabidopsoid lineage and is closely associated with *Crucihimalaya*, *Transberingia*, and *Boechera*. This position was also found for other nuclear genes (Joly et al. 2009) and corresponds to the phylogenetic position of *Pachycladon* determined using the nrITS region (see Sect. 14.2.5.2). Yet, the other genome copy present in *Pachycladon* (genome-B) has a much deeper position in the Brassicaceae phylogeny, likely at the base of the Arabidopsoid clade. No species sequenced for the *CHS* gene have high affinities with this B-genome copy. Molecular clock estimates infer that the two genome copies diverged about 8.18 Mya, but that the hybridization event that led to the formation of *Pachycladon* occurred between 0.8 and 1.61 Mya (see Fig. 14.3b; Joly et al. 2009). The two genome copies of *CHS* present in the *Pachycladon* genome share a most recent common ancestor with *A. thaliana* approximately 7 and 10 Mya, respectively.

14.4.1.2 Maternal History

To investigate the maternal origin of the genus, Joly et al. (2009) used the chloroplast gene *rbc*L, which is maternally inherited, to construct a phylogeny that included *Pachycladon* and Brassicaceae sequences available in GenBank. This analysis showed that the *Pachycladon rbc*L sequences were relatively basal in the *Arabidopsoid* clade, although the species sampling in this analysis was limited. More recently, four chloroplast genes (*rbc*L, *nad4*, *mat*K, *ndh*F; Couvreur et al. 2010) were sequenced from some *Pachycladon* species and analyzed with 55 representative Brassicaceae species. A Bayesian analysis in BEAST 1.4.8 showed that *Pachylcadon* fell within a well-supported clade (posterior probability = 1.0) that consisted of the tribe Boechereae and some genera of the tribe Camelineae, including *Crucihimalaya* and *Transberingia* (Mandáková et al. 2010). In light of this more comprehensive and better-supported analysis, it is likely that the A-genome was the maternal parent in the allopolyploid event that gave rise to *Pachycladon*.

14.4.1.3 Hybridization and Adaptive Radiation

While the question of whether hybridization acted as a trigger for the adaptive radiation of *Pachycladon* is still the subject of ongoing research, it is clear that an allopolyploid event preceded the radiation of the genus (Joly et al. 2009). Because the allopolyploidization

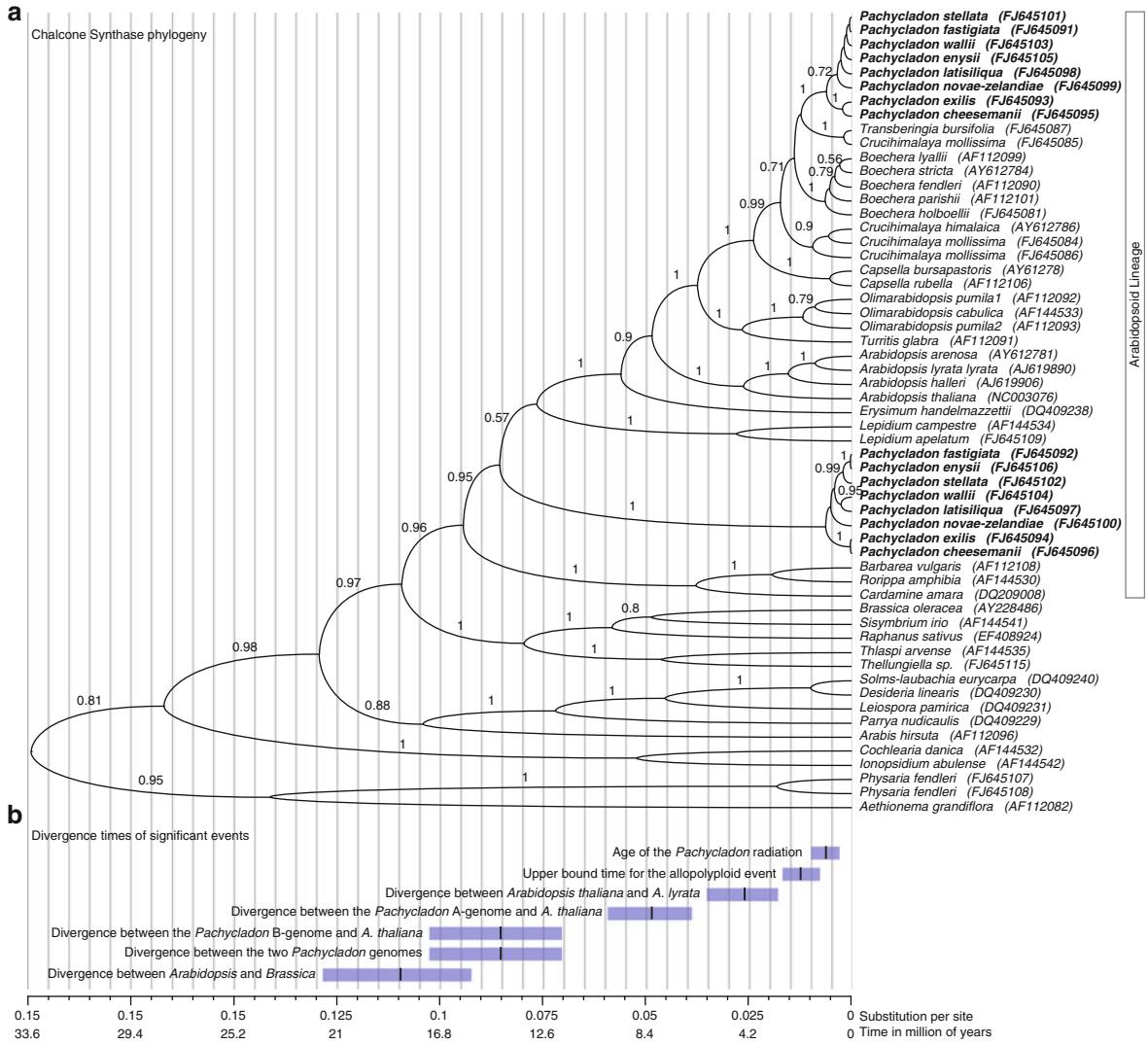

Fig. 14.3 Chalcone synthase (*CHS*) phylogeny for *Pachycladon* species and other Brassicaceae species and estimates of important divergence times. (**a**) Chronogram obtained from a Bayesian phylogenetic analysis in BEAST vers. 1.4.8 (Drummond and Rambaut 2007) using a sample of the accessions available in GenBank (*accession numbers in brackets*). The analysis used a GTR + Γ + I substitution model (selected using Model test 3.7; Posada and Crandall 1998) and a lognormal relaxed molecular clock with the mean substitution rate fixed to 1. Two independent runs of 4×10^7 were run and trees were sampled every 1,000 generations for the second half of the run. Trees sampled from these two runs were combined to obtain a maximum clade probability tree. Posterior probabilities are shown above nodes when these were greater than 0.5. (**b**) Median and 95% credible intervals for important divergence times. The time scale has been obtained using a divergence time of 18.51 million of years between *Arabidopsis* and *Brassica* (see Joly et al. 2009)

event occurred during the early Pleistocene (Early Quaternary), it is possible that the major geological and ecological changes in the Late Tertiary (see Sect. 14.1) have played a major role in driving hybridization, but also speciation and rapid radiation of the neo-species in a new environment of diverse geological substrates and altitudinal range. It is also likely that trans-oceanic dispersal of modern-day *P. radicatum* from New Zealand to Tasmania occurred during this radiation as well.

14.4.2 Pachycladon *as a Model System for Polyploid Evolution in the Brassicaceae*

Polyploidy is known to be a major influential force in the evolution of higher plant genomes (Otto and Whitton 2000; Wendel 2000) with repeated cycles of polyploidization, followed by diploidization appearing to have occurred throughout the evolutionary history of flowering plants (Vision et al. 2000). Polyploids have the inherent advantage of possessing more than one copy of the genome, which allows the plants to tolerate major changes, at both the gene and genomic levels, and this may confer selective evolutionary advantages. In theory, the presence of two copies (homeologous copies) of a gene in an allopolyploid could allow subfunctionalization (Lynch and Conery 2000) and lead to adaptation. Indeed, previous studies on homeologous gene expression have provided examples of gene subfunctionalization in *Gossypium* (Adams et al. 2003) and have demonstrated that variation in expression levels of homeologous genes can affect the phenotype of allopolyploids (Wang et al. 2006) and potentially offer adaptive benefits (Finnegan 2001). These mechanisms, if demonstrated to have been important in the evolution of a group by connecting differential gene expression to selectable changes driving the origin of species (Kellogg 2003), could lend support to the hypothesis that hybridization can act as a trigger for adaptive radiation (Seehausen 2004). The genus *Pachycladon* represents a perfect model to investigate these hypotheses given that a recent allopolyploid event preceded its radiation and that its ecophysiology is currently under in-depth study.

Understanding the molecular basis of evolutionary processes responsible for the speciation of *Pachycladon* might also help to add to our understanding of the impact of polyploidy on the whole family. The "paradox" of the small and narrow range of genome sizes for most Brassicaceae (Lysak et al. 2009), given the preponderance of polyploid taxa in the family, suggests that Brassicaceae genomes are dynamic and that polyploidization (specifically, hybridization in the case of allopolyploids) is crucial to genome evolution in the Brassicaceae. For instance, diploid *A. thaliana* appears to be an ancient polyploid that may have experienced at least three whole-genome duplication events that predate the origin of the Brassicaceae (Vision et al. 2000; Blanc et al. 2003; de Bodt et al. 2005) but nevertheless possesses a very small genome ($n = 5$; 1C ~157 Mbp). Recent evidence from studies of *A. thaliana* and its close relatives suggests that in the last ~5 million years, the ancestral Brassicaceae karyotype [$n = 8$; 1C ~490 Mbp] (Dolezel et al. 2003; Schranz et al. 2006; Lysak et al. 2009) underwent rapid chromosomal rearrangement and genome reduction to give this $n = 5$ karyotype of modern-day *A. thaliana* (Kuittinen et al. 2004; Koch and Kiefer 2005; Yogeeswaran et al. 2005; Lysak et al. 2006). Ancestral genome-wide and partial duplication events may have enabled its genome to tolerate the concomitant loss of chromosomal content (1C ~ 490 Mbp to 157 Mbp) (Yogeeswaran et al. 2005; Lysak et al. 2006). Similar evidence of lineage-specific changes in genome structure and content have been suggested for genome reduction in other Brassicaceae species following polyploidy (Lysak and Lester 2006; Lysak et al. 2006; Schranz et al. 2006; Lysak et al. 2007) and is likely one of the crucial mechanisms of diploidization in the Brassicaceae family. This ability to tolerate such drastic changes to the genome (genomic plasticity), of Brassicaceae species appears to be a direct consequence of polyploidization, and has been suggested to enhance their ability to survive and adapt to environmental change during alternating glacial–interglacial cycles in Eurasia, and possibly drive speciation (McClintock 1984; Kianin and Quiros 1992; Kowalski et al. 1994). It is conceivable that similar comparative genome studies between *Pachycladon* and its close relatives, and between species of *Pachycladon*, may add to our growing knowledge of the evolutionary history of modern-day Brassicaceae, but with special reference to allopolyploid evolution. The latter is clearly crucial as many economically important Brassicaceae species are allopolyploids.

14.4.3 Allied Crop Species

The *Brassica* lineage within the Brassicaceae, the origin of which the B-genome of *Pachycladon* is more closely ascribed, contains many economically important crop plants. These include diploid crops like radish (*R. sativus*), turnip and Chinese cabbage (*B. rapa*), cabbage, kale, broccoli, cauliflower, kohl rabi,

and Brussels sprouts (all subspecies of *B. oleracea*), and black mustard (*B. nigra*) as well as polyploid crops like white mustard (*Sinapis alba*), Indian mustard (*B. juncea*), Ethiopean mustard (*B. carinata*), and canola (oilseed rape) (*B. napus*) (UN 1935).

14.5 Role in Classical and Molecular Genetic Studies

14.5.1 Genetic Studies

14.5.1.1 Forward Genetics: Mapping of Genes and Polygenic Clusters

A number of classical genetic studies are being envisaged for *Pachycladon*. Given the young age of the radiation, species remain largely interfertile. This has enabled the successful generation of hybrids between several *Pachycladon* species tested (see Fig. 14.4b) (Heenan unpublished data). These F_1 hybrids are fertile, so there is the potential to generate F_2 mapping populations segregating for traits of interest.

One such F_2 mapping population derived from parents *P. cheesemanii* and *P. fastigiatum* has been generated in order to help identify the genetic basis of morphological, physiological, and biochemical traits of interest in *Pachycladon*. These two species differ in a number of traits including trichome density (dense, sparse, or absent), trichome complexity (simple or branched), altitudinal preference (generalist vs. alpine), substrate preference (generalist vs. greywacke specialized), number of times they flower before death (polycarpic vs. monocarpic), seed production levels (high vs. low), and dispersability of seeds (wingless vs. winged). They also differ in their profile of glucosinolates, compounds that discourage herbivory, and flavonoids, compounds that help protect against UV damage that can occur at high altitudes (see Sect. 14.5.2 for more details). These traits are all likely to confer adaptive advantage by helping these plants cope with biotic or abiotic stress in their habitats or by improving reproductive success. Many of these traits are also of great interest for crop improvement. Therefore, molecular characterization and improved understanding of any of these traits in the *Pachycladon* system may potentially be useful for the improvement of allied Brassicaceae crops.

Trichome density, geological substrate tolerance, and secondary metabolism are traits that will be the main focus of the mapping project in progress, in order to complement various other studies that are currently underway or are being planned for the near future. Single nucleotide polymorphism (SNP) markers are to be developed for *P. cheesemanii* and *P. fastigiatum* from expressed sequence tag (EST) libraries that are currently under construction (see Sect. 14.7.1 for more details) to facilitate linkage mapping. A quantitative trait loci (QTL)-based approach will be taken to map polygenic clusters of interest (Symonds personal communication). Through comparative profiling (see Sect. 14.5.2) and QTL studies, it is hoped that candidate adaptive gene sets will be identified. These will be subjected to studies of molecular evolution and population genetics.

14.5.1.2 Reverse Genetics: Candidate Gene Approach

The availability of the full genome sequence of the closely related model plant, *A. thaliana*, and the fact that a great deal of functional information is available about its genes, allows a candidate gene approach to be used to look for *Pachycladon* homologs that can then be subject to reverse genetic strategies to determine whether they are responsible for variation in morphological traits of interest. A number of genes have been characterized in *A. thaliana* that have been implicated in trichome development, glucosinolate biosynthesis, glucosinolate hydrolysis, and flavonoid biosynthesis, and their homologs in *Pachycladon* represent candidate genes that might be responsible for the observed variation in these traits. Direct PCR-based approaches or probing of *Pachycladon* EST libraries with *A. thaliana* probes might allow sequencing of *Pachycladon* orthologs and paralogs of these genes. Preliminary evidence suggests that the former approach is tractable in *Pachycladon* (Symonds personal communication). Application of the latter approach was effective in determining the sequence of a candidate rapidly evolving gene from an EST library derived from close relative *A. lyrata* (Yogeeswaran unpublished data) and therefore may be effective for this system as well, at least for more conserved genes. Sequence alignment may reveal differences in the genotype that may be responsible for phenotypic variation between species and can be tested

for associations with phenotypic traits of interest. Ultimately, knock-out or knock-down experiments will be needed to confirm functional roles for candidate genes in *Pachycladon*.

14.5.2 Comparative Transcript and Metabolite Profiling in Pachycladon

Since speciation and adaptive radiation are predicted to have occurred very recently in *Pachycladon*, it is possible that the observed differences in traits of interest between species may not have been fixed in the genotype. In this case, mapping and sequencing efforts may not reveal stably inherited differences at the gene level (true allelic variants) between species that can be exploited for population genetic studies or that explain the observed differences in morphology, physiology, and substrate preference. Instead epigenetic mechanisms may be responsible for differential expression of genes or gene silencing. Therefore, comparative transcript and metabolite profiling have been undertaken in parallel to mapping efforts, to help elucidate the genetic basis of the observed differences in phenotype. Signatures of phenotypic divergence predicted by such comparisons may be indicative of forces driving speciation or secondary adaptation following speciation. Given the young age of the *Pachycladon* radiation, secondary adaptation may be less likely to account for species-specific adaptive phenotypes. Two such comparative profiling studies have been performed (see Sects. 14.5.2.1 and 14.5.2.2) that have already yielded interesting insights into the evolution of this alpine genus. Ecophysiological, morphological, and ecological studies to complement these studies are also being planned or are in progress.

14.5.2.1 Native Population Study

Gene expression differences between the two greywacke specialists, *P. enysii* and *P. fastigiatum*, were investigated using heterologous microarrays from *A. thaliana* and sampling wild populations from each species (Voelckel et al. 2008). The microarray platform was produced by spotting the *A. thaliana* AROS version 1.0 genome set (Operon Biotechnologies, operon.com) to glass slides (Plant and Food Research, Auckland, New Zealand). Each of the 26,282 70-mer oligonucleotides was spotted once. In total, there were 26,880 probes on the array including controls. In this study, results could be obtained for 76% of all probes (Voelckel et al. 2008). Two biological processes found to be differentially expressed between *P. enysii* and *P. fastigiatum*, viz., glucosinolate and flavonoid biosynthesis, were further investigated through biochemical assays. These investigations led to the characterization of twelve glucosinolate (GLS) compounds in both species (Voelckel et al. 2008).

Glucosinolate Biosynthesis

P. enysii produces C4 and C3 GLS, whereas *P. fastigiatum* only produces C3 GLS and *P. enysii* and *P. fastigiatum* predominantly produces alkenyl GLS and methylsulfinylalkyl GLS, respectively. These patterns were in accordance with expression patterns of glucosinolate biosynthetic loci such as methylthioalkylmalate synthase 1 (*MAM1*) and 2-oxoglutarate-dependent dioxygenase 2 (*AOP2*). Moreover, glucosinolate profiles from wild *P. enysii* and *P. fastigiatum* individuals did not group into two species-specific profiles, but rather into five distinct profiles referred to as chemotypes, two specific to *P. fastigiatum* and three specific to *P. enysii* (see Sect. 14.2.5.4 for more on *Pachycladon* chemotypes).

Glucosinolate Breakdown

The differential expression of two myrosinase-associated genes, the epithiospecifier protein gene (*ESP, At1g54040*) and the epithiospecifier modifier 1 gene (*ESM1, At3g14210*), predict that *P. enysii* and *P. fastigiatum* would markedly differ in the formation of glucosinolate breakdown products. In line with the up-regulation of *ESP* and *ESM1* in *P. enysii* and *P. fastigiatum*, respectively, the former was found to produce nitriles, whereas the latter produced isothiocyanates following myrosinase-mediated glucosinolate hydrolysis. In *A. thaliana,* it has been unambiguously demonstrated that ecotypes producing isothiocyanates are more toxic to generalist herbivores than ecotypes producing nitriles (Lambrix et al. 2001; Burow et al. 2006; Zhang et al. 2006). Given the differences in gene expression and hydrolysis product formation, it has

been suggested that *P. enysii* and *P. fastigiatum* resemble *Arabidopsis* ecotypes Ler and Col-0 with *P. fastigiatum* being better protected against generalist herbivores than *P. enysii*. Further studies are necessary to determine whether this presumed difference in toxicity can be explained by greater herbivory pressures in *P. fastigiatum* environments, some unknown selective agent for nitrile formation in *P. enysii* environments, or a combination of both.

Flavonoid Biosynthesis

P. enysii is assumed to have adapted to its high-altitude alpine habitat with the evolution of trichomes and UV-protective compounds. The differential expression of three flavonoid synthesis genes (flavonol synthase, flavonoid 3′-hydroxylase, and ferulate-5-hydroxylase) led to the prediction of increased levels of quercetin and sinapic acid esters in *P. enysii* leaves. Both types of compounds have been implicated in UV-B defense in *Arabidopsis* and other plant species (Landry et al. 1995; Bharti and Khurana 1997; Ryan et al. 2002). Quercetin-glycoside levels were not found to be significantly different between species, but *P. enysii* leaves contained significantly more cinnamic acid derivatives than *P. fastigiatum* leaves. Detailed analyses of individual cinnamic acid derivatives are needed to test if this difference is caused by sinapic acid esters, a subclass of cinnamic acid derivatives.

14.5.2.2 Common Garden Study

Differential expression and metabolite profiling studies suggest that interactions with herbivores and pathogens were important in the differentiation of *P. enysii* and *P. fastigiatum* (Voelckel et al. 2008). A common garden comparison involving *P. cheesemanii*, *P. exile*, and *P. novae-zelandiae* was performed to test if this inference can be extended to additional species within the *Pachycladon* radiation (Voelckel et al. 2010).

Transcript and Protein Profiling

Evidence from heterologous microarrays and shotgun proteomics revealed differential expression of genes not only involved in glucosinolate hydrolysis and biosynthesis but also involved in the interconversion of carbon dioxide and bicarbonate, water use efficiency, and other stress-related genes in *P. cheesemanii*, *P. exile*, and *P. novae-zelandiae* (Voelckel et al. 2010). Thus, it was suggested that the three species diverged in physiological processes that affect carbon and water balance in addition to divergence in glucosinolate metabolism. Experiments to test these predictions are currently underway. Predicted differences in glucosinolate hydrolysis products were directly confirmed and resembled those found in *P. enysii* and *P. fastigiatum* (see metabolite profiling). Given the differential expression of *ESP* and *ESM1* and other myrosinase-associated loci across several *Pachycladon* species (Voelckel et al. 2008, 2010), a characterization of the genetic architecture of the *ESP* gene cluster (*At1g54000* to *At1g54040*) and the *ESM1* gene (*At3g14210*) in *Pachylcadon* has been initiated.

Metabolite Profiling

When comparing glucosinolate profiles in the leaves of *P. cheesemanii, P. exile*, and *P. novae-zelandiae*, patterns did not reflect the phylogenetic relationships of the three species (Voelckel et al. 2010). *P. cheesemanii* and *P. novae-zelandiae*, despite being less closely related than *P. cheesemanii* and *P. exile*, had more similar glucosinolate profiles than *P. cheesemanii* and *P. exile*. They shared their main two compounds allyl and *S*-2-hydroxy-3-butenyl glucosinolate. *P. exile* produced neither alkenyl nor C4 glucosinolates, a profile most similar to the one described for *P. fastigiatum* chemotype 2 (Voelckel et al. 2008). Hence, chemotypes are likely to be the product of convergent evolution and are, therefore, not useful as taxonomic markers. Overall, 14 GLS of different classes were identified suggesting the segregation of various GLS biosynthetic loci across the three *Pachycladon* species. For example, between-species differences in chain length of methionine-derived glucosinolates, methylsulfinylalkyl GLS, alkenyl GLS, and hydroxyalkenyl GLS suggest segregation at the *GLS-elong*, *GLS-AOP*, and the *GLS-OH* loci, respectively. In contrast to *P. cheesemanii* and *P. exile*, *P. novae-zelandiae* produced a complex blend of glucosinolates. One of its major leaf GLS was found to be glucoraphanin (4MSOB). Glucoraphanin is the GLS precursor of the

isothiocyanate, sulforaphane, which is an anticancer compound. Glucosinolates in *P. cheesemanii* were hydrolyzed into isothiocyanates, whereas the two major glucosinolates of *P. novae-zelandiae* were converted into their corresponding nitriles and epithionitriles. Similar to *P. enysii* (nitrile producing) and *P. fastigiatum* (isothiocyanate producing), this polymorphism in glucosinolate hydrolysis was associated with the expression of the ESP protein in *P. novae-zelandiae* but not *P. cheesemanii* (Voelckel et al. 2010). However, *P. enysii* and *P. fastigiatum*'*s* glucosinolate profiles were different from those of *P. cheesemanii* and *P. novae-zelandiae* in that both of the latter produced S-2-hydroxy-3-butenyl, a compound neither present in *P. enysii* nor present in *P. fastigiatum*.

In addition to GLS, 18 flavanoid compounds were identified with distinct patterns in each species. *P. exile* and *P. novae-zelandiae* both produced six different flavonoids, whereas *P. cheesemanii* produced eight different compounds. Similar to glucosinolate profiles, flavonoid profiles of *P. cheesemanii* and *P. novae-zelandiae* were more similar than those of *P. cheesemanii* and *P. exile* as the former shared two of the flavonoid compounds, whereas the latter shared none (Voelckel and Reichelt unpublished data).

14.5.3 Ecophysiological Studies

Initial comparative studies on physiological parameters such as photosynthetic carbon and light-response curves, stomatal and mesophyll conductance, water use efficiency, and carbon isotope discrimination found *P. enysii*, *P. fastigiatum*, and *P. cheesemanii* to differ markedly in these parameters (Bickford and Barbour unpublished data). In future work, the common garden physiological comparisons will be extended to more species and complemented by physiological studies in natural *Pachycladon* populations. Monitoring of environmental conditions such as photosynthetic active radiation, soil and air temperature, and humidity has begun at *P. cheesemanii*, *P. enysii*, *P. stellatum*, and *P. fastigiatum* sites. Collection of these environmental data is expected to lead to a better understanding of the differences in ecophysiology and microclimate in the alpine habitats of these species.

14.6 Potential for a Role in Crop Improvement

14.6.1 Interspecific Hybridization

Heenan (1999) successfully produced interspecific F_1 hybrids between *P. novae-zelandiae* (female parent) × *P. cheesemanii* (male parent) and *P. novae-zelandiae* (female parent) × *P. exile* (male parent) that produced moderate to high levels of viable pollen and could be selfed to generate F_2 populations. Morphological traits of the F_1 hybrids were predominantly intermediate to the parental types. Pollen viability appeared to segregate in a Mendelian fashion in the F_2 population where approximately 1/4th of F_2 plants were male sterile. Interspecific crosses between many species of *Pachycladon* have since been generated and in some cases, natural hybrids have also been found in the wild, and these are summarized in Fig. 14.4b (Bicknell et al. 2009; Heenan unpublished data). Morphological traits of these artificial and natural hybrids were also found to be intermediate to the parent plants as seen in Fig. 14.4a, in the example of leaf size and morphology of hybrids between *P. enysii* × *P. fastigiatum* and *P. novae-zelandiae* × *P. fastigiatum*. These F_1 hybrids are fertile; hence, there is potential to create mapping populations segregating for traits of interest between members of this genus. One such F_2 population derived from a cross between *P. cheesemanii* and *P. fastigiatum* is currently being used for QTL mapping (see Sect. 14.5.1).

14.6.1.1 Apomixis and Matromorphy

While making interspecific crosses using either *P. cheesemanii* or *P. exile* as the female parent and any other *Pachycladon* species as the male parent, it was noted that progeny seedlings frequently resembled the maternal parent rather than showing the usual intermediate phenotype expected of *Pachycladon* F_1 hybrids or segregation for parental traits in the F_2 generation (Heenan unpublished data). This suggested that these seedlings might be a product of apomixis. Apomixis is the production clonal embryos without fertilization of egg cells or in essence, embryo production by asexual reproduction (Asker and Jerling 1992). Many

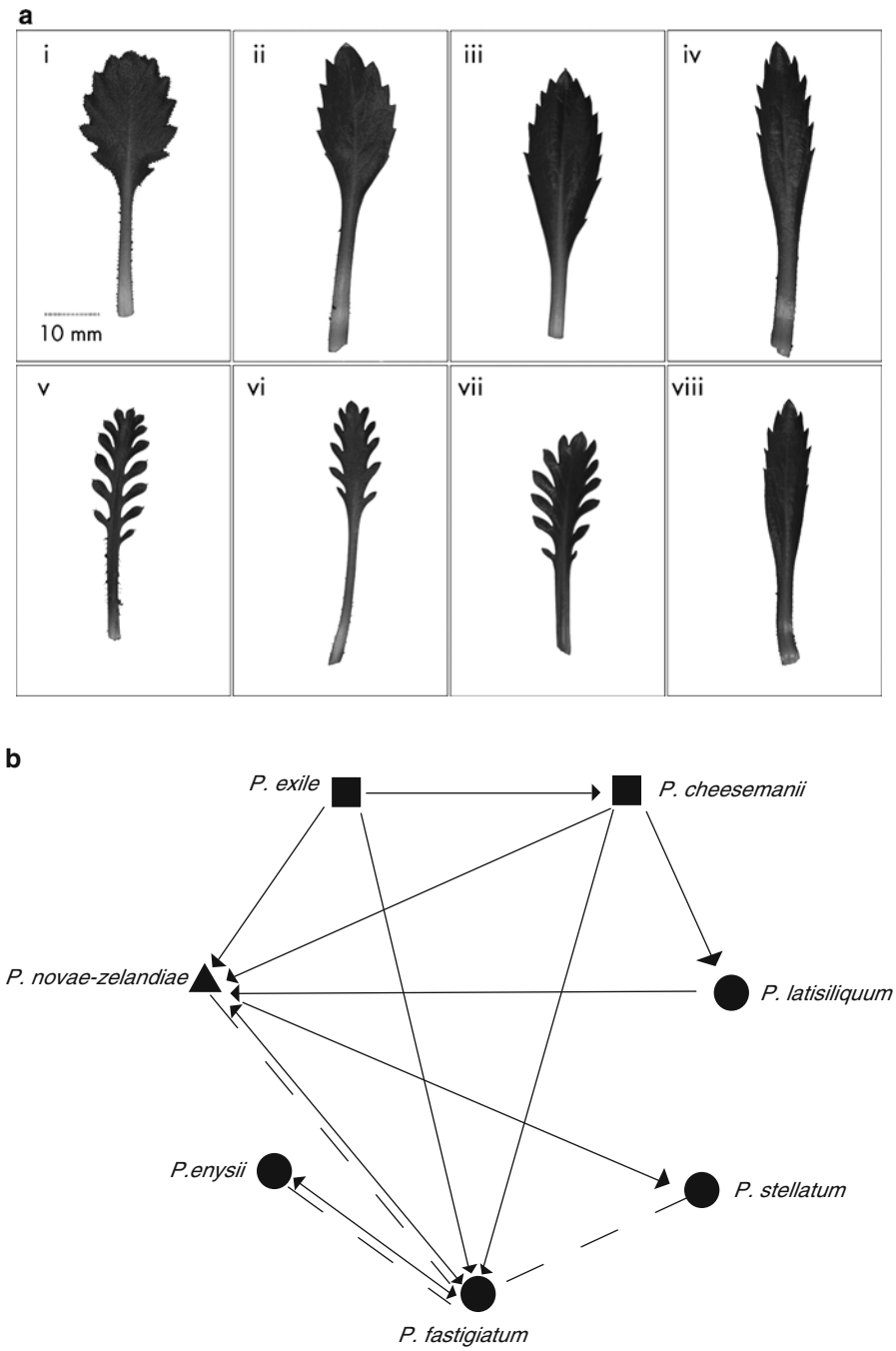

Fig. 14.4 Wild and artificial interspecific hybrids between *Pachycladon* species. (**a**) Leaf morphology (i) *P. enysii*, (ii) *P. enysii* × *P. fastigiatum* (artificial hybrid), (iii) *P. enysii* × *P. fastigiatum* (wild hybrid), (iv) *P. fastigiatum*, (v) *P. novae-zelandiae*, (vi) *P. novae-zelandiae* × *P. fastigiatum* (artificial hybrid), (vii) *P. novae-zelandiae* × *P. fastigiatum* (wild hybrid), and (viii) *P. fastigiatum*. (**b**) Interspecific hybrid combinations between *Pachycladon* species. Species assigned to *Pachycladon* in earlier and current taxanomic assignments (*solid triangle*) and *Pachycladon* species earlier assigned to segregate genera *Cheesemania* (*solid circle*) and *Ischnocarpus* (*solid square*). *Solid lines* represent artificial hybrids with the *arrow head* indicating the female parent, *two headed arrows* indicate that reciprocal crosses were possible and *dash lines* represent wild hybrids

crop plants are products of F_1 hybrid seed that demonstrate desirable heterotic effects, but F_2 seed derived from these are less favorable as they segregate for traits of interest. Apomixis is a highly desirable agricultural trait, as such an F_1 hybrid or indeed any plant with favorable traits, however complex and unstable, could be fixed through the production of clonal seed (Hanna and Bashaw 1987; Savidan 2000). Apomixis and other related processes such as androgenesis, matrocliny, and matromorphy have been previously reported in other Brassicaceae members (e.g., Barabas and Redei 1971; Eenick 1973, 1974; Chen and Heneen 1989; Schranz et al. 2005).

P. cheesemanii and *P. exile* transformants, with single copies of the green fluorescent protein gene (*GFP*), were emasculated and not pollinated, self-pollinated, or crossed with other species of *Pachycladon*, *A. thaliana,* and *B. rapa* to determine the nature of the earlier apomictic observation (Bicknell et al. 2009). *P. exile*, the maternal parent in the crosses with *Arabidopsis* and *Brassica*, could not cross-fertilize with these distant species, indicating that it is capable of recognizing "self" at the genus vs. non-genus level. It was determined that several F_1 generation plants derived from crosses with *Pachycladon* species showed maternal phenotype and these, and all F_2 generation plants derived from them, scored positive for the GFP marker and retained the maternal phenotype (no segregation in the F_2 generation). However, maternal-like seedlings were not produced in the absence of pollination or when the maternal plants were selfed; hence, autonomous apomixis did not occur. The type of apomictic response observed in these *Pachycladon* species is called matromorphy and is triggered by wide hybridization, resulting in seedlings expressing the maternal phenotype, derived asexually from maternal reproductive tissue (Bicknell et al. 2009). Matromorphic plants and natural hybrids derived from *P. exile* or *P. cheesemanii* have not been observed in the wild to date and matromorphy has not been observed in the laboratory or in the wild for other species of *Pachycladon*.

14.6.2 Intergeneric Hybridization

Heenan et al. (2008) successfully generated an artificial intergeneric hybrid by crossing a tetraploid *A. thaliana* accession (female parent, $2n = 4x = 20$) with *P. cheesemanii* (male parent, $2n = 2x = 10$). The hybrid status of this plant, formally named × *Pachydopsis hortorum*, was confirmed by flow cytometry, chromosomal counting, and AFLP analysis and was found to be $2n = 15$. The ×*Pachydopsis* hybrid is a robust perennial herb (see Fig. 14.5a, b) showing profuse vegetative growth, large leaves (see Fig. 14.5c), and floral traits intermediate to both parents (see Fig. 14.5d) but does not produce viable pollen. As a female parent, it allowed pollen hydration, pollen tube germination, possibly fertilization and some embryogenesis, by both *A. thaliana* and *Pachycladon* species. However, embryo development was arrested at the torpedo stage upon which the seeds were aborted. When inflorescences were treated with colchicine, polyploidy was successfully induced ($2n = 30$) and flowers from these inflorescences were backcrossed to *A. thaliana* Ler ($2n = 10$) and *P. cheesemanii* and crossed to *P. novae-zelandiae*. The former resulted in seed that produced backcross plants with the expected genotype ($2n = 20$), while the crosses to *Pachycladon* species failed to produce any seed.

Nasrallah et al. (2000) were able to produce a viable hybrid by ovule rescue from a wide interspecific cross between *A. thaliana* Col and *A. lyrata* ssp. *lyrata*, but these parent plants are only about 5 million years diverged. *A. thaliana* pistils permit hydration and pollen tube germination, even by distantly related pollen like *Brassica* (~20 million years diverged); however, there are no reports of this leading to successful fertilization and embryogenesis outside the *Arabidopsis* genus. *A. thaliana* and *Pachycladon* are more distantly related than *A. thaliana* and *A. lyrata*, with *CHS* genes sharing a most recent common ancestor between ~7 and 10 MYA. The fact that they could produce a viable F_1 hybrid by sexual hybridization is rather remarkable given the distance between these species. It also suggests that their mechanisms of fertilization, embryo development, and endosperm formation are highly conserved and can function in the hybrid background.

In ×*Pachydopsis*, many aborted seeds in both the backcrosses with the F_1 plant and the polyploid were arrested during advanced stages of embryogenesis. In *A. thaliana*, embryogenesis commences just hours after fertilization, and by about 9–10 days after anthesis, the embryos are in the torpedo stage (Mansfield and Briarty 1991; Faure et al. 2002). In contrast,

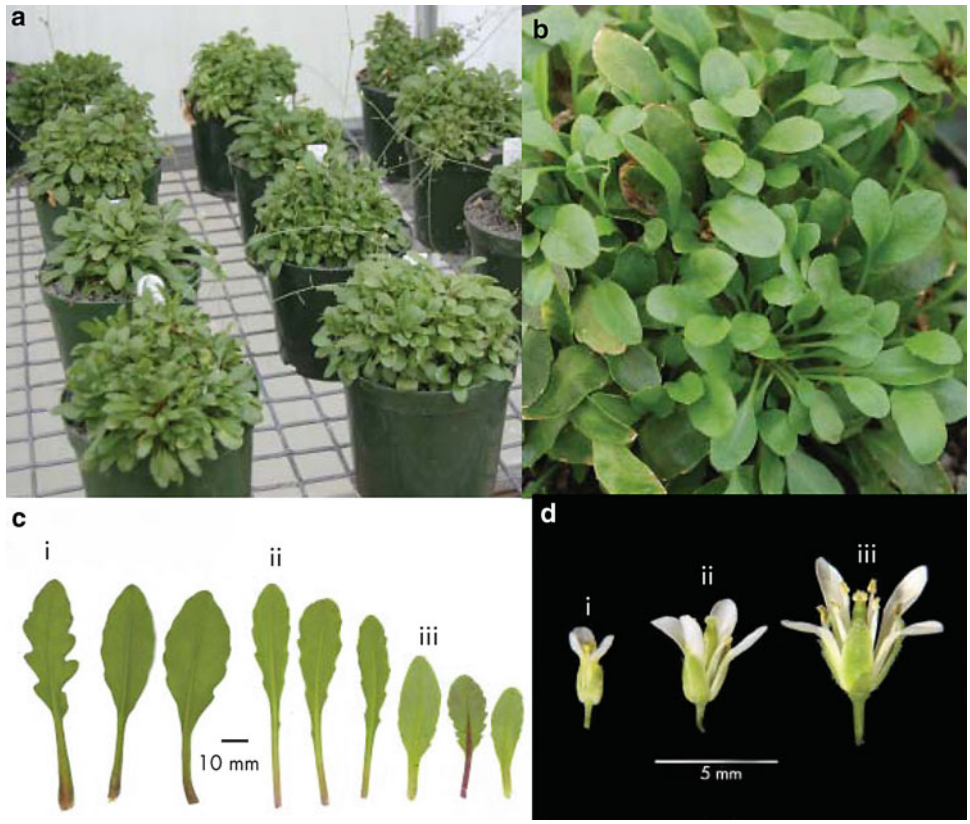

Fig. 14.5 × *Pachydopsis hortorum* intergeneric hybrid. (**a**) Cultivated plants 18 months old. (**b**) Vigorous rosette production in the hybrid (**c**) Leaf morphology of the intergeneric hybrid and parents (i) *Pachycladon cheesemanii*, (ii) × *Pachydopsis*, and (iii) *Arabidopsis thaliana* (**d**) Floral morphology of the intergeneric hybrid and parents (i) *Pachycladon cheesemanii*, (ii) × *Pachydopsis*, and (iii) *Arabidopsis thaliana*

embryogenesis in *P. cheeesemanii* is delayed by about 9 or 10 days post-anthesis (Luo et al. 2003). Otherwise, the processes in both species are very similar. It is possible that during the first 10 days post-anthesis, in the hybrid background, embryogenesis proceeds under the direction of the *A. thaliana*–derived genes. When the *Pachycladon*-derived genes become activated after the delay, the developmental program might become confounded by conflicting signals, thereby leading to arrested development. In the case of successful seed production, some epigenetic mechanism may operate to silence either the *A. thaliana* or the *Pachycladon*-derived embryogenesis genes. This intergeneric hybrid system may be useful for molecular characterization of genes responsible for initiation of embryogenesis and for understanding formation of allopolyploids from divergent genomes, such as those that gave rise to *Pachycladon*.

Finally, evidence for both independent chromosomal assortment (Nasrallah et al. 2000) and intergenomic recombination (Yogeeswaran 2005), despite divergence and differences in chromosomal and genomic structure, were obtained by molecular analysis of two backcross populations derived from the *A. thaliana*–*A. lyrata* hybrid and linkage mapping of *A. lyrata* (Yogeeswaran et al. 2005). If independent chromosomal assortment and/or intergenomic recombination occurred in the × *Pachydopsis* background, it might be possible to introgress genes from *Pachycladon* into the *A. thaliana* background or vice versa.

14.6.3 Genetic Transformation

Since *Pachycladon* species are relatively interfertile, somatic hybridization has not been attempted for this genus. However, some effort has been made in the arena of genetic transformation. Floral dipping, a transformation strategy developed for *A. thaliana* (Clough and Bent 1998) and successfully used in several other Brassicaceae members, was found to be ineffective for *Pachycladon* species. Instead, a transformation protocol using hypocotyl explants from 14-day-old seedlings resulted in transgenic plants, by regeneration through tissue culture, following cocultivation with *Agrobacterium tumefaciens*. This protocol was used to successfully transform *P. cheesemanii* and *P. exile* with *GFP* (Bicknell et al. 2009).

14.7 Genomics Resources Being Developed for *Pachycladon*

In the course of current and future projects planned for *Pachycladon*, several genomic resources will be created at the Allan Wilson Center for Molecular Ecology and Evolution, Massey University (Lockhart personal communication), that will facilitate future study of these plants. These resources can also be used for comparative genome studies with other Brassicaceae species, which are already sequenced, or are currently being sequenced, including *A. thaliana*, *A. lyrata*, *Capsella rubella*, *Brassica oleracea*, and *B. rapa* (Jackson et al. 2006) and therefore will be of great use to the research community at large. A few of the major resources are briefly outlined below.

14.7.1 EST Libraries, SNPs, and Microsatellites

EST libraries are being developed as a source for generating microarray probes, SNP, and microsatellite markers, for designing primers specific to candidate genes and providing a reference transcriptome for the annotation of short-sequence tags generated by gene expression tag profiling studies. A preliminary library of *P. enysii* roots and leaves has been developed using 36-base pair (bp) single-end Solexa reads, an approach to transcriptome analysis that could be applied to other non-model genomes (Collins et al. 2008). From a total of 40 million reads, 22,631 *Pachycladon* contigs were assembled de novo under the optimal assembly parameter k-mer = 19 (Collins et al. 2008). BLAST results with the assembled contigs identified 4,283 potential *Pachycladon* genes that matched *A. thaliana* ESTs. For 1,155 of these genes, mapped contigs showed a varying degree of overlap amongst themselves enabling the analysis of SNPs between both *Pachycladon* copies and between *Pachycladon* and *Arabidopsis*. There were 141 *Pachycladon* genes with a >100-nucleotide (nt) overlap, and amongst these, there were nine genes with a >300-nt overlap. Subsequently, 36-bp Solexa transcriptome reads were obtained for *P. cheesemanii* and 454 reads (40–320 bp) were obtained for *P. fastigiatum*. The recent development of paired-end sequencing (Fullwood et al. 2009) also enabled another Solexa run with 75-bp reads from both ends of an EST for *P. fastigiatum*. Assembly and annotation parameters for the EST libraries are currently being explored.

14.7.2 Establishment of Sequencing-Based Gene Expression Profiling *in* Pachycladon

A pilot digital gene expression study performed on the Solexa Genome Analyzer is currently underway to explore the potential of tag-based profiling in *Pachycladon*. As a temporary measure, tag annotation is realized by mapping tags to the most recent *A. thaliana* transcriptome. After a successful build of *Pachycladon* EST libraries, the *Pachycladon* transcriptome itself is then intended to serve as the annotation template. In the pilot study, which involved RNA from *P. enysii* and *P. fastigiatum*, over 44 million tags of 18 bp in length were sequenced in total. A quarter of all tags mapped uniquely with either no, or one, or two mismatches to the *Arabidopsis* transcriptome, whereas 19% did not map (because of more than two mismatches) and 56% mapped repeatedly (at more than one location) to the *Arabidopsis* transcriptome. A statistical analysis of uniquely mapped tags is currently underway (Voelckel, Biggs and Lockhart unpublished data). However, since tag profiling only

captures transcripts with particular restriction sites and unambiguous mapping of 18 bp tags may be problematic, mRNA sequencing is also being explored as an alternative to tag profiling for genome-wide gene expression profiling in *Pachycladon*.

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Chapter 15
Ricinus

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15.1 Introduction

This chapter focuses on *Ricinus*'s (1) basic botanical aspects, origin, distribution, and conservation; (2) cultivation, general utilization, and improvement by breeding programs; (3) traditional genetics and new genomic resources developed; and (4) challenges faced for further exploitation and future research directions.

15.2 Origin, Distribution, and Conservation

15.2.1 Taxonomy

Castor bean (*Ricinus communis* L.) belongs to the Euphorbiaceae family (spurge family), which consists of about 280 genera and 8,000 species of plants and trees worldwide. However, *Ricinus* is a single-species genus. Sometimes, these plants contain white latex, which may be irritable (Purseglove 1981). Close relatives of castor bean include *Cnidoscolus* Pohl. species (spurge nettle), *Croton* L. species, *Crotonopsis* Michx. species (rushfoil), *Mercurialis* L. species (mercury), *Acalypha* L. species (copperleaf), *Tragia* L. species, *Stillingia* L. species (Garden), *Phyllanthus* L. species, *Andrachne* L. species, and *Euphorbia* L. species (spurge) (Fernald 1950). Other close relatives include *Antidesma bunius* L. Spreng., *Baccaurea motleyana* Muell.-Arg., *B. sapida* Muell.-Arg., *Phyllanthus acidus* (L.) Skeels, and *P. emblica* L., which are cultivated for their edible fruits. Sources of drying oil include castor bean and *Aleurites* spp. The close relative, cassava (*Manihot esculenta*) is an important root crop. Rubber producers include *Manihot* spp. and *Hevea brasiliensis*. Common ornamental shrubs in the tropics include *Acalypha hispida* Burm. f., *A. wilkesiana* Muell.-Arg., *Codiaeum variegatum* (L.), *Euphorbia milii* Ch. Des Moulins, *E. pulcherrima* Willd. ex Klotzsch (poinsettia), *E. tirucalli* L., and *Jatropha* spp. (Purseglove 1981).

15.2.2 Morphology

Castor bean is a short-lived perennial in the tropics; however, it is an annual plant in Texas and California. It is predominantly wind-pollinated. Leaves range from 10 to 76 cm wide, alternate, palmately divided into 5–11 lobes, and reaches heights of 91–366 cm. The leaves are spirally arranged, stipules 1–3 cm long, united, and deciduous. The petiole ranges from pale green to red, round, and 8–50 cm long with two nectiferous glands at the lamina junction. There are two glands on both sides at the base and one or more glands on the upper surface toward the base. The leaves vary in color from green, purple to red and some are used as ornamental plants in home gardens. The stems are green or red becoming hollow with age and have well-marked nodes. A single stem terminates into an inflorescence at the sixth to tenth node in dwarf and early maturing cultivars. However, stem termination occurs at the 8th to 16th node in later maturing cultivars and at the 40th or more node in tall and wild type plants. Two to three sympodial branches grow out from each node as the panicle develops. The inflorescence develops from these branches.

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Flowers are green to yellow without petals borne in a terminal with many flowered panicles ranging from 10 to 40 cm long. The flowers are unisexual with male flowers at the base and female flowers on 30–50% of the upper inflorescence (Purseglove 1981). The plant is monoecious with the pistil in the upper raceme and stamens below resulting in frequent cross-pollination (Martin and Leonard 1967). The plants are protogynous with most female flowers setting seeds and fruits prior to the male flowers opening on the same inflorescence. The anthers burst when dry or touched scattering the pollen and the male flowers abscisse soon after pollen shed. As the flowers open, glands on young leaves below the inflorescence exude nectar, which attracts insects (Purseglove 1981). The fruit is spiny, smooth, dehiscent or indehiscent, and contains three to four celled capsules with each cell containing a mottled seed. The seed is obovoid with a prominent hilum or caruncle (Martin and Leonard 1967). *Ricinus* is the latin word for tick and the seeds resemble an *Arachnid* in morphology (Tyler et al. 1976).

15.2.3 Origin and Distribution

Castor bean (*R. communis* L.) has several common names including castor, castor oil plant, and palma christi (National Plant Germplasm System 2010). It probably originated in tropical Africa, was then taken to India and China, and through gradual spreading it is now grown worldwide from temperate to tropical zones (Martin and Leonard 1967; Purseglove 1981).

15.2.4 Conservation

There are several conservation centers for castor bean in the world. In the United States, 1,043 castor bean accessions, collected or donated from 51 countries worldwide, are curated at the USDA, ARS, Plant Genetic Resources Unit, Griffin, Georgia. The majority of the castor bean accessions were acquired from the United States (665), India (105), Iran (43), Brazil (39), South Africa (30), Argentina (25), Turkey (23), Peru (16), Pakistan (10), Israel (9), and Cuba (8). Five accessions originated from both Algeria and Mexico while four were acquired from the former Soviet Union and Paraguay. One to three accessions originated from Afghanistan, Albania, Bahamas, Benin, Botswana, Bulgaria, Cambodia, China, Colombia, Costa Rica, Ecuador, Egypt, El Salvador, Ethiopia, Greece, Guatemala, Hungary, Indonesia, Jamaica, Jordan, Kenya, Madagascar, Mongolia, Morocco, Nepal, Portugal, Puerto Rico, Sri Lanka, Syria, Uruguay, Virgin Islands, Yugoslavia, Zaire, and Panama (National Plant Germplasm System 2010).

Genetic erosion is the loss of genetic diversity within a species. A recent assessment (Allan et al. 2008) suggested that castor bean germplasm contains relatively low levels of genetic diversity. Some castor bean accessions may have originated from a single sampled plant, which could lead to within-population variation and potentially causing some genetic erosion. Another possible cause of genetic erosion in castor bean could be from selfing as the primary reproductive mode. Selection of castor beans by farmers for a trait of interest could also lead to narrow genetic diversity. Human selection for high oil producing castor beans may have reduced the genetic diversity of this species. Selection for similar traits has altered the genetic makeup and diversity of other crops including soybean (Hyten et al. 2006), rice (Zhu et al. 2007), and hard red winter wheat (Harlan 1987). The Euphorbiaceae may be typically lower in genetic diversity than other dicots, which could explain low levels of diversity using amplified fragment length polymorphism (AFLP) or simple sequence repeats (SSRs) (Allan et al. 2008).

Ex situ conservation efforts are conducted at the USDA, ARS, Plant Genetic Resources Conservation Unit, Griffin, Georgia. Its mission is to acquire, conserve, regenerate, characterize, evaluate, document, and distribute castor bean accessions. The goal of this collection is to conserve in a quality manner, the range of genetic variation, and diversity identified in castor bean. Efficient preservation, seed regeneration, germination, secure backups, and distribution of castor bean germplasm are part of this mission. Seed regeneration is a very important curation activity. Viable castor bean seeds are monitored and reevaluated using standard seed germination methods. Typically, castor beans are scheduled for regeneration if germination percentages drop below 70% or if few seeds per accession exist. Regeneration of accessions strives to preserve the gene and genotypic structure of castor bean populations (Breese 1989). Castor bean plants are mainly self-pollinated by wind and/or insects and

require either isolation by distance or bagging the inflorescence prior to fertilization to avoid contamination because castor bean can cross pollinate in excess of 80% (Dick Auld, Texas Technical University). Even though castor beans are wind-pollinated, they are still considered mainly as self-pollinating. We regenerate 50 plants per castor bean accession per 6 m^2 plot. Plant morphology, phenotype, and reproduction are characterized and recorded for regenerating each castor bean accession. At maturity, castor bean plants are hand-harvested, dried at 21°C and 25% RH for about 1 week, and threshed. Seeds are then counted, weighed, and stored at 4°C for distribution while seeds for long-term storage are stored at −18°C. For distribution, castor bean germplasm have been sent to various researchers worldwide. Most of the research is devoted to seed oil evaluations for biodiesel, industrial, and pharmaceutical uses.

15.3 Production and Utilization

15.3.1 Production

Castor bean was cultivated in Egypt as long ago as 4000 BC. It subsequently migrated to India and China during the Tang period (618–906 AD). Castor bean entered North America soon after Columbus discovered the New World and has been naturalized in many tropical to semi-tropical areas of the world (Purseglove 1981). Castor bean was grown on more than 35,000 acres in the United States during 1960. Seed production exceeded 30 million pounds and averaged more than 840 lb/acre. Most of the production was centered in Texas (Martin and Leonard 1967). It can become a weed in second year cropland. However, castor bean can be rotated or plowed under the soil prior to setting mature seed to eliminate the seed from overwintering in second year crops. Castor bean is also grown in California. The leading production countries include Brazil, India, and China whereas Mexico, Italy, Argentina, Indonesia, African countries, and South America have limited production (Martin and Leonard 1967). Castor bean is usually grown as a dry land crop in India and often rotated with finger millet (*Eleusine coracana*). However, castor bean production in the southwestern United States occurs with the use of irrigation (Purseglove 1981). Currently, most production in the US occurs in the Texas high plains. Many countries have used castor bean to treat various health ailments (UK Cropnet 2008) (Table 15.1).

Table 15.1 Ethnobotannical uses of castor bean

Use	Countries
Abscess	China
Head ache	China, Ghana, Guatemala, Ivory Coast, Mexico, Philippines, Sudan
Stomach ache	South Africa, Trinidad
Arthritis	China
Asthma	Haiti
Bactericide	Trinidad
Dog bite	China
Blister	Java
Boil	China, South Africa, Tonga
Burn	China, India
Cancer	Canada, Indochina
Cholera	India
Cold	Bahamas, Haiti
Colic	Mexico
Convulsion	India
Dermatosis	Malaya
Expectorant	China
Fever	Ghana, Hawaii, Ivory Coast, Mexico
Flu	Haiti, Trinidad
Gout	Java
Hair oil	Africa, Guatemala, Haiti
Inflammation	Trinidad
Insecticide	Guatemala, Haiti, South Africa
Laxative	Africa, China, Samoa, Venezuela
Leprosy	India
Moles	Australia
Palsy	China
Purgative	China, Ghana, Haiti, Mexico, Spain, Turkey, Venezuela, Trinidad
Rheumatism	Somalia
Scald	China
Skin	China
Soap	Haiti, Mexico
Sore	China, India, Java, Philippines, South Africa
Swelling	West Africa, Java
Tonic	Bahamas
Toothache	South Africa
Tuberculosis	Bahamas
Tumor	Brazil, Chile, Tonga, India
Wound	Java, Rhodesia, China
Rash	Tonga
Rheumatism	Haiti, Java
Sprain	Java

15.3.2 Uses of Castor Bean

Castor bean seeds contain 35–55% oil and 1–5% of the protein toxin, ricin, by weight. Castor bean's growth and yield increases in response to elevated CO_2 levels; however, the castor oil content is unaffected (Vanaja et al. 2008). The properties and uses of ricin will be discussed later. After the extraction of the oil and the inactivation of ricin, the defatted mash (castor bean meal) is used as animal feed while the seed husks are used as a high nitrogen fertilizer. Castor bean meal contains about 40% crude protein; chemical analysis indicates that it is deficient in the essential amino acids lysine, methionine, and tryptophan (Vilhjalmsdottir and Fisher 1971).

15.3.2.1 Castor Oil

Castor oil from castor bean seeds is used as a medicine (a purgative); an ingredient in shampoo, soap, hand lotion; and as a high speed lubricant (aviation and automotive). Among its medical uses, 1.25% of castor oil emulsion has recently been shown to be effective in the clinical management of evaporative dry eye (Khanal et al. 2007) and homogenized castor oil eye drops are effective in the treatment of meibomian gland dysfunction, a major cause of lipid deficiency dry eye (Goto et al. 2002). Castor oil together with balsam of Peru and trypsin has been shown to reduce edema and scabbing of patient wounds (Gray and Jones 2004). Castor oil is considered safe and effective when used as a stimulant laxative (i.e., purgative) (FDA 2003). According to a recent safety assessment, castor oil, hydrogenated castor oil, glyceryl ricinoleate, ricinoleic acid, potassium ricinoleate, sodium ricinoleate, zinc ricinoleate, cetyl ricinoleate, ethyl ricinoleate, glycol ricinoleate, isopropyl ricinoleate, methylricinoleate, and octyldodecyl ricinoleate are considered safe as cosmetic ingredients (Johnston 2007). Castor oil is used in a number of other products. For example, hydrogenated castor oil is used in water repellent coatings, candles, shoe polish, carbon paper, and ointments (Merck Index 1989). Constituents of castor oil include ricinoleic acid, zinc ricinoleate, and methyl ricinoleate (TNO BIBRA International Ltd 1999). Ricinoleic acid is used in textile finishing, and as a source of sebacic acid, heptanol, ricinoleate salts, and 12-hydroxystearic acid (Lewis 1997). Zinc ricinoleate is used as a fungicide, emulsifier, grease, lubricant, water proofing agent, lubricating oil additive, and stabilizer in vinyl compounds (Lewis 1997). Methyl ricinoleate is used as a plasticizer, lubricant, cutting oil additive, and wetting agent (Lewis 1997).

15.3.2.2 Proteins (Ricin and Agglutinin)

Purified ricin is a white powder that is soluble in water and stable over a wide pH range (Audi et al. 2005). Aqueous solutions of ricin are inactivated by heat (80°C for 1 h); however, ricin powder or crude preparations require higher temperatures or longer periods for inactivation. Ricin is a heterodimeric glycoprotein toxin with a molecular weight of 65 kDa, which is extracted from castor bean seeds. It consists of two polypeptides (A chain 32 kDa and B chain 34 kDa) linked by a single disulfide bond (Olsnes and Pihl 1973). The B chain is a lectin and binds to galactose-containing glycolipids and glycoproteins expressed on the surface of cells, facilitating the entry of ricin into the cytosol. The A chain inhibits protein synthesis by irreversibly inactivating eukaryotic ribosomes through removal of a single adenine residue from the 28S ribosomal RNA loop contained within the 60S subunit. This process prevents chain elongation of polypeptides and leads to cell death. The $K_{CAT} = 1{,}500\ min^{-1}$. One molecule of ricin is sufficient to inactivate 1,500 ribosomes per minute leading to rapid inhibition of protein synthesis and cell death (Vietta and Uhr 1985; Olsnes and Sandvig 1988). The estimated lethal oral dose of ricin for human is 1,000–20,000 μg/kg body weight (ca. 8 beans). Castor bean seeds also contain a closely related lectin termed *R. communis* agglutinin (RCA) (Roberts et al. 1985). RCA is a tetramer consisting of two ricin-like heterodimers held together by noncovalent forces (Olsnes et al. 1974). Each heterodimer contains an A chain (32,000 kDa) disulfide linked to a galactose-binding B chain (37 kDa). Although the nucleotide sequences of ricin and RCA are similar, these proteins are the products of distinct genes, and it has been suggested that the ricin gene evolved first and then duplicated to give rise to the RCA gene (Ready et al. 1984). RCA is not directly cytotoxic; however, binding of RCA to galactose residues on red blood cells can lead to agglutination and subsequent hemolysis (Audi et al. 2005).

15.3.2.3 Phytochemicals for Health

Many of the phytochemicals found in castor bean plants and seeds have potential medicinal uses (Morris 2004) and several have been shown to be clinically useful. For example, chlorogenic acid (found in the castor bean plant) has a clinically significant effect on the absorption and utilization of glucose in the diet and may reduce body mass and fat when added to instant coffee (Thom 2007), and can reduce blood pressure in people with mild hypertension (Watanabe et al. 2006). The antioxidant, flavonoid quercetin was shown to reduce blood pressure in hypertensive people (Edwards et al. 2007), while the flavonoid isoquercitrin, when used as an ingredient, is clinically effective and safe in people with chronic venous insufficiency (Schaefer et al. 2003). A fucose rich oligo- and poly-saccharide was demonstrated in a clinical study to significantly attenuate periorbital wrinkles in women with a total regression of crow's feet observed in some women (Robert et al. 2005). These phytochemicals from castor bean have not yet been introduced into phamarceutical markets. Perhaps, the most significant medical use of compounds obtained from castor bean involves the toxic protein, ricin. Even before the mechanism of action of ricin was understood, it was shown to have anticancer properties (Mosinger 1951; Lin et al. 1971). Once it was realized that ricin consists of two subunits with different roles in the intoxication process, the idea appeared that the receptor-binding subunit of the toxin molecule might be replaced by another binding moiety, such as an antibody directed against a cell surface antigen (Olsnes and Pihl 1981). These chimeric molecules have been termed immunotoxins. Immunotoxins can be used to deliver tumor cell specific toxins by targeting specific tumor cell surface antigens while not harming normal cells that lack these surface antigens (Lam et al. 2004). Scadden et al. (1998) demonstrated that an immunotoxin prepared by linking an anti-B4 (anti-CD-19) antibody with a modified ricin could be used to target cells bearing surface CD-19. This immunotoxin was used successfully in combination with chemotherapy to treat patients with AIDS-related non-Hodgkin's lymphoma. In another study, 18 children received an autologous hematopoietic stem cell transplant purged with B-4 blocked ricin. Of these, four remained in remission (Sandler et al. 2006). The results of another clinical trial demonstrated a durable remission (>4 years) of non-Hodgkin's lymphoma in 40% of patients treated with chemotherapy followed by an infusion of anti-B4-blocked ricin immunotoxin (Longo et al. 2000). Another immunotoxin was constructed by linking an anti-CD25 monoclonal antibody to deglycosylated ricin A chains. In a clinical trial, this immunotoxin showed moderate efficacy in patients with Hodgkin's lymphoma (Schnell et al. 2003) and in those with relapsed Hodgkin's lymphoma (Schnell et al. 2000).

15.3.3 Genetic Improvement and Cultivar Development Through Traditional Breeding

The primary breeding objectives are to increase yield or oil content. Many traits (e.g., disease resistance or drought tolerance) can contribute to yield. Therefore, efforts for disease resistance, semi-dwarf, drought tolerance, and other biotic and abiotic stress have been initiated into castor bean breeding programs. Hybrid castor beans were also developed for increasing castor bean yield in India (Classen and Hoffman 1950). During 1991–1995, a cooperative research program for increasing yield and oil content was developed by the European Union (participated by eight countries). Thirty-six varieties were surveyed to determine the variability of oil content and fatty acid composition (Ramos et al. 1984). A large variability of oil content was observed, ranging from 39.6 to 59.5%, whereas less variability of ricinoleic acid (a major fatty acid in castor bean) ranging from 83.65 to 90.0% was detected by gas chromatography analysis of fatty acid composition. Therefore, the potential exists for castor bean breeding programs to increase oil content of the seeds. Castor beans normally have a high level of ricinoleic acid (~900 g/kg of seed) and low levels of oleic acid (~30 g/kg of seed). A natural mutant line OLE-1 (~780 g/kg oleic acid) producing high levels of oleic acid was identified by screening 191 castor bean germplasm accessions (Rojas-Barros et al. 2004). This line could be a good parent for use in developing new castor bean cultivars with a high level of oleic acid. More recently, breeding research has focused on selection of accessions for divergent ricin and agglutinin concentrations (Pinkerton et al. 1999). Efforts toward decreasing the amount of ricin in seeds are of paramount importance because castor bean is a highly

valued oil producing crop and has recently drawn increased attention in research on biofuels. Future efforts for increasing oil content and improving castor bean as a biodiesel feedstock will probably be the highest priority for castor breeding programs (Da Silva et al. 2006; Meneghetti et al. 2006; Conceicao et al. 2007; Moser et al. 2008).

15.4 Current Biotechnology and New Genomic Resources Available for Castor Bean

15.4.1 General Genetics

R. communis is a diploid species with a chromosome number of $2n = 20$ although the basic number could be $x = 5$ (Singh 1976; Paris 1981). Its genome size was estimated to be about 425 Mbp/1C (Arumuganathan and Earle 1991). From the diploid species, different euploids, including haploids, triploids, and tetraploids were created by altering the ploidy level. The gene dosage effects on cellular proteins from euploidization of the nuclear genome were examined. In comparison with diploid types, there was no difference in the component of proteins analyzed; however, differences in protein band intensity (i.e., relative amount of protein) have been detected. Analysis of the upload series revealed that the photosynthetic CO_2 fixation rates decreased in leaf tissues with increasing size of the nuclear genome (Timko and Vasconcelos 1981). Traditional cytogenetic analyses of castor bean were also carried out by observation of the relative length of chromosome arms, the size and length of heterochromatic segments on particular chromosomes, and the presence and absence of a secondary constriction (Paris et al. 1980; Paris 1981). To our knowledge, there have been no aneuploids reported in castor bean.

15.4.2 Genetic Diversity and New Genomic Resources Developed

Although there are more than a thousand castor bean accessions in the US castor bean germplasm collection, there is limited genomic information (e.g., DNA markers and sequence information) indicating that the genetic diversity of castor bean has not been well assessed. Recently, the castor bean genome has been sequenced in low depth (4× of the genome equivalent) by a shotgun approach. Approximately, ~50,000 expressed sequence tags (ESTs) from different tissues have been produced by The Institute of Genome Research (TIGR). In comparisons with sequences available from other Euphorbiaceae species, castor bean showed the highest sequence similarity to cassava. As more sequence information becomes available, greater opportunities for studying comparative genomics between castor bean and cassava will be evident. The available genomic information for castor bean was jointly reported at the Plant and Animal Genome XIV Conference by TIGR and Northern Arizona University (Chan et al. 2006). From the available sequence information, SSRs have been mined and SSR markers have been developed. These same two groups have assessed the genetic diversity of castor bean using SSR markers and AFLPs (Allan et al. 2008). From this study, nine markers from SSRs and three primer combinations (ACG_CTA, ACC_CGT, and ACC_CTA of *Eco*RI_*Mse*I) for AFLPs were employed with 41 accessions representing worldwide distribution from five continents and 35 countries. To assess the genetic diversity within accessions, five seeds from each accession were selected. A summary of the results from this study indicate that (1) there was no clear geographic structuring of genotypes across continents or countries within continents; (2) SSR markers yielded a higher percentage of polymorphic loci, higher heterozygosity, and a greater range of genetic distance and therefore were more informative than AFLP markers; (3) the correlation between two SSR and AFLP data sets was low ($R^2 = 0.19$), but each marker type showed a similar pattern of genetic diversity and a lack of geographic structure; (4) a higher genetic variation within accessions than among accessions was revealed by both AFLPs and SSRs; and (5) from the accessions investigated, the genetic diversity within the US castor bean collection was restricted, compared to the results from other species. As more sequence information becomes available, more DNA markers will be identified from that database. To further assess the genetic diversity of castor bean, more accessions and more DNA markers should be employed in future studies. The genes for ricin have been cloned (Lamb et al. 1985; Tregear and Roberts 1992) and the mechanism for ricin toxicity has

been well elucidated. Cloning functional genes for ricin and understanding the regulation of ricin synthesis will provide the opportunity to manipulate these genes for reducing the amount of ricin in castor bean seeds by genetic engineering. This may be difficult as the ricin genome encodes seven full-length ricin family members that have the ability to inhibit translation and that may contribute to the toxicity of *R. communis* (Leshin et al. 2010).

15.4.3 Plant Tissue Culture and Transformation

Establishing a good tissue culture system (or procedure) is a prerequisite for successful transformation of any plant species. Tissue culture of castor bean has been successfully initiated from mature endosperm tissue (Mohan Ram and Satsangi 1963). On the basis of several reports, callus initiation and plantlet regeneration from explants were restricted to young seedling tissues. Although regeneration from callus has been reported from different tissues (including young leaves, stem, and hypocotyl sections), the probability of callus differentiation into shoots was very low. For successful plant transformation after foreign DNA has been integrated into plant cells or organs, the transformed cells must be able to produce mature plants and carry the foreign gene to the next generation. Therefore, it is critical to establish an efficient regeneration procedure in tissue culture for transformation in castor bean. A highly efficient and reproducible method of in vitro shoot proliferation from meristematic explants has been developed in castor bean by the addition of a cytokinin (e.g., thiadiazuron) to Murashige and Skoog (MS) medium (Sujatha and Reddy 1998). Continuous improvements of regeneration procedures in tissue culture have enabled genetic transformation of castor bean. Through vacuum infiltration of wounded castor flower buds, an *Agrobacterium tumefaciens*-mediated transformation method was established and patented (McKeon and Chen 2003). Using embryo axes from castor bean mature seeds, a stable genetic transformation system has been established using either *A. tumefaciens*-mediated or particle gun-mediated gene transfer (Sujatha and Sailaja 2005; Sailaja et al. 2008). Using *A. tumefaciens*-mediated genetic transformation, a synthetic delta endotoxin gene *cryIAb* and the herbicide resistance gene *bar* have been successfully introduced into castor bean. In T_1 plants, *cryIAb* and *bar* genes were cosegregated and the Southern-positive plants for *cryIAb* exhibited resistance to semilooper (Malathi et al. 2006). The establishment of stable genetic transformants of castor bean will lead to further development of transgenic castor beans in the future.

15.5 Challenges for Further Exploitation and Future Research Directions

To promote castor bean as a useful crop, there are several hurdles, which need to be overcome, such as reducing the content of ricin and RCA in seeds, preventing seeds from shattering before harvesting, and making seed harvest easier.

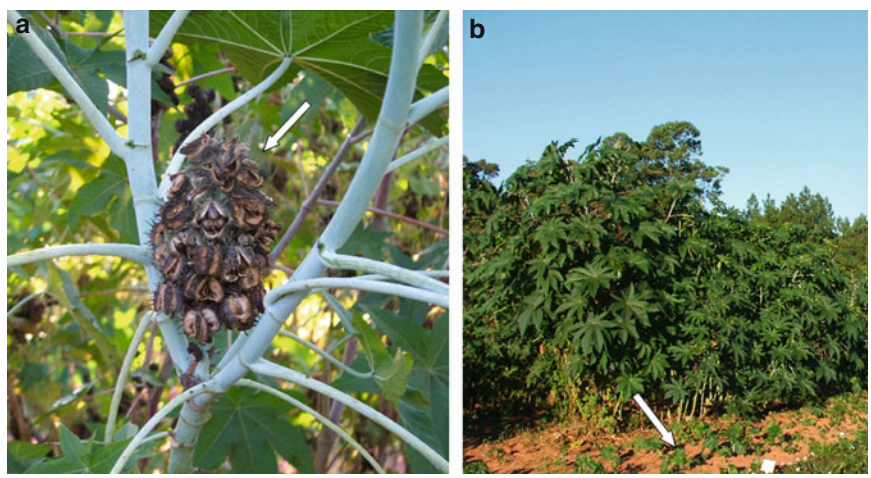

Fig. 15.1 Castor beans shattering and germinating before harvest. (**a**) Several mature capsules shattered their seeds with empty capsules remaining on the spike. (**b**) Many of the shattered seeds germinated

Reduce ricin and haemagglutinin in castor bean seeds: Since a significant amount of variation in ricin and RCA exist in the USDA, ARS germplasm collection, castor bean accessions with low ricin, and RCA contents have been used in crosses with a high yield cultivar for developing new cultivars (Pinkerton et al. 1999). A more effective way may involve a transgenic approach to down-regulate or knockout the key genes involving ricin biosynthesis.

Overcoming seed-shattering problems and harvesting difficulties: Seed shattering and harvesting are linked to each other. After seeds mature, they will shatter if they are not harvested in time (Fig. 15.1). Seed shattering reduces yield and causes problems for the next cultivation. If temperature and soil moisture are optimal, shattered seeds will germinate causing problems during the next cultivation. Seedlings need to be removed or they will compete with newly planted crops. This competition may lead to yield reduction for the new crops. Plant stature (height and size) of castor bean can make harvesting difficult either by hand or by combine. If the castor bean plant is too tall or wide (large canopy), a combine cannot successfully harvest the plant. Developing semi-dwarf castor beans should be one of the targets for castor bean breeding. Moreover, synchronizing the maturation date of castor bean plants is also important. This can decrease harvesting frequency and increase seed quality. Harvesting seeds after frost can greatly reduce seed quality because both mature and immature seeds will be harvested. Traditional breeding methods can achieve the goal of developing semi-dwarf and mature-synchronized castor bean cultivars.

Optimizing oil yield for biodiesel production: As castor bean seeds contain a high percentage of oil, castor bean may become one of the feedstocks for biodiesel production. Both grain yield and oil content can be directly contributed to oil production (oil content % $\times$ grain yield kg/acre $=$ oil yield production gallon/acre). Breeding materials (parents) with a high percentage of oil can be selected by screening castor bean germplasm. However, developing castor bean cultivars with a high grain yield is not easy to achieve because many traits contribute to yield. Utilization of heterosis in hybrid castor bean may be the most appropriate approach for development of high oil yield. To select high oil content and high yielding parents, one may also rely on exploiting the genetic diversity of castor bean germplasm in breeding programs.

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Chapter 16
Sesamum

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16.1 Introduction

Sesame (*Sesamum indicum* L.) is one of the oldest oilseed crops known to mankind and its cultivation dates back to 3050–3500 BC (Bedigian and Harlan 1986). It is grown in tropical and subtropical regions (Ashri 1998) of the world. Evidence indicates that Ethiopia is sesame's center of origin. There is considerable dispute regarding the Afghan/Persian region as the place of origin, with secondary centers in India and China, as sesame was an important crop in the Persian region during 2130–2000 BC (Weiss 1971). Sesame was grown during the ancient Harappan, Mesopotamian, and Anatolian eras for its edible seed and its oil (Bedigian 2004).

Sesame contains about 35–60% oil and 19–30% protein (Ashri 1998; Uzun et al. 2002; Arslan et al. 2007) that vouches for its superior quality, nearly matching that of olive oil (Kapoor 1990). Sesame oil is highly stable (Uzun et al. 2007) compared with the other edible oils, mainly due to the presence of antioxidants (Davidson 1999), including sesamin, sesaminol, sesamol, sesamolinol, and squalene contents in the seed (Mohamed and Awatif 1998; Shyu and Hwang 2002). Hiremath et al. (2007) studied genetic diversity in seed oil content and fatty acid composition in six wild species of the genus *Sesamum*, viz., *S. mulayanum*, *S. capense*, *S. laciniatum*, *S. latifolium*, *S. occidentale*, and *S. schinzianum* and compared it with that in cultivated sesame. This study further noted the seed oil content to be in the range of 46.13–53.8% in the cultivated *Sesamum* species and 20.3–33.9% in wild *Sesamum* species. Palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acids were the principal fatty acids in all the wild species and cultivated *Sesamum*. Wild *Sesamum* species exhibited a wide range of variation in palmitic and stearic acid contents. Stearic acid content in all the wild species was significantly higher than in the cultivated sesame. Lower oleic acid and higher linoleic acid contents were apparent in the wild species.

Sesamum belongs to Pedaliaceae, a small family of 17 genera and 80 species of annual and perennial herbs that occur in the Old World tropics and subtropics (Cronquist 1981; Mabberley 1997). Other genera that are considered to be related to *Sesamum* are *Ceratotheca*, *Martyniaceae*, *Anthadenia*, and *Volkameria* (Nayar and Mehra 1970; Kobayashi 1991). Various numbers of species have been reported by different workers. Joshi (1961) reported 36 species based on index kewensis. Nayar and Mehra (1970) reported 34 species and Kobayashi (1981) listed 37 species. New species, such as *S. indicum* var. *sencottai* and var. *yanamalai* (Devarathinam and Sundaresan 1990) and *S. mulayanum* Nair (Mehetre et al. 1993) were also added. According to Kobayashi (1991), 22 of the species were localized only in Africa, 5 only in Asian countries (India, Sri Lanka or East Indies or in all areas), 7 species were reported to be found in both the African and Asian countries, and one each was reported in Crete and Brazil. It was concluded by Ashri (1998) that taxonomy and cytogenetics of the genus *Sesamum* require more detailed investigations. Based on a study by Bedigian, the number of species in the genus *Sesamum* was revised back to 23 (Table 16.1). Few of the wild species (*S. angustifolium*, *S. calycinum* ssp. *Baumii*, *S. malabaricum*, and *S. radiatum*) are edible. As there exist several

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Table 16.1 Revised list of *Sesamum* species and their chromosome number ($2n$)

$2n = 26$	$2n = 32$	$2n = 64$	Unknown
***S. indicum* L.** (*S. africanum*; *S. auriculatum*; *S. brasiliense* Vell.; *S. edule* Hort ext Steud.; *S. hopkinsii; S. javanicum*; *S. lamiifolium* Engl.; *S. luteum* Retz.; *S. occidentale* Heer & Regel; *S. oleiferum* Moench; *S. orientale* L.; *S. somalense*; *Anthadenia sesamoides* Van Houtte; *Dysosmon amoenum* Rafinesque; *Volkameria sesamoides* O. Kuntze.) ***S. alatum* Thonn.** (*S. ekambaramii* Naidu; *S. gracile* Endl; *S. pterospermum* R. Br.; *S. rostratum* Hochst; *S. sabulosum* A.Chevalier) ***S. capense* Burm.f.** (*S. gibbosum* Brem. & Oberm.; *S. grandiflorum* Schinz; *S. pentaphyllum* E. Mey.; *S. schenckii*) ***S. malabaricum* Burm.** (*S. mulayanum* Nair)	***S. angolense* Welw** (*S. macranthum* Oliver) ***S. angustifolium* Engl.** (*S. calycinum* ssp. *angustifolium,* *S. indicum* var. *angustifolium*) ***S. laciniatum* Willd.** ***S. latifolium* Gillett** ***S. prostratum* Retz.**	***S. radiatum* Schum. & Thonn.** (*S. biapiculatum* de Wild.; *S. caillei* A. Chev.; *S. foetidum* Afzel; *S. mombazense* De Wild. & Th. Dur.; *S. occidentale* Heer & Regel; *S. talbotii* Wernham; *S. thonneri*)	*S. abbreviatum* Merxm. *S. calycinum* Welw. ssp. *calycinum* *S. calycinum* ssp. *baumii* (Stapf.) Seidenst. Ex Ihlenf. *S. calycinum* ssp. *pseudoangolense* Seidenst. Ex Ihlenf. *S. capense* Burm. f. ssp. *lepidotum* Schinz. *S. marlothii* Engl. *S. parviflorum* Grabow-Seidensticker *S. pedalioides* Hiern *S. rigidum* Peyr. ssp. *rigidum.* *S. rigidum* ssp. *merenksyanum* Ihlenf. & Seidenst. *S. schinzianum* Aschers. ex Schinz *S. triphyllum* Welw ex-Aschers. *S. triphyllum* Welw.ex Asch. var. *grandiflorum* (Schinz) Merxm.

Species (Bold); Synonym in parenthesis (unbold)
Source: IPGRI and NBPGR (2004), Descriptors for Sesame (*Sesamum* spp.), IPGRI, Rome; NBPGR, New Delhi

ambiguities in sesame species, more detailed studies are required to distinguish them on the basis of cytogenetic and molecular characteristics.

16.2 Wild Species as Resources for Stress Resistance

The wild species of sesame are known to exhibit tolerance and resistance to different pests and diseases as they can grow under adverse conditions (Table 16.2). Several wild *Sesamum* species have proved resistant to biotic and abiotic stress (Joshi 1961; Nayar and Mehra 1970; Weiss 1971; Brar and Ahuja 1979; Mazzani 1983; Kolte 1985; Prabakaran and Rangasamy 1995). Uzo (1985) reported that *S. angustifolium* is the most drought resistant species as it shows a high percentage of fruit set (89%) during the dry season. Some of the adaptive features, for example, in wild species are fleshy roots, small linear leaves, a large number of stomata located at the adaxial surface, hairiness, and increased fruit set in the dry season, when the cultivated sesame in the vicinity had dried out. Species *agustifolium* with gray seed is reported to be a drought resistant donor and species *radiatum,* a foliar disease resistant source to improve commercial sesame.

According to Srinivasulu (1991), *S. alatum* is immune to phyllody, and two other species – *S. laciniatum* and *S. radiatum* – were resistant to *Antigastra catalaunalis* (shoot webber). The species *S. malabaricum* has the unique character of withstanding heavy rainfall when compared with cultivated sesame (John et al. 1950). *S. prostratum* was also observed to be resistant to many pests and diseases (Ramanujam 1942). This species is drought resistant and suitable for sandy tracts. *S. laciniatum* Klein ($2n = 32$) is resistant to drought and also to *Antigastra*.

Thangavelu (1994) reported shoot webber resistance in *S. malabaricum*, *S. alatum*, *S. laciniatum*, and *S. radiatum*; powdery mildew resistance in

Table 16.2 *Sesamum* species as sources of resistance to biotic and abiotic stresses

Sesamum species	Chromosome number (2*n*)	Brief morphological description	Desirable attributes
S. indicum	26	Annual, erect, moderately branching, white flowers, anthers yellowish white, yellow glands present, medium long cylindrical capsules, smooth seeds with thin testa.	High quality oil and seed
S. alatum	26	Annual, erect, highly branching, linear, trim, penta- lobed entire leaves, dark purple flowers, anthers purple, purple glands present, deeply grooved cylindrical capsules, winged seeds with thick testa.	Resistant to phyllody and *antigastra*
S. malabaricum	26	Annual, erect, profusely branching, heteromorphic linear to three lobed entire leathery leaves, purple flower with dark purple lip, anthers yellowish white, prominent yellow glands, long cylindrical capsules, rough seeds with thick testa.	Resistant to water logging, donor for cytoplasmic male sterility
S. mulayanum	26	Annual, erect, profusely branching, purple flower with dark purple lip, anthers yellowish white, prominent yellow glands, long cylindrical capsules, rough seeds with thick testa.	Resistant to fusarium wilt and gall fly
S. prostratum	32	Perennial, prostrate, profusely branching, coarse leathery leaves with serrated margin, dark purple flowers with purple anthers, yellow glands absent, medium laterally compressed tough capsules seeds with thick testa.	Resistant to *antigastra,* and salinity. Non-shattering capsules
S. laciniatum	32	Perennial, prostrate, profusely branching, deeply dissected coarse leaves, deep purple flowers with purple anthers, yellow glands absent, small laterally compressed tough capsules, deeply reticulate seeds with thick testa.	Resistant to a*ntigastra* and drought. Non-shattering capsules
S. occidentale	64	Annual, erect, profusely branching, coarse linear entire leaves, purple flowers with light purple anthers, yellow glands present, long cylindrical capsules, rough seeds with thick testa.	Resistant to drought
S. radiatum	64	Annual, erect, moderately branching, coarse broader leaves, purple flowers and anthers, yellow glands present, long laterally compressed capsules, rough seeds with thick testa.	Resistant to drought

S. malabaricum, *S. alatum*, and *S. occidentale*; and phyllody resistance (mycoplasma disease) in *S. alatum*. Mehetre et al. (1993) reported phyllody and wilt resistance in *S. mulayanum* and *S. prostratum*. Lee et al. (1991) reported that species *S. alatum* and *S. radiatum* possessed resistance to Phytophthora blight, Fusarium wilt, leaf blight, and seedling blight.

16.3 Interspecific Hybridization

Sesame has the advantage of epipetalous flowers, and thus, emasculation and pollination are easy. A high number of seeds (40–50) are produced per flower. In addition, low seed rate (2.0–2.5 kg/ha) and high seed multiplication ratio (1:300) facilitate ease of hybridization and recombination breeding programs. Insects favor cross-pollination in sesame, facilitating natural hybridization (Duhoon 2004). Hence, incorporation of desirable genes from wild to cultivars was attempted by several researchers, using controlled crossing. The details of interspecific crosses attempted among various species are furnished in Table 16.3. Close affinity of compatibility or incompatibility among the interspecific crosses is well established (Table 16.4).

On the basis of the information of chromosome number in various species of *Sesamum*, three broad groups could be recognized, as given below.

Group I	$n = 13$	*S. indicum*, *S. alatum*, *S. capense*, *S. malabaricum*, *S. mulayanum*
Group II	$n = 16$	*S. prostratum*, *S. laciniatum*, *S. angolense*, *S. angustifolium*
Group III	$n = 32$	*S. radiatum*, *S. occidentale*

Table 16.3 Interspecific crosses of *Sesamum* species

Details of intraploidy crosses			
Between 2*n*:26 chromosome species			
S. indicum	*S. alatum*	Capsules developed but seeds non-viable Normal fruit set, F_1 viable	Joshi (1961), Sundaram (1968), Subramanian (1972), Ramalingam et al. (1992)
S. alatum	*S. indicum*	Capsule set normal-seeds, non-viable Viable seeds through embryo rescue	Joshi (1961), Sundaram (1968), Ramalingam et al. (1992), Rajeswari et al. (2010)
S. indicum	*S. malabaricum*	Fertile hybrids	John et al. (1950), Nimmakayala (1997)
S. malabaricum	*S. indicum*	F_1 sterile, cytoplasmic male sterile source	Prabakaran (1996), Nimmakayala (1997), Kavitha (1998)
S. indicum	*S. capense*	Fruit set normal; seeds non-viable	Sundaram (1968), Nayar and Mehra (1970)
S. indicum	*S. mulayanam*	F_1 hybrids viable, and resembling wild parent	Biswas and Mitra (1990), Nimmakayala (1997)
Between 2*n*:32 chromosome species			
S. laciniatum	*S. prostratum*	F_1 fertile and F_2 segregation normal	Joshi (1961), Nayar and Mehra (1970), Kobayashi (1991)
S. angolense.	*S. latifolum*	F_1 sterile	Nagamura and Sato (1958)
S. prostratum	*S. latifolum*	F_1 sterile	Joshi (1961)
Between 2*n*:64 chromosome species			
S. radiatum *S. occidentale*	*S. occidentale* *S. radiatum*	Good capsule and seed set; F_1 meiosis normal and F_2 segregation normal with high fertility	Ramanathan (1950), Nayar and Mehra (1970), Subramanian (1975)
Details of interploidy crosses			
Between 2*n*:26 and 2*n*:32 chromosome species			
S. indicum	*S. prostratum*	Viable seeds – F_1 sterile – Amphidiploid fertile	Ramanathan (1950), Kedharnath et al. (1959), Sundaram (1968)
S. indicum	*S. laciniatum*	Viable seeds – F_1 sterile – Amphidiploid fertile	Kedharnath et al. (1959), Aiyadurai et al. (1963), Subramanian and Chandrasekaran (1977)
S. laciniatum	*S. indicum*	No seed set	Biswas and Mitra (1990), Kirija (1992)
Between 2*n*:26 and 2*n*:64 chromosome species			
S. indicum	*S. occidentale*	Rarely developed capsules had shriveled and non-viable seeds	Ramanathan (1950), Devaratnam (1965), Sundaram (1968), Subramanian (1972)
S. indicum	*S. radiatum*	Capsules set; but no seeds	Garu (1934), Dhawan (1946), Mazzani (1952), Subramanian (1972)
Between 2*n*:32 and 2*n*:64 chromosome species			
S. occidentale	*S. laciniatum*	Non-viable seeds	Ramanathan (1950), Subramanian (1972)
S. radiatum	*S. laciniatum*	Non-viable and shriveled seeds	Nayar and Mehra (1970), Subramanian (1972)
S. radiatum	*S. prostratum*	Non-viable seed	Nayar and Mehra (1970)
S. occidentale	*S. prostratum*	Non-viable seeds	Ramanathan (1950), Dadlani (1956), Joshi (1961)
S. radiatum	*S. angolense*	Viable seeds and hybrids with 2*n*:48 and 64	Nagamura and Sato (1958)
Between 2*n*:58 and other species			
S. indicatum (2*n*:58)	*S. indicum* (2*n*:26)	Good fruit set with 2.9% viable seeds	Kedharnath (1954)
S. indicatum (2*n*:58)	*S. prostratum* (2*n*:58)	Good fruit set with 1.0% viable seeds	Kedharnath (1954)
S. lacinientale (2*n*:58)	*S. radiatum* (2*n*:64)	Shriveled, non-viable seeds	Subramanian (1972)
S. indicatum (2*n*:58)	*S. lacinientale* (2*n*:58)	F_1 fertile, non-viable seeds	Mohammed and Dorairaj (1968)

Modified from Subramanian (2003)

Table 16.4 Cross compatibility of sesame species collected in India

Male parent								
Female parent	*S. indicum*	*S. alatum*	*S. malabaricum*	*S. mulayanum*	*S. prostratum*	*S. laciniatum*	*S. occidentale*	*S. radiatum*
S. indicum	Selfed	NS	H	H	H	H	NS	NS
S. alatum	NS	Selfed	NS	NS	NS	NS	NS	NS
S. malabaricum	H	NS	Selfed	H	H	H	NS	NS
S. mulayanum	NS	NS	H	Selfed	–	–	NS	NS
S. prostratum	H(S)*	NS	H	–	Selfed	H	NS	NS
S. laciniatum	H(S)*	NS	H	–	H	Selfed	NS	NS
S. occidentale	NS	NS	NS	NS	NS	NS	Selfed	H
S. radiatum	NS	NS	NS	NS	NS	NS	H	Selfed

Modified from Nimmakayala (1997)
H viable hybrids, *NS* no seed set, *H(S)** sterile hybrids, – no reports available

16.3.1 Intraploidy Compatibility Relationship

16.3.1.1 Crosses Among the Species with 2*n* = 26

Kedharnath (1954) attempted crosses between the species *indicum* and *alatum* and found that when *S. indicum* was used as the pistillate parent, the fruit set was normal, but the seeds were shriveled and non-viable. He noted the early abortion of young embryos. In reciprocal cross, with *S. alatum* as pistillate parent, very few fruits were set with normal looking seeds, and these were found to be non-viable. These results were confirmed by Sundaram (1968) and Subramanian (1972). Earlier attempts to transfer phyllody resistance from wild to cultivated sesame were not successful (Subramanian 1972). Ramalingam et al. (1992) was the first to report a successful hybrid between *S. alatum* and *S. indicum*. This study reported that out of the 1,102 flowers that were pollinated, capsule set was only 0.04% and only one crossed seed germinated to produce a single F_1 plant that was morphologically distinct from either of the parents. Ram et al. (2006) reported crossability barriers at the level of pollen–pistil interactions in the cross of *S. alatum* × *S. indicum*. Rajeswari et al. (2010) have optimized a simple and efficient protocol for producing an interspecific hybrid between *S. alatum* and *S. indicum* through ovule culture. In this cross, capsule retention without embryo abortion was extended up to 7 days after pollination by spraying a mixture of growth regulators. Phenotypically, the *S. alatum* × *S. indicum* hybrid plants were intermediate to parents for the majority of traits. Cytological studies revealed normal meiosis in the hybrid without any chromosomal abnormalities. Screening against phyllody disease under greenhouse conditions revealed that the hybrids were moderately resistant.

Hybrids of *S. indicum* and *S. capense* yielded a few seeds, but they were non-viable (Kobayashi 1949). Devaratnam (1965) and Sundaram (1968) also reported shriveled and non-viable seeds when these two species were crossed.

S. indicum and *S. malabaricum* were cross-compatible (Table 16.4), and their F_1 hybrids had normal meiosis and were fertile (Kobayashi 1991; Nayar 1995; Nimmakayala 1997). In the crosses of *S. malabaricum* as female and *S. indicum* as male parent, the F_1s were male sterile (Prabakaran 1996; Nimmakayala 1997) and their reciprocal hybrids were found to be fully fertile, indicating nuclear–cytoplasmic interactions controlling fertility. Kavitha (1998) developed stable male sterile plants by backcrossing F_1 hybrids of *S. malabaricum* and *S. indicum*. The sesame genotypes Si 1525 and SVPRI restored pollen fertility in F_1s, and a dominant gene was found to control the fertility restoration. Wide hybridization was thus likely to result in the development of cytoplasmic– genetic sterile lines, which can be useful for exploitation of hybrid vigor.

Crosses of *S. indicum* and *S. mulayanum* were successful, with partly fertile hybrids, whereas the reciprocal crosses set no seeds (Biswas and Mitra 1990). Nimmakayala (1997) made crosses involving four species (*S. malabaricum*, *S. mulayanum*, *S. alatum*, and *S. indicum*) to develop male sterile lines and to transfer pest and disease resistant genes from wild to cultivated types (Fig. 16.1). The crosses that involved *S. alatum* did not set capsules. The capsule setting percentage ranged from 38 to 51% in crosses involving *S. malabaricum* as male or female parent, and fruit set was

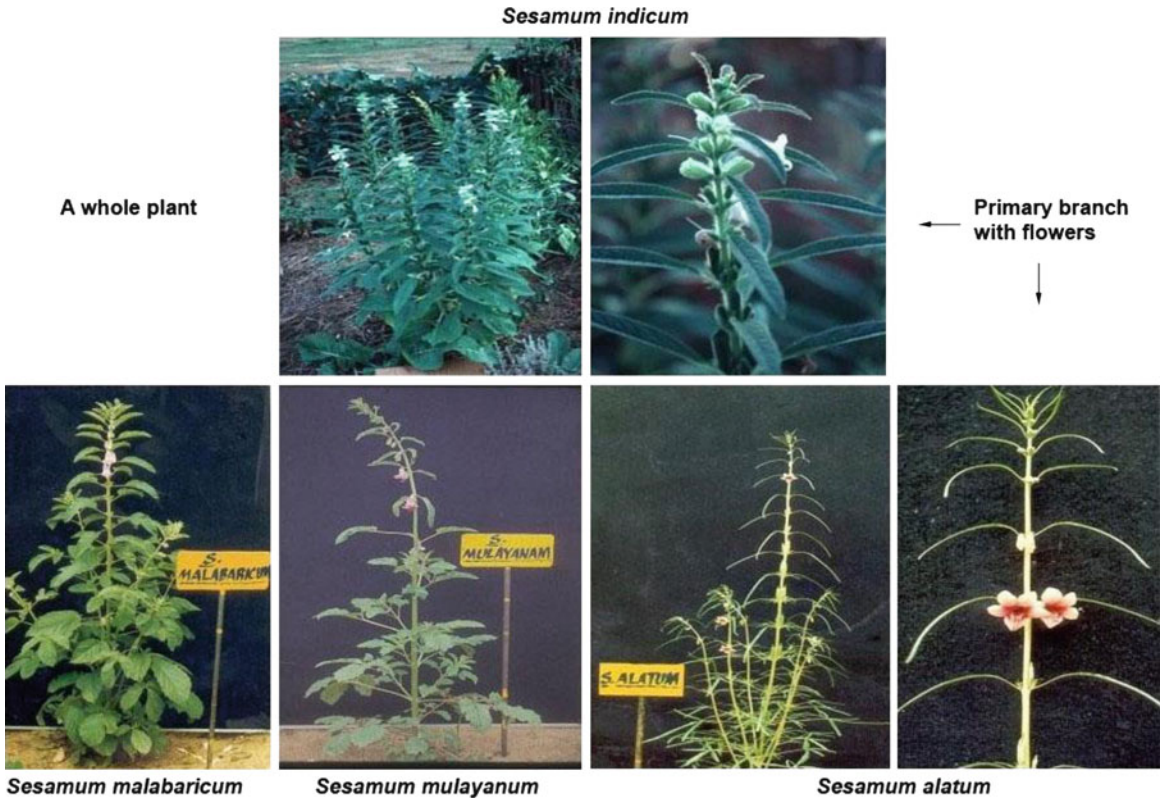

Fig. 16.1 Sesame species (Group I) having $2n = 26$

very low in *S. mulayanum* × *S. indicum* crosses. In addition, *S. mulayanum*, which had earlier been reported to be resistant to phyllody, powdery mildew, and *Antigastra*, was found to be susceptible under field conditions (Fig. 16.2). Segregation data pertaining to various morphological characters (purple corolla and size of capsules) were found to be under the control of dominant genes. The pollen sterility ranged from 79 to 94% and 53 to 88% in the F_1s of *S. malabaricum* × *S. indicum* and *S. indicum* × *S. mulayanum* crosses, respectively. The F_1s of *S. malabaricum* × *S. indicum* that showed more than 90% pollen sterility were used for backcrossing with *S. indicum* parent to develop cultivated male sterile lines.

16.3.1.2 Crosses Among the Species with 2*n* = 32

Crosses between *S. laciniatum* (Fig. 16.3) and *S. prostratum* were made by Ramanathan (1950), and he noticed good fruit and seed set. Kedharnath (1954) made both direct and reciprocal crosses and found that the characters of *S. prostratum* were dominant in the F_1s. The hybrids were fully fertile and had good seed set. In the F_2 simple Mendelian segregation was observed for two traits: lobed leaves and fruit shape. Fertile F_1 hybrids were also reported between these two species by Joshi (1961), Nayar and Mehra (1970), and Kobayashi (1991). In crosses of *S. angolense* × *S. prostratum* and *S. angolense* × *S. laciniatum*, capsule setting was very low, with shriveled and non-viable seeds. No fruit set was reported in their reciprocal crosses (Joshi 1961).

16.3.1.3 Crosses Among the Species with 2*n* = 64

In this group (Fig. 16.4), fertile reciprocal crosses yielded good viable seeds, and the F_1 hybrids appeared similar to either of the parents (Kedharnath 1954; Joshi 1961; Subramanian 1972). In a study involving the progenies of *S. radiatum* × *S. occidentale*, normal

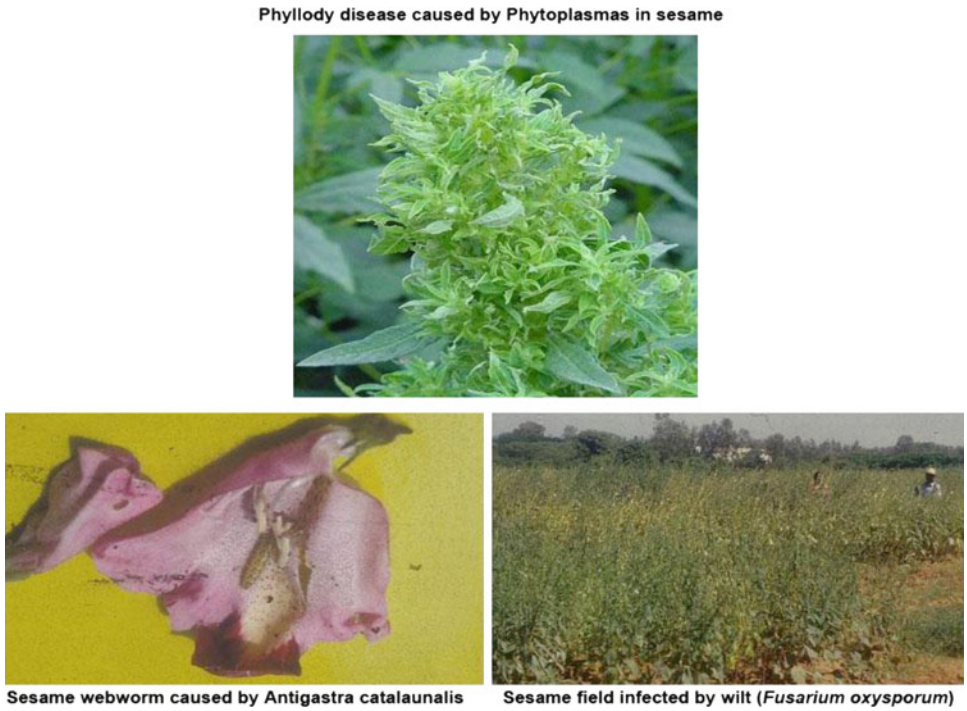

Fig. 16.2 Major pest and diseases of Sesame

Fig. 16.3 Sesame species (Group II) having $2n = 32$

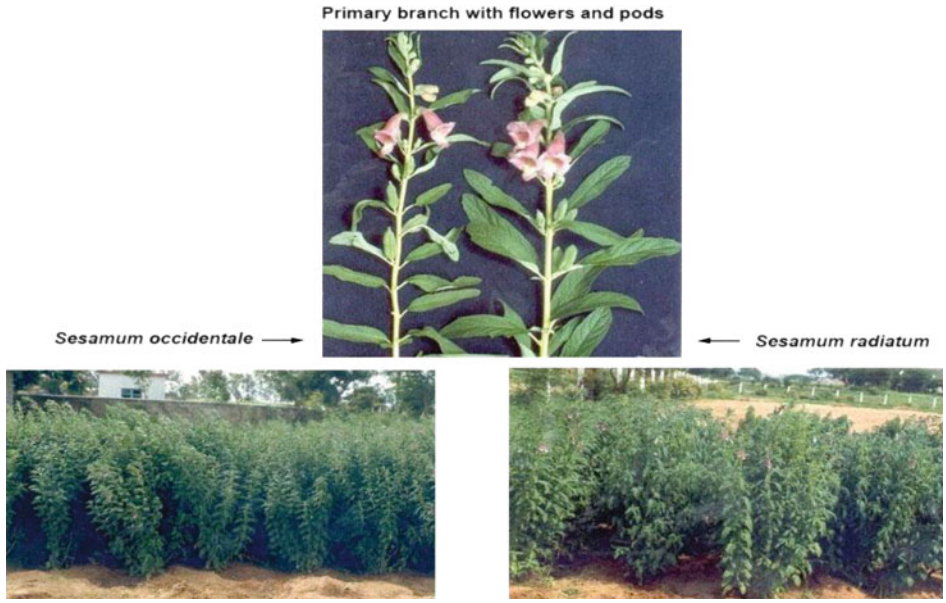

Fig. 16.4 Sesame species (Group III) having $2n = 64$

fruit and seed set were observed, with regular meiosis (Subramanian 1975).

16.3.2 Interploidy Compatibility Relationship

The main interest in attempting wide crosses among *Sesamum* species by researchers across the world is the introgression of desirable genes from various wild species into the cultivated species. So far, there have not been any success stories reporting desirable gene transfer from wild species to the cultivated one.

16.3.2.1 Crosses Between 2*n* = 26 and 2*n* = 32 Species

Reciprocal crosses involving *S. indicum* and *S. prostratum* produced good seed set and the F_1s were vigorous, semi-spreading, profusely branching and flowering, but completely sterile (Ramanujam 1942; Kedharnath 1954). The sterile hybrids ($2n = 29$) were doubled using colchicine, and the amphidiploids were named *S. indicatum* by Ramanujam (1944). The amphidiploid ($2n = 58$) not being of any use, backcrosses were made with both the parents (Kedharnath 1954).

Both direct and reciprocal crosses between *S. indicum* and *S. laciniatum* were successful, with good viable seed set (Aiyadurai et al. 1962; Subramanian 1972). The hybrids had $2n = 29$ chromosomes and were found to be sterile, exhibiting intermediate features between parents. The amphidiploids ($2n = 58$) were obtained by colchicine treatment and turned into highly fertile plants; the amphidiploid was named *S. lacinientale* (Ramanathan 1950). Backcrosses were made to both the parents (Sundaram 1968; Subramanian 1972), and the resulting seeds appeared shriveled and non-viable. Crossing of *S. lacinientale* with *S. alatum* also resulted in ill-developed capsules with no seed set (Subramanian 1972).

16.3.2.2 Crosses Between 2*n* = 26 and 2*n* = 64 Species

Garu (1934) and Dhawan (1946) reported shriveled and non-viable seeds when *S. indicum* was crossed with *S. radiatum*, caused by early collapse of hybrid endosperm and subsequent starvation of the proembryo. Lee et al. (1991) tried embryo rescue and obtained 6 F_1 hybrids out of 1,630 cultured embryos, while none survived in the reciprocal hybrid embryos. However, reciprocal crosses involving these produced some viable seeds (Nayar and Mehra 1970; Lee et al. 1991).

The crosses between *S. indicum* and *S. occidentale* were reported to be successful (Ramanathan 1950), but the reciprocal crosses produced shriveled and non-viable seeds (Subramanian 1972).

16.3.2.3 Crosses Between 2*n* = 32 and 2*n* = 64 Species

Ramanathan (1950) and Subramanian (1972) obtained only shriveled and non-viable seeds in a cross with *S. occidentale* as female parent and *S. laciniatum* as male parent, whereas in the reciprocal cross, there were no capsules formed. Crosses between *S. occidentale* and *S. prostratum* did not produce any viable seeds.

16.3.2.4 Crosses Between 2*n* = 58 and Other Species

The cross between *S. indicatum* ($2n = 58$) × *S. indicum* ($2n = 26$) resulted in good fruit set, but only 2.9% of seeds were viable (Kedharnath 1954). Seedlings raised from these viable seeds were sesquidiploids ($2n = 45$) or di-*indicum*-mono-*prostratum* ($2n = 42$). By backcrossing the amphidiploids *S. indicatum* ($2n = 58$) to *S. prostratum* ($2n = 32$), using the latter as the pollen parent, good fruit set was noted. However, only 1% of these seeds were viable, and the resulting plants were sesquidiploids ($2n = 45$) with two sets of *S. prostratum* chromosomes and one set of *S. indicum* chromosomes. Subramanian (1972) made crosses between the amphidiploid *S. lacinientale* with *S. radiatum* and *S. occidentale*, but the seeds produced were empty. The two synthesized amphidiploids *S. lacinientale* ($2n = 58$) and *S. indicatum* ($2n = 58$) were hybridized and F_1, F_2, F_3, and F_4 were studied; their variances for different characters have been reported (Subramanian 2003). When the di-*indicum* and mono-*laciniatum* plants identified in C_5 progenies of *S. lacinientale* ($2n = 58$) were backcrossed with *S. indicum*, plants with $2n = 26$ and 34 were obtained (Kavitha 1995). Plants with $2n = 34$ were again crossed with *S. indicum* ($2n = 26$), and plants with $2n = 26$ and 38 chromosomes were segregated (Jaisankar 1996). When the seeds of plants with $2n = 38$ chromosomes were raised, they produced sterile plants with $2n = 40$, which indicated the transgression of genes between *S. indicum* and *S. laciniatum* (Bindu 1997).

16.4 Intergeneric Hybridization

Apart from interspecific crosses, a good number of intergeneric crosses (Table 16.5) have also been attempted to assess the relationship among the genera *Sesamum*, *Martynia*, and *Ceratotheca*. Kedharnath (1954) made crosses between *Ceratotheca sesamoides* and various species of *Sesamum*. No viable seeds were obtained in any of the crosses, except *S. indicatum* ($2n = 58$, a synthetic amphidiploid) × *C. sesamoides* ($2n = 32$). About 28 seedlings with $2n = 45$ were produced. The plants were semi-erect and appeared intermediate in features when compared with their parents, but were found to be completely sterile. It is also interesting that the primary species *S. indicum* and *S. prostratum* did not produce viable seeds in crosses with *C. sesamoides*; however, the amphidiploid of *S. indicum* × *S. prostratum* could produce some viable seeds when crossed with *C. sesamoides*. Successful crosses between *S. indicum* and *C. sesamoides*, however, were reported by Falusi et al. (2002). These crosses were made to study the inheritance of hairiness

Table 16.5 Intergeneric crosses in *Sesamum*

Between *Sesamum* and *Martynia*			
S. indicum (2*n*:26)	*Martynia diandra* (2*n*:32)	Developed capsules but seeds are non-viable	Srinivasan (1942)
S. laciniatum (2*n*:32)	*Martynia annua* (2*n*:32)	No capsules setting Flowers dropped after 4 days of hybridization	Subramanian (1995)
S. radiatum (2*n*:64)	*Martynia annua* (2*n*:32)	No capsules setting Flowers dropped after 4 days of hybridization	Subramanian (1995)
Between *Sesamum* and *Ceratotheca* species			
S. indicatum (2*n*:58)	*Ceratotheca* Sesamoides (2*n*:32)	F_1 mostly sterile with 1–3 IIIs	Kedharnath (1954)
S. indicum (2*n*:26)	*Ceratotheca* Sesamoides (2*n*:32)	F_1 fertile Evaluated F_2 and backcross progeny	Falusi et al. (2002)

on stem and petiole. All the F_1 plants had medium sized hairs on their stem and petiole. The F_2 and backcross data indicated that the inheritance of many hairs on stem and petiole was controlled by two independently assorting dominant alleles. The possibility of gene exchange between these two genera indicated that they are closely related.

Martynia, though grouped under a different family, is very closely related to Pedaliaceae, and successful crosses could be made between *Sesamum* and *Martynia*. Richharia (1937) observed that pollen grains of *Martynia diandra* (= *Martynia annua* Linn.) had good germination in the styles of *S. indicum*. Srinivasan (1942) obtained non-viable seeds in the cross between *S. indicum* × *M. diandra*. Subramanian (1995) reported that the pollen grains of selfed plants of *S. indicum* entered the ovary after 6 hrs of pollination, and in the intergeneric crosses, the pollen grains of *M. annua* when pollinated on the stigmatic surface of *S. laciniatum* ($2n = 32$), *S. radiatum* ($2n = 64$), and *S. occidentale* ($2n = 64$) individually, the pollen germinated without inhibition and grew in the styles and entered the ovary. But the pollinated flowers dropped after 4 or 5 days of hybridization in all the crosses.

16.5 Molecular Genetics and Breeding

Information on the use of molecular markers for the characterization of genetic diversity in cultivated and wild *Sesamum* species is limited. Isshiki and Umezake (1997) reported isozyme studies in 41 accessions of cultivated sesame from three countries, viz., Japan, Korea, and Thailand. Nanthakumar et al. (2000) studied the genetic relationships among seven cultivated and wild *Sesamum* species, using isozymes and random amplified polymorphic DNA (RAPD) markers. Bhat et al. (1999) used the RAPD technique to determine genetic diversity among Indian sesame germplasm. RAPD markers were used to determine the extent and distribution of genetic diversity in 38 populations of sesame collected from four regions of Turkey (Ercan et al. 2004). Kim et al. (2002) used intersimple sequence repeat (ISSR) marker assays for characterization of the genetic diversity in the collection of Korean and worldwide sesame accessions. The ISSR method enabled separation between genetically diverse accessions based on historical–geographical origin. The results of this study were in agreement with the other studies that showed that geographical separation did not result in creating wider genetic distance. This is because sesame was introduced to many countries and materials from widely separate locations were exchanged for use in breeding.

A microsatellite enriched library was developed for capturing repeat motifs that contained genomic fragments in sesame (Nimmakayala et al. 2005). These microsatellite markers were useful to assess the sesame molecular diversity and/or to integrate with the other markers that were used to make a genetic map of sesame.

A set of 124 genotypes collected from different parts of the world were tested for polymorphisms, using 14 microsatellite primers and 10 amplified fragment length polymorphism (AFLP) primer combinations. A total of 168 alleles could be scored from SSR data obtained using the LICOR genotyper. The largest number of alleles (26) was amplified by SSR primer PRU 26 that has a repeat motif of $(CA)_{16}$ and the least by PRU4 with a repeat motif of $(CA)_6T(CA)_5$ that amplified only three alleles. The AFLP analysis that used ten primer combinations resulted in more than 2,896 polymorphisms, which resolved genome wide diversity among 124 genotypes.

16.6 Future Prospects and Research Priorities

Genetic diversity from the available and unexploited wild sesame species are rich resources for many desirable traits, such as erect branching, linear/deeply palmate/lanceolate leaves, number of capsules per axil, capsule size, number of seeds per capsule, days to maturity, determinate habit, resistance to pests, in particular to *Antigastra,* and the other diseases (powdered mildew, wilt complex, bacterial blight), wider adaptability, cytoplasmic–genic male sterility, and tolerance to heavy rainfall. Moderate- to high-level of resistance may be available in various wild species, but it has not yet been exploited. In addition, the cytogenetics of the genus and the interspecific relations at the molecular level are poorly known. Phyllody, caused by a mycoplasma-like organism (MLO), which is transmitted by leafhoppers (*Orosius albicinctus* Distant), induces vegetative proliferation of the

flower buds and can be most destructive. A breakthrough in breeding for resistance to phyllody will have a very significant effect on sesame yields, especially in Southeast Asia. Screening is very difficult because it depends on field conditions, vector population build-up, and MLO-infestation levels. The development of reliable, reproducible screening methods for the key diseases and pests as well as rapid molecular disease identification, are urgently required. Priority objectives of research should be the morphological, cytological, and molecular characterization of wild species. Though the wild species are the potential sources for major pest and disease resistance genes, transfer of these into cultivated species has not been accomplished over the past 50 years due to several reasons, as discussed above. With present day molecular and genomic tools, the identification and transfer of desirable genes from wild to cultivated species would be much faster and less cumbersome.

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Chapter 17
Sinapis

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17.1 Introduction

The genus *Sinapis* belongs – together with *Brassica*, *Coincya*, *Diplotaxis*, *Eruca*, *Erucastrum*, *Raphanus*, *Sinapidendron*, and *Trachystoma* – to the core genera of the subtribe Brassicinae within the tribe Brassiceae (Schulz 1919, 1923, 1936; Janchen 1942; Warwick et al. 2009).

This classical subdivision of the tribe Brassicae, one of the 13–19 tribes of the Brassicaceae family, based somewhat arbitrarily on morphological characters, is considered by many taxonomists to be highly artificial. The same applies to the generic circumscriptions within the subtribe Brassicinae. One of the best examples for the need to reevaluate relationships is the polyphyletic genus *Sinapis*. Here, especially the close relationship between *Sinapis arvensis* L. (in contrast with other *Sinapis* species) and *Brassica nigra* (L.) Koch (genome BB) is claimed by several authors (Song et al. 1988; Warwick and Black 1991, 1997; Tsukamoto et al. 1993; Kapila et al. 1996; Oshima 2000; Warwick and Sauder 2005; Agerbirk et al. 2008). However, this statement is not supported by data from Flannery et al. (2006) and simple sequence repeat (SSR) analysis of plastid DNA. The latter study, including *S. alba* and *S. arvensis*, provides evidence for a clear separation of the genus *Sinapis* from *Brassica*.

Taxonomic confusion with extensive synonymy and species subdivisions (subsp./var.), which can be found throughout the Brassiceae, is also typical for the genus *Sinapis*. The polyphyletic nature of *Sinapis* was corrected, in part, by the transfer of *S. aucheri*, a member of the *Rapa*/*Oleracea* phylogenetic lineage to *Brassica* (Al-Shehbaz and Warwick 1997; Warwick and Hall 2009). According to Warwick et al. (2009), it comprises four annual species, all belonging to the *Nigra* lineage: *Sinapis alba* L., *S. arvensis*, *S. flexuosa* Poir., and *S. pubescens* L. Among them, *S. alba* and *S. arvensis* are by far the most important breeding resources. That is why the special focus of this chapter is on these two species. Recent changes like the placement of the genus *Hirschfeldia* in *Sinapis* and other genera (Warwick et al. 2006) are beyond the scope of this chapter.

If not stated otherwise the information given in this chapter refers to Warwick et al. (2009), an outstanding collection of data available for wild crucifers.

17.2 Basic Botany of the Species

White mustard (also known as "yellow mustard"), ***S. alba*** L., Sp. Pl. 2: 668. (1753), is a plant of the Mediterranean (Irano-Turanian, Euro-Siberian) floral region (phytogeographical zone). The species probably originates from the Mediterranean region and can nowadays be found cosmopolitically (not described for Australia) on coastal plains and hills on chalk, gypsum slopes, open woodlands, brush, alluvium, and damp steep rock faces. The populations of *S. alba* comprise wild and cultivated genotypes and weedy escapes. Weedy *S. alba* occurs on roadsides and waste places and also in fields, vineyards, and olive groves. Calcareous and nitrous soils are preferred.

In general, *S. alba* is more limited in distribution than *S. arvensis* as described by Daniels et al. (2005)

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for Great Britain. It is most common in southern England. However, at sites where it is present it can form large populations.

Synonyms for *S. alba* are *Brassica alba* Boiss., *B. hirta* Moench, *Sinapis dissecta* Lag., and *S. mairei* H. Lindb. According to Warwick et al. (2009), *S. alba* can be divided into three subspecies: subsp. *alba* L., subsp. *dissecta* (Lag.) Simonk., and subsp. *mairei* (H. Lindb.) Maire.

The pods, which contain four to eight yellowish seeds (Rothmaler 1988), or at least the beaks reveal white bristly trichomes. The seedless beak is flattened, curved, and equalling to or exceeding the length of the valves (Mulligan undated). Hairs can also be found on cotyledons (lamina and petioles). Very dense hairs occur on juvenile leaves (see survey of tribes in Gómez-Campo 1980).

The diploid chromosome number is $2n = 24$ (Karpetchenko 1924). Fluorescence in situ hybridization (FISH) performed by Schrader et al. (2000) showed that the chromosomes of *S. alba* carry four 5S and six 25S rRNA gene sites. In agreement with similar findings for *Raphanus sativus*, it has one chromosome pair with both rRNA genes; the two are closely located on a short arm. These results were confirmed in FISH analyses by Ali et al. (2005). These authors found four (terminal/subterminal) chromosomes carrying loci for 5S rDNA. Six chromosomes showed 18S–5.8S–25S (45S) rRNA genes. Ali et al. (2005) identified two of them harboring both, 5S and 45S rDNA, on opposite chromosome arms.

A few genome size data are available for *S. alba* (genome abbreviation: SS, according to Wei et al. 2007). Arumuganathan and Earle (1991) report a nuclear DNA content of 1.02 pg/2C (N) and 492 Mbp/1C for *S. alba*. Moreover, a more recent paper claims 0.57 pg/2C (N) and 553 Mbp/1C, respectively (Johnston et al. 2005).

Wild *S. alba* accessions usually reveal high levels of erucic acid (>45–50%; Yaniv et al. 1994, 1995). However, over the past decade, white mustard has become an oil crop species in Canada. Breeding for double low (low content of erucic acid and glucosinolates) or even canola quality is an important goal (cf. Brown et al. 1999; Rakow 2002; Rakow and Raney 2003; Pietka et al. 2007). With improved quality it also might become an alternative to spring oilseed rape, especially under drought conditions. Furthermore, white mustard is widely used as a source of mustard condiments along with *Brassica* species like *B. juncea* and *B. nigra*.

Wild mustard (charlock, field mustard), ***S. arvensis*** L., Sp. Pl. 2: 668. (1753) [syn. *Brassica arvensis* (L.) Rabenh., *B. sinapistrum* Boiss., *B. kaber* (DC.) L.C. Wheeler, *B. xinjiangensis* Y.C. Lan and T.Y. Cheo, *Raphanus turgidus* Pers., *Sinapis allionii*, *S. turgida* (Pers.) Delile, *S. schkuhriana* Rchb: (cf. Warwick et al. 2009); $2n = 18$ (Karpetchenko 1924); genome SarSar (cf. Snowdon et al. 2000; Winter 2004)], is an annual and cosmopolitically distributed wild crucifer belonging to the Mediterranean, Irano-Turanian, and Saharo-Sindian (questionable: Euro-Sibirian, American) floral regions (Warwick et al. 2009). It was found to have occurred in the northeastern United States as early as 8000 BP and was abundant and widespread by 2000 BP (Warwick et al. 2009). *S. arvensis* occurs along coasts and plains (montane to 1,800 m). It prefers loamy fields rich in nutrients and moderately dry to fresh ruderal places (Rothmaler 1988). Moreover, the species can be found in dry stream beds, roadsides, oases, and mainly on calcareous soils. The seeds of this old medicinal plant contain glucosinolates and are rich in erucic acid (Bettach et al. 1996; Daun et al. 2003).

Daniels et al. (2005) describe *S. arvensis* for Great Britain as a widespread and abundant arable weed. It is the most common of the oilseed rape relatives found in and around arable fields. According to Warwick et al. (2009), two subspecies of *S. arvensis* can be differentiated: subsp. *allionii* (Jacq.) Baillarg and subsp. *arvensis* L.

The pods of this species contain 8–13 black seeds (Rothmaler 1988), and are glabrous or with bristly trichomes. The glabrous beaks are long conical, straight, slightly winged, one-seeded, and considerably shorter than the length of valves (Mulligan undated).

Arumuganathan and Earle (1991) report a nuclear DNA content of 0.76 pg/2C (N) and 367 Mbp/1C for *S. arvensis*.

S. flexuosa Poir., Lam., Encycl. Méth. Bot. 4: 341. (1797), is an annual wild species belonging to the Mediterranean floral region. It has a limited geographical distribution with reports only for southern Spain, the Canary Islands (Tenerife, Gomera), and parts of northern Africa. It can be found along non-arid to semi-arid coasts, in plains (montane to 1,600 m), on cliffs, beaches, and sandy fields. It prefers dry

pastures, open woodlands and brush, and chalky soil. Hairs can also be found on cotyledons (lamina and petioles), juvenile leaves, and fruits (Gómez-Campo 1980; Warwick et al. 2009). Warwick et al. (2009) mention no synonyms and no subspecies division. Genomic data of this species with $2n = 12$ (Harberd 1972) are not known to the author.

The fourth species of the genus is ***S. pubescens*** L., Mantissa Pl.: 95. (1767). It is a perennial or suffrutescent (slightly woody or obscurely shrubby at the base of stem; usually a short-lived perennial, unbranched stem) wild crucifer of the Mediterranean floral region. The geographical distribution is similarly limited as that of *S. flexuosa*: Albania, southeastern France, southern Italy including Sicily, the Canary Islands (La Palma), and northern Africa including Egypt. It occurs along non-arid to semi-arid coasts, plateaus, hills (montane to 2,300 m), rocks and cliffs, on shaded grassy slopes, gullies, rubble, scrub, in open woodlands and brush, dry pastures, fields, and meadows. It also prefers chalky soil.

Very dense hairs occur on juvenile leaves of *S. pubescens* (Gómez-Campo 1980; Warwick et al. 2009).

Synonyms for *S. pubescens* are *S. aristidis* Pomel, *S. boivinii* Baillarg., *S. indurata* Coss., *Brassica palmensis* Kuntze, and *Sinapidendron palmense* (Kuntze) O.E. Schulz. *S. pubescens* can be divided into three subspecies: subsp. *aristidis* (Pomel) Maire and Weiller, subsp. *indurata* (Coss.) Batt., and subsp. *pubescens* L. (Warwick et al. 2009).

The diploid chromosome number is $2n = 18$ (Manton 1932). Genomic data are not available.

17.3 Conservation Initiatives

According to the author's knowledge, there are no special conservation initiatives for species of the cosmopolitically abundant genus *Sinapis*. Gene bank conservation is usually performed via seeds.

Currently, the gene bank at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) at Gatersleben, Germany (*http://gbis.ipk-gatersleben.de*), for example, hosts 198 accessions of *S. alba*. Moreover, 42 and 5 samples of *S. arvensis* and *S. pubescens*, respectively, are also stored and described there. No hit can be obtained for *S. flexuosa*. It is not likely that this is due to synonymy because no synonyms are listed in Warwick et al. (2009) as mentioned in Sect. 17.2. The taxon with limited geographical distribution (see Sect. 17.2) is also not listed in the Missouri Botanical Garden's electronic database available at *http://www.tropicos.org*.

17.4 Role in Elucidation of Origin and Evolution of Allied Crop Plants

As described earlier, although all four *Sinapis* species, *S. alba*, *S. arvensis*, *S. flexuosa* and *S. pubescens*, belong to the *Nigra* lineage, there are several molecular studies indicating the close relationship between the *S. arvensis* (Sar) genome and the *Brassica* B-genome (see Sect. 17.1). This also corresponds with important agronomical traits like blackleg resistance, which occur in both, in the Sar and in the B-genome. By interspecific hybridization and embryo rescue techniques applied in early generations of a backcross program such traits can be transferred into the related crop Brassicas without the B-genome, like oilseed rape (*B. napus*, genome AACC), cabbage (*B. oleracea*, CC), and turnip rape (*B. rapa*, AA; see Sect. 17.6).

For example, based on internal transcribed spacer (ITS) DNA sequences, Agerbirk et al. (2008) showed that the genus *Sinapis* is polyphyletic, as it forms three separate clades in the phylogenetic tree: the "Arvensis" (with *S. arvensis* as a direct sister to *B. nigra*), the "Alba" (including not only *S. alba* and *S. flexuosa*, but also *Kremeriella cordylocarpus*, *Hemicrambe fruticulosa*, and *Coincya* spp.), and the "Pubescens" (with *S. pubescens* as well as *Hirschfeldia incana* and *Erucastrum canariens*) clade.

Moreover, these authors also identified two separate occurrences of sinalbin. The first in a group of species related to *S. alba* (including members of the genera *Coincya* and *Kremeriella*); and the second in *S. arvensis*, nested among sinalbin deficient species.

17.5 Role in Classical and Molecular Genetic Studies

An integrated genetic map of *S. alba* has been described in Nelson et al. (2005). This genetic map consists of 382 loci arranged in 12 substantial linkage

groups with no unassigned loci. The genetic markers were based on restriction fragment length polymorphisms (RFLPs) detected using a set of 160 *Brassica* cDNA and genomic clones as probes (Nelson et al. 2005; see also Nelson and Lydiate 2006).

For the remaining three species of the genus *Sinapis*, no genetic linkage maps are known to the author.

17.6 Role in Development of Cytogenetic Stocks and Role in Crop Improvement

Thirty-eight interspecific hybridizations, mainly obtained by sexual crosses and embryo rescue techniques, between *S. alba* as one parent and *Brassica* species and *R. sativus*, respectively, as the other parent are documented by Warwick et al. (2009). However, protoplast fusions, both symmetric and asymmetric, have been widely used to transfer genes of interest from white mustard into *Brassica* crops (e.g., Wang et al. 2005; see also Plümper 1995 for more details).

S. alba possesses important agronomic traits (see Wei et al. 2007; Warwick et al. 2009 for more details). For example, it is highly resistant to many insect pests of crucifers like flea beetles (*Phyllotreta cruciferae* and *P. striolata*; Gavloski et al. 2000; Henderson et al. 2004), cabbage aphid (*Brevicoryne brassicae*; Thompson 1963), cabbage root fly or cabbage maggot (*Delia radicum*; Jyoti et al. 2001), and cabbage seedpod weevil (*Ceutorhynchus obstrictus* = *C. assimilis*; Ulmer and Dosdall 2006; Carcamo et al. 2007).

Resistance of *S. alba* to the beet cyst nematode (*Heterodera schachtii*) has been described by Thierfelder et al. (1991) and Lelivelt et al. (1993), whereas Pattison et al. (2006) showed resistance to the root-knot nematode (*Meloidogyne* spp.). Moreover, there are reports of resistance to important pathogens like turnip mosaic virus (Mamula et al. 1997) and the blackleg fungus *Leptosphaeria maculans* (Plümper 1995; Gugel and Séguin-Swartz 1997).

Resistance of white mustard to *Alternaria* black leaf spot (e.g., *Alternaria brassicae*, *A. brassicicola*) has been detected by various authors, but in many cases it has been described as a moderate one (Kolte 1985; Brun et al. 1987; Tewari et al. 1987; Ripley et al. 1992; Sharma and Singh 1992; Plümper 1995; Hansen et al. 1995; Hansen and Earle 1997; Sharma et al. 2002). Several experiments have been performed to transfer this resistance to *B. napus* (Ripley and Arnison 1990; Ripley et al. 1992; Chèvre et al. 1994; Brown et al. 1997), *B. oleracea* (Nothnagel et al. 1996; Hansen and Earle 1997), *B. rapa* (Chinese cabbage; Gong et al. 1994), and *B. juncea* (Mohapatra and Bajaj 1987) by intergeneric (sexual or somatic) hybridizations (see Plümper 1995 and Klewer 2005 for more details).

A good example for the difficulties of *Alternaria* resistance transfer from white mustard to *Brassica* species are somatic hybrids of *S. alba* (+) *B. napus* produced via asymmetric protoplast fusions by Plümper (1995; not mentioned in Warwick et al. 2009). Some hybrids with $2n = 52–60$ (no complete chromosome addition of the parental genomes) revealed the resistance level of *S. alba* in detached leaf tests. Klewer (2005) used some of these hybrids ($2n = 45–54$, including 9–13 *S. alba* chromosomes) as the female parent in backcrosses with *B. napus*. Their offspring (BC_1, BC_2), however, lost the moderate resistance of *S. alba* [Fig. 17.1; for comparison see also absolute resistance ("complete immunity") of *Diplotaxis erucoides* also in Fig. 17.1] and showed only a weak and instable resistance expression. The same applies to sexual hybrids between the two parents and their backcross offspring (Klewer 2005; not mentioned in Warwick et al. 2009). On the basis of her results, Klewer (2005) claims that *Alternaria* resistance in *S. alba* is likely to be polygenic.

Furthermore, white mustard is also tolerant to high temperatures and drought stress (Brown et al. 1997). In addition, a lot of data on seed fatty acids and their genetics, based on examinations of Ecker and Yaniv (1993), are summarized in Séguin-Swartz et al. (1997).

All those traits mentioned earlier make *S. alba* a valuable germplasm resource for Cruciferae crop breeding. However, in comparison with the potential of *S. arvensis*, the wider distance of the *S. alba* genome to the *Brassica* genomes, especially the B-genome (see Sect. 17.1), is likely to limit the intergeneric trait transfer via recombination events.

Warwick et al. (2009) list 44, for the most part sexual hybridizations between *S. arvensis* and other Brassiceae species (*Brassica* spp., *R. sativus*, *Erucastrum gallicum*). Hybrids from *B. napus* × *S. arvensis* crosses obtained using embryo rescue are described,

Fig. 17.1 Leaf symptoms of the four species with the strongest resistance expression (significantly better than *Brassica napus*) in a wet chamber test after inoculation with *Alternaria brassicicola*. From left to right: *B. napus* cv. "Ceres," *Diplotaxis tenuifolia* (from India), *B. elongata* ssp. *integrifolia*, *Sinapis alba* cv. "Emergo" and *D. erucoides* (from India). The brown spots on *D. erucoides* are caused by the injury of the leaf and are not disease symptoms (Figure from Klewer (2005), permission of reproduction is gratefully acknowledged, legend slightly modified and translated by the author)

for example, by Harberd and McArthur (1980), Inomata (1988), Kerlan et al. (1992, 1993), Bing et al. (1991, 1995), Lefol et al. (1996), and Moyes et al. (1999). Moreover, reports on Inomata (1997) and Moyes et al. (2002) on BC_1 plants (respective backcrosses with *B. napus*) are included in this survey. Protoplast fusions leading to somatic hybrids were performed by Toriyama et al. [1987; *S. arvensis* (+) *B. nigra* and *S. arvensis* (+) *B. oleracea*] and Hu et al. [2002; *B. napus* (+) *S. arvensis*].

However, the original hybrids *B. napus* × *S. arvensis* produced by Plümper (1995), the basis for one of the most advanced backcross programs (e.g., BC_1–BC_4, BC_3S_1, BC_3S_2, BC_4S_1; see below and Fig. 17.2 for details) reported by Snowdon et al. (2000) and Winter (2004) are not mentioned in the valuable list of Warwick et al. (2009).

The same applies to a recent publication of Wei et al. (2010). The authors developed a disomic, cytologically stable *B. napus–S. arvensis* addition line, which contains one pair of chromosomes from *S. arvensis* and 19 pairs from *B. napus*. This line displays very strong restoration ability to the *Nsa* cytoplasmic male sterile (CMS) line, high resistance to *Sclerotinia sclerotiorum*, and a low incidence of pod shattering. As Wei et al. (2010) also report, the characterization of mitochondria from the *Nsa* CMS line (from the same somatic hybridization) has indicated that this line mainly contains the *S. arvensis* cytoplasm. Thus, the cytoplasmic sterile gene in *Nsa* CMS line is likely to be derived from the *S. arvensis* parent.

Beside the mentioned resistance to *Sclerotinia sclerotiorum*, genotypes of *S. arvensis* show resistances to many other important pathogens like *Alternaria* spp. causing black leaf spot (Siemens 2002) and turnip mosaic virus (Mamula et al. 1997).

Wild mustard exhibits strong blackleg (*L. maculans*) resistance, which is effective in all parts and all developmental stages of the plant (see studies of Plümper 1995; Winter 2004 and Fig. 17.2a, b). With the exception of an old, unconfirmed report of Hughes (1933), to the author's knowledge, there are no data showing any susceptible genotype of *S. arvensis* or resistance breakthrough worldwide. Since the pathogen is known for its evolutionary potential and the ability to adapt quickly to a given resistance source (Kuswinanti et al. 1999; Howlett 2004), this is surprising. On the other hand, this phenomenon is promising for interspecific resistance transfer. Moreover, it is in significant contrast with reports of Australian isolates overcoming the *Brassica* B-genome resistance (Salisbury and Ballinger 1993; Purwantara et al. 1998; Winter 2004), which was formerly considered to be absolute and stable.

On the basis of the above mentioned work of Plümper (1995), Winter (2004) succeeded to transfer oligogenic resistances to blackleg from *S. arvensis*

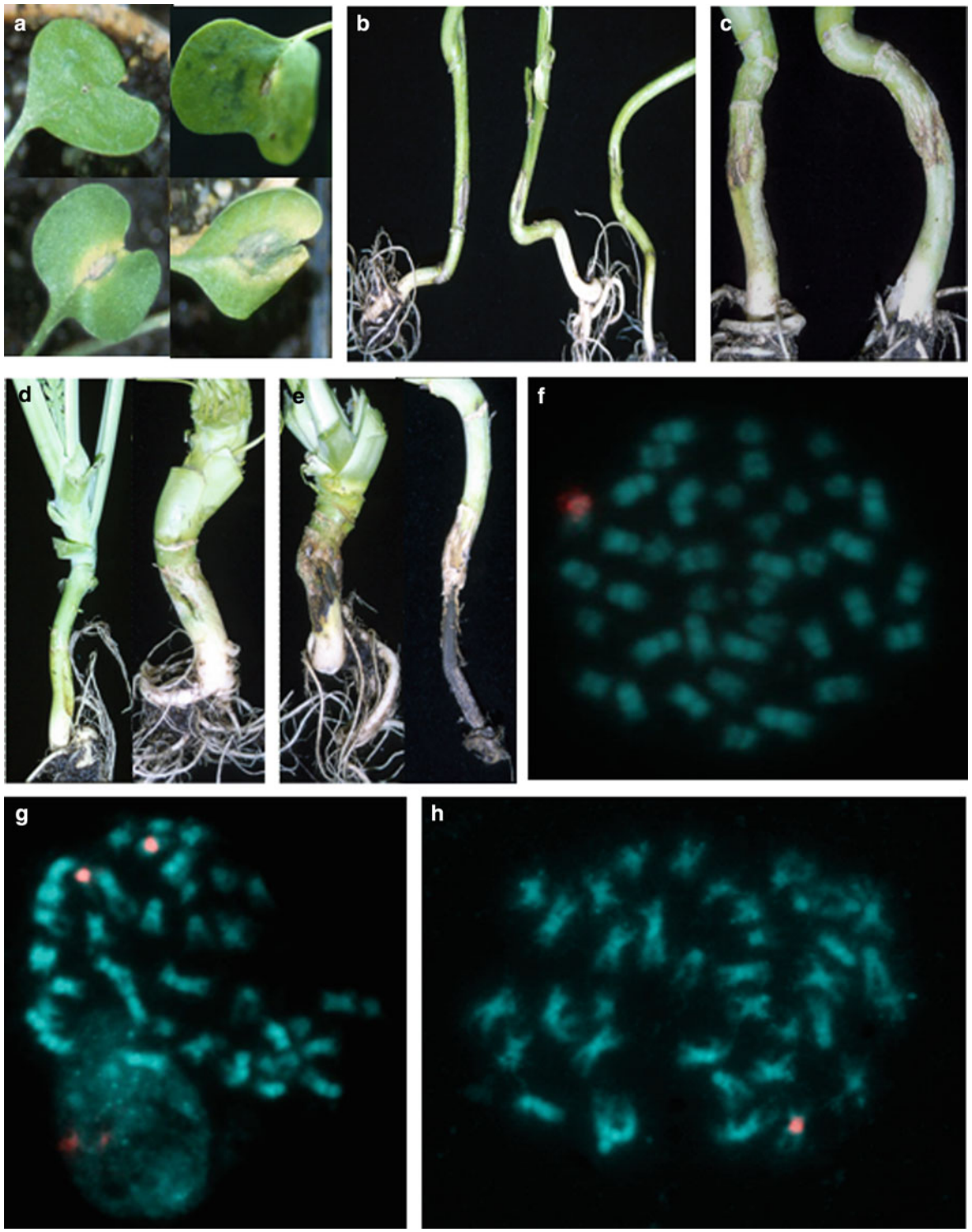

Fig. 17.2 Phytopathological and molecular cytogenetic characterization of *B. napus–S. arvensis* addition lines and control genotypes. Resistance behavior in tests with double inoculation with *Leptosphaeria maculans* isolate W4. (**a**) Cotyledon resistance (*above*) and susceptibility (*below*) phenotypes in BC_3S_1 progeny. The *above-left* phenotype is typical for *S. arvensis*, while the *below-right* symptoms are typical for the *B. napus* cultivars "Ceres," "Madora" and "Lesira." (**b**) Adult plant resistance of

to *B. napus*. Preliminary results were published by Snowdon et al. (2000; Fig. 17.2a–h) The authors, for the first time reported monosomic and double monosomic *B. napus*–*S. arvensis* addition lines and putative recombination lines with $2n = 38$. As this backcross program illustrates the potential of interspecific hybridizations involving *S. arvensis*, it is summarized in the following section.

Tests with double inoculation (early inoculation of the cotyledons followed by a later inoculation at the stem base) were performed in the greenhouse for accurate evaluation of plant resistance levels, both at the seedling (Fig. 17.2a) and adult plant stages (Fig. 17.2b–e). Genomic in situ hybridization (GISH) applied to BC_3, BC_3S_1, and BC_3S_2 *B. napus*–*S. arvensis* progenies revealed monosomic and double monosomic *S. arvensis* addition chromosomes in the *B. napus* background (Fig. 17.2g, h). These lines included fertile plants exhibiting high cotyledon (Fig. 17.2a, above-left) and adult plant (Fig. 17.2d) resistance associated with the presence of an acrocentric addition chromosome from *S. arvensis* (for monosomic acrocentric addition, see Fig. 17.2f; for acrocentric plus metacentric addition, see Fig. 17.2g). Plants with only a metacentric addition (Fig. 17.2h) showed the adult plant susceptible phenotype (Fig. 17.2e).

Moreover, from BC_3 onwards, other resistant individuals were observed to have a normal *B. napus* karyotype with no visible GISH signals (probably because of its localization on the distal part of the chromosome arms rather than its small size), indicating possible resistant introgression lines. Furthermore, indications for allosyndetic bivalent pairing have been found in this study. Such events, generally considered to be rare, have also been reported by other authors. Mizushima (1950) detected only three allosyndetic bivalents in ASar and CSar hybrids, but seven in BSar hybrids (see close relationship between Sar and B-genome discussed earlier). These data are in agreement with examinations by Kerlan et al. (1993) and Chèvre et al. (1996), who found just a few paired chromosomes in ACSar hybrids.

All *S. arvensis* plants examined showed resistance to both very aggressive (PG 4) isolates used in the study, W4 (Germany) and M1 (Australia, known to be able to overcome the *Brassica* B-genome based blackleg resistance, see above), on the cotyledon (Fig. 17.2a, above-left) and the adult plant (Fig. 17.2b) stages. *B. napus* cv. "Ceres," the backcross parent, revealed to be only moderate adult plant susceptible (Fig. 17.2c) with some individuals scored resistant. This is due to the quantitative, partial, and polygenic resistance from the genes of the French cultivar "Jet Neuf" it carries. This influence in the intergeneric offspring is limited by the severe *S. arvensis* resistance used here.

Resistance data from selfing progenies of double resistant plants show that cotyledon and adult plant resistance are conferred by different loci. Adult plant resistance is assumed to be oligogenically inherited (1–2 genes). Moreover, non-segregating, adult plant resistant lines were obtained.

In addition, data of Bowers et al. (1997) show evidence for an anti-mosquito effect of *S. arvensis*. Mithila and Hall (2007) successfully produced microspore-derived doubled haploid wild mustard. Data on fatty acids, isozymes, and their respective genetics in *S. arvensis* are reported in Séguin-Swartz et al. (1997).

Two publications on the genetics of self-incompatibility are summarized in Séguin-Swartz et al. (1997). In a study using a wild population of *S. arvensis* from South Wales, UK, Ford and Kay (1985) confirmed a single-locus, multi-allelic sporophytic incompatibility system similar to that found in other crucifers. Fourteen

Fig. 17.2 (continued) S. arvensis. (**c**) Moderate adult plant susceptibility of *B. napus* cv. "Ceres." (**d–h**) Plants of the BC_3S_1 generation: adult plant resistance (**d**) und susceptibility (**e**). The phenotype on the left of (**e**) is representative for the majority of adult plant susceptible *B. napus*–*S. arvensis* plants examined, while the stem on the right of (**e**) shows plant collapse, typical for *B. napus* cv. "Lesira." (**f–h**) Genomic in situ hybridization results in three BC_3S_1 offspring plants. *S. arvensis* chromosomes are labeled red with Cy3, while *B. napus* chromosomes show no hybridization signals and are stained blue with DAPI (from a cooperation with Rod Snowdon, University of Giessen, Germany). (**f**, **g**) Individuals showing both cotyledon and adult plant resistance (double resistance) and corresponding respectively to the left and right plants in (**d**), with (**f**) a monosomic, acrocentric addition, and (**g**) one acrocentric and a second, metacentric *S. arvensis* addition chromosome. (**h**) An individual susceptible on cotyledon and adult plant stages with a monosomic, metacentric addition chromosome, corresponding to the right-hand plant in (**e**). Figure slightly modified and legend modified and translated from Winter (2004); based on Snowdon et al. (2000)

different *S*-alleles were found in a sample of ten plants collected from a single field, which would give a minimum estimate (Paxman's maximum likelihood estimate) of 24 *S*-alleles in the population. Both dominance and independent action of alleles occurred in the pollen and stigma. A second report of Stevens and Kay (1988) comprises analyses conducted on diallel crosses within F_1 and F_2 families derived from crosses between plants from geographically remote populations (Crete × Lincolnshire, UK) and (Crete × South Wales, UK) and within self families derived from the parental plants. Control by a single locus was established. The expression of individual *S*-alleles could be modified as shown by incomplete dominance in a selfed family and mutual weakening in a F_1 family.

For *S. flexuosa*, no data for interspecific hybridizations and valuable agronomic traits are known to the author. In a study of Durkeet and Harborne (1973), *S. flexuosa*, together with *B. tournefortii*, revealed to be exceptional in having flavonol 3-monosides and 3-diglycosides instead of 7-glucosides and 3,7-diglucosides of kaempferol and isorhamnetin as most other *Brassica* and *Sinapis* species examined. The authors claim that these glycosidic patterns are likely to be of taxonomic value.

Fifteen intergeneric sexual hybridizations with *S. pubescens* are reported by Warwick et al. (2009). These include crosses not only with *Brassica* species, but also with *D. erucoides*, *E. gallicum*, *E. virgatum*, *Sinapidendron frutescens* (for all see Harberd and McArthur 1980), and *R. sativus* (Bang et al. 1996). With the exception of the study of Bing et al. (1991) on a cross *Brassica carinata* × *S. pubescens* embryo rescue has been used in all experiments. Interestingly, in all 15 successful hybridizations *S. pubescens* was the male parent. Reciprocal crosses were always tried, when embryo rescue was used, but failed. The only report on successful backcrosses (*Brassica napus* × *S. pubescens*) in Warwick et al. (2009) is from Inomata (1994). Through ovary culture the author developed F_2, BC_1, and BC_2 plants with $2n = 38$.

There are very few reports on valuable agronomic traits and introgression attempts. Kumar et al. (2003) successfully transferred white rust resistance (*Albugo candida*) from this species into somatic hybrids *S. pubescens* (+) *B. nigra*. In contrast, Gavloski et al. (2000) showed the susceptibility of *S. pubescens* to flea beetle (*Phyllotreta cruciferae*) feeding.

17.7 Genomics Resources Developed

Only very limited data on genomics of *Sinapis* species are known to the author. Homann and Link (2003) showed transcription characteristics of three cloned sigma factors from *S. alba*. In a proteomics approach, Pfannschmidt et al. (2000) describe the multisubunit chloroplast RNA polymerase A from the same species. In addition, a study of von Lintig et al. (1997) revealed that the light-dependent regulation of carotenoid biosynthesis in *S. alba* occurs at the level of phytoene synthase expression and is mediated by phytochrome.

17.8 Scope for Domestication and Commercialization

S. alba is grown for condiments (mustard) and – with ongoing breeding efforts – for its valuable oil (see Sect. 17.2). The latter is especially the case in less favorable conditions, where oilseed rape (canola) and other oil Brassicas cannot be grown efficiently.

Historically, native peoples of North America have used a number of "wild" crucifers for both food and medicinal purposes, for example, the Malecite and Micmac Indian Tribes used *S. alba* to treat tuberculosis (Arnason et al. 1981; Jacobson et al. 1988).

Moreover, white mustard is regarded as a potential (alternative) biocrop. For example, there is a report from the University of New Hampshire about a pilot research project commencing in 2007 (one year before it started with sunflower) "to test the feasibility of small-scale oil pressing and biodiesel production. The project will measure the yield of oil that can be processed into biodiesel for use on farms, the feed value of the meal that remains after the oil has been pressed from the. . .seeds, and the food quality of the oil." *S. alba* cv. "Ida Gold" is one of the genotypes included. The other three cruciferous oilseed crops are *Camelina*, canola, and *Brassica juncea* (Anonymous 2010). To the author's knowledge preliminary results have not yet been reported.

In addition, over the past years the beneficial effects of Brassicaceae use in agricultural practice have been investigated in detail. Among *Brassica*

species, this also applies to research on *Sinapis* spp., like *S. alba*. In the focus of interest is the myrosinase–glucosinolate system common for crucifers. For example, Palmieri (2007) points out that this enzymatic system is important not only because it is one of the most significant protection structures in the plant kingdom, but also because it provides chemoprotection when assimilated with diet or useful outcomes in crop protection, particularly when used in green manure. The utilization of green manure includes a soft method to fight plant and soil pathogens (biofumigation). This appears to be practical because of its good agronomic, economic, and environmental attributes (Palmieri 2007).

17.9 Some Dark Sides and Their Addressing

Among other crucifer species *S. arvensis* has become a naturalized weed in Canada and the United States, representing both a potential source of germplasm and agricultural problems (Warwick et al. 2009).

A point of concern – for the most part in public, to a less extend also in the scientific community – coming along with the cultivation of transgenic oilseed rape is potential interbreeding between genetically modified *B. napus* and non-transgenic plants of the same or a related species (vertical gene transfer). Potential gene flow between transgenic oilseed rape and weedy *S. arvensis* is of special interest, because of the ability of these crucifers (and of many others as well) to hybridize across species borders. In Sect. 17.6, this has been shown, with some examples, for the lab level.

Most assessment studies related to this problem show a low hybridization risk (for a detailed summary see Winter 2004). Moreover, in crossing experiments, it is a widely known phenomenon that even with extensive use of embryo rescue techniques the production of early backcross offspring from interspecific hybridizations is much more difficult than the development of the original hybrids (Sacristán, Berlin, Germany, personal communication; see also Winter 2004; Warwick et al. 2009). So, even if F_1 hybrids occur in the field in very rare cases, a BC_1 plant (backcrossed with *B. napus* or the wild crucifer parent) is very unlikely to become established. To the author's knowledge, this has never been described for the hybridization system discussed here.

In this context, beside the appearance of sporadic volunteer plants in the later vegetation periods, especially the ability of *B. napus* pollen to get dispersed up to distances of 3 km (Rieger et al. 2002) has to be considered. This, potentially, opens chances for hybridizations with closely related wild species (Snow 2002). However, examinations by Rieger et al. (2002) made clear that gene flow from herbicide resistant oilseed rape to *B. napus* grown on neighboring fields leads to a remarkably low average content of 0.07% "contaminated" seed samples. This is significantly lower than the European Union threshold value of 1.0%.

Studies on the likelihood of outbreeding of *B. napus* with wild crucifers under agronomic conditions showed very low hybridization frequencies in both directions for *Raphanus raphanistrum* [Chèvre et al. 2000 (with one hybrid *R. raphanistrum* × *B. napus*); Rieger et al. 2001]. In contrast, frequent hybridizations between the more closely related species *B. napus* and *B. rapa* have been described by several authors (Jørgensen and Andersen 1994; Bing et al. 1996; Wilkinson et al. 2003).

These results, based on studies with non-transgenic plants, have been confirmed in investigations from oilseed rape fields in Canada by Warwick et al. (2003). These authors, for the first time, showed evidence for a transgene introgression from a genetically modified (GM) crop into a related wild species. They detected F_1 plants from *B. rapa* × *B. napus* (genome AAC, $2n = 29$, morphology like *B. rapa*; hybridization frequencies between 7 and 13.6%). In contrast with this, among 32,821 seedlings Warwick et al. (2003) found only one hybrid between *R. raphanistrum* and herbicide resistant *B. napus*. Moreover, analyses of 42,828 and 21,841 seedlings in commercial *B. napus* fields revealed no hybrids with *S. arvensis* and *E. gallicum*, respectively. That is why, the probability of gene flow between GM oilseed rape on the one side and *R. raphanistrum*, *S. arvensis*, or *E. gallicum* on the other side has been estimated by Warwick et al. (2003) as being very low (less than $2–5 \times 10^{-5}$).

In summary and taking into account the generally low probability of hybridization, Stewart et al. (2003) consider oilseed rape to be a "moderate risk crop." This is the case, despite of many closely related wild species of the tribe Brassiceae (see Sect. 17.6 for

relevant hybridization experiments on the lab level), that occur as free-living populations and as agricultural weeds.

Later a field study of Daniels et al. (2005) drew much attention. The authors identified hybridization between oilseed rape and *S. arvensis*. In a "reviewer's comment," published together with the report of Daniels et al. (2005), it has been pointed out that "such a finding needs to be interpreted with caution. The frequency of such an event in the field is likely to be very low, as highlighted by the fact it has never been detected in numerous previous assessments." (see e.g., the above mentioned reports). "Furthermore, the conditions where the hybrid was found appear to be quite unusual, restricted as it was to a case where *Sinapis* was sufficiently abundant in a crop to act as a significant conspecific pollen donor. The consequences of the transfer of the herbicide tolerance trait on the fitness and persistence of *S. arvensis* were not assessed in this study but are presumed to be negligible (Hails and Morley 2005)." Nevertheless, the author of this chapter agrees with the reviewer's comment that "this unusual occurrence merits further study in order to adequately assess any potential risk of gene transfer."

Another big issue and point of concern are herbicide resistance of *S. arvensis*, which has been reported by several authors (see also Séguin-Swartz et al. 1997; Warwick et al. 2009).

Inheritance studies of the resistance of wild mustard to the auxinic herbicide dicamba, based on three reciprocal crosses between herbicide resistant and herbicide susceptible (wild type) plants, have been reported by Jasieniuk et al. (1995). The data indicate that the trait was controlled by a single, dominant nuclear gene. Resistance to dicamba, a synthetic auxin belonging to the group 4 herbicides, has been described for 1990 in Canada for the first time (e.g., Heap and Morrison 1992; Jasieniuk et al. 1995).

Resistance of *S. arvensis* to acetolactate synthate (ALS) inhibitors (group 2 herbicides) have been reported from Australia for 1996 (Heap 2010), from Canada for 1992 (e.g., Warwick et al. 2005), from Turkey for 2001 (Heap 2010), and from the United States for 1999 (Christoffers et al. 2006).

In Canada, among the photosystem II inhibitors (group 5 herbicides, triazines), resistance of wild mustard to triazine has been found already in 1983 (Ali et al. 1986; Heap 2010), whereas metribuzin resistance has been reported for the first time in 1994 (Heap 2010).

17.10 Recommendations for Future Actions and Conclusions

From the author's point of view, with ongoing breeding efforts toward canola oil quality, *S. alba* will become an alternative oil crop to *B. napus*. This will be especially the case under less favorable climatic conditions.

Although, as described in Sect. 17.9, gene flow between transgenic oilseed rape and weedy *S. arvensis* is a very unlikely event, it is possible, as Daniels et al. (2005) showed. This has to be considered in risk assessment studies.

Both, *S. alba* and *S. arvensis*, are important gene resources for intergeneric trait transfer to *Brassica* crops. Considering its close relationship with the *Brassica* B-genome species, *S. arvensis* is likely to be even more relevant than *S. alba* for such breeding approaches. The significance of *S. arvensis* as a gene resource has been shown in many above mentioned examples. One of the most advanced and promising backcross programs led to the blackleg resistance transfer from the wild species into putative recombinant *B. napus*–*S. arvensis* lines with oilseed rape karyotype (Winter 2004). Wild mustard is a valuable alternative to current sources of blackleg resistance in oilseed rape breeding.

In comparison, the two remaining species, *S. pubescens* and, especially, *S. flexuosa*, are currently only of limited importance for breeding purposes. More screening against pathogens and pests, as well as studies on further traits of agronomic value, are needed to evaluate the potential of these species sufficiently.

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