Chittaranjan Kole *Editor*

Wild Crop Relatives: Genomic and Breeding Resources

Industrial Crops

Springer

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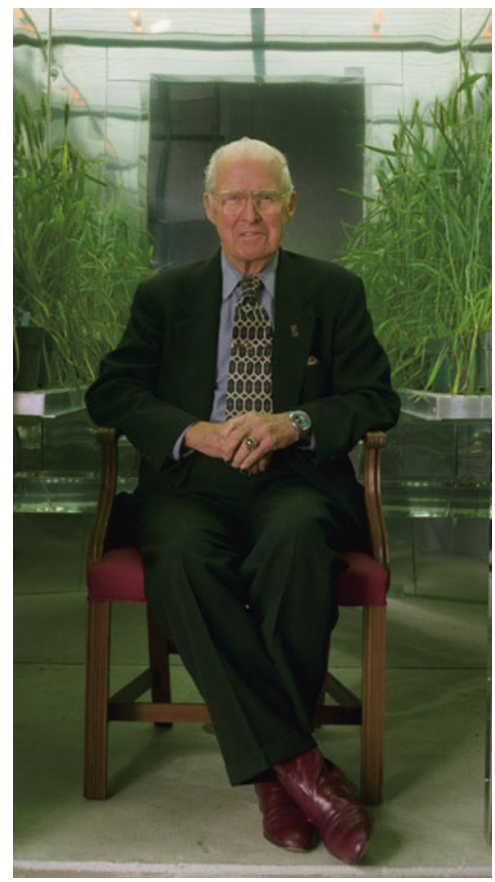
Dedication



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: Industrial Crops" includes 10 chapters dedicated to *Beta, Corchorus, Crotalaria, Dioscorea, Erianthus, Gossypium, Ipomoea, Manihot, Miscanthus, and Saccharum*. The chapters of this volume were authored by 31 scientists from 7 countries of the world, namely Australia, Germany, Ghana, India, Japan, Nigeria, and the 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 industrial crops with an intention of serving science and society.

Clemson, USA Chittaranjan Kole

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Abbreviations

ACP	Acid phosphatase
AFLP	Amplified fragment length polymorphism
ARS	Agricultural Research Service
BAC	Bacterial artificial chromosome
BJRI	Bangladesh Jute Research Institute
BNYVV	Beet necrotic yellow vein virus
BSA	Bulked segregant analysis
CAM	Crassulacean acid metabolism
CAPS	Cleaved amplified polymorphic sequence
CBB	Cassava bacterial blight
cDNA	Complementary-DNA
CENARGEN	Centro Nacional de Pesquisas de Recursos Genéticos e Biotecnologia
CGR	Centralized Germplasm Repository
CIAT	International Center for Tropical Agriculture
CIP	International Center for Potato
cM	Centi-Morgan
CMA	Chromomycin A3
CMD	Cassava mosaic disease
CMS	Cytoplasmic male sterility
cpDNA	Chloroplast-DNA
CRIJAF	Central Research Institute for Jute and Allied fibers
CSIRO	Commonwealth Scientific and Industrial Research Organization
CTCRI	Central Tuber Crops Research Institute
DAP	Days after pollination
DAPI	4′-6-Diamidino-2-phenylindole
DB	Database
DDPSC	Donald Danforth Plant Science Center
DHS	Deoxyhypusine synthase
DMSO	Dimethyl sulphoxide
DOE-JGI	Department of Energy-Joint Genomic Institute
DPA	Days postanthesis
EMS	Ethylmethane sulfonate
EPA	Environmental Protection Agency
EST	Expressed sequence tag
Est	α,β-Esterase
F_1	First filial generation

F_2	Second filial generation
F_4	Fourth filial generation
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FES	Friable embryogenic callus
FISH	Fluorescent in situ hybridization
GA	Gibberellic acid
GBIF	Global Biodiversity Information Facility
GC	Gas chromatography
GCP	Global Cassava Partnership
GISH	Genomic in situ hybridization
GM	Genetically modified
GMZ	Gene management zone
GOT	Glutamate oxalacetic transaminase
GP-1	Gene pool-1
GRIN	Germplasm Resources Information Network
GRU	Genetic Resources Unit
HCN	Hydrogen cyanide
HPLC	High performance liquid chromatography
IBPGR	International Board for Plant Genetic Resources
ICAR	Indian Council of Agricultural Research
IDBB	International Database for *Beta*
IIRB	Institute for Beet Research
IITA	International Institute for Tropical Agriculture
IJO	International Jute Organization
ILTAB	International Laboratory for Tropical Agriculture Biology
INIBAP	International Network for Improvement of Banana and Plantain
INIEA	Instituto Nacional de Investigaciony Extension Agraria
IPA	Pernambuco Agriculture Research Institute
IPGRI	International Plant Genetic Resources Institute (now Biodiversity International)
ISSR	Intersimple sequence repeat
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
LC	Liquid chromatography
LD	Linkage disequilibrium
L-DOPA	L-3,4-Dihydroxyphenylalanine
MAS	Marker-assisted selection
mRNA	Messenger-RNA
mtDNA	Mitochondrial DNA
NATP	National Agricultural Technology Project
NBPGR	National Bureau of Plant Generic Resources
NCBI	National Center for Biotechnology Information
ndhF	NADH dehydrogenese gene
NOR	Nucleolus organizer region
NPGS	National Plant Germplasm System
ORF	Open reading frame
PA	Pyrrolizidine alkaloid

PAGE	Polyacrylamide gel electrophoresis
PAL	Phenylalanine ammonia lyase
PCA	Principal component analysis
PCR	Polymerase chain reaction
PIC	Polymorphism information content
POX	Peroxidase
PPB	Participatory plant breeding
PVS2	Plant vitrification solution 2
QTL	Quantitative trait loci
RAPD	Random(ly) amplified polymorphic DNA
rDNA	Ribosomal-DNA
RFLP	Restriction fragment length polymorphism
RGA	Resistance gene analog
SBCN	Sugar beet cyst nematode
SCAR	Sequence characterized amplified regions
SDRF	Single-dose restriction fragments
SDS	Sodium dodecyl sulfate
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
STMS	Sequence tagged microsatellite
STS	Sequence tagged sites
TILLING	Targeted induced local lesions in genomes
UPGMA	Unweighted pair group method with arthmetic mean
USAID	Unites States Agency for International Development
USDA	United States Department of Agriculture
WB	Wild beet
YAC	Yeast artificial chromosome
YMV	Yam mosaic virus

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Chapter 1
Beta

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1.1 Basic Botany of the Species

Beet is classified taxonomically as Dicotyledoneae, Caryophyllidae (Centrospermae), Amarantheaceae (formerly Chenopodiaceae), *Beta vulgaris* L. Linneaus recognized one wild and two cultivated types (table and foliage), which have been domesticated since the earliest beginnings of agriculture (Ford-Lloyd and Williams 1975). The genus *Beta* comprises four sections and 12 well-defined species; *Beta* (formerly *Vulgares*), *Corollinae*, *Procumbentes* (formerly *Patellares*), and *Nanae*, represented by a single species endemic to Greece. With the exception of Section *Beta*, species of other sections have a more limited geographic distribution, and are found on European islands of the Atlantic Ocean and coastal and inland locations from Greece to Iran (Ford-Lloyd and Williams 1975; de Bock 1986). The wild taxa within the genus *Beta* are an important genetic resource for disease resistance breeding of cultivated beet, in particular *B. vulgaris* subsp. *maritima*, the closest wild relative. This subspecies is common along the Mediterranean coastline and the central and northern Atlantic coasts of Europe and to a lesser extent inland. Dissemination of its seed may often be by ocean currents because the fruit is buoyant and most extant wild populations are found within 10 m of mean sea level (Doney et al. 1990; Fievet et al. 2007).

The Section *Beta* includes the cultivated beets (*B. vulgaris* subsp. *vulgaris*), which is divided into four cultivar groups (leaf beet group, garden beet group, fodder beet group, and sugarbeet group) (Lange et al. 1999). The wild maritime beet (or wild sea beet) and cultivated beet groups are cross-compatible. The Section *Beta* is indigenous to the Mediterranean coastal area, extends westward as far as the Cape Verde Islands and Moroccan coast, east through the Middle East to India, and north along the Atlantic coast to Scandinavia. Letschert et al. (1994) revised the taxonomy of Section *Beta*. Since then, it is generally accepted that the Section *Beta* consists of two wild species and *B. vulgaris*, which is further subdivided into two wild subspecies and the cultivated forms (Table 1.1). Ford-Lloyd (2005) summarized the current taxonomic knowledge and published a taxonomy that was used in the International Database for *Beta* in 2002 (Germeier and Frese 2004), and was a pragmatic approach to facilitate communication among *Beta* germplasm users.

The taxonomy of wild beets was re-evaluated by Kadereit et al. (2006) and Hohmann et al. (2006), and these studies indicate that Section *Procumbentes* should be separated from the genus *Beta*. Examining the phylogeny of subfamily *Betoideae*, a small subfamily of the *Amaranthaceae*/*Chenopodiaceae* alliance, they provided strong evidence for a monophyletic development of this subfamily comprising the *Beta*, *Patellifolia* A.J. Scott et al. (= *Beta* section *Procumbentes* Ulbr.), *Aphanisma* Nutt. ex Moq., *Hablitzia* M. Bieb, and *Oreobliton* Durieu. Kadereit et al. (2006) analyzed with 29 nuclear ribosomal ITS1 sequences a representative sample of *Beta* species, as well as four representatives of *Hablitzieae* as outgroup, and found *Beta nana* to be closely related to *Beta* section *Corollinae* species. They also suggested merging *Beta* section *Nanae* into section *Corollinae*. This chapter will, however, use the taxonomic system as described by Ford-Lloyd (2005) since this is the

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Table 1.1 The taxonomy of Ford-Lloyd (2005) compared with the changes suggested by Kadereit et al. (2006)

System after Ford-Lloyd (2005)	System after Kadereit et al. (2006)[a]
Beta sect. *Beta*	*Beta* sect. *Beta*
B. vulgaris L.	*B. vulgaris* L.
B. vulgaris L. subsp. *vulgaris* (all cultivated forms)	
B. vulgaris L. subsp. *maritima* (L.) Arcang.	*B. vulgaris* L. subsp. *maritima* (L.) Arcang.
B. vulgaris L. subsp. *adanensis* (Pamuk.) Ford-Lloyd & Williams	*B. vulgaris* L. subsp. *adanensis* (Pamuk.) Ford-Lloyd & Williams
B. macrocarpa Guss.	*B. macrocarpa* Guss.
B. patula Ait.	
Beta sect. *Corollinae*	*Beta* sect. *Corollinae* (incl. sect. *Nanae*)
B. corolliflora Zos. ex *Buttler*	*B. corolliflora* Zos. ex Buttler
B. macrorhiza Stev.	*B. macrorhiza* Stev.
B. lomatogona Fisch. et May.	*B. lomatogona* Fisch. et May.
B. intermedia Bunge	
B. trigyna Waldst. et Kit.	*B. trigyna* Waldst. et Kit.
Beta sect. *Nanae*	
B. nana Boiss. et Heidr.	*B. nana* Boiss. et Heidr.
Beta sect. *Procumbentes*	*Patellifolia* A. J. Scott et al.
B. procumbentes Sm.	*P. patellaris* (Moq.) A. J. Scott et al.
B. patellaris Moq.	*P. procumbens* (Sm.) A.J. Scott et al.
B. webbiana Moq.	

[a]This research material lacked *Beta patula*. They did discuss species relationships, however, without final conclusions. In fact, Kadereit et al. (2006) proposed a phylogenetic study with the aim to fully resolve relationships within the genus *Beta* and support Curtis (1968) who fist suggested that *B. procumbens* and *B. webbiana* may not be distinct species

context of virtually all of the wild species utilization literature. The differences between the taxonomy (Ford-Lloyd 2005) and the major changes suggested by Kadereit et al. (2006) are summarized in Table 1.1.

There is evidence that *Beta* was a part of the human diet as long ago as the late Mesolithic period (Kubiak-Martens 1999). The beet was probably domesticated as a pot herb, and leaves first harvested from wild plants to use for food (Coons 1936; Ford-Lloyd and Williams 1975; de Bock 1986; Lange et al. 1999). Selection likely transformed the annual habit into the biennial habit characteristic of all current crop types, conserving and propagating germplasm for their leaf quality, the only part originally utilized. As a leafy pot herb, beet was mentioned in early Greek and Roman texts. It was not, however, until the Middle Ages that we find reference to the root being used both as a vegetable and medicinal herb (Pink 1992; Biancardi 2005; Goldman and Navazio 2008). In 1822, William Morgan provided a historical account of the development of fodder beet suggesting that sweet, swollen roots were probably selected from leafy beets, likely bearing resemblance to the chard of today (cited in Ford-Lloyd and Williams 1975, original not found). However, Zossimovich (1940) suggests the swollen root was introduced into Europe from Persia. By the eighteenth century, the use of beet root had expanded to include animal feed, and the fodder beet had become an important component of European agriculture by the nineteenth century.

Beets grown exclusively for sucrose are of relatively recent origin. Economic production of sucrose was accelerated by edict in Napoleonic France under British blockade of sucrose from tropically grown sugarcane (Winner 1993). The first sugarbeets with higher levels of sucrose were selected from a white fodder beet variety, the White Silesian beet (Fischer 1989), and this variety is still considered to be the primary source of sugarbeet germplasm grown today. However, there was speculation that Knauer's *Beta Imperialis*, developed in 1858 and also known as the mother of modern sugarbeet, originated from unintentional crosses between the Silesian beet and wild sea beet from the North Atlantic Coast or from spontaneous crosses between fodder beet and chard (Zossimovich 1940; Fischer 1989).

1.2 Conservation Initiatives

By the 1980s, public and private plant breeders began more seriously to consider the wild sea beet (and other wild *Beta* species) as a genetic resource in which to find resistance to increasing pressure from insects, nematodes, and diseases, and as a source of genes for greater productivity. This raised the awareness around the world that important genetic resources of the wild sea beet were being lost (Pignone 1989; Doney et al. 1995; Frese 2003). Habitat destruction continues to be reported for species in section *Beta* (Frese et al. 1990; Bartsch et al. 2002; Tan and Inal 2004) and of major concern are the deleterious effects of overgrazing mountain habitats of species from other sections of

the genus *Beta* (Frese et al. 2001a, 2009; Arjmand et al. 2004). And of special interest is the potential effect of global warming on the survival of *B. nana*, which is the only alpine species of *Beta* having a narrow altitudinal distribution range above 1,800 m (Frese et al. 2009). Changes in day-length sensitivity in wild beets are also suggested as a consequence of climate change (van Dijk and Hautekeete 2007).

Early plant exploration missions from the US were undertaken in 1925 and 1935 throughout Europe and the Near East to collect potential sources of disease resistance in wild sea beet and other *Beta* species (Coons 1954, 1975). Although there was some effort to evaluate this material, it ended up in unsuitable storage conditions; however, the seed that germinated was increased and a cursory evaluation made. As the realization that these wild relatives could be of great value in the improvement of the sugarbeet crop has grown (De Bock 1986; Doney 1993; Doney and Whitney 1990; Van Geyt et al. 1990; Stevanato et al. 2001) so has the activity in collection of seed for ex situ storage. Throughout the 1980s and 1990s, IPGRI (International Plant Genetic Resources Institute – now Biodiversity International) and USDA-ARS (United States Department of Agriculture-Agriculture Research Service) funded collection missions, which collected wild beets from the Atlantic and Mediterranean coasts of Europe (Ford-Lloyd 1989; Doney et al. 1990, 1995; Frese et al. 1990; Frese 2003). Later collections have filled in the geographic gaps and collected more species from within different Sections of *Beta* (El Manhaly et al. 1998; Frese et al. 2001a, 2009).

The USDA-ARS National Plant Germplasm System (NPGS) is a cooperative effort by public (State and Federal) and private organizations to preserve the genetic diversity of plants. These genetic resources are available to researchers to develop new varieties that are able to resist stresses (biotic and abiotic) and are more productive (Janick 1989). The USDA-ARS NPGS is responsible for acquiring, preserving, evaluating, documenting, and distributing crop germplasm. Information on this collection is available through the Germplasm Resources Information Network (GRIN), which is accessible through http://www.ars-grin.gov/npgs/index.html (accessed August 2010). The USDA-ARS NPGS *Beta* collection is housed at the Western Regional Plant Introduction Station in Pullman, WA, and backed up with the base collection in the USDA-ARS National Center for Genetic Resources Preservation at Fort Collins, CO. This ex situ collection has 2,550 accessions with 1,895 accessions (74.3%) available. Table 1.2 contains a breakdown of the collection by species.

Table 1.2 Total number of accessions and number backed up per species in the NPGS *Beta* collection

Taxon	Total accessions	Accessions backed-up
Beta corolliflora	4	3
Beta hybrid	2	1
Beta lomatogona	29	4
Beta macrocarpa	15	13
Beta macrorhiza	20	2
Beta nana	21	0
Beta patellaris	29	12
Beta patula	3	3
Beta procumbens	15	5
Beta sp.	16	5
Beta trigyna	48	5
Beta vulgaris	24	16
Beta vulgaris subsp. *maritima*	572	391
Beta vulgaris subsp. *vulgaris*	1,722	1,437
Beta vulgaris subsp. *vulgaris* (*NCGRP and CSR*)	14	
Beta webbiana	8	1
Beta × *intermedia*	8	1
Total	2,550	1,899

The International Data Base for *Beta* (IDBB) was developed beginning in 1987 at the Center for Genetic Resources, Netherlands (Wageningen) as a Dutch–German collaboration to enhance and streamline genetic resources activities within the international community of collection curators, researchers, and other users. Today, the IDBB is maintained by the Julius Kühn-Institute, Quedlinburg, Germany. The IDBB is considered a central information system within a global network of decentralized germplasm holdings. One of them, the Dutch–German *Beta* collection, is the second-largest *Beta* holding worldwide with 2,320 accessions housed at the Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany (http://eurisco.ecpgr.org/). The IDBB was begun with three major objectives (1) provide an inventory of international *Beta* genetic resources held in genebanks; (2) to identify duplicates among genebank holdings; and (3) provide coordination in collecting missions, seed regeneration, seed increases, and other activities common to all genebanks (Frese and van Hintum 1989).

The IDBB also was designed to serve as an international repository of metadata on *Beta* accessions within germplasm collections throughout the world. By 1989, the IDBB had information on 6,875 accessions from 18 genebanks of more than 26 with *Beta* holdings worldwide (Frese and van Hintum 1989). In late 1991, the responsibility for the database management was assigned to the genebank of the Institute of Crop Science and Plant Breeding of the Federal Agricultural Research Center (FAL) located in Braunschweig, Germany (Frese 1993). In 1996, the genebank's affiliation changed to the Federal Research Center for Cultivated Plants (formerly Federal Center for Breeding Research on Cultivated Plants – BAZ) (Germeier and Frese 2000). The number of holders (genebanks and other collections containing *Beta* germplasm) grew, as did the total number of accessions with information in the IDBB (Fig. 1.1; Frese and van Hintum 1989; Frese 1993; Germeier and Frese 2000). Most recently, the IDBB was completely redesigned and upgraded, providing users with comprehensive and readily accessible passport data from 28 collections (European and non-European) along with characterization and evaluation data (Germeier and Frese 2004). The information content, however, has not increased since the year 2002 after the end of the evaluation project GENRES CT42 funded by the European Commission (2006). Today the IDBB is a static system.

Although there still are gaps in the collections, much of the wild *Beta* germplasm within the section *Beta* is well represented in ex situ holdings of genebanks around the world. This is not necessarily true of the other sections of the genus *Beta* (*Corollinae*, *Procumbentes,* and *Nanae*). Species from these sections often have hard, dormant seeds, which make germination difficult (De Bock 1986). More recent plant exploration trips to Azerbaijan and Iran (Frese et al. 2001a) and Greece (Frese et al. 2009) have been focused on collecting species from these other sections.

The maintenance of accessions, and especially core collection entries (which ideally would be available in quantity), of sections *Corollinae, Nanae* and *Procumbentes* are difficult because of the problems in germination and because these species are not at all adapted to the climate in central Europe or in the US. If grown in an alien environment, strong selection pressure would favor those genotypes, which were adapted to genebank management practices and climate, rather than maintain the population's original genetic diversity.

The report of ECP/GR task force on a *Beta* core collection strongly recommended in situ management

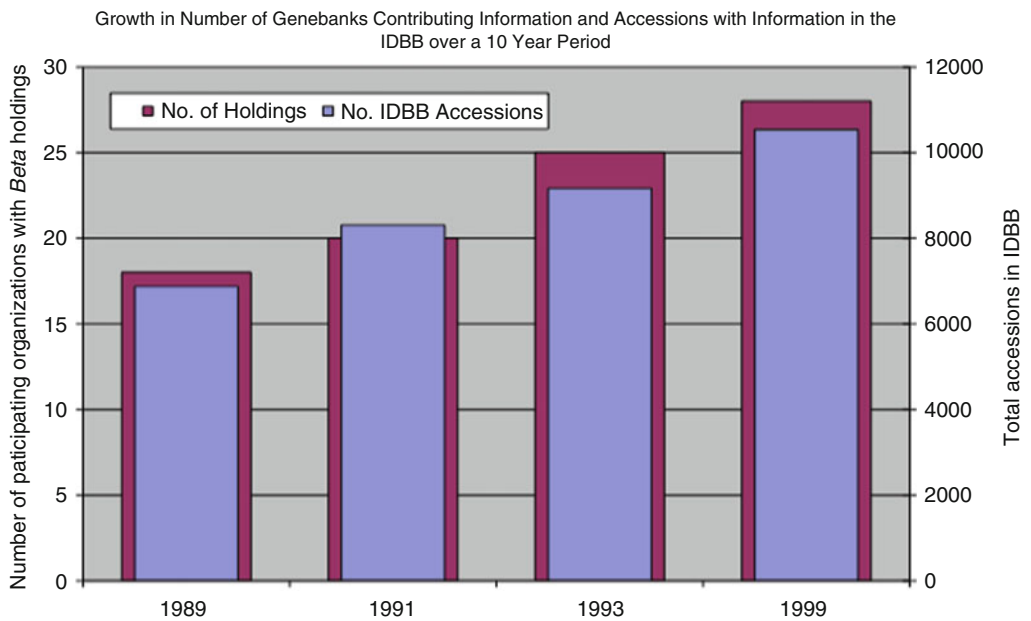

Fig. 1.1 International database for *Beta* statistics – 1989–1999

of species from these *Beta* sections, which would complement the current ex situ collections (Ford-Lloyd et al. 2000), and this recommendation was discussed at the second joint meeting of a Working Group on *Beta* and World *Beta* Network at Bologna, Italy in 2002 (Frese 2004). Nonetheless, there are few implemented efforts to develop in situ conservation of wild *Beta* species. An important exception to this is Turkey, which is one of the centers of origin for beet (Harlan 1992; Vavilov 1992), and contains widely distributed species of section *Beta* and *Corollinae* (Tan and Inal 2004). The establishment of in situ Gene Management Zones (GMZs) began in 1993, to target globally significant wild crop relatives originating in Turkey and, although *Beta* is not included, some *Beta* species are found in GMZs associated with target species (Tan and Inal 2004). Additionally, *B. vulgaris* subsp. *adanensis* is one of the endangered herbaceous plant species listed in the Bern Convention and is, therefore, a target species for conservation and management for sustainable protection in Turkey. The establishment of in situ management activities clearly is a responsibility of countries located in the natural distribution area of *Beta* species. The discussion of initiating in situ conservation efforts has been raised in Germany, Italy, Greece, France, and Spain, but only small efforts, if any, are being made (Bartsch et al. 2002; Frese 2004; Frese et al. 2004, 2009; Stevenato et al. 2004), probably due to the lack of national in situ conservation strategies. Up to now, efforts include the establishment of an in situ preserve on for *Beta lomatogona* at the Ardabil Research station of the Sugarbeet Seed Institute in Iran (Arjmand et al. 2004) and the use of on-farm multiplication in environments similar to those in which *Beta* accessions were collected, by the National Gene Bank in Hungary (Simon 2004).

Iriondo et al. (2008) published a guide to assist in the establishment of reserves for the conservation and management of genetic diversity of wild plant species. Their methodologies are being tested by the EU project AGRI GENRES 057 (2007–2011) using among other genera *Beta* for a case study (http://aegro.jki.bund.de, accessed June 2011). Detailed plans are worked out aiming at the establishment of a genetic reserve for *Beta patula* within an already existing formal protected area on Madeira (http://aegro.jki.bund.de/aegro/index.php?id=103, accessed June 2011).

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

Sugarbeet is the most economically valuable crop species in the Order *Caryophyllales*. Unusual features of *Caryophyllales* include carbohydrate seed storage reserves deposited from maternally derived perisperm (vs. endosperm), and pigments are generally alkaloid betalains, rather than the more familiar anthocyanins (excepting two families). Successive cambia are common and perhaps carried to extreme in beet where 10–15 rings of vascular tissue develop early, leading to a swollen storage root. C_4 photosynthesis is common in some families, and Crassulacean acid metabolism (CAM) photosynthesis is inducible in some taxa by salt stress. As a group, *Carophyllales* are often found in marginal and stressful environments, perhaps best exemplified by the cacti (Behnke and Mabry 1994).

Caryophyllales are thought to have diverged from other core eudicots around 100 million years ago. Recent molecular phylogenetics of nuclear 18S rDNA and plastid genes indicate that *Caryophyllales* are a unique group with affinity to the Asterid clade (Cuènoud et al. 2002; Fior et al. 2006; Jansen et al. 2007; Soltis et al. 2009). No reference genome yet exists for, or is phylogenetically near, this large family representing 7% of eudicot diversity (Stevens 2001 and onwards). Consequently, little comparative genomics is available within the *Caryophyllales*. Gene synteny conservation between species representing major eudicot clades (caryophillids, rosids, asterids) represented by sugarbeet, potato, sunflower, *Prunus* spp., and *Arabidopsis* was estimated to be 16–33%, with a conclusion that conserved synteny blocks extend among unrelated dicot plant families (Dominguez et al. 2003).

The *Caryophyllales* as circumscribed by Angiosperm Phylogeny Group (1998, 2003) contains 31 families, 692 genera, and 11,155 species. Family-level taxonomic relationships are not completely resolved. The *Caryophyllaceae* (notable for having anthocyanins rather than betalains) contribute carnations (*Dianthus* spp.) and *Silene latifolia* (notable for sex chromosome dimorphism). The *Amaranthaceae* (now includes *Chenopodiaceae*) contributes several ornamentals (Celosia, Amaranth), several noxious weeds (pigweed, lamb's quarter's, Russian thistle),

spinach (dioecious with homomorphic chromosomes), quinoa, and beets (both leaf and root crops). Spinach is an oft-used model for photosynthesis research. The *Aizoaceae* contributes ornamentals such as common ice plant and *Lithops* spp. (living stones) and the leafy vegetable New Zealand spinach, the *Portulacaceae* contributes purslane as both an ornamental and weedy species, and the Cactaceae contributes *Opuntia* spp. as ornamental and food (prickly pear) as well as drugs (peyote). Phylogenetically less tightly associated are the *Polygonales* including *Droseraceae* (venus fly trap, sundew), *Nepenthaceae* (old world pitcher plants), and *Polygonaceae* contributing weeds (smartweed), ornamentals, and foods (buckwheat, rhubarb). Also loosely associated within *Caryophyllales* are the *Santalales* (where families lack seed coats) including *Loranthaceae* and *Viscaceae* (epiphytic mistletoe, witches' broom).

DNA content (C-value) of *B. vulgaris* is reported as 714–758 Mbp per haploid genome (Bennett and Smith 1976; Arumuganathan and Earle 1991), and these are the only values available for the genus. Highly repetitive DNA sequences comprise ca. 60% of the beet genome (Flavell et al. 1974). The highly repetitive fraction consists of ribosomal DNA repeats, numerous families of short (140–160 nt) repeating units present at high copy number (10^5–10^6 copies), and various classes of transposable elements (Schmidt and Metzlaff 1991; Schmidt et al. 1991, 1995, 1998; Schmidt and Heslop-Harrison 1993, 1996b; Morchen et al. 1996; Kubis et al. 1997, 1998, Gindullis et al. 2001b; Staginnus et al. 2001; Dechyeva et al. 2003; Jacobs et al. 2004; Dechyeva and Schmidt 2006; Menzel et al. 2006, 2008; Kuykendall et al. 2008a, b, 2009). Each chromosome has a characteristic pattern of repeat-sequence distribution, supporting the model of sugar-beet as a true diploid with little or no duplication of the primary chromosome set (Halldén et al. 1998). Diversification of highly repetitive sequences among *Beta* spp. genomes has proven advantageous for characterizing interspecific hybrids (Schmidt et al. 1990, 1997; Schmidt and Heslop-Harrison 1996a; Gao et al. 2000; Desel et al. 2002).

In beet, the terminal constriction on Chromosome 1 carries the major cluster of 18S–5.8S–25S ribosomal RNA genes (Schmidt et al. 1994). An additional site was observed using labeled *Arabidopsis* DNA via in situ hybridization to chromosome spreads (Zoller et al. 2001). Ribosomal DNA repeats have been informative phylogenetic markers for the genus (Santoni and Bervillé 1992). 5S ribosomal RNA genes of *B. vulgaris* have been cytologically and genetically located to an interstitial site near the centromere on Chromosome IV (Schmidt et al. 1994; Schondelmaier et al. 1997), although not all appear functional (Turner and Brown 2005). Terminal and subterminal repeat units in the genus show both similarities and differences, and may be informative for examining chromosome evolution in *Beta* (Dechyeva and Schmidt 2006). Organization of centromeric regions has been of interest to understand the molecular processes of chromosome segregation and the process of non-disjunction and to create plant artificial chromosomes (Gindullis et al. 2001a, b; Menzel et al. 2008; Jacobs et al. 2009). A generalized picture of beet chromosome structure and organization indicates that *Beta* chromosomes are substantially similar to most other dicot chromosomes, at a gross level (Salentijn et al. 1994; Schmidt and Heslop-Harrison 1998).

A number of *B. vulgaris* genetic maps have been constructed with molecular markers (Barzen et al. 1992, 1995; Pillen et al. 1992, 1993; Boudry et al. 1994, Uphoff and Wricke 1995; Halldén et al. 1996; Schondelmaier et al. 1996; Nilsson et al. 1997; Schumacher et al. 1997; Hansen et al. 1999; Weber et al. 1999; Rae et al. 2000; Schneider et al. 2001, 2007; Möhring et al. 2004; Grimmer et al. 2007a). Maps have been constructed using anonymous genomic restriction fragment length polymorphic (RFLP), random amplified DNA polymorphic (RAPD), amplified fragment length polymorphism (AFLP) markers, and also gene-specific simple sequence repeat (SSR), and single nucleotide polymorphism (SNP) markers, and where possible maps have included morphological and isozyme markers. The number of markers ranged from 85 to 413 markers, and the genetic distance summed across all nine linkage groups (corresponding to the basic chromosome number of nine in *Beta*) for each map ranged from 621 to 1,057 cM. The reason for a large difference in genetic map length is not clear, but it is not related to the number of markers mapped. Most maps have shown a strong clustering of markers in one or two regions of each linkage group, suggesting restricted genetic recombination (Nilsson et al. 1997), however, this trend is less pronounced using markers derived from expressed genes.

All but three maps (Yu 2004; Laurent et al. 2007; McGrath et al. 2007) have been constructed from

sugarbeet, and other crop types or wild species used as a direct parent are not yet represented. The fundamental genetic basis is unlikely to be much different; however, allele frequencies likely vary and fixation of "crop use specific" or "domestication" alleles might be expected. Interestingly, the extreme segregation distortion for linkage group 5 in the Sugar × Red Table beet maps of Laurent et al. (2007) and McGrath et al. (2007) were opposite in their transmission despite both using sugarbeet as the maternal parent, with table beet alleles favored in the former and sugarbeet alleles in the latter. Distorted segregation ratios are common in beet linkage maps, presumably due to the high heterozygosity and presence of sublethal alleles and genetic interactions as might be expected from an obligate outcrossing species.

Molecular marker evidence suggests greater diversity is present in wild *B. vulgaris* subsp. *maritima* relative to cultivated germplasm. It is clear that molecular markers are useful for characterizing *Beta* germplasm (Jung and Herrmann 1991; Mita et al. 1991; Jung et al. 1993; Senda et al. 1995; Raybould et al. 1996a, b, 1997, 1998; Reamon-Buttner et al. 1996; Kraft et al. 1997, 2000; Shen et al. 1998; Tufto et al. 1998; Bartsch and Ellstrand 1999; McGrath et al. 1999; Wang and Goldman 1999; Raybould and Clarke 2000; Driessen et al. 2001; Hansen et al. 2001; Laporte et al. 2001; Cureton et al. 2002; Richards et al. 2004; Fievet et al. 2007; Poulsen et al. 2007; Fenárt et al. 2008). Ninety-five percent of the markers tested discriminated between Sections *Beta* and *Procumbentes* and 43% of the genomic clones detected variation between *B. vulgaris* cultivars. A relatively large number of *Beta* accessions were examined at the ribosomal RNA encoding genes (Santoni and Bervillé 1992). Most *B. vulgaris* germplasm was monomorphic for a particular type, and interestingly, variants were found in Swiss chard, while *B. vulgaris* subsp. *maritima* contained the greatest diversity among all accessions analyzed. Genetic diversity in cultivated sugarbeet germplasm was found to be low compared with other beet types and wild species using 111 polymorphic fragments across 41 diverse accessions of cultivated and wild beet (Jung et al. 1993). Cultivated beets may contain only one third of the genetic diversity present in sea beets (Fenárt et al. 2008; Saccomani et al. 2009). Diversity within crop types has been investigated most intensively within sugarbeet, and while diversity is reduced in sugarbeet relative to sea beets, evidence is consistent with all crop types having been selected from within the wild sea beet germplasm pool. Wild species diversity is viewed as a potential source of novel agronomic alleles (Frese et al. 2001b), and it is clear that much genetic and allelic diversity remains to be exploited in crop beet improvement. Interestingly, a complete class of disease resistance genes, the TIR-type, is completely lacking in *B. vulgaris* (Tian et al. 2004).

A system of cytoplasmic male sterility (CMS) makes hybrid sugarbeet production practical, with three genes (two linked) in the recessive state conferring the male sterile phenotype in a sterile cytoplasm, a condition, along with the recessive allele for monogerm seed, which has led to a narrowing of the genetic diversity of sugarbeet relative to wild types. Beets are normally allogamous, governed by a complex gametophytic self-incompatibility system, which prevents self-pollination but allows almost any two plants to cross-pollinate (Owen 1942; Bruun et al. 1995). Occurrence and distribution of novel mitochondrial types within crop and wild beet populations has been characterized (Saumitoulaprade et al. 1991; Cuguen et al. 1994; Forcioli et al. 1998; Desplanque et al. 2000; Sadoch et al. 2000, 2003; Fenárt et al. 2006; Nishizawa et al. 2007) in addition to the current economically important Owen (1945) S- (Sterile) and N- (Normal, fertile) cytotypes. Twenty-three mitochondrial cytotypes have been described, and non-random associations between chloroplast and mitochondrial cytotypes may indicate common cytoplasmic ancestry in some populations of sea beets. Expression of genes between normal and sterile cytoplasms has shown extensive differences but the same complement of genes (Ducos et al. 2001a, b; Ivanov et al. 2004; Satoh et al. 2004).

The sugarbeet N-type mitochondrial genome is sequenced (Kubo et al. 2000) and compared with other sequenced angiosperm mitochondrial genomes (Kubo and Mikami 2007). The sugarbeet mitochondrial genome is 368,799 bp and has a 43.9% G + C content. The beet mitochondria genome, as represented by a single male fertile genotype, is over twice as large as the chloroplast genome (368 kb), and encodes 59 recognizable genes (Satoh et al. 2004). Duplicated sequences, introns, unidentified open reading frames, and foreign sequences imported from the chloroplast and nucleus comprise much of the mitochondrial genome, and extensive rearrangements

between N- and S-type cytotypes are evident (Kubo et al. 1999), suggesting extensive repatterning mediated by short repeats, as well as incorporation of novel, nuclear, and plastid sequences (Satoh et al. 2006). Chloroplast features present in some *Beta* mitochondrial genomes may be phylogenetically informative (Kubo et al. 1995), and some unusual features of RNA editing in beet mitochondria have been described (Mower and Palmer 2006).

Loci involved in restoration of male fertility in a sterile cytoplasm, *X* and *Z*, have been located on Chromosomes 3 and 4, respectively (Schondelmaier and Jung 1997), with locus *X* located terminally on Chromosome 3 (Pillen et al. 1993; Uphoff and Wricke 1995), and likely confirmed independently by Hagihara et al. (2005a, b). A third putative locus was found ca. 15 cM from Z on Chromosome 4 by quantitative trait loci (QTL) analyses (Hjerdin-Panagopoulos et al. 2002). Using bulked segregant analysis and AFLP markers, Touzet et al. (2004) described a novel restorer locus for the G-cytotype on Chromosome 8. Previously, Laporte et al. (1998) demonstrated linkage of RAPD markers with monogermity (*m*) and a restorer for the H-cytotype, and suggested that this may not be novel since Owens gene *Z* and *m* are on Chromosome 4 (Schondelmaier and Jung 1997). Potential gene functions that could be "Implicated in fertility restoration of CMS are cytochrome-C oxidase and alternative oxidase (Ducos et al. 2001b, 2001a), and expression of an anther-specific lipid transfer protein (Matsuhira et al. 2007).

1.4 Role in Development of Cytogenetic Stocks and Their Utility

Tetraploids have been a staple tool for sugarbeet hybrid seed production, and there is no reason to assume that artificially induced polyploids in Section *Beta* would not behave in the same manner (Lange and De Bock 1989). With one exception noted to date, e.g., the Canary Island tetraploid forms of *B. macrocarpa* (Buttler 1977), species in Section *Beta* are uniformly diploid. Ploidy levels have been manipulated, and a low degree of autosyndetic pairing among at least three chromosomes is evident in haploids of sugarbeet (Yu 1980, cited in Nakamura et al. 1991). Pachytene chromosome morphology has been described in diploid sugarbeets (Nakamura and Tsuchiya 1982). Sugarbeet tetraploid pollinators have been used frequently to create triploid hybrids (often with >10% anisoploid plants) for commercial sugarbeet production in crosses with diploid CMS seed parents; however, current diploid hybrid germplasm has similar yield potentials and is less costly to develop, so fewer triploid varieties are being produced (Bosemark 2006). Two trisomic series have been produced in beets (Butterfass 1964; Romagosa et al. 1987, 1988) and each has a unique phenotype; however, these have been difficult to fix in a homozygous condition since the extra chromosome is rarely transmitted through pollen, making their application for genetic mapping difficult (Nakamura et al. 1991). Molecular markers were applied to the Butterfass series (Schondelmaier and Jung 1997; see also Lange et al. 1993; Oleo et al. 1993), and this has allowed the essential standardization of linkage group nomenclature among laboratories; however, discrepancies still exist in the older literature.

All *Beta* species are based on $x = 9$ chromosomes. Most species within Section *Beta* are presumed to be diploid ($2n = 18$), although cytogenetic evidence for most species is incomplete. No reports of cytogenetic differences or abnormalities have been reported within crop types or between hybrids within Section *Beta*, suggesting the group is a well-defined biological taxon (Abe and Tsuda 1987; Abe et al. 1987; Lange and De Bock 1989). Within *B. vulgaris*, increasing genetic diversity appears to promote genetic recombination (Kraft et al. 1998). The nine chromosomes of *Beta* spp. are morphologically similar at mitotic metaphase, with the exception of centromeres either metacentric or submetacentric and the presence of a terminal constriction, or satellite, on Chromosome 1 from sugarbeet and *Beta corolliflora* (Bosemark and Bormotov 1971; Nakamura et al. 1991; Gao et al. 2001). Monosomic and nullisomic plants have not been recovered, indicating that the true diploid nature of the crop and cytogenetic results are supported by linkage analyses of molecular markers where a lack of extensive chromosome duplication is documented (Halldén et al. 1998).

Hybridization between Section *Beta* and Sections *Corollinae* or *Procumbentes* is more difficult, with numerous meiotic abnormalities and high levels of male and female sterility described uniformly across interspecific hybrids where attempted (Filutowicz et al. 1971), with the former often described as easier

to hybridize than the latter; however, only specific interspecies crosses have been considered more useful for sugarbeet breeding, specifically *B. corolliflora* (Savitsky 1969) and both *B. procumbens* and *B. patellaris*. From these, monosomic addition lines have been created to attempt transfer of a number of useful disease resistances into beets, with some limited success to date.

There is great interest in Section *Procumbentes* since the three species (*B. procumbens, B. webbiana,* and *B. patellaris*) are immune to the sugarbeet cyst nematode (SBCN), and are also highly resistant to Cercospora leaf spot, the curly top virus, and rhizomania (Coons et al. 1955; Paul et al. 1992; Reamon-Ramos and Wricke 1992; Mesbah et al. 1997a). Species in this section are endemic to the western range of *Beta* species distribution. A great deal of effort has been applied in transferring nematode immunity into sugarbeet, and early efforts through 1987 are well summarized (Nakamura et al. 1991; Mesbah et al. 1997b), with many problems encountered, including male sterility and low female fertility, degeneration of root tissues in hybrids requiring grafting, and ultimately, little or no legitimate chromosome recombination between Section *Beta* and Section *Procumbentes* chromosomes. These efforts did result in translocations of nematode resistance into diploid sugarbeet (Savitsky 1978; De Jong et al. 1986; Jung and Wricke 1987; Nakamura and Tsuchiya 1988), and led to the cloning of the nematode resistance gene *Hs*1 (Cai et al. 1997), although additional genes are involved in expression of immunity (Jung et al. 1992a; Sandal et al. 1997; Kleine et al. 1998). Interestingly, translocations from all three Section *Procumbentes* species mapped to Chromosome 9 of sugarbeet, indicating a preferential recombination hotspot between chromosomes of these two sections (Heller et al. 1996), and the nucleotide sequence of this region has been determined but the precise chromosome breakpoints have not yet been confirmed (Schulte et al. 2006).

Interspecific hybrids between *B. vulgaris* and Section *Corollinae* have been created (Filutowicz et al. 1971 and references cited therein; Cleij et al. 1976) however, none have led to commercial application to date. In interspecific hybrids with *B. corolliflora*, fertility is reduced but an almost complete set of monosomic alien addition lines have been made, minus one attributed to lethal traits carried by that chromosome (Gao et al. 2001). Traits such as cold tolerance, curly top resistance, and Cercospora leaf spot resistance have been targets for these interspecific hybrids (Gao and Jung 2002). Apomixis is common in *B. lomatogona* and *B. corolliflora*, and one monosome showed a >95% transmission through seed, suggesting this monosomic addition line harbors genes involved in apomixis (Li et al. 2007).

Species-specific repeats and other DNA markers have been very useful to characterize interspecific hybrids, advanced generation materials, and cytogenetic stocks, as it partially obviates the need for cytological characterization, and provides a tool for cytological localization of sequences to chromosome regions using fluorescent in situ hybridization (FISH) (Schmidt et al. 1993, 1997; Kubis et al. 1997; Mesbah et al. 2000; Desel et al. 2002), including identification of particular chromosomes based on their hybridization signatures. Such techniques have been deployed in various approaches for examining interspecific *B. vulgaris* interspecific hybrids with *B. procumbens* and *B. patellaris* (Schmidt et al. 1990; Jung and Herrmann 1991; Jung et al. 1992b; Salentijn et al. 1992; Mesbah et al. 1996; Schmidt and Heslop-Harrison 1996a; Mesbah et al. 1997b; Dechyeva et al. 2003), as well as with *B. corolliflora* (Gao et al. 2000, 2001; Fang et al. 2004).

1.5 Role in Classical and Molecular Genetic Studies and in Crop Improvement Through Traditional and Advanced Tools

Much of the research pertaining to Sugarbeet breeding and genetics research has been summarized recently (Biancardi et al. 2005; Draycott 2006). Less information is available for the other crop types; however, much of the information is transferrable, especially as it pertains to disease resistance. Early breeding objectives were to increase the concentration and extractability of sucrose and little effort was placed on finding and maintaining high levels of host-plant resistance to insect, nematode, and disease pests. As sugarbeet production spread, diseases endemic to these new areas of cultivation were encountered and limited sucrose production, in some cases severely. Plant breeders

initiated the first systematic attempts to screen exotic and wild beet germplasm for resistance to these new diseases early in the twentieth century (Lewellen 1992; Frese et al. 2001b; Panella and Lewellen 2005, 2007).

As for most crops, the inheritance of color is both instructive and simple to phenotype. *Beta* is unusual in that it contains betalain pigments instead of the more familiar anthocyanins. Betalain pigments are red-violet betacyanins and yellow betaxanthins. Both are derived from betalamic acid following the cleavage of L-DOPA (Fischer and Dreiding 1972; Clement et al. 1992). Alleles at two linked loci (R and Y) control pigmenation in the beet plant (Keller 1936). Red roots are observed only in the presence of dominant alleles at the R and Y loci. White roots are conditioned by recessive alleles at both loci, which is characteristic of most sugarbeet cultivars. Betalain pigments extracted from red beet roots provide a natural alternative to synthetic red dyes and are used as commercial food coloring (von Elbe et al. 1974). Additional loci play influence the amount of betalain synthesized and stored by the beet root (Watson and Gabelman 1984)

Beet was domesticated, perhaps more than once, as a leaf pot herb along the coast of the Persian Gulf (Campbell 1976; Biancardi 2005), and, also perhaps, along the Mediterranean coast (Ford-Lloyd and Williams 1975; Ford-Lloyd 1995, 2005; Goldman and Navazio 2008). Leaf beet has been reported in China as long as 2,500 years ago (Shun et al. 2000). But the first preserved written record appears in classical Greek and Roman texts (Ford-Lloyd and Williams 1975; Biancardi 2005; Francis 2006). Beets with swollen roots as a table vegetable and herbal remedy appear in the sixteenth century and the use of beets for feeding cattle is described in the late eighteenth century (Ford-Lloyd 1995). Sugarbeet is a relatively new crop, and its development in the late 1700s followed Andreas Marggraf's demonstration that the crystalline sugar from beet (white mangold and red garden beet) was the same sweet substance, sucrose, that came from cane (Winner 1993).

As with most domesticated crops, continued gene flow was likely between the new domesticates and the progenitor (sea beet, *B. vulgaris* subsp. *maritima*) grown in that area (Harlan 1992). However, gene flow was increasingly restricted as the crop plant moved into areas outside the range of the wild progenitor. In areas where beets are grown for seed sympatrically with wild relatives, we still see evidence of gene flow between the crop and wild relatives (Bartsch and Schmidt 1997; El Manhaly et al. 1998; Desplanque et al. 1999; Arnaud et al. 2003; Alibert et al. 2005a, b; Andersen et al. 2005; Sukopp et al. 2005; Cureton et al. 2006). Fodder beet (White Silesian Rübe; Fischer 1989) probably provided the germplasm for early sugarbeet varieties; however, speculation exists that sea beet germplasm from the North Atlantic coast was unintentionally introgressed into Knauer's *Beta Imperialis* (the "mother of modern sugarbeet") (Bosemark 1979, 1989). Some have also claimed that sugarbeet to was derived from spontaneous crosses between chard and fodder (Zossimovich 1940; Fischer 1989). By the late 1800s, some began looking into sea beet and other wild species in the genus *Beta* as potential reservoir of genetic diversity to be introgressed into sugarbeet (von Proskowitz 1893).

The first example of tapping the genetic diversity of sea beet was in the Po Valley of Italy. Here, warm night temperatures and high humidity supply a perfect environment for Cercospora leaf spot (CLS) (caused by *Cercospora beticola* Sacc.). O. Munerati was the first plant breeder to collect sea beet germplasm and successfully incorporate it into a sugarbeet breeding program, when he utilized populations of sea beet growing in the Po estuary to introgress CLS resistance into sugarbeet (Munerati et al. 1913). This program produced, "Cesena," "Mezzano" and the "Rovigo" series (R 148, etc.) of cultivars that have formed the foundation of much of the *Cercospora*-resistant beet germplasm used worldwide (Munerati 1932; Biancardi and De Biaggi 1979). It is difficult to estimate the extent *B. vulgaris* subsp. *maritima* germplasm was used in commercial beet breeding programs of the times, especially because the undesirable traits from the wild beet that were introduced into these varieties, made the commercial plant breeders reluctant to use them. Nonetheless, European researchers and plant breeders crossed sea beet to cultivated beet (Rasmussen 1932, 1933; Tjebbes 1933), and there were additional attempts at introgressing CLS resistance (Stehlik 1947; Schlösser 1957), or other disease resistances into sugarbeet (Margara and Touvin 1955; reviewed by Asher et al. 2001). As a sugarbeet seed industry developed in the United States, interest in sources of resistance to the endemic pests and diseases expanded. Collection trips in 1925 and 1935 to look for CLS resistance and curly top resistance in wild beet

accession were led by G. H. Coons, who was familiar with Munerati's success (Coons et al. 1931). Other *Beta* species were collected as well (Coons 1975). Efforts were made by researchers to evaluate this material for resistance to CLS (Bilgen et al. 1969), Rhizoctonia root rot, and black root (Schneider and Gaskill 1962). Again, although there was continual contact and germplasm exchange among sugarbeet breeding programs worldwide, most of the exchanges were informal and it is impossible to document the use of material derived from wild species in commercial breeding programs (Lewellen 1992).

Species from sections of the genus outside of section *Beta* are outside the primary gene pool (Harlan and de Wet 1971) and do not easily hybridize to cultivated beet (Coons 1954; Van Geyt et al. 1990). Still researchers in the mid-twentieth century attempted to cross these species with species from within section *Beta*. In the US, Gaskill (1954) used cultivated Swiss chard as a bridging species for crosses with *Beta webbiana* and *B. procumbens*, and Oldemeyer (1954) used tetraploid sugarbeet (colchicine doubled), *B. macrocarpa*, *B. v.* subsp. *maritima*, and *B atriplicifolia* as parents from section *Beta* to cross with *B. webbiana* and *B. procumbens*. Oldemeyer and Brewbaker (1956) used a red table beet and sea beet as a bridge to cross with *B. webbiana* and *B. procumbens* and also made crosses with species of the section *Corollinea*. Seeds were produced but most of the F_1 hybrids were sterile, and many had degenerated root systems, and could only be grown to maturity by grafting onto healthy sugarbeet stems (Coe 1954; Johnson 1956). At this time, European researchers also were making interspecific hybrids, e.g., Seitz (1935) with *B. trigyna* and Margara (1953) with *B. lomatogona* (both section *Corollinea*). Again there were problems in fertility and survival. This work has been thoroughly reviewed by Coons (1975), de Bock (1986), and Van Geyt et al. (1990). The most important contribution to commercial germplasm of species outside section *Beta* is nematode resistance (Savitsky 1975; Cai et al. 1997). Although offering near immunity, the linkage drag of undesirable traits with this resistance has hindered its use in commercial hybrids (Yu 2005a).

In the 1960s, production of sugarbeet underwent a transformation. True hybrid varieties emerged utilizing CMS (and genetic fertility restoration) discovered by Owen (1945), and monogerm seed (Savitsky 1952). An initial single source of monogerm and CMS caused a genetic bottleneck about this time, which aggravated growing disease pressure, resulting from increased cultivated acreage and a shortened rotation between sugarbeet crops. In the 1980s, there was a renewed interest in wild beet germplasm as a source of disease resistance, overcoming the prevailing reluctance of commercial breeders to utilize exotic germplasm, perhaps because of earlier experiences with the undesirable traits accompanying resistance from this germplasm (Lewellen 1992; Frese et al. 2001b). In 1983 in the US, the Sugarbeet Crop Advisory Committee (CGC) began aggressively evaluating the *Beta* germplasm collection of the USDA-ARS NPGS (Doney and Whitney 1990; Panella and Lewellen 2007). In Europe, Bosemark (1989) created the theoretical framework to incorporate exotic germplasm into elite breeding programs, and Frese based his strategy to broaden the germplasm base of the sugarbeet gene pool on this work (see Frese 1990, 2000). The theoretical framework was put into practice through the World *Beta* Network, organized around the objective of improving international collaboration among users and curators of germplasm collections throughout the world.

Public plant breeders in the US (USDA-ARS) began utilizing sea beet germplasm, evaluated by the CGC, to develop sugarbeet germplasm, which was released to sugarbeet, table beet, leaf beet, and fodder beet seed companies, internationally (Lewellen and Whitney 1993; Doney 1998; Panella 1998; Yu et al. 1999). Currently, evaluation data from the NPGS *Beta* collection are contained in the GRIN Database. This database is publically accessible through http://www.ars-grin.gov/npgs (accessed August 2010) (Panella et al. 2003; Panella and Frese 2003). Concurrently, European public and private plant breeders, working collaboratively began a massive evaluation effort on an international core collection comprising 805 accessions of the IDBB (Ford-Lloyd et al. 2000; Frese 2000). Between 300 and 700 accessions of the core collection were used and screened by project partners for resistance to seedling diseases (caused by *Aphanomyces cochlioides, Phoma betae*), leaf diseases (caused by *Cercospora beticola, Erysiphe betae*, *Beet yellows virus*, *Beet mild yellowing virus*), Rhizomania (caused by *Beet necrotic yellow vein virus*), Rhizoctonia root and crown rot (caused by *Rhizoctonia solani*), as well as drought tolerance.

Evaluation data can be accessed at http://idbb.bafz.de/CCDB_PHP/idbb/ (accessed August 2010) (Panella and Frese 2003). Private and public plant breeders in Europe and throughout the world have used these results to introgress newly discovered sources of disease resistance into cultivated beet (Asher et al. 2001, 2009; Biancardi et al. 2002; Francis and Luterbacher 2003; Luterbacher et al. 2004, 2005; Panella and Lewellen 2007).

As the utilization of genes from wild beet genetic resources has grown, a tremendous increase in tools for genetic analyses has also become more available over the past 15 years. These techniques require genetic diversity between parents, and breeding has narrowed down the level of heterozygosity in beet germplasm (McGrath et al. 1999), although we know genetic diversity within *B. vulgaris* and among all section *Beta* species is high (Mita et al. 1991; Jung et al. 1993; Kraft et al. 1997; McGrath et al. 1999; Wang and Goldman 1999; Richards et al. 2004; Fievet et al. 2007). Many of the mapping population parents have some exotic germplasm, especially where prebreeding has been used to introgress pest resistance (Lange and De Bock 1994), disease resistance (Campbell and Bugbee 1993; Giorio et al. 1997; Lewellen and Schrandt 2001), plant architecture traits (Theurer 1993), and combining ability (Doney 1993), and already some marker discovery has been done on hybrids from exotic sources and sugarbeet parents (Friesen et al. 2006; Grimmer et al. 2007b; Asher et al. 2009). As we continue to develop more efficient methods to transfer genes from wild relatives into cultivated beet crops, accurate screening of exotic germplasm at an early stage will become more important to determine not only the magnitude of the allelic effect at specific loci but also pleiotropic effects of allelic interactions at different loci (Panella 2008).

Rhizomania ("crazy root," caused by beet necrotic yellow vein virus – BNYVV) has caused major reduction in sugar production wherever it has occurred. As soon as rhizomania was identified, an extensive screening of genetic resources to identify host-plant resistance and to incorporate resistance into elite sugarbeet germplasm was initiated (Biancardi et al. 2002). Erichsen discovered a single dominant resistance gene, the "Holly" gene, at Tracy, CA in 1983 (Lewellen et al. 1987). This gene confers strong resistance to BNYVV and was named *Rz*1. The resistance in the hybrid "Rizor" (developed by SES Europe, now SESVanderHave, in Italy) (De Biaggi 1987; Biancardi et al. 2002) and *Rz*1 were the only major gene resistances discovered in commercial sugarbeet hybrids (Scholten and Lange 2000; Biancardi et al. 2002).

WB42, a *B. vulgaris* subsp. *maritima* accession from Denmark carries resistance to rhizomania that was shown to be different from *Rz*1, and confers a higher level of resistance in growth chamber testing, and was considered a different gene, *Rz*2 (Scholten et al. 1996, 1999; Reza et al. 2003). It has been incorporated into released sugarbeet germplasms C37 (PI 590715), C48 (PI 538251), and C79-3 (PI 593662) (Lewellen et al. 1985; Lewellen and Whitney 1993; Lewellen 1997). Most sources of resistance from *B. vulgaris* subsp. *maritima*, conditioned by a single gene have been shown to be either *Rz*1 or *Rz*2 (Biancardi et al. 2002); however, there may be more sources as yet undiscovered. Another resistance gene on Chromosome III, linked to *Rz*1 and *Rz*2, has been reported by Gidner et al. (2005) and assigned *Rz*3. *Rz*3 has shown varying resistance expression as a heterozygote, and was mapped from WB41 (*B. vulgaris* subsp. *maritima* from Denmark) in crosses with sugarbeet. Gidner et al. (2005) described plants with combined *Rz*1 and *Rz*3 or *Rz*2 in a heterozygous condition having a lower virus titer than with *Rz*1 alone. Earlier, WB41 resistance was backcrossed into sugarbeet and released as C79-2 (Lewellen 1995, 1997). Mapping evidence suggests that C79-4 and C28 resistance (Lewellen 1991, 1995, 1997) may be different than *Rz*1, *Rz*2, or *Rz*3 (Panella and Lewellen 2007). Two additional genes, also on Chromosome III, *Rz*4 and *Rz*5, were mapped to the same chromosomal region as *Rz*1 and *Rz*3, using molecular markers (Grimmer et al. 2007a, 2008). However, there has not yet been a concerted effort to determine if these single gene rhizomania resistances are different genes, or alleles at, perhaps, a complex resistance-gene locus (Hunger et al. 2003).

In 2003, a new strain of rhizomania that defeated *Rz*1 resistance was discovered in California (Liu et al. 2005) and then Minnesota (Rush et al. 2006). *Rz*2 and *Rz*3 conferred partial resistance, which was significantly influenced by minor host-reaction genes (Rush et al. 2006). Fortunately, progeny families of C79-9 (derived from PI 546397 – another *B. vulgaris* subsp. *maritima* accession from Denmark) appeared to have high resistance to this new strain of rhizomania (Lewellen 1995, 1997). The inheritance and allelism

of this resistance has not been determined; however, it is clear that these accessions represent an important reservoir of rhizomania resistance genes (Panella and Lewellen 2007).

The sugarbeet cyst nematode (SBCN) (*Heterodera schachtii* Schm.) is an important soil-borne sugarbeet pest worldwide. Until recently, near immunity to SBCN damage was obtained by a terminal translocation of Section *Procumbentes* chromatin with a gene, $Hs1^{pro}$, onto sugarbeet (see above, and Jung et al. 1994; Kleine et al. 1995). Commercial hybrids with this source of resistance have a serious (10–15%) yield penalty in the absence of nematode infestation. Problems associated with $Hs1^{pro}$ caused breeders to reevaluate the partial resistance to SBCN known from *B. vulgaris* subsp. *maritima* (Heijbroek 1977). Hybrids have been commercialized with this resistance e.g., "Beta 8520N," Betaseed, Inc. and "Pauletta," KWS GmBH. USDA-ARS researchers in Salinas have identified and introgressed sources of apparent moderate SBCN resistance (Lewellen 2006, 2007). Moderate resistance had been reported in PI 546413 (WB 242 – *B. vulgaris* subsp. *maritima* from Loire river estuary in France) (Heijbroek et al. 1977) and has been introgressed into public germplasms (Lewellen 2004a, 2006). Another *B. vulgaris* subsp. *maritima* accession (N499, PI 599349) provided population CN72 (PI 636339) (Lewellen 2006). Greenhouse and field tests in infested soil indicate that moderate to high SBCN resistant plants segregate within these materials (Lewellen 2006, 2007). Another source was from C50 (Lewellen and Whitney 1993) and C51 (PI 593694) (Lewellen 2000). C51 families segregated for SBCN resistance in the Imperial Valley of California (Lewellen 2004b, 2007). Screening of the NPGS *Beta* collection's genetic resources has indicated a number of accessions with resistance or segregating for resistance to SBCN, and these are being introgressed into sugarbeet (Panella and Lewellen 2007).

Root-knot nematode (RKN) (*Meloidogyna* ssp.) may cause severe yield loss in sugarbeet cultivated in warmer temperate areas. Evaluation of *B. vulgaris* subsp. *maritima* germplasm has yielded two unique RKN resistance sources (single gene) (Yu et al. 1999). PI 546426 (WB 258 from the Po Delta of Italy) was developed and then released as the germplasm, Mi-1 (PI 593237) (Yu 1997). Yu (2002) developed additional germplasms from this source (M1-2 and M1-3), and a linked isozyme marker was developed (Yu et al. 2001). Another germplasm was developed from PI 546387 (WB66) (Yu 1996), released and, then, improved as M6-1 and M6-2 (Yu and Lewellen 2004). Weiland and Yu (2003) identified a cleaved amplified polymorphic sequence (CAPS) marker linked to this resistance gene (see also review by Yu 2005b).

1.6 Genomics Resources Developed

A total of 150,290 records for *Caryophyllales* were in the NCBI database as of 1 May 2009 with a total of 97,862 expressed sequence tags (ESTs), 80% of these from only three species, *B. vulgaris* (29,654 ESTs), *Mesembryanthemum crystallinum* (common ice plant, 27,385 ESTs), and *Tamarix hispida* (17,401 ESTs), the latter two are model halophyte species. Only nine of the families in the order have any EST representation. Most records are from numerous phylogenetically informative species (e.g., Fior et al. 2006; Cuènoud et al. 2002), most with only 1–10 records. Other sequences have been derived for various biologically relevant questions, including salt and drought stress ESTs, sex dimorphism or sperm cell development ESTs, jojoba (oil) ESTs, and spinach photosystem genes as a model photosynthesis organism. Clearly there is a need for additional comparative sequencing because this depth is insufficient to represent diversity among the >11,000 recognized species.

All *B. vulgaris* ESTs are from sugarbeet, and represent a reasonable cross-section of important tissue types, including genes induced upon nematode infection (Samuelian et al. 2004). The majority of ESTs were generated after oligo-fingerprinting of cDNA libraries (Bellin et al. 2002; Herwig et al. 2002), and there is good breadth of coverage (>18,000 contigs) but little depth for assessing the level of gene expression changes. In addition, 31,138 genome survey sequences have been deposited, primarily derived from paired-end bacterial artificial chromosome (BAC) and fosmid clones (McGrath et al. 2004; Lange et al. 2008). A total of 187 other sequences are available in GenBank for *Beta* species other than *B. vulgaris*.

A number of yeast artificial chromosome (YAC) and BAC and other DNA libraries of beet have been

made for various purposes, including cloning of genes controlling nematode resistance, flowering, bolting gene, apomixes, CMS restoration and centromeres (Jung et al. 1990, 1992a; Eyers et al. 1992; Delfavero et al. 1994; Klein-Lankhorst et al. 1994; Kleine et al. 1995; Gindullis et al. 2001a; Hohmann et al. 2003; Fang et al. 2004; McGrath et al. 2004; Hagihara et al. 2005a, 2005b; Schulte et al. 2006; Reeves et al. 2007; Jacobs et al. 2009). An oligo fingerprinting approach to physical map construction is underway, and a draft *B. vulgaris* genome sequence should be available in the near future. A 130 kb BAC clone containing the NPR1 disease resistance locus has been sequenced (Kuykendall et al. 2008b), as has the entire *B. procumbentes* region translocated into *B. vulgaris*, of which ca. 480 kb of contiguous nucleotide sequence is reported (Schulte et al. 2006). Evident among the total of ca. 600 kb of sequence are 150 open reading frames (ORFs), which at a first level of approximation represent a potential to be expressed genes, or four ORFs per kb. However, relatively few expressed genes represented by ESTs are represented by these ORFs (4.25% in the case of Kuykendall et al. (2008b), the actual number of EST similarities in the *B. procumbens* translocation was not given although 12 were described with a relationship to stress and transcription factors). The majority of ORFs represent unknown gene-like sequences, retro-transposons, or DNA transposons, ranging from 16.1% to 43.3% of sequence depending on the particular BAC clone analyzed.

1.7 Scope for Domestication and Commercialization

According to Alexander (1971), a "typical" sugarbeet taproot can be divided in water (75%) and dry matter (25%), and ca. 75% of the dry matter is sucrose. Nitrogenous compounds compose only 1.8% of non-sugars. Sucrose is perhaps the most abundant, chemically pure, renewable resource on the planet, and can be used directly for fuel production (ethanol) or as an adjuvant to facilitate fermentation of more complex carbohydrate mixtures. Small changes to the sucrose molecule, described as over-functionalized with reactive groups, could create new industrial uses for such sucrose-like molecules, and such changes conceivably could occur in planta. Such uses would create a new market. All parts of the vegetative beet currently have an economic outlet (seeds, e.g., fruits, are not currently used, but have potential applications). Coproducts of factory processing are (1) molasses used as an animal feed ingredient or for the production of ethanol, glutamate, glycine–betaine, and as a nitrogen source in bioreactors, (2) pulp for animal, including pet, food, and (3) lime ($CaCO_3$) used for improving acid soils.

An impressive modification of the plant morphology is evident, not only among the cultivated types themselves, but also among extant wild beet populations. In garden, fodder, and sugarbeets, the shape and the composition of the root became completely different from wild types, whereas in the leaf beet, only the foliar apparatus has been remarkably modified (Biancardi 1999). Common pathogens do not discriminate between crop types, so breeding for resistance is a common feature of all beet improvement programs. Often, resistances have been identified in sugarbeet and then transferred to other crop types (Goldman and Navazio 2008). Wild beets will unlikely be directly exploited for new products or uses, rather they will continue to be used as a resource to improve the current cultivated germplasm wherever possible. While a complete list of secondary metabolite compounds is not available for *Beta* species, one can speculate that the *Carophyllales* as a whole contain some undiscovered and potentially useful novel phytochemicals.

Sucrose and pigments accumulate in vacuoles of parenchyma cells, located in between concentric cortical rings that are a unique and distinguishing feature of beets (Artschwager 1926; Hayward 1938). Accumulation and storage of biochemicals to economically recoverable levels, the reason for the sugarbeet, is limited to sucrose, but other industrial chemicals (betalain pigments, sucrose esters, fructans, specialty lipids, ascorbate) theoretically could be produced economically in beets (see Gurel et al. 2008). Protein concentrations are generally low in storage roots, and recovery of high-value bio-ceuticals could be facilitated. Fresh beet products are good dietary sources of potassium and folic acid, low in protein, and the betalin pigments have potential as antioxidants (Cai et al. 2003; Stintzing and Carle 2004).

1.8 Some Dark Sides and Their Addressing

1.8.1 Gene Flow

The existence of crop–weed complexes is well known and described in the literature (Pickersgill 1981; Harlan 1992). Weed beet has been characterized as fully interfertile with domestic beet and wild sea beet and, therefore considered taxonomically as a subspecies in *B. vulgaris* (De Bock 1986; Ford-Lloyd 1986; Ford-Lloyd and Hawkes 1986). This has been a long-term problem in Europe (Longden 1976; Boiteau and Christmann 1977; Hornsey and Arnold 1979), especially in areas where sea beet and domestic beet seed production both occur (Boudry et al. 1993; Desplanque et al. 1999). The wild relatives of sugarbeet are not native to the United States (De Bock 1986). Weed beets generally have not been a persistent problem in the United States (except for California). Growers are careful to remove flowering (bolting) plants from their fields, and chemical weed control is used to control weed beets in rotation crops. Weed beets are only a problem when present in the cultivated beet crop. The story in California is a little bit different due to the introduction of sea beet and *B. macrocarpa*. California's climate is a Mediterranean climate with hot dry summers and cool wet winters, ideal for both *B. macrocarpa* and *B. v.* subspecies *maritima*. Carsner (1938) described annual beets along the coast and in the San Joaquin Valley that appeared to be "hybrids between cultivated sugar beets and wild forms of foreign origin" (*B. v.* subsp. *maritima*), and a wild beet in the Imperial Valley that was different from the sea beet hybrids (*B. macrocarpa*). The sea beet weedy types proved to be compatible with sugarbeet (Dahlberg and Brewbaker 1948), and the major concern was the harboring of sugarbeet disease in these weed beets (Carsner 1938; Johnson and Burtch 1959).

In 1975, McFarlane (1975) determined the two types of wild beet to be *B. macrocarpa* and *B. v.* subspecies *maritima* and noted types with an intermediate morphology between *B. vulgaris* and *B. macrocarpa*. More recently, allozyme works has given a clearer picture of the origin of the wild beets in California (Bartsch and Ellstrand 1999). The *B. macrocarpa* found in the Imperial Valley were speculated to have come as a contaminant of chard or red beet, in the ballast sand of ships from Spain, or as contaminants in feed grain brought by Spanish in the late eighteenth or early nineteenth century (Dahlberg and Brewbaker 1948; McFarlane 1975; Bartsch and Ellstrand 1999). For the sea beet types, Bartsch and Ellstrand (1999) postulated three potential origins, introduced sea beets, feral descendants of chard and red beet, or hybrids between sea beet and cultivated beet. Currently these *B. macrocarpa* populations are of great interest.

The renewed interest in gene flow between wild and cultivated beet is due the commercial introduction of transgenic sugarbeet in the United States with tolerance to the herbicide glyphosate, and other anticipated products of biotechnology in the future. This discussion has been ongoing (Boudry et al. 1993; Dietz-Pfeilstetter and Kirchner 1998; Ellstrand et al. 1999; Saeglitz et al. 2000; Bartsch et al. 2001; Bennett et al. 2004; Cureton et al. 2006; Fenárt et al. 2007; Fievet et al. 2007; Wozniak 2007; Sester et al. 2008; Darmency et al. 2009). Much of the research has focused on whether there is gene flow and how to quantify it (Bartsch and Schmidt 1997; Bartsch et al. 1999; Desplanque et al. 1999; Arnaud et al. 2003; Alibert et al. 2005a, b; Cuguen et al. 2005; Fenárt et al. 2008). Additionally, the direction and distance of pollen dispersal has been given much attention (Pohl-Orf et al. 1999; Saeglitz et al. 2000; Fenárt et al. 2007; Darmency et al. 2009). The fact that there is documented gene flow between weedy, wild, and domestic beets in Europe is one of the barriers to the commercial use of transgenic sugarbeet in Europe. Risks associated with growing transgenic beets were recently summarized (OECD 2008).

1.8.2 Databases

Data on cultivated beets and wild species are generated worldwide. These data can be grouped into four classes (1) taxonomic, (2) ecogeographic, (3) characterization and evaluation data, and (4) genetic and genomic data. The majority of passport, characterization, and evaluation data relate to genebank accessions managed by the USDA/ARS NPGS. In particular, evaluation data are continuously generated in screening programs coordinated by the sugarbeet crop germplasm committee and uploaded to the GRIN.

Additionally, taxonomic portion of GRIN provides the classification and nomenclature for these genetic resources and many other economic plants on a worldwide basis. Included in GRIN TAXONOMY are scientific names for 26,521 genera (14,132 accepted) and 1,230 infragenera (1,199 accepted) and 91,233 species or infraspecies (54,881 accepted) with common names, geographical distributions, literature references, and economic impacts. Because Europe lacks a comparable coordinated system, not only in the field of *Beta* genetic resources, evaluation data do not arrive in a central system, and new information on wild beet genetic resources cannot be accessed readily, if at all, by the scientific user community or by policy and decision makers responsible for the conservation and management of biological diversity.

Currently, data on *Beta* are distributed over a number of global, European, and national multispecies information systems. Frese and Germeier (2009) highlighted the complementarity of the in situ and ex situ genetic resource conservation approaches and stressed that both management strategies often rely on identical data sources such as taxonomic systems or geographic data. Despite this obvious synergistic potential, all systems are operated separately. The insufficient compatibility between the systems appear at the genus/crop level only when crop experts wish to work with data hosted by these different systems. A medium quality of taxonomic data is one obvious weakness of multispecies information systems.

The Global Biodiversity Information Facility (GBIF, http://www.gbif.org/, accessed August 2010) holds information on occurrences (specimens and living material) of *Beta*. GBIF hold an array of interesting distribution maps but also a striking diversity of taxonomic names (more than 300 different designations such as accepted names, synonyms, illegible names, and names with typing mistakes). In addition, GBIF contains duplicated information at the accession level which is hard to identify. The European Environment Agency (*EUNIS*, http://eunis.eea.europa.eu/, accessed August 2010) keeps records on *Beta* occurrences within formal protected areas, the European PGR Search Catalog (*EURISCO*, http://eurisco.ecpgr.org/static/index.html, accessed August 2010) contains passport data on *Beta* accessions held in European genebanks, but only those that were officially notified by the national plant genetic resources program coordinators with the effect that the International Database for *Beta* may report more accessions available in a national genebank system than EURSICO.

Characterization and evaluation data are kept in European national genebank systems or as files in the scientific community. Access to these data is not straightforward. Genomic data on *Beta* are generated by GABI (genome analysis within the biological system plant) in Germany and by the French partner program Genoplant. Public access to the information platform (GABIPD – Primary Database http://www.gabipd.org/, accessed August 2010) is possible. Under these circumstances, it is not easy for a lab scientist to acquire from the international germplasm conservation and management system a set of accessions or in situ population samples best meeting the needs. It is not possible for a conservation biologist to learn about the specific value of a population for BNYVV resistance breeding growing in situ just by querying an information system or to take rational management decisions on *Beta* populations occurring within a protected site based on genetic distance measurements. This information is mainly available in literature. For instance, knowledge on the exact geographic origin of the BNYVV resistant wild beet sources WB41 and WB42 is traced in papers of Gidner et al. (2005) and Lewellen et al. (1987) but is difficult to find in the database. Similarly, genetic distance information between wild beet populations have been published for *B. vulgaris* subsp. *maritima* by Fievet et al. (2007) and Fenárt et al. (2008), but are not readily web-available. Frese and Germeier (2009) outlined steps to build a comprehensive global and crop-specific information system on *Beta* genetic resources. However, the implementation of the concept would require a broad support from the genetic resources management, the scientific and user community, as well as policy, and is therefore pending.

1.9 Recommendations for Future Actions

A beet genome sequence would impact science and society, in part leading to enhancement of rural economies by creating specialty chemical production opportunities, advancing bioinformatics of plant genomes and their evolution, and uncovering potential uses for

beets not available in other crops due to beets' unique taxonomic position. Further, comparative genomics opportunities could allow design and testing environmentally benign but effective herbicides for Chenopod weeds and contribute to agricultural sustainability. The Caryophyllales are a large group of plant often found in marginal, stressful habitats (Stevens 2001 and onwards). A few species within this group have achieved notoriety, notably sugarbeet and carnation, and many others are used routinely. The problem is how to take this unique group beyond molecular taxonomy to discovering useful genetic diversity. There is no *Caryophyllales* community yet to provide guidance, but some species have well-developed networks of academic or commercial interests. The first task is to provide circumstances for genomic unification among this group, e.g., a readily accessible, comprehensive, state-of-the-art descriptive gene and genome summary for the *Caryophyllales*. Next is to expand the repertoire of species and families represented, and foster cross-species discovery of genome similarities and differences.

Evaluation of germplasm continues to be critical in identifying those accessions that have genes of interest. This is clearly illustrated in the screening for pest and disease resistance, including development of disease and stress-screening techniques, in the field, greenhouse, and laboratory (e.g., Ruppel and Gaskill 1971; Ruppel et al. 1979; Panella 1998, 2000; McGrath et al. 2000, 2008; Scholten and Lange 2000; Scholten et al. 2001; Büttner et al. 2004; Stark et al. 2006). Evaluation methodology (streamlined phenotyping) is another piece of information that commercial seed companies want with enhanced germplasm, and such methods can be developed in conjunction with screening wild and cultivated germplasm with the rationale that dissecting the molecular basis of traits leads to more precise phenotyping methods.

As we attempt to utilize exotic germplasm, it is important to characterize and understand the genetic diversity of those populations. Molecular markers provide a useful tool to supplement characterization by morphological traits and disease response. Although neutral markers may be used to quantify diversity, in looking at genetic diversity along the Atlantic coast of France, a key vernalization regulatory gene, BvFL1, was mined from an EST database (Reeves et al. 2007).

Gene discovery, be it in wild or domesticated populations, provides a new way to characterize genetic resources as well as providing the most useful marker – the gene itself. SNPs within genes (and having a phenotypic effect) will become more common and more useful as we characterize them and understand their pleiotropic effects. They are useful tools to describe alleles and measure allelic diversity. Already, important progress is being made in developing useful SNPs in sugarbeet (Grimmer et al. 2007a; Schneider et al. 2007). As more markers (protein or DNA) are developed, and more genes influencing important traits are cloned and characterized, pre-breeders will be introgressing characterized genes rather than empirical phenotypes. They also will be going outside of sugarbeet's primary gene pool and into the secondary and tertiary gene pools (Harlan and de Wet 1971). Evaluation will not be concentrated only on whether the trait is present but on the biochemistry and physiology of how a particular phenotype is manifest. Mapping genes is important, but more important may be the discovery and knowledge of allelic diversity in both cultivated and wild beets, as well as the allelic interactions (at crucial loci) and their effects on phenotype.

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Chapter 2
Corchorus

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Please note the Erratum to this chapter at the end of the book

2.1 Introduction

2.1.1 Origin, Diversity, and Distribution

The genus *Corchorus* belonging to the family Malvaceae (formerly under Tiliaceae) is distributed throughout the tropical and subtropical regions of the world (Kundu 1951; Purseglove 1968; Chang and Miau 1989). Although 215 species, subspecies, varieties, and forms have been reported under the genus *Corchorus* (Global Biodiversity Information Facility 2008: http://www.gbif.org), precise number of good species is approximately 100 (Saunders 2006). Out of 100 good species, *Corchorus capsularis* and *Corchorus olitorius* were selected and domesticated in the wake of civilization and are the commercially important cultivated species of *Corchorus*. The others were found wild in nature, but most of them are now extinct or in endemic condition. However, being an extremely variable genus, its natural distribution, genetic and evolutionary relationships, as well as center of origin are poorly documented, virtually controversial and yet to be resolved. Wild *Corchorus* taxa are mostly distributed in the tropical/subtropical regions of Africa, America (including Brazil, Mexico, Bolivia, Venezuela, and West Indies), Australia, China, Taiwan, India, Myanmar, Bangladesh, Nepal, Sri Lanka, Japan, Indonesia, Thailand, Malaysia, and Philippines (Fig. 2.1; Kundu 1951; Brands 1989–2007).

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The primary center of origin and diversity of the wild taxa of *Corchorus* appears to be Africa, where large numbers of species have been reported includingthe two cultivated species *C. capsularis* and *C. olitorius* (Kundu 1951; Singh 1976; Edmonds 1990). Africa represents approximately 46 species (Aluka 2006–2008: http://www.aluka.org), viz., *C. acutangulus, C. aestuans, C. africanus, C. angolensis, C. antichorus, C. asplenifolius, C. baldaccii, C. brevicornutus, C. brickchettii, C. capsularis, C. cinerascens, C. confusus, C. depressus, C. discolor, C. echinatus, C. fascicularis, C. gillettii, C. gracilis, C. hirsutus, C. hochstetteri, C. junodii, C. kirkii, C. longipedunculatus, C. malchairii, C. merxmuelleri, C. microphyllus, C. mucilagineus, C. muricatus, C. olitorius, C. parvifolius, C. pinnatipartitus, C. pongolensis, C. psammophilus, C. pseudo-capsularis, C. pseudo-olitorius, C. quadrangularis, C. quinquenervis, C. saxatilis, C. schimperi, C. serrifolius, C. somalicus, C. sulcatus, C. tridens, C. trilocularis, C. urticifolius*, and *C. velutinus*. Though *Corchorus* species occur throughout the Africa, the largest numbers were reported from the eastern and southern part of the continent. The Republic of South Africa is the richest source of diversity with 16 species followed by Tanzania, which represents 13 species. Ethiopia, Mozambique, and Zimbabwe represent 12 species each, while 11 species of *Corchorus* were reported from Kenya. Zambia has nine species of the wild taxa of *Corchorus* (Edmonds 1990; Plants of South Africa 2009: http://www.biodiversityexplorer.org/plants/malvaceae/corchorus.htm). The dominating *Corchorus* species showing wide range of adaptability are *C. tridens*, *C. trilocularis*, *C. aestuans,* and *C. olitorius*. *Corchorus fascicularis*, *C. pseudo-olitorius,* and *C. urticifolius* are scarce in distribution, but *C. baldaccii*, *C. brevicornatus*, *C. pseudo-capsularis,* and *C. schimperi* are rare and endemic.

C. Kole (ed.), *Wild Crop Relatives: Genomic and Breeding Resources*, *Industrial Crops*,
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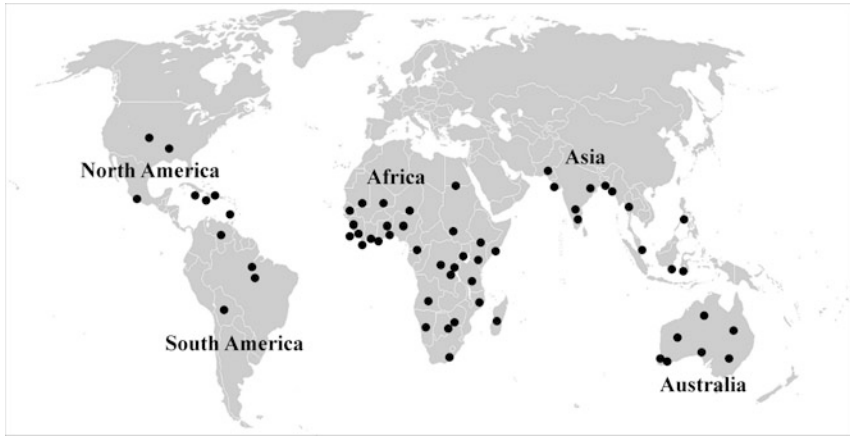

Fig. 2.1 Distribution pattern of wild species of *Corchorus* in Africa, Australia, South America, Caribbean Island, and Indian subcontinent

Interestingly, 53 species of the genus *Corchorus* including the two cultivated species *C. capsularis* and *C. olitorius* have been recently reported from Australia and out of which 21 are considered to be endemic. Among these endangered species, *Corchorus cunninghamii* and *C. pascuorum* are natural tetraploids ($2n = 4x = 28$), which are rare in the genus *Corchorus* (Australian New Crop Website 2008: http://www.newcrops.uq.edu.au). The *Corchorus* species are well distributed and occur mainly in New South Wales, northern Territory, Queensland, southern Australia, western Australia, northern Botanical Province, and Eremaean Botanical Province (Halford 2004; Coleman 2008). The native of 32 species of wild *Corchorus* appears to be Australia (Hinsley 2006, 2008; Kew Science Directory 2009: http://www.kew.org/epic) and perhaps it is the secondary center of origin of the wild taxa of *Corchorus,* viz., *C. allenii*, *C. aulacocarpus*, *C. australis*, *C. carnarvonensis, C. congener, C. cunnighamii, C. elachocarpus, C. edderi, C. hygrophilus, C. incanus, C. interstans, C. lasiocarpus, C. leptocarpus, C. lithophilus, C. macropetalus, C. mitchellensis, C. obclavatus, C. pascuorum, C. puberulus, C. pumilio, C. reynoldsiae, C. rothii, C. saxicola, C. sericeus, C. sedoides, C. subergentus, C. sublatus, C. tectus, C. thozetii, C. tomentellus, C. vermicularis,* and *C. walcottii*. The two cultivated species *C. capsularis* and *C. olitorius* and other wild flora like *C. acutangulus, C. aestuans, C. argutus, C. hirsutus, C. hirtus, C. tridans, C. trilocularis, C. echinatus, C. pseudo-capsularis,* and *C. longipes* were either migrated or introduced from Indo-Burma region, Africa, and Latin America.

Around 30 wild taxa of *Corchorus* are well distributed in the America (USA, Brazil, Mexico, Bolivia, and Venezuela) and Caribbean Island. Wild species including *C. aquaticus, C. biflorus, C. campestris, C. hirtus, C. siliquosus, C. terresianus, C. pilosus, C. orinocensis, C. argutus, C. coreta, C. lanuginosus, C. mompoxenis, C. tortipes, C. americanus,* and *C. neocaledonicus* are the native of this region.

In Asia, wild species are distributed in India, Bangladesh, Pakistan, Thailand, Indonesia, but none of them are native to this continent. The exact number of native Indian *Corchorus* species is yet to be known, but the two cultivated species are distributed throughout India and *C. capsularis* is assumed to be native of Indian subcontinent although there are still controversies (Kundu 1951). *C. aestuans* L., *C. depressus* Stooks L., *C. fascicularis* Lam., *C. pseudo-olitorius* Islam and Zaid, *C. tridens* L., *C. trilocularis* L., *C. urticifolius* Lam., and *C. velutinus* Her. were reported from India (Mahapatra et al. 1998) and most of the wild species were reported to be migrated from Africa. The existence of *C. velutinus* is still doubtful as the herbarium specimen is yet to be collected or reported (Mahapatra et al. 1998), but there is no doubt about the presence of *C. pseudo-capsularis* that has not been reported earlier. *C. aestuans*, the most dominating wild species of India is distributed from southern peninsular of Tamil Nadu and Kerala to mountainous region of Jammu and Kashmir in the extreme north. It is followed by *C. tridens, C. trilocularis,* and *C. fascicularis. Corchorus tridens* and *C. trilocularis* were found to be restricted to central, western, and southern part of the country. *Corchorus urticifolius* and *C. pseudo-capsularis*

are also restricted to Tamil Nadu, while *C. pseudo-olitorius* is distributed in the western boundary of the country. *C. depressus* occurs mainly in the semi-arid region of Rajasthan, Gujarat, Tamil Nadu, and Punjab (Mathur and Sundaramoorthy 2008).

2.1.2 Genetic Resources of Corchorus Species

The International Jute Organization (IJO), Dhaka, Bangladesh initiated a project in 1987 to enrich the germplasm in collaboration with its member countries, viz., Bangladesh, China, India, Indonesia, Nepal, and Thailand. The IJO appointed Dr. J. M. Edmonds, a herbarium consultant, to conduct an in-depth survey of the major herbaria in Europe and East Africa to provide the basis for a series of IJO germplasm exploration missions. This effort resulted in the publication of a technical report on distribution pattern of *Corchorus* in Africa. It also described the germplasm potential of different *Corchorus* wild species. Based on species richness of *Corchorus*, Tanzania (13 species) and Kenya (11 species) were selected as the target for the IJO exploration missions. A total of 374 seed samples representing 12 *Corchorus* species (*C. aestuans, C. baldaccii, C. brevicornutus, C. fascicularis, C. olitorius, C. pseudo-capsularis, C. pseudo-olitorius, C. shimperi, C. tridens, C. trilocularis, C. urticifolius,* and one unknown species) were collected. Explorations were also made in different countries including China, Indonesia, Nepal, Thailand, and Pakistan to collect wild *Corchorus* germplasm from their natural habitats. IJO has also acquired more than 300 germplasm accessions through correspondence with USDA (USA), CSIRO (Australia), CENARGEN (Brazil), and IBPGR (Italy). As a result, a total of 2,300 accessions were collected by IJO and distributed to different countries for evaluation, conservation, and utilization in *Corchorus* breeding programs.

The Gene bank of the Germplasm Division, Bangladesh Jute Research Institute (BJRI) has been designated as the IJO Centralized Germplasm Repository (CGR). This Gene Bank has the capacity of preserving 100,000 accessions of jute germplasm. As of 2007, 4,081 accessions representing 15 species of *Corchorus* are under preservation in two sets, one set at −20°C and another under +4°C in the Gene Bank of BJRI.

In India, till early 1970s, plant breeders had worked with a gene pool of mere 300 accessions, mainly of local collections, of both cultivated and eight wild relatives of *Corchorus*. Systematic studies on distribution and diagnostic features of *Corchorus* species were initiated in 1977 through direct explorations and correspondence (Mahapatra et al. 1998). Majority of the collections in India were made by the Central Research Institute for Jute and Allied fibers (CRIJAF) of the Indian Council of Agricultural Research (ICAR) and National Bureau of Plant Generic Resources (NBPGR). Later on, during 1999–2004 under the National Agricultural Technology Project (NATP) 655 accessions covering landraces and wild relatives of *Corchorus* and allied fiber crop species from different agroclimatic regions were collected and characterized. As of 2008, CRIJAF had a working collection of 2,899 *Corchorus* accessions comprising 939 *C. capsularis*, 1,647 *C. olitorius,* and 313 wild relatives representing eight species. Active collections of all *Corchorus* species are conserved in the mid-term gene bank of CRIJAF and the base collections are in the National Gene Bank at NBPGR, New Delhi.

2.1.3 Cytology and Karyotype

The base number of chromosomes in almost all the species of *Corchorus* is 7 ($2n = 2x = 14$). The haploid number of chromosomes in *C. capsularis, C. olitorius, C. aestuans, C. tridens, C. trilocularis, C. fascicularis, C. depressus, C. pseudo-capsularis, C. baldaccii* Mattei, *C. gilletti* Bari, *C. sidoides, C. neocaledonicus* Schlechter, *C. hrisutus, C. walcottii, C. elachocarpus,* and *C. asplenifolius* Burch Trav is 7 and are more or less equal sized metacentric chromosomes (Banerjee 1932; Bhaduri and Chakravarti 1948; Rao and Datta 1953; Sarma and Datta 1953; Datta 1954; Basak 1958; Islam and Qaium 1961; Goldblatt 1984, Goldblatt and Johnson 2006; Arangzeb 1979, 1989; Alam and Rahman 2000). Hypo- and hyperploidy were reported in six species of *Corchorus*. These phenomena were first recorded in *C. olitorius* and *C. capsularis* (Datta 1952a, b), and subsequently in *C. fascicularis* (Rao and Datta 1953),

C. trilocularis, *C. tridens* and *C. aestuans* (R.M. Datta, unpublished observations), and in *C. sidoides* F. Muell, a native of Australia (Basak 1958).

Seven wild species of *Corchorus,* viz., *C. junodii* (Africa); *C. pascuorum, C. cunnighamii* (Australia); *C. hirtus, C. argutus, C. siliquosus, C. orinocensis* (America and Caribbean Island) are natural tetraploids ($2n = 4x = 28$) (Rao and Datta 1953; Roy 1962; Islam et al. 1975). Tetraploids are abundant in American and Australian taxa, but rare in African *Corchorus* species (Islam et al. 1975). At metaphase I or diakinesis the 28 chromosomes are mostly present as univalent and one quadrivalent. From the recent meiotic studies, it appears that *C. junodii* is a segmental allopolyploid. Hexaploid ($2n = 6x = 42$ chromosomes) and aneuploid ($2n = 4x - 2 = 26$ chromosomes) species are also reported in the genus *Corchorus* (Kubitzki and Bayer 2003).

Normal mitotic chromosomal behavior was observed in *C. aestuans*. All metaphase plates were found to contain chromosomes. Usually one nucleolus was seen in interphase stage, but the presence of two or more nucleoli at interphase was not uncommon. At late prophase or pro-metaphase stage four chromosomes were found attached with the nucleolus (secondary constricted chromosomes). All the 7 pairs of chromosomes appeared median with variable arm length and they were observed to be medium in size except a small pair of chromosomes (Arangzeb 1989). The fluorescent banded somatic karyotype of *C. capsularis*, *C. olitorius,* and *C. trilocularis* showed 14 metacentric chromosomes of almost equal in size (Alam and Rahman 2000). These chromosomes could easily be identified with CMA (chromomycin A3) and DAPI (4′-6-diamidino-2-phenylindole). The base specific banding similarity of their chromosomes suggests that their genomes might be derived from a common ancestor.

CRIJAF has recently initiated a detailed study on karyotyping of *Corchorus* species available in the genebank. The chromosomes of *C. olitorius, C. capsularis, C. aestuans, C. trilocularis, C. tridens, C. pseudo-olitorius, C. pseudo-capsularis, C. fascicularis,* and *C. urticifolius* are metacentric (F% range: 40.00–50.00) to submetacentric (F% range: 33.63–39.15) with $2n = 2x = 14$ and the length of the chromosomes varies between 1.5 and 3.5 μm. The total haploid chromatin length is in the range of 12.68–17.72 μm. Moreover, the haploid chromatin length did not show any variation among the species as evident from χ^2 test of heterogeneity. The total complement length (TCL) percent of the species ranges from 10.15 to 20.07%. A long chromosome pair in the species was found to possess secondary constriction. Meiotic analysis of these nine species also confirmed that the $2n = 14$; however, *C. fascicularis, C. aestuans*, *C. pseudo-olitorius,* and *C. pseudo-capsularis* showed aneuploid variations in their meiocytes. Hypoploid and hyperploid variations were found to be more in M1 cells than A1 cells and persistent cytomixis occurring in those species accounted for such aneuploidy. *C olitorius, C. capsularis, C. tridens*, *C. trilocularis,* and *C. urticifolius* form nearly 7II per cell in MI, which widely vary from 6.60 per cell to 7.08 per cell in other species. Univalent per cell was recorded to be higher in *C. aestuans* and *C. fascicularis* (Fig. 2.2).

2.1.4 Taxonomic Position

Kingdom: Plantae; Division: Magnoliophyta; Subdivision: Angiosperm; Class: Magnoliopsida; Order: Malvales; Family: Malvaceae; Subfamily: Grewioideae; Tribe: Grewieae; Genus: *Corchorus*.

The genus *Corchorus* belongs to the subfamily Grewioideae of the family Malvaceae (formerly Tiliaceae). The chloroplast genes *rbc*L and *ndh*F were sequenced and phylogenetic analyses were performed to clarify the taxonomic placement of this genus (Whitlock et al. 2003). The results strongly supported the inclusion of Oceanopapaver in a clade of former Tiliaceae, the Grewioideae (Malvaceae). Within *Grewioideae*, Oceanopapaver forms a well-supported clade with representatives of the pantropical genus *Corchorus* and the endemic Malagasy genus *Pseudocorchorus*. Morphological characters consistent with this placement include stellate indumentum, mucilage canals, valvate sepals with stellate hairs on the adaxial surface, and pollen morphology. The most closely related genus is probably *Pseudocorchorus*, which differs in having zygomorphic (bilaterally symmetric) flowers and unilaterally inserted stamens. The six species of *Pseudocorchorus*, may well be nested within *Corchorus*, and may be reclassified in *Corchorus* in the subsequent revision of the genus.

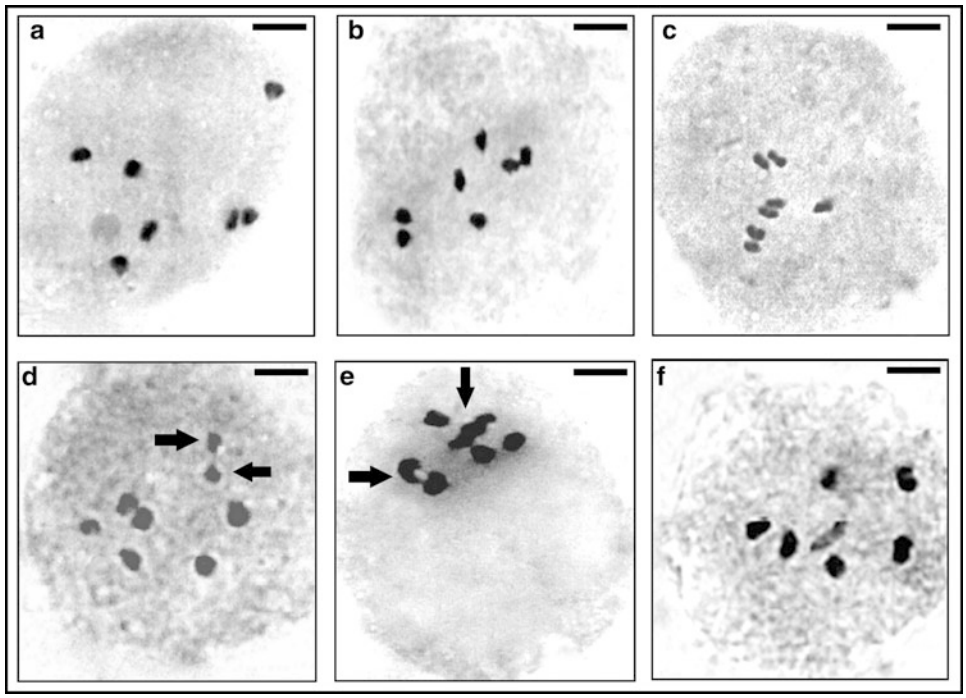

Fig. 2.2 Meiosis in *Corchorus* species ($2n = 2x = 14$) at diplotene stage. (**a**, **b**, **c**, **f**) Formation of seven bivalents (7II). (**d**) Formation of six bivalents (6II) and two univalent (2I, marked). (**e**) Formation of two quadrivalents (2IV, marked) and three bivalents (3II)

2.1.4.1 A Key to South Asian and African Species of *Corchorus* (Edmonds 1990; Khan 1997)

Capsule globose or short elliptic (Not more than twice as long as broad)
- Capsule globose dehiscing into 5 valves
  - Capsules rough with short tubercular excrescences, leaf margin coarsely dentate. ---------- ***capsularis***
  - Capsules covered with short soft blunt prickles, leaf margins coarsely serrate---------- ***pseudo-capsularis***
- Capsule short elliptic, dehiscing into 3 eristate valves, stellate-fomantose, A perennial woody herb up to 1 m tall. -------***gillettii***

Capsule elongated (3 or more times as long as broad)
- Capsule sausage shaped, straight, 0.9-cm × 0.4–0.5 cm, splitting into 3 valves, leaf merging coarsely dentate; spreading to ascending perennial herb become woody below. ---------- ***baldaccii***

- Capsule narrowly elongate, straight or slightly curved
  - Capsule horns spreading
    - Capsule dehiscing into 3–4 valves, horns usually forked
      - Capsule 1–2.6 cm × 0.4–0.7 cm, Splitting into 3–4 valves ending in 3–4 spreading horns, seeds pitted, brownish-black, a prostrate or ascending annual up to 25 cm long. ---------- ***aestuans***
      - Capsule 2–4.2 cm × 6.1–0.2 cm, splitting into 3 valves ending in 3 small spreading horns, seeds, dull brown, a suberant or decumbent annual up to 1 cm lay. --- ***tridens***
    - Capsules dehiscing into 5 valves, horns usually simple
      - Capsules 0.6–2 cm × 0.1–2 cm, seeds smooth glossy, dark brown, annual up to 60 cm with decumbent annual up to 1 cm lay. ---------- ***africannus***
      - Capsules 0.5–1.7 cm × 0.3–0.4 cm, seeds glossy, black an annual up to 30 cm tall.---------- ***brevicornutus***

Capsule horns, if present straight not spreading, although the valves often reflexed
- Capsule splitting into 5 valves, leaf margin serrate into 2 basal serration prolonged into 2 setae, seeds irregularly. ribbed, black. an annual up to 2.5 m tall. -------- ***olitorius***
- Capsule splitting into 3–4 valves, A much branched woody perennial with prostrate branched leaves roundish, crenate – serrate/3 ---------- ***antichorus***
  - Erect prostrate or semi-prostrate annuals . . .
    - Capsule banana shaped (0.9–2 cm × 0.5 cm), the muricate valves after dehiscence with dentate ridges giving capsule a varity appearance, seed punctate, brown to black ---------- ***schimeri***
    - Capsule straight/slightly curved, not banana shaped
      - Capsule 2.5 cm × 0.1–0.4 cm when young, densely covered with shining palmate hairs, glabrous when mature seed smooth, pruinose black. an annual herb up to 1 m tall. --------- ***trilocularis***
      - Capsule when young, not covered by palmate hairs.

Capsule 0.8–1.6 cm lay, seeds glossy, dark brown with tan colored raphe, erect to semi-prostrate annual up to 1 m long. ---------- ***fascicularis***

Capsule 1.3–3.5 m long

Seeds reticulate, with concave sides, sepals and petals 4, erect to semi-prostrate annual up to 25 cm ----------***urtricifolius***

Seeds verrucose, sepals and petals 5, a spreading annual up to 0.75 m tall -------- ---------- ***pseudo-olitorius***

2.1.4.2 Partial Synonymy of *Corchorus*

According to Hinsley (2008), *Antichorus* Linn., *Caricteria* Scop., *Coreta* P. Bri., *Ganja* Reichb., *Maerlensia* Vell., *Nettoa* Baill., *Oceanopapaver* Guillaumin, *Palladia* Lam., *Rhizanota* Lour. ex Gomes, *Riddelia* Rafin., *Scorpia* Ewart & A.H.K. Petrie were synonymized with the accepted genus *Corchorus* L.

Similarly, several *Corchorus* species were synonymized with already accepted species. The information on synonymy is of immense use in *Corchorus* taxonomy and practical plant breeding as well. Lack of information on synonymy may lead to inadvertent crossing of same species and subsequent failure of the crop breeding programs. Hinsley (2008) has documented detailed account on the synonymy of *Corchorus* species (Table 2.1). According to Hinsley (2008), there are 87 accepted species in the genus *Corchorus*; however, the inclusion of *C. orinocensis*, *C. hochstetteri*, *C. quadrangularis*, and *C. muricatus* under the accepted species needs further clarification as these species were synonymized with *C. hirtus, C. pseudocapsularis*, *C. trilocularis,* and *C. schimperi*, respectively. Further details on synonymy of *Corchorus* may be obtained from http://www.malvaceae.info/Synonymy/Synonymy.php?genus=Corchorus.

2.1.5 Morphology

Most of the wild *Corchorus* species are erect to suberect, sometimes decumbent annual to perennial in their growth habit. Profuse branching of most of the wild species (Fig. 2.3) differentiates them from the cultivated species, which are herbaceous, erect, nonbranching annuals. At maturity, cultivated varieties of *C. capsularis* attain a height of about 1.5–4.0 m and those of *C. olitorius* 1.5–4.5 m or more, but the plant height of wild *Corchorus* varies greatly. *C. kirkii* and *C. merxmuelleri* attain a height of about 2.0–2.5 m, while *C. fascicularis*, *C. pseudo-capsularis*, *C. trilocularis*, *C tridens*, *C. gillettii*, *C. junodii, C. cunninghami*, and *C. velutinus* grow up to 1.0 m or little more. *C. africanus*, *C. pseudo-olitorius*, *C. longipedunculatus, C. angolensis, C. cinerascens, C. confuses,* and *C. depresus* are medium in height (0.5–0.6 m) and *C. baldaccii, C. schimperi, C. brevocornutus, C. urtricifolius, C. aestuans, C. erodoides, C. pinnatipartitus, C. salacatus,* and *C. saxatilis* are exceptionally dwarf in stature (0.2–0.3 m). The stems are reddish to yellowish or purplish green and are cylindrical. Leaf blades are lanceolate, oblong-lanceolate, linear, linear-lanceolate, or narrowly oblong. Leaf margin varies widely across the species; it may be serrate or serrate-dentate or sometimes dentate-crenate with or without basal setae. Petiole length varies from 0.2 to 4.0 cm and may be sparsely or densely pubescent. Flowers of all the species are yellow, small in size, and occur in condensed cymes. In general, the flowers possess 4–5 sepals and petals, and the number of stamens varies from 5 to numerous. All the species are mostly self-pollinated with the exception of *C. cunninghamii*, which is a cross-pollinated one. The ovary is cylindric to ellipsoid with 2–6 locules and the seeds are numerous. Seed of *C. gillettii*, *C. erinoceus*, *C. kirkii*, and *C. merxmuelleri* are comparable with *C. capsularis* measuring about 2.0 mm in length. Seed color varies from blackish red to brown or dark brown or sometime black (Fig. 2.4) as in *C. saxatilis* and *C. cunninghamii*. The detailed morphology of wild taxa is presented in Table 2.2 (Edmonds 1990).

A key to the identification of wild *Corchorus* species had been documented based on the morphology of stamens. Pollens in these species are subprolate (prolate *C. pseudo-capsularis* having); tricolporate (both tricolporate – 90.0% and tetracolporate – 10.0% in *C. trilocularis*), colpi margin normal or incurved. Size of colpi was found to be medium to relatively longer and varies from 29.98 ± 0.64 to 36.72 ± 0.92 μm. Pore elongate with edges raised or inconspicuous; exine surface reticulate, reticulation indistinct or distinct, tri- to pentagonal or variously gonals, shallow to alveolate or pitted, sometimes angular with raised irregularly walls, junction knobbed or unknobbed. Pollen size was observed to vary among the species

Table 2.1 List of species synonymized with accepted species of the genus *Corchorus* (Hinsley 2008)

S. No.	Synonym	Accepted species
1.		*Corchorus depressus* (L.)
	Antichorus depressus L.	*Corchorus depressus* (L.) Stocks
	Corchorus antichorus Raeusch.	*Corchorus depressus* (L.) Stocks
	Corchorus microphyllus Fresen.	*Corchorus depressus* (L.) Stocks
2.		*Corchorus orinocensis* Kunth
	Corchorus argutus Kunth	*Corchorus orinocensis* Kunth
	Corchorus argutus var. *benthamii* K. Schum	*Corchorus orinocensis* Kunth
	Corchorus argutus var. *longicarpus*	*Corchorus orinocensis* Kunth
	Corchorus argutus var. *prismatocarpus* K. Schum.	*Corchorus orinocensis* Kunth
	Corchorus hirtus var. *argentinensis* Rodrigo	*Corchorus orinocensis* Kunth
	Corchorus hirtus var. *pilolobus* auct. non (Link) K. Schum.	*Corchorus orinocensis* Kunth
	Corchorus hirtus var. *orinocensis* (Kunth) K. Schum.	*Corchorus orinocensis* Kunth
	Corchorus prismatocarpus A. St.-Hil., A. Juss. & Cambess.	*Corchorus orinocensis* Kunth
3.		*Corchorus hirtus* L
	Corchorus orinocensis Kunth	*Corchorus hirtus* L
	Antiphyla serrata Rafin.	*Corchorus hirtus* L.
	Corchorus hirtus var. *cuyabensis* K. Schum.	*Corchorus hirtus* L.
	Corchorus hirtus var. *orinocensis* (Kunth) K. Schum.	*Corchorus hirtus* L.
	Corchorus pilolobus Link	*Corchorus hirtus* L.
	Riddelia antiphyla Rafin.	*Corchorus hirtus* L.
4.		*Corchorus hirtus* var. *glabellus* Gray
5.		*Corchorus hirtus* var. *hirtus*
6.		*Corchorus aestuans* L.
	Corchorus acutangulus Lam.	*Corchorus aestuans* L.
7.		*Corchorus aestuans* var. *brevicaulis* (Hosok) Liu & Lo
	Corchorus brevicaulis Hosok	*Corchorus aestuans* var. *brevicaulis* (Hosok) Liu & Lo
8.		*Corchorus africanus* Bari
9.		*Corchorus angolensis* Exell & Mendonça
10.	*Corchorus arenicola* Hochr.	*Corchorus argillicola* M.J. Moeaha & P. Winter
11.		*Corchorus asplenifolius* Burch.
	Corchorus serrifolius Burch.	*Corchorus asplenifolius* Burch.
	Corchorus mucilagineus Gibbs	*Corchorus asplenifolius* Burch.
12.		*Corchorus aulacocarpus* Halford
13.		*Corchorus baldacii* Mattei
14.		*Corchorus brevicornatus* Vollesen
15.		*Corchorus cinarescens* Deflers
	Corchorus bricchettii Weim.	*Corchorus cinerascens*
	Corchorus erinaceus Weim.	*Corchorus cinerascens*
	Corchorus hirsutus var. *stenophyllus* K.Schum.	*Corchorus cinerascens*
	Corchorus stenophyllus (K.Schum.) Weim.	*Corchorus cinerascens*
16.		*Corchorus capsularis* L.
	Rhizanota cannabina Lour. ex Gomes	*Corchorus capsularis* L.
17.		*Corchorus carnarvonensis* D.A. Halford
18.		*Corchorus confusus* Wild
19.		*Corchorus congener* D.A. Halford
20.		*Corchorus crozophorifolius* (Baill.) Burret
21.		*Corchorus cunninghamii* F. Muell.
	Corchorus cavaleriei Lévl.	*Helicteres glabriuscula*
22.		*Corchorus pseudocapsularis* Schweinf.
23.		*Corchorus hochstetteri* Milne-Redh.
	Corchorus hochstetteri Milne-Redh	*Corchorus pseudocapsularis* Schweinf.
	Corchorus echinatus Hochst. ex Garcke	*Corchorus pseudocapsularis* Schweinf
24.		*Corchorus elachocarpus* F. Mueller

(*continued*)

Table 2.1 (continued)

S. No.	Synonym	Accepted species
25.		*Corchorus elderi* F. Muell.
26.		*Corchorus fascicularis* Lam.
27.		*Corchorus gillettii* Bari
28.		*Corchorus trilocularis* L.
29.		*Corchorus quadrangularis* G. Don
30.	*Corchorus quadrangularis* J.A.Schmidt	*Corchorus trilocularis* L.
	Corchorus gracilis R.Br.	*Corchorus trilocularis* L.
	Corchorus triflorus Bojer	*Corchorus trilocularis* L.
	Corchorus somalicus Gand.	*Corchorus trilocularis* L.
	Corchorus greveanus Baill.	*Pseudocorchorus greveanus*
31.		*Corchorus hirsutus* L.
	Maerlensia hirsuta Vell.	*Corchorus hirsutus* L.
32.		*Corchorus incanus* D.A. Halford
33.		*Corchorus interstans* D.A. Halford
	Corchorus japonicus Houtt.	*Kerria japonica*
34.		*Corchorus junodii* (Schinz) N.E. Br.
	Corchorus discolor N.E. Br.	*Corchorus junodii* (Schinz) N.E. Br
35.		*Corchorus kirkii* N.E. Br
	Corchorus pongolensis Burtt Davy & Greenway	*Corchorus kirkii* N.E. Br
36.		*Corchorus laniflorus* Rye
37.		*Corchorus lasiocarpus* D.A. Halford
38.		*Corchorus lasiocarpus* ssp. *lasiocarpus* D.A. Halford
39.		*Corchorus lasiocarpus* ssp. *parvus* D.A. Halford
40.		*Corchorus leptocarpus* (A. Cunn.) Benth
41.		*Corchorus lithophilus* D.A. Halford
42.		*Corchorus longipedunculatus* Mast.
	Corchorus longipes Tate	*Gilesia biniflora*
43.		*Corchorus macropetalus* (F. Muell.) Domin
44.		*Corchorus macropterus* G.J. Leach & Cheek
45.		*Corchorus merxmuelleri* Wild
46.		*Corchorus mitchellensis* Halford
47.		*Corchorus neocaledonicus* Schlecter
	Oceanopapaver neo-caledonicum Guillamin	*Corchorus neocaledonicus*
48.		*Corchorus obclavatus* Halford
49.		*Corchorus olitorius* L.
50.		*Corchorus olitorius* var. *incisifolius* Asch. & Schweinf.
51.		*Corchorus olitorius* var. *malchairii* (De Wild.) R. Wilczek
	Corchorus malchairii De Wild.	*Corchorus olitorius* var. *malchairii* (De Wild.) R. Wilczek
52.		*Corchorus olitorius* var. *olitorius*
53.		*Corchorus parviflorus* (Benth). Domiin
54.		*Corchorus parvifolius* Sebsebe
55.		*Corchorus pascuorum* Domin
56.		*Corchorus pilosus* Kunth
57.		*Corchorus pinnatiparititus* Wild
58.		*Corchorus psammophilus* Codd
59.		*Corchorus pseudoolitorius* Islam & Zaid
60.		*Corchorus puberulus* D.A. Halford
61.		*Corchorus pumilio* R. Br. ex Benth.
62.		*Corchorus urticifolius* Wight & Arn.
	Corchorus quinquenervis Hochst. ex A. Rich.	*Corchorus urticifolius* Wight & Arn
63.		*Corchorus reynoldsieae* Halford
	Corchorus rostratus Danguy	*Pseudocorchorus rostratus*

(*continued*)

Table 2.1 (continued)

S. No.	Synonym	Accepted species
64.		*Corchorus saxatilis* Wild
65.		*Corchorus saxicola* D.A. Halford
66.		*Corchorus schimperi* Cufod.
67.		*Corchorus muricatus* Schumach. & Thonn.
	Corchorus muricatus Hochst. ex A. Rich.	*Corchorus schimperi* Cufod.
68.		*Corchorus sericeus* Ewart & O.B. Davies
69.		*Corchorus sidoides* F. Mueller
70.		*Corchorus sidoides* ssp. *sidoides*
71.		*Corchorus sidoides* ssp. *vermicularis* (F. Muell.) D.A. Halford
72.	*Scorpia simplicifolia* Ewart & A.H.K. Petrie	*Corchorus sidoides* ssp. *vermicularis* (F. Muell.) D.A. Halford
73.		*Corchorus siliquosus* L.
74.		*Corchorus sp. Hammersley Range*
75.		*Corchorus sp. Hammersley Range hilltops*
76.		*Corchorus sp. Meentheena*
77.		*Corchorus subargentus* Halford
78.		*Corchorus sublatus* Halford
79.		*Corchorus sulcatus* I. Verd.
80.		*Corchorus tectus* D.A. Halford
81.		*Corchorus thozetii* Halford
82.		*Corchorus tomentellus* F. Muell.
83.		*Corchoropsis crenata*
	Corchorus tomentosa Thunb.	*Corchoropsis crenata*
	Corchorus tomentosa var. *tomentosicarpa* P.L. Chiu	*Corchoropsis crenata*
84.		*Corchorus torresianus* Gaudich.
85.		*Corchorus tridens* L.
86.		*Corchorus velutinus* Wild
87.		*Corchorus walcottii* F. Mueller

(evidenced from χ^2-test of heterogeneity; $p < 0.01$ for polar axis and $p < 0.001$ for equatorial diameter) and it ranged from 38.30 ± 0.54 μm × 30.45 ± 0.54 μm (*C. capsularis*) to 31.17 ± 0.64 μm × 24.2 ± 0.55 μm (*C. trilocularis*).

2.1.5.1 Key to Wild *Corchorus* Species on the Basis of Stamen Morphology

A. Stamens 10
B. Flower buds pyriform, fruits elongated.
C. Leaves lanceolateb .....***C. fascicularis***.
C′. Leaves ovate to ovate lanceolate .....***C. pseudo-olitorius.***
B′. Flower buds rounded, fruits rounded .....***C. capsularis.***
A′. Stamens 15 or more
D. Stamens within 20.
E. Stamens 15; stigma inconspicuously 2–3 lobed.
F. Pedicel glabrous; bract glabrous; stigma 3-fid. each again 2-fid.
G. Flowers to 12 mm long; angles of fruits acute . . . .***C. aestuans.***
G′. Flowers to 4 mm long, angles of fruits winged .. ..***C. tridens.***
F′. Pedicel hairy; bracts hairy; stigma 2-fid ....***C. urticaefolius***.
E′. Stamens more than 15, within 20; stigma distinctly 3-fid each again bifid and horney .....***C. pseudo-capsularis.***
D′. Stamens more than 25.
H. Ovary oblong, angular; stigma bristle ....***C. olitorius.***
H′. Ovary oblong, not angular; stigma bifid ....***C. trilocularis***.

2.1.6 Physiological Pathways

2.1.6.1 Development of Fibers

Corchorus species are potential sources of fiber consisting of many sclerenchyma cells united together into small or large strands or bundles, arranged in the

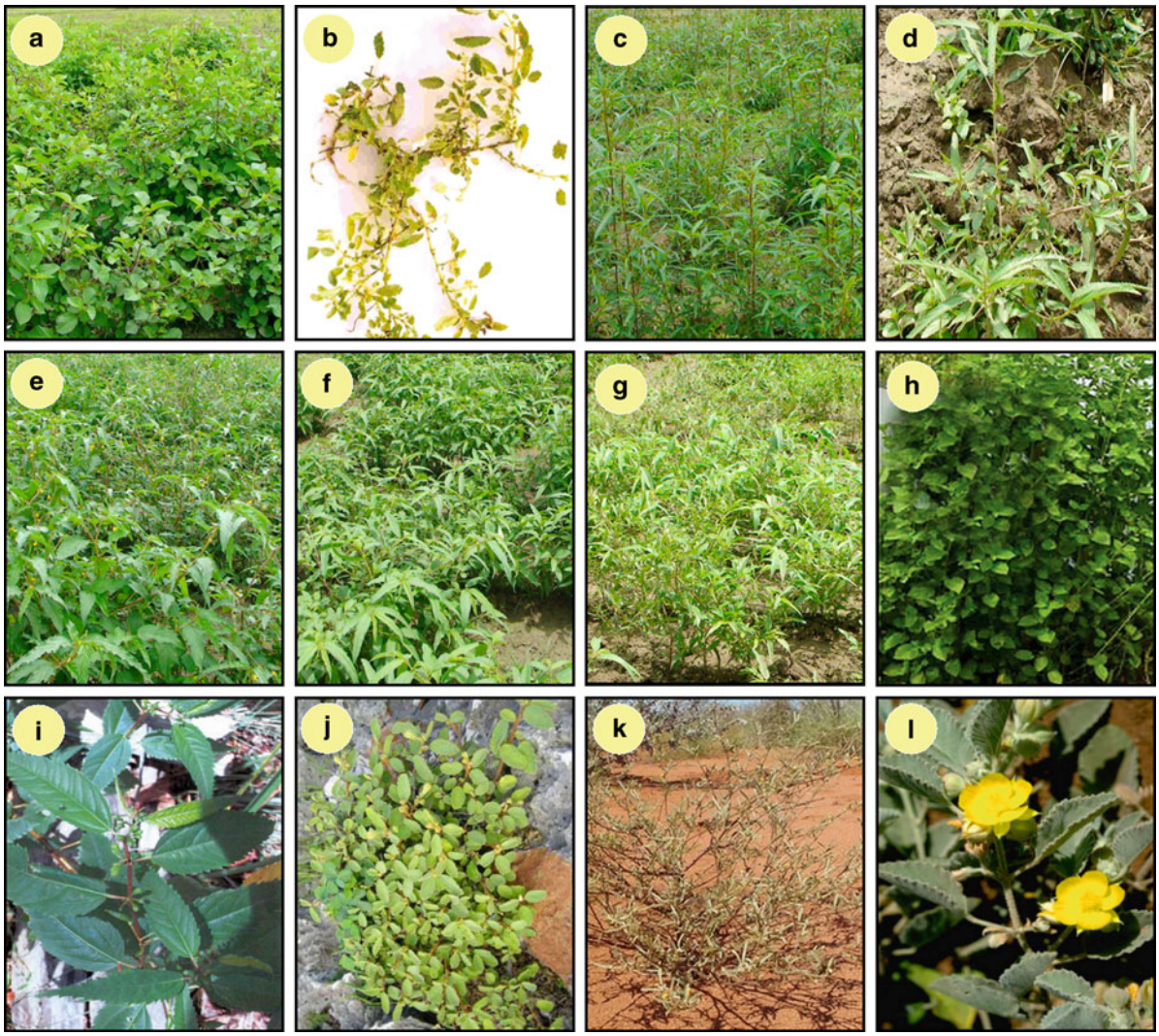

Fig. 2.3 Representative wild species of *Corchorus* including available Indian species (**a**) *Corchorus aestuans*, (**b**) *Corchorus depressus*, (**c**) *Corchorus fascicularis*, (**d**) *Corchorus pseudo-capsularis*, (**e**) *Corchorus pseudo-olitorius*, (**f**) *Corchorus tridens*, (**g**) *Corchorus trilocularis*, (**h**) *Corchorus urticifolius*, (**i**) *Corchorus cunnighamii*, (**j**) *Corchorus hirsutus*, (**k**) *Corchorus sedoides*, (**l**) *Corchorus walcottii*

form of a layer or layers, in the bast or precisely inside the stem. Fiber consists of pyramidal wedges (triangular shaped association of several bundles arranged radially in the secondary phloem and separated by thin walled parenchyma cells) tapering outwards. The fiber bundles in each wedge are arranged in 8–24 layers, alternately with groups of thin walled phloem. The outermost rows of fibers are developed from the elements of the pro-cambium in the proto-phloem region by cell division and modifications resulting in proto-phloic fibers (10%). All the sieve tubes and companion cells are obliterate and the entire pro-phloem region is converted into a solid patch of fiber. The main bulk (90%) fibrous region is developed by two types of fiber initials, viz., fusiform and ray initials of cambium. For this reason these are often termed as secondary fiber. Elongation of pro-phloem fiber continues symplastically with stem internode, but the elongation of secondary fiber results from the cambial activity of the region stops. The stem apical growth ceases with initiation of flowering, but cambial activity for secondary fiber development continues. The fiber strength and fiber fineness, which measures the quality of fibers depend upon the differential

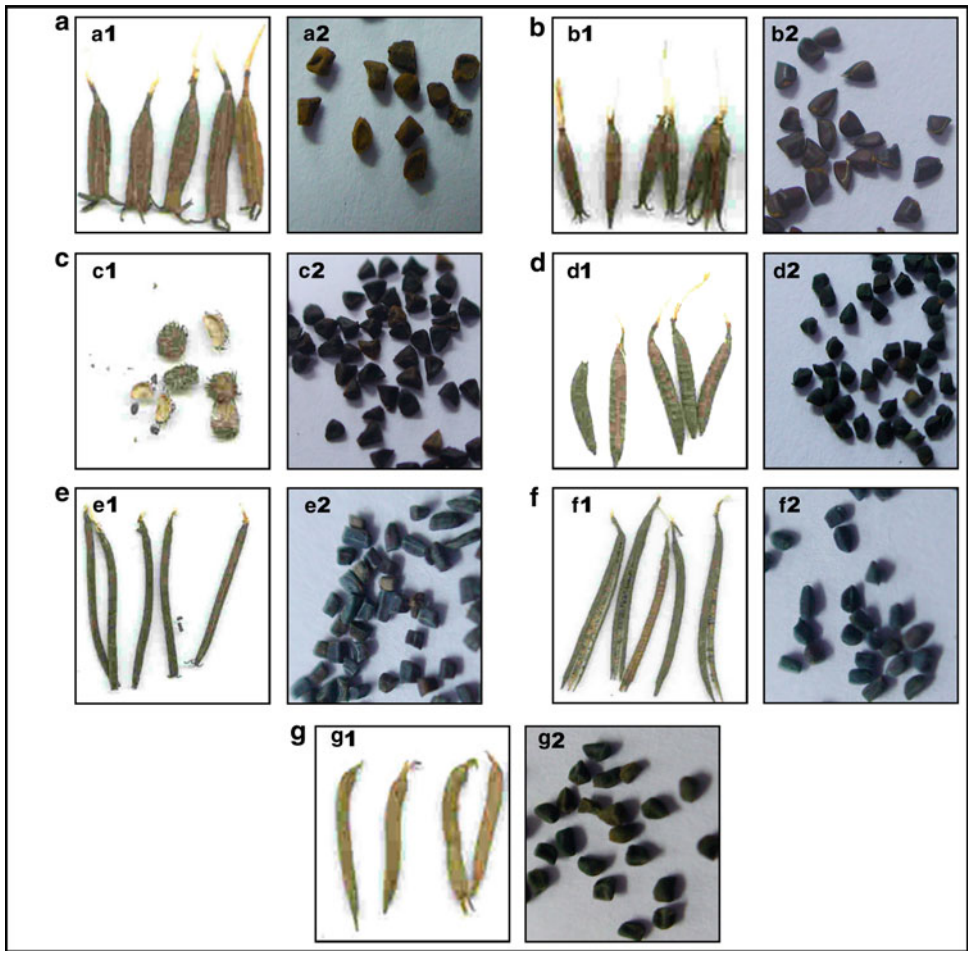

Fig. 2.4 Variation of capsule and seed morphology of available Indian wild *Corchorus* species (**a**) *Corchorus aestuans*. a1: capsule; a2: seed (**b**) *Corchorus fascicularis*. b1: capsule; b2: seed (**c**) *Corchorus pseudo-capsularis*. c1: capsule; c2: seed (**d**) *Corchorus pseudo-olitorius*. d1: capsule; d2: seed (**e**) *Corchorus tridens*. e1: capsule; e2: seed (**f**) *Corchorus trilocularis*. f1: capsule; f2: seed (**g**) *Corchorus urticifolius*. g1: capsule; g2: seed

cambial activity among species. Therefore, the quality measurement gives the overall performance of several traits, viz., the number of fiber cells, the number of fiber bundles in each fiber wedge, number of wedges along the stem length, and so on.

2.1.6.2 Fiber Composition of *Corchorus* Species

The *Corchorus* fiber has three principal chemical constituents, viz., α-cellulose (60–61%), hemi-celluloses (15–16%), and lignin (12.5–13.5%). The α-cellulose form the bulk of the ultimate fiber cell walls chaining broadly parallel to the fiber axis. The hemi-cellulose and lignin, however, are located mainly in the area between neighboring ultimate cells, where they form the cementing material of the middle lamella, providing strong lateral adhesion to the ultimate cells. It is although not clear about the equilibrium among these components and role of middle lamella, but the prominent role of hemi-cellulose compared to lignin as an intercellular building material is well documented.

Color instability or yellowing of *Corchorus* fiber is a major concern for manufacturing diversified *Corchorus* fiber products. This is due to lignin content of the fiber. Abundance of lignin may affect yarn quality also. Therefore, reduction of lignin content is a major breeding objective while utilizing wild relatives of *Corchorus*. In lignin biosynthesis, the enzyme

Table 2.2 Comparative morphology of wild *Corchorus* spp. (Source: Edmonds 1990)

Characters		*C. fascicularis*	*C. pseudo-capsularis*	*C. pseudo-olitorius*	*C. africanus*	*C. trilocularis*	*C. tridens*
General	Type (annual/ biennial/ perennial) and branching habit	Much branched erect to semi-prostrate annual herb	A multibranched, annual herb	A spreading annual herb	Annual herb with decumbent ascending branches	Annual herb	Annual herb suberect or decumbent
	Height	Up to 1 m	Up to 1 m	0.75 m	60 cm	1 m, rarely up to 1.5 m	Up to 1 m
Stem	Color and glabrescent/ pubescence	Reddish, glabrescent, smooth	Reddish, moderately pilose	Reddish, glabrescent	Yellowish-green pilose/villose	Often purplish, setulose pubescence when young, but on older stem the pubescence is confined to only one side	Red, glabrous to sparsely pilose
Leaf blade	Shape	Elliptic or obovate, or narrowly elliptic	Ovate to ovate lanceolate to elliptic	Narrowly lanceolate to ovate or ovate – lanceolate	Ovate, elliptic to oblong	Narrowly elliptic to elliptic, ovate to lanceolate	Narrowly ovate, ovate or narrowly oblong
	Size	3.7–5.2 m long, 0.4–2.1 m wide	3.0–9.7 cm long, 0.9–4.0 cm wide	3.0–11.5 cm long, 0.3–3.3 cm wide	2.5–7.4 cm long, 1.4–4.2 cm wide	4–9 (−13) cm long, 0.6–3.0 cm wide	2.8–11.2 cm long, 0.6–2.0 (−3.0) cm wide
	Glabrescent/ pubescence	Glabrous above and beneath	Scattered pubescence above and beneath, mainly on veins and mid rib	Glabrous apart from odd hairs on midrib and veins beneath	Pilose especially on midrib and vein above and beneath	Pubescence beneath especially prominent on midrib and veins	Glabrous above, pubescent on midrib beneath and occasionally on blade
	Margin	Serrate, basal setae absent	Serrate with a pair of basal setae of up to 1 mm long	Serrate, usually with a pair of basal setae	Serrate, ciliate usually with a pair of basal setae	Coarsely serrate (occasionally crenate) with a pair of basal setae (0.6–10.0 mm)	Coarsely serrate usually with a pair of basal setae (2.9 mm)
	Leaf base	Cuneate, acute to rounded	Rounded or cuneate, acute to acuminate	Truncate or cuneate, acute to acuminate	Cuneate or rounded, acute or rounded	Rounded or broadly cuneate, acute to subacute	Rounded, acute to subacute
	Petioles	Moderately pilose on the upper side	Pilose on the upper side	Pilose on the upper side	Pilose	Covered with setaceous hairs	Pilose, especially on the upper side
	Petiole length	0.3–1.9 cm	0.5–3.5 cm	0.7–2.0 cm	0.4–1.1 (−2.5) cm	0.3–2.0 cm	0.3–1.4 cm
	Stipule length	3–6 mm	3–12 mm	1–3 mm	2–10 mm	6–12 mm	Up to 5 mm
	Stipule texture	Glabrous	Pilose	Glabrous	Pilose	Pubescent	Glabrous
Inflorescences	Type	(1−) 2–5 flowered fascicles	1–3 flowered fascicles	1–3 flowered fascicles	Contorted pedunculate cymes of up to 6 flowers	1–3 flowered cymes	1–4 flowered, glabrous fascicles
	Peduncle and pedicel length	Up to 1 mm	1–3 mm, 1.5–2.0 mm	1–3 mm, 0.3 (−0.8) mm	Up to 3 mm, up to 5 mm	1–2 mm	–
	Peduncle texture	Sparsely pubescent	Pilose	Glabrous	Pilose	–	Glabrous
	Bract length	Up to 1 mm	1–5 mm	Up to 1 mm	Up to 6 mm	Up to 3.3 mm	Up to 4 mm
	Bract texture	Glabrous	Pilose	Glabrous	Pilose	Ciliate	Glabrous
Sepals	Number of sepals	5	5	5	5	4–5	4–5
	Size of the sepals	1.8–2.5 mm long, 0.4–0.6 mm wide	4–5 mm long, 1 mm wide	4.5–5.3 mm long, 1 mm wide	4.5–6.3 mm long, 1.0–1.5 mm wide	5.5–6.3 mm long, 1 cm wide	3.5–4.3 mm long, 0.5–0.6 mm wide
	Sepals texture	Ciliate at upper third margin, torulose	Turulose	Ciliate at basal margin, torulose	Pilose on dorsal side	Dorsal surface pubescent especially at base, torulose	Glabrous or with odd hairs, torulose

(*continued*)

Table 2.2 (continued)

Characters		*C. fascicularis*	*C. pseudo-capsularis*	*C. pseudo-olitorius*	*C. africanus*	*C. trilocularis*	*C. tridens*
Petals	Number of petals	5	5	5	5	4–5	4–5
	Size of the petals	2.5–2.7 mm long, 0.5–0.8 mm wide	3.5–5.0 mm long, 2.0–2.2 mm wide	4.3–5.0 mm long, 1–2 mm wide	4.3–6.5 mm long, 2.0–3.8 mm wide	5–7 mm long, 2.0–2.5 mm wide	3.5–4.8 mm long, 0.5–1.5 mm wide
	Shape	Spathulate, without basal claw	Obovate with ciliate claw at base wide pale yellow	Obovate, with ciliate claw at the base	Obovate, with a sparsely ciliate claw at base	Obovate with a short ciliate claw	Obovate, tapering to a short ciliate claw
Stamens	Number of stamens	5–10	c. 25	20	Numerous	Numerous	c. 10
	Stamen length	1.5–1.8 mm	3.0–4.0 mm	3.2 mm	5.3 mm, anther pitted	–	–
Ovary	Placement/texture	Oblong, pubescent, 1.0–1.3 mm long, 0.5–0.6 mm wide	Spherical echinulate, 1 mm wide	Linear, setulose, 2.8–3.0 mm long, 1 mm wide	Cylindric, pilose, 1 mm long, 0.7 mm wide	Cylindric, 3 mm long, shortly pubescent	Cylindric, 2–3 mm long, papillose
Capsules	Arrangement	Occasionally solitary, usually in 2's to 5's	Solitary or in 2's rarely 3's	Solitary or in 2's borne erect deflexed	Solitary or in 2's or 3's	–	–
	Size	0.8–1.6 cm long, 0.3 cm wide	0.5–0.8 cm wide, 0.7–1.0 cm wide	2.0–3.5 cm long, 2–3 mm wide	0.6–2.0 cm long, 1.0–2.5 mm	2.5–7.3 (−9.2) cm long, 1.0–2.5 (−4.0) mm wide	2.0–4.2 cm long, 1.0–1.5 (−2.0) mm wide
	Shape	Straight or slightly broader in basal half, erect, splitting into 3 valves, ending in 3 breaks which are 1.5 mm long	Spherical, strongly echinate splitting into 5 valves	Cylindric, torulose, straight or slightly curved splitting into 8 valves, tapering to short beak (1.5 mm)	Cylindric, constricted between the seeds, straight or slightly curved, splitting into 5 valves	Cylindric torulose, held erect or straight or slightly carved spitting into 3, rarely, 4 valves	Slender cylinders held erect, straight or slightly curved splitting into 3 valves
	Texture	Pubescent when young, often becoming glabrous	Glabrous	Young capsules pilose, mature capsules glabrous	Shortly pubescent ending in 5 spreading simple horns	Glabrous when mature, covered with scattered forked or palmate hairs which are dense when young	Glabrous to sparsely setulose, ending in 3 small spreading horns (1 mm long)
Seeds	Shape	Diolabriformic or angular	Angular,	Angular	Pyramidal to diolabriformic	Oblong – ovoid	Angular, oblong
	Length	c. 1.5 mm	up to 2 mm	up to 1.2 mm	c. 1.0–1.5 mm	Up to 1 mm in diameter	Up to 0.5 mm in diameter
	Color and texture	Glossy, dark brown, with tan colored	Reticulate black	Brownish black, verrucose	Glossy dark brown, smooth	Blackish and pruinose	Dull dark brown, finely reticulate
Fruiting		March –July, November	January to August	January, March, May, November	January to May	Throughout the year, January to December	Throughout the year, January to December

Characters		*C. baldacciii* Mattei	*C. gillettii* Bari	*C. schimperi* Cufod	*C. brevicornutus* Vollesen	*C. urtricifolius* Wight & Arnold	*C. aestuans* L.
General	Type (annual/biennial/perennial) and branching habit	A spreading to ascending perennial woody herb	Perennial woody herb	Semi-prostate annual	Erect annual herb	Much branched, erect to semi-prostate annual herb	A prostate to ascending annual herb
	Height	30 cm	Up to 1 m	Up to 25 cm	Up to 30 cm	Up to 25 cm	Up to 25 cm
Stem	Color and glabrescent/pubescence	Dark red, densely stellate tomentose	Densely stellate tomentose when young, becoming glabrous and dark red	Reddish glabrous to moderately pilose, more so down one side	Reddish, glabrous to slightly pubescent	Sparsely to moderately pilose, denser down one side	Moderately pilose, more so on one side
Leaf blade	Shape	Narrowly ovate or oblong or elliptic	Ovate, narrowly elliptic to narrowly oblong	Oblong or elliptic to narrowly elliptic	Ovate to elliptic	Narrowly ovate to ovate-lanceolate	Ovate to ovate-lanceolate to broadly ovate
	Size	1.0–5.8 cm long, 0.5–1.3 cm wide	2.0–6.7 cm long, 0.7–3.6 cm wide	0.8–4.5 (−8.0) cm long, 0.3–1.8 (−2.0) cm wide	2.7–4.5 cm long, 1.1–2.5 cm wide	4.0–8.3 cm long, 1.5–4.3 cm wide	2.0–8.0 cm long, 1.6–4.4 cm wide

(*continued*)

Table 2.2 (continued)

Characters		*C. baldacciii* Mattei	*C. gillettii* Bari	*C. schimperi* Cufod	*C. brevicornutus* Vollesen	*C. urtricifolius* Wight & Arnold	*C. aestuans* L.
	Glabrescent/ pubescence	Stellate pubescent to tomentose and paler beneath	Stellate pubescent to tomentose above, densely stellate tomentose beneath	Scattered pubescence beneath mainly on veins and midrib	Scattered pubescence on midrib above and on midrib and veins beneath	Sparsely setulose above, more setulose beneath mainly on veins and midrib	Scattered pubescence above and beneath mainly on veins and midrib
	Margin	Coarsely dentate	Dentate with large acute teeth	Crenate occasionally dentate and shortly ciliate, without basal setae	Crenate, without basal setae	Coarsely serrate (rarely crenate) usually without basal setae	Usually serrate (occasionally crenate) usually with a pair of basal setae (3–5 mm)
	Leaf base	Rounded, acute or rounded	Rounded	Cuneate, rounded or acute at the apex	Unequal at the base, subacute or rounded	Rounded, acuminate to acute	Rounded, acute or subacute
	Petioles	Stellate tomentose	–	Pilose on upper side	Pilose more so on upper side	Pilose especially on one surface	Pilose on upper side
	Petiole length	0.4–1.0 cm	0.5–1.8 cm	2–8 mm	0.3–0.7 cm	0.3–1.7 (−3) cm	0.8–2.7 (−5.3) cm
	Stipule length	1.5–3.5 mm	2 mm	1.5–3.5 mm	1.5–2.8 (−5) mm	1–5 mm	5–8 mm
	Texture	Pilose with a mixture of stellate or simple hairs	Stellate tomentose	Sparsely pilose	Pilose	Ciliate	Pilose
Inflorescence	Type	1–2 (−3) flowered fascicles	1–3 (−4) flowered fascicles	1–2 (−3) flowered fascicles	1–2 flowered fascicles	1–2 (−4) flowered pilose fascicles	1–2 (−3) flowered fascicles
	Peduncle length	0.4–1.5 cm	Epedunculate	Up to 2 mm	Up to 1 mm	Up to 2 mm	Up to 2 mm
	Texture	Both stellate pubescent	Stellate tomentose	Glabrous to pilose	Glabrous	Both pilose or sparsely so,	Pilose pedicels glabrous
	Bract length	Up to 1 mm	1.0–1.5 mm	Up to 2 mm	0.5 mm	Up to 1 mm	Up to 2.7 mm long
	Bract texture	Pilose with a mixture of simple and stellate hairs	Stellate tomentose	Glabrous	Glabrous sparsely pilose	Ciliate, membranous	Ciliate
Sepals	Number of sepals	5	5	5	5	4	5
	Size of the sepals	7.5–8.4 mm long, 1.0–1.5 mm wide	9–11 mm long, 2–3 mm wide	4.3–4.5 mm long, up to 1 mm wide	3.6–3.8 mm long, 1 mm wide	3.2–6.4 mm long, 0.5–1 mm wide	3.0–3.9 mm long, 0.6–0.8 mm wide
	Sepals texture	Dorsal surface covered with stellate hairs	Dorsal surface covered with stellate hairs	Scattered pilose hairs on dorsal side, torulose	Sparsely pilose, torulose	Pilose	Ciliate at basal margin, torulose
Petals	Number of petals	5	5	5	5	4	5
	Size of the petals	7.3–8.0 mm long, 3.0–4.0 mm wide	6.5–11 mm long, 3–6 mm wide	4.0–4.5 mm long, 1.5 mm wide	2.6–2.8 mm long, 1.0–1.1 mm wide	3.5–5.3 mm long, 1–2 mm wide	3.3–3.8 mm long, 0.8–1.0 mm wide
	Shape	Obovate, with ciliate claw at base,	Slightly ciliate claw at base,	Oblanceolate with ciliate claw at base	Obovate with ciliate claw at base	Obovate with ciliate claws at base,	Oblanceolate with ciliate claws at base
Stamens	Number of stamens	Numerous	Numerous	c. 10	c. 10	c. 15	c. 10
	Length of the stamen	Up to 6 mm	Up to 7 mm	Up to 2.8 mm	2.3 mm	4 mm	3 mm
Ovary		Sausage shaped, tomentose, 2.0–2.3 mm long, 1.0–1.3 mm wide	Cylindrical, tomentose 2.5–3.0 mm long, 1.2–1.5 mm wide	Trigonal-cylindric, pilose on angles, 2.0–2.3 mm long, 0.7–1.1 mm wide	Cylindric, densely strigose, 1.5 mm long 1 mm wide	Oblong, densely strigose, 2 × 1 mm	Cylindric, puberulous 2 mm long, 1 mm wide

(*continued*)

Table 2.2 (continued)

Characters		*C. baldacciii* Mattei	*C. gillettii* Bari	*C. schimperi* Cufod	*C. brevicornutus* Vollesen	*C. urtricifolius* Wight & Arnold	*C. aestuans* L.
Capsules	Arrangement	Solitary or in pairs	Solitary or in 2's or 3's	Solitary or in pairs (occasionally in 3's)	Solitary or in 2's	Solitary or in 2's or 3's rarely 4's	Solitary or in 2's or 3's
	Size	0.9–2.0 cm long, 0.4–0.5 cm wide	0.8–1.4 cm long, 0.4–0.5 cm wide	0.9–2.0 cm long, 0.5 cm wide	0.5–1.7 cm long, 0.3–0.4 cm wide	1–3 (−3.0) cm long, 2.0–3.0 cm wide	1.0–2.6 cm long, 0.4–0.7 cm wide
	Shape	Sausage shaped to elliptic, straight, splitting in 3 valves, borne erect or at right angles to stem on spreading pedicles	Straight or slightly curved, splitting into 3 cristate valves, borne erect or at right angles to stem	Slightly curved (banana shaped), splitting into 3 muricate valves with dentate ridges giving capsules a warty appearance, borne at right angles to stem or deflexed	Straight, splitting up to 5 valves, borne erect	Cylindric to trigonous, straight or slightly curved often reflexed, splitting into 3 valves often tapping to a small beak	Straight or slightly curved, splitting into 3 winged valves, borne erect and adpressed to stem
	Texture	Stellate tomentose	Stellate tomentose	Glabrous to sparsely pilose	Scabrid, ending in 5 spreading horns	Moderately pilose or sparsely	Glabrous, ending in 3 spreading horns with 2–3 mm long and usually forked
Seeds	Shape	Somewhat angular	Angular	Somewhat angular	Somewhat angular	Angular	Somewhat angular
	Length	c. 1.5 mm	1.8–2.0 mm	c. 1.5 mm	c. 1 mm	c. 1 mm long	c. 1 mm
	Color and texture	Striate black	Dark brown, striate	Mid-brown to black, punctate	Glossy black	Blackish, reticulate with concave side	brownish black
Fruits		March to October November–December	May to July, Throughout the year	November–December (February–December)	January, June, August, December	February	February–May,

Characters		*C. erinoceus*	*C. erodiodes*	*C. kirkii*	*C. merxmuelleri*	*C. longipedunculatus*	*C. pinnatipartitus*
General	Type (annual/ biennial/ perennial) and branching habit	Perennial herb or shrub	Delicate looking, perennial, arising from woody tap root	Small shrub	Shrub with numerous spreading branches, young branchlets	Annual delicate herb	Small perennial with a woody rootstock
	Height	Up to 1 m	c. 0.3 m	Up to 2.5 m	About 2 m	Up to 0.6 m	Up to 0.2 m
Stems	Color and texture	Yellowish brown to orange red, densely covered with a stellate pubescence, later becoming reddish brown and glabrescent	Flattened forming a rosette, sparsely pilose, becoming glabrescent	Woody, branches grayish or yellowish, densely tomentose hair stellate	Gray-tomentellous with small compact stellate hairs, late glabrescent with a brown bark	Slender stems, branches angular or compressed at first, glabrous	Suberect or somewhat trailing, glabrous
Leaf blade	Shape	Ovate, ovate-oblong, oblong to elliptic, or ochraceous	Oblong, obovate or ovate-oblong	Oblong to oblong-lanceolate	Ovate, apex acute	Linear	Somewhat lanceolate to linear lanceolate or narrowly oblong
	Size	2.2–3.8 cm long, 0.8–1.1 (1.5) cm wide	1.5–2.1 cm long, 0.5–0.8 cm wide	2.4–10.0 cm long, 0.8–2.7 cm wide	1.7–2.4 cm long, 1.3–1.6 cm wide	1.0–8.0 cm long, 0.2–0.4 cm wide	1.8–2.6 cm long, 0.6–1.2 cm wide
	Texture	Densely covered with a velvety stellate pubescence	Pilose/strigose above, moderately strigose beneath	Thick textured, densely gray green or yellowish tomentose on both sides, hairs stellate	Densely and stellately pubescent on both sides	Glabrous or minutely setulose-pubescent on midrib below	Glabrous on both sides or minutely and sparsely pubescent on the midrib below.
	Margin	Serrate	Serrate to pinnatifid with serrate to bipinnate lobes	Crenate, coarsely serrate	Deeply dentate to coarsely serrate-dentate, every tooth veined	Denticulate	Deeply pinnatipartite, sometime palmately lobed

(*continued*)

Table 2.2 (continued)

Characters		*C. erinoceus*	*C. erodiodes*	*C. kirkii*	*C. merxmuelleri*	*C. longipedunculatus*	*C. pinnatipartitus*
	Leaf base	Cuneate, obtuse at apex	Cordate to subhastate, apices rounded	Rounded or broadly cuneate	Shallowly cordate, apex acute	Cuneate or narrowly truncate, acute to acuminate at apex	Truncate or shallowly cordate, basal setae absent
	Petioles	Densely stellate-pubescent	Moderately pilose	Grayish or yellowish tomentose	Grayish – tomentose on all sides	Very shortly pubescent on upper side	Lanceolate with a subulate apex
	Petiole length	0.8–1.0 cm	(1.2) 1.8–3.2 cm	Up to 1.3 cm long	–	Up to 0.5 mm	0.3–0.7 cm
	Stipule length	1.5–(2.0) mm	–	5 mm	2 mm	5–7 mm	c. 1.5–2.0 mm
	Stipule texture	Densely stellate pubescent	Moderately pilose	Stellately tomentose on the back, subulate	Densely and stellately tomentellous	Glabrous, setaceous	Line of hairs on the upper side
Inflorescence	Type	2–3 flowered	1-flowered	3–8(−10) flowered cymes	1–2 flowered cymes	1–3 flowered cymes	1–3 flowered fascicles
	Peduncle length	1–8 mm	Epedunculate pedicels	2–10 mm	3 mm	20 mm	c. 0.5 mm epedunculate or puberulous
	Peduncle texture	Densely stellate pubescent	Moderately pilose with long hairs	Stellately tomentose	–	Hair-like glabrous	
	Bract length	–	–	1.5–8.0 mm	1.5(−3.5) mm	2.0–3.0 mm	1 mm
	Bract texture	–	–	Tomentose or pilose on the back	Grayish tomentellous, subulate	Setaceous glabrous	Setaceous
Sepals	Number of sepals	5	5	5	5	5	5
	Size of the sepals	c. 3–4 mm long, c. 1.5 mm wide	–	Up to 10 mm long lanceolate–acuminate to linear	5–6 mm long, 0.75–1.0 mm wide narrowly oblanceolate, apex long acuminate, slightly keeled at the back	About 4 mm long, very narrowly elliptic to linear, caudate or setaceous at the apex	Often purplish, or pinkish, about 7 mm long, oblanceolate, apex subacute, or acute
	Sepals texture	–	–	Stellately pilose or tomentose on the back	Grayish tomentellous outside, glabrous inside	Glabrous, margins somewhat inrolled	Glabrous
Petals	Number of petals	5	5	5	5	5	5
	Size of the petals	c. 3 mm long, 2 mm wide	c. 4.5 mm long, 2.0 mm wide	The same length as the sepals	9.0 mm long, 4.5 mm wide	3–4 mm long	The same length as the sepals
	Shape	–	Moderately strigose	Narrowly obovate to linear oblong, with a short basal claw ciliolate on the margin	Yellow, narrowly obovate with a short basal claw minutely ciliate on the margin	Linear to narrowly oblanceolate, not clawed or ciliate at the base	Yellowish or tinged pinkish purple, narrowly obovate, claw minutely ciliate on the margin
Stamens	Number of stamens	Numerous	Numerous	20–30	Numerous	About 12	Very numerous
	Length of the stamen	Filamentous c. 3.0 mm long		Filiform		Filamentous	Filamentous
Ovary		–	Binocular seeded	Cylindric, 3 or occasionally 4 locular, many sected, densely stellate pilose	3-locular, trigonously cylindric, very shortly tomentellous	Trigonously subcylindric, 3-locular, very minutely setulose pubescent	Trigonously ellipsoid, 3 locular, very minutely glandular papilose
Style		–	–	About 2 mm long, glabrous	4 mm long, slender, glabrous	1.0–1.5 mm long, glabrous	About 3 mm long, slender
Capsules	Arrangement	Solitary or in 2's or 3's	Usually borne beneath rosette or recurved pedicels and often buried in sand	Solitary or in cluster of up to 7	Usually solitary, occasionally in 2's	Borne on erect pedicels, usually solitary, occasionally in 2's	Solitary or in 2's or 3's

(*continued*)

Table 2.2 (continued)

Characters		*C. erinoceus*	*C. erodiodes*	*C. kirkii*	*C. merxmuelleri*	*C. longipedunculatus*	*C. pinnatipartitus*
	Size	0.9–2.0 cm long, 7.0–11.1 cm wide	7–9.8 mm long, 2 mm wide	2–3 cm long, c. 3.5 mm wide	3.0–4.0 cm long, 1.0–1.5 mm wide	1.8–5.0 cm long, c. 2 mm wide	1.6–2.0 cm long, 1.5–2.0 mm wide
	Shape	Longitudinally ovoid to ovate, splitting into 3 or 5 valves	Shortly silique form, often tapering and curving towards the apex	Narrowly ovoid to linear cylindric not beaked, splitting into 3 valves	Subcylindric slightly torulose, slender straight or slightly curved, valves grayish	Cylindric, slender, trigonous, narrowing towards base, tapering into an individual beak, splitting into 3 valves	Subcylindric, straight or curved (banana shaped) often on recurved or twisted pedicels
	Texture	Wooly and densely covered with silky, stellate complex hairs	Moderately pilose and covered with short glistening hairs	Densely covered with soft stellate-haired shortish bristles	Tomentellous, moderately covered with small stellate hairs.	Smooth and glabrous	Sparsely glandular to glabrescent
Seeds	Shape	–	–	Compressed ellipsoid	–	Subcylindric	Subcylindric angular
	size	c. 2.25 cm long, 1.2 mm wide	–	c. 2.5 mm long, 1.3 mm wide,	c. 2 mm long, 1 mm wide	c. 0.8 mm long, 1.5 mm wide	1.5 mm long, 1 mm wide
	Color and texture	Blackish red	–	Brown	Brown	Brown	Dark brown

Characters		*C. angolensis*	*C. asplenifolius*	*C. cinerascens*	*C. confusus*	*C. depressus*	*C. junodii*
General	Type (annual/ biennial/ perennial) and branching habit	Fine stemmed shrublet	Perennial herb with prostrate or suberect annual stem from a woody root stock	Perennial herb or shrublet	Perennial herb with prostrate or spreading branches	Prostrate annual or perennial herb, often developing a thick long and woody rootstock	Small shrub
	Height	Up to about 0.6 mm	–	Up to 0.5 m	Up to 0.6 m long	Up to 30 cm long	Up to 1.6 m
Stem	–	Finely pubescent on all sides	Glabrous or with a line of short curly hairs on one side only or with spreading hairs all around the stem as on the line of short curly hairs	Stem covered with dense stellate pubescence, branchlets tomentellous	Young branchlets with spreading pubescence covering all side of the stem	Mostly adpressed to ground, glabrous to sparsely pubescent	Young branches grayish pubescent with stellate hairs, older branches glabrescent and brown
Leaf blade	Shape	Lanceolate	Lanceolate, oblong-lanceolate, linear, linear-lanceolate or narrowly oblong	Lanceolate, narrowly ovate or narrowly elliptic	Linear lanceolate, narrowly lanceolate to ovate, apex acute, or subobtuse	Ovate to elliptic or oblong, without setae	Lanceolate, oblong elliptic or ovate, rounded or acute at the apex
	Size	2.0–7.0 cm long, 1.4–2.8 cm wide	1.5–8.0 cm long, 0.2–1.6 cm wide	1.3–7.5 cm long, 0.4–2.2 cm wide	1.0–2.7 cm long, 0.5–2.6 cm wide	0.3–2.5 cm long 0.2–1.5 cm wide	1.5–8.5 mm long, 0.6–3.1 cm wide
	Glabrescent/ pubes cence	Crenate glabrescent to moderately pilose on both sides	Glabrescent to moderately hispid with tubercular-based hairs	Pubescent to tomentellous beneath, sparsely to densely pubescent above, all hairs stellate		Glabrous to sparsely setulose on veins	Glabrescent above, densely whitish, or yellowish tomentose below, all hairs stellate
	Margin	Deeply serrate, serrate or more rarely serrate	Dentate-crenate, serrate or serrate-dentate, toothing very variable in size, sometimes irregular or biserrate, basal setae absent	Serrate	Margin crenate-serrate, pilose on both sides especially on the nerves, hair not tubercle based, strongly 3-nerved from the base	Crenate to serrate	Margin coarsely dentate or crenate, shortly and densely greenish tomentose
	Leaf base	Obtuse to slightly cordate	Rounded or broadly cuneate	–	Base rounded, or slightly cordate, sometimes with a pair of setaceous basal lobes	–	Rounded or broadly cuneate at the base

(*continued*)

Table 2.2 (continued)

Characters		*C. angolensis*	*C. asplenifolius*	*C. cinerascens*	*C. confusus*	*C. depressus*	*C. junodii*
	Petioles	–	–	–	Setulose-pilose on all sides	–	Petiole, densely stellate, gray pubescent
	Petiole length	5–25 mm	4.0–10.0 mm	3–20 mm	3–8 mm	0.2–4.0 cm	Up to 1.6 cm long
	Stipule length	About 4 mm	2.0–10.0 mm	1.0–2.0 (−3.5) mm	Up to 6 mm	Up to 2 mm long	
	Glabrescent/pubescence of the stipule	Pubescent	Glabrescent, setaceous or setulose pubescent	Stellate pubescent	Setaceous, pubescent	Sparsely pubescent	Densely stellate, gray pubescent
Inflorescence	Type	Small (1–)2–5 flowered cymes	Single, 1–4 flowered cymes	(2–)3–8 flowered tomentellous fascicles (always some with 4 or more flowers)	Small (1–) 2–3 flowered cymes opposite the upper leaves, pedicels similar, 3–8(−13) mm long,	1–2(−4) flowered glabrous, fascicles	Leaf opposed, 3–6 flowered cymes, peduncles
	Peduncle length	Up to 25 mm	1–7 mm	0–2(−3) mm	3–25 mm long	Up to 2 mm	6–10 (−21) mm long
	Glabrescent/pubescence	Pubescent	Pubescent or glabrous	Densely stellate pubescent	Patent pubescence on all sides	Glabrous	Densely stellate, gray pubescent, pedicels similar, 5–9 mm long
	Bract length	Filiform, similar To The Stipules	–	–	1–5 mm long	1.5–2.0 mm	3–4 mm long
	Glabrescent/pubescence	–	–	–	Setaceous similar to the stipules	–	Subulate, gray stellate pubescent
Sepals	Number of sepals	5	5	5	5	5	5
	Size of the sepals	10 mm long, 2.5 mm wide	–	3–8 mm long, 1.0–1.5 mm wide	Up to 10 mm long, 1.5 mm wide	2–3 mm long	Up to 10 mm long, and with a cordate apex up to 4.5 mm long but often less
	Glabrescent/pubescence of the sepals	Pubescent outside		Tomentellous	Linear-lanceolate to narrowly lanceolate, apex acuminate, setulose-pilose outside	Glabrous	Linear, densely pubescent outside glabrous within
Petals	Number of petals	5	5	5	5	5	5
	Size of the petals	8 mm long, 4.5 mm wide	–	3.5–6.0 mm long, 2.0–4.0 mm wide	–	–	7–8 mm long
	Shape	Narrowly obovate with a short basal claw ciliate on the margin	–	–	Yellow slightly shortened than the sepals, oblanceolate, to obovate, with a short basal claw ciliate at the margin	–	Yellow, oblanceolate, with a basal claw up to 1 mm long with ciliolate margin
Stamens	Number of stamens Length of the stamen	Numerous, filamentous	Very numerous, filamentous		About 50	10–15	Numerous, filiform
Ovary		3 loculars, trigonously cylindric, pubescent	Trigonously subcylindric, very shortly setulose-pubescent, 3-locular	–	Trigonously cylindric, densely pubescent	Densely covered with bulboius based bristly hairs	Ovoid, densely pubescent, 5-locular, ovules, very numerous
Style		About 3 mm long, slender, glabrous	2–7 mm long, glabrous	–	About 2.5 mm long, slender or glabrous	–	3–4 mm long, glabrous
Capsules	Arrangement		2's, 3's or 4's	Occasionally solitary, usually often borne in clusters of up to 4	Solitary or 2's or 3's	Solitary or in 2's	Borne on erect pedicels, in clusters up to 5

(*continued*)

Table 2.2 (continued)

Characters		*C. angolensis*	*C. asplenifolius*	*C. cinerascens*	*C. confusus*	*C. depressus*	*C. junodii*
	Size	2.2–6.0 mm long, 1.5–2.0 mm wide held on straight fruiting pedicels	1.1–4.0 cm long, c. 2 mm wide	0.8–3.5 cm long, 2.5–3.5(−6) mm wide	2.0–5.0 cm long, 1.5–3.0 mm wide	0.8–2.0 cm long, C. 1.5 mm wide	1.4–2.0 cm long, 1.1–1.5 cm wide
	Shape	Cylindric, torulose, straight tapering slightly towards undivided apex, trigonous splitting into 3 valves	Subcylindric not 3 angled, usually curved, many seeded often or rather twisted or curved pedicels and therefore variable in presentation	Cylindric to slightly torulose, straight to slightly curved on spreading pedicels	Cylindric trigonous undivided at the apex, surface somewhat tapering to a small beak 0.2 mm long, Valves hollowed out to receive seeds within	Cylindric, often tapering to short beak, up to 2 mm long	Spherical to ovoid, (including bristles), splitting into 3(−5) valves
	Glabrescent/pubescence	Glabrescent when mature with scattered palmate hairs, especially towards the apex	Sparsely setulose-scafrid	Floccose tomentose with stellately hairy bristles	Usually scabrous on the angles or hispidulous and covered with scattered palmate and simple hairs		Densely covered with rather stiff, stellately plumose, bristles of up to 5 mm long
Seed	Shape	–	Shortly cylindric	–	–	–	Irregularly ellipsoid
	Length	1.5–2.2 mm long, 0.9–1.2 mm wide	1.3–2.0 mm long, 0.75–1.0 mm broad,	c. 2 mm long	c. 2.0 mm long, 1.2 mm wide	1–1.5 mm long, Very finely reticulate	2 mm long, 1.3 mm wide,
	Color	Dark gray	Dark brown	–	Dark brown to gray	–	Brown

phenylalanine ammonia lyase (PAL) in the bark tissue is the gatekeeper enzyme for the phenylpropanoid pathway and was found to be developmentally controlled (Sengupta and Palit 2004). Therefore, attempts should be made through biotechnological approach to down-regulate this enzyme to reduce lignin content in *Corchorus* fiber. Lignin composition is also important in paper and pulp industry because very low lignin content may compromise the structural integrity of the cell wall and stiffness and strength of the stem. Lignin, which is a complex racemic aromatic heteroploymer, is composed of guaiacyl (G) and syringyl (S) monolignol units; G units are derived from caffeic acid, while S units are from sinapic acid. In the pulp and paper industries, the S:G ratio is important because S monolignols are chemically degraded more easily than G monolignols, thus avoiding the use of more expensive and environmentally hazardous chemicals. There is usually a high degree of genetic variability in the S:G ratio, not only between species, but also within species. Various developmental and environmental factors also cause variability in the S:G ratio. The extent of variability in the S:G ratio among wild relatives of *Corchorus* is not known. Therefore, characterization and genetic manipulation of the S:G ratio could be a major objective for conditioning *Corchorus* species as donor of genes for pulp and paper processing.

2.2 Utilization of Wild Species of *Corchorus* in Crop Improvement Through Traditional and Advanced Tools

The development of cultivated *Corchorus* so far has been confined to intraspecific hybridization. There is limited scope for further improvement of cultivated varieties of jute in the absence of requisite variability and genetic diversity due to narrow genetic base. Therefore, additional genetic variability is required to provide new phenotypes in breeding populations. Interspecific hybridization is an excellent tool for the improvement of cultivated species and to broaden the genetic base. Most of the wild species of *Corchorus* are poor yielder, but potent sources of biotic and abiotic stress tolerance coupled with finest quality of fiber (Palve et al. 2004; Mahapatra and Saha 2008). *C. depressus, C. asplenifolius, C. cinerascens, C. erinoceus,* and *C. erodiodes* exhibited a higher degree of

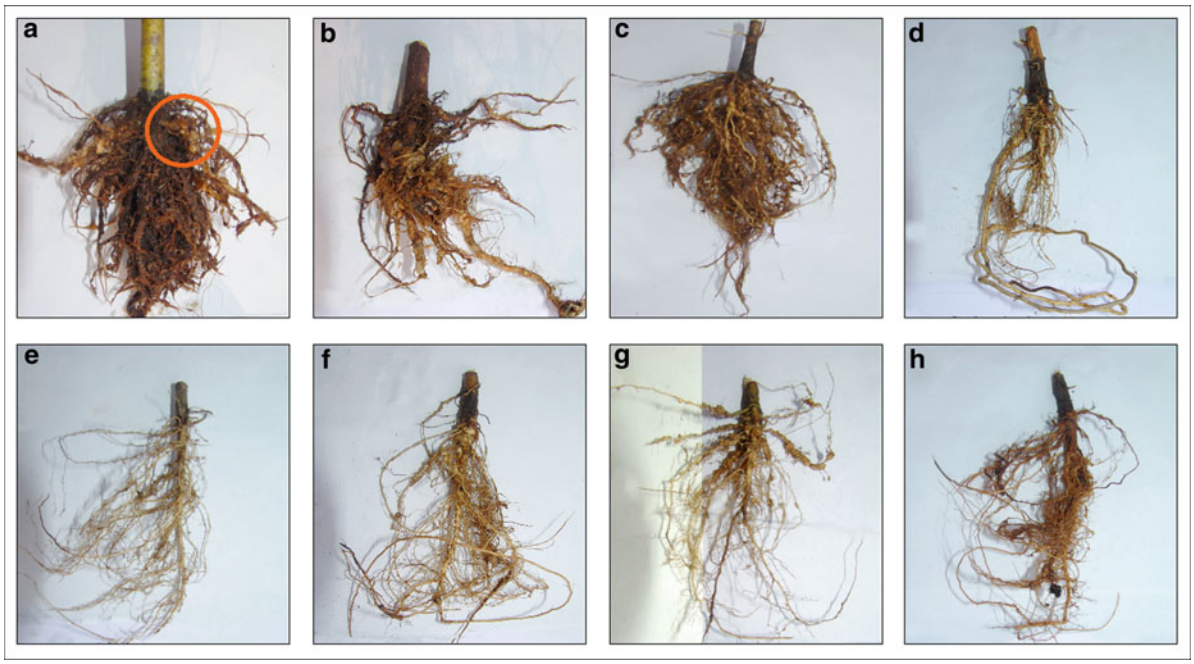

Fig. 2.5 Reaction of *Corchorus* spp. to root-knot nematode *Meloidogyne incognita*. (**a**) *Corchorus capsularis* var. JRC-212, (**b**) *Corchorus olitorius* var. JRO-8432, (**c**) *Corchorus aestuans*, (**d**) *Corchorus fascicularis*, (**e**) *Corchorus pseudo-capsularis*, (**f**) *Corchorus pseudo-olitorius*, (**g**) *Corchorus tridens*, (**h**) *Corchorus urticifolius*

drought tolerance and *C. trilocularis* and *C. tridens* (WCIJ-046) showed a significant level of tolerance to water stagnation. *C. hirtus, C. sulcatus,* and *C. siliquosus* have the ability to colonize on extremely shallow soil. *C. pseudo-olitorius* showed immune reaction to fungal diseases including stem rot, root rot, black band, soft rot, and anthracnose. Similarly *C. urticifolius* and *C. pseudo-capsularis* exhibited resistance reactions to all diseases except soft rot and anthracnose, respectively. Besides these, *C. junodii, C. pinnatipertitus, C. saxatilis,* and *C. gillettii* are also potent sources of biotic stress resistance. *C. urticifolius, C. pseudo-capsularis,* and *C. pseudo-olitorius* were found to be moderately resistant to root-knot nematode, *Meloidogyne incognita* (Cofoid & Chitwood) Chitwood. Nematode galls were observed only in the lateral roots, but the main root was free of galls. The number of egg masses and galls per plant were 7 and 12 in *C. pseudo-capsularis* as against 310 and 403 in *C. capsularis* cv. JRC 212 (SK Laha personal communication; Fig. 2.5; Table 2.3). *C. pseudo-capsularis* produces finest fiber (0.20 tex) followed by *C. urticifolius* (0.30 tex), *C. aestuans* (0.51 tex), *C. trilocularis* (0.77 tex), and *C. pseudo-olitorius* (0.95 tex). *C. angolensis* and *C. merxmuelleri* were found to be higher yielder along with quality fibers. So it becomes imperative to introgress the desirable genes from wild germplasm into the bast fiber producing cultivated species of *Corchorus*. Being vastly divergent from the cultivated types, wild species need to be used as unique source of variability and breeding material for initiating interspecific hybridization program through traditional and advanced tools of biotechnological approaches to increase the productivity and quality of jute fiber.

Table 2.3 Reaction of *Corchorus* spp. against root knot nematode *Meloidogyne incognita*

Corchorus spp.	No. of galls per plant	No. of egg mass per plant	Reaction
C. fascicularis	120	100	HS
C. pseudo-olitorius	15	10	MR
C. urticifolius	14	11	MR
C. tridens	90	70	S
C. pseudo-capsularis	12	7	R
C. aestuans	111	100	HS
C. olitorius (cv. JRO 8432)	350	230	HS
C. capsularis (cv. JRC 212)	403	310	HS

MR Moderately resistant; *S* Susceptible; *HS* Highly susceptible

2.2.1 Traditional Approach

Several attempts on interspecific hybridization through multidirectional crossing, followed by selection amongst the more promising types between the two cultivated species of *Corchorus* (*C. capsularis* and *C. olitorius*), and simultaneously between the cultivated and wild species were made over the last five decades by many workers, but the recovery of fully fertile hybrid is yet to be reported. Reports of interspecific hybridization in this direction are rather meager: *C. tridens* ($2n = 2x = 14$) × *C. siliquosus* ($2n = 4x = 28$) (Anonymous 1952); *C. siliquosus* and *C. capsularis* (Patel and Datta 1960); *C. sidoides* ($2n = 2x = 14$) × *C. siliquosus* ($2n = 4x = 28$) (Datta and Sen 1961); *C. aestuans* ($2n = 2x = 14$) × *C. capsularis* ($2n = 2x = 14$) (Islam and Sattar 1961; Arangzeb and Khatun 1980); *C. trilocularis* ($2n = 2x = 14$) × *C. capsularis* (Faruqi 1962); *C. capsularis* × *C. trilocularis* at the tetraploid level (Chaudhuri and Mia 1962); *C. olitorius* and *C. trilocularis* (Basak 1966); *C. siliquosus* × *C. olitorius* colchi-tetraploid (Islam and Abbasi 1966); *C. trilocularis* × *C. olitorius* (Islam and Feroza 1967); *C. olitorius* and *C. aestuans* (Islam and Haque 1967); *C. hirtus* ($2n = 4x = 28$) × *C. trilocularis* (Mughal 1967); *C. aestuans* × *C. olitorius* (Haque and Islam 1970; Arangzeb and Khatun 1980); *C. olitorius* cv. O-4 × *C. trilocularis* India (Arangzeb 1994).

Many workers claimed to have succeeded in producing interspecific hybrids; however, all those putative hybrids showed dominance of the female parent in F_1 and F_2 generations. Improvement of jute through conventional sexual hybridization between two species could not be achieved due to the absence of intermediate characters in F_1 hybrids. This was confirmed by isozyme and simple sequence repeat (SSR) profiles, where F_1 plants showed bands similar to female parent, but unique hybrid bands or those of male parent were missing. Although it is suspected that failure to obtain true hybrids is either due to apomixis or chromosome elimination or some sort of genetic disharmony and imbalance prevailed in the development of pods and seeds the cellular and/or genetic basis for this phenomenon is yet to be unraveled.

Successful interspecific hybrid was, however, obtained from *C. trilocularis* × *C. capsularis* cv. D-154 at the diploid level with the application of gibberellic acid (GA) at 75 ppm. The hybrid was intermediate between the parents and F_2 population segregated in the parental and hybrid types. Hybrid plants were also obtained between *C. capsularis* cv. Atom-5 × *C. trilocularis* India; *C. aestuans* × *C. trilocularis* India at the diploid level by applying GA at 75 ppm (Arangzeb 1994). Another successful hybrid was obtained between *C. trilocularis* Africa × *C. septentrionalis* at the diploid level in bud pollination without application of any growth hormone and the F_1 was intermediate for most of the characters with meiotic irregularities (Arangzeb 1994). A successful interspecific hybrid between *C. pseudo-olitorius* (WCIJ-34) × *C. capsularis* (Tripura) was obtained to introgress diseases resistance (Palve and Sinha 2005). This interspecific hybrid exhibited partial to full male sterility due to non-homology between the parents. The resultant semi-fertile hybrids, which are now in F_4 generation, are expected to have considerable values with respect to breeding for disease resistance and fiber quality. Close genetic harmony between *C. capsularis* and *C. trilocularis* has been established in a recent report on cytomorphological studies in F_1 hybrids (*C. capsularis* L. × *C. trilocularis* L.) (Maity and Datta 2008).

A total of 212 accessions of seven wild species (*C. pseudo-capsularis, C. pseudo-olitorius, C. aestuans, C. tridens, C. fascicularis, C. urticifolius,* and *C. trilocularis*) together with four varieties of two cultivated species (JRO 524, JRO 878, JRC 321, and JRC 212) were evaluated at the Central Research Institute for Jute and Allied Fibers, Kolkata (India) for quantitative traits, qualitative traits like fiber fineness, fiber strength, and biotic stress resistance with a view to carrying out the crossing program for fertile and stable interspecific hybrids. Fiber strength of *C. pseudo-olitorius* is the highest (10.96 g tex^{-1}) among the wild species, although it possesses comparatively coarse fiber (0.93 tex). On the contrary, *C. pseudo-capsularis* possesses lowest fiber strength, but finest fiber (Table 2.4). Among the seven wild species, several accessions of *C. trilocularis, C. fascicularis, C. pseudo-olitorius,* and *C. aestuans* are resistant against stem rot caused by *Macrophomina phaseolina*. Several accessions among all the wild species are resistant to stem weevil, *Apion corchori* Marshall, the most devastating pest of jute crop. Interspecific crosses were attempted between cultivated

Table 2.4 Reaction of eight *Corchorus* species (6 wild sp. + 2 cultivated sp.) for quantitative traits, qualitative traits and biotic resistance

Characters		*Corchorus aestuans* (77)	*C. tridens* (31)	*C. trilocularis* (65)	*C. pseudo-olitorius* (17)	*C. pseudo-capsularis* (3)	*C. fascicularis* (17)	*C. olitorius* (2)	*C. capsularis* (2)
Quantitative traits									
Plant height (cm)	Mean	111.64	123.62	122.82	89.80	99.50	140.26	352.25	316.22
	Range	79.75–141.95	74.25–150.40	95.45–160.15	69.55–145.40	70.75–111.15	104.75–165.17	328.00–376.50	312.84–319.60
	SD	19.87	21.49	21.15	20.47	17.26	18.32	28.56	18.16
Basal diameter (cm)	Mean	0.80	1.02	0.64	0.73	0.82	0.84	1.69	1.66
	Range	0.40–1.10	0.47–1.48	0.52–0.95	0.36–0.86	0.49–1.06	0.42–1.32	1.62–1.75	1.63–1.69
	SD	0.23	0.34	0.24	0.16	0.21	0.32	0.27	0.25
Days to first flowering	Mean	71.60	37.90	30.20	24.80	28.4	54.70	150.50	136.50
	Range	39.86–113.64	32.36–40.27	26.80–32.48	20.64–31.76	28.00–29.60	34.58–115.92	147.00–154.00	125.00–148.00
	SD	32.14	5.81	2.27	6.43	1.28	40.80	18.90	9.82
Number of nodes	Mean	31.05	30.87	29.50	29.21	28.40	40.67	59.50	53.55
	Range	16.10–45.54	26.86–39.06	23.75–38.13	25.38–33.98	27.06–28.78	26.34–52.43	57.20–61.80	52.72–54.38
	SD	8.67	4.36	4.02	4.65	1.28	8.28	13.84	7.26
Qualititative traits									
Fiber strength (g tex^{-1})	Mean	8.38	6.20	5.85	10.96	5.56	7.32	26.37	22.49
	Range	7.96–8.74	5.72–7.04	5.60–5.98	8.40–12.18	5.39–5.68	7.26–7.46	25.80–25.94	21.97–23.02
	SD	9.07	1.08	0.99	1.38	1.92	1.35	4.22	2.14
Fiber fineness (tex)	Mean	0.54	0.72	0.75	0.93	0.23	0.82	2.88	1.56
	Range	0.48–0.65	0.68–0.84	0.71–0.78	0.87–0.96	0.19–0.27	0.72–0.95	2.74–3.01	1.52–1.58
	SD	0.26	0.65	0.38	1.07	0.19	0.74	1.84	1.02
Biotic resistance									
Stem weevil	Mean	0.12	0.23	0.78	0.10	0.49	0.34	7.42	6.10
	Range	0.06–0.14	0.14–0.32	0.64–0.95	0.03–0.15	0.36–0.62	0.26–0.47	6.98–7.86	5.46–6.74
	SD	3.17	0.13	0.22	0.09	0.18	0.15	0.62	0.91
Stem rot	Mean	0.86	1.64	1.02	0.08	1.15	0.14	3.98	3.81
	Range	0.63–1.02	1.49–1.86	0.67–1.11	0.02–0.11	1.06–1.63	0.10–0.16	3.74–4.10	3.66–3.97
	SD	0.28	0.29	0.31	0.06	0.40	0.04	0.25	1.21

and wild species (JRO-524 × *C. trilocularis*, JRO-524 × *C. pseudo-olitorius, C. trilocularis* × JRO-524, *C. trilocularis* × JRC-321, *C. pseudo-olitorius* × JRO-524, *C. pseudo-olitorius* × JRC-321, *C. aestuans* × JRO-524, *C. aestuans* × JRC-321, *C. tridens* × JRO-524, *C. tridens* × JRC-321, *C. fascicularis* × JRO-524 and *C. fascicularis* × JRC-321) with the aim of introgressing these desired traits from wild to cultivated species and good pod setting was observed in JRO-524 × *C. pseudo-olitorius*, the true hybridity, is however, yet to be confirmed.

2.2.2 Advanced Approaches

To overcome the limitations of traditional crop improvement approaches of *Corchorus* spp. modern molecular genetics and biotechnology have long been recognized as having the potential to increase productivity; improve fiber quality; and broaden the tolerance to drought, salinity, and resistance against various biotic stresses. Advances in molecular biology and genetic engineering would relieve the scientific constraints on the growth of jute production by targeted exploitation of the potential of wild species for desired agronomic traits.

2.2.2.1 Application of Molecular Markers

Genetic Diversity Among the Wild Species

For any meaningful crop breeding program, an accurate estimate of genetic diversity within and between gene pools is a pre-requisite. Molecular markers provide a direct measure of genetic diversity and go beyond indirect diversity measures based on morphological traits or geographical origin (Métais et al. 2002). However, very few efforts were made in the past to develop molecular markers to study the genetic variability among the *Corchorus* spp. (Hossain et al. 2002, 2003; Qi et al. 2004; Basu et al. 2004; Roy et al. 2006; Haque et al. 2007; Mir et al. 2008b). Different authors had determined the genetic diversity of various jute varieties and accessions collected from diverse locations, using random amplified polymorphic DNA (RAPD; Qi et al. 2003b; Haque et al. 2007), intersimple sequence repeat (ISSR; Qi et al. 2003a), RAPD and amplified fragment length polymorphism (AFLP; Hossain et al. 2002, 2003), AFLP and SSR (simple sequence repeat; Basu et al. 2004), sequence tagged microsatellite (STMS), ISSR and RAPD (Qi et al. 2004; Roy et al. 2006) and SSR (Akter et al. 2008; Mir et al. 2008a, b) markers. Based on morphology and DNA classification, it was found that *C. urticifolius* could be one of the original wild species and *C. tilacularis* was a variant of *C. tilacularis* (Qi et al. 2003a, b). The availability of large number of SSR or microsatellite markers offered the scope for estimating the genetic diversity between the wild and cultivated species of jute at the DNA level. The highest similarity (81.67%) was observed between *C. trilocularis* and *C. tridens*. In contrast, only 55.42% similarity was recorded between *C. trilocularis* and *C. fascicularis*. The wild species *C. urticifolius* was found to be closely related with both the cultivated species of jute because it has more than 70% similarity with *C. olitorius* (cv. JRO 524 and PPO 04), and *C. capsularis* (cv. JRC 321 and CMU 010) at DNA level. On the other hand, among the wild species utilized for the experiment, *C. fascicularis* was found to be distantly related to both the cultivated species of jute (Fig. 2.6). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of peroxidase and acid phosphatase of seven *Corchorus* species (five wild species, viz., *C. aestuans, C. paseudo-capsularis, C. pseudo-olitorius, C. tridens, C. trilocularis* and two cultivars JRC-321, JRO-524) showed distinct polymorphism in their banding patterns (Fig. 2.7). Seed protein content is higher in *C. pseudo-olitorius* (24.5 mg g^{-1}) and *C. tridens* (21.3 mg g^{-1}) than in other wild and cultivated species and lowest in *C. aestuans* (6.96 mg g^{-1}). Protein profiling also showed distinct variability among the wild species and between wild and cultivated species (Fig. 2.8). The results clearly indicate that all the *Corchorus* species are genetically distinct and their exploitation in crop improvement program may be effective in broadening the genetic base.

Marker-Assisted Breeding

Recently, it has been observed that the SSR polymorphism in *Corchorus* is higher than in other crop species (Mir et al. 2009). Based on this large-scale assessment of SSR allelic polymorphism, it now appears to be feasible to construct genetic linkage

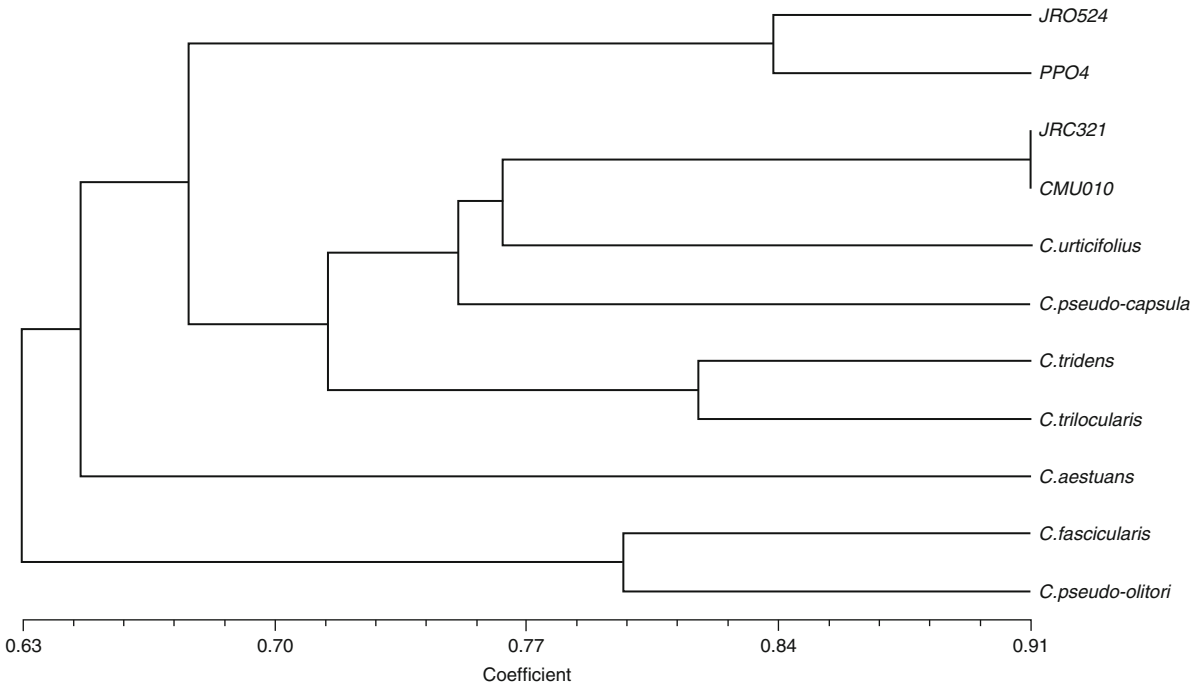

Fig. 2.6 A *dendrogram* showing clustering pattern of 11 jute genotypes (7 wild species + 4 cultivars of two cultivated species) based on simple matching coefficient from SSR polymorphism

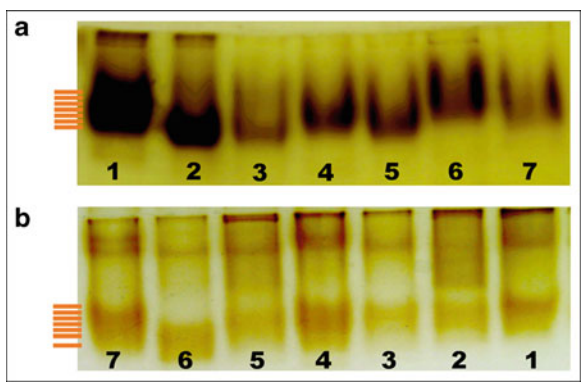

Fig. 2.7 Diversity among wild *Corchorus* species based on Isozyme. (**a**) SDS-PAGE of acid phosphatase showing distinct polymorphism. (**b**) SDS-PAGE of peroxidase showing distinct polymorphism; in both the cases, *lane 1*: *C. capsularis* var. JRC-321; *lane 2*: *C. olitorius* var. JRO-524; *lane 3*: *C. aestuans*; *lane 4*: *C. fascicularis*; *lane 5*: *C. pseudo-olitorius*; *lane 6*: *C. tridens*; *lane 7*: *C. trilocularis*

maps for quantitative trait loci (QTL) analysis of desirable traits. In a collaborative project between Central Research Institute for Jute and Allied Fibers, Kolkata (India) and Choudhury Charan Singh University, Meerut (India) more than 1,000 SSRs and AFLPs were developed to embark upon a program of gene tagging combined with the construction of a framework linkage map and QTL interval mapping specifically for fiber fineness and low lignin content. Simultaneously, genotyping of 324 diverse accessions of *Corchorus* is being carried out using 100 polymorphic SSRs for determining the level of linkage disequilibrium (LD) between the marker loci and the association of marker loci with yield and quality traits to identify linked markers. The results of marker-trait associations following association analysis will be confirmed following QTL analysis to validate the markers. The validated markers will be recommended for marker-assisted selection (MAS) in different *Corchorus* species.

2.2.2.2 Cell and Tissue Culture

To overcome the barrier of sexual incompatibility, which limits the scope for improvement through conventional genetic introgression, the culture of plant cells, tissues, and organs under defined laboratory

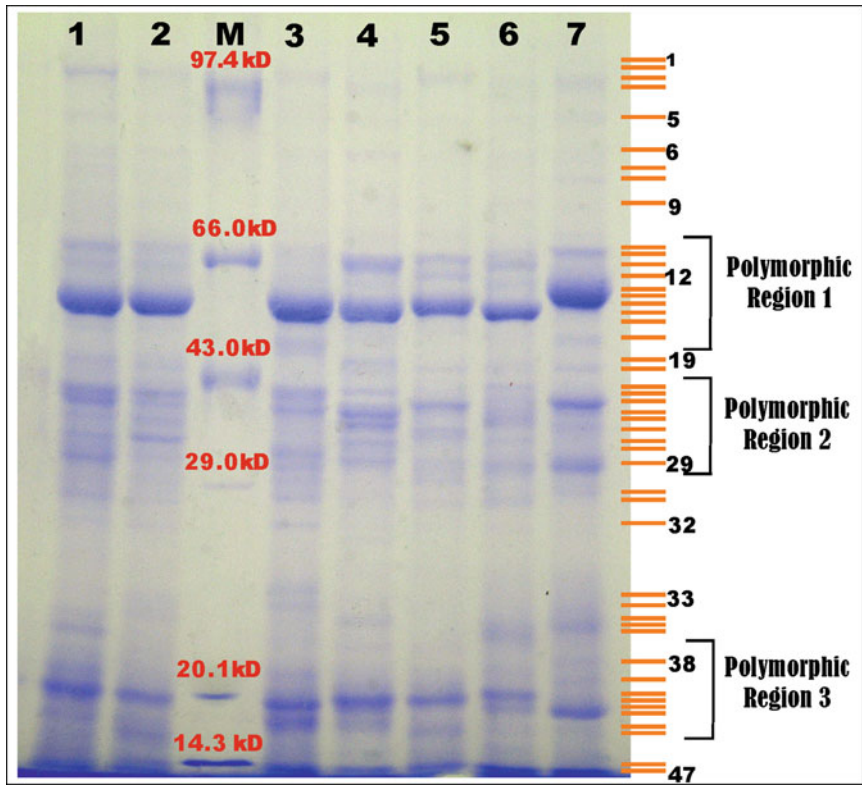

Fig. 2.8 Diversity among wild *Corchorus* species based on protein. *Lane 1*: *C. capsularis* var. JRC-321; *lane 2*: *C. olitorius* var. JRO-524; *lane 3*: *C. aestuans*; *lane 4*: *C. fascicularis*; *lane 5*: *C. pseudo-capsularis*; *lane 6*: *C. tridens*; *lane 7*: *C. trilocularis*; *M* Molecular weight marker (range: 14.3–97.4 kDa)

conditions has been applied. Totipotency exhibited by plant cells enables intact, fertile plants to be regenerated from tissues, which are given the appropriate stimuli from exogenously supplied growth regulators in the culture medium. The ability to regenerate plants is fundamental to the multiplication of elite individuals by micropropagation. Additionally, the genetic engineering of plants, through exposure of somaclonal variation, somatic hybridization by symmetric and asymmetric protoplast fusion and transformation involving *Agrobacterium*-mediated gene delivery or direct DNA uptake, also necessitates reproducible plant regeneration protocols.

There are some reports on successful regeneration in both the cultivated jute species: multiple shoot regeneration from hypocotyls, cotyledons, and leaves (Ahmed et al. 1989; Seraj et al. 1992; Khatun et al. 1993; Saha et al. 2001; Huda et al. 2007; Sarker et al. 2007); in vitro clonal propagation (Abbas et al. 1997); root organogenesis (Khatun et al. 2002); and somatic embryogenesis (Saha and Sen 1992) from protoplast derived calluses. Protoplast fusion was also tried to generate interspecific hybrid cell lines. A chloroplast DNA (cpDNA) marker was developed in jute, which showed species-specific hybridization patterns with *Eco*RI-digested total genomic DNA of *C. capsularis* and *C. olitorius*. This cpDNA marker was used in the characterization of the somatic hybrid cell lines at their early stages of growth. Evidence for the presence of both types of cpDNA in the hybrid cell lines was obtained when the total genomic DNA of 4–7-month-old hybrid cell lines was challenged with the chloroplast DNA marker through southern analysis. It was shown that the early segregation of the parental chloroplasts did not occur in jute, although this is common in other plant species (Saha et al. 2001). So, dimensions of tissue culture research have to be exploited through somatic hybridization both in case of cultivated and wild species. Therefore, molecular genetics and biotechnology in jute in combination with conventional breeding and other areas will guide future research for improving quality and productivity of *Corchorus* with enhanced biotic and abiotic stress tolerance.

2.3 Genomic Resources Developed

More than 200 DNA sequences of *Corchorus* species were deposited in the GenBank till December 2005 (NCBI 2009: http://www.ncbi.nih.gov/index.html). The first DNA sequence of *C. olitorius* was submitted by Alverson et al. (1999). Partial DNA base sequences of NADH dehydrogenese (*ndhF*) gene of a number of wild species of *Corchorus,* viz., *C. bricehaettii*, *C. argutus*, *C. siliquosus*, *C. sidoides* as well as cultivated species of *C. olitorius* and *C. capsularis* were published by Whitlock et al. (2003). Subsequently, Basu et al. (2003a, b) determined complete cDNA sequences of cafteoyl-CoA-*O*-methyl transferase and cinnamyl-alcohol dehydrogenase, which are two of the three major genes involved in lignin biosynthesis. Liu et al. (2005) deposited base sequence of 18S ribosomal RNA gene of wild species *C. aestuaus* and *C. olitorius*. Islam et al. (2005) constructed both cDNA and genomic DNA library in the plasmid vector pSMART and pBluescript, respectively. They submitted complete sequence for tRNA-Leu and partial sequence of DNA fragment encoding several proteins such as RNA polymerase β- subunit -1, 18S rRNA, mitochondrial DNA directed RNA polymerase and carboxyltransferase β- subunit to the GenBank.

2.4 Commercialization of *Corchorus*: Unexploited Potentials Beyond Fiber

Despite the fact that jute is better known as fiber crop, it has also some unexploited potentials as a source of pharmaceutical compounds and dietary supplements due to higher amount of β-carotene. For commercialization of these unexploited potentialities of jute, it is the right time to introduce and domesticate *Corchorus* as an alternative crop for leafy medicinal vegetables.

2.4.1 Therapeutic Uses

The renewed interest in non-orthodox medicine in the West has dramatically increased the demand for plant-based medicine in global market (Natesh 2001). Different plant parts of *Corchorus* can contribute to this aspect and may be used directly as pharmaceuticals. They may also serve as templates for chemical synthesis of bioactive principles (Hazra and Saha 2004; Table 2.5). *Corchorus* species containing important bioactive compounds such as cardiac glycosides, stropanthidin, β-sitosterol, terpenoid-corosin, flavone glycoside, urasolic acid, vitamin C, β-carotene, mucilage, and others are potential candidates for developing plant-based drugs (Chopra et al. 1986; Sen 2002).

2.4.2 Dietary Supplements

Leaves of *Corchorus* are consumed as leafy vegetables in various parts of the world especially in Asia, the Middle East, and part of Africa. Besides adding a distinct flavor to food, jute leaves also have nutritional value, and dried leaves act as thickeners in soups, stews, and sauces. It is a popular vegetable in West Africa and nowadays in Japan, China, Caribbean Island, and some European countries also. The Yoruba of Nigeria calls it "ewedu" while the Songhay of Mali calls it "fakohoy." It is made into a common mucilaginous (somewhat "slimy") soup sauce in some West African culinary traditions and Arabian countries where it is called "Molukhiya." In India, the leaves, "Pat pata" and tender shoots are eaten as dietary supplement to rice from ancient time. It is also a popular dish in the northern provinces of the Philippines, where it is known as "Saluyot." The seeds are also used as flavoring agents, and herbal tea is also made from the dried leaves. In Syria and Jordan, it is typically made into a stew served over white rice and boiled chicken. Recently, noodles made of Saluyot puree is getting popular among Chinese and Japanese for its nutritional value. The leaves are rich in protein, β-carotene, iron, calcium, vitamin B, and vitamin C. The plant has an antioxidant activity with a significant α-tocopherol equivalent vitamin E (Table 2.6). Leaves also contain oxydase and chlorogenic acid. The folic acid content is substantially higher than that of other folacin-rich vegetables, ca. 800 μg per 100 g (ca. 75% moisture) or ca. 3,200 μg per 100 g on a zero moisture basis (Chen and Saad 1981; Duke 1983).

Table 2.5 Therapeutic compounds of different species of *Corchorus* used as herbal drugs

Name of the species	Plant parts	Therapeutic compounds	Implication/mode of application
C. olitorius L. (wild)	Leaves, seeds	Cardiac glycosides olitoriside, corchoroside A, B, stropanthidin, β-sitosterol, olitorin, stropanthidiol, helveticoside (seeds); erysimoside, ursolic acid (roots); vitamin C (fruits); mucilage (leaf, stem, and seed coat)	*Leaves:* Astringent, alexiteric (long lasting effect); remove tumors, ascites, pain (accumulation of serous fluid in peritoneal cavity), abdominal tumors and piles (ayurvedic formulations); demulcent, tonic, diuretic, chronic cystitis, gonorrhea, piles, laxative (dried) *Seeds:* Tonic, fever drink, appetizer (infusion); purgative (raw)
C. capsularis L. (wild)	Leaves, roots, and unripe fruits, seeds	Corchotoxin, olitoriside, corchoside A, B, and C, erysimoside, stropanthidin, helveticoside, β-sitosterol (roots and leaves), ursolic acid, terpinoid-corosin (roots); oligosaccharides, mucilage (leaf, seed coat, and stem), vitamin C (fruits)	*Leaves:* Dysentery, fever, dyspepsia (indigestion); liver disorder, tonic, stomachic (a gastric stimulant); laxative, carminative, appetizer, antiperiodic, anthelmintic (expels intestinal warms); astringent (contract organic tissues); demulcent (snoothing); bilious trouble (excess bile production); heptic (liver inflammation); intestinal colic, gastriligia (stomachache); gastric catarrh (inflammation of mucus membrane) and skin diseases (infusion); dysentery (dried) *Roots and Unripe fruits:* Diarrhea (decoction) *Seeds:* Purgative (raw)
C. aestuans L.	Whole plant, seeds	Vitamin C, β-sitosterol, cardenolides (glucoside A, B and C)	*Whole plant:* Used in fever, stomachache *Seeds:* Gonorrhea, tonic
C. depressus L.	Whole plant, roots, leaves, seeds	2α, 3β, 20β, -urs-12-en-23β-18-dioic acid 2, 3-diacetate	*Whole plant:* Acrid, remove tumors and pain, cures piles (ayurvedic formulation) *Roots:* Tonic, gonorrhea, cooling medicine in fever (ayurvedic formulation) *Leaves:* Emollient (snoot skin irritation and mucus membrane) *Seeds:* Tonic, gonorrhea (decoction)
C. fascicularis Lam.	Leaves, whole plant, seeds	Flavones glycoside	*Leaves:* Cooling, laxative, stimulant tonic, aphrodisiac (arousing sexual desire); destroy "tridosha" (ayurvedic formulation) *Whole plant:* Acrid (bitter); astringent to the bowls; removes tumors, ascites, piles, cure dysentery, anthelmintic, discharging ulcers, restorative (raw) *Seeds:* Alexipharmic (antidote to poison); remove tumors, pain, stomach troubles, skin diseases, scabies and tonic (ayurvedic formulations)
C. humilis M.	Whole plant		Cooling medicine, demulcent, gonorrhea (macerated in water)
C. siliquosus L.	Leaves		Pot-herb (raw)

(*continued*)

Table 2.5 (continued)

Name of the species	Plant parts	Therapeutic compounds	Implication/mode of application
C. tridens L.	Leaves	Glycosidal fraction	Depress the central nervous system, lower the blood pressure, and stimulate smooth muscles (ayurvedic formulations)
C. trilocularis	Seeds, whole plant	Cardenoide-trilocilarin and corchoroside B (seeds)	*Seeds*: Fever, remove obstruction in abdominal viscera, specific in rheumatism (raw) *Whole plant*: Mucilage is demulcent (ayurvedic formulations); astringent to the bowls; removes tumors, ascites, piles, cure dysentery, asthelmintic, disxharging ulcers, restorative (raw)
C. acutangulus Lam.	Leaves		Pot-herb (raw)
C. antichorus Raeusch	Whole plant		Pot-herb (raw after boiling)
C. urticifolius L.	Leaves		Pot-herb (raw)
C. pseudo-olitorius Islam & Zaid	Leaves	Mucilage	Appetizer (boiled in hot water)

Table 2.6 Nutrient content of *Corchorus* leaf

Nutrients	Nutrient composition per 100 g
Energy	43–58 kcal
Water	80.4–84.1 g
Protein	4.5–5.6 g
Carbohydrate	7.6–12.4 g
Fat	1.0–1.3 g
Calcium	266–366 mg
Iron	7.2–7.7 mg
Potassium	440–444 mg
β-carotene (vitamin A)	6,410–7,850 μg
Thiamin (vitamin B)	130–150 μg
Riboflavin (vitamin B)	260–530 μg
Niacin (vitamin B)	1,100–1,200 μg
Ascorbic acid (vitamin C)	53–80 mg

2.4.3 Wild Species as a Source of Biofuels

Biofuels represent the second largest source of energy used after fossil fuels all over the world. In developing countries, about 2,000 million people depend almost entirely on biofuels for their energy needs. Stick and caddies of wild *Corchorus* species can well be utilized as substitute for fossil fuel by briquetting and gasification (Paul 2008). Woody type wild species like *C. fascicularis*, *C. baldaccii*, and *C. hirsutus* could be effective for this purpose.

2.4.4 Wild Corchorus as Raw Material for Pulp and Paper Industry

Pulp and paper industry is one of the core industries, and its growth has a direct impact on the socioeconomic development of any country. The sustainability of paper industry is very important and depends on (1) availability of cellulose raw material, (2) how the industry will address environmental issues, and (3) ability of the industry to keep pace with the modernization. Sustained availability of cellulose raw material is the single most important factor, which will determine the healthy growth of the paper industry. In the last 30 years, due to depleting forest-based resources, availability of good quality cellulose raw materials has been one of the major challenges before the paper industry. It is estimated that the world demand of pulp and paper will rise to 620 million tons by the year 2010. The shortage of raw material required to

fulfill the required growth rate of medium and high quality paper cannot be met with forest resources and the pulp from agro-residues of inferior quality. Hence, the *Corchorus* spp. with similar strength of hard wood have emerged as potential pulp and paper material. Along with cultivated species of jute, the wild taxa of *Corchorus* can also be exploited for pulp and paper industry.

2.5 Dark Side of Wild *Corchorus* Species

2.5.1 Constraints as Weeds and Invasive Species

Most of the wild species of *Corchorus* are common weeds in Australia, Egypt, Mozambique, Philippines, Senegal, USA, European countries, Thailand, Afghanistan, India, Kenya, Nepal, Turkey, and Zambia (Holm et al. 1979). Some of the wild taxa, being native/invasive species have emerged as real threat to the cultivated crops (Randall 2007). *C. aestuans* (weed in Australia, Pakistan; invasive spp. in India; mainly found in cotton field), *C. angolensis*, *C. antichorus*, *C. argutus*, *C. asplenifolius*, *C. depressus* (weed in Pakistan, found in cotton field), *C. fascicularis* (weed in sugarcane, Sudan; invasive spp. in India from tropical America), *C. hirsutus*, *C. hirtus*, (weed in Australia, Sudan, Japan, France, Turkey, Afghanistan, USA, Philippines, Thailand), *C. orinocensis*, *C. siliquosus* (known as Broom weed, America and common in sugarcane field), *C. tridens* (weed in cotton field, Pakistan; invasive spp. in India from Africa), *C. tilocularis* (weed in Australia, Tanzania, Pakistan; invasive spp. in India; weed in maize, beans, and wheat in Tanzania; weed in cotton in Pakistan), *C. sidoides* (native weed, pasture land of Australia), *C. walcottii* (native weed, pasture land of Australia), *C. pseudo-olitorius* (invasive spp. India, weed in sugarcane), *C. baldaccii* (weed in Kenya).

2.5.2 Alternative Host and Potential for Super Weeds

Wild species being alternative hosts for viruses as well as vectors have direct or indirect effect on crop loss and they have the potential to become super weeds due to gene flow from transgenic crops. The wild species, which were found as alternative hosts are listed in Table 2.7.

Table 2.7 Wild species of *Corchorus* as alternative hosts for viruses and vectors

Virus/vector	Wild *Corchorus* spp.	Remarks
Tobacco streak virus	*Corchorus tilocularis*	Weed host in sunflower field in Australia (GRDC 2008)
Bemisia tabaci Genn.	*Corchorus tridens*	Found in northern Nigeria; heavily infested plants were distorted, chlorotic, and stunted (Alegbejo and Banwo 2005)
White fly	*Corchorus aestuans*	Found in Kenya (Edmonds 1990)
Spiny bollworm (*Earias* spp.) of cotton	*Corchorus tilocularis*	*Corchorus tilocularis* is the main host for larvae in South Africa (Green et al. 2003)
Cotton leaf curl virus	*Corchorus fascicularis*	Found in Nigeria (Brunt et al. 1990)

2.6 Future Thrust

The UNO declared 2009 as "The Year of Natural Fibers." Jute is the cheapest and the second most important vegetable fiber following cotton in terms of global production, consumption, and availability. Jute fiber is 100% biodegradable and recyclable and thus, environment friendly. It is one of the most versatile natural fibers that has been in use as raw material for packaging, textiles, non-textiles, construction, and agricultural sectors. It helps to make best quality industrial yarn, fabric, net, and sacks. In the era of growing awareness about environmental pollution and ecological degradation due to synthetic polymers, the importance of natural fiber like jute is gaining momentum.

It has been estimated that to produce one ton of synthetic fiber, at least 31 kg of nitrogen dioxide and 12 kg of sulfur dioxide are emitted into air when fossil fuel is used. These figures increase to 155 kg and

70 kg, respectively, when crude petroleum is used. On contrary, one ton of jute fiber comes from 4 tons of friendly biomass, besides adding nearly 6 tons of carbon dioxide. It is also estimated that from jute, 3 million tons of dry leaves go to the soil incorporating 90, 30, and 80 thousand tons of nitrogen, phosphorus, and potash, respectively. In addition, 4.5 million tons of the biomass produced goes into retting water, which further fertilize the soil. Further, production of one tons of synthetic material consumes 90 GJ (Giga Joule), while the same amount of natural fiber consumes only 7.8 GJ (Sinha et al. 2004). All these facts make the natural plant fibers environment friendly right from the field to industrial processing and even in the disposal of waste. As a consequence, the demand for natural fiber is increased tremendously and at least threefold for jute. The earlier concept of "jute for packaging only" has gone, but the diversified products are gaining more importance nowadays. In jute (1) non-technical textile sector, viz., (a) geo-textile, (b) automobile textile, (c) medico-textile is emerging at a faster rate. The jute geo-textile is gaining momentum for road construction and to check the erosion. It is proved that the roads are getting more strength and durability. (2) In the textile sector, jute can be used alone for the preparation of clothes, if the fiber fineness is increased. Wild species of jute possess finest fibers (0.5 tex) and low strength (4–6 g tex^{-1}), which may be exploited by interspecific hybridization to develop variety for textile industry. Moreover, wild *Corchorus* spp. also showed resistance to biotic (*Macrophomina*, *phaseolina*) and abiotic stresses (drought and waterlogging), which may be exploited through interspecific hybridization with elite cultivars. Jute fiber contains cellulose (60–61%), hemi-cellulose (15–16%), and lignin (12.5–13.5%). Though lignin gives more strength to the fiber, it increases the coarseness of the fiber. Hence, efforts need to be taken to develop variety with optimum lignin, which helps to enhance the strength as well as fineness. At present jute is being blended with cotton, but in near future it can be utilized directly for textile purpose. This can be achieved through hybridization of cultivated species with wild species. Since the wild *Corchorus* spp. are potential sources for better fiber quality, and biotic and abiotic stress resistance, necessary efforts should be taken to conserve them either in situ (perennial spp. like *C. baldaccii*, *C. depressus,* etc.) or through regeneration at regular intervals (annual spp. like *C. trilocularis*, *C. tridens,* etc.). In general, the viability of wild species ranges from 2 to 3 years. Since lower moisture content at storage may lead to hard-seededness, which ultimately hinders germination, the accessions of wild *Corchorus* species should not be conserved below 10% moisture during medium- to long-term storage.

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Chapter 3
Crotalaria

Jorge A. Mosjidis and Ming Li Wang

3.1 Basic Botany of the Species

The genus *Crotalaria* belongs to the Fabaceae family and contains approximately 600 species that grow wild in tropical and subtropical areas (Polhill 1981). Sunn hemp (*Crotalaria juncea* L.), the main species in the genus, is grown worldwide in tropical areas and is being bred to grow and produce seeds in temperate climates (Mosjidis 2006). Sunn hemp is probably native to the Indo-Pakistan subcontinent (Wiersema et al. 1990). It belongs to the family Fabaceae, subfamily Papilionoideae, tribe Crotalarieae, section *Calycinae* (van Wyk and Schutte 1995). *C. juncea* is a diploid species ($2n = 2x = 16$) (Purseglove 1981). The haploid nuclear content of *C. juncea* L. is approximately 1C = 1.23 pg (Gupta 1976; Bennett and Leitch 1995).

Sunn hemp is an erect herbaceous annual that branches in the upper portion of the stem. Dense plantings reduce branching. Stems are cylindrical with ridges that end on an inflorescence. Deep yellow flowers develop acropetaly on the inflorescence, which is a terminal open raceme. Plants produce an indeterminate number of flowering stems. The number of flowering stems is much influenced by water availability, temperature and daylength. Flowers are visited by a variety of insects and humming birds. Flowers of sunn hemp are cross-pollinated by several bee species. In Brazil, it has been reported to be visited by native stingless bees *Xylocopa* spp. (Nogueira-Couto et al. 1992; Heard 1999). Bees visit fully open flowers and prefer to collect pollen in the afternoon (Maiti 1997). Fertilization occurs only after the stigmatic surface has been damaged by bees or by mechanical means (Purseglove 1981).

Sunn hemp has as base chromosome number of 8. A karyotype characterization by chromosome banding was recently conducted by Mondin et al. (2007). They found that chromosomes can be identified during metaphase based on small differences in their morphology when Feulgen-stained. There is a secondary constriction only in the largest chromosome. This secondary restriction is the major nucleolus organizer region (NOR) in sunn hemp. However, chromosome 4 may have nucleolar activity in some stages of the cell cycle.

The genus *Crotalaria* has about 600 species distributed mostly in tropical and subtropical areas of the southern hemisphere and Africa (Polhill 1981). Chromosome number of most *Crotalaria* spp. is $2n = 16$ (Polhill 1981), but species in the section *Chrysocalycinae* subsection *Incanae* usually have $2n = 14$ and polyploids ($2n = 32$) have been reported among the American species (Mondin et al. 2007).

Sunn hemp is the fastest growing species of the genus *Crotalaria*. It has multipurpose use as green manure, fiber and animal fodder crop (Purseglove 1981) and has potential as an ornamental plant. Sunn hemp is an important source of fiber for the production of paper and ropes in the Indo-Pakistan subcontinent (White and Haun 1965). *C. spectabilis*, *C. paulina* and *C. mucronata* may have as much biomass production as *C. juncea* (Mes et al. 1957). However, *C. spectabilis* is toxic. Another species that has potential use in Africa is *Crotalaria ochroleuca* Don. known as slender leaf rattlebox (Sarwatt 1990).

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3.2 Conservation Initiatives

Genetic erosion is not a major issue for the cultivated species at this time, but germplasm stored in banks is limited. It is necessary to collect seeds of landraces and ecotypes in the Indo-Pakistan subcontinent and other countries of southeastern Asia to increase the number of accessions presently stored in germplasm banks. Seed longevity seems to be good and many species have hard seeds (Kak et al. 2007). *C. juncea* can have a small percentage (about 10% or less) of hard seed (J Mosjidis unpublished results).

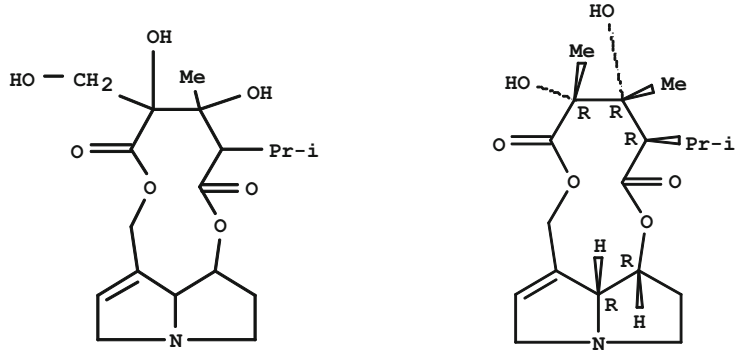
3.3 Roles in Elucidation of Origin and Evolution of Sunn Hemp

The tribe Crotalarieae has two genera, *Crotalaria* and *Lotononis,* which include species that currently have agronomic use. These two genera also form a group characterized by containing pyrrolizidine alkaloids (van Wyk and Verdoorn 1990). Pyrrolizidine alkaloids are N-containing compounds derived from ornithine, an intermediate in arginine biosynthesis.

It has been reported that sunn hemp seeds contain various pyrrolizidine alkaloids such as junceine, riddelliine, senecionine, seneciphylline and trichodesmine (Smith and Culvenor 1981). However, Ji et al. (2005) determined that trichodesmine and junceine (Fig. 3.1) were the only pyrrolizidine alkaloids present in the seeds of nine *C. juncea* populations that originated in different parts of the world. The amounts of pyrrolizidine alkaloids reported by Ji et al. (2005) were small that agrees with other reports (Adams and Gianturco 1956; Pilbeam and Bell 1979b; Williams and Molyneux 1987). This was confirmed again by Nurhayati and Ober (2005) who did not detect alkaloids in cotyledons, leaves, flowers or roots of *C. juncea*, which suggests that alkaloid production is limited to the seeds.

A study of free amino acids in *Crotalaria* seeds indicated a high correspondence between free amino acid distribution and taxonomic relationships in the genus (Pilbeam and Bell 1979b). *C. juncea* had an amino acid pattern different from all other *Crotalaria* species. It had low levels of pyrrolizidine alkaloids and a high content of δ-hydroxynorleucine in the seeds, up to 2% of seed weight (Pilbeam and Bell 1979a), whereas species in the section *Crotalaria* subsection *Crotalaria* contain γ-glutamyltyrosine. Species in the section *Calcynae* also lack γ-glutamyltyrosine. Thus, *C. juncea* seems to be intermediate between sections *Calcynae* and *Crotalaria* (Pilbeam and Bell 1979b). Some *Crotalaria* species have the toxic amino acids α-amino-β-oxylaminopropionic acid, α-amino-γ-oxylaminobutyric acid and/or α,γ-diaminobutyric acid in the seeds (Pilbeam and Bell 1979b).

Another compound reported to be in sunn hemp seeds is cardenolide cardiogenin 3-*O*-beta-D-xylopyranoside (Yadav and Thakur 1994), but its significance has not been assessed. Hamana et al. (1996) determined that *C. juncea* and *C. spectabilis* seedlings had a high content of polyamides. Mangotra and Bhargava (1987) used carbohydrates present in the plants to identify taxonomic relationships, but they found them to be of limited taxonomic use in the genus *Crotalaria*. However, Anuradha et al. (1989) determined that the presence of a number of secondary metabolites in several *Crotalaria* spp. can be used to identify taxonomic relationships within the genus.



Junceine Trichodesmine

Fig. 3.1 Pyrrolizidine alkaloids junceine and thrichodesmine

In most angiosperms, mitochondrial and chloroplast genomes are inherited maternally. Variation detected from mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA) can be used for population genetic studies and phylogenetic analysis at both intra- and interspecific levels (Netwon et al. 1999; Desplanque et al. 2000). Recently, Boatwright et al. (2008) used nucleotide sequences from the internal transcribed spacer (ITS) of nuclear ribosomal DNA (the plastid gene *rbc*L) and morphological data to study phylogenetic relationships in the tribe Crotalariae. They determined that this tribe is monophyletic and is sister to the tribe Genisteae. As far as we know, there are no DNA markers developed from mitochondrial genome of *Crotalaria* that can be used for population and phylogenetic studies within the genus *Crotalaria*.

3.4 Roles in Genetic Studies and Development of Cytogenetic Stocks

Lack of genomic resources such as DNA markers, large insert-size clones (e.g., bacterial artificial chromosomes) and known-function genes in *Crotalaria* has hindered advanced genetic studies, such as genetic and physical mapping, in the genus.

Most species from the genus *Crotalaria* are diploids with 16 chromosomes ($2n = 2x = 16$). However, a diploid with 14 chromosomes ($2n = 2x = 14$, for example, *C. incana*) and tetraploids with 32 chromosomes ($2n = 4x = 32$, for example, *C. paulina* and *C. stipularia*) were also found within *Crotalaria* (Oliveira and Aguiar-Perecin 1999). Karyotyping by C-banding revealed that two secondary constrictions (NOR-heterochromatin) were on the distal region (major nucleolus organizer regions) of chromosome 1 and the proximal region of chromosome 4. The secondary constriction regions on the chromosomes were also confirmed using 45S rDNA as probed by fluorescent in situ hybridization (FISH) analysis (Mondin et al. 2007). Identification of the variation of basic chromosome number among species within *Crotalaria* may help to understand and explain the process of evolution and divergence for speciation.

Classical genetic studies are almost non-existent. Miranda et al. (1989) determined that anthocyanin pigmentation in flowers and hypocotyl of *C. juncea* was due to a pair of alleles. The allele in the dominant form ($A-$) was required for color presence and the recessive (*aa*) for absence. However, no genetic work has been accomplished in *C. juncea* using its wild allies.

3.5 Genomics Resources

As most *Crotalaria* species are not major crops, there have been no public efforts on sequencing any species within the genus. Therefore, there is very limited sequence information available. By searching NCBI database, only 152 entries are available related to *Crotalaria* and over one-third of the sequences have a high homology either to ribosomal or to chloroplast DNA. Nucleotide sequences of cpDNA (*rbc*L) and ncDNA (ITS 1 and 2) of rDNA have been used for phylogenetic analysis within the family Leguminosae. It was found that Crotalarieae shares the same ancestry with Genisteae, Thermopsideae/Podylyrieae (Käss and Wink 1997).

Seeds from *Crotalaria* contain large amounts of pyrrolizidine alkaloid (PA), which are chemicals related to plant defense. Deoxyhypusine synthase (DHS) is one of the specific enzymes involved in PA biosynthesis. Two DHS genes (*DHS1* and *DHS2*) have been identified and cloned in *C. juncea*. Genes for DHS and pseudogenes with homology to the DHS-coding genes were also identified and sequenced in other *Crotalaria* species including *C. anagyroides*, *C. retusa* and *C. scassellatii* (Nurhayati and Ober 2005). There were 12 sequence entries generated through sequencing cDNA and genomic DNA of DHS genes.

From a phylogenetic point of view, some legumes are more closely related to *Crotalaria* than others. In one study (Choi et al. 2006), 274 unique gene sequences were selected from six model (or cultivated) legumes and tested on 94 legume species (including *Crotalaria cunninghamii* R. Br.). From this study, six sequences from *C. cunninghamii* have been deposited into Genbank. The successful rate of amplification across the legume family is definitely related to the phylogenetic distance with the model or crop legumes from which the primers were derived. Limited sequence resources within the *Crotalaria* genus are greatly hindering the genetic research within the genus. Although DNA markers are not available

Fig. 3.2 Selection for adaptation to humid temperate climate of the Southeast USA. In the *foreground* are the selections adapted and able to produce seed in temperate regions. In the *background* is Tropic Sun, a tropical cultivar unable to produce seed in the Southeast USA

for *Crotalaria*, they can be developed by a transferable approach (i.e., testing whether DNA markers developed from other related species will work on species within the *Crotalaria* genus). Fifty-eight primer sets from *Medicago* and soybean were tested on *Crotalaria* species and 28 (48%) generated polymorphic amplicons. Using these transferred DNA markers, the genetic diversity of *Crotalaria* from four species was assessed, their phylogenetic relationships were analyzed and even some misclassified accessions were identified and renamed (Wang et al. 2006). For genetic studies of *Crotalaria* species, new genomic and genetics resources need to be developed by sequencing and mutagenesis (Fig. 3.2).

3.6 Some Dark Sides and Their Addressing

Pyrrolidizidine alkaloids ingested in sufficient amount can be toxic to animals and birds. Various animal species have different susceptibility to pyrrolizidine alkaloids. Susceptibility was determined to be highest for pigs, followed by chickens, horses and cattle, rats, mice, sheep and the least susceptible were goats (Hooper 1978). Many *Crotalaria* species have been found to be toxic to animals particularly because of high pyrrolizidine alkaloid content. In fact, Strickland et al. (1987) determined that about 50% of the *Crotalaria* species they tested were toxic. This information has been indiscriminately extended to *C. juncea*, but has not been substantiated. Exceptions are the reports of Ritchey et al. (1941) and Nobre et al. (1994). Ritchey et al. (1941) found that feeding a high dose of seeds to sheep for 26 days was toxic to them. Nobre et al. (1994) reported that feeding a diet that included 40% *C. juncea* seeds to horses for 30 days caused their death. The recent study of Hess and Mosjidis (2008) where *C. juncea* whole or ground seeds were included in the diet of broiler chicken (highly sensitive to pyrrolizidine alkaloids) showed that the seeds did not affect bird mortality. However, when seeds were fed at a feed ingredient level (2–5% of the diet) it reduced body weight and feed consumption. Inclusion at a contaminant level (0.5%) had no negative impact on the birds (Hess and Mosjidis 2008). A rat bioassay conducted by Strickland et al. (1987) indicated that neither leaves, or

Table 3.1 Seed toxicity of *Crotalaria* species introduced to the USA

Species	Common Name	Acute toxicity	Reference
C. juncea	Sunn hemp	No	Purseglove (1981), Rotar and Joy (1983), Williams and Molyneux (1987), Strickland et al. (1987), Hess and Mosjidis (2008)
C. pallida	Smooth crotalaria	No	Williams and Molyneux (1987)
C. retusa	Wedge-leaf rattle box	Yes	Hooper and Scanlan (1977), Williams and Molyneux (1987)
C. spectabilis	Showy crotalaria	Yes	Johnson et al. (1985), Williams and Molyneux (1987)

stems or seeds of *C. juncea* were toxic; however, other *Crotalaria* species were toxic. Showy crotalaria (*C. spectabilis*), wedge-leaf rattle box (*C. retusa*) and smooth crotalaria (*C. pallida*) were introduced into the USA as green manure crops to improve soil fertility (Williams and Molyneux 1987).

There are two types of evidence indicating that *C. juncea* seeds do not cause acute toxicity as reported for other *Crotalaria* species (Asres et al. 2004) and particularly some species introduced to the USA such as *C. spectabilis* (Williams and Molyneux 1987) (Table 3.1). The first set of evidence comes from the reports that found low pyrrolizidine alkaloids levels or lack of them in seeds and other plant parts in a large number of populations developed in several countries (Adams and Gianturco 1956; Pilbeam and Bell 1979b; Strickland et al. 1987; Williams and Molyneux 1987; Ji et al. 2005; Nurhayati and Ober 2005). Second, toxicological studies conducted with animals (Purseglove 1981; Rotar and Joy 1983), particularly chicken that are very susceptible to pyrrolizidine alkaloids, demonstrated lack of acute toxic effects (Williams and Molyneux 1987; Hess and Mosjidis 2008). However, the results of Hess and Mosjidis (2008) and the reports of Ritchey et al. (1941) and Nobre et al. (1994) point out that seeds should not be considered foodstuff and they should not be included in large amounts and for long periods of time in an animal diet.

3.7 Recommendations for Future Actions

Most *Crotalaria* species are weedy, but a few have been used as a crop with agricultural, economic and pharmaceutical importance. They can be used as cover crops for providing green manure and preventing soil erosion, as rotation crops for nitrogen fixation, as forage crops for grazing, as a source of long fiber to produce high-quality paper, as intercepting crops for nematode control, and as medicinal plants for chemical compound extraction. The following aspects are recommended for future actions.

1. *Expanding the germplasm stored in banks: Crotalaria* species are distributed worldwide but their germplasm has not been well collected. For example, there are approximately 600 species, but there are only 242 accessions in the USDA collection representing about 30 species. More accessions need to be curated from different regions of the world.
2. *Generation of genomic resources:* There are very limited genomic resources (only 152 sequence entries in NCBI). As sequencing technology advances and sequence cost drops sharply, low-pass genomic sequencing (even with 2–3*x* genome coverage) should be initiated within *Crotalaria* species. Low-pass genomic sequencing will provide not only the genome feature of *Crotalaria* but also a plenty of DNA markers for genetic studies.
3. *Generation of genetic resources:* Application-oriented hybridization (crossing within and among species) and mutagenesis (irradiation or EMS-induced mutation) should be conducted in *Crotalaria*. New populations from hybridization and mutation populations will not only help to develop new cultivars and breeding materials but will also provide materials for genetic studies.
4. *Breeding for photoperiod insensitivity:* Photoperiod sensitivity is a major limitation for growing *C. juncea* in diverse environments. Sowing the plants at a later time in the season has the potential to increase the use of this plant as a green manure crop. Lack of sensitivity to photoperiod would allow plants to grow taller and produce more biomass when planted in late summer.
5. *Biochemical and biological analysis:* Some biochemical compounds are unique in *Crotalaria*. To exploit their utilization, these compounds need to be extracted, identified and characterized biochemically (such as using HPLC, GC, GC-mass

spectrometry, LC-mass spectrometry). Metabolomics could fill the gap in our knowledge of chemicals present in *Crotalaria*. On the basis of the compound property, bioassays need to be designed and to test biological activity, for example, elucidation of the mechanism of nematode resistance using characterized compounds from *Crotalaria* by bioassay.

6. *Further exploiting of its utilization: Crotalaria* are under-exploited species and many of its utilizations have not been well mined. Some species such as *C. medicaginea* have been reported to have mucilaginous polysaccharides in the seeds (Gupta and BeMiller 1990) that have a variety of industrial and medicinal applications. *C. ochroleuca* has the potential to be used as a forage plant (Sarwatt 1990). *C. juncea* has a rapid growth (120 days), large biomass production of the tropical types (8,900–13,000 kg ha^{-1}) and efficient nitrogen accumulation (135–285 kg ha^{-1}); sunn hemp (*C. juncea*) has the potential to be widely used as cover crop in the USA southeastern regions (Schomberg et al. 2007). Furthermore, its potential to be used as a feedstock for bioethanol production has not been explored yet. Because of its large biomass production, sunn hemp may need to be evaluated as one of the feedstock candidates for cellulosic ethanol production, particularly in tropical areas.

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Chapter 4
Dioscorea

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4.1 Introduction

After the cereal grains, majority of the world's population depends on root and tuber crops, which include stems, rhizomes, corms, bulbs, tubers, and other types of organs as well as true roots. Among these, yams – a multispecies crop – are considered as one of the most important staple food crops of sub-Saharan Africa that provide valuable source of dietary carbohydrate and income. They are mainly grown in tropical and subtropical Africa, Central and South America, parts of Asia, and the Caribbean and South Pacific Islands (Coursey 1967; Adelusi and Lawanson 1987). Yams are monocotyledonous classified under the genus *Dioscorea*, family Dioscoreaceae, and order Dioscoreales. More than 600 species have been reported in the genus *Dioscorea* (Coursey 1969), which has been reestimated to comprise about 250–400 species distributed throughout the tropics and subtropics growing wildly as climbing vines (Caddick et al. 2002). Less than 50 species have been domesticated for food and industrial use (Hahn 1995), of which only ten are important as staples in the tropics (Coursey 1969; Hahn 1995), while many of the wild species are also a reliable source of food during food scarcity. They have been the main food source for the Mbuti pygmies of eastern Zaire (present Democratic Republic of Congo) (Milton 1985; Hart and Hart 1986), the Batek of Peninsular Malaysia (Endicott and Bellwood 1991), the Baka pygmies in the forests of southern Cameroon (Dounias 2001; Sato 2001), and people at Kuk Swamp of Papua New Guinea (Fullagar et al. 2006). The economically important species worldwide are *Dioscorea rotundata* (white guinea yam), *D. cayenensis* (yellow guinea yam), *D. dumetorum* (trifoliate or bitter yam), *D. alata* (yellow yam), *D. esculenta* (Chinese yam), *D. trifida* (cush-cush yam), and *D. bulbifera* (water or greater yam). Several wild species are also used as a source of food particularly during famines due to failure of staple food crops (Hahn 1995). About 30 species are grown on a minor scale for steroidal compounds such as sapogenin, dioscorin, and diosgenin for the pharmaceutical industry (Martin and Degras 1978; Orkwor 1998; Hahn 1995).

4.1.1 Origin and Distribution

The genus *Dioscorea* is considered to be among the most primitive of the Angiosperms and differentiated as Old and New world species (Coursey 1967; Hladik and Dounias 1993). The occurrence of *Dioscorea* spp. in southern Asia, Africa, and South America long predates human history, and domestication of the different species in these areas appears to have been by aboriginal man. The formation of the Atlantic ocean at the end of Cretaceous era seems to have separated the Old and New world species of yams (Coursey 1967). The desiccation of the Middle East during the Miocene period probably separated the African and Asian species (Coursey 1967). Yams are also believed to have originated in the tropical areas of three separate continents: Africa (mainly West Africa for *D. rotundata*, *D. cayenensis* and *D. dumetorum*), Southeast Asia (for *D. alata* and *D. esculenta*), and South America (for *D. trifida*). The Asiatic yam, *D. alata*, might have originated in tropical Burma

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and Thailand and *D. trifida*, the South American yam, is believed to date back to pre-Columbian times (Ayensu and Coursey 1972). Although *D. alata* reached the east coast of Africa at about 1500 BC from Malaysia, there is no evidence that it played any significant role in the evolution of cultivated African yams.

In West Africa, domestication of yam started as early as 50000 BC, during the Paleolithic era (Davies 1967). However, archeologists believe that actual cultivation of yam started about 3000 BC, about the same time when it started in Southeast Asia (Coursey 1967; Davies 1967; Alexander and Coursey 1969). The earliest domesticated yams in West and Central Africa are *D. rotundata*, *D. cayenensis,* and *D. dumetorum*, while in Southeast Asia, it was *D. alata* that was first cultivated. *D. alata* moved to India and Pacific Ocean more than 2,000 years ago (Coursey and Martin 1970). It is believed that there has been an east-to-west movement of yam species, wherein *D. alata* and *D. esculenta* moved westward to Africa and America, and the African species, *D. rotundata* and *D. cayenensis* moved westward to the Americas. The West African yam belt (Fig. 4.1) comprising Nigeria, Republic of Benin, Togo, Ghana, Cameroon, and Côte d'Ivoire is believed to have the oldest yam culture and constitute the largest yam biodiversity. About 90% of world yam is cultivated in this belt with *D. rotundata* and *D. cayenensis* accounting for most of the production. They are mostly preferred in West Africa owing to their organoleptic properties of the tubers but *D. alata* has the widest geographical distribution among the food yams (Martin 1976).

Currently yams are cultivated in about 50 tropical countries on 4.6 million ha worldwide with an annual production of about 52 million tons (FAO 2007). However, not all the countries (such as China) are recorded under Food and Agriculture Organization (FAO) statistics. More than 96% of world supply of fresh yam tubers comes from Africa, while four countries in West Africa namely Nigeria (72%), Cote d'Ivoire (9.5%), Ghana (6.6%), and the Republic of Benin (4.3%) account for about 92% of this output with 48.5 million tons of fresh tuber production per year (FAO 2007; Table 4.1). Most of the production in these regions comes from *D. rotundata* with the exception of Cote d'Ivoire, where *D. alata* accounts for 70% of yam production (Doumbia 1998), although 75% of domestic yam trade involves *D. rotundata* (Touré et al. 2003). World production of yams is reported to have tripled between the periods 1961–1963 and 1994–1996 (Lev and Shriver 1998). This has been attributed principally to increases in area of cultivation although yield increases were also recorded. In West Africa in general, and in Nigeria in particular, the increase in area planted with yams corresponds to an expansion of yam cultivation from the humid forest to more favorable conditions in the moist savanna (Manyong et al. 1996).

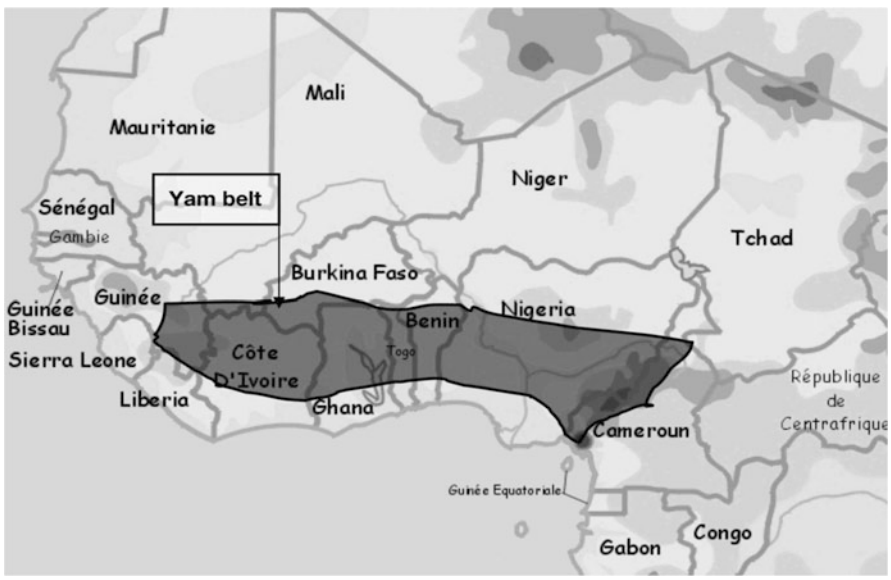

Fig. 4.1 The West African Yam belt. Source: http://www.cirad.org

Table 4.1 Area, production, and productivity of yam

Regions/Countries	Area (m ha)	Fresh tuber production (m tons)	Average yield (t/ha)
World	**4.6**	**51.5**	11.2
Africa	**4.4**	**50.1**	11.4
East Africa	0.04	0.25	7.0
North Africa	0.06	0.14	2.3
Middle Africa	0.1	1.1	6.8
West Africa	4.2	48.5	11.5
Benin	0.2	2.2	11.4
Burkina Faso	0.003	0.02	6.7
Cote d'Ivoire	0.5	4.9	9.8
Ghana	0.3	3.4	11.9
Guinea	0.004	0.04	10.9
Liberia	0.002	0.02	8.3
Mali	0.003	0.07	22.6
Mauritania	0.0004	0.003	6.3
Nigeria	3.1	37.2	12.1
Togo	0.06	0.6	10.0
Asia	**0.02**	**0.2**	15.0
East Asia	0.01	0.17	21.6
Southeast Asia	0.01	0.03	5.1
America	**0.2**	**1.2**	8.0
Central America	0.06	0.07	6.0
Caribbean	0.07	0.5	6.3
South America	0.07	0.6	10.2

Source: http://www.faostat.org

4.1.2 Importance of Yams

Species in the genus *Dioscorea* is extremely widespread in most of the tropical and subtropical regions. They are principally grown for food and have organoleptic qualities that make them the preferred carbohydrate food where they are grown. However, their storage organs (underground and/or aerial tubers) are also sources of proteins, fats, and vitamins for millions of people in West Africa. In countries where yams are generally cultivated, wild yams are used as food in times of shortage or famine (Coursey 1967; Fig. 4.2). The wild forms of *D. dumetorum* along with other species such as, *Dioscorea praehensilis*, *D. latifolia*, *D. preussii,* and *D. smilacifolia* are used as food in emergency throughout West Africa (Dalziel 1937; Labouret 1937; Irvine 1952). Similarly, in East and Central Africa, a number of wild species including *D. sansibarensis*, *D. preusii*, *D. cochleari-apiculata*, *D. schimperiana,* and *D. minutiflora* are used as famine food (Burkill 1939; Walker 1952). In African tropical rain forests, *D. praehensilis*, *D. mangenotiana*, *Dioscorea burkilliana,* and *D. semperflorens* are used as food (Sato 2001). The Asiatic species, *D. hispida*, which is closely related to *D. dumetorum*, is also used as food when there is shortage in parts of India and China (Burkill 1939). Certain wild species, such as, *D. sylvatica* Ecklon, are sold in the markets in Zimbabwe for treating skin diseases and chitsinga (physical disorder characterized by pain and swelling of the joints) (Gelfand et al. 1985). These wild species, although consumed only under famine conditions, also makes enormous contribution to human welfare. Apart from food, *Dioscorea* species are also used in pharmaceutical industries as sources of biologically active compounds or their precursors. Important but neglected species such as *D. villosa, D. praehensilis,* and *D. togoenensis* are known to have medicinal properties. *D. villosa,* for instance, is believed to benefit the liver and endocrine system. It regulates the female reproductive system, particularly during menstrual distress and menopause, and is also used in treating infertility. It is an effective treatment for morning sickness when used with chaste berry and dandelion. It is also famed

Fig. 4.2 Wild *Dioscorea* plants and tubers, mostly used as food. Photos by BJ Park and H Kikuno

for its steroid-like saponins, which can be chemically converted to progesterone contraceptives and cortisone. Similarly, *D. praehensilis*, variously referred to as Bush Yam or forest yam, has bitter tuber, which can only be eaten after careful preparation. Its young shoots are eaten in Bas Congo (DR Congo). In Gabon, the tuber is only eaten when young and after long cooking, all aimed at detoxifying it. In northern Nigeria, it is eaten as famine food.

Madagascar is very unique in both numbers of species that exists and their uniqueness in forms. They are unique in three ways (1) in their degree of endemism (most belong to an endemic clade, which represents one of the main lineages within *Dioscorea*); (2) almost all species have edible tubers; and (3) they are extracted from forest as wild plants. Studies have shown that there exists about 40 species of *Dioscorea* in this region, with 32 of them endemic to this region (Wilkin et al. 2007). Some of these species are *D. alatipes, D. arcuatinervis, D. bemarivensis, D. hexagona, D. karatana, D. maciba, D. namorokensis, D. ovinala*, *D. proteiformis,* and *D. ambrensis* (documented and conserved at Royal Botanic Gardens, Kew, UK). These species are very important to Malagasy people, especially on a local scale, as food providers or as medicines derived from the forests or from small-scale cultivation. The tubers are used as a starch source and can be eaten raw (*D. soso*, *D. fandra*), others are simply boiled or baked (*D. nako*), while some need extensive preparation (*D. antaly*). Traditional medicinal uses are a feature of *Dioscorea*, since the genus is rich in steroidal saponins. The most frequently encountered medicinal use of yams in Madagascar is the treatment of burns, ulcers, and other skin complaints with the bulbils of *D. bulbifera*.

Despite their economic and socio-cultural importance, there is limited knowledge about the origin, phylogeny, diversity, and genetics of these wild yams due to research neglect and several biological constraints (Mignouna et al. 2007). These wild species may serve as an important source of genetic variation in yam breeding work especially for resistance to pests and diseases. Further genetic improvement to reduce the bitter constituents in some of the species may render them more palatable and popular. It is therefore imperative to clarify the cytogenetic status, e.g., chromosome number of wild yams to enhance their usage in future breeding work.

4.1.3 Domestication of Yams in West Africa

The domestication of wild yams is a common practice mainly in West Africa that offers an insight into how farmers tap wild genetic resources to create products suitable for agriculture. However, until recently, breeders or agronomists have not focused enough attention to understand such an organized process of generating on-farm biodiversity through introduction of relatively new material that could be exploited through participatory plant breeding (involving farmers and breeders together). It is believed that farmers collect the tubers of wild yams (or natural interspecific hybrids) from forest areas during hunting and brought under cultivation with intense vegetative multiplication and selection procedure (at different periods of time making it a lengthy procedure) that induced changes in plant characteristics (both morphological and biochemical changes), mainly in tuber characteristics, making it a completely different variety (Mignouna and Dansi 2003). However, only limited research has been done to understand this process of domestication followed by these farmers to generate agricultural biodiversity. In West Africa, yam is basically subjected to monocropping followed by societies that practice something called "civilization of the yam" (Miège 1952). These societies are highly organized and well structured with their food needs mainly covered through production from *D. rotundata*. Burkill (1939) was convinced that *D. rotundata* resulted from the process of domestication that African farmers practiced to bring wild forms into agriculture. This hypothesis was not clear until recently when numerous studies using powerful tools (such as enzymatic and molecular markers; flow cytometry) were carried out to understand the relationship between *D. rotundata* and wild yams. In addition, social surveys carried out in Benin (Dumont and Vernier 1997) and Nigeria (Vernier et al. 2003) showed similar practices of domestication by farmers in these regions. Hildebrand (2003) also reported a similar kind of domestication process in southwestern Ethiopia where several wild yams have been brought under cultivation. In addition, Baco et al. (2007) hypothesized that high level of diversity of yam varieties exist in West Africa and this is more related to farmers' ethnic group. This practice differs from one ethnic group to another within a given area, but remains constant for a given ethnic group independent of its geographical location.

In the yam belt of West Africa, the situation is more confusing with the occurrence of a species complex, *D. rotundata* Poir. and *D. cayenensis* Lam., also known as Guinea yams. These two species are phenotypically distinguishable with *D. rotundata* (white yam) having white-fleshed tubers and 6–8 months growth period, and *D. cayenensis* (yellow yam) with yellow-fleshed tubers and 8–12 months growth period. However, the descriptions of both Lamarck (1792) and Poiret (1813) seem inadequate to separate these two species clearly (Miège and Lyonga 1982). In 1936, Chevalier created a new subsection, Cayenenses, under the section Enantiophyllum to include Guinea yams and all their wild relatives. However, the studies based on morphological characters are not conclusive enough to distinguish these two species clearly and the debate continues. Miege regarded *D. rotundata* as a subspecies of *D. cayenensis* in his book *Flora of West Tropical Africa* (1968 edition). Hamon (1987) pooled all West African cultivated yams that are not bulbiferous under this species complex. *D. rotundata* and *D. cayenensis* were domesticated from plants belonging to wild Dioscoraceae of the Enantiophyllum section (Burkill 1939; Miège 1952; Hamon 1987; Terauchi et al. 1992).

D. cayenensis is found in West and Central Africa. In West Africa, it coexists with *D. rotundata* but not widely cultivated, while it is grown along with *D. alata* in most of the forest areas in central Africa where *D. rotundata* is rather limited. Based on morphological characteristics, Miège (1982) proposed *Dioscorea abyssinica* Hochest ex. Knuth, *D. lecardii* De Wild., *D. liebrechtsiana* De Wild., *D. praehensilis* Benth. and *D. sagittifolia* Pax. as the possible wild progenitors of Guinea yams. Other wild yams that are morphologically related to Guinea yams are *D. burkilliana* J. Miege, *D. mangenotiana* J. Miege, *D. minutiflora* Engl., *D. smilacifolia* De Wild. and *D. togoensis* Knuth (Chevalier 1936; Miège 1982). Of all these species, *D. burkilliana, D. liebrechtsiana, D. minutiflora, D. mangenotiana, D. smilacifolia,* and *D. praehensilis* are found in rain forests, while the rest are found in the Savannas. Within each ecological zone, these wild species are distributed widely without any geographical isolation. Studies were further conducted using molecular markers to find a solution to this controversy. Dansi et al. (2000a) reported that

isozymes (leaf proteins) could differentiate the accessions of the two species, supporting the idea that *D. rotundata* and *D. cayenensis* are two distinct species. Studies conducted using chloroplast DNA showed that *D. rotundata* and *D. cayenensis* bear the same chloroplast genome, type A (which should make them the same species), as three other wild species *D. praehensilis*, *D. liebrechtsiana,* and *D. abyssinica* (Terauchi et al. 1992; Ramser et al. 1997; Chaïr et al. 2005). Based on nuclear ribosomal DNA, Terauchi et al. (1992) suggested that *D. cayenensis* is an interspecific hybrid with male parent being either *D. burkilliana*, *D. minutiflora,* or *D. smilacifolia* and the female parent being either *D. rotundata*, *D. abyssinica*, *D. liebrechtsiana,* or *D. praehensilis*. Mignouna et al. (2004) used the PCR-based marker, random amplified length polymorphism (RAPD), to establish the relationship between wild and cultivated yams, and showed that the accessions of *D. rotundata* could be clearly separated from the accessions of *D. cayenensis*. The accessions of *D. rotundata* showed a higher degree of polymorphism and were more closely related to *D. praehensilis* and *D. liebrechtsiana*.

Several researchers have suggested the phylogenetic proximity of *D. cayenensis* to *D. burkilliana* (Akoroda and Chheda 1983; Onyilagha and Lowe 1985; Mignouna et al. 1998; Dansi et al. 2000b). There are several morphological characteristics that support this suggestion. Hamon (1987) reported that the tubers of both the species appear very similar when grown in Cote d'Ivoire. In Cameroon too, *D. cayenensis* tubers have been reported to appear similar to those of *D. burkilliana* (Dumont et al. 1994). However, various arguments have been put forward regarding the morphological variability in tuber characteristics of *D. cayenensis* observed throughout West and Central Africa, suggesting that *D. cayenensis* probably has multiple origins or may have originated from two probable ancestors, *D. burkilliana* or *D. minutiflora*. In other studies, *D. minutiflora* has been considered as a form of *D. burkilliana* (Mignouna and Dansi 2003; Chaïr et al. 2005), indicating that the species is highly polymorphic. Hamon (1987) described the existence of two genetic forms of *D. minutiflora* using isozymes. The debate continues and the polyploid nature of *D. cayenensis* makes it more difficult to make a clear conclusion about the kinship of this species with *D. burkilliana* and *D. rotundata,* indicating the need for further investigation.

4.1.4 Germplasm Collection and Conservation

Yam has great economic and social significance in sub-Saharan Africa representing greatest genetic diversity of this crop in this region. The diversity under cultivation is further enhanced by the ongoing domestication of wild yam in various countries (Mignouna and Dansi 2003; Scarcelli et al. 2006). Although many authors suggested that most of the pre-domesticated yams are wild because they were collected from non-cultivated areas (Hamon et al. 1992; Dumont and Vernier 2000; Mignouna and Dansi 2003; Tostain et al. 2003), it has recently been concluded that a constant gene flow occurs between the cultivated species complex (*D. cayenensis–D. rotundata*) and its wild related species making it difficult to clearly identify the varieties as either wild or cultivated.

Wild yams are sexually propagated while cultivated yams are vegetatively propagated; however, farmers carry out intense vegetative propagation of the plants collected from forest areas (which could be wild or interspecific hybrids) for long periods of time, contributing to the domestication of species. Furthermore, yam domestication has been a factor for the degradation of forests and fertile lands, and also contributing to the loss of landraces. Therefore, it is of paramount importance to invest in yam conservation for food security and preservation of genetic diversity in the tropical and subtropical areas.

There are two approaches for plant genetic resources conservation, namely, (1) in situ conservation that involves maintaining plants in the area where they developed their distinctive properties, i.e., in the wild or in farmer's field. This is certainly the optimal option as it allows germplasm to evolve with its natural environment. (2) Ex situ conservation that involves conservation of full plants or propagules out of their natural environment and includes seedbank, field bank, arboretum, botanical gardens, etc. The International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, maintains the largest world collection of yams, accounting for over 3,087 accessions of West African origin. Eight species are represented in the collection comprising accessions of *D. rotundata*, *D. alata*, *D. dumetorum*, *D. cayenensis*, *D. bulbifera*, *D. mangenotiana*, *D. esculenta,* and *D. preusii*. Two

species, *D. rotundata* and *D. alata* account for 67% and 25% of the total collection, respectively. These accessions are held in trust at Food and Agricultural Organization (FAO) and are distributed without restriction for use in research for food and agriculture. Other collections have been reported in Africa (Burkina Faso, Cameroun, South Africa and Uganda); West Indies (Barbados, Cuba, Guadeloupe, Jamaica, Saint Dominique, Trinidad-Tobago); America (Brazil, Colombia, Costa rica, Guatemala, Mexico, Panama, USA); Pacific (Cook Islands, Fiji, Niue Island, New Caledonia, Papua New Guinea, Solomon Islands, Tonga, Vanuatu, western Samoa); and Asia (Bangladesh, India, Indonesia, Japan, Nepal, Malaysia, Philippines, Sri Lanka, Thailand, Vietnam) (Malaurie 1998; Lebot 2009). In addition, the Global Crop Diversity Trust (http://www.croptrust.org) is presently supporting the regeneration of several national collections in West Africa such as in Benin, Togo, Ghana, and Cote d'Ivoire.

The easiest and cheapest ex situ conservation strategy involves collection of germplasm accessions in the form of seeds to produce orthodox seeds. Several yam species have been reported to produce such seeds (Daniel 1997). However, the varieties with the most desirable traits quite often do not flower (Lebot 2009) and hence do not produce seeds. Because of this, traditional yam conservation is achieved via the establishment of field banks. At IITA, a field gene bank has been established wherein yam germplasm is vegetatively propagated by planting setts from underground or aerial tubers. Plants are grown in the field for about 6–9 months, depending on the species or genotype. Mature tubers are then dug up or aerial tubers are plucked and stored in a traditional yam barn under ambient temperature or at 15°C for 4–5 months. However, there is a high risk of genetic erosion in the field and in storage due to occurrence of diseases and attack of pests, adverse climatic conditions and also likeliness of theft. In addition, maintenance of field banks are expensive and laborious. In vitro conservation offers an alternate approach for ex situ conservation of yam. There are added advantages as it reduces the risk of germplasm loss due to above listed factors and allows maintenance of disease-free germplasm in a limited space under pest and pathogen-free condition, and facilitates safe exchange of germplasm at the international level (Hanson 1986; Ng 1988). In vitro conservation is followed in *Dioscorea* spp. through nodal cutting or meristem culture. However, the combination of optimal mineral and growth regulators varies depending on species and genotypes (Mantell et al. 1978; Saleil et al. 1990; Malaurie et al. 1995a, b; Sedigeh et al. 1998; Myouda et al. 2005). Malaurie et al. (1993) established an in vitro germplasm collection for 16 *Dioscorea* species from Africa and Asia. The collection consisted of ten wild species (*D. abyssinica, D. bulbifera, D. burkilliana, D. dumetorum, D. hirtiflora, D. mangenotiana, D. minutiflora, D. praehensilis, D. schinzperana, and D. togoensis*), five edible species (*D. alata, D. bulbifera, D. cayenensis–D. rotundata* complex, *D. dumetorum,* and *D. esculenta*), and one interspecific hybrid (*D. cayenensis–D. rotundata* complex, cv. Krengle × *D. praehensilis*). Similarly, an in vitro genebank has been established at IITA that conserves over 1,500 accessions of yam wherein 5–10 seedlings of each accession are stored at 16 ± 2°C under a 12 h photoperiod (Dumet et al. 2007). Each seedling is either maintained in a test tube or a polyethylene bag on solid Murashige and Skoog-based medium (Dumet et al. 2007). Under such storage conditions, accessions are subcultured every 10–24 months depending on species and genotype.

In order to further rationalize ex situ conservation of plant tissues for long periods, several groups have investigated cryopreservation. Cryopreservation is conservation of plant tissues at very low temperature, generally using liquid nitrogen (−196°C) so that tissues could be stored in perpetuity. Two approaches are followed for cryopreservation of plant tissues. One is based on evaporative desiccation of plant tissue prior to freezing, which often involves encapsulation of tissues before dehydration treatment. Tolerance to natural or induced dehydration is the key factor for success of this method. The other approach involves the use of cryoprotectants such as DMSO (dimethyl sulphoxide), ethylene glycol, or glycerol. In addition to an osmo-dehydration effect, these compounds stabilize the plant tissues when submitted to freeze/thaw cycles. Successful cryopreservation has been reported for different species of yam such as *D. bulbifera*, *D. oppositifolia*, *D. alata*, *D. cayenensis*, *D. wallichii,* and *D. floribunda* (Mandal et al. 1996; Malaurie et al. 1998; Mandal 2000; Leunufna and Keller 2003). To date, there is no universal cryopreservation process for plant tissues, and in many cases, adjustments are made to suit a species or genotypes. Although cryopreservation is a promising approach for ex situ conservation, cryobank is yet to be

established for yam. It is assumed that in vitro storage, including cryopreservation, induced somaclonal variation, i.e., genotypic or/and phenotypic variations would be the best bet for long-term conservation in yams. At IITA, work is in progress to investigate integrity of cryopreserved germplasm.

Any ex situ germplasm collection (national, regional or international) is expected to capture maximum diversity and the knowledge on genetic diversity is obtained through proper characterization of germplasm collection using morphological descriptors or molecular markers for further utilization in crop improvement programs. At IITA, two types of databases are maintained for each accession. The passport data consist of information on a unique identifier for each genotype (accession number), its taxonomic data (genus, species, pedigree), geographical information (latitude, longitude, altitude), environmental data (market, farmer field, topography, soil type etc.), and collection data (collector's name, year of collection, collection number, etc.) (IPGRI/IITA 1997). The characterization data consist of information on agro-morphological characters recorded following internationally standardized morphological descriptors for yam (IPGRI/IITA 1997). The passport and characterization data are maintained as databases and can be retrieved through online search using the URL http://genebank.iita.org/search. A core collection of yam (391 accessions) has also been established at IITA representing 75% of genetic diversity. Data on 99 morphological descriptors were used to stratify the global collection based on species and country of origin to define the core collection (Mahalakshmi et al. 2006). However, the analysis did not take into consideration the sex and ploidy status of the accessions, two attributes important to breeders who may want to use the core accessions in yam improvement programs. The passport or characterization data of most of the accessions are also incomplete and duplicates are yet to be eliminated. Yam germplasm has been characterized for morphological characters (Dansi et al. 1998, 1999, 2000a, b), physico-chemical characteristics (Lebot et al. 2006), organoleptic properties (Egesi et al. 2003), soluble tuber protein profiles (Ikediobi and Igboanusi 1983), or isozyme patterns (Lebot et al. 1998; Dansi et al. 2000a; Mignouna et al. 2002a). Similarly, use of molecular markers such as amplified fragment length polymorphism (AFLP), RAPD and simple sequence repeat (SSR) markers have also been reported (Mignouna et al. 2003; Malapa et al. 2005; Egesi et al. 2006; Scarcelli et al. 2006; Tamiru et al. 2007; Tostain et al. 2007) in diversity studies of yams. Efforts are underway at IITA to use molecular markers, such as SSRs, to fingerprint the entire collection, mainly to identify the duplicates in the collection and establish a core collection using information on morphological and molecular data.

Another important aspect of conservation strategy is the distribution of germplasm to various users. In case of yam, the distribution is driven by the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), an international agreement with the overall goal of supporting food security via germplasm exchange and benefit sharing. At IITA, germplasm exchange is carried out following the phytosanitary procedures during which germplasm is rendered free of regulated quarantine pests and pathogens. Efforts are also being made to establish virus-free germplasm through meriseming and selection of virus-free germplasm through virus indexing.

4.1.5 Botany

The word "Yam" is applied only to members of the genus *Dioscorea* (Alexander and Coursey 1969) that belong to family Dioscoreaceae in the order Dioscoreales classified under the monocotyledons. Most of the wild species of African origin are included within this section. The order Dioscoreales has been redefined and now comprises three families: Burmanniaceae, Dioscoreaceae, and Nartheciaceae (Chase et al. 1995; Caddick et al. 2000, 2002). The genus *Dioscorea* is the largest genus within Dioscoraceae comprising about 600–850 species (Knuth 1930; Burkill 1960), although Ayensu (1972) estimated that there are less than 200 species that are distributed across the tropics and subtropics. Currently, the world checklist in Royal Botanic Gardens, Kew includes 644 accepted species in Dioscoreaceae in five genera, which are *Dioscorea*, *Rajania*, *Tacca*, *Stenomeris,* and *Trichopus* (Govaerts et al. 2007). In a taxonomic status based on gross morphological characters, the genus *Dioscorea* is divided into five sections: Enantiophyllum, Combilium, Opsophyton, Macrogynodium, and Lasiophyton (Burkill 1960). The most important edible yam

species – *D. rotundanta*, *D. alata*, and *D. cayenensis* – and the minor economic species in temperate zone – *D. opposita* and *D. japonica* – belong to the section Enantiophyllum. *D. dumetorum*, *D. hispida,* and *D. pentaphylla* belong to the section Lasiophyton; *D. bulbifera* to the section Opsohyton; *D. esculenta* to the section Combilium; and *D. trifida* to the section Macrogynodium.

The yams are Angiosperms, or flowering plants, and are twining climbers, and produce dry capsules. All species of economic importance are tuberous, sometimes producing aerial tubers called bulbils. Majority of *Dioscorea* species are distributed throughout the tropics, while a few economically important species are also found in the warmer regions of the temperate zones (Bai and Ekanayake 1998). Wild species are either annuals (with aerial and underground tubers growing annually) or semi-perennials (aerial part growing in 12–24 months cycle, along with perennial underground part) or perennials (aerial and underground parts growing over several years). Cultivated species are generally grown as annuals. There are huge differences in size, shape, and number of tubers per plant within and between species.

The tuber of yams is a storage organ, which forms a new tuber and shrivels away simultaneously when the regrowth is induced. As the organ lacks the typical characteristics of a modified stem structure, the tuber has no pre-formed buds or eyes, no scale leaves, and no terminal bud at the distal end of the tuber (Hahn et al. 1987). Some perennial species produce tubers that become larger and more lignified as the plant ages. Some species, such as *D. bulbifera*, *D. alata*, *D. opposita,* and *D. japonica* produce bulbils in leaf axils on vine of matured plant in addition to underground tubers. The Enantiophyllum species usually produce one to three large tubers, while *D. esculenta* (Combilium) produce 5–20 tubers; *D. dumetorum* (Lasiophyton) produces 3–12 tubers and *D. trifida* (Macrogynodium) produces a larger number of tubers that are small in size. The number and shape of yam tubers vary depending on the species and genotype (Martin and Sadik 1977; Okonkwo 1985).

The yam plant possesses shallow fibrous root system that is normally unbranched and concentrated within the top 0.3 m of the soil and very few actually penetrate up to 1 m depth (Onwueme 1978). Several roots rapidly develop and reach 3–4 m in radius around the plant after sprouting. According to Onwueme (1978), yam possesses three different types of adventitious roots (1) adventitious roots arising from the corm-like structure at the base of the stem, whose function is to absorb minerals, nutrients, and water from the soil; (2) adventitious roots arising from the body of the yam tubers; and, (3) adventitious roots originating from the exposed lower nodes of the growing yam plants. Fibrous roots are smooth in general but some species have stems and roots with spines. The wild relatives of yams have more spiny roots than the cultivated species (Onwueme 1978).

The stems of all *Dioscorea* species, except for few, climb by twining, and the direction of twining of the vine, i.e., anticlockwise or clockwise, is a characteristic peculiar to the particular section within the genus *Dioscorea*. Species of the Enantiopyllum section twine to the right (clockwise) and those of the Combilium, Opsophyton, Macrogynodium, and Lasiophyton sections twine to the left (Onwueme 1978). In many species, for instance, *D. cayenensis*, *D. esculenta,* and *D. nummularia*, vines have spines which support the twining habit while also deterring animal predators (Okonkwo 1985). The wings present in some species, such as *D. alata* and *D. colocasiifolia,* also support the twining habit. The length of stems generally varies depending on the species. *D. esculenta* rarely climbs more than 2–3 m while some forms of *D. rotundata* may climb to 10–12 m under favorable conditions (Coursey 1967). Some wild species, such as, *D. sansibarensis*, *D. preussii,* and *D. mangenotiana* may even be longer.

Most of *Dioscorea* species have simple, cordate, or acuminate leaves borne on long petioles but in some species, they could be lobed or palmate with pointed tips. *D. trifida* has lobed leaves consisting of three leaflets while *D. dumetorum* has compound leaves (Okonkwo 1985). In general, the leaves vary in shape, size, and color (pigmentation) from one species to another or even between individuals of same species. Yam leaf lamina generally has three primary nerves joining at the tip of the lamina. The area of lamina in cultivated species is about 50–200 cm^2, although in some wild species such as, *D. sansibarensis*, it may be much larger. The leaf arrangement on the stems is usually described as opposite or alternate depending on species or growth of the stem, i.e., alternate on the lower part of the stem and opposite on the upper (younger) part (Onwueme 1978). The leaf petiole is long and depending on species could be

winged or spined. Both stems and leaves of many *Dioscorea* species are covered with hair.

The flowers of yam are basically dioecious, with male (staminate) and female (pistillate) flowers borne separately or on separate plants. In some cases, monoecious plants with staminate and pistillate flowers are occasionally observed among the genotypes of *D. rotundata* (Sadik and Okereke 1975a, b). Generally, the female plants are less in number with fewer flowers than male plants. The male or female flowers are borne on axillary spikes in the leaf axils of yam vines. Flowering in the major edible yams has been reported to be sparse, irregular, or absent. The male flowers are sessile, glabrous, and spherical and are borne axially or terminally. These flowers consist of a calyx of three sepals and a corolla of three petals, arranged regularly and almost similar in size and appearance, with three or six stamens (Onwueme 1978). The ovary of female flowers is trilocular with each locule having 2–3 ovules and is located below the corolla (Miége 1968; Sadik and Okereke 1975a, b). The flowers of all *Dioscorea* spp. are entomophillous and are pollinated mainly by insects (Coursey 1967; Fig. 4.3). It is assumed that the sweet scent of the flowers mainly attract these insects, although the species involved have not yet been identified (Govaerts et al. 2007).

The flowers are succeeded with the formation of dry, dehiscent, trilocular capsules (1–3 cm long) with each fruit producing six seeds. The seed in each capsule is small and has wings that vary in shape in different species (Coursey 1967; Lawton 1967; Onwueme 1978). The seeds are flat and light and the wings help in wind dispersion. The seed germination process is well explained by Lebot (2009).

Some *Dioscorea* spp. such as *D. alata*, *D. bulbifera, D. pentaphylla*, *D. opposita,* and *D. japonica* produce bulbils in the axils of leaves. They are specifically adapted for vegetative propagation and have the appearance and morphology of condensed stems (Coursey 1967). They are smaller than the underground tubers, but in *D. bulbifera*, these are the main storage organ and are larger in size. Bulbils can be toxic in some species, while in others they are fine-textured and are appreciated for taste (*D. bulbifera*). Short day length generally accelerates formation of bulbils.

4.1.6 Constraints to Yams

Low soil fertility, weed competition, pests, and diseases in the field (foliar and soil borne) and in storage, and attack by animals (pigs and rodents) limit the production and productivity of yam cultivation wherever they are grown (Kenyon et al. 2003; Baimey et al. 2006; Coyne et al. 2006; Tchabi et al. 2008; Lebot 2009). These factors, and intensive cultivation of improved cultivars, are the factors responsible for genetic erosion of wild yams and landraces. Although most of the documented information is related to the cultivated species, in general, the information on the economic significance of pests and diseases under farmers' conditions is often lacking or inadequate although yield losses of up to 100% have been attributed to them in experimental trials (Lebot 2009). The economic importance of insect pests (e.g., leaf and tuber beetles, mealy bugs and scales), fungal diseases (e.g., anthracnose, leaf spot, leaf blight and tuber rots), and viral diseases (caused by poty- and badnaviruses), as well as nematodes also vary depending on the agro-ecological zone, cropping system, and production practices. For instance, anthracnose disease caused by *Colletotrichum gloeosporiodes*, mosaic disease caused by yam infecting poty- and badnaviruses, root rot nematodes (*Meloidogyne* spp.), tuber rot, and scale insects are considered as most economically important pests and diseases in West African yam belt (Kenyon et al. 2003; Coyne et al. 2006). Integrated pest management and crop improvement are being pursued at IITA to manage these constraints in West Africa.

Availability of "clean" planting material is the greatest limitation in West Africa. Farmers in West Africa bulk planting material (tubers, veins and setts) from both infected (symptomatic) and asymptomatic plants and use them indiscriminately for planting. This practice not only contributes to the spread of pests and pathogens along with the planting material but also affects the yields and gradual decline in source material. Efforts are being made to harmonize clean seed yam systems in West Africa which would improve the quality of farmer-preferred materials. A similar system needs to be conceived to conserve the wild yams and landraces and protect them from pests, diseases, and soil erosion.

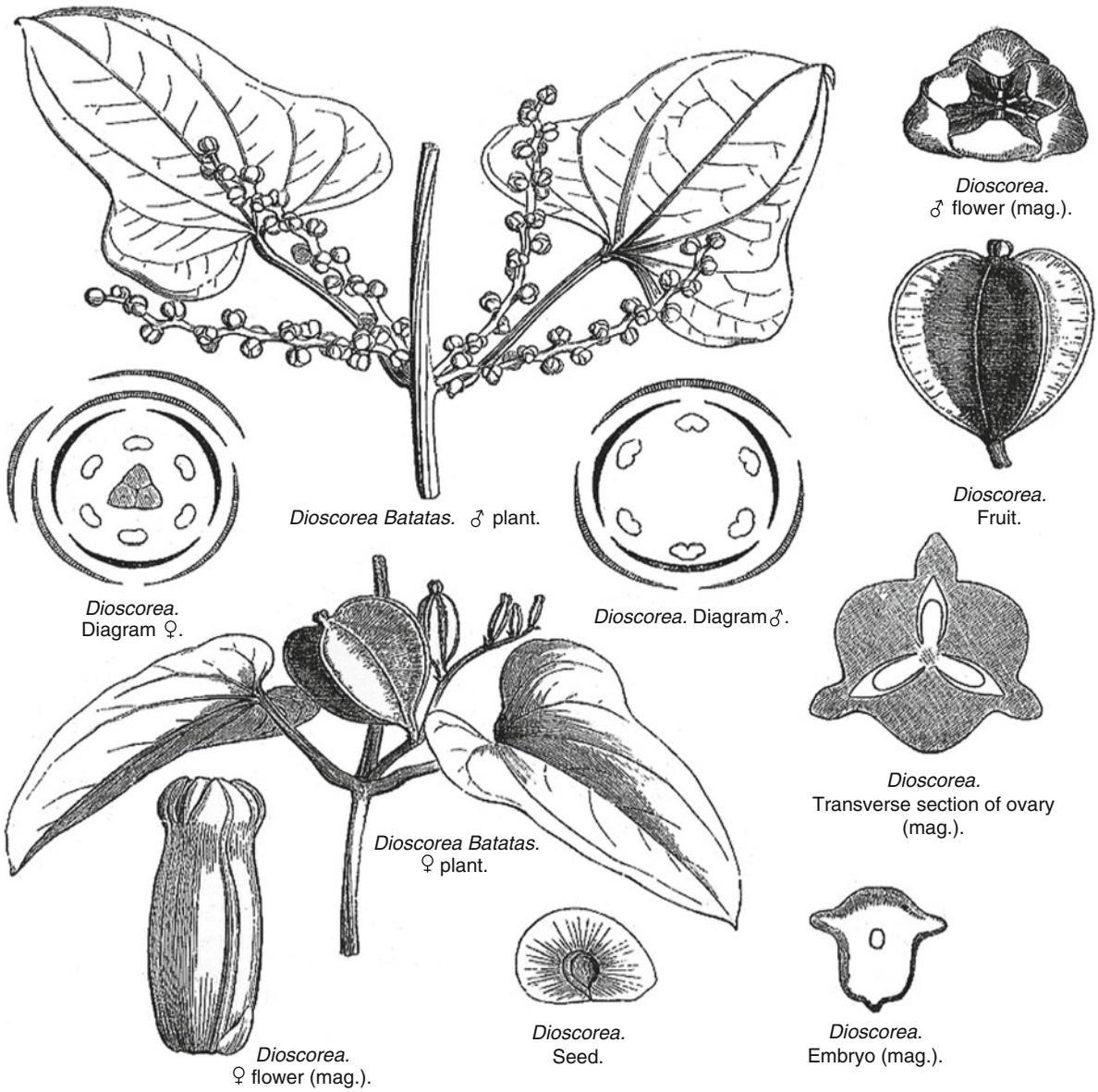

Fig. 4.3 The plant, male and female flower, embryo, and fruit of a wild *Dioscorea* species. Source: Watson and Dallwitz (1992)

4.1.7 Cytogenetics

Dioscorea is one of the most difficult genera for cytotaxonomic and cytogenetic studies (Essad 1984) because of small size of the chromosomes and flowering in some species, hence chromosome counts of only about 55 species have been reported so far (Essad 1984; Degras 1993). However, the genus offers an attractive model for the investigation of ploidy events and chromosome evolution in wild and cultivated species in relation to their dioecious nature, vegetative/sexual propagation, and the process of domestication (Bousalem et al. 2006). The existence of various ploidy levels and the lack of a diploid relative to the cultivated polyploid yam leads to various complications in cytogenetic studies in yams. Observations have been restricted in most cases to the determination of chromosome numbers and chromosome pairing

from mitotic (Sharma and De 1956; Raghavan 1958, 1959; Ramachandran 1968; Essad 1984) and meiotic cells (Abraham and Nair 1990; Abraham 1998). Many authors have reported the difficulties encountered in chromosome counting in cultivars of *D. cayenensis/D. rotundata* complex (Miège 1954; Baquar 1980) and other *Dioscorea* species (Ramachandran 1968; Baquar 1980; Essad 1984; Gamiette et al. 1999).

The basic chromosome number of yam species is controversial, although it has been acknowledged as being $x = 10$ or $x = 9$, with a high frequency of polyploid species. Tetraploid species are the most frequent, followed by diploids ($2x$), hexaploids ($6x$), and octaploids ($8x$) (Essad 1984). The base chromosome number $x = 10$ (found in all Asian species) is present in only 52% of the African species and 13% of the American species examined so far (Bousalem et al. 2006). Recent data have revealed a basic chromosome number of $x = 20$ (Scarcelli et al. 2005), but this is based on a small number of yam species. The highest chromosome number and the smallest chromosome sizes are reported to occur more in tropical *Dioscorea* species, while the smallest number and largest sizes occur more in temperate species. This coupled with the fact that two new basic chromosome numbers $x = 6$ (Segarra-Moragues and Catalán 2003; Segarra-Moragues et al. 2004) and $x = 20$ (Scarcelli et al. 2005) have also been found in recent years, suggest that more work will be needed to understand the cytogenetics of both cultivated and wild yams. Furthermore, it is important for yam breeders to determine the ploidy status of clones, especially of new introductions before they can be utilized in a breeding program (Egesi et al. 2002). These fundamental data are, therefore, essential for sexual breeding of yams, which will enable ploidy manipulations in intraspecific crosses, effective breeding and conservation of the species, and for elucidating the phylogeny and the origins of the yam and the evolution of the genus *Dioscorea*. For this purpose, flow cytometry has been recently used to determine ploidy levels in yams (Gamiette et al. 1999). The method is non-destructive (one sample can be prepared from a few milligrams of leaf tissues), exceptionally rapid, sensitive and convenient, does not require dividing cells, and can detect both mixoploidy and aneuploidy (Galbraith et al. 1983; De Laat et al. 1987; Arumuganathan and Earle 1991a, b; McMurphy and Rayburn 1991; Dolezel 1997). Ploidy determination of *D. alata*, *D. cayensis-rotundata* and some wild yam species by flow cytometry and conventional chromosomes counting revealed variable ploidy levels of $4x$, $6x$, and $8x$ in the species (Gamiette et al. 1999) with majority being tetraploid (Dansi et al. 2000a). Hamon et al. (1992) showed a continuous range of ploidy ($3x$, $3.5x$, $4.5x$, $6x$, $7x$, $8.5x$) in *D. alata* by flow cytometry, with occurrence of high proportion of hexaploids (Egesi et al. 2002). Other studies have classified *D. rotundata* as diploid with a basic chromosome number of 20 (Daïnou et al. 2002; Scarcelli et al. 2005), but evidence of Scarcelli et al. (2005) is based on progenies from a monoecios plant rather than dioecious plants.

The inheritance of ploidy in yams, that is, allo- or, autoploid, is also not clear. More studies are necessary to identify the inheritance patterns of the polyploid yams. Identification based on multivalent formation will be misleading since autotetraploid species do not always exhibit multivalent formation (Soltis and Riesberg 1986) and allotetraploid species have been shown to form multivalents at times (Watson et al. 1991). Therefore, analyses of segregating populations have been extensively used to assess inheritance patterns (Demarly 1958; Jackson and Casey 1982). Recently, segregation analysis using isozyme and SSR markers led to the conclusion that *D. rotundata* ($2n = 4x = 40$) is a diploid species (Scarcelli et al. 2005), while that based on AFLP markers reflected a disomic inheritance in *D. alata* and *D. rotundata* (Mignouna et al. 2002b, c), revealing an allotetraploid structure for the two species. Segarra-Moragues et al. (2004) also concluded on the basis of SSR patterns that the wild species, *D. pyrenees* and *D. chouardii*, are allotetraploid. SSR segregation analysis and cytogenetic evidence revealed a tetrasomic behavior and an autotetraploid structure of the genome of the American species, *D. trifida* (Bousalem et al. 2006). The situation in other *Dioscorea* species could also have a base chromosome number of $x = 20$ rather than $x = 10$ and most of them could be di-, tri- or tetraploid in nature. It is confirmed that polyploidy is common among *Dioscorea* species and accessions having 40 chromosomes are most common, followed by accessions with 20, 60, and 80 chromosomes (Lebot 2009). There are reports of accessions having 100 chromosomes (*D. bulbifera* and *D. esculenta*), 120 chromosomes (*D. hastata*, *D. minutifolia*, and *D. smilacifolia*), and 140 chromosomes (*D. opposita*, *D. pentaphylla* and *D. cayenensis*).

Further studies based on genomic in situ hybridization (GISH) and florescent in situ hybridization (FISH) will probably provide better information on the inheritance of ploidy in yams. These techniques can be used to localize the presence or absence of specific mRNA or DNA sequences on chromosomes.

4.1.7.1 Relevance of Cytogenetics and Wild Yams to Cultivated Species

The cultivated yam species *D. rotundata* ($2n = 40$) has been considered as a tetraploid species with a basic chromosome number of ten (Scarcelli et al. 2005), suggesting that in diversity studies, one should anticipate the detection in individual genotypes of up to four alleles per DNA marker locus. Recent studies by various authors have challenged this assertion and the most parsimonious hypothesis from their work is to conclude that *D. rotundata* is a diploid (Zoundjihekpon et al. 1994; Daïnou et al. 2002; Mignouna et al. 2002a, b, c, d) since only two alleles have been commonly found in this species. Similar contentions have been observed in *D. alata* where Egesi et al. (2002) noticed that majority of plants used in their study were hexaploid (84.9%) with a smaller percentage of tetraploids (15.7%), contrary to the accepted theory that *D. alata* is a tetraploid. A higher number of male plants were also found to be hexaploid than tetraploids, again at variance with earlier findings, which reported that hexaploid male plants are rare. Higher ploidy levels were not directly related to sparse or erratic flowering as previously reported as profuse flowering occurred in some male hexaploid accessions (Egesi et al. 2002). Octoploidy, which is commonly noticed in both *D. rotundata* and *D. alata* (Hamon et al. 1992), or mixoploidy (Dansi et al. 2001) was not observed in this analysis. Similarly, there was no association of ploidy level to geographic origin of materials as was asserted by Miège (1954) and Essad (1984). Ploidy level in yam was shown to be now easy and rapidly determined using high-resolution flow cytometry (Egesi et al. 2002), making a case for revisiting the cytogenic information available especially in case of wild yams.

D. abyssinica and *D. praehensilis* are considered by Hamon et al. (1997) and Terauchi et al. (1992) as wild relatives of *D. rotundata*. These two species have been assumed to be tetraploid, with $2n = 40$ chromosomes (Miège 1952). It has been suggested that *D. praehensilis* is one of the parents of the cultivated *D. cayenensis* Lam., together with other species of the section *Enantiophyllum* such as *D. abyssinica* Hochst. ex Kunth and *D. burkilliana* J. Miège. It produces viable seeds even after long period of storage. The major challenge, therefore, is to trace the ploidy level of the cultivated species by looking at their ancestry, especially when Scarcelli et al. (2005) found that the cultivated species of *D. rotundata* and wild species, *D. abyssinica* and *D. praehensilis*, did not differ in number of alleles per locus.

4.2 Classical and Molecular Genetic Studies

The genetics of yams is least understood among the major staple food crops (Martin 1966; Zoundjihekpon et al. 1994) due to several biological constraints including, a long growth cycle (about 8 months or more), dioecy, poor- to non-flowering plants, polyploidy, vegetative propagation, heterozygous genetic background, and poor knowledge about the organization of the crop (Mignouna et al. 2007). In addition, although yams are monocots, they are very distantly related to grasses such as wheat, maize, rice, and sorghum, whose genomes are well studied. For example, banana and wheat are more closely related to each other than either is to yam. Hence there is no convenient model system for yam genomics and not much research effort has been made to understand the same. In recent years, some progress has been made in germplasm characterization and in the development of molecular markers for genome analysis in wild or cultivated yam species, and all the available information is presented in this section.

Yams have a relatively small genome size, estimated at 550 Mbp/1C for *D. alata* and 800 Mbp/1C for *D. rotundata* (Mignouna et al. 2002b, c). Molecular genetic analysis of the yam genome is gaining momentum. The initial effort in yam genomics was devoted to the development of polymorphic DNA markers and assessment of their potential application in yam. Framework genetic linkage maps based on amplified fragment length polymorphism (AFLP) markers have been constructed for *Dioscorea tokoro*, a wild relative of yam (Terauchi and Kahl 1999), and

for two cultivated species, *D. rotundata* (Mignouna et al. 2002b) and *D. alata* (Mignouna et al. 2002c). The saturation of these maps with polymorphic markers, such as simple sequence repeat (SSR) is necessary for full utilization of their potentials. The identification of key traits that are related to yield and quality of yams, and the gene action associated with the traits are paramount for the understanding of the genetics of this important crop. Previous studies have focused mainly on the analysis of disease resistance, and the identification of genomic regions associated with resistance to yam mosaic virus and anthracnose diseases in *D. rotundata* and *D. alata*, respectively (Mignouna et al. 2002a, b, c, d). Results of these analyses, however, are yet to be confirmed in populations of different genetic background and also in other environments. Presently, IITA is involved in the identification of key traits that affect yield and quality of yam, with the hope of identifying DNA markers that are linked to these traits for their subsequent use in yam breeding. Various mapping populations are being developed for quantitative trait loci (QTL) analysis of yield and quality-related traits in *D. rotundata* and *D. alata*.

4.3 Crop Improvement Through Traditional and Advanced Tools

There has been very limited use of wild *Dioscorea* spp. in yam improvement program, although farmers, mainly in West Africa, practice domestication of wild species to develop cultivated forms (Dumont et al. 2006). In addition, wild-related species possess useful traits. *D. abyssinica* and *D. praehensilis*, the wild relative of guinea yams (*D. rotundata–D. cayenensis* species complex) are believed to be reservoir of resistance genes for virus diseases such as Yam mosaic virus; however, their use in breeding programs has been almost negligible. Similarly, the related species of Asiatic yam (*D. alata*) such as *D. nummularia* and *D. transversa*, may possess resistant genes for anthracnose disease, but has been seldom used in breeding programs. It is believed that there may be introduction of deleterious characteristics into the cultivated species if crossing is made using wild species. Additionally, there may be problems of interspecific hybridization owing to differences in ploidy level between different species, and this may require embryo rescue technique for the success of such crosses. In general, few efforts have been made to use wild species in breeding programs.

Traditional breeding efforts in yam have resulted in substantial achievement leading to release of high-yielding and diseases-resistant cultivars. For instance, through classical breeding, IITA has developed several clones and populations of *D. rotundata* and *D. alata*, and disseminated these for further evaluation and selection under local environmental conditions in partnership with national programs in Africa. Through collaborative evaluation of IITA-derived breeding lines with National Research Institutes (National Root Crop research Institute, Umudike, Nigeria, and the Crops Research Institute of Ghana), eight varieties of *D. rotundata*, seven during 2001 and 2003 and one in 2007, have been released in Nigeria and Ghana, respectively, while three varieties of *D. alata* were released in Nigeria in 2009. More lines are in the pipeline to be released by these institutions and other root crop programs in other yam-producing countries including Benin, Burkina Faso, Ivory Coast, Sierra Leone, Togo, and Liberia. These varieties have multiple pest and disease resistance, wide adaptability, and good organoleptic attributes (Mignouna et al. 2007).

Yam-breeding programs have focused on clonal selection from landraces and hybridization of elite genotypes within and between species. IITA yam research focuses mainly on the most important species cultivated in Africa, *D. rotundata* and *D. alata*. There are many wild yam species available, some of them with edible tubers, which may be potential sources of useful traits, which can be used in breeding programs.

4.3.1 Interspecific Hybridization

Interspecific hybridization in yams is desirable for the genetic improvement of the crop, but it is faced with a lot of challenges, including cross-compatibility and synchronization of flowering, and very little work has been done in these areas. Some species can hybridize easily, while others do not. For instance, *D. rotundata* can be crossed to *D. cayenensis*, but crossing either of the two to *D. alata* has not been successful

(IITA unpublished data). Research effort in interspecific hybridization at IITA is geared toward the genetic improvement of yam, primarily on *D. rotundata*, *D. cayenensis,* and *D. alata* by transferring complementary traits from one to the other, e.g., higher carotenoid in *D. cayenensis* could be transferred to *D. rotundata* by interspecific hybridization.

4.3.2 Advanced Biotechnological Techniques for Yam Improvement

Research on biotechnology of yam has been limited to tissue culture, but has focused on disciplines including genetic transformation and molecular marker application. Xinhua et al. (1986) and Schafer et al. (1987) attempted to transform *D. opposita* and *D. bulbifera* using *Agrobacterium* followed by Tor et al. (1993, 1998) who transformed cultures of *D. alata* using particle bombardment. However, genetically modified yam is yet to be developed although this approach could be successfully used in transferring virus resistance and anthracnose resistance genes into commercial varieties. Similarly, some progress has been made using isozymes (Hamon and Touré 1990a, b; Dansi et al. 2000a, b) and DNA markers (Terauchi et al. 1992; Ramser et al. 1996, 1997; Mignouna et al. 1998; Chaïr et al. 2005) for germplasm characterization and phylogenetic studies in yam. Mignouna et al. (2007) demonstrated the relationship between cultivated yams (*D. cayenensis* – *D. rotundata*) and wild species from West Africa using DNA markers.

4.3.2.1 Callus Culture and Plant Regeneration

Direct plant regeneration from explant materials, somatic embryogenesis, and plant regeneration from callus culture have been very useful for rapid clonal propagation and obtaining somaclonal variants. In yam, regeneration rate is very low in some cases, and there are genotype-dependent differences between species in their ability to generate plantlets in vitro (Asiedu et al. 1998). These techniques have been conducted on several species: *D. rotundata* (Ng 1984; Osifo 1988), *D. alata* (Mantell et al. 1978; Fautret et al. 1985; Twyford and Mantell 1990), *D. composita* and *D. cayenensis* (Sinha and Chaturvedi 1979; Viana and Mantell 1989), *D. abyssinica* (Ng et al. 1994) and *D. trifida* (Fautret et al. 1985).

4.3.2.2 Embryo Culture

Embryo rescue, a technique to save immature embryos from a hybrid and to enhance germination of seeds that are dormant or cannot germinate easily under normal situation, has been reported in Chinese yam (Yakuwa et al. 1981) and *D. rotundata* (Okezie et al. 1983).

4.3.2.3 Protoplast Culture

Protoplast isolation and culture technique is very useful in the production of yam varieties, especially those that do not produce flowers. These techniques offer the greatest promise for the production of somatic hybrids, but very little work has been reported in this research area. They have been successfully applied to *D. rotundata* (Onyia et al. 1984) and *D. alata* (Twyford and Mantell 1990).

4.3.2.4 Development of Expressed Sequence Tags

The lack of DNA or express sequence tag (EST) sequences hampered fundamental studies such as gene characterization and genetic linkage mapping in yams. The dearth of genomic data in yam species prompted IITA to take up initiative in generating fundamental molecular genomic data useful to enhance the conventional yam improvement program. Most of the currently available molecular markers for the yam genomes are based on amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD). For marker-assisted breeding to be feasible, it is important to develop user-friendly and high-throughput markers such as EST-SSR and single nucleotide polymorphism (SNP) markers. The application of genomic tools in yam breeding and germplasm characterization will accelerate the development of improved yam germplasm in Africa and elsewhere.

In an effort to develop the yam genomics resources, an initial attempt was made to sequence ESTs from a

cDNA library constructed from floral tissue (Mignouna et al. 2003). However, the first several hundred sequences were found to be predominantly house-keeping genes suggesting a better approach in construction of cDNA library. Another project has been launched recently to generate several thousand ESTs in a collaborative project between IITA and the University of Virginia (based on USAID-Linkage fund). The objective of the project was to generate cDNA libraries from yam leaf tissues challenged with *Colletotrichum gloeosporioides*, the fungal pathogen responsible for yam anthracnose disease, and perform the sequencing of cDNA clones to subsequently identify ESTs with differential gene expression for marker development. In addition, SSR markers were developed in *D. alata* using sequence resources from heterologous crop species. Some of these markers were polymorphic in the test panel and are presently being tested in IITA's Central Biosciences Center.

4.4 Linkage Mapping and QTL Analysis

Two framework linkage maps were constructed for wild yam species, *D. tokoro*, using 271 AFLPs, five sequence-tagged microsatellite sites, one isozyme, and one morphological marker. For the two parents DT7 and DT5 used in the cross, 13 and 12 linkage groups, respectively, were identified. The total map lengths were 669 and 613 cM, respectively, for DT7 and DT5, which covered more than 75% of the *D. tokoro* genome (Terauchi and Kahl 1999). Similarly, maps were constructed in *D. alata* using AFLP markers that included 338 markers on 20 linkage groups with a total map length of 1,055 cM (Mignouna et al. 2002b) and in *D. rotundata* in which 107 markers were mapped on 12 linkage groups (585 cM) for male and 13 linkage groups (700 cM) for female (Mignouna et al. 2002c). They identified three quantitative trait loci (QTLs) and one QTL for resistance to Yam mosaic virus on male and female parent linkage groups, respectively. Similarly, one AFLP marker was found to be associated with anthracnose resistance on linkage group 2 explaining about 10% of total phenotypic variance (Mignouna et al. 2002a). Another linkage map was generated for *D. alata* based on 508 AFLP markers that covered a total length of 1,233 cM on 20 linkage groups accounting for about 65% of the entire genome of yam (Mignouna et al. 2007). Similarly, Petro et al. (2009) developed a linkage map of *D. alata* using 523 polymorphic markers from 26 AFLP primer combinations that covered a total length of 1,627 cM and included 20 linkage groups. They detected 10 QTLs for anthracnose resistance explaining 7–40% of the phenotypic variance. One of the major objectives of future research on linkage mapping should be integration of all the available maps of *D. alata* and *D. rotundata* with that of wild diploid species, *D. tokoro*. Although AFLP markers have been used for generating linkage maps so far, efforts are underway to saturate the maps with SSRs or EST-SSRs for use in yam breeding programs.

Bulked segregant analysis (BSA) has been successfully used in *D. rotundata* and *D. alata* to identify Yam mosaic virus (YMV) and anthracnose resistance genes, respectively (Mignouna et al. 2002a, b). Two RAPD markers OPW18850 and OPX15850 closely linked in coupling phase with the dominant YMV-resistance locus *Ymv-1* were identified. Similarly, two RAPD markers, OPI171700 and OPE6950, closely linked in coupling phase with anthracnose resistant gene, *Dcg-1*, were identified.

4.5 Application of Genomic Tools and Gene Discovery

Few laboratories around the world work on the molecular aspects of yam species in general and uncultivated yams, in particular. With respect to genomic resources, yam is considered as one of the understudied crops. Yam scientists who intend to embark on marker-aided breeding will begin the step with searching for nucleotide sequence data on the web. In this genomic era when genome sequences of many plants are completed or being completed every now and then and web-accessible databases are growing exponentially, it is hard to find new entries of nucleotide or protein sequences of any of the yam species. A number of peer-reviewed public web accessible databases offer the tool to seek information on specific genomic resources and tools (Galperin 2008). Entrez (http://www.ncbi.nih.nil.gov) is one of the popular web resources that comprise a wide array of primary and secondary databases and tools for data mining. According to the latest record of Entrez, draft genomes have been completed or are near completion for a

number of crop plants and model plants. Unfortunately, yam is not one of the 137 species listed in the Entrez plant genome project database. However, the Germplasm Resources Information Network (GRIN) database (USDA-ARS 2009) comprises 126 species of *Dioscorea*, the largest of the five genera in the family Dioscoreaceae. A summary of the data records for the family Dioscoreaceae in the GRIN database is shown in Fig. 4.4. These figures match the Genbank records but slight variation exists in the number of nucleotides and protein accessions in the genus *Dioscorea*, which is 576 and 588, respectively, in Genbank. Most of the genomic data in the family Dioscoreaceae come from *Dioscorea elephantipes* (a wild species), whose entire plastid genome has been sequenced (Hansen et al. 2007). Hence, most of these accessions represent house-keeping genes or photosynthesis-related genes of non-nuclear genome origin.

Nucleotide sequence search in the most recent release of genbank database (GenBank release 169.0) showed a total of 771 nucleotide sequences and 634 protein sequences for the entire family of Dioscoreaceae. Of these, while only 31 are mRNA (EST) sequences, the remaining genomic sequences are predominantly partial sequences of house-keeping genes derived from organelle genomes – chloroplast and mitochondria (Table 4.2). All of the 31 EST sequences (acc DN792550–DN792580) were obtained from subtracted cDNA library of *D. nipponica* root. In the absence of nuclear genome data, the plastid genome sequences can be utilized for applications such as DNA bar coding for species identification (details below).

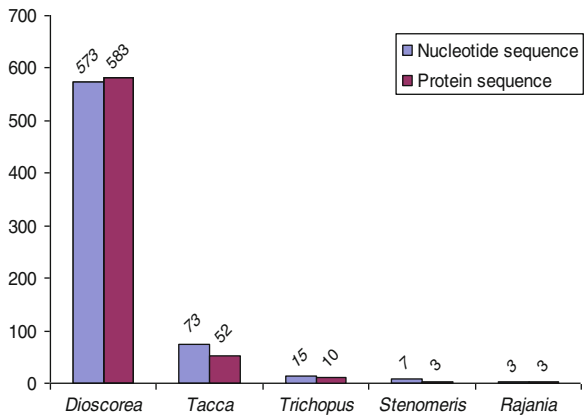

Fig. 4.4 Nucleotide and protein sequences in different genera within the family Dioscoraceae

To date, less than 20 microsatellite markers are available in yam species. Tostain et al. (2006) have isolated a total of 16 microsatellites containing sequences from *D. alata* (5 SSRs), *D. abyssinica* (6 SSRs), and *D. praehensilis* (5 SSRs). These SSR markers were tested and found to be transferable to other *Dioscorea* species. The remaining cultivated species such as *D. rotundata* and *D. cayenensis* have significantly low number of sequence data (3 and 6, respectively). Furthermore, most of the Genbank accessions in these two species were non-nuclear genomes. In general, a large number of the available genomic data in *Dioscorea* were obtained from non-cultivated species (Table 4.2). This information can still be utilized in cultivated species by applying homology-based techniques such as cluster of orthologous groups (COG) for primer design and other applications (Tatusov et al. 1997, 2003).

4.5.1 Genome Size

Plant DNA C-values database (Bennett and Leitch 2005; http://data.kew.org/cvalues/) contains DNA C-values for 11 yam species, all from the genus *Dioscorea*. It is notable that the Mbp values span a wide range of values from as low as 466 (1C = 0.48 pg) in *D. togoensis* to 2,352 (1C = 2.4 pg) in *D. villosa* (a wild *Dioscorea* species). The difference in genome size is partly attributable to the ploidy level even though values are not available for some species to conclude with certainty (Table 4.3). Despite the apparent variation in the estimated values, in general, the genome size of yam species is relatively low.

4.5.2 Plastid Genome

The *D. elephantipes* annotated chloroplast complete genome was reported by Hansen et al. (2007) (Genbank: EF380353; RefSeq: NC_009601). The entire plastid size is 152,609 nucleotides consisting of 129 genes of which 83 are protein-coding genes. These entries represent the most annotated records of the

Table 4.2 The number of nucleotide and protein entries in Genbank for the genus *Dioscorea* as of August 2010

Dioscorea sps.	No of nucleotide entries	Species	No of protein entries
Wild			
D. tokoro	87	*D. tokoro*	40
D. elephantipes	12	*D. elephantipes*	241
D. polystachya	73	*D. polystachya*	7
D. communis	22	*D. communis*	10
D. nipponica	18	*D. nipponica*	8
D. schimperiana	21	*D. schimperiana*	12
D. zingiberensis	13	*D. zingiberensis*	4
D. chouardii	10	*D. caucasica*	5
D. abyssinica	9	*D. gracillima*	4
D. sansibarensis	8	*D. sansibarensis*	4
D. praehensilis	8	*D. tenuipes*	6
D. sylvatica	8	*D. sylvatica*	4
D. pyrenaica	8	*D. decipiens*	4
Cultivated			
D. bulbifera	50	*D. bulbifera*	45
D. japonica	34	*D. japonica*	11
D. alata	87	*D. alata*	7
D. trifida	11	*D. sp. Qiu 94044*	4
D. rotundata	3	*D. rotundata*	0
All other taxa (including 31 EST sequences)	351	All other taxa	207
Total	771	Total	634

Table 4.3 Estimated genome sizes of *Dioscorea* species listed in Plant DNA C-values Database release 4.0, October 2005 (Bennett and Leitch 2005; http://www.kew.org/cvalues/homepage.html)

Genus	Species	Chromosome No.	Ploidy level	1C (Mbp)	1C (pg)	Original Reference
Wild						
Dioscorea	*togoensis*	40[a]	4	466	0.48	Hamon et al. (1992)
Dioscorea	*abyssinica*	40[a]	4	613	0.63	Hamon et al. (1992)
Dioscorea	*mangenotiana*	40[a]	4	613	0.63	Hamon et al. (1992)
Dioscorea	*praehensilis*	40[a]	4	613	0.63	Hamon et al. (1992)
Dioscorea	*sylvatica*	NA[b]	NA	833	0.85	Bharathan et al. (1994)
Dioscorea	*villosa*	NA[b]	NA	2352	2.4	Bharathan et al. (1994)
Dioscorea	*elephantipes*	NA[b]	NA	6615	6.75	Zonneveld et al. (2005)
Cultivated						
Dioscorea	*alata*	40	4	564	0.58	Arumuganathan and Earle (1991a, b)
Dioscorea	*cayenensis-rotundata*	40	4	613	0.63	Hamon et al. (1992)
Dioscorea	*cayenensis-rotundata*	60	6	858	0.88	Hamon et al. (1992)
Dioscorea	*cayenensis-rotundata*	80	8	1274	1.3	Hamon et al. (1992)

[a]Numbers refer to references listed at http://data.kew.org/cvalues/updates.html#REFERENCES of the C-values database (Bennett and Leitch 2005)
[b]Data not available

genus *Dioscorea* in public database. Information from the plastid genome has been successfully utilized for phylogenetic studies such as estimation of relationships among the major angiosperms, and provided an insight into the evolution of gene and intron content (Jansen et al. 2007; Hansen et al. 2007). Additional sequences and annotation of the genus *Dioscorea* can be found on the GOBASE database (http://gobase.bcm.umontreal.ca/). A search for *Dioscorea* in this database returns 615 chloroplast genes.

4.6 Future Perspective of Genomic Research

The paucity of genomic resources in yam species calls for a concerted effort from the research community to enrich genomic resources of yam so as to accelerate the germplasm enhancement endeavor. The rate of bolstering yam genomic resources is steadily growing at IITA. EST-derived SSR markers are already in use to characterize germplasm. Furthermore, this EST project is anticipated to result in the development of microarrays for high-throughput expression analysis and gene discovery. However, the outcome of this project is still too little to impact the deployment of molecular markers in advanced molecular-assisted breeding. The importance of yam in sub-Saharan Africa warrants the application of relevant cutting-edge technologies for germplasm enhancement. The steadily declining cost of the new generation sequencing technologies provides an impetus to embark on yam genome-sequencing initiative. Presently, a staggering number of plants are undergoing genome sequencing. As one of world's most important food crop, it is imperative to consider genome sequencing of yam. Successful completion of genome sequencing and annotation will trigger other investigators involved in evolution, taxonomy, physiology, systems biology, and comparative genomics. In parallel with development of genomic resources, thorough cytogenetic studies are of paramount importance to understand the genome structure and to pave the way for successful and effective subsequent genomic studies such as gene/QTL mapping and genome sequencing. Some of the ongoing initiatives in molecular technology are discussed below.

4.6.1 Microarray

Generation of sufficient nucleotide sequences paves the way for global gene expression analysis via microarray. The current EST project in yam is anticipated to generate sufficient ESTs to build microarray chips for transcript analysis. However, a concerted effort to generate DNA, mRNA, and protein data is the best way for accelerated development of genomic tools in yam species.

4.6.2 Identification of Candidate Genes by Comparative Genomics

In order to overcome the paucity of gene level knowledge in yam, approaches such as resistance gene analogs (RGAs) can be deployed to identify genes involved in plant defense (Moroldo et al. 2008). The rapid accumulation of genome sequence data sparked an array of functional genomics tools that are being employed to understand the complex pathways involved in host plant–pathogen interaction. In the absence of yam genome sequences, such homology-based identification of RGAs can be utilized as a shortcut method for the identification of gene-targeted markers of economically important diseases such as yam mosaic virus and yam anthracnose.

Application of comparative genomics will further allow the transfer of knowledge from thoroughly studied model plants to yam. Discovery of genes involved in flowering in model plants such as *Arabidopsis* have been successfully utilized to identify homologous genes in garlic (Rotem et al. 2007) and in cauliflower (Saddic et al. 2006). Such approaches can be adopted for discovery of genes regulating the flowering signaling pathways in yam. Dormancy, described as the temporary suspension of growth in stored yam tubers, is a persistent challenge that could be tackled by genomic intervention.

4.6.3 Reverse Genetics: Tilling

Targeting induced local lesions in genomes (Tilling) is increasingly becoming a popular technique of reverse genetics for detection of mutation in a target gene followed by assignment of the phenotypes to the gene sequence (Gilchrist and Haughn 2005). Tilling has been applied to crops with insufficient DNA sequences information by comparative genomics. Application of Tilling seems very prudent for yam researchers by capitalizing on advances in functional genomics of model plants. Knowledge of gene function in highly investigated plants sheds light on the genetic mechanism and pathways of key physiological traits in under-researched crops such as yam. Some of the traits that can be targeted by tilling could be flowering, dormancy, and resistance to diseases caused by fungi and viruses such as *Yam mosaic virus*.

4.6.4 DNA Barcoding

Species identification in the genus *Dioscorea*, the most important and the largest in the family, has remained a daunting task and the consequences of domestication on species identification has been described above. In IITA, there is a growing interest to apply DNA barcoding not only to address the issues with mislabeling and understanding interspecific crosses, but also to get an insight into the ongoing domestication process in *Dioscorea*. A DNA barcode is an aid for taxonomic identification that uses short, standardized DNA sequences (mostly 400–800 bp) present universally in target lineages and has sufficient sequence variation to discriminate among species of a particular organism. This provides a rapid and accurate procedure for unambiguous species identification by having sufficient sequence variation among species and low intraspecific variation. The universally accepted genes for plant DNA barcoding are of plastid origin. Polymorphism of chloroplast DNA especially *trn*K, *mat*K, and intergenic *trn*L–*trn*F regions have been used to study the phylogeny of various plants (Wolfe et al. 1987; Kress et al. 2005; Selvaraj et al. 2008). Fortunately, yam has relatively well-developed chloroplast genome information that can be tapped (Hansen et al. 2007).

4.7 Recommendations and Way Forward

Advances in cytogenetics such as molecular cytogenetics including techniques such as comparative genomic hybridization arrays (CGH), SNP-array based karyotyping, and automated systems for counting the results of standard FISH preparations, promise easy, accurate, and fast cytogenetics studies. These tools must be employed as a matter of urgency to re-examine the cytogenetics of yams. SSR markers, chromosome counts, and flow cytometry have been used with success to determine the mode of inheritance and the level of ploidy and provide new evidence for a base chromosome number (Bousalem et al. 2006). This approach must be adopted to analyze all yam species especially the wild types to clarify once for all the cytogenetic status to pave way for accelerated improvement of the yam crop.

Efforts for ex situ conservation of yam need to be augmented to protect the diversity of landraces and wild yams which are under threat of extinction due to agriculture intensification, erosion of forests, and increase in incidence of pests and diseases. There is a need for understanding the diversity in pathogen population responsible for important diseases such as "anthracnose" and "mosaic" in yam in order to establish an efficient breeding tactics. This information will also help in characterization of wild species for resistance to pests and diseases. Promising germplasm that has broad-specific resistance identified during this process could augment pest- and disease-resistance breeding programs.

Numerous questions meanwhile remain to be answered as far as the cytogenetics of yams is concerned. For instance, as Scarcelli et al. (2005) queried, "could the coexistence within the genus *Dioscorea* of the diploid $2n = 40$ *D. rotundata*, with diploid $2n = 20$ *D. tokoro* and *D. gracillima* be due to diploidization of genomes after their polyploidization for wild and cultivated plant genomes as documented for soybean?" What is the inheritance pattern for polyploidy in yams? Could diploidy and monoecy of parental plants be related? What is the chromosome number? A wide range of biotechnological and bioinformatics tools could be adopted to address these recalcitrant issues that will unravel the genetic potential of this orphan crop, which offers food and income security to millions of subsistence farmers in tropical and subtropical world.

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Chapter 5
Erianthus

P. Jackson and R.J. Henry

5.1 Basic Botany of the Species

5.1.1 Taxonomy

Erianthus represents a group of large grasses belonging to the Andropogoneae tribe, within the Poaceae family. *Erianthus* is very wide in distribution, occurring in America (New World species), China, India, Southeast Asia, New Guinea, and the Mediterranean region. The Old World (Asian) species are of importance in the evolution of sugarcane.

Within Andropogoneae, *Erianthus* belongs to the Saccharainae subtribe. Within the latter, two natural groupings, Saccharastrae and Eulaliastrae, have been identified, which although not recognized under the International Code of Botanical Nomenclature, are useful aids to discussion (Daniels and Roach 1987a). The former is generally found distributed in relatively warm and moist regions, and is often tall growing, while the latter are generally smaller plants adapted to drier areas. *Erianthus* belongs to the Saccharastrae grouping. This group is of direct concern to sugarcane improvement, and a key to the genera of this group, modified from Celarier (1956) was given by Daniels and Roach (1987a).

The Andropogoneae are frequently polyploid, and the evolution, speciation, and taxonomy are complex and in many cases unclear and confusing, with the monophyletic status of many genera being in doubt (Hodkinson et al. 2002). In addition, it is difficult and arbitrary to impose taxonomic boundaries because of the likelihood of interspecific and intergeneric hybridization and chromosome doubling. This situation has contributed a state of change and lack of clarity among some genera including *Erianthus*.

The genus and name *Erianthus* was established by Michaux in 1803 (cited in Alfonse Amalraj and Balasundaram 2006) based on the Greek word "Erion" meaning wool and "anthos" meaning flower, in reference to its wooly glumes. Hooker (1896, cited in Alfonse Amalraj and Balasundaram 2006) referred 11 species in *Erianthus*, and separated these from the *Saccharum* genus (Bonnett and Henry 2011) on the basis of presence of an awned fourth glume. Later authors, including Jesweit (1925, cited in Daniels and Roach 1987a) and Grassl (1946) maintained this separation. Mukherjee (1957) considered *Erianthus* to be a primitive genus of the *Saccharum* complex. Grassl (1972) went further and split *Erianthus* into two genera, placing the Old World species (of which he identified at least seven) into the genus *Ripidium*, and retaining the New World (American) species in *Erianthus*. Daniels and Roach (1987b) pointed out that a fern genus was listed previously by the same name, making the *Ripidium* name nominated by Grassl (1972) illegitimate. However, the "Ripidium" group is regarded as being meaningful and useful for those working in sugarcane breeding, and Daniels and Roach (1987a) validated the use of Ripidium as a section of *Erianthus* for seven Old World species, following Henrard (1926).

Erianthus has been regarded by some taxonomists as being synonymous with *Saccharum*. Bor (1960) treated the nine Old World *Erianthus* species he considered under *Saccharum* as well as *Erianthus*, because he was uncertain of how these should be classified. Botanists at Kew (Clayton and Renoize 1986; Phillips and Chen 2005) have treated the Old

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World species of *Erianthus* under *Saccharum*. The prevailing view among taxonomists in recent years appears to have been for *Erianthus* to be treated in the synonym of *Saccharum*, as seen in the 2003 Flora of North America (http://herbarium.usu.edu/webmanual/), and the 2006 Flora of China (http://www.efloras.org/florataxon.aspx?flora_id=620&taxon_id=128968).

By contrast, Grassl (1972) working with materials at the USDA world sugarcane germplasm collection, argued for differentiation of *Erianthus* and *Saccharum* on the basis of different floral structures and growth type. He argued that all Old World *Erianthus* are tufted bunch grass types with strong aggressive rhizome systems. Although rhizomes can be very aggressive and deep in some forms of *S. spontaneum*, they have a much longer internode, resulting in new culms being spread out much more than the dense bush of *Erianthus*. In addition, he argued that the culms of the Old World species of *Erianthus* are different to *Saccharum* in that the pith is dry and extends all the way to the rind. More definitively, DNA studies by Sobral et al. (1994), Al-Janabi et al. (1994), Besse et al. (1996), Hodkinson et al. (2002), and Nair et al. (2005) also suggest that *Erianthus* has quite a different lineage to other members of the *Saccharum* complex and some of these studies suggest evolutionary separation may have occurred almost as long ago as the *Saccharum–Sorghum* separation.

Mukherjee (1957) first used the term "*Saccharum* complex" to refer to members of *Saccharum*, *Narenga* Bor, *Schlerostachya* (Anderss. Ex Hackel) A. Camus, and *Erianthus* Michx. section Ripidium. These genera are grouped together on the basis that they can potentially interbreed, have been implicated in the evolution of *Saccharum*, and have been classified as *Saccharum* on some occasions. This term has been widely adopted and accepted by sugarcane researchers and breeders (Daniels and Roach 1987a). Daniels et al. (1975) included *Miscanthus* Anderss. Section *Diandra* Keng species in a revision of the complex.

5.1.2 List of Species and Characteristics

Based on review of literature, the following species of *Erianthus* appear to have been commonly accepted.

5.1.2.1 Old World Species

1. *Erianthus arundinaceus* (Retz.) Jeswiet. (= *Saccharum arundinaceum*, *Ripidium arundinaceum* Retz. Grassl) ($2n = 30, 40, 60$). Found in India, China, Indonesia, and New Guinea. This species is characterized by the presence of vegetative stalks and broad leaves, growing to a height of up to 7 m (Mukherjee 1958). It is considered to be closely related to *E. procerus* and considered by some authorities to be synonymous. However, *E. arundinaceus* produces vegetative stalks, which elongate before flowering, in contrast to *E. procerus* (Mukherjee 1958) and the two species differ in the presence of the chemotaxonomic marker F8, which is always present in *E. procerus* (Daniels et al. 1980). Besse et al. (1996) found a close similarity between *E. arundinaceus* from India and *E. procerus*, consistent with the suggestion by Daniels and Roach (1987a) of the close relationship between these two species, but only in relation to Indian sources of *E. arundinaceus*. Besse et al. (1997) in a study using restriction fragment length polymorphism (RFLP) markers found that *E. arundinaceus* collected from India and Indonesia were clearly separated. They also found that *E. procerus* and *E. bengalense* from India to be more like Indian *E. arundinaceus* than to *E. arundinaceus* from Indonesia.
2. *Erianthus procerus* (Roxb.) ($2n = 40$). (= *E. cilaris* (Anderss.) Jesw., *E. elegans* (Jesw. Ex. Backer), *Saccharum ciliare* Anderss.). Distributed from Northeast India, Burma, Malaysia, Kalimantan, China (Mukherjee 1958; Daniels and Roach 1987b). This species resembles *E. arundinaceus* but lacks vegetative stalks and has large droopy panicles (Alfonse Amalraj and Balasundaram 2006).
3. *Erianthus kanashiroi* (Ohwi) (=*Ripidium kanashiroi, Saccharum kanashiroi*) ($2n = 60$). Distributed from Southeast Asia to China. This species was previously suggested as being a hybrid of *Miscanthus sinensis* and *E. arundinaceus* (Adati and Shiotani 1962), but Grassl (1972) disagreed, and Daniels et al. (1980) showed a distinctive *Erianthus* flavonoid marker present.
4. *Erianthus bengalense* (Retz.) (= *Saccharum bengalense, Ripidium bengalense, Saccharum munja*) ($2n = 20, 30, 40, 60$). Hooker (1897) included this

in *S. arundinaceus*, but Mukherjee (1958) argued the case for this being a separate species, using the name *Erianthus munja*, after the common name of "Munja grass" used in North India (Alfonse Amalraj and Balasundaram 2006). *E. bengalense* is smaller and found in drier areas than *E. arundinaceus* (Daniels and Roach 1987a).

5. *Erianthus ravennae* (L.) P Beauv. (=*E. purpuraccens* Anderss., *Ripidium ravennae* (L.) Trin., *Saccharum ravennae* (L.) Murray) ($2n = 20$). Distributed from India to the Mediterranean (Bor 1960). This species is said to resemble *E. bengalense* but is distinguished on the basis of awned spikelets, broader, erect, dark green leaves, and hairy leaf sheaths (Hole 1911). Besse et al. (1997) using RFLP markers found that *E. ravennae* to be distinct from the other species from Section Ripidium they studied. In this study and another by Besse et al. (1998) using amplified fragment length polymorphism (AFLP) markers, *E. ravennae* was depicted in classification dendrograms as being most similar to *E. elephantinus*.
6. *Erianthus hostii* Griseb. (=*Ripidium strictum* (Host.) Trin, *Saccharum strictum* (Host.) Spreng) ($2n = 20$). Distributed Mediterranean to Iraq. Is characterized by short callus hairs and a creeping rhizomatous habit (Mukherjee 1958).
7. *Erianthus elephantinus* (Hook.f.) (=*Ripidium elephantinum* (Hook.f.) Grassl) ($2n = 20$). It has a limited distribution and found in Assam and eastern Himalaya. Panje (1954) reported that it is found in mainly wet areas with a high water table. Several workers (cited in Daniels and Roach 1987a) suggest that it could be a specialized race of *E. ravennae*, but based on flavonoid studies Daniels et al. (1980) suggested it was very distinct and could have contributed to the origin of *S. officinarum*.
8. *Erianthus rockii* (Keng). (chromosome number unknown). Found in southwestern regions in China, and grows in dry and cold areas and described by Keng (1939). DNA studies (Hodkinson et al. 2002; Cai et al. 2005b) suggest this species is relatively more similar to *Miscanthus* species as compared with other *Erianthus*, and Hodkinson et al. (2002) proposed membership of the genus *Miscanthidium*.

Chromosome numbers for various *Erianthus* species as indicated above have been reported by Rao and Raghavan (1951), Celarier (1956), Mehra et al. (1968), Jagathesan and Devi (1969), and Mohan and Sreenivisan (1983). The Royal Botanical Gardens (http://data.kew.org/cvalues) lists *E. arundinaceus* as having a genome size of 3.63 pg (1C).

Information given in the descriptions above about distribution has been taken mostly from Daniels and Roach (1987a). Some morphological characteristics of the *Erianthus* sect. Ripidium species were also given by Daniels and Roach (1987a) and some of this data is given in Table 5.1. While plant sizes will clearly vary depending on environmental conditions, the data provided may provide an approximate guide to relative growth and size of plants of each species. Generally, *E. arundinaceus* is regarded as having the most significant vegetative stalks, with other species apart from *E. kanashiroi* only having stalks which elongate on flowering (Mukherjee 1958).

Table 5.1 Some characteristics of Old World species of *Erianthus*, copied substantially from Daniels and Roach (1987a)

Species	Stalk height (m)	Stalk thickness (mm)	Inflorescence length (cm)
E. ravennae	<6[a]	<18[b]	30–90[c]
E. elephantinus	<4[c]	<20[d]	<40[c]
E. hostii	1–2[c]	<7[c]	15–30[c]
E. bengalense	3.5–5[c]	<12[c]	30–75[c]
E. procerus	4–6[e]	<14[c]	60–120[c]
E. arundinaceus	7[c]	<25[c]	60–75[c]
E. kanashiroi	5[e]	<20[e]	30[f]

[a]Bor (1960)
[b]Hole (1911)
[c]Mukherjee (1958)
[d]Hooker (1897)
[e]Daniels and Roach (1987a)
[f]Walker (1976)

5.1.2.2 New World Species

Webster and Shaw (1995) recognized five species of sugarcane native to North America traditionally referred to as *Erianthus*. However, following Clayton and Renoize (1986) they did not recognize *Erianthus* as a separate genus and considered it a synonym of *Saccharum*, although these have traditionally been placed under *Erianthus*. These species are: *S. alopecuroideum* L. Nutt (= *E. alopecuroides* (L.) Ell.), *S. balwinnii* Spreng. (= *E. strictus* Baldw), *S. brevibarbe* (Michx.) (= *E. contortus* Ell.), *S. coarctatum* Fern. (*E. coarctatus* Fern.), and *S. giganteum* (Walt.) Pers. (= *E. gigantus* (Walt.) C.E. Hubb.). A description of these species is provided in http://herbarium.usu.edu/webmanual/. In a study done with random amplified polymorphic DNA (RAPD) markers, Burner et al. (1997) suggested that sugarcane was genetically more similar to the New World *Erianthus*/*Saccharum* than to the Old World *Erianthus*. However, the utility of RAPD markers for assessing relationships between different genera has been questioned, and the finding of Burner et al. (1997) was later contradicted by a study by Besse et al. (1998) using AFLP markers, who showed that *E. giganteus* was more different to *Saccharum* species than the Old World species included (*E. arundineus, E. procerus, E. bengalense, E. ravennae, E. elephantinus*).

5.2 Conservation Initiatives

5.2.1 Germplasm Collections

Collections of *Erianthus* germplasm exist in two sugarcane related germplasm collections recognized as "world collections" by the International Society of Sugarcane Technologists (ISSCT). One is maintained in India by the Sugarcane Breeding Institute, within the Indian Council of Agricultural Research, at Kannur, Kerala and at Coimbatore, Tamil Nadu. An account of the *Erianthus* accessions in this collection is given by Sreenivasan et al. (2001). The second is maintained in the USA by the US Department of Agriculture at Miami, Florida (Schnell et al. 1997), with *Erianthus* accessions listed in the database (at http://www.ars-grin.gov/npgs/searchgrin.html) for this collection under the genus name of *Saccharum*. Most accessions in these two collections were sourced originally from Indonesia and India. Several collections exist in China, consisting of germplasm collected predominately within China. One of these, at Kunming, Yunnan, is described by He et al. (1999). The Chinese national sugarcane germplasm collection, containing *Erianthus* accessions, is maintained by the Yunnan Sugarcane Research Institute, Kaiyuan, Yunnan and in China another collection maintained by Guangzhou Sugar Industry Research Institute, at Hainan. Other germplasm collections maintained by individual sugarcane breeding programs containing *Erianthus* accessions are maintained in Australia, Brazil, and Thailand, although in the former two, most of the *Erianthus* accessions overlap considerably with accessions maintained in the ISSCT sanctioned collections.

5.2.2 Erosion of Germplasm Resources and Maintenance

The erosion of *Erianthus* spp. germplasm resources in the wild was noted by He et al. (1999). They reported that 10 years prior to their collection (which started in 1985) a great diversity of wild species was observed, but because of widespread development, very few specimens could be found during their collection expeditions. This example points to the likely significant erosion of genetic diversity in *Erianthus* and related species in rapidly developing regions such as those within China, and possibly mirrors other parts of Asia.

In all collections, *Erianthus* accessions, as with other related species in these collections, have been maintained as clonally propagated plants. This method of maintenance is clearly more expensive than storing seeds, with plants requiring periodic cutting (usually yearly) and occasional replanting. Plants are also susceptible to environmental catastrophes such as hurricanes or flooding. However, given the hardiness and vigor of most *Erianthus*, maintenance of accessions in this genus is considerably easier compared

with less vigorous species such as *S. officinarum*. True seed production for long-term storage has also been undertaken for the USDA collection (Schnell et al. 1997).

Erianthus species are not subject to specific intellectual property rights issues. *Erianthus* species are not included in the FAO treaty on plant genetic resources for food and agriculture and are therefore still distributed under the rules of the Convention on Biodiversity. This would allow claims to ownership of germplasm by states or countries with wild *Erianthus* populations.

5.3 Role in Development of Cytogenetic Stocks and Their Utility

Crosses between *Erianthus* and *Saccharum* as a source of new genetic variation for sugarcane improvement have been reported as discussed in Sect. 5.5. However, to our knowledge these have not yet been used to develop addition or substitution lines and only to a limited extent in basic genetics studies, as discussed in Sect. 5.4.

In situ hybridization studies have been done to examine chromosome transmission in hybrids between *S. officinarum* and *E. arundinaceus* (D'Hont et al. 1995; Piperidis et al. 2000) and in backcrosses derived from such hybrids and sugarcane (Piperidis et al. 2010). The latter study found $2n$ chromosome transmission in the hybrid clone derived from *S. officinarum* × *E. arundinaceus* when crossed with a sugarcane cultivar (containing chromosomes derived from *S. officinarum* and *S. spontaneum*), consistent with other studies in *Saccharum*.

In the study of Piperidis et al. (2010) there was also no apparent recombination between *Saccharum* and *Erianthus* chromosomes. Therefore, although it has not yet been done, it should be possible to generate backcross populations from repeated crossing to sugarcane, which would be segregating for individual *Erianthus* chromosomes from each *Erianthus* homology group. DNA markers could then be used to assign a breeding value (either positive or negative) for *Erianthus* chromosomes within a sugarcane background. If successful, such information could be used in subsequent sugarcane breeding for targeting retention or elimination of desirable or undesirable *Erianthus* chromosomes, respectively.

5.4 Role in Classical and Molecular Genetic Studies

There have been very few basic genetic studies using *Erianthus*. D'Hont et al. (1995) and Piperidis et al. (2000, 2010) used genomic in situ hybridization (GISH) to examine chromosome composition in progeny derived from *Saccharum* × *E. arundinaceus*. In both studies between 25 and 30 chromosomes were reported in the hybrids compared with 30 expected assuming n transmission. D'Hont et al. (1995) used in situ hybridization to detect an rDNA site at a terminal position on six chromosomes in *E. arundinaceus* to provide evidence supporting a basic chromosome number of $x = 10$ in this $2n = 60$ species. Lalitha and Premachandran (2007) reported on chromosome behavior during meiosis in hybrids between *S. spontaneum* and *E. arundinaceus*. They found predominantly bivalent formation in pollen mother cells of the hybrids, and at later meiotic stages abnormalities such as lagging chromosomes were present.

We are not aware of published reports on molecular genetic linkage maps or quantitative trait loci (QTL) detection studies utilizing *Erianthus*.

5.5 Role in Crop Improvement Through Traditional and Advanced Tools

5.5.1 Attributes of Commercial Value

There are a number of comments in the scientific literature, particularly in introductory comments to research reports, about the favorable attributes of *Erianthus*, and the consequent interest by sugarcane breeders in using *Erianthus* for imparting these attributes into sugarcane breeding programs. In particular, a generally high level of vigor, drought and waterlogging resistance, good ratooning ability, and disease

resistance have been attributed to *Erianthus* and to *E. arundinaceus* particularly (e.g., D'Hont et al. 1995; Piperidis et al. 2000; Cai et al. 2005a, b; Aitken et al. 2007; Lalitha and Premachandran 2007). However, while these comments are probably valid it would appear that there is little supporting data available, and most comments probably originate from observations by breeders and others of the growth of *Erianthus* spp. plants in germplasm collections or in the wild. Published reports showing data from well-controlled field experiments appear rare.

Matsuo et al. (2001) report on a study showing superior performance of *Erianthus* (species not indicated) compared with Napier grass and maize under both dry and wet field conditions. *Erianthus* roots were observed as deep as 2.5 m, despite the presence of both hard pans and shallow water tables, compared with Napier grass with roots rarely less than 1.35 m. These authors also reported results from pot experiments with adjusted water tables suggesting favorable growth of *Erianthus* under waterlogged conditions and ability of roots to grow well-developed aerenchyma (which could assist in root oxygenation). The ability of *Erianthus* to extend its root system during the rainy season and to take up water from deeper soil layers during the dry season was suggested as the mechanism for greater drought tolerance in *Erianthus*. The same study also suggested superior adaptation of *Erianthus* spp. under low N conditions and low soil pH.

There are several reports of high levels of resistance in *E. arundinaceus* to insects, which are commercially destructive in sugarcane including for major borer pests (Sardana 2002), and white grub (Allsopp and Cox 2002; Mukunthan and Nirmala 2002). These sources of insect resistance could be potentially important in an introgression breeding program targeting sugarcane improvement. However, to our knowledge no work in sugarcane breeding programs has yet progressed aiming to exploit these sources of resistance.

5.5.2 *Use of* Erianthus *for Sugarcane Improvement*

There have been several published reports of production of hybrids between *Saccharum* spp., especially *S. officinarum* and sugarcane cultivars (which are principally derived from *S. officinarum* and *S. spontaneum*, discussed further by Bonnett and Henry 2011) and *E. arundinaceus* (D'Hont et al. 1995; Piperidis et al. 2000; Ram et al. 2001; Cai et al. 2005a, b; Nair et al. 2006; Lalitha and Premachandran 2007), *E. rockii* (Aitken et al. 2007), *E. ravennae* (Janakiammal 1941), and *E. cilaris*. We are also aware of unpublished work in this area through personal communications with sugarcane breeders in several countries. The main motivation for these efforts has been to support longer term sugarcane improvement through introduction of new genetic diversity and/or genes contributing to desirable traits. However, use of *Erianthus* for successful development of sugarcane cultivars has not been reported or occurred so far to our knowledge.

Several factors have limited the contribution of *Erianthus* in sugarcane improvement programs to date. First, production of *Saccharum* × *Erianthus* hybrids has been complicated by the difficulty in identifying true hybrids amongst populations of seedlings arising from crosses between sugarcane (*Saccharum* spp.) and *Erianthus*. In many cases, putative hybrids have been later being shown with DNA markers to be self or arising from pollen contamination (D'Hont et al. 1995; L. McIntyre, K. Aitken, N. Berding, personal communication and experience of first author). In sugarcane breeding programs aiming to utilize *Erianthus*, these experiences demonstrate the importance of being able to accurately and quickly distinguish true hybrids with markers, otherwise efforts may be wasted on propagating and evaluating unwanted materials, and rare true hybrids may be lost. Second, while some true hybrids have been produced, some breeders have reported difficulty in producing fertile crosses between these resulting hybrids and *Saccharum* (Piperidis et al. 2000; Q-W Li, G. Piperidis, personal communication and experience of first author). Difficulties in producing fertile hybrids, or any hybrids at all, may be attributed to the apparently relatively large genetic distance between *Saccharum* and *Erianthus*, even larger than for other genera such as *Miscanthus* (Sobral et al. 1994; Alix et al. 1998; Cai et al. 2005a).

The ability to detect numerous specific *E. arundinaceus* markers allows for early and reliable identification of true hybrids during the breeding process (Piperidis et al. 2000; Cai et al. 2005a). Further, there is at least one successful example reported of fertile hybrids between *Saccharum* and *Erianthus,* and progeny produced from these hybrids and sugarcane,

opening up opportunity for introgression of *Erianthus* genome components into sugarcane breeding programs (Cai et al. 2005a).

Other factors suggested as potentially contributing to the lack of success of introgression of *Erianthus* into sugarcane breeding programs include chromosome erosion during crossing and backcrossing, and lack of recombination between the chromosomes of the two genera (D'Hont et al. 1995). Reduced transmission of chromosomes in crosses between *Saccharum* and *Erianthus* or the hybrids and *Saccharum* were reported by D'Hont et al. (1995) and Piperidis et al. (2000, 2010).

Interestingly, all reports of successful hybrids between *Saccharum* and *Erianthus* in recent decades that have been validated using DNA markers have been between *S. officinarum* and *Erianthus*, rather than modern sugarcane cultivars (which are complex hybrids containing both *S. officinarum* and *S. spontaneum* chromosomes).

To date there have been few reports backed with statistically supported data demonstrating performance of *Saccharum* × *Erianthus* hybrids or their derivatives. Grassl (1972) reported vigorous and good looking plants arising from a cross between *E. kanashiroi* and *S. spontaneum*. Sugarcane clones produced by crossing an *E. arundinaceus* clone with a *S. officinarum* clone were also reported by Ram et al. (2001) as having superior low temperature tolerance and red rot resistance. However, sugar contents in the progeny in the latter study were higher than that may have normally been expected from first-generation *Erianthus* hybrids. This raises questions about if the progeny were true hybrids and it would seem important to confirm parentage with DNA markers before making conclusions.

5.6 Genomics Resources Developed

Erianthus species have not been the subject of genomics resource development. Microsatellite or simple sequence repeat (SSR) markers from sugarcane have been found to be transferable to *Erianthus* (Cordeiro et al. 2001). Repeat sequences that are unique to *Erianthus* and allow distinction from other genera in the *Saccharum* complex have been reported (Besse and McIntyre 1998; Alix et al. 1999; Cai et al. 2005a, b). These have been applied to identification of hybrids in intergeneric crosses. Ribosomal gene sequences have also been applied to these distinctions (Besse et al. 1996; Pan et al. 2000). Molecular analyses have been used to establish the relationships between *Erianthus* and other related genera as discussed in Sect. 5.1.

The extensive genomic resources for *Saccharum* and *Sorghum* are likely to be the most useful for work with *Erianthus*. The *Sorghum* genome sequence has been reported (Paterson et al. 2009) and large numbers of expressed sequence tag (EST) sequences are available for these species. *Erianthus* is well supplied with genomic resources from work in the *Saccharum* complex despite that lack of *Erianthus* specific research.

5.7 Scope for Domestication and Commercialization

The main potential economic use for *Erianthus* considered to date is as a genetic resource for *Saccharum* improvement for sugar production, as discussed in Sect. 5.5. The development of efficient ligno-cellulosic biomass energy crops from the *Saccharum* complex may include the use of germplasm from *Erianthus*. *Erianthus* species are also grown as ornamentals, for stockfeed (Matsuo et al. 2001) and as a paper source (Amalraj et al. 2008).

Amalraj et al. (2008) advocated the increased use of wild relatives of sugar cane, particularly *E. arundinaceus,* as a feedstock for the paper industry, especially for sugarcane farmers who face problems such as drought, poor marginal land, and difficulties with supply to sugar mills. They argue that demand for paper feedstock will increase particularly in Asia, while bagasse (the fiber residue after crushing cane in sugar mills) supply will decrease to a trend in utilizing this for electricity generation. While details were not given, some results were cited suggesting superior pulp properties in *Erianthus* compared with sugarcane, as well as favorable energy value.

Several studies have indicated the potential for *Erianthus* to produce high yields of biomass and therefore potential for renewable energy production (e.g., Mislevy et al. 1987, 1997; Matsuo et al. 2001).

Tropical grass taxa such as *Erianthus* with a C4 photosynthetic pathway are highly efficient at converting solar energy into biomass and are generally more water use efficient than most other plants operating with a C3 pathway. However, apparently few studies have been conducted exploring the agronomic potential of *Erianthus*, in comparison with some other species such as switchgrass and *Miscanthus*. Studies on biomass accumulation by Mislevy et al. (1997) showed how some yield components changed with crop age, while also highlighting the negative impact of harvesting regularly (>once per year) prior to crop maturity.

5.8 Some Dark Sides and Their Addressing

Erianthus species may have potential to become weeds. Weed potential has been studied also in the related *Saccharum* and *Miscanthus* genera. This may pose a problem for use of *Erianthus* as an ornamental or energy grass crop improvement. Randal (2002) lists four species as weeds: *E. angustifolius*, *E. arundinaceus, E. formosanus,* and *E. rufipilus* (*Miscanthus*).

5.9 Recommendations for Future Actions

Based on review of literature and the current status of *Erianthus* in sugarcane breeding programs, the following priorities for future research and use of *Erianthus* are suggested.

5.9.1 *Evaluation of* Erianthus *for Potential Bioenergy Production Systems*

There is increasing interest and development across the world in cost-effective production of bioenergy, either in the form of electricity generation or liquid biofuel. If as expected the technologies and economics underlying bioenergy production systems improve and become more widely applied in future, increasing attention will turn to finding the best feedstocks for lowest cost biomass supply. *Erianthus* species, especially *E. arundinaceus*, have had a reputation amongst sugarcane breeders for being vigorous, tolerant to adverse environmental conditions including water stress and waterlogging, and resistant to diseases and insects. Some of these materials may, therefore, offer value feedstock for future bioenergy production systems, and be competitive (or better) than other candidate feedstock crops being considered, at least in some environments. However, despite this reputation, there have been few studies, backed with sound data, published to support this reputation. Further studies, particularly comparing *Erianthus* accessions with other high profile candidates for biomass production such as Switch grass and *Miscanthus* could help validate and, if appropriate, more widely publicize, the value of *Erianthus* for potential future bioenergy production systems.

5.9.2 *Support Better Utilization of Existing Germplasm Collections*

Several good collections of *Erianthus* germplasm, together with other sugarcane related germplasm, exist in several countries. However, most accessions have not been utilized for crop improvement programs or evaluated for commercial deployment. There is a case for more effective and efficient utilization of *Erianthus* germplasm resources already collected. This depends on level of funding for breeding programs. However, better utilization may also be supported via an internet-based database network, aiming to efficiently manage and publish availability of materials across different collections and countries. Such a database network may help broker mutually beneficial partnerships between institutions and investors involved in crop improvement and germplasm collection curators. Involvement and coordination by a group such as the International Plant Genetic Resources Institute (Bioversity International) or other group with an international mandate to foster deployment of germplasm resources could help facilitate initiation of such a network.

5.9.3 *Extend the Utilization of* Erianthus *for Sugarcane Improvement*

There has long been a strong interest among sugarcane breeders in using *Erianthus* in sugarcane improvement. However, this has been challenging to date, partly because of the difficulty in producing and efficiently identifying true hybrids. The advent of DNA markers and recent validation of fertile hybrids between sugarcane and *Erianthus* should now open up greater opportunities for using *Erianthus* germplasm in sugarcane breeding programs.

5.9.4 *Apply DNA Markers to Support Introgression of* Erianthus *in Sugarcane Breeding Programs*

As with all wild germplasm, it is likely that there will be both favorable and unfavorable genome components and genes in relation to contributing toward improvement of commercially important traits in sugarcane breeding. The use of DNA markers in QTL mapping study in advanced backcross populations has been suggested as a way to tag favorable and unfavorable QTL in plant breeding programs. In *Erianthus*, this could be effectively done in advanced backcross generations in which segregation of chromosomes representative of each homology group occurs. In this way, a breeding value (positive or negative) for each chromosome may be assigned, which could then be easily selected for or against using DNA markers in subsequent breeding. Advances in genomics and knowledge of the sequences of the genomes of sugarcane and related species could provide new tools to support these objectives.

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Chapter 6
Gossypium

Chandrakanth Emani

6.1 Introduction

Gossypium is an important genus of the plant kingdom due to the presence of the economically important species of cotton in its family Malvaceae. The complex genus comprises 51 perennial species distributed all over the planet (Fryxell 1992) with four primary cultivated species and the rest being diploid wild species. The wild species are a veritable wealth of exotic germplasm resources as they are important sources of genetic diversity. Classical plant breeding and modern molecular breeding exploited many valuable agronomic traits and abundant gene resources present in the wild species for improving cultivated cotton. Cotton is the most important source of natural fiber globally and it is also a multipurpose crop that is a rich source of oil, seed meal, and hulls. Despite the advent of synthetic alternatives such as rayon, nylon, and polyester, the cotton lint (fiber) is still an important produce for the textile industry. Even the short fibers called "linters" left on seed after ginning are removed at the oil mills to serve as valuable sources of cellulose plastics and synthetic fibers (Bajaj 1998a), medical products, paper industry, photographic film, and cosmetics (Rathore et al. 2008). The oil content of various cotton cultivars ranges from 16 to 25% of dry seed weight (Lawhon et al. 1977) that leads to a substantial global produce utilized in edible oil extraction. The cottonseed meal comprises 16–22% protein rich in essential amino acids such as lysine, methionine, and tryptophan (Beradi and Cherry 1980), and is utilized as a cattle feed and fertilizer. Seed hulls are also used as cattle feed and as field soil covering called mulch (Bajaj 1998a). Thus, cotton is a globally important economic crop and the cotton plant itself shaped many historical, cultural, social, and economic events on the planet for centuries (Yafa 2004). In more recent times, a renewed effort by researchers across the globe concentrated on focused and collaborative projects to improve cotton and the most significant of these efforts centered around extensive sequencing of genomes, isolation, and characterization of marker genes for various agronomically important characters and creating virtual databases on the world wide web as rich exchange resources for information on the wealth of cotton germplasm (Chen et al. 2007). Against this background, it would be a worthwhile endeavor to comprehensively examine the complex taxon or genus of *Gossypium* for its origins, the various species within the genus and how classical as well as biotechnological approaches utilizing the wealth of wild germplasm can further expand the existing opportunities to globally improve the economically important cotton crop.

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6.2 Basic Botany of the Genus

6.2.1 Botanical Distribution and Genomic Groups

Gossypium is a complex genus that includes 51 perennial species distributed globally of which 46 are diploid ($2n = 2x = 26$) and the other five are tetraploid ($2n = 4x = 52$) (Fryxell 1992). All of the species exhibit disomic patterns of inheritance

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Table 6.1 *Gossypium* genomes and their geographical distribution

Genome	Number of species
African clade	
1. A genome	2
2. B genome	4
3. E genome	4
4. F genome	1
American clade	
1. D genome	12
Australian clade	
1. C genome	9
2. G genome	1
3. K genome	12

(Chen et al. 2007). The genus comprises four primary cultivars, namely, *G. arboreum* L. ($2n = 2x = 26$), *G. herbaceum* L. ($2n = 2x = 26$), *G. hirsutum* L. ($2n = 2x = 52$), and *G. barbadense* L. ($2n = 2x = 52$). All others are wild species that are diploid. Based on cytogenetic studies and interspecific hybrid viability/fertility analysis, the diploid species are classified into eight genomic groups, A–G and K (Table 6.1; Percival et al. 1999). The A, B, E, and F genomes comprise the African clade that is distributed naturally in Africa and Asia; while the D genome is the clade indigenous to the Americas; and the C, G, and K genomes comprise the Australian clade (Wendel and Cronn 2002).

6.2.2 *Evolution of* Gossypium *Species*

A concerted worldwide collaborative effort by cotton researchers aims to provide a comprehensive description of many poorly described diploid species and these efforts sometimes result in discovery of many newer *Gossypium* species (Chen et al. 2007). The diversity of wild *Gossypium* species distributed across Australia, Africa, Asia, and the Americas led researchers to conclude that the lineage of *Gossypium* may have had its origins 10–20 million years ago (Brubaker et al. 1999). The important cultivated species of cotton, whose English name is a derivative of the Arabic "*al qutn*" (Metcalf 1999) was known to have been cultivated during the Indus Valley civilization dating back as early as the fifth millennium BC (Stein 1998). The earliest cotton species may have evolved as a

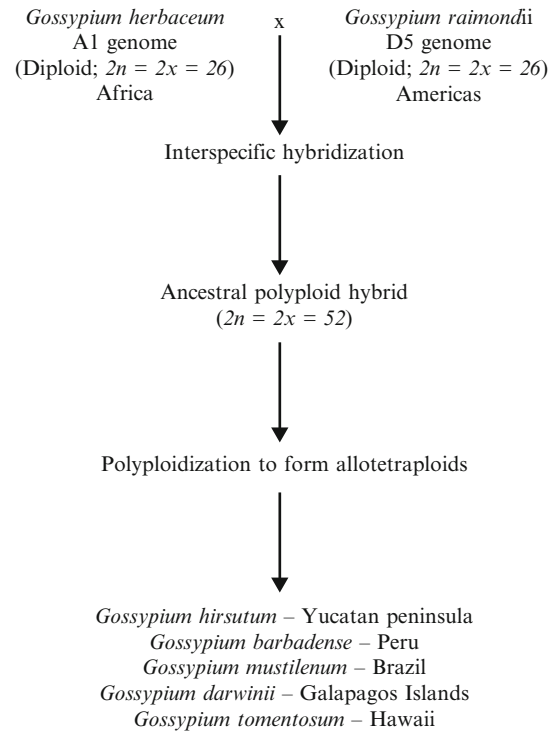

Fig. 6.1 Evolution of *Gossypium* species

diploid species in Africa as the first *Gossypium* line to produce the hairs or trichomes on the seed and then migrated to various parts of the world (Fig. 6.1). The primary cultivar *G. herbaceum* L. ($2n = 2x = 26$) was found wild in southern Africa, representative of the A-genome-like ancestral African species. Another A-genome primary cultivar *G. arboreum* L. ($2n = 2x = 26$) was established in southern Asia and a third D-genome diploid species *G. raimondii* migrated to the western hemisphere or the New World 1–2 million years ago (Brubaker et al. 1999). The 52 chromosome species including *G. hirsutum* and *G. barbadense* are classic natural allotetraploids that evolved in the New World owing to a critical interspecific hybridization that occurred during the migration of diploid species between the A-genome-like African ancestral *G. herbaceum* (A1) and the D-genome-like American ancestral *G. raimondii* (D5) (Wendel and Cronn 2002), a fact recognized in earlier cytogenetic (Phillips 1963) and seed electrophoretic (Cherry et al. 1970) studies. The first of the polyploid hybrids retained the ability of fiber production that was more vigorous than the parental diploid species and subsequent polyploidization gave rise to five extant allotetraploid species,

namely, *G. hirsutum* in the Yucatan peninsula, *G. barbadense* in Peru, *G. mustelinum* in Brazil, *G. darwinii* in Galapagos Islands, and *G. tomentosum* in Hawaii (Wendel and Cronn 2002). The categorization of different genomic groups based on cytology of interspecific hybrids (Table 6.1) shows that the A-genome contains two diploid cultivars (*G. arboreum* and *G. herbaceum*), and the B, C, D and E genomes have 4, 9, 12, and 4 species, respectively. The F genome has one species *G. longicalyx* and the G genome has the cultivated type *G. bickii*. The tetraploid species are natural amphidiploids that have a mixture of A and D genomes (for a detailed description of the categorization and speciation with all the specific names, the reader may refer to Bajaj 1998a).

Only the A genome species produce spinnable fiber, a feature absent in the D genome species (Applequist et al. 2001). *G. hirsutum*, the upland or American cotton accounts for 95% of the annual global cotton crop with the only known cotton with spinnable lint that grows wild being *G. herbaceum* var. *africanum*, that accords a status of a probable ancestor to all linted cottons of the Old and New World (Bajaj 1998a). *G. barbadense*, the extra-long staple or Pima cotton accounts for less than 2% of the cotton fiber production (National Cotton Council, http://www.cotton.org, 2006). While earlier studies showed clear indications that New World cotton tetraploids contain a set of chromosomes from one of the two A genomes and another chromosome set from a D genome species (Beasley 1940), more recent studies worldwide are striving to decipher in greater detail the contribution of A and D subgenomes to gene expression in the allotetraploids with a long-term goal of designing effective strategies to improve economically important characters such as improving fiber traits (Saha et al. 2006; Yang et al. 2006).

6.2.3 Genome Size and DNA Content

Polyploidy and genome size variation are two important properties within the *Gossypium* genus that warrant a thorough and comprehensive understanding in terms of both functional and agronomic perspectives, and the decoding of cotton genomes may prove to be a step toward the right direction in achieving this goal (Chen et al. 2007). Examination of the haploid genome sizes revealed that the New World diploid *G. raimondii* measures about 880 Mbp, the Asian diploid cultivar *G. arboreum* measures 1.75 Gbp, and the popular cultivar *G. hirsutum* measures 2.5 Gbp (Hendrix and Stewart 2005). The 2C nuclear DNA content of the AD tetraploid cotton ($2n = 4x = 52$) was estimated to be 4.91 pg or 2,401 Mbp (Hendrix and Stewart 2005) and this was an approximate additive for the A genome donor *G. herbaceum* and D genome donor *G. raimondii*. The vast variation observed in the DNA content across the diploid species is reflective of the copy numbers of the dispersed repetitive DNA that spread to the newly formed cotton genomes during the polyploid formation (Zhao et al. 1998). Hawkins et al. (2006) showed that differential lineage-specific amplification of retrotransposon-like elements is responsible for genome size variation in *Gossypium*. In general, the DNA content of the allopolyploids approximately matched the sum of the A and D genome progenitors, a fact corroborated by Liu et al. (2001) who determined that about 22,000 amplified length polymorphism (AFLP) fragments were additive in allopolyploids. Studies to decipher the polyploidy formation (Jiang et al. 1998) and the genome evolution during polyploidy (Wendel 2000) suggested the role of many genetic and epigenetic mechanisms for gene expression in the phenotypic variation and selection of the allotetraploid species (Chen 2007). The accumulation of genome-specific transcripts, transcription factors, and phytohormonal regulators during early stages of fiber development in cotton (Yang et al. 2006) laid further credence to the variation seen in allotetraploid species.

6.2.4 Agricultural Status and Status as a Model Species

Gossypium genus comprises the economically important species of cotton that is the single most important natural fiber of the world that generates a $25 billion produce and accounts for nearly half a million jobs in the industrial sector affecting 100 million families in over 150 countries (Chen et al. 2007). In terms of basic plant science research, cotton fiber is an outstanding model for the study of plant cell elongation, and cell wall and cellulose biosynthesis (Kim and Triplett 2001).

A single cottonseed has approximately 25,000 cotton fibers that are cellular elongations of the ovule epidermal layers, and the fiber comprises pure cellulose, the largest component of plant biomass (Chen et al. 2007). In the present era of alternative biofuel generation from the biomass, cotton fiber may prove a cheaper alternative for biofuel conversion as compared to other related sources such as lignin that is more difficult for biofuel conversion compared to cellulose. The importance of cotton as an edible oil source and cattle feed was mentioned earlier and collectively, it shows that focused research studies on the translational genomics of properties such as fiber, oil, and cellulose in the model species of cotton may contribute to improvement of diverse biomass crops (Chen et al. 2007).

The "wealth" of wild germplasm in *Gossypium* is in terms of many valuable agronomic traits and abundant genetic resources among the diverse species. Some specific examples are: immunization to bacterial diseases, cotton wilt and of resistance to microzyme, aphid, black arm, jassid, and red spermidite in *G. anomalum*; resistance to low temperatures (−8°C) in *G. sturtianum* and *G. thurberri*; resistance to pests with sucking mouthparts in *G. rainmondii*; alkali and salt tolerance in *G. davidsonii*; drought resistance in *G. ardidum*, *G. harkensii*, *G. rainmondii*, *G. stocksii,* and *G. tomentosum*; high lint quality in *G. anomalum*, *G. thurberi,* and *G. tomentosum*; bollworm resistance in *G. raimondii*, *G. thurberi*, *G. somalense*; insect resistance in *G. harkensii*, *G. bickii,* and gossypol-free seeds in *G. bickii* (Bajaj 1998a, b, c; Sun et al. 2006). All these traits make the wild germplasm useful for the genetic improvement of cultivar cotton species.

6.3 Conservation Initiatives

Gossypium germplasm is traditionally conserved through maintaining seed stocks and cultivar cotton as an annual crop, is generally propagated through seed. The traditional method of conserving germplasm through seed collections is most effectively overseen by the USDA-ARS Southern Crops Research Laboratory located at College Station, Texas that maintains the largest collection of *Gossypium* germplasm with over 5,000 accessions (Percival and Kohel 1990). In addition, Mexico, the center of the origin and diversity of the widely cultivated *G. hirsutum*, is now overseeing the establishment of a government-controlled *Gossypium* species nursery with seed accessions of *G. hirsutum, G. barbadense, G. gossypoides, G. aridum, G. laxum, G. lobatum, G. schwendimanii*, *G. trilobum*, and one undescribed diploid taxon to enable the in situ conservation of some of these species that are threatened by environmental extinction (Ulloa et al. 2006).

Natural crossing, mechanical mixtures during ginning, and long-term seed storage causes all the varieties to rapidly deteriorate in quality, and hence researchers always focused on developing suitable means of vegetative propagation. The advantage of the clones obtained by vegetative propagation is that they are true to type and can be utilized for the production of nucleus seed that maintains the purity of a variety (Bajaj 1998b). Conventional methods of vegetative propagation by cuttings (Parmar et al. 1978) were hampered by slow multiplication rates that led to the adaptation of in vitro micropropagation (Bajaj 1998b) that showed reasonably high multiplication rates. Micropropagation was successfully employed in *Gossypium* species for collection and maintenance of germplasm, rapid multiplication of novel seed varieties, clonal propagation for evaluating diseases, and also for production of male sterile lines (Bajaj and Gill 1992). The advent of tissue culture and biotechnology especially in the area of genetic transformation to induce genetic variability in cotton called for renewed attempts to develop effective methods for storage of desirable cell lines as well as germplasm.

6.3.1 Micropropagation Through Tissue Culture

Tissue culture for short- and medium-term storage and cryopreservation for long-term conservation were seen as effective alternatives for the conservation of *Gossypium* species for the past two decades (Bajaj 1998b). The earliest reports of in vitro culture of excised meristems of *G. hirsutum* (Chappel and Mauney 1967) were successfully extended to shoot tip and meristem cultures of *G. arboreum*, and interspecific hybrids of *G. arboreum* × *G. stocksii*, and *G. herbacium* × *G. stocksii* with regeneration rates of 41.6–72.7% (Bajaj 1998b). Altman et al. (1990) combined a novel

tissue culture and field growth protocol for collecting germplasm of various wild *Gossypium* species, especially for those varieties where viable seed production was the biggest challenge. For plants with difficulty in obtaining viable seeds, embryo, and ovule culture from 3 days after pollination (DAP), ovules and 15 DAP embryos were successfully employed as a method of micropropagation in *G. hirsutum, G. arboreum, G. herbaceum, G. Stocksii,* and *G. anomalum* along with interspecific hybrids among these species (Bajaj 1998b).

Somatic embryogenesis was considered as a method of choice for the fastest large-scale propagation in crop plants (Zimmerman 1993). The first report in cotton was from suspension cultures of a wild species *G. klotzschianum* (Price and Smith 1979) where the somatic embryos failed to regenerate. Further attempts in the same species (Finer and Smith 1984) resulted in plants with abnormal leaves and shoots. Sun et al. (2003) finally achieved plant regeneration in *G. klotzschianum* that now serves as a model regeneration system for wild cotton. This method was successfully extended to include other wild varieties such as *G. davidsonii*, *G. klotzschianum*, *G. raimondii*, and *G. stocksii* (Sun et al. 2006). *G. hirsutum* is by far the most successful cotton tissue culture system (Davidonis and Hamilton 1983; Shoemaker et al. 1986; Trolinder and Goodin 1987; Firoozabady and DeBoer 1993; Kumria et al. 2003), where it was shown to be highly genotype dependent and highly efficient in the coker varieties (Firoozabady and DeBoer 1993). The coker varieties are now the varieties of choice for genetic transformation (Firoozabady et al. 1987; Umbeck et al. 1987; Rathore et al. 2006). The first successful somatic hybridization through protoplast fusion was achieved between *G. hirsutum* Coker 201 and *G. klotzschianum* (Sun et al. 2004) and such methodologies represent potential and novel tools for transferring the agronomically important characteristics of wild cottons to cultivated cotton in global breeding programs.

6.3.2 *In Vitro Conservation of Germplasm*

To tide over the challenges of stored seeds undergoing deterioration over time, various alternative methods of in vitro cultures for short and medium-term storage were successfully utilized in cotton. One of the earliest alternative methods was the shoot-tip culture storage where culture could be maintained at room temperature for more than 6 months (Bajaj and Gill 1986). The field tissue culture method of Altman et al. (1990) enabled storage of cotton varieties where availability of viable seeds cannot always be assured especially during botanical expeditions. In this method, cuttings were cultured in media directly in the field and after being transported outdoors for almost three weeks, brought to the lab where they were transferred to soil after sterilization. Cryopreservation that entails storing cell culture, embryos, and meristem tips in liquid nitrogen that can be later regenerated into plants was successfully utilized to prevent genetic erosions or the instability of plants to maintain desired cell types that usually arises due to periodic subculturing (Bajaj 1995). This method will be highly desirable for conservation of rare, elite, and desirable cotton cultures and establishing germplasm banks and international exchange of useful germplasm (Bajaj 1979).

6.4 Role of Wild Relatives in Elucidation of Origin and Evolution of Cotton: Cotton Fiber as a Model

As mentioned earlier in this chapter, the highly elongated structure and the exceptional chemical makeup of cotton fibers have established them as an exceptional and ideal model for elucidating the origin, evolution, and gene expression of important plant developmental processes such as cell wall biogenesis and plant cell elongation, and the associated metabolic pathways that accompany such processes (Kim and Triplett 2001). The cotton fibers (the more appropriate term is "trichomes") are unicellular in nature and arise from ovule epidermis and not as part of regular vascular tissue. These single-celled structures are present in all *Gossypium* species and have a long history of evolution with both human and environmental-mediated changes with a 10 million year history resulting in 51 species (Brubaker et al. 1999; Wendel and Cronn 2002). The remarkable human interventions of domesticating four cultivated species from the diverse array of wild species make the wild species a truly invaluable genetic resource to examine and elucidate

vital plant developmental processes. Added to this, since the fiber has an ovule-epidermal origin and is not from a regular vascular tissue, the cell elongation process can be evaluated independently from cell division. The fiber development comprising four overlapping developmental stages, namely, fiber initiation, cell elongation, secondary cell wall deposition, and maturation is timed conveniently starting from initiation at various stages that can be measured parallel to the days post anthesis (DPA). Since the culture method for cotton ovules was perfected almost three decades ago (Beasley 1971), the fiber formation is a convenient in planta as well as an in vitro system uniquely suited to study the plant cell wall–cytoskeleton continuum and the parallel plant developmental processes (Kim and Triplett 2001). Since the cotton germplasm has a wealth of wild plant relatives, the process of fiber formation if analyzed between the wild and cultivar varieties by mutant analysis, cytogenetics, and various molecular marker applications may throw valuable insights into the origin, evolution, and gene expression of various plant developmental processes involved with plant cell wall and cellulose biosynthesis. The resulting knowledge may also prove useful in improving diverse biomass crops in exploiting them as biofuel alternatives.

Hovav et al. (2008) examined the morphological changes of fiber formation between the short, tightly adherent fibers of the wild *G. longicalyx* with the drived long, spinnable fibers of its close cultivar relative *G. herbaceum* taking into account the genetic and evolutionary background. A comparative gene expression profiling across a developmental time-course of fibers in both species was conducted using microarrays with 22,827 genes. The observed changes in gene expression were temporally stretched out over time in *G. herbaceum* relative to *G. longicalyx* reflecting a prolongation of the ancestral developmental program. In course of the gene comparison at various stages, the Blast2GO program (http://www/blast2go.de/) was used to identify biochemical pathways involved in a given comparison. Results indicated that many genes involved with stress responses were upregulated early in *G. longicalyx* fiber development. Several candidate genes upregulated in *G. herbaceum* were related to regulating redox levels and cell elongation processes. Three genes involved in modulating hydrogen peroxide levels were constantly expressed in both wild and domesticated species with long fibers, but not in wild species with short fibers. Since hydrogen peroxide is important for cell elongation, and is toxic at high concentrations, the reduced cell elongation may be due to stress responses seen in short fiber species. These observations suggest that long spinnable fiber evolution is aided by novel expression of genes that assist in regulating reactive oxygen species. The study thus throws valuable insights in proposing a model for evolutionary origin of a novel morphology through differential gene regulation in plant developmental and evolutionary processes.

Future researchers can thus use similar approaches in examining gene profiling comparisons between the diverse arrays of wild relatives with corresponding closely related domesticated species to gain valuable insights into various agronomic and economically important characteristics like disease resistance, lipid biosynthesis and the evolution of protein components in *Gossypium* species.

6.5 Role in Development of Cytogenetic Stocks and Their Utility: Haploid Production by Anther Culture

Cotton researchers have for long emphasized on the importance of haploids in breeding and genetic studies as haploid production is useful in obtaining homozygous lines in a single generation that can be evaluated in a field (Harland 1936). Haploids are also valuable to increase the efficiency of existing breeding methods through an improved reliability of selection (Bajaj and Gill 1998a). Haploids occur naturally in *G. barbadense* and *G. hirsutum* (Harland 1936), *G. davidsonii* (Skovsted 1935), *G. arboreum,* and *G. herbaceum* (Bergach 1971). Haploids were induced by conventional methods such as semi-gamy in the tetraploids *G. hirsutum* and *G.barbadense* (Chaudhari 1978) and rarely by polyembryony (Blank and Allison 1963). Due to the low frequency of haploid induction by conventional methods, researchers undertook other methods such as anther culture (Bajaj and Gill 1998a) and induced parthenogenesis (Zhou et al. 1991).

Early studies to induce androgenesis in cotton resulted only in callus formation and later studies in *G. arboreum* were successful in inducing haploid cells ($2n = x = 13$) and a wide range in chromosome numbers that included diploids, triploids, tetraploids,

pentaploids, and hexaploids, with a few aneuploid cells (Bajaj and Gill 1985). The occurrence of low frequency of haploid cells notwithstanding, which may be attributed to the derivation of haploid cells mostly from somatic tissue, the induction of gametoclonal variation through the pollen-derived callus would be useful for inducing genetic variability by somaclonal variation in cotton (Bajaj 1998c). The genetically unstable haploid cell cultures tend to revert back to diploid form by endomitosis, and hence it is imperative that methods be developed for their preservation. Cryopreservation was an ideal method, where the anther-derived callus from *G. hirsutum* and *G. arboreum* was freeze preserved in liquid nitrogen (−196°C) for 2–3 h and still could resume active growth as seen in highly cytoplasmic aggregates of suspension cells after retrieving the frozen callus (Bajaj and Gill 1998a). Cold treatment (4°C) of anthers was found to promote nuclear divisions in pollen leading to the formation of multinucleate pollen and pollen embryos in *G. arboreum* (Bajaj and Gill 1989).

6.6 Role in Classical and Molecular Genetic Studies

The wealth of wild species in *Gossypium* are a rich source of genetic variability, and in spite of being short-fibered and lintless, have a number of traits such as fiber quality, insect resistance, drought tolerance, and disease resistance (Hutchinson et al. 1947; Prentice 1972). The importance of introgressing the glandless wild species *G. sturtianum* that are gossypol-free with upland cultivated *G. hirsutum* using ovule culture was pointed out by classical breeders (Altman et al. 1987). Earlier classical breeders also stressed on production of interspecific hybrids between diploid and tetraploid *Gossypium* species for introducing improved agronomic and quality traits into commercial cotton (Meyer 1974).

6.6.1 Agronomic Traits of Interest in Classical Breeding

The earliest classical breeders chalked out their objectives based on a long-term goal of increasing the economic gains at field level cotton crop production and strategies centered on improving agronomic traits such as host plant resistance, abiotic stress tolerance, fiber and seed qualities, and agronomic adaptability (Rathore et al. 2008). Insect, nematode, and soil-borne fungal diseases were the major factors of importance for breeding cotton varieties with improved host-plant resistance. The most important and notorious pest that created both economic and social upheavals in the US cotton fields and cotton industry was *Anthomonus grandis* (boll weevil). Breeders focused on developing early maturing varieties that produce fruits early in the growing season so that the plants avoid the reproducing second and third generation post-diapause boll weevils in the field. Biochemical and morphological traits were also identified to aid in breeding host-plant resistance (for details see Rathore et al. 2008). Extensive screening of early-generation plants for various stresses such as drought, heat, and cold was conducted to select the most productive plants resistant to abiotic stresses, with more sophisticated methods being developed till more recently for drought-tolerant selections (Longenberger et al. 2006). Fiber quality, fiber strength, and techniques to break the negative linkages among the properties of the fiber and the crop yield are the major points of interest to breed cotton with better fiber (Culp and Harrell 1973). Since the cottonseed represents only 10% of the crop's economic value, breeders focused on minor strategies in this trait with the major attention given to seed size as related to the seedling vigor (Quisenberry and Gipson 1974). In terms of seed quality, an important component that breeders focused on was the secondary metabolite gossypol, the substantial chronic effect of which prevents non-ruminant and human consumption of cottonseed (Abou-Donia 1976). The presence of gossypol diminishes the protein quality of cottonseeds (Jaroszewski 1998) and also makes oil extraction an expensive affair. Early breeding efforts to develop glandless gossypol-free cotton (McMichael 1959) led to commercial varieties (Halloin et al. 1978), but since gossypol was an allelochemical that inhibits insect growth, the developed lines were not successful in attracting the interest of farmers due to poor survival against pest attacks.

6.6.2 Limitations of Conventional Breeding

Genetic diversity is an important factor in advancing the breeding populations and the wealth of wild *Gossypium* germplasm with desirable agronomic characters needs to be properly exploited, especially by private breeders who tend to utilize more in-house elite germplasm when compared to public breeders (Bowman 2000). The numerous attempts made by conventional breeders to transfer desirable agronomic traits from wild to cultivated cotton were hampered by limitations such as low frequency of crossing and an eventual hybrid breakdown (Gerstel and Phillips 1958), and early abortion of the hybrid embryos due to incompatibility (Pundir 1972). Added to these are the methodical limitations mainly due the slow process of breeding, the marginal retail values of the non-transgenic varieties, the increasing costs of nurseries and the fallible phenotypic screening procedures that ultimately led to the rapid adaptation of transgenic breeding, and other molecular breeding applications in cotton improvement (Rathore et al. 2008).

6.6.3 Interspecific Hybridization Through Embryo Rescue Methods

The early efforts of hybridization through embryo rescue methods were between *G. herbaceum* and *G. arboreum* cultivars with the wild *G. stocksii* and *G. anomalum* (Bajaj and Gill 1998b). In the interspecific crosses between these species, hybrids were obtained by preventing the degeneration of embryos by treating the flowers with growth regulators and then rescuing the immature embryos by tissue culture. The effect of growth regulators on boll retention, effective manipulation of tissue culture media for growing the rescued embryos and finally the successful regeneration of plants was achieved in all hybrid combinations (Bajaj and Gill 1998b). The hybrids were also utilized for backcrossing, and the dominant characters of the wild parents were seen to express in most cases. The early embryo abortion seen in hybrids was thus delayed by application of growth hormones, and traits were successfully transferred from wild species through a meticulous combination of tissue culture and conventional backcross genetics.

6.6.4 Molecular Breeding to Assist Introgression from Wild Species

The advent of molecular biology especially the techniques of plant DNA isolation, construction of gene libraries, and finally the revolutionary technique of polymerase chain reaction (PCR) paved the way to the path-breaking tools and strategies of molecular marker-assisted selection (MAS). MAS was successfully integrated into cotton plant breeding programs to select agronomically important traits such as fiber quality. To increase the reliability and effective utilization of MAS breeding, the first step of molecular breeders was to develop polymorphic molecular markers among the plant genomes.

The earliest DNA-based procedures for detecting polymorphism in plants were restriction fragment length polymorphism (RFLP) (Botstein et al. 1980) and random amplified polymorphic DNA (RAPD) (Williams et al. 1990). RFLP was used to study the origin of domesticated cotton at a molecular level (Brubaker and Wendel 1994) and also to develop a detailed molecular map of cotton to study the chromosome organization and evolution as it relates to a disomic polyploid genome (Reinisch et al. 1994). Cotton genome is relatively large, with an 1C content of 2,230 Mbp (Arumuganathan and Earle 1991) that makes it imperative to generate approximately 5,000 molecular markers to effectively understand and quantitatively interpret the cotton genome (Lacape et al. 2003). The requirement of radioactive Southern blotting and extensive use of restriction enzymes in RFLP made molecular breeders to look for cost-effective technologies to develop the enormous numbers of molecular markers to study the cotton genome. The discovery of PCR led to the development of the RAPD technique that uses short 10 bp universal oligonucleotide primers and the PCR-mediated amplification of random fragments from genomic DNA that proved more economical than RFLP in terms of developing molecular markers. The earliest studies reported in utilizing RAPD in cotton were to monitor the transfer of wild diploid cotton genes into *G. hirsutum* by

hybridization and backcrossing (Mergeai et al. 1998). RAPD markers specific to wild cotton species were quickly and efficiently generated and proved to be very effective in developing DNA fingerprinting of cotton species and interspecific hybrids. Mergeai et al. (1998) demonstrated that RAPD markers reliably differentiate between *G. hirsutum* varieties and are effective molecular tools to assess the introgression from several wild diploid species (*G. sturtianum, G. thurberi and G. raimondii*). RAPD also proved effective in detecting useful specific markers in remote genomes such as the C cytotype and were efficient in bulk segregant analysis method (Michelmore et al. 1991) to identify markers associated with glandless-seed and gland-plant trait of *G. sturtianum*. To limit the problems that may hinder proper analysis associated with minor changes in RAPD-amplification processes, Paran and Michelmore (1993) proposed the sequencing of the RAPD products and their conversion to normal PCR products by using longer primers. This led to the evolution of a novel technique based on sequence tagged sites (STS) called the sequence characterized amplified regions (SCAR) technology (Olson et al. 1989) that was effectively used in determining accurately the genetic relationships between BC genome cotton hybrids and their parents comprising cultivars of *G. hirsutum* and the wild species *G. sturtianum, G. thurberi* and the resulting trispecific hybrids G405 and G 376 (Mergeai et al. 1998). The clear and reliable detection of RAPD-mediated polymorphism among many upland cotton species, cultivars, and backcross populations made it a method of choice for introgressing agronomically useful traits from wild species to cultivated cotton.

Molecular marker technology threw open the doors to effective diagnostic technologies enabling more effective utilization of polyploidy and quantitative trait loci (QTL) analysis to study agronomically important morphological characters in *Gossypium* species (Jiang et al. 1998, 2000; Ulloa and Meredith 2000). To date, many other types of DNA markers such as amplified fragment length polymorphisms (AFLP) (Lacape et al. 2003), simple sequence repeats (SSR) and sequence-tagged sites (STS) (Zhang et al. 2002; Mei et al. 2004; Rong et al. 2004) have been developed for cotton research.

The demand for constructing saturated genetic maps resulted in the evolution of new sources of molecular markers and numerous novel SSRs or microsatellite markers have been developed in *Gossypium* (Connell et al. 1998; Reddy et al. 2001; Saha et al. 2003; Han et al. 2004). These markers were generated from cotton genomic or express sequence tag (EST) sequences and most have been used successfully in the construction of genetic maps and molecular tagging. The bioinformatic analysis of the ESTs generated from various cotton cDNA libraries would generate valuable information for functional classification of the *Gossypium* genus as very few cotton genes have been experimentally identified (Han et al. 2006).

A 10 days post-anthesis (dpa) wild-type cotton fiber represented as a tester and a fuzzless-lintless mutant as driver were used to isolate cDNA that was subjected to subtractive PCR, from which 280 independent cDNA fragments and 172 genes were significantly upregulated in elongating cotton fibers as confirmed by in situ hybridization in representative cases by cDNA macroarrays (Ji et al. 2003). In another study, Arpat et al. (2004) identified more than 2,500 stage-specific "expansion-associated" genes that are downregulated coincident with the termination of fiber elongation and 81 novel genes newly identified that are preferentially expressed during secondary cell wall synthesis from comparison of 10 versus 24 dpa fiber transcripts. Recently, Han et al. (2006) employed BLASTX to survey 489 SSR-containing cotton ESTs and mapped some important functional genes. The mapped genes together with other ESTs or genes mapped in various other studies comprehensively increases our understanding of the structure and function of the cotton genome that open novel technological avenues aiding in improved cotton production and quality. The development of EST-SSR mapping is thus one of the many effective ways to achieve a saturated genetic map of *Gossypium* to map genes or QTLs for cotton yield, fiber quality, and disease resistance, and for integrating physical and genetic maps (Han et al. 2006).

6.7 Cotton Genome: Worldwide Resources and Databases

A comprehensive worldwide collaborative effort by the cotton research community to decode the various *Gossypium* genomes will lay a vital foundation for

gaining a better understanding of the functional and agronomic significance of polyploidy and genome size variation within the *Gossypium* genus, and recent efforts have called for such initiatives (Chen et al. 2007).

A decade's worth of path-breaking research studies in cotton genomics has yielded a cotton molecular map comprising 3,347 loci detected by 2,669 probes (Rong et al. 2004). The cotton map has been linked to at least 295 QTLs controlling 26 traits related to various important agronomic characters such as plant growth, development, and morphology reproductive biology, fiber yield and quality disease resistance, and the preservation of productivity and quality under drought stress (Gingle et al. 2006). At least four high quality bacterial artificial chromosome (BAC) libraries exist, for *G. barbadense*, *G. raimondii*, and two strains of *G. hirsutum* (Tomkins et al. 2001).

BACs, ESTs, and genomic maps provide vital clues for molecular breeders and the scientific community in general for sequence analysis and assembly. At least a dozen genetic maps of crosses between diverse cotton species and genotypes are available, most made to map specific traits and QTLs. Some of these maps collectively include approximately 5,000 DNA markers (approximately 3,300 RFLPs, approximately 700 AFLPs, approximately 1,000 SSRs, and approximately 100 single nucleotide polymorphisms) (For details, see Gingle et al. 2006).

The avalanche of data stemming out of many such studies needs resources both at laboratories and the virtual worldwide web so that the cotton research community can take advantage and also keep track of the day-to-day changing scenario in cotton genomics research. One of the earliest attempts to foster such collaborative and resource-friendly developments occurred in 2001, when like-minded scientists representing many of the cotton-producing nations met in Montpellier, France, and developed an agenda to increase communication, limit redundancy, and foster accelerated progress toward characterizing the cotton genome for the benefit of the public sector. This major event evolved into the International Cotton Genome Initiative (http://algodon.tamu.edu/icgi/icgi.html) resulting in a new level of organization to the public efforts of mapping the cotton genome. Efforts are on to identify a single-community web site to develop a newsgroup list-server that will allow researchers to express and discuss their ideas about cotton genome sequencing and genomic research (Chen et al. 2007).

The collaborative movement brought forth renewed efforts worldwide when a coalition of cotton genome scientists developed a strategy for sequencing the cotton genomes, which will vastly expand opportunities for cotton research and improvement worldwide (Chen et al. 2007). As an illustrative example, "The Cotton Diversity Database" (http://cotton.agtec.uga.edu) evolved as a Web resource for *Gossypium* species' phenotypic and genomic data. A primary goal for this resource is to provide both a useful management tool for classical and molecular breeders and a research tool for geneticists and genomic researchers.

Looking at the enormous amount of data generated from various sequencing projects that will be extremely large and difficult to comprehend for many prospective end users, it was deemed essential to develop a data management system for an easier access and utilization of genomic and sequence data. More recent efforts by scientists from China, the United States, and France resulted in RecentTropGENE-DB (http://tropgenedb.cirad.fr/en/cotton.html) using molecular software called the CMap comparative map viewer (Nguyen et al. 2004). Databases such as the CottonDB (http://cottondb.org) and the Cotton Microsatellite Database (http://www.cottonmarker.org) that contains approximately 8,000 microsatellites (Blenda et al. 2006) adapted the same software. In addition, CottonDB provides genomic, genetic, and taxonomic information, including germplasm, markers, genetic and physical maps, trait studies, sequences, and bibliographic citations (Chen et al. 2007). The Cotton Portal (http://gossypium.info) is an example of a single port of entry to participating Cotton Web resources. A participating resource in this database called the Cotton Diversity Database (http://cotton.agtec.uga.edu; Gingle et al. 2006), is a vital interface providing information on field trials, phylogenetic, genetic, and comparative data, and is closely integrated with comparative physical, EST, and genomic (BAC) sequence data, expression profiling resources, and the capacity for additional integrative queries (Chen et al. 2007).

Microarray technology that recently developed as a molecular strategy of choice to explore genome-wide gene expression and function in a single platform

added a new dimension to cotton genomics research. Presently, cotton oligo-gene microarrays consisting of approximately 23,000 70-mer oligos designed from 250,000 ESTs can be found at the Web site http://cottonevolution.info/microarray (Chen et al. 2007).

The continued efforts to develop BAC libraries for several *G. hirsutum*, *G. barbadense*, *G. arboreum* as also *G. raimondii, G. longicalyx*, and an outgroup *Gossypioides kirkii* found its way into public domains on the internet through a Web finger-printed contig (FPC) site and an existing BACMan resource at the Plant Genome Mapping Laboratory (http://www.plantgenome.uga.edu) (Chen et al. 2007).

In an effort to understand the *Gossypium* genome across species, the whole-genome shotgun sequence of the smallest *Gossypium* genome, *G. raimondii* (approximately 880 Mbp) was carried out to gather fundamental information about gene content and organization. This research was under the auspices of the US Department of Energy Joint Genome Institutes (http://www.jgi.doe.gov/). The partial or complete sequencing of *G. raimondii* genome is expected to establish the critical initial template for characterizing the spectrum of diversity among the eight *Gossypium* genome types and three polyploid clades (Wendel and Cronn 2002).

Cotton researchers can take a leaf out of the book of model community database of their fellow researchers who developed the *Arabidopsis* Information Resource (http://www.arabidopsis.org/). Vital lessons in terms of hosting and managing information resources using a community accepted genome annotation, nomenclature, and gene ontology can be modeled on this data portal to develop a one-stop cotton sequencing and genomics database (Chen et al. 2007).

6.8 Recommendations for Future Actions

6.8.1 Educating the General Public

As research in biotechnology slowly advanced from the baby steps taken in 1980s to commercial applications in the twenty-first century, various misinterpretations and unwarranted media propagation of half-baked information led to a general fear among the elite and the general public that translated into a backlash against plant biotechnology research. As much as it is important to regulate biotechnology research, it is also vital for the various research institutions, industries and federal agencies to develop educational portals for the layman to simplify the biotechnological aspects among the general public to make them better informed of the technology. Coordinated efforts by the US government in the 1980s ensured the development of a framework to regulate agricultural biotechnology so that the general public was ensured of the fact that genetically modified products are safe to consume. Various federal agencies like United States Department of Agriculture (USDA), Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) are in place to regulate the research (Rathore et al. 2008), but these need to design effective strategies to educate the general public about the safety, applicability and larger benefits of plant biotechnology.

6.8.2 Better Exploitation of the Wealth of Wild Germplasm in Cotton

Gossypium genus boasts of an enormous and diverse variety of wild germplasm with beneficial agronomic traits that are yet to be fully exploited by successful introgression into the cultivated varieties. Since genetically modified (GM) cotton has been one of the success stories in terms of wider acceptance by the farming community, it would be an easy effort to continue the trend to harness the recent biotechnological and molecular research efforts to realize the goal of utilizing the wild germplasm for cotton improvement. Cantrell (2005) proposed a "Rational Fiber Design" approach, where plant biotechnology is integrated with textile chemistry to create novel value-added fibers for future applications traversing diverse agronomic traits. As cotton researchers put forth newer information in terms of novel genes, a better understanding of cotton genome evolution and saturated genetic and molecular maps, it is up to the cotton scientific community to collaborate and design effective strategies to exploit the wealth of wild germplasm to improve cultivated cotton.

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Chapter 7
Ipomoea

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7.1 Introduction

7.1.1 Origin and Distribution

The genus *Ipomoea* (family Convolvulaceae) contains 600–700 species including cultivated sweetpotato [*Ipomea batatas* (L.) Lam.]. Over half of them are concentrated in the Americas, where there may be 400 taxa, classified within the subgenera *Eriospermum*, *Quamoclit*, and *Ipomoea*. These three subgenera contain ten sections; seven of them are mainly confined to the northern and southern America, where they are distributed as cultigens, medicinal plants, and weeds (Austin and Huaman 1996). Many *Ipomoea* species have considerable importance either as medicinal plants or as ornamental plants. Because of new discovery of more species and increased information on morphology, the series has been revised several times (Austin 1978, 1988, 1991; McDonald and Austin 1990; Austin et al. 1993) and currently the series *Batatas* contains, in addition to *I. batatas*, 13 wild species classified as closely related to sweetpotato (Table 7.1). All of these species except *I. littoralis* are endemic to the Americas. Two are considered to be of hybrid origin. *Ipomea leucantha* has been determined to be a cross derivative of *I. cordatotriloba* × *I. lacunosa* hybrids, and *I. grandifolia* is known to be a hybrid derivative of *I. cordatotriloba* × *I. batatas* (Austin 1978). Cytological investigations have shown that there are three different ploidy levels within the series. The cultivated sweetpotato is a hexaploid with $2n = 90$, *Ipomea tiliacea* and *Ipomea tabascana* are tetraploids with ploidy level of $2n = 60$, and the rest of the species are diploids containing $2n = 30$.

Sweetpotato was known to be originally domesticated in the New World (Austin 1988; Zhang et al. 2004) and further spread between latitude 42°N and 35°S, from sea level up to an altitude of 3,000 m. The region between the Yucatán Peninsula of Mexico and Orinoco River in Venezuela is postulated as the center of diversity because of its rich morphological variation in cultivated sweetpotato collections as well as in four other species in the *Batatas* group (Austin 1988). Secondary centers of diversity are Peru and Ecuador. Archeological remains showed that the cultivated sweetpotato existed in Peru and Mexico during 2500–2000 BC (Austin 1988). In addition, carbon-dated (wild) specimens found in Peru were dated back to 10000–8000 BC (Source: Natural History Museum). The cultivated sweetpotato is known to be dispersed from the Americas in two waves. The earliest migration was to Polynesia, although it is not known when and how (Austin 1988). The second movement was by Columbus, who first introduced sweetpotato to Europe during 1492, then to Africa in 1500. In the sixteenth century, Portuguese explorers introduced sweetpotato to Africa, India, southeastern Asia, and the East Indies. Later on, Spanish trading galleons introduced to Philippines (Austin 1988). Studies on assessment of genetic diversity by using amplified fragment length polymorphism (AFLP) markers traced pre-historic introduction to Oceania from Mesoamerica (Rossel et al. 2000).

Nearly 26,000 accessions of *Ipomoea* spp. are available in 83 gene banks around the world (Rao et al. 1994). Out of these, about 8,000 accessions belong to the cultivated sweetpotato (Takagi 1988).

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Table 7.1 Ploidy, genome type, and self-compatibility of *Ipomoea* species from series *Batatas*

Ipomoea species	2*n* (*x* = 15)[a]	Genome type[b]	Self-compatibility
I. triloba	2*x* = 30	A	C
I. cordatotriloba (previously *I. trichocarpa*)	2*x*, 4*x*	A	C
I. cynanchifolia	2*x* = 30	A	C
I. lacunosa	2*x* = 30	A	C
I. × *leucantha*	2*x* = 30	A	C
I. umbraticola	2*x* = 30	A	C
I. ramosissima	2*x* = 30	A	C
I. tenuissima	2*x* = 30	A	C
I. grandifolia	2*x* = 30	A	C
I. littoralis	2*x* = 30	B	I
I. tiliacea	4*x* = 60	X	I
I. tabascana	4*x* = 60	B	C
I. trifida	2*x*, 3*x*, 4*x*, 6*x*	B	I
I. batatas	6*x* = 90	B	I
I. gracilis (outgroup)	4*x* = 60	X	I

C Self-compatible, *I* self-incompatible (Nishiyama et al. 1975)
[a]Chromosome number from Jones (1974), Austin (1988) and Jarret et al. (1992)
[b]Genome type from Jarret et al. (1992)

A comprehensive analysis of genetic variation and distribution pattern is essential for sound conservation strategies including sampling of extant genetic resources in germplasm collections, identification of duplicates, selection for core collection, and future explorations. About 200 accessions of sweetpotato are maintained in China consisting of Chinese landraces, new introductions, breeding lines, and cultivars (Wang et al. 1998). The sweetpotato genebank held at CIP, Peru, maintains a total of 5,526 cultivated accessions from 57 countries, of which 2,589 are from Latin America (Huamán and Zhang 1997).

7.1.2 Economic Importance

Sweetpotato is the seventh largest food crop with about 122 Mt produced annually worldwide (FAOSTAT 2006) and the fifth nutritional contributor to human diets in the developing countries (Plucknett 1991). It produces stable crop yields under a wide range of environmental conditions and one of the staple diets in many countries. Over 95% of the global sweetpotato crop is produced in the developing countries, where it is the fifth most important crop on fresh weight basis after rice, wheat, maize, and cassava. Asia is the world's largest in sweetpotato production, with China alone producing 90% of total world production. The nutrient composition of the storage root starch is up to 33.3% (on fresh matter basis) and storage root sugar is as low as 0.6% (on fresh matter basis). Additionally, storage root contains β-carotene, calcium, magnesium, iron, and zinc upto 154, 4,091, 1,815, 10, and 6.3 ppm, respectively. This corresponds to 15.4-mg β-carotene, 409-mg calcium, 181-mg magnesium, 1-mg iron, and 0.6-mg zinc in 100 fresh storage roots (CIP 2009).

7.2 Phylogenetic Relationships Among *Ipomoea* Species in *Batatas* Section

In spite of importance, information pertaining to evolution and phylogeny of cultivated sweetpotato is still obscure. Several hypotheses have been put forward to explain the origin of sweetpotato. Sweetpotato was thought to be originated from the diploid *I. leucantha* Jacq., and further by polyploidization into a tetraploid species, *I. littoralis* Blume (Nishiyama 1971). The subsequent hybridization between these two species might have generated triploid *Ipomeoa trifida* (H.B.K.) Don., which finally settled to hexaploid by chromosome doubling. Another hypothesis for the origin of sweetpotato may be through hybridization between *I. trifida* and *I. triloba*, resulting in wild ancestor of *I. batatas* (Austin 1988). On the basis of

morphology, ecology, cytology, and cluster analyses, *I. trifida* and *I. triloba* were considered being the closest extant relatives of the cultivated sweetpotato, which had supposedly arisen through an allopolyploid (Austin 1988). In a third hypothesis, the autopolyploidy of *I. trifida* was discussed as an alternative pathway of origin (Shiotani 1987), and particularly the occurrence of 2*n* gametes (diplogametes) might have been involved in the origin of the polyploidy series, and allowed sexual interconnection among different ploidy levels. In fact, the production of 2*n* pollen was reported in diploid *I. trifida* and tetraploid *I. batatas* (Orejda et al. 1990; Freyre et al. 1991). Therefore, a mechanism of sexual polyploidization through fertilization by non-reduced gametes is a strong possibility of gene flow among the species with different ploidy levels. In summary, interspecific hybridization involving the species, *I. trifida* (2*x*, 4*x*, 6*x*), *I. leucantha* (2*x*), and *I. littoralis* (4*x*), and subsequent domestication gave rise to the cultivated form of *I. batatas* (Nishiyama 1982).

Many studies were performed using molecular genetic markers to resolve the phylogenetic relationships of sweetpotato and its related species. The use of restriction fragment length polymorphism (RFLP, Jarret et al. 1992), random amplified polymorphic DNA (RAPD, Jarret and Austin 1994), and microsatellites or simple sequence repeat (SSR, Buteller et al. 1999; P. Nimmakayala et al. unpublished) markers revealed the close relationship between *I. trifida* and *I. batatas*. A total of 220 microsatellites (Nimmakayala et al. Unpublished results) were used to understand the phylogenetic relationship of individual species (Fig. 7.1) in the *Batatas* complex [*I. batatas* (6*x*), *I. tabascana* (4*x*), *I. tiliacea* (2*x*), *I. trifida* (4*x*), *I. triloba* (2*x*), and *I. leucantha* (2*x*)]. The number of alleles as amplified by various species is presented in Fig. 7.2. The number of alleles amplified in diploids, tetraploids, and hexaploids was comparable based on the knowledge of their ploidy levels. This result indicated that the polyploidization in *Batatas* complex followed massive genome reorganization and rearrangements through processes such as gaining or losing the alleles and genomic fragments. Principal component analysis (PCA) was carried out and the results indicated distinct clustering pattern of various species (Fig. 7.3). The construction of genetic relationships using unweighted pair group method with arithmetic mean (UPGMA) and PCA demonstrated further use of SSRs in sweetpotato genotypic identification and classification of genetic relationships.

The recent cpDNA data indicated that *I. trifida* is the most likely one of the diploid progenitors of hexaploid *I. batatas* (McDonald and Mabry 1992). On the basis of the available information on β-amylase, a fairly conserved nuclear gene, Rajapakse et al. (2004) cloned and sequenced β-amylase gene to study phylogenetic relationships on several species. In this study, *I. tabascana*, a tetraploid species with B genome is sharing close relationship with *I. trifida*. *I. tabascana*, a species discovered by McDonald and Austin (1990), was shown also to be closely related to sweetpotato. This study classified *I. tiliacea* as one of the ancestral species, and the species *I. triloba* as not related to the cultivated sweetpotato as previously thought. Putting together various possible conclusions based on investigations using genomic and organelle DNA, most closely related species of sweetpotato are *I. trifida* (2*x*) and *I. tabascana* (4*x*). In addition, fluorescence in situ hybridization (FISH) data indicated that polyploidization was followed by decrease in the number of 18S rDNA loci in higher ploidy level and provided evidence for major genomic rearrangements and/or diploidization of polyploid *I. batatas*. On the basis of chromosome morphology, tetraploid *I. trifida* appeared to be more closely related to sweetpotato than *I. tabascana* (Srisuwan et al. 2006).

7.3 Cross-Compatibility Relationships among *Ipomoea* Species

The *Ipomoea* species in the *Batatas* complex were divided into three subgroups (A, B, and X) based on their selfing abilities, interspecific crossing capabilities as well as morphological and cytogenetic characteristics (Ting et al. 1957; Jones 1965; Jones and Deonier 1965; Martin and Jones 1973; Nishiyama et al. 1975; Oracion et al. 1990). The A group includes *I. triloba* and three closely related species, viz., *I. lacunosa, I. trichocarpa*, and *I. ramoni*. The species within the A group are self-compatible and cross-compatible with each other (Jones and Deonier 1965). The B group comprises the species *I. batatas, I. littoralis*, and *Ipomea trifida*, and these species are self-incompatible but cross-compatible with each

Fig. 7.1 *Ipomoea* species grown in the green house (WVSU). (**a**) *I. batatas* (6*x*); (**b**) *I. tabascana* (4*x*); (**c**) *I. tiliacea* (2*x*); (**d**) *I. trifida* (4*x*); (**e**) *I. triloba* (2*x*); (**f**) *I. leucantha* (2*x*)

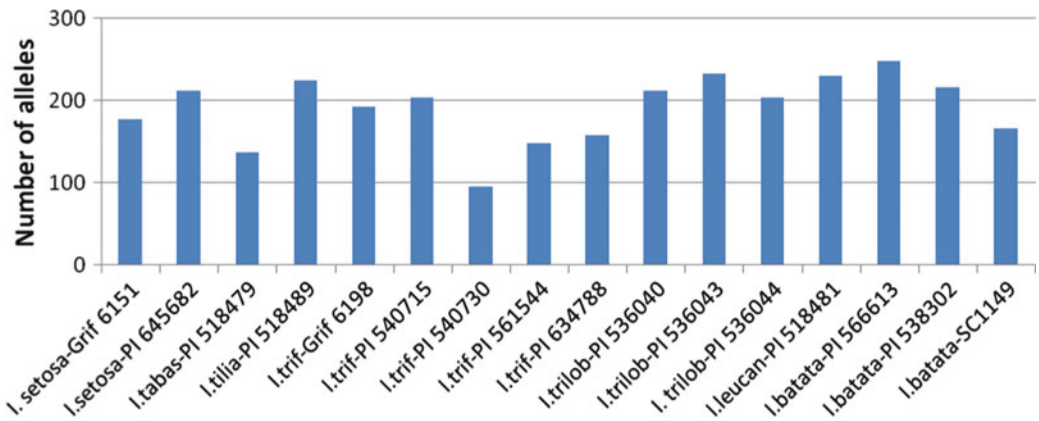
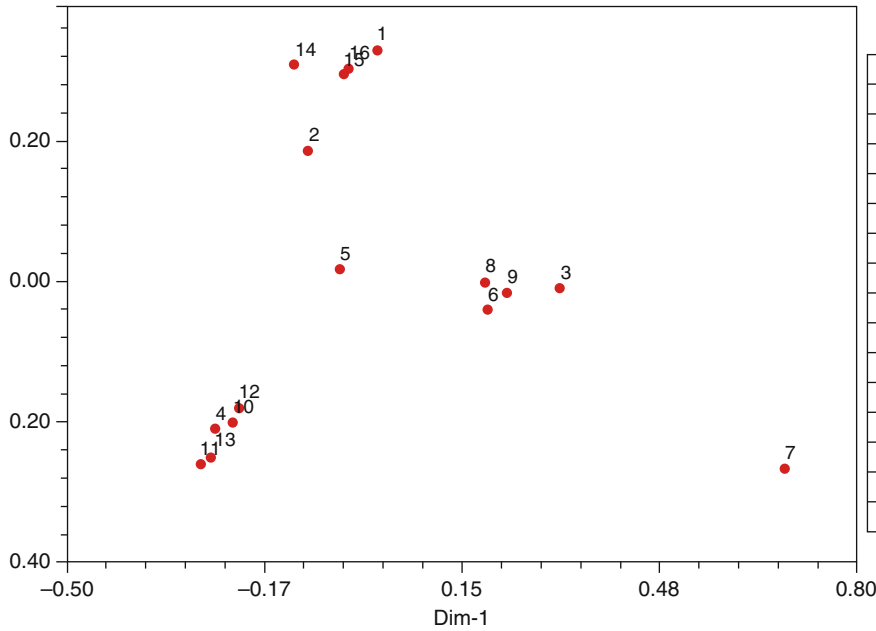


Fig. 7.2 Number of alleles amplified using 220 *I. Batatas* specific SSRs in *Batatas* complex



W1	Ipomoea setosa
W2	Ipomoea setosa
W3	Ipomoea tabascana
W4	Ipomoea tiliacea
W5	Ipomoea trifida
W6	Ipomoea trifida
W7	Ipomoea trifida
W8	Ipomoea trifida
W9	Ipomoea trifida
W10	Ipomoea triloba
W11	Ipomoea triloba
W12	Ipomoea triloba
W13	Ipomoea leucantha
SP-2	Ipomoea batatas
SP-3	Ipomoea batatas
SP-4	Ipomoea batatas

Fig. 7.3 Principal component analysis of *Batatas* complex using microsatellite amplifications

other. The X group includes two well-known tetraploids, *I. tiliacea* (4*x*) and *I. gracilis* (4*x*), which are self-incompatible but cross-compatible (Nishiyama et al. 1975). The B group is cross-incompatible with the A and X group, but the A group is cross-compatible with the X group when the latter is used as the pollen parent. Genome types of *Ipomoea* species as currently classified as presented in Table 7.1. Former Phylogenetic studies of the group based on morphological traits (Austin 1988) indicated that cultivated races of *I. batatas* (6*x*) share close relations with *I. trifida* (HBK) G. Don (2*x*) and *I. triloba* L. (2*x*). However, the study of morphology in the series is difficult due to the overlapping of traits between species, interspecific hybridization, and homoplasy. Austin (1988) reported that, in the literature and in germplasm collections, *I. trifida* is often confused with sweetpotato and that the two species are closely related. Tetraploid samples previously identified as *I. trifida* were later classified as *I. batatas*, based on flower morphology (Bohac et al. 1993).

Utilization of wild germplasm in sweetpotato breeding began in the year 1956 in Japan immediately after introduction of the wild hexaploid plants ($2n = 90$), designated K123 from Mexico (Nishiyama and Teramura 1962; Nishiyama 1971). Subsequently, K123-derived lines had attracted major attention across the world mainly because of their high starch content and resistance to two major nematodes. Besides K123, many wild species related to sweetpotato have been introduced from Mexico, Guatemala, Colombia, Ecuador, and the United States. There are many excellent disease- and pest-resistant traits in these species that can be used to improve sweetpotato (Iwanaga 1988; Komaki 2004; Zhang and Liu 2005). Therefore, many researchers tried to make wide cross to obtain interspecific hybrids by controlled pollinations or somatic cell culture techniques.

However, interspecific hybrids between sweetpotato and its related species have been scarce, mainly due to the genome differentiation and interspecific incompatibility (Martin 1970, 1982; Teramura 1979; Shiotani et al. 1990; Lu and Li 1992). A few B-genome species have been used to improve sweetpotato quality and disease resistance (Hozyo and Kato 1973; Iwanaga 1988; Iwanaga et al. 1991). Species in the A-genome group may also be sources of genes for sweetpotato improvement. A-genome species such as *I. triloba,* which can be found in both very dry and extremely wet habitats (Martin and Jones 1973), may be a potential source of drought tolerance and resistance to root rots and foliar fungal diseases. So far, evaluation and interest in species belonging to the A-genome group has been limited due to

cross-incompatibilities, which have restricted their use in sweetpotato breeding (Wedderburn 1967). A-genome species such as *L. triloba,* which can be found in both very dry and extremely wet habitats (Martin and Jones 1973), may be a potential source of drought tolerance and resistance to root rots and foliar fungal diseases. Ting et al. (1957) performed more than 3,000 interspecific crosses between the sweetpotato plant and its 24 related wild species of the genus *Ipomoea*, yet not a single viable F_1 hybrid was obtained. Wedderburn (1967) made attempts at hybridization between *I. batatas* and *I. trichocarpa* or *I. gracilis*. Only the initiation of embryo development occurred when *I. trichocarpa* as female parent was crossed with *I. batatas* as male parent, and the seeds produced by this hybridization were not viable.

7.4 Description of Wild Species of Sweetpotato

Wild species of sweetpotato are propagated by seeds. No tuber formation has been found in the species of A and B groups of classification by Nishiyama et al. (1975). The major problem in seed germination tests is the presence of the seed coat, which can delay or prevent imbibition, that is, hardseededness is a common problem. The seeds are non-endospermic with axile foliar embryos within hard seed coats (Steinbauer 1937). Consequently, scarification and chipping treatments are useful in promoting germination. The scarification experiments with exposure of seeds to sulfuric acid for 30 min, followed by washing in running water, gave 100% germination of seeds across different species. Six species of *Ipomoea* are characterized as per the descriptors for sweetpotato (Huamán 1991) as described below.

- *I. batatas* ($2n = 4x = 90$): Cultivated sweetpotato with deep purple flowers (Fig. 7.1a).
- *I. tabascana* ($2n = 4x = 60$): Greenish purple vines. The leaves are lobed with three deep lobes, sparse pubescence and linear shaped. Flowers are pentagonal and purple colored with obovate and obtuse sepals. Flowering is very scarce (Fig. 7.1b).
- *I. tiliacea* ($2n = 2x = 30$): Spreading plant type with deep purple vines. The leaves are glabrous triangular in shape with no lobes with scarce flowering (Fig. 7.1c).
- *I. trifida* ($2n = 4x = 60$): A tetraploid species, very twining, spreading with dark purple leaves with the green tips. Leaves are cordite with sparse tip pubescence. The flowering is limited (Fig. 7.1d).
- *I. triloba* ($2n = 2x = 30$): Spreading type with dark purple spots. The leaves are with sparse hairs and no lobes. The leaf petiole pigmentation was greenish purple. The flowering is scarce (Fig. 7.1e).
- *I. leucantha* ($2n = 2x = 30$): Mostly twining, spreading type with purple colored vines. The leaves are triangular in shape with no pubescence. The leaves were with slightly lobed with purple pigmentation on abaxial surface. The flowering is very scarce (Fig. 7.1f).

7.4.1 Confirmation of Ploidy Level

Ploidy level is determined directly by somatic chromosome counts (Lower and Johnson 1969; Karp et al. 1982). Unfortunately, chromosome counts in sweetpotato are difficult, due to the small size of chromosomes and special difficulties associated with chromosome staining and metaphase arrest (Jones et al. 1986). Ploidy level in sweetpotato, however, can also be indirectly determined by flow cytometry and counting chloroplasts in the guard cells. Among the different methods, flow cytometry is the most powerful technique for measuring the DNA content. It is an expensive, but quick and reliable tool to accurately determine the ploidy levels. We determined ploidy levels of various species in *Batatas* complex using flow cytometry and counting chloroplasts in guard cells (UK Reddy and coworkers personal communication).

7.4.1.1 Chloroplast Count

Chloroplast number per guard cell pair was counted using the methodology developed by Compton et al. (1996). Using fine forceps, the lower epidermis was removed from fully expanded leaves of seedlings and transferred to a microscopic slide in a drop of water covered by a cover slip, and chloroplasts per guard cell pair were counted under 400× magnification. Chloroplasts were counted in ten guard cell pairs per leaf and totally three different leaves per plant were used

for counting. A total of five different diploid, tetraploid, and hexaploid accessions were included in the study to minimize experimental error. The mean number and standard error of chloroplasts were calculated for each accession in various species of the *Batatas* complex (Fig. 7.4). Chloroplast counts across various ploidy levels of different species of the *Batatas* complex indicated that the diploids had chloroplasts in the range of 9–12 per guard cell pair, and the tetraploid range was 14–22 per guard cell pair. This observation is quite logical as the number of chloroplasts per guard cell pair in the tetraploids is expected to be more than the diploids. In contrast, the expected chloroplast number is approximately 2.5× in the hexaploids than the diploids. Intriguingly, in the current study, Nimmakayala and colleagues (unpublished) observed chloroplasts per guard cell pair in the cultivated hexaploid species to be in the range of 10–14, which is similar to the chloroplast number of diploid species.

7.4.1.2 Flow cytometry

The ploidy level of various *Ipomoea* species was determined by flow cytometry using Ploidy Analyzer PA I (Partec, Germany). The Ploidy Analyzer determines ploidy level by the precise measurement of total DNA content of the individual nuclei. It can measure thousands of individual nuclei with a single pass. The Partec flow cytometer uses the DAPI (4, 6-diamidino-2-phenylindole)-based staining solution, Cystain UV for staining the DNA of individual nuclei. The UV excitation of Cystain is provided by a mercury arc lamp and the blue emission of DAPI is collected by filter. The Ploidy Analyzer displays the result as a DNA histogram at different channel representing different ploidy levels in real time on a large LCD screen. The machine was calibrated using a machine standard, Partec DNA control UV (trout red blood cells) and the nuclei isolated from leaf tissues of diploid species served as an internal standard. The sample was prepared following the standard protocol supplied by the manufacturer (http://www.partec.de). The ploidy levels of various species in *Batatas* complex were determined by flow cytometry. The results of various ploidy levels are presented in Fig. 7.5. The results obtained from five biological replications of diploids, tetraploids, and hexaploids were analyzed. Interestingly, the peak, which indicates the genome content, is at the same position in all the samples of diploids and tetraploids (Fig. 7.5). In this experiment, when various samples of hexaploids were analyzed, a peak was noted at a different position than the diploids as expected to be in the hexaploids. The position of the tetraploid peak at the diploid position is not typical.

Out of the 13 species of *Ipomoea* section *Batatas*, *I. trifida* (HBK) Don (6*x*, K123), *I. littoralis* Blume (4*x*, K233), and *I. leucantha* Jacq (2*x*, K221) could

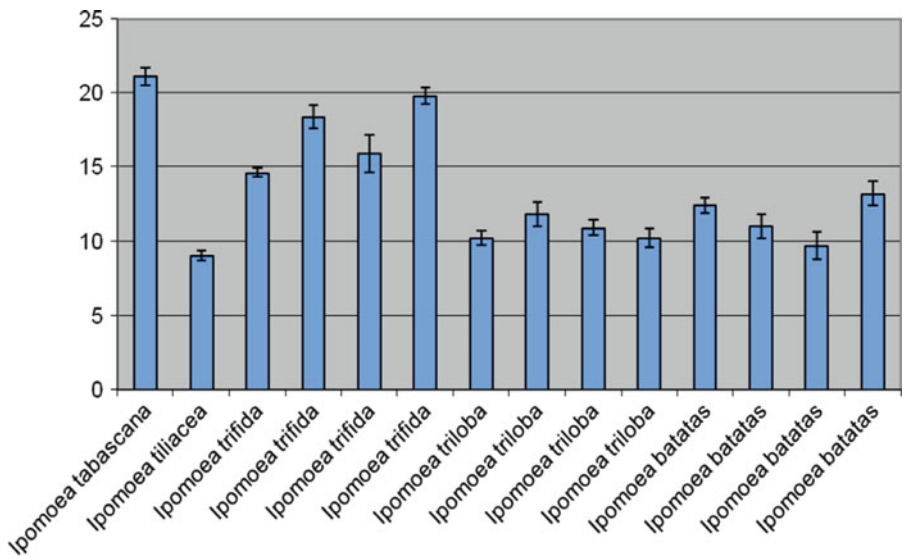


Fig. 7.4 Number of chloroplasts of ten guard cell pairs (Mean and standard error) across the *Batatas* complex

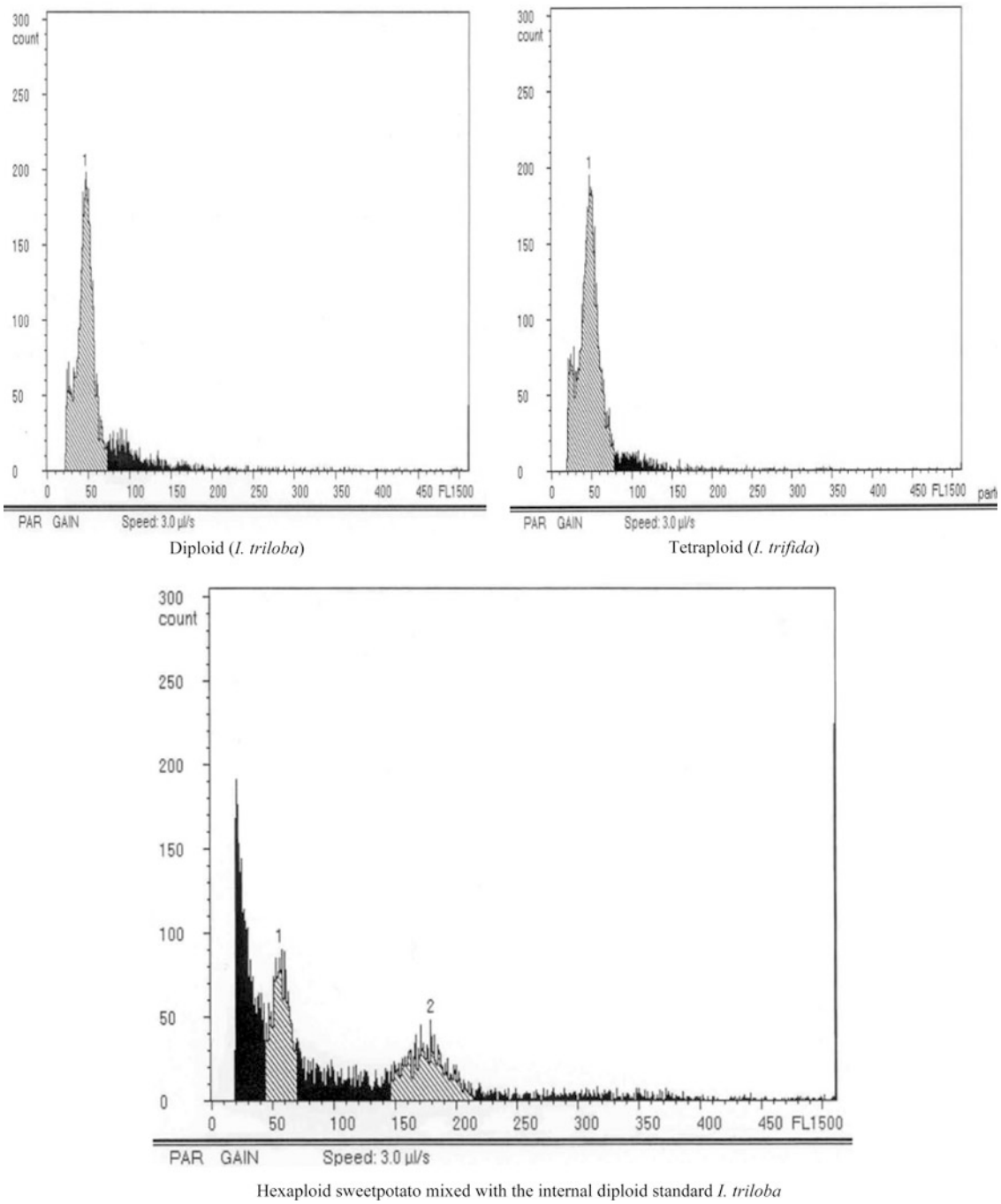


Fig. 7.5 *Histogram* showing *peaks* generated by flow cytometry of diploid, tetraploid, and cultivated hexaploid sweetpotato

be crossed with sweetpotato (Nishiyama et al. 1975; Shiotani and Kawase 1987); however, no promising breeding lines could be selected. Kobayashi et al. (1994) obtained two interspecific hybridization combinations from *I. triloba* × *I. trifida*, and *(I. triloba* × *I. lacunosa)* × *I. batatas* (4*x*) by ovule culture methods. The hybridization between sweetpotato and diploid *I. trifida* showed very low crossing rate

(Komaki 2004). Somatic cell hybridization was also exploited to produce hybrids but only a few combinations were reported to be successful. For those hybrids obtained, wild parents were among the following species: *I. triloba* (Liu et al. 1994), *I. lacunose* (Zhang et al. 2002), and *I. cairica* (Guo et al. 2006). Nevertheless, these somatic cell hybrid plants varied substantially in their chromosome numbers, were morphologically leaned to their wild parent, and a few could set storage roots. By applying plant growth hormones to stimulate pollination, Cao et al. (2009) produced two novel F_1 interspecific hybrids between *I. batatas* (L.) Lam. ($2n = 6x = 90$) and two wild species *I. grandifolia* ($2n = 2x = 30$) and *I. purpurea* ($2n = 2x = 30$). *I. purpurea* is highly resistant to sweetpotato stem nematode (SSN) caused by *Ditylenchus destructor* and sweetpotato virus (SPV), while *I. grandifolia* is resistant to SSN and moderately resistant to SPV. Both the hybrids yielded storage roots of intermediate size, much larger than that of their wild parents. These results demonstrated that it is quite possible to integrate outstanding characters from both sweetpotato cultivars and their wild relatives. The F_1 hybrids produced may be used as "bridge" role to transfer elite genes from wild germplasm to the cultivars.

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Chapter 8
Manihot

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8.1 Introduction

Global food demand has been projected to double by the year 2050 and research for increased agricultural production has been suggested as the key to feed the increasing world population. Cereal crops have been the major and preferred food sources across many nations; however, there is tremendous amount of pressure on these crops to meet the increasing demand which is in turn associated with changing climate, poor soil conditions, and increased disease and pest incidences. Root crops such as cassava currently fits as the best alternative to cereals as it has the ability to tolerate longer periods of drought and disease and insect pressure while producing economic yields for the poor farming communities. Owing to these attributes, cassava plays a very important role as a food security crop in Africa. It is believed that bringing marginal lands and poor soils under cassava cultivation and exploring the additional uses of cassava and its wild relatives can help meet global food demand. Undoubtedly, increased cassava production not only for human consumption and industrial use, but also as feed in poultry and livestock industry will ease the global pressure on cereal crops.

Cassava, *Manihot esculenta* Crantz, a starchy root crop, is one of the major staple of about 800 million people worldwide (Lebot 2009). It is cultivated between 30°N and 30°S of the equator, having a mean annual temperature greater than 18°C (Nassar and Ortiz 2007). It is a major source of carbohydrate in sub-Saharan Africa, ranking sixth among major food crops of the world after wheat, rice, maize, potato, and barley. Native to neotropics, it is mainly cultivated in tropical and subtropical regions in Africa, Asia, and Latin America. The world production of cassava root was 238.5 million tons in 2008 (FAO 2008) with majority of it being produced in Africa, with Nigeria being the world's largest producer (49 million tons in 2008). Worldwide cassava production has increased by 15% between 2005 and 2008 (FAO 2009). This increase is due to elevated prices of cereal and other staple food crops, improved cultivars, improved cassava root processing and productions techniques, alternative utilization of cassava as bio-energy (ethanol), and animal feed resource. These positive attributes made the farmers in cassava growing areas turn toward the cultivation of cassava thus increasing the area under cassava cultivation while utilizing the improved cultivars for production with government support (FAO 2008). Cassava ranked first in production in Nigeria followed by yam and sorghum (FAO 2010) (http://ndn.nigeriadailynews.com).

Cassava – *M. esculenta* Crantz – is classified under kingdom Plantae, subkingdom Tracheobionta, superdivision Spermatophyta, division Magnoliophyta, class Magnoliopsida, subclass Rosidae, order Euphorbiales, family Euphorbiaceae, and genus *Manihot*. Synonyms of this species are *Manihot utilissima* Phol., *Manihot aipi* Phol., *Jatropha manihot* L. and related wild species is *Manihot dulcis*. Common names of *M. esculenta* are "cassava," "yuca," "bitter cassava," "tapioca," "tapioca plant," "manioc," "Brazilian arrowroot," and "sweet potato tree." It has many vernacular names – *yucca*, *dang noi*, *man sum palung*, *pearks sakhoo*, *tapioca*, *manioca*, and *huacamote*. Cassava relatives in the Euphorbiaceae family include several commercially important plants, such as rubber

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trees (*Hevea brasiliensis*), castor oil plants (*Ricinus communis*), and ornamental plants (*Euphorbia* spp.).

There are 12 species in genus *Manihot* viz., *M. angustiloba* (Torr.) Müll. Arg. (desertmountain *Manihot*), *M. caerulescens* Pohl (manicoba), *M. catingae* Ule (manicoba brava), *M. davisiae* Croizat (Arizona *Manihot*), *M. dichotoma* Ule (manicoba rubber), *M. esculenta* Crantz (cassava), *M. glaziovii* Müll. Arg. (ceara rubbertree), *M. grahamii* Hook. (Graham's *Manihot*), *M. subspicata* D.J. Rogers & Appan (spiked *Manihot*), *M. tristis* Müll. Arg. (*Manihot*), *M. tristis* Müll. Arg. ssp. *saxicola* (Lanj.) D.J. Rogers & Appan (*Manihot*), and *M. walkerae* Croizat (Walker's *Manihot*) reported in USDA plant data base (http://plants.usda.gov). These wild species provide a rich source of genes for cassava improvement in the breeding programs.

The wild *Manihot* species are gaining importance due to the presence of potential interspecific variations with useful genes for morphological and physiological traits as well as biotic and abiotic stresses (Allem 1984). The useful gene for low cyanide content was observed in *M. pringlei,* resistance to African cassava mosaic virus from *M. glaziovii*, resistance to cassava bacterial blight from *M. pseduglaziovii* and *M. reptans*, high starch content from *M. tristis* and *M. augustiloba*. Genetic resistance to pests was found in *M. glaziovi* and *M. dichotoma* (mealy bug); *M. neusana*, *M. pohlii,* and *M. grahami* (Stem borer). The abiotic stress-tolerant genes were present in *M. chlorostica* for salinity, *M. pseudoglaziovi*, *M. carthaginesis,* and *M. dichotoma* for drought while *M. attenuata* and *M. rubricaulis* for cold temperatures. The outstanding contribution for tolerance to aluminum toxicity, acid soils, and low phosphorous levels is from *M. irwinii, M. tripartita,* and *M. orbicularis*. In Africa, using wild crop relatives through classical breeding, a variety of elite genotypes with improved starch, dry matter, resistance to biotic, and abiotic stress resistance/tolerance as well as with reduced cyanogenic level have been already developed (Hahn 1989). About 98 species have been reported for the genus *Manihot* with cassava (*M. esculenta* ssp. *esculenta*) as the only cultivated staple food crop in the tropics (Rogers and Appan 1973).

In addition to human consumption, cassava also has many industrial uses and cultivated cassava is the cheapest known source of starch, producing more than 300 industrial products (Tonukari 2004). Of these, one promising application is fermentation of the starch to produce ethanol used in biofuel (Nguyen and Gheewala 2008). Despite its growing demand and production potential, it has received less attention from the researchers compared to other globally important crops and is still considered as an "orphan crop." It is grown mainly by small-scale farmers in areas that have little or no access to improved varieties, fertilizer and other production inputs and are often cut off from the marketing channels and agro-processing industries. This chapter presents a brief overview of the origin, evolution, and botany of *Manihot* species; collection and conservation strategies followed to protect landraces from extinction; germplasm characterization using morphological, cytological, biochemical, and molecular markers; limitations of present strategies; and future directions.

8.2 Basic Botany of the Genus

8.2.1 Origin, Geographical Location, and Distribution

The evolutionary origin of cassava (*M. esculenta* ssp. *esculenta*) is still under debate even though several researchers put forth different hypothesis on this topic. Most hypotheses were made around three important questions that probably could determine the actual botanical origin of cassava, such as, the wild species from which it descends; the geographical origin (the area where the progenitor existed); and the agricultural origin (the area of original cultivation of wild ancestor) (Allem 2002). The available knowledge show that there is enough evidence already available on botanical origin of this crop and evidence on geographical origin is in conjecture with the areas where cultivation has begun. The traditional hypotheses suggest that cassava is a "compilo-species," i.e., the result of hybridization events among several species including *Manihot aesculifolia*, a species endemic to Central America (Olsen and Schaal 2001; Olsen 2004), which was considered as the probable center of origin for cultivated cassava. On the other hand, it was reported that all the wild taxa of cassava have a broad ecological range from southwestern Amazonia to the Savannas of the Guianas (Allem 2002).

Allem (1994) proposed that the modern cultivated cassava, *M. esculenta* ssp. *esculenta*, originated from two primitive forms having three subspecies. These are *M. esculenta* ssp. *esculenta* (in which all known cultivars and landraces are included), and two wild types such as *M. esculenta* ssp. *peruviana* (occurring in eastern Peru and western Brazil) and *M. esculenta* ssp. *flabellifolia* (with a distribution from Goiás in Brazil to Venezuelan Amazonia). Allem (1999) considered *M. esculenta* ssp. *flabellifolia* as the wild ancestor of cultivated cassava and included it in the primary gene pool GP-1 together with *M. peruviana* (Allem et al. 2001). This close relationship has since been supported by studies of Roa et al. (1997, 2000) using amplified fragment length polymorphism (AFLP) markers to elucidate the genetic relationships. A detailed molecular analysis based on the single-copy nuclear gene encoding glyceraldehyde 3-phosphate dehydrogenase (Olsen and Schaal 1999) indicated that cassava was domesticated specifically from populations of *M. esculenta* ssp. *flabellifolia* occurring along the southern rim of the Amazon basin in the Brazilian states of Acre, Rondônia, and Mato Grosso, and likely extending south into similar conditions in Bolivia. The premise of a southern Amazonian domestication has been further supported by subsequent studies, which consistently showed that genetic variation in cassava is almost entirely a subset of the genetic variation occurring in the wild *M. esculenta* populations from this geographical region (Olsen and Schaal 2001; Léotard and McKey 2004; Olsen 2004). Duputie et al. (2007) further demonstrated, based on molecular studies, that domesticated cassava probably has a single progenitor rather than a pool of hybridizing unidentifiable wild species. They also confirmed that crop has undergone hybridization in nature with the only probable wild relative *M. esculenta* ssp. *flabellifolia* at several places in French Guiana with a single domestication center, rather than multiple sites throughout the neotropics, and hybrids (F_1, F_2 and probable backcrosses) are all fertile.

Different centers of origin have been reported for the genus *Manihot*. Vavilov (1951) assumed that the center of diversity for cassava is in the Brazilian–Bolivian region, which probably is the origin of this crop. However, Harlan (1961) proposed more than one center of diversity for all cultivated species and he also suggested that all centers of diversity may not represent centers of origin of a particular cultivated species. This also applies in case of cassava and its wild progenitors. Therefore, four centers of diversity have been identified for *Manihot* species (Nassar 1978a) such as Mexico, Northeast and Central Brazil, Southwest Brazil and Bolivia following the model described by Dobzhansky (1973) for species formation in Iris, Eucalyptus, Liatris, Penstemon, and Tragopogon. Microcenters of diversity also exist for these species, which probably arose from frequent hybridization between species and heterogenic topography of their habitats (Nassar 2003). About 80 species are considered to have originated from two centers of diversity in Brazil, one being central Brazil and the other as Northeast Brazil (Olsen and Schaal 1999, 2001; Olsen 2004). Another 17 species have been found in Mexico and Central America (Rogers and Appan 1973; Nassar 2001). Sometimes Africa has been also considered as an additional center of diversity for cultivated cassava (Gulick et al. 1983; Pickersgill 1998). Table 8.1 lists the wild species of *Manihot* collected from different localities of northeastern Brazil. It is apparent that

Table 8.1 Wild species of *Manihot* collected from different localities in northeastern Brazil

Species	Locality
M. caerulescens Pohl	Aparipina, PE
M. heptaphylla Ule	Seabra, BA
M. cichotoma Ule	Jequié, BA
M. catingae Ule	Itaberaba, BA
M. brachyandra Pax et Hoffmann	Petrolina, PE
M. maracasensis Ule	Itambé, BA
M. epruinosa Pax et Hoffmann	Bentecoste, Fortaleza, CE
M. glaziovii Mueller	Arcoverde, Ouricure, Serratalada, PE
M. quinquefolia Pohl	Senhor do Bonfim, Juazeiro, BA
M. jacobinensis Mueller	Vitória da Conquista, BA

Source: Nassar (2000)

Table 8.2 Primary and secondary centers of origin for *Manihot*

Center of diversity	Species included
Primary center: Brazil	*M. acuminatissima* Mueller; *M. sparsifolia* Pohl; *M. pruinosa* Pohl; *M. alutacea* Rogers et Appan; *M. divergens* Pohl; *M. cecropiaefolia* Pohl; *M. triphylia* Pohl; *M. pentaphylla* Pohl; *M. anomala* Pohl; *M. procumbens* Mueller; *M. crotalariaformis* Pohl; *M. pusilia* Pohl; *M. logepetiolata* Pohl; *M. tomentosa* Pohl; *M. purpureo-costata* Pohl; *M. attenuata* Mueller; *M. orbicularis* Pohl; *M. tripartita* (Sprengel) Mueller; *M. pilosa* Pohl; *M. sagittato-partita* Pohl; *M. falcata* Rogers et Appan; *M. quinqueloba* Pohl; *M. violacea* Pohl; *M. irwinii* Rogers et Appan; *M. mossamedensis* Taubet; *M. fruiculosa* (Pax) Rogers et Appan; *M. gracilis* Pohl; *M. warmingii* Mueller; *M. replans* Pax; *M. stipularis* Pax ; *M. oligantha* Pax; *M. nana* Mueller; *M. stricta* Baillon; *M. salicifolia* Pohl; *M. weddelliana* Baillon; *M. peltata* Pohl; *M. janiphoides* Mueller; and *M. handroana* N.D. Cruz.
Secondary center: southwestern Mexico	*M. pringlei* Watson; *M. aesculifolia* Pohl; *M. oaxacana* Rogers et Appan; *M. rhomboidea* Mueller; *M. easkerae*; *M. waskearae* Croizat; *M. divisiae* Croizat; *M. michaelis* McVaugh; *M. websterae* Rogers et Appan; *M. aurivulata* Mcvaugh; *M. rubricaulis* I.M. Hohnson; *M. chlorosticta* Standley & Goldman; *M. subspicata* Rogers et Appan; *M. caudata* Greenman; *M. angustiloba* (Torrey) Mueller; *M. tomatophylla* Standley; *M. foetida* Pohl.
Third center: northeastern Brazil	*M. zentneri* Ule; *M. surinamensis* Rogers et Appan; *M. quinquefolia* Pohl; *M. pseudoglaziovii* Pax et Hoffmann; *M. maracasensis* Ule; *M. quinquepartita* Huber; *M. caerulescens* Pohl; *M. marajoara* Chermont de Miranda; *M. tristis* Mueller; *M. glaziovii* Mueller; *M. epruinosa* Paz et Hoffmann; *M. brachyandra* Pax et Hoffmann; *M. dichotoma* Ule; *M. leptophylla* Pax; *M. reniformis* Pohs; and *M. heptaphylla* Ule.
Fourth Center: western Mato Grosso do Sul and Bolivia	*M. guaranitica* Choda et Hassier; *M. pruinosa* Pohl; *M. jacobinsis* Mueller; *M. condesata* Rogers et Appan; *M. xavantinensis* Rogers et Appan; and *M. flemingiana* Rogers et Appan.

western Pernambuco and central Bahia present the greatest variability of *Manihot*. The primary center of diversity is central Brazil, the secondary center is southwestern Mexico, while the third center of diversity is northeastern Brazil, and the fourth center of diversity is western Mato Grosso do Sul and Bolivia (Table 8.2).

Ekanayake et al. (1997) suggested that the major center of origin for cultivated *Manihot* is located between Brazil and Paraguay and the minor center of origin is Central America including Colombia, Venezuela, Guatemala, and southern Mexico since a large number of wild *Manihot* species are present there (Sauer 1952; Rogers 1965). Presently, cultivated *Manihot* is distributed throughout tropical America and in Asia and Africa. It was introduced into Africa and Asia by the Portuguese travelers in the fifteenth century (Jennings and Hershey 1985; Allem 1994). Further studies on *Manihot* species found in the Central American region suggested that they are only distantly related to the cultivated *Manihot* species (Fregene et al. 1994; Schaal et al. 1994; Roa et al. 1997).

A primary gene pool (GP-1) for cassava was established based on the degrees of cross transferability between cassava and its wild relatives (Allem et al. 2001). The GP-1 of a crop is composed of gene reservoirs that cross easily with the domesticated species, while the crosses regularly produce fertile offspring. The GP-1 is further subdivided into cultivated and wild gene pools. The cultivated gene pool encompasses commercial stocks of the crop, as well as landraces. The wild GP-1 of the crop comprises putative ancestors and closely related species that show a fair degree of fertile relationships with the domesticated species. Two South American wild subspecies of cassava (*M. flabellifolia* and *M. peruviana*) were proposed as natural members of the wild GP-1 of the crop. Another Brazilian species (*M. pruinosa*) is morphologically so close to both wild subspecies that it may turn out as another member of the wild GP-1 (Allem et al. 2001).

The debate still continues, however, it has been generally accepted that cultivated cassava originated from a single progenitor, *M. esculenta* ssp. *flabellifolia*, with probable hybridizations among them and the stretch from Brazil to Bolivia is the center of origin for this crop with many microcenters existing in different places.

8.2.2 Morphology and Taxonomy of Manihot

Rogers and Appan (1973) classified *Manihot* species into 19 sections, with trees in the section *Glaziovianae* to subshrubs, nearly acaulescent, in the section *Stipularis*. Other sections, like *Tripatitae* and *Graciles*, are perennial subshrubs with large woody roots (Nassar 1980). The roots of all *Manihot* species are tuberous, fleshy, farinaceous, and can grow up to 90 cm in length and 15 cm in diameter. The roots can weigh up to 40 kg (average is between 4 kg and 7 kg). These roots are rich in starch and contain a venomous volatile chemical compound, hydrocyanic acid (HCN), that can be eliminated by cooking or heat treatment to make them edible (Rickard 1985; Ravindran 1993; Bunyeth and Preston 2006). Contrary to those of the cultivated cassava genotypes, roots of wild species are fibrous and slender; some species frequently exhibit a limited number of tuberous roots. Root surface is smooth or rough, and subepidermis varies from red or yellow to white color. The cortex of tuberous rooted species is white, cream, or yellow colored.

Stem height varies from almost acaulescent in subshrubs to about 20 m in tree species. Shrubs native to the Brazilian savanna frequently have their stem die back to the crown in the dry season. Stem color varies from gray or brown to reddish. The branching pattern is typically dichotomous or trichotomous with branching point exhibiting a terminal inflorescence. In wild species, the young stem frequently has a varying degree of pubescence, a character rarely encountered in the cultigen (Nassar and Grattapaglia 1986).

Leaves are alternate varying from subsessile to long petiolated. They are spirally arranged on the stem with petioles ranging from 5 to 30 cm long, usually longer than the blades. The young leaves vary in color from yellowish green to deep purple. All species produce palmately lobed leaves. The inflorescence of *Manihot* species is terminal and monoecious except in few rare species that are native to Central Brazil with dioecious inflorescence and grouped in racemes or panicles (Watson and Dallwitz 1992). Flowers have a single perianth composed of five petals, with length ranging from 0.5 to 2.0 cm. Buds of staminate flowers are ovoid or spheric, and those of pistillates are conic. All *Manihot* species outcross and pollination is by insects. Fruits are capsules with three locules and can be elliptic, conical, smooth, or with small wings (Watson and Dallwitz 1992). Seeds have caruncles varying in size, playing a major role in water exchange, enhancing germination in dry areas. The cotyledons are flat and large.

8.2.3 Cytology, Karyotype, and Genome Size

All *Manihot* species have a chromosome number of $2n = 36$ and behave like diploids in meiosis. Thirty-nine cultivars of cultivated *Manihot* species and eight related wild species were analyzed for number, morphology and size of chromosomes, prophase condensation pattern and the structure of the interphase nucleus. All investigated accessions showed a similar karyotype with $2n = 36$, small metacentric to submetacentric chromosomes (Bai 1987; Reginaldo and Marcelo 2002).

The current review on chromosome number of *Manihot* species revealed the segmental allopolploid origin of cassava cultivars as suggested by Jennings (1963) and Magoon et al. (1969). Magoon et al. (1969) studied the pacyhytene chromosomes of cassava cultivars and found completely paired pachytene bivalents varying in length from 19.3 to 40.0 μm. These authors also observed the haploid chromosomal complement inter alia has three functional nucleolar chromosomes and six chromosomal types represented in duplicate during pachytene. This study supported the putative tetraploid nature of current cultivar and suggested its diploid ancestor with similar karyotype (Magoon et al. 1969). Umanah and Hartman (1973) analyzed the karyograms of *M. esculenta* and *M. glaziovii* and concluded that the tetraploid level of both species with similar satellite chromosome pairs was observed in each karyotype.

Studies conducted with isozymes and codominant markers also support the hypothesis and show a disomic inheritance at 12 loci (Umanah and Hartman 1973; Jennings and Iglesias 2002). Similar pachytene studies have been carried out in *M. glaziovii* and a comparison with the karyotype of cassava showed many common features, including the same number and a similar morphology of chromosomes (Krishnan

et al. 1970). Further studies on the genetics of *Manihot* species have been very limited and breeders have concentrated on obtaining the basic information required for effective genetic improvement of the crop. A more complete genomic characterization of the species and its relatives is needed to be focused soon. However, some authors have described it as a segmental allotetraploid with basic chromosome number of $x = 9$. Studies of Jos and Nair (1979) on the meiotic behavior of several cassava genotypes revealed regular 18 bivalent formation of the chromosomes typical of its diploid ($2n = 2x = 36$) status. Cassava breeders also observed a similarity in breeding behavior of allotetraploids to that of diploid (Wricke and Weber 1986).

Although most cultivated *Manihot* genotypes studied are diploid, spontaneous polyploids, such as triploids ($3n$) and tetraploids ($4n$) have been reported in some genotypes (Hahn et al. 1980, 1999). Triploid and tetraploid plants differ from diploid plants in vigor and leaf shape and size. Triploid plants usually grow and yield better than tetraploid and diploid plants. The nucleic acid content of diploid cultivated *Manihot* is 0.83 pg per nucleus equivalent to 800 Mbp in the haploid genome (Awoleye et al. 1994). The heterozygosity levels of different *Manihot* accessions have been further confirmed from diversity studies using isozyme (Lefevre and Charrier 1993a, b), restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), AFLP, and simple sequence repeat (SSR) markers (Sánchez et al. 1999; Fregene et al. 2001, 2003).

8.2.4 Agricultural Status

Historically a number of wild *Manihot* species were agronomically evaluated and had genes transferred to cassava, particularly for resistance to diseases such as mosaic virus and bacterial blight and to pests such as green spider mites and mealy bugs, but these hybrids rarely reached commercialization. They have also been used to increase protein content and decrease cyanide content. These species, for example, include *M. catinga* and *M. dichotoma* for resistance to mosaic virus, *Manihot pseudoglaziovii* for drought tolerance, *M. tristis* ssp. *saxicola* for high protein content, *M. tripartite* for compact roots, white flesh and high protein content, *M. stipularis* for drought resistance, adapted to low temperature and dwarfiness, *M. caerulescens* for drought resistance, tolerance to toxic soils and adaptation to low temperature. An exception is the species *M. glaziovii*, which hybridize naturally with *M. esculenta* and have been successfully crossed with it in Africa that provided high-yielding, locally adapted commercial varieties with genes for resistance to cassava mosaic virus, bacterial blight, as well as low cyanide content (Hahn et al. 1980).

The wild *Manihot* species are not cultivated for human consumption and are mostly grown in forests. The consumption of cultivated cassava in Brazil's semi-arid northeast region is among the world's highest, with a per capita consumption at just under 100 kg per year (http://www.fao.org/docrep/007/y2413e/y2413e0c.htm). Because cassava cultivation is growing steadily as an important staple food crop, indigenous species are more likely to experience the thinning out or eventual extinction of their populations. There are, therefore, recommendations to conserve these wild forms for their potential contribution toward improvement of cultivated cassava. Rogers and Appan (1973) described 98 species related to cassava; another new species, *M. neusana*, has also been described (Nassar 1985); and few more are being described. Each and every species is considered to possess traits of potential interest to plant breeders along with high interspecific variation for morphological and physiological traits that could be exploited for sustainable production of cassava.

8.3 Conservation Initiatives

8.3.1 Genetic Erosion

Although cassava is more important in the food and industrial economies of Africa and Asia than in the Americas, there is potential for competition between the cultivated and wild *Manihot* species for arable land. The largest producing countries (e.g., Brazil, Colombia and Paraguay) have the most potential for cassava cultivation to displace wild species (http://www.fao.org/docrep/007/y2413e/y2413e0c.htm) as presented in Table 8.3. The Brazilian Amazon, home to an estimated seven forest species of *Manihot*, has lost 15% of its

Table 8.3 Species of *Manihot* of particular concern for humankind

1[a]	2[b]	3	4	5
M. aesculifolia	*M. anomala*	*M. caerulescens*	*M. flabellifolia*	*M. brachyloba*
M. angustiloba	*M. baccata*	*M. diamantinensis*	*M. peruviana*	*M. pilosa*
M. auriculata	*M. brachyloba*	*M. dichotoma*	*M. pruinosa*	*M. triphylla*
M. caudata	*M. compositifolia*	*M. glaziovii*	–	–
M. chlorosticta	*M. flabellifolia*	*M. jacobinensis*	–	–
M. crassisepala	*M. flemingiana*	*M. janiphoides*	–	–
M. davisiae	*M. hassleriana*	*M. maracasensis*	–	–
M. foetida	*M. mossamedensis*	–	–	–
M. michaelis	*M. peruviana*	–	–	–
M. oaxacana	*M. pilosa*	–	–	–
M. pringlei	*M. pruinosa*	–	–	–
M. rhomboidea	*M. quinquepartita*	–	–	–
M. rubricaulis	*M. sagittato-partita*	–	–	–
M. subspicata	*M. tripartita*	–	–	–
M. tomatophylla	*M. triphylla*	–	–	–
M. walkerae	*M. violacea*	–	–	–
M. websterae	–	–	–	–

Source: http://www.fao.org/docrep/007/y2413e/y2413e0c.htm

[a]1 = Mexican species threatened because of development; 2 = Brazilian species threatened because of development and cassava cultivation; 3 = Species of maniçobas economically valuable to dwellers of Brazil's NE semi-arid region; 4 = Species involved in the ancestry of cassava and constituting the wild primary genepool of the crop; 5 = The putative closest wild relatives of cassava and assumed to participate in the secondary genepool of the crop

[b]Includes species of column 3

original vegetation (Walker and Holmes 1996). The importance of cassava in slash-and-burn agriculture in traditional Amazonian communities is well established (Salick et al. 1997; Emperaire et al. 1998). Thus, the threat of cassava cultivation per se to wild *Manihot* populations is not negligible.

The clearing of land to cultivate cassava in the Brazilian semi-arid region has been most prevalent in areas inhabited by seven wild *Manihot* species known as maniçobas. These species live in the thorny bushy vegetation called carrascos and in the harsh conditions of the innermost area called sertão. They are: *M. caerulescens*, *M. diamantinensis*, *M. dichotoma*, *M. glaziovii*, *M. jacobinensis*, *M. janiphoides*, and *M. maracasensis*. The clearing and burning of the vegetation in drought-plagued areas of the Caatinga has the potential to hit hard local populations of *Manihot* because they are usual components of the vegetation. About 100 municipalities of the area have annual rainfall rates below 500 mm, and seldom between 0 and 250 mm (http://www.fao.org/docrep/007/y2413e/y2413e0c.htm). The urgent need of collection and preservation of wild germplasm is also supported by the fact that considerable genetic erosion is currently taking place among wild *Manihot* species in nature. Nassar and Cardenas (1985) described that *M. walkerae, M. guaranitica, M. subspicate, M. angustiloba, M. longipetiolata,* and *M. pringlei* are extremely endangered species.

Socio-economic data as well as farmers' estimation of genetic erosion were the potential indicators of genetic erosion or diversity (Guarino 1995; Brush 1999) in cassava. The study area, the Ucayali region of the Peruvian Amazon, was surveyed through interviews with 285 cassava farmers in 50 communities, while diversity was assessed based on agro-morphological characterization of 295 cultivated *Manihot* accessions (Willemen et al. 2007). The study revealed also that farmers are a good direct source of information on the diversity present at community level, which can contribute to the development of methodologies to assess diversity more rapidly.

8.3.2 Germplasm Conservation

The international institutes of the Consultative Group for International Agricultural Research (CGIAR) such as Centro Internacional de Agricultura Tropical (CIAT) and International Institute of Tropical

Agriculture (IITA) along with national institutes made several collection efforts to conserve landraces, improved cultivars, and wild relatives of cassava. About 20,000 accessions of cassava and its wild relatives are presently conserved as ex situ germplasm collection in CIAT, IITA, and national institutes of more than 45 different countries worldwide (Bonierbale et al. 1997). All the accessions in the two CGIAR institutes are held "in trust" under the auspices of Food and Agricultural Organization (FAO) of the United Nations for public access and are freely distributed to all the users through Standard Material Transfer Agreement (SMTA). However, these collections may include duplicates and the total number of unique accessions is likely to be much smaller. South America has the largest collection, followed by West and Central Africa. Such collections are the reservoir for genetic improvement in cassava worldwide (Kawano 2003), although only a sample of these accessions have been thoroughly assessed by the breeders for their useful variation. Hence, a core subset of 630 accessions from 23 countries was established by CIAT (Bonierbale et al. 1997) to provide an entry point and access to the entire collection. A total of 295 cultivated and wild *Manihot* accessions are maintained in Peru by Instituto Nacional de Investigaciony Extension Agraria (INIEA) (Fukuda and Guevara 1998). More than 1,635 accessions (785 exotic and 850 indigenous) have been collected and characterized at Central Tuber Crops Research Institute (CTCRI), India (Pillai et al. 2002). Plant quarantine regulations for cassava made it clear in the 1980s that only in vitro plants would be accepted for distribution worldwide, and technical guidelines for the safe movement of *Manihot* germplasm were formulated (Frison and Feliu 1991).

Various conservation methods are followed such as field gene bank, seed storage, in vitro storage, and cryopreservation of shoot tips and pollen. On farm conservation methods are yet to be established but would be of great advantage in complementing conventional ex situ conservation. Even DNA bank can be one of the options for cassava germplasm conservation.

8.3.3 Conservation Methods

There are two basic conservation methods for plant genetic resources, ex situ and in situ, and the method adopted depends mainly on cost as well as the targeted gene pool of a species. Cassava is an outcrossing species and produces botanical seeds, however, it is mainly vegetatively propagated using stem cuttings or by shoot tips in in vitro cultures to maintain genotypes. The wild relatives are also predominantly outcrossing, and are propagated mainly by botanical seeds, and in some cases, through stem cuttings (Iglesias 1994; Ng and Ng 1997).

Field genebanks are another conservation method wherein cassava germplasm are maintained in the field, as living collection, which are relatively easier to establish and cheaper to maintain with plant materials readily available for evaluation/characterization and cross-pollination. However, germplasm conserved in field are under constant pressure of prevailing diseases and pests, which could lead to a loss of germplasm accessions or even genetic drift (Ng and Ng 2002). Similarly, seed banks are also maintained for cassava seeds, which are orthodox and stored best in cool and dry conditions. The in vitro propagation and techniques are well established in cassava and are routinely used in many genebanks, particularly in CIAT, IITA and in national programs in Brazil, Argentina, Paraguay, and Cuba (Bonierbale et al. 1997; Ng et al. 1999). Shoot tip cultures of cassava clonal accessions are usually conserved under reduced growth or slow growth culture media or reduced incubation conditions (temperature and light intensity). Cultures are checked regularly that undergoes virus indexing for certification by Plant Quarantine Services for multiplication and distribution.

Cryopreservation has been recently gaining importance as it enables long-term conservation of germplasm. Methods to cryopreserve cassava germplasm were developed by Escobar et al. (1997) using classical protocols (chemical dehydration and programmed freezing). New protocols such as encapsulation, dehydration, and quick-freezing have also been developed and validated in cassava. More than 82% of the accessions tested have recovery rates of more than 30%, the minimum required for cryopreservation. Protocols are now being tested for wild relatives of cassava, species of which sometimes behave very poorly in vitro or even in the field, making their conservation troublesome. Plants have been recovered for *M. esculenta* ssp. *flabellifolia*, *M. esculenta* ssp. *Peruviana,* and *M. carthaginensis*.

The droplet freezing method that uses dimethyl sulphoxide (DMSO) as the cryoprotectant, and is termed droplet-vitrification when applied in combination with PVS2 vitrification solutions (Panis et al. 2005; Sakai and Engelmann 2007), has also been used in cassava. In this case, shoot-tips require pregrowth treatments before proceeding to the cryoprotectant stage. In the case of droplet-freezing, 10% (v/v) DMSO is dispensed as 2.5–20 μL droplets of cryoprotectant onto sterile aluminum foil strips of 2–3 × 0.5–1.0 × 0.003 to 0.005 cm^3 dimensions with a loading of 5–10 droplets/strip. The shoot-tip meristems are added to each droplet using liquid nitrogen (LN)-tolerant sterile forceps and the foils are directly exposed to liquid phase LN and transferred to cryovials at two foils per vial and stored in the liquid phase of LN. For rewarming, the foils are removed from the vials and placed directly into liquid medium at ambient room temperatures (ca. 25°C). The shoots dislodge on rewarming and are plated onto recovery medium. The cost estimates show that USD5 per year per accession is required for maintaining field collections, whereas the in vitro collection costs USD4.20 per year per accession and cryopreservation maintenance costs are USD1 per accession per year.

The in situ or on-farm conservation of cassava is still not fully operational as it requires broad knowledge on social, biological, and environmental factors. Generally in situ conservation involves maintenance of genetic materials, mainly wild relatives in their natural ecosystems. There is lack of proper documentation of information on cassava and its wild relatives to develop effective in situ conservation strategy. However, the demand for wild *Manihot* species on the part of cassava breeders had a positive effect on their conservation and study. The plants became better known and this in turn raised interest in further collection and conservation efforts. In addition, the wild *Manihot* germplasm assembled in research institutions in Africa and Asia from the early 1930s through the late 1950s was multiplied for generations and made available to the community for other characterization studies. Ex situ conservation will still remain the most effective and reliable way of conserving cassava genetic resources.

Genetic resources of cassava comprise local or introduced landraces, improved cultivars, and related wild species. The CIAT cassava germplasm collection consists of 6,000 accessions with landraces from Latin America and Asia, elite clones selected by CIAT, and the International Institute of Tropical Agriculture (IITA) in Nigeria conserves about 3,000 accessions including several wild *Manihot* species. These are mainly conserved in the form of slow-growth in vitro plantlets and also as living collection in the field. Efforts are underway in IITA to develop a DNA bank for cultivated cassava accessions, which will further be extended to other *Manihot* species.

8.4 Role of Wild *Manihot* Species on Elucidation of Origin and Evolution of the Cultivated *Manihot* Species

One of the questions that remained unsolved until recent times is about the evolutionary and geographical origin of cultivated cassava, and the actual wild progenitors of this crop. In general, *Manihot* species possesses morphological traits that could be reliable for species delimitation but most species show tremendous intraspecific morphological variability. These led to conclusions that hybridization is extensive among co-occurring (sympatric) species in nature (Rogers and Appan 1973). On the basis of morphological traits, the Central American species, *M. aesculifolia* (Kunth, Pohl) and *M. carthaginensis*, was considered as the closest wild relatives and putative ancestors of the crop, although populations were observed in South America that have closer physical appearance to cassava. Tracing cassava's origin through ethno-botanical studies has complicated it further, owing to the crops long-term geographically widespread use (Sauer 1993). Studies using DNA markers such as RFLPs (Fregene et al. 1994), AFLPs (Roa et al. 1997) and DNA sequences (Schaal et al. 1997) indicated that South American and Central American species form two distinct evolutionary lineages, and cultivated cassava is genetically more closer to South American lineage. During the same time, Allem (1994) indicated that the naturally occurring *Manihot* populations (*M. esculenta* ssp. *flabellifolia*) in South America are morphologically more similar to cassava, and could be the wild progenitor of cassava. However, work with AFLP markers and microsatellite analysis showed that *M. aesculifolia, M. carthaginensis,* and *M. brachyloba* are the most distant relatives of the crop, whereas the

wild forms *M. esculenta* ssp. *flabellifolia* and *M. esculenta* ssp. *peruviana* appear to be the closest (Roa et al. 2000). Later, Olsen and Schaal (2001) confirmed, based on DNA sequences and SSR markers that cultivated cassava has a single wild progenitor, *M. esculenta* ssp. *flabellifolia* and the geographical origin of the crop lies along the southern border of the Amazonian basin. The findings of this study also indicated that hybridization between other wild species, such as *M. pruinosa,* might be occurring in nature but it is unlikely that this hybridization would have played any role in the origin of this crop. There could be other progenitors involved in the evolution of cassava; however, strong evidences are lacking. The polymerase chain reaction (PCR)-based markers, such as SSRs and EST-SSRs, also indicated a strong grouping of varieties related to the region of cultivation in Brazil (Carvalho and Schaal 2001). The occurrence of pubescent cassava cultivars in Peru that might have descended from *M. peruviana* indicate that cassava might have been domesticated in eastern Peru (Hahn 1993). It can be summarized that the regions where cassava and wild relatives co-occur, there is possibility of hybridization and introgression; however, it is well proved now that *M. esculenta* ssp. *flabellifolia* is the single progenitor of cassava and its geographical origin lies in southern America.

8.5 Role of Wild *Manihot* Species in the Development of Cytogenetic Stocks and Their Utility for Cassava

8.5.1 Addition and Substitution Lines

Cultivated cassava is a diploid with $2n = 36$ chromosomes based on regular meiosis (Allem 1984). However, meiotic irregularities such as laggards, delayed separation of bivalents, non-orientation, and non-congression of bivalents, monads, dyads, and polyads were also reported (Bai 1987). Interspecific hybrids of cassava with its wild relatives show fair regular meiosis and the backcrossed generations exhibit high fertility (Nassar 2000). Similarly, polyploids were found to be produced in *Manihot* by unreduced gamete fertilization (Nassar 1992) playing a vital role in plant evolution as a means of preserving favorable hybrid combinations during sexual reproduction. The formation of unreduced microspores, which are gametes with somatic chromosome number, appears to be a common phenomenon in angiosperms (de Wet 1980).

Unreduced microspores are very important in plant breeding as they may lead to the development of highly productive triploids and tetraploids by sexual reproduction resulting in preservation of their heterozygosity (Mendiburu and Peloquin 1977). Unreduced microspores occur in interspecific hybrids of cassava involving wild species as one of the parents, as a consequence of meiotic irregularity, and now there is a general agreement that dyads form due to spindle abnormalities, which may be visible at meiotic metaphases, I and II (Nassar 1992; Nassar and Freitas 1997). The causes of formation of unreduced microspores in cassava are variable. It was reported that unreduced microspore formation is due to simple recessive genes (Mok and Peloquin 1975) and also due to the occurrence of non-functional spindles, due to which all the resulting metaphase chromosomes remain in the center instead of separating to the poles (Vorsa and Bingham 1979). Triploidy has been used successfully in cassava improvement especially for the production of high starch varieties in India. In Brazil, the most drought-tolerant cultivar is a natural triploid (Manebeba Branca). Spontaneous tetraploids, triploids and 2*n* pollen (unreduced gametes) were also obtained in IITA (Hahn et al. 1990) from diploid interspecific crosses and from open-pollinated interspecific hybrids, involving female diploid plants of *M. esculenta* crossed with male parents of *M. pruinosa* or *M. glaziovii*. These two related species are highly apomictic but can also hybridize naturally with cassava. Spontaneous sexual and asexual polyploids occur in polyploid breeding of cassava. Sexual polyploidization has advantages over asexual polyploidization in terms of variability of gametes, heterosis, vigor, plant architecture, and productivity of the crop (Hahn et al. 1990). Many polyploid cassava clones have been generated at IITA utilizing bilateral and unilateral polyploidization. Triploids obtained from the cross between normal diploid and colchicine-induced autotetraploids outyielded the autotetraploid parents (Jos et al. 1987). A natural hybrid of *M. pseudoglaziovii* and cassava was collected and multiplied vegetatively and studied cytogenetically (Nassar 1991). A triploid was selected from the selfed progeny of this hybrid and its evaluation showed a high

productivity of tuber roots and resistance to stem borer pest. All these results indicate that triploids are more promising than tetraploids in cassava improvement.

Breeding for characters governed by recessive genes is difficult in cassava owing to its allotetraploid nature; therefore, production of haploids utilizing microspore cultures or dihaploids from $4x \times 4x$ matings in *Manihot* could be useful for cassava breeding in various ways. Reducing ploidy levels from tetraploidy to diploidy level could be useful and be efficiently applied for cassava improvement because genetic manipulation is much easier at diploid level than at tetraploid level (Hahn et al. 1990). Thus wild relatives have traditionally been used as sources of useful traits and their use will continue to introgress new genes into cassava.

8.5.2 Aneuploids

Availability of aneuploids greatly facilitates the construction of cytogenetic maps in which it is possible to locate the marker loci not only on the chromosomes but also on specific regions of the chromosomes. The meiotic division in interspecific hybrids may lead to a higher frequency of aneuploid gametes, making it possible to select polyploids from their progeny (Nassar et al. 1995). Progenies of two interspecific hybrids of cassava with wild *Manihot* species were studied meiotically as well as mitotically (Nassar et al. 1996). A tetrasomic aneuploid from the progeny of the above-mentioned hybrids had a very large starchy root. These results indicated that root formation in *Manihot* species is controlled by additive polygenes that are distributed on more than one chromosome (Nassar et al. 1996). Apomixis in *Manihot* is frequently associated with aneuploidy but it does occur in some diploid types. It is due to the formation of aposporic sacs, which can easily be detected by clearing tissue preparations (Young et al. 1979; Nassar and Santos 2002).

8.5.3 Interspecific Hybridization

Most species of the *Manihot* genus can be easily crossed with cultivars of *M. esculenta* and desirable alleles can be transferred for cassava improvement (Hahn et al. 1980). However, only limited number of possible interspecific crosses has, so far, been attempted. Crosses were made between *M. esculenta* and *M. glaziovii* for resistance to cassava mosaic disease (CMD), hybrids from *M. esculenta* and *M. melanobasis* crosses had superior seed set and root yield, and crosses between cassava and *M. saxicola* resulted in hybrids with better yield and higher protein content. Successful crosses were also obtained between cassava and *M. dichotama* and *M. catingae*. Four interspecific hybrids were obtained between cassava and *Manihot* species for polyploidization. These are *M. neusana* × *M. esculenta*, *M. glaziovii* × *M. esculenta*, *M. aesculifolia* × *M. esculenta* and *M. pohlii* × *M. esculenta* (Nassar 2000).

Interspecific hybrids of several wild *Manihot* species with cassava through controlled crosses by insect vectors have been reported (Nassar 1978b, c, d, 1989, 1994). These were identified by the dominant morphological markers from cassava such as noded stem, setaceous bracteole, ribbed fruit, and tuberculated root. Wild *Manihot* species were hybridized with cultigens in order to incorporate their desirable genes for drought tolerance, high protein content, apomixis, high yield and pest and disease resistance into the cultivars (Nassar 1997, 1999), and also for nutritional quality and yield of roots (Nassar and Dorea 1982).

8.6 Role of Wild *Manihot* Species in Classical and Molecular Genetic Studies of Cassava

8.6.1 Classical Genetic Studies

Not much research has been carried out on cassava genetics and the earliest work in the field of genetics was done in Indonesia, Zaire, East Africa, Madagascar, India, and Brazil. During the 1970s, with the establishment of CIAT, Columbia, and IITA, Nigeria research was focused more on cassava classification and variability. These two international centers collaborated with national programs such as EMBRAPA, Brazil, CTCRI, etc. to increase yield per unit area and also root quality. The breeding strategies varied from one country to another and depended mainly on the

final use of the crop, whether for human consumption or industrial use or for subsistence. In Africa, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) are the major constraints. Bacterial blight caused by *Xanthomonas axonopodis* pv. *Manihotis* is prevalent in Asia, Africa, and Latin America, and is also a major constraint. There were other difficulties with cassava being allogamous and highly heterozygous in nature because of which its sexual progenies are highly heterogeneous with wide morphological variation among them, something which is not preferred for commercial cultivation. The breeding strategy for Africa is higher yield coupled with resistance to diseases and pests, and IITA continued its research on crossing selected wild species, particularly *M. glaziovii*, with cassava with the aim of producing improved genotypes, especially with resistance to African mosaic virus (Hahn et al. 1980). In South America, breeders had access to a much broader range of genetic variability within cassava, and did not resort to use of wild species for obtaining novel traits. However, the African institutes crossed cassava and the Brazilian species *M. dichotoma*, *M. catingae*, and *M. glaziovii*, besides the Surinamese species *M. saxicola* and *M. melanobasis* (Nichols 1947; Jennings 1959) for targeted traits.

Achievements in cassava breeding using its wild relatives in Africa includes the development of a range of elite cassava genotypes, such as TMS 30572 and TMS 4(2)1425, that combined high stable yields, agronomic traits, and consumer quality with acceptable levels of resistance to CMD and cassava bacterial blight (CBB). These genotypes are widely cultivated (Hahn et al. 1989) and adopted (Otim-Nape et al. 1994) in Africa. The introduction of Latin American germplasm into the breeding programs in Africa has resulted in significant broadening of the genetic base of cassava in Africa. Classical breeding has contributed to improve dry matter and starch content as well as reducing the cyanogenic potential in cassava. Subsequently, a farmer participatory plant breeding (PPB) scheme was employed to evaluate recombinant progenies and select several varieties adapted to the semi-arid region of northeastern Brazil (Fukuda and Saad 2001).

Cassava mosaic disease (CMD) in Africa, a disease that is not found in Latin America, limited immediate use of the African germplasm but required introgression of CMD resistance into the Latin American germplasm. Resistance to CMD was introduced by backcrosses and thousands of recombinant seeds were produced and distributed to participating countries in West and Central Africa. The improved cassava germplasm has extended considerably the range of cultivation of cassava beyond its traditional area in the humid and subhumid tropics into the semi-arid zone of West and Central Africa by more than 100,000 ha between 1989 and 1999 (IITA 2000b, c, d).

Conventional breeding programs in Africa, Asia, and South America have been successful in developing and releasing varieties with enhanced harvest index and resistance to prevailing diseases. However, the rate of improvement in cassava yields has been much lower than its potential except for some Asian countries (Kawano 1978). The classical breeding in cassava is also complicated due to strong heterozygosity and inbreeding depression coupled with lengthy breeding cycles and the need for making large number of crossings to obtain the first generation progenies, which are then screened for desired traits. Selected individuals are then propagated vegetatively to generate sufficient number of clones for further screening and multilocation trials (Ceballos et al. 2004). There is a general acceptance that better progress can be made in cassava improvement through incorporation of modern biotechnological tools and molecular marker-assisted selection in breeding programs.

8.6.2 Molecular Marker Studies

In recent times, genetic or DNA markers have become one of the fundamental tools for understanding the inheritance and diversity present in any crop. The availability of markers has made it possible to construct genetic/linkage maps, cloning of genes, and quantitative trait loci (QTL) analysis along with whole genome sequencing. The earliest markers used in cassava were morphological and Graner (1942) described leaf shape and root color as two morphological markers to study their inheritance. Later many more morphological markers were described based on stem, leaves, and root characteristics (Harshey and Ocampo 1989). The second generation markers were biochemical ones such as isozymes, which provided a useful tool for genetic fingerprinting and to study genetic diversity in cassava (Ramirez et al. 1987; Lefevre and Charrier 1993a). Isozymes have also been used to determine genetic

relationships among African cassava germplasm (Lefevre and Charrier 1993b; Wanyera et al. 1994) and also to fingerprint the CIAT germplasm (Ocampo et al. 1992). However, with the advent of DNA/molecular markers, they gained importance rapidly in the studies involving inheritance, genomics, and genetic diversity. The most prominent molecular markers systems included minisatellites (Jeffreys et al. 1985), RFLPs (Botstein et al. 1980), RAPDs (Williams et al. 1990), and more recent ones are microsatellites/SSRs (Litt and Lutty 1989), AFLPs (Vos et al. 1995), DNA sequencing of internal transcribed spacer (ITS) of ribosomal DNA, DNA chips or oligonucleotide arrays, and single nucleotide polymorphisms (SNPs); the list is ever-growing. Currently, the cassava research community has developed several hundreds of molecular markers of which SSRs account for the largest proportion (Fregene et al. 1997; Mba et al. 2001; Okogbenin et al. 2006; Raji et al. 2009). Recently, following development of ESTs in various labs around the world (Anderson et al. 2004; Lokko et al. 2007; Sakurai et al. 2007), SNP markers are becoming the markers of choice owing to their suitability to the latest high-throughput genotyping platforms (Gedil and Sartie 2010).

8.6.2.1 Genetic Diversity Studies

The germplasm collection of cassava consisting of cultivated and wild accessions is a very important resource for the future improvement of the crop, and understanding the genetic relationships among accessions using molecular markers has made it possible to select diverse parents in the breeding programs. The genetic relationships among cassava accessions and wild *Manihot* species was studied using DNA markers (Jeffreys et al. 1985; Ocampo et al. 1995) and possible duplicate accessions were identified. The molecular analysis of *M. aesculifolia* contradicted its origin in Meso-America as described based on morphological characters and suggested a possible domestication of this species from Brazil along with some other close wild relatives such as *M. trsitis* and *M. esculenta* ssp. *flabellifolia*. Another study using AFLPs demonstrated that *M. esculenta* ssp. *flabellifolia*, *M. trsitis* and *M. peruviana*, all originating from Brazil, are more closely related to cassava than its Mexican close relative, *M. aesculifolia* (Roa et al. 1997; Carvalho and Schaal 2001).

Molecular markers have made immense contribution to cassava breeding and genetics, in assessment of genetic diversity, taxonomical studies, understanding the phylogenetic relationships in the genus, confirmation of ploidy, and development of genetic maps (Fregene et al. 1997, 2001; Lokko et al. 2004).

8.6.2.2 Construction of Genetic Maps

As most of the economic traits studied in cassava are polygenic, efforts were made to identify important QTLs through molecular mapping studies (Hahn et al. 1989; Rajendran 1989; Amma et al. 1995; Bryne et al. 1997; CIAT 2003).

First Generation Maps

Angel et al. (1993) initiated work on a detailed genetic map of cassava for tagging agronomically important traits and to clone cassava genes using a range of cassava accessions and a wild *Manihot* species. It was concluded that a combined use of RFLP and RAPD markers would lead to the construction of a detailed map of cassava. Gomez et al. (1996) used 328 RAPD markers for linkage analysis in cassava. Following this, the first cassava linkage map was developed by Fregene et al. (1997). This map was based on an F_1 population of two geographically divergent parents. The female parent TMS I30572, with resistance to CMD, was derived through introgression from *M. glazovii* (a wild relative of cassava), while the South American male parent CM 2177–2 (ICA-Cebucan) was susceptible.

In a heterozygous species, the segregating F_1 population is obtained by crossing a wild relative with a cassava cultivar (Pillay and Kenny 1996; Fregene et al. 1997). In a cross with three isoenzyme loci segregating as single-dose restriction fragments (SDFs, for more details read Wu et al. 1992) in the gametes of the female parent plus a total of 150 RFLPs, 30 RAPDs, and five microsatellites, 20 linkage groups were defined spanning 950 cM map length with an average marker density of one per 6 cM. In another study with 120 RFLPs, 50 RAPDs, four microsatellites and one isoenzyme single dose marker in the gametes of the male parent, 24 linkage groups were defined with a total length of 1,220 cM and

average marker density of one marker every 8 cM. Intervals were observed to be larger in the male-derived map than in the female-derived map and a paired *t*-test showed significantly ($P = 0.01$) greater distances in the male-derived map, suggesting a reduced recombination rate in gametes of the female parent. The mean interval length between adjacent allelic bridges (markers common to both parents) in the female-derived map was 38% less than in the male derived map. The male- and female-derived maps together covered 300 markers and 80% of the cassava genome requiring more number of markers to complete the genetic map.

Second-Generation Maps

Most of the markers present in first-generation map are based on RFLPs or RAPDs, which are not the best for large-scale, high-throughput marker-assisted breeding of plant populations. Therefore, attempts were made to develop second-generation map using PCR-based, highly polymorphic markers such as SSRs and sequence-tagged sites (STSs), which can be easily integrated into the existing genetic maps or can be used to develop new linkage maps. Mba et al. (2001) developed and characterized 172 SSR markers to saturate the existing linkage map of TMS I30572 × CM 2177–2 based on 150 progenies. Currently, the total number of RAPD, RFLP, and SSR markers on the cassava genetic map is 830 and for the first time 18 analogous linkage groups representing 18 chromosomes of cassava were identified (Fregene et al. 2001). With over 1,000 markers available, further efforts have been made to generate linkage maps in cassava from diverse genetic backgrounds and saturate the map (Akano et al. 2002; Lokko et al. 2003, 2004).

8.7 Role of Wild *Manihot* Species in Cassava Improvement Through Traditional and Advanced Tools

According to Allem (1999), wild genetic resources of *Manihot* have five important utilities (1) agricultural value (agrobiodiversity – direct or indirect economic application either as food suppliers or as income generators); (2) scientific value (enlargement of knowledge); (3) social value (recreation); (4) cultural value (broader spectrum of interest to the community); and (5) ecological value (keystone species or as participants in nature's food chains).

8.7.1 Traditional and Molecular Breeding Efforts

The importance of cultivated *Manihot* as a global food source demands the production of improved germplasm (Scott et al. 2000). Clonal selection was the predominant method in conventional breeding for cassava improvement at the national centers in Africa and Brazil. The only exception to this was the production of the CMD-resistant clones by hybridizing *M. glaziovii* with cassava (Storey and Nichols 1938). However, traditional breeding systems for cassava are hindered by its highly heterozygous nature, asynchronous flowering, and inbreeding depression. In addition, traditional breeding techniques for cultivated *Manihot* are cumbersome, requiring screening of approximately 100,000 seedlings after the first sexual crossing and at least 10 years for the improved product to reach the farmer. The CIAT, Columbia established a cassava breeding program in early 1970s with the aim of improving yield potential and resistance/tolerance to pests and diseases. As a part of this program development, the following processes were accomplished: germplasm collection and evaluation, generation of advanced breeding materials, and varietal selection and dissemination (Kawano 2003).

The development of transgenic technologies in cassava could circumvent many of the problems inherent in traditional improvement programs. Cassava cultigens are deficient in many desirable agronomic characters such as pest and disease resistance, drought tolerance, and low protein content (Nassar 2000). Developing interspecific hybrids and employing genetic transformation systems could help facilitating introgression of heterologous genes that could potentially improve the genetic diversity and revolutionize the improvement of cassava breeding program. The capacity to integrate transgenes into cassava is now established and being utilized to generate plants expressing traits of agronomic interest (Nigel et al. 2005). In 2000, CIAT initiated a program to introgress

genes for several traits such as root yield, quality, and dry matter content from wild cassava relatives into its germplasm collection (Ojulong et al. 2008).

Apomixis is the alternative means of reproduction in cassava, which helps in avoiding the systemic pathogens, excluding the genetic segregation in the progeny, and facilitating rapid speciation in this genus. Apomixis is present at very low level in cassava (1–2%). Facultative apomixis was discovered in the wild relatives of cassava (Nassar et al. 1998; Nassar 2001) and interspecific hybridization was carried out to transfer the useful genes to the cultigens (Nassar and Collevatti 2005).

Polyploidization of interspecific hybrids was attempted by colchicine treatment, which resulted in the production of tissues having different ploidy levels (Nassar 1991). In addition, a somatic polyploidization technique has also been proposed for cassava breeding in which in vitro plantlets were treated with colchicine and oryzalin (Awoleye et al. 1994). Thus interspecific hybridization, apomixis, and polyploidy contributed to the evolution of the *Manihot* in which interspecific hybridization and polyploidy produced the genetic variability necessary for speciation, while apomixis was responsible for perpetuating new hybrid types adapted to various environments.

8.7.2 Genetic Transformation

Efforts to develop a genetic transformation system for cassava were initiated in early 1990s, but remained elusive until 1996 because of its recalcitrance to in vitro manipulation. Initial efforts reported a system of somatic embryogenesis in cassava (Stamp and Henshaw 1982). For this purpose, embryogenic cultures are induced to develop from immature leaf explants after 3 weeks culture on medium supplemented with growth hormones (Stamp 1987) and also by particle bombardment or *Agrobacterium tumefaciens*-mediated transformation (Raemakers et al. 1996, 1997). However, only chimeric tissues were recovered because of the multicellular nature of the morphogenic events and highly organized nature of the embryonic structures (Schöpke et al. 1993).

By manipulating the embryonic culture systems of cassava, the genetic transformation capability has been improved. Four different techniques have been reported for recovering transgenic cassava plants with integration of transgenes for beneficial agronomic traits (Li et al. 1996; Schöpke et al. 1996; Taylor et al. 2004). All the four systems are reliant on the production of embryogenic tissues from in vitro mother plants (leaf explants). These four transformation systems are: production of somatic embryos from friable embryogenic callus, cotyledon fragments, embryogenic cultures, and immature leaf explants (Taylor et al. 2004).

Thus, cassava improvement continues to tap genetic variation from wild *Manihot* through conventional breeding and advanced techniques such as transgenics. However, novel sources of variation are required to genetically advance this important food crop of Africa and other areas in the tropics of the developing world.

8.8 Genomics Resources in *Manihot* Species

To augment the cost-effectiveness of achieving the required goals, a number of genomic resources and molecular tools have been developed during the recent years to advance breeding of cassava cultivars.

The incentive for the genome sequence of cassava began in 2003 but the whole genome project gathered momentum in early 2009 utilizing 454 sequencing technology. More than 61 million sequencing reads were generated and assembled into a draft genome that contains an estimated 95% of cassava genes. It is one of the first large genome projects to primarily use 454 Life Sciences' long-read sequencing platform, which enabled both improved quality of the draft, and its rapid generation. The annotated draft genome sequence is available at the US Department of Energy-Joint Genomic Institute (DOE-JGI) Phytozome Web site (http://www.phytozome.net/cassava). The other genomic resources of cassava include expressed sequence tag (EST) databases, which provide resources for producing Euphorbiaceae-specific DNA microarrays and cross-species comparisons. A full-length cDNA library of cassava plants under normal, heat, drought, aluminum, and post-harvest physiological deterioration conditions was built (Cortés et al. 2002). As of August 2010, there are 83,537 nucleotide and EST sequences at public genomic databases such

as GenBank, which is a very small number compared to the number of sequences from maize (4,459,127), rice (4,430,606), soybean (2,097,140), potato (385,881), or sugarcane (295,578).

In order to characterize the library and find the number and putative functions of the transcripts that were captured, nearly 20,000 clones were sequenced from both ends (Sakurai et al. 2007). These studies were able to determine the information about 5′ UTR's of 1,949 sequences and 3′ UTR's of 2,241 sequences as well as the complete coding sequence of 732 genes, all of which is an essential resource for gene discovery, characterization, and cloning, and to assist in the annotation of the cassava genome. The ESTs were assembled into 6,355 contigs and 9,026 singletons that were further grouped into 10,577 scaffolds. About 4,621 new cassava sequences and 1,521 sequences were found with no significant similarity to plant protein databases. Transcripts of 7,796 distinct genes were captured and functional classification was assigned to 78% of them, while more than half of the enzymes annotated to metabolic pathways in *Arabidopsis* (Sakurai et al. 2007).

To increase the tools for understanding and manipulating drought tolerance in cassava, another group of scientists generated ESTs from normalized cDNA libraries prepared from dehydration-stressed and control well-watered tissues yielding a total of 18,166 ESTs with an average read length of 586 nucleotides (Lokko et al. 2007; Raji et al. 2009). Analyses of these ESTs resulted in the identification of 8,577 unique gene clusters, which can be utilized for the development of microarrays and gene-derived molecular markers to further dissect the molecular basis of drought tolerance in cassava.

Another genomic resource for cassava includes bacterial artificial chromosome (BAC) library from a wild relative of cassava that was constructed with the objective of map-based cloning of disease and pest resistance and root quality genes (Fregene et al. 2001). This library has 5*x* coverage of the genome and a 95% probability of finding any desired clone. This BAC library is publicly available through CIAT.

The other area where progress has been made is to target the resistance gene analogs (RGAs). The rapid accumulation of genome sequence data has paved a way to develop arrays of functional genomic tools to understand the complex pathways of host–pathogen interactions. A study is underway where several pairs of degenerate primers matching the conserved domains of *R*-genes to amplify putative RGAs in cassava and its wild relatives such as *M. glaziovii* and *M. eprunosa* along with castor bean (*R. communis*) has been identified (M Gedil et al. personal communication). In addition, a cassava DArT (diversity array technology) chip with 735 polymorphic markers has been used to fingerprint a diverse range of cassava populations including genotypes from Africa, Latin America, Asia, and breeder lines maintained at IITA along with few wild relatives (IITA unpublished data).

8.9 Gene Flow Between *Manihot* Species

The major constraint in *Manihot* is interbreeding between the species that can cause a "swamping" of the rarer species' gene pool, creating hybrids that drive the originally purebred native stock to complete extinction. It has been confirmed that cassava varieties can cross-pollinate naturally with wild relatives, as a result of which, in South America, transgenes could move easily from a genetically modified variety to other species. The cultivated *Manihot* is developed from wild *Manihot* through controlled selection and hybridization; therefore it is expected that there is highly successful cross-breeding between cassava and its wild relatives, which might pose issues related to gene flow in near future. The gene flow has been closely observed in Africa between cassava (*M. esculenta* ssp. *esculenta*) and *M. glaziovii* in Nigeria and Cote d'Ivoire (Beeching et al. 1993). Therefore, cross breeding between a transgenic cassava resistant to stem borer and the wild relative *M. glaziovii* could take place in regions such as Northeast Brazil where cassava is widely cultivated.

The wild relative of cassava, *M. esculenta* ssp. *flabellifolia* is another potential source to carry genetically modified traits. It was revealed in Central America that only five seeds (very few) formed from two crosses made between cassava and *M. aesculifolia,* a distant relative of cassava (http://books.google.com). Therefore, it is not accountable information on gene flow. The gene flow was well explained by Chavarriaga-Aguirre et al. (1999) between Mexican and Guatemalan cassava accessions using molecular markers. Based on this preliminary data, the authors concluded that there is no concrete information

about gene flow between the cultivated cassava and wild *Manihot* in the actively growing regions of cassava. Further, the biosafety issues of transgenic cassava and potential weed problems still need to be studied in-depth. It was reported by Allem (1984) that the new genes for resistance to African mosaic virus have been incorporated from *M. glaziovii* to *M. esculenta*.

8.10 Recommendations for Future Actions

To boost the role of cultivated *Manihot* as a food security and biofuel crop, there is need for increased research to improve and stabilize yields by developing genetic resistance to major pests and diseases (IITA 2000b, c, d). Part of this could be achieved through increased efforts toward collection and conservation of wild species that are reservoirs of desirable genes for resistance and tolerance to prevailing insects and diseases and also newly emerging diseases and pests owing to changing environment. The 10–12 wild *Manihot* species native to Brazil's southern (subtropical) and southeastern (Cerrado or Savanna) regions are under tremendous threat of erosion due to excessive deforestation over the last five decades for the cultivation of cash crops (http://www.fao.org/docrep/007/y2413e/y2413e0c.htm). Therefore, it is recommended that wild *Manihot* species needs protection from extinction through on-farm conservation in their natural habitat, mainly in the Caatinga region of Northeast Brazil and the Cerrado of central-west Brazil.

Furthermore, wild *Manihot* species should be collected and conserved ex situ, especially the six species most closely related to *M. esculenta*, for possible future interspecific breeding to transfer desirable traits to the cassava. The urgent need for collection and preservation of germplasm was also supported by the reports of genetic erosion by Nassar and Cardenas (1985) for extremely endangered species such as *M. walkerae, M. guaranitica, M. subspicate, M. angustiloba, M. longipetiolata,* and *M. pringlei*.

The conservation efforts should also target the group of species known as "the cassava species complex," which is composed of the wild progenitor of the crop and four other closely related species from the Brazilian tropics. Study of this complex, from the perspectives of taxonomy, biosystematics, and cladistics, will shed new light on the origin, phylogeny, and evolutionary patterns of cultivated *Manihot*. It was only through recent systematic studies of the biodiversity that the long-searched origin of cassava seems close to completion (Allem 1994, 1999; Olsen and Schaal 1999). Similarly, pest and disease resistance genes can be easily introgressed from these valuable wild species into cultivated genotypes by traditional and molecular breeding approaches (Charoenrath et al. 2006). High starch content in *Manihot* species makes it one of the best alternative candidates for ethanol production (Ziskaa et al. 2009). The technology for converting *Manihot* into biofuel ethanol source is currently being perfected and will be very soon applicable anywhere in the world (FAO 2008).

The second conservation effort should select the so-called species, maniçobas, from Northeast Brazil's semi-arid Caatinga region. Three of them in particular merit attention: *M. caerulescens* ("maniçoba do piauí," a source of cheap latex), *M. dichotoma* ("maniçoba de jequié," a minor supplier of latex and domestic utensils such as wooden spoons), and *M. glaziovii* ("maniçoba do ceará," a source of resistance to diseases and pests). The latter is a most versatile species. It provides commercially useful latex and wood, and has been studied for potential use as feed for goats and cattle in Brazil's semi-arid northeast (Ravindran 1993). Most importantly, *M. glaziovii* supplied African cassava breeders with genes for resistance to the African cassava mosaic virus and the cassava bacterial blight (Hahn et al. 1980). A more far-reaching implication is the possibility that commercial cassava stocks worldwide share much of their genome with that of the wild progenitor. Wild materials related to the ancestry of the crop, and making up the wild primary gene pool, should be in storage to enable comparative tests.

Breeding efforts to increase the yield potential and disease/pest resistance of cassava have in some cases reduced the number of commercially grown varieties and thus eliminated the use of certain farmers' landraces. However, extensive collections of *M. esculenta* varieties in the centers of origin in Latin America, and their conservation in field-grown germplasm banks, in vitro culture, cryopreservation, have helped to prevent the loss of biodiversity in *M. esculenta*.

Cassava production has had a minimal effect on the biodiversity of *Manihot* in the center of origin of the species, i.e., in Mexico and Brazil, with a possible exception of the semi-arid northeast of Brazil where intensive monocropping of cassava may threaten the survival of seven *Manihot* species native to that area. Narrowing of the genetic base of commercial cassava varieties by the use of a small number of widely-adapted, high-yielding varieties should be avoided, by the continued release of new varieties with a broad genetic background. This will reduce the risk of widespread crop failure, for example, in case of adverse climatic conditions or appearance of new diseases or pests.

Global organizations such as FAO, CIAT, and IITA actively advocating for cassava crop improvement and effective utilization of biological control agents for mealy bug and green mite in Africa are two examples of such global efforts. Global collaborative and conservation efforts will help preserve the natural diversity of cassava; enhance the development of high-yielding, disease and pest tolerant varieties; improve crop management; and post-harvest practices while avoiding duplicated efforts. To bring the fruits of these global collaborations to farmers, multiplication and distribution of the improved cassava vegetative stocks should be taken up by local, national, and international government agencies.

Cultivation of transgenic crops is rapidly spreading across the world. Accessibility of novel transgenic technology at a nominal licensing fee from commercial and academic institutions around the world to researchers would help to trap the resources from wild *Manihot* to enhance the current crop improvement efforts. Increased investment and international collaboration in global *Manihot* research will definitely help meet the global food and biofuel demand at a rapid pace.

The completion of whole genome sequencing in cassava (*M. esculenta* ssp. *esculenta*) has been a major and most significant achievement for the entire cassava research community. This will not only accelerate the development of large number of new and efficient molecular markers for the characterization of other *Manihot* species but will also enhance the use of next-generation technologies to identify genes involved in many important traits. To meet the demand for rapidly growing population and to mitigate the impact of climate change that has resulted in global food crisis, use of wild relatives and integration of newer and innovative technologies in breeding programs will accelerate the process of improving multiple traits in a more precise way and in turn improve the productivity of this major staple crop.

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Chapter 9
Miscanthus

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9.1 Basic Botany of the Species

9.1.1 Origin and Distribution

The word *Miscanthus* derives from the Greek word "michos" to designate the inflorescence (panicle and florets) and "anthos" that refers to the flower (Clifton-Brown et al. 2008). However, *Miscanthus* has other names regionally. For example in Japan, *Miscanthus* is called "Susuki" (Adati 1958).

It has been reported previously that *Miscanthus* spp. was originally native to Asia, mainly in its eastern and southeastern parts (Clifton-Brown et al. 2008). The natural geographic distribution is largely in temperate and subtropical zones, extending westward to central India and eastward to Polynesia, with a few species in Africa (Hodkinson et al. 2002c). It is also found in the boreal zone (northeastern Siberia 50°N) (Scally et al. 2001). The genus is widely adapted to various habitats, and is also able to survive at both low and high altitudes (>3,000 m) (Chou 1989), which explains its tolerance capability (Chen and Renvoize 2006). These features would have represented important advantages, which might have contributed not only to *Miscanthus* being trialed through the world, but would have facilitated the understanding of genetic × environmental interactions and allowed empirical breeding (Chou et al. 2001). Consequently, some *Miscanthus* species, mostly hybrids, have been grown throughout Europe and northern America for their ornamental value as garden plants and later as a source of paper fiber material (Hodkinson et al. 2002c; Chou 2009). Since the 1970s, the genus has been intensively studied in Europe as a biomass crop (Clifton-Brown and Lewandowski 2000; Clifton-Brown et al. 2000, 2004; Jones and Walsh 2001; Lewandowski and Schmidt 2006; Sims et al. 2006; Stamyf et al. 2007) and also in academic research programs in the United States and Botanic Garden International organization (http://www.tropicos.org). In a recent publication, Stewart et al. (2009) reviewed the ecological study of *Miscanthus sinensis* in Japan.

9.1.2 Taxonomic Position

The taxonomy of the *Miscanthus* genus was studied firstly in 1855 by Andersson (1856), thereafter followed the works of Honda (1930), then Adati (1958), Hirayoshi et al. (1955, 1957, 1959, 1960), and Adati and Shiotani (1962) in Japan. Generally, the taxonomy of the genus is complex and confusing because of the level of diversity. However, a wide range of species, hybrids, and cultivars have been identified. The cytogenetic and phylogenic studies combined distinguished a group of 14–20 species recognized by most horticulture organizations such as the Royal Horticulture Society and member of the international botanic nomenclature (Clifton-Brown et al. 2008). In spite of that, unintentionally in some cases, some *Miscanthus*

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species are classified with *Erianthus* or *Saccharum* (sugarcane) as the most allied plants due to their close similarity (Hodkinson et al. 2002c; Chou 2009). On the basis of the description of Clayton and Renvoize (1986) and Hodkinson et al. (2002c), it appears that *Miscanthus* species belongs to the Poaceae family, in a subfamily of Panicoideae placed in the tribe of Andropogoneae (Daniels and Roach 1987; Greef and Deuter 1993). Whether the *Miscanthus* genus belonged effectively to Andropogoneae tribe was unclear, because of their bisexuality (having both male and female florets) (Ibaragi 2003; Ibaragi and Ohashi 2004; Chen and Renvoize 2006). Clifton-Brown et al. (2008) opined on the bisexual nature being characteristic in the Andropogoneae tribes, with a few genera unusually male or sterile. On the basis of their long experience (since 1972), some scientists have attempted to propose an evolutionary trend in the *Miscanthus* genus (Chou and Lee 1991; Greef and Deuter 1993; Lee 1995; Chou 2009). Their works represented substantial progress taxonomically and in the nomenclature of species. Consequently, *Miscanthus* has been defined as containing the following species (Ibaragi 2003; Ibaragi and Ohashi 2004; Chen and Renvoize 2006):

- *Miscanthus floridulus* (Labill.)
- *Miscanthus intermedus* (Honda) Honda
- *Miscanthus longiberbis* Nakai
- Miscanthus lutarioparius
- *Miscanthus oligostachyus* Stapf.
- *Miscanthus paniculatus* (B.S. Sun) Renvoize and S.L. Chen
- *Miscanthus sinensis* Andress
- *Miscanthus trinctorius* (Steud.) Hack
- *Miscanthus sacchariflorus* (Maxim.) Hack
- *Miscanthus transmorrisonensis* Hayatta
- Hybrid *Miscanthus* × *giganteus* Greef and Deuter ex Hodkinson and Renvoize
- *Miscanthus sinensis* spp. *condensatus* (Hackel) T. Koyama

New entries are continuing to be added because of the large diversity within the genus so that the nomenclature is likely to be subjected to ongoing revision. However, the available collections can be exploited as basic information for future genetic resource banks or genetic improvement. Within the current collection, the most clearly distinguished species are *M. sinensis* and *M. sacchariflorus*, as well as a sterile triploid hybrid *M.* × *giganteus*, which are found primarily in China, Korea, and Japan (Jones and Walsh 2001; Hodkinson et al. 2002a, b, c). It has been reported that the hybrid was obtained from natural cross between *M. sinensis* and *M. sacchariflorus*, and that it was imported from Yokohama (Japan) by Aksel Olsen in 1935, and was subsequently adapted to many agricultural regions of Europe (Nielsen 1990) and northern America where it generated considerable interest (Scally et al. 2001; Chou 2009).

9.1.3 *The Morphology of* Miscanthus

The distinguishing characteristics of *Miscanthus* species were primarily based on morphological analysis (Adati 1958). This has been reviewed with significant progress since the first descriptions by Andersson (1856), Adati (1958), and Clifton-Brown et al. (2008). The genus is recognized as a typical tall grass. Its vertical growth ranges between 2 and 7 m in height depending on species and environment. Leaf blades (drop, erectile, thin, and thick), usually broad and sometimes narrow, 30–100 cm length and 1–4 cm wide, vary largely depending on species, position on the stem, stem type, and growth stage (Adati 1958). In general, leaf morphology decreases with plant age, and the flag leaf being the latest to emerge before the flower, is small and erectile. The leaves are supported by erect stems, on which emerges an inflorescence (panicle, 30 cm above the flag leaf) from August to September or late autumn (October) for the latest species. The panicle is also variable in terms of size. *Miscanthus* species are also recognized for their remarkable differences in tillering capacity, another important morphological trait (Adati 1958), allowing the plants to be annually harvestable at the end of season.

M. sinensis has more bunched compact shoots than *M. sacchariflorus,* which is strongly rhizomatous; and the triploid hybrid *M.* × *giganteus* shows intermediate in morphology (Fig. 9.1).

Generally *Miscanthus* produces very light and small paired spikelets, which are persistent thorough the winter (Hayashi and Numata 1971; Hayashi 1979). Spikelets are awnless for *M. sacchariflorus* (Watson and Dallwitz 1992). Many more details are mentioned in the work of Hirayoshi et al. (1957), Nakagoshi

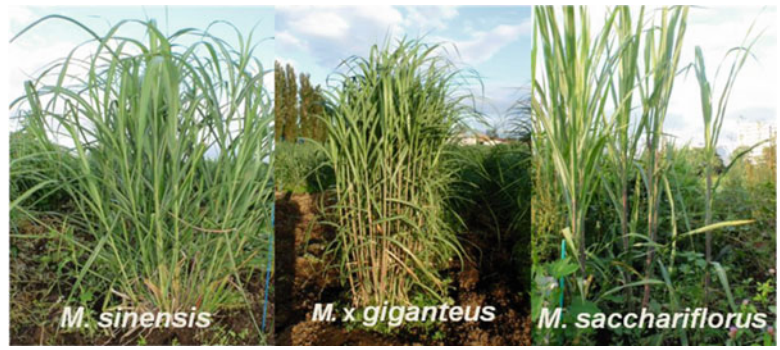

Fig. 9.1 Three different types of *Miscanthus* under a field experiment condition. Plants were grown side by side in June 2010 at the Field Science Center for Northern Biosphere, at Hokkaido University in Sapporo, Japan

(1984), and Hsu and Chou (1992). The morphological traits of *Miscanthus* are sometimes unstable for certain phenotypic traits (Lee 1964a, b, c), allowing the International Plant Name Index (IPNI) and taxonomists to wrongly describe and identify plants. *M.* × *giganteus* was wrongly called *Miscanthus sinensis* "*giganteus*" or *ogiformis*. To prevent confusion in the literature, Hodkinson and Renvoize (2001) have corrected the "name" as *M.* × *giganteus* "Greef et Deuter." Evidence from several studies in the United States and in Europe showed that *M.* × *giganteus* is the most strongly vigorous in terms of morphology. Morphological analysis has been beneficial for taxonomy by reducing synonymic problems, but it has been less exploited for genetic improvement. This is more complicated when integrating large number of species. Molecular studies and DNA sequencing may contribute substantially to the clarification of genetic backgrounds (Chou et al. 2001; Hodkinson et al. 2002a, b, c).

9.1.4 Cytology, Karyotype, and Genome Size

The genus *Miscanthus* has a series of ploidy level (Adati 1958). On the basis of cytogenetic analysis, it was revealed that *M. sinensis* is diploid, *M. sacchariflorus* is tetraploid, and the hybrid *M.* × *giganteus* is triploid, with a chromosome number of 57 (Lafferty and Lelley 1994). In attempts at genetic recombination, satellite chromosomes have been found, suggesting an allotriploid genome, which indicates an origin based upon closely related species (Głowacka et al. 2009), while the triploid character is supposed to be the major cause of species sterility (Lafferty and Lelley 1994). Other studies, in the *Miscanthus* genus showed that effective polyploidization is highly dependent on genotype. The tetraploids generated were fertile, and useful in plant breeding (Głowacka et al. 2009). Recently Rayburn et al. (2009) observed the genome size of *Miscanthus* to be consistent with expectations based upon these understandings.

9.2 Conservation Initiatives

9.2.1 Germplasm Collection and GenBank

Miscanthus is an unimproved plant, simply because of the lack of information and genetic resources required to facilitate both fundamental research and breeding work. A number of germplasm collections exist and are described within the Botanic Garden Conservation International and its satellites worldwide (http://www.bgci.org). Traditionally and empirically, the species were collected from their natural environment based on their ornamental advantage and other perceived valuables. Attempts to construct genetic banks for conservation have been mostly limited by the lack of knowledge of the reproductive biology and genetic characterization (Renvoize 2003). Germplasm conservation is mainly ex situ. Germplasm banks in the current form are collections of accessions principally in the form of rhizomes rather than seeds. Consequently, this conservation method has been criticized, because it requires much space even if it is not costly.

Since it has been recognized that plant collections provide excellent materials for research linked to many disciplines, the principal scientific uses of this material have aimed at obtaining information to help resolve problems regarding conservation, analysis of genetic diversity and population dynamics. For instance, the molecular approach was introduced to manage the conservation of genetic resources (Chou et al. 1987, 1999; Greef et al. 1997; Hodkinson et al. 2002a, b, c; Iwata et al. 2005).

9.2.2 Evaluation Genetic Erosion and Maintenance

Miscanthus, like other valuable plants such as food crops, requires conservation efforts to prevent decline in genetic diversity and ensure ability to breed for specific traits in the future. *Miscanthus* has been described as a potential non-food crop, mainly limited to three species: *M. sinensis*, *M. sacchariflorus*, or sterile hybrid *M.* × *gigantheus*. For the time being, priority is being given to evaluating the genetic resource, which has been revised and discussed thoroughly (Hodkinson and Renvoize 2001; Hodkinson et al. 2002c). Agronomically, *Miscanthus* has been evaluated as a perennial crop harvestable in long-term cropping systems (up to about 22 years after planting) (http://www.bical.net/france), for its efficiency in carbon sequestration (Hansen et al. 2004; Clifton-Brown et al. 2007), nitrogen cycling (C-FAR Biofuel Symposium 2009), and photosynthetic efficiency (Weng and Ueng 1997; Lewandowski et al. 2000). The genetic variability of the genus has also been evaluated, but in a limited number of investigations (Linde-Laursen 1993; Lafferty and Lelley 1994; Chou 2009). However, it is still difficult to speculate about the genetic erosion of *Miscanthus* while the genetic background characterization is so limited.

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

The plants most closely related to *Miscanthus* are *Erianthus* and *Saccharum* as mentioned above. In a phylogenetic study based on analysis of DNA sequences to assess the interrelationship between the presumably allied plants, Hodkinson et al. (2002a, b, c) have reported that *Miscanthus* s.l. and *Saccharum* s.l. are polyphyletic with a basic chromosome number of 19. Moreover, their results indicated that *Saccharum* species including *Saccharum officinarum* and *Saccharum spontaneum* are sister to *Miscanthus*. The multiple attractive features and the promising advantages suggest the need for more research to elucidate the genetic background and to identify more details of the interrelationships between the species.

9.4 Role in Crop Improvement

Miscanthus is an attractive plant for diverse interests. With the development of the bioenergy concept, this attractiveness has increased with particular interest for scientists and industrialists as a resource for energy security, coping with and adapting to global climate change and providing economic sustainability (Jorgensen and Muhs 2001; Heaton et al. 2004, 2008; Powlson et al. 2005; Chou 2009; Mantineo et al. 2009). The qualities most required for a suitable bioenergy crop are observed with *Miscanthus*: high and good biomass combustion quality, which depends largely on minimizing moisture (easy and safe storage), and low inorganic chemical content (ash, potassium, chloride, nitrogen, and sulfur) (Jones and Walsh 2001; Lewandowski et al. 2003). Increased attention on *Miscanthus* has resulted from the recognition that bioenergy represents an important alternative renewable energy source available but not yet fully explored. Compared with food crops, which produce first generation bioenergy by fermentation of sugars and starch, the development of bioenergy from *Miscanthus*, a non-food crop, requires an entirely different approach based upon conversion of the cell wall to bioenergy. Thus, a specific focus on genetic improvement of energy crops is needed. However, the genetic resources are largely unavailable.

Miscanthus has also been grown as a forage by selecting suitable species in some countries such as Japan. But there is no genetic diversity over large areas and the crop is totally unimproved. A germplasm collection of the parent species has been

developed with propagation by seed in some cases. This will provide a diverse germplasm collection of *Miscanthus* parent lines characterized by molecular methods, breeding capability, genetic diversity in the crop, new higher yielding and adapted lines, improved low temperature tolerance, and fall senescence characteristics.

9.5 Genomics Resource Developed

Miscanthus species are largely unimproved crops. For instance, *Miscanthus* has not been the subject of traditional genomic resource development but this is now likely to change rapidly as new techniques are applied. Breeding in *Miscanthus* is in its infancy, beginning largely in the 1990s (Clifton-Brown et al. 2008). However, simple sequence repeat (SSR) and restriction fragment length polymorphism (RFLP) from maize have been found to be transferable to *Miscanthus* (Hernandez et al. 2001). Hodkinson et al. (2002a, b, c) have used amplified fragment length polymorphism (AFLP) and intersimple sequence repeat (ISSR) markers to characterize genetic resource collections of *Miscanthus*. AFLP markers distinguished species, intraspecific taxa (varieties and cultivars), and putatively clonal material. They were also able to assess the interrelationship of the taxa, to investigate the origin of *Miscanthus* hybrid plants and to estimate the overall level of genetic variation in the collection. The AFLP markers detected a high degree of intraspecific variation and allowed subdivisions of the genetic resource collection to be made, particularly within *M. sinensis*.

9.6 Scope for Domestication and Commercialization

Until recently, some *Miscanthus* species were mainly used as ornamental garden plants and had a potential market in Europe and North America, and in some cases for thatching and animal feed in Asia. Currently, the main potential economic use of *Miscanthus* is for energy (biofuel) production due to its high biomass potential (Heaton et al. 2008). Clifton-Brown et al. (2008) reported that breeding programs at the Aberystwyth University in the UK and Plant Research International in the Netherlands was initiated in 2004 and are focusing on improvement of *M. sinensis* and using their *M. sinensis* selections to develop improved versions of *M.* × *giganteus*. A German breeding program led by M. Deuter at Tinplant was established in 1992 and released two cultivars of *M.* × *giganteus* in 2006, viz. "Amuri" and "Nagara" (http://www.tinplant-gmbh.de/). Although the studies on genetics and breeding for this crop are very recent, many early priorities are domestication of the traits, for which information is available in sorghum and/or sugarcane and for which the locations of controlling genes or quantitative trait loci often correspond across divergent grasses. The major problem with improving *Miscanthus* species is the complexity of its genetic resource and the fact that the productive hybrid *M.* × *giganteus* is sterile. Interest in this species is high as a source of biomass for its high cellulose content that is useful for conversion into biofuel. Current research efforts on domestication of *Miscanthus* focus strongly on selection for high potential biomass production, based on empirical selection.

9.7 Some Dark Sides and Their Addressing

Miscanthus does not have only positive and beneficial interest depending on location. Some species are considered to be invasive and aggressive weeds in the United States (Darke 1999). In the UK, a study from the Wales Biomass Centre (Cardiff University Llysdinam Field Centre, Newbridge-on-Wye, Llandrindod Wells, Powys, D1 6AS) and Missouri Botanical Garden (2009) has pointed out some negative impacts of *Miscanthus* on the environment.

Miscanthus is a perennial crop, and plant stand can be maintained for 20–25 years. Like short-rotation coppice (SRC) willow, this may create a visual impact on the rural landscape. Therefore, when selecting sites for *Miscanthus*, one should take into account the landscape esthetics and public foot path access, as well as local archeology.

There have been some concerns whether *Miscanthus*, as an introduced species, might be an

invasive plant. If so, this is not a problem because most *Miscanthus* varieties used for biomass production are sterile hybrids. To date, there is no evidence that *Miscanthus* is invasive. Even so, it has been shown that *Miscanthus* is easy to get rid off by harvesting the rhizomes using a modified potato harvesters or killing the crop using glyphosate herbicides.

On the ecologogical impacts of *Miscanthus*, canopy cover by mature *Miscanthus* is dense at the late stage of its growing period. Consequently, the number of other wild animal species such as skylarks and lapwings decline with time from late spring to early autumn.

Miscanthus leaves are not palatable to insects, and as such most invertebrate populations are dependent on the weed vegetation within the crop. Therefore, if *Miscanthus* fields are kept weed-free at all times, their effects on invertebrate population is bound to resemble that of arable crops.

9.8 Recommendations for Future Actions

Miscanthus species has a long tradition of use. They have been used for different purposes. The rapid increase in consideration of these species demonstrates that their potentialities have not yet been fully explored. However, based on the scientific literature, genetically, *Miscanthus* remains an unknown crop. This demonstrates the difficulties of advancing *Miscanthus* improvement. In order to fully and efficiently exploit the potential of *Miscanthus*, priorities need to be adopted for future research in relation to bioenergy uses. Bioenergy production is likely to depend essentially upon cellulosic biomass; thus *Miscanthus* will play a potentially important role as a major feedstock because of suitability economically, agronomically, and in the ability to respond to various environments. It is necessary to evaluate *Miscanthus* in this context.

9.9 Genetic Background for Better Utilization

Germplasm of *Miscanthus* has been collected. However, genetically the background remains unclear so that it is difficult to predict the potential for utilization of this material. It will, therefore, be helpful to clarify the genetic origin of existing collections. This is possible by exchanging materials from different research centers (Botanic Garden Conservation International, BGCI-UK) under international guidelines. This will help constructing a gene bank in order to avoid erosion or loss of materials.

An agronomic point of view is also crucial to ensure the genetic potential of *Miscanthus* is realized. Growing *Miscanthus* as an ornamental garden addition may not need any specific advanced technology. However, the use of *Miscanthus* as an energy crop will need more consideration of the need for specific individual traits. Identification of these agronomic priorities may guarantee and reinforce the strength of *Miscanthus* utilization (Schwarz et al. 1994). Generally, *Miscanthus* requires low input of nutrients, is efficient in carbon sequestration, and has efficient photosynthesis to convert radiation and nutrients captured to deliver high biomass production. The crop stand and productive period can be extended up to 20–25 years. To increase the economic gain in terms of biomass, Jorgensen (1997) suggested harvesting in spring, while Lewandowski et al. (2003) suggested it may be optimal in autumn. As *Miscanthus* is becoming an important crop, particularly for its biomass yield, several approaches are being tested to predict long-term biomass yield in Europe (Clifton-Brown et al. 2004; Stamyf et al. 2007; Clifton-Brown et al. 2008).

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Chapter 10
Saccharum

G.D. Bonnett and R.J. Henry

10.1 Basic Botany of the Species

Plants of the *Saccharum* genus have been used for thousands of years as a source of sweetener. The genus is placed in a group of genera often described as the *Saccharum* complex (Mukerjee 1957) within the Andropogoneae tribe of the Poaceae family.

10.1.1 Taxonomic Position

The *Saccharum* genus has been in existence since it was established by Linnaeus in 1753 (Mukerjee 1957). The genus has been subjected to frequent revision, often between the genera included in the *Saccharum* complex (Whalen 1991). There is not yet consensus to how many species the genus comprises. At one extreme, Irvine (1999) based on a thorough analysis of cytology, morphology, and history condenses the genus to two species, *S. officinarum* and *S. spontaneum*. The remainder of the five (Whalen 1991) or six (Daniels and Roach 1987; Alfonse Amalraj and Balasundaram 2006) species accepted by these other authors are included by Irvine (1999) in *S. officinarum* L. (Table 10.1). At the other extreme in common usage are classifications that incorporate a larger number of species, e.g., http://plants.usda.gov. In this classification, the plume grasses, which other authorities include in the genus *Erianthus* are included in *Saccharum,* a situation that was acknowledged by Daniels and Roach (1987) who stated most grass taxonomists include *Erianthus* under *Saccharum*.

The species currently classified as *S. spontaneum* have been subdivided in the past. Hackel (1889) divided *S. spontaneum* into three subspecies based on geographical origin, *indium*, *aegyptiacum,* and *luzonian* from India, Africa, and Asia, respectively. While the use of subspecies has not been continued by taxonomists, the terminology has sometimes been used, even at the species level (e.g., *Saccharum aegyptiacum* Willd; Poljakoff-Mayber 1959).

In this chapter, the germplasm contained in the six species of Daniels and Roach (1987; Table 10.1) will be discussed as this is the range of material available for future genetic improvement of sugarcane within the genus *Saccharum*, irrespective of how it is divided internally. Other genera of the *Saccharum* complex, *Erianthus* (Jackson and Henry 2011) and *Miscanthus* (Anzoua et al. 2011) are covered in separate chapters of this volume.

10.1.2 Geographical Distribution

The distribution of the *Saccharum* genus is contributed most significantly by *S. spontaneum*. This species and *S. robustum* are the only two species found growing outside of cultivation (Irvine 1999). Mukerjee (1957) showed the range of the genus extending between the equator and 40°N from central North Africa through to Far East Asia and then around the equator through New Guinea. Panje and Babu (1960) reported the range of *S. spontaneum* extending further into West Africa and in the east of the distribution to the islands

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Table 10.1 Species included in the genus *Saccharum* by various authors

Authors	Species accepted in *Saccharum*
Alfonse Amalraj and Balasundaram (2006)	*barberi, edule, officinarum, robustum, sinense, spontaneum*
Brandes (1958)	*barberi, officinarum, sinense, spontaneum*
Daniels and Roach (1987)	*barberi, edule, officinarum, robustum, sinense, spontaneum*
Irvine (1999)	*spontaneum, officinarum*
Whalen (1991)	*officinarum, robustum, edule, spontaneum, sinense*[a]

[a]Whalen (1991) groups *S. barberi*, under *S. sinense*

on the edge of the Philippines Sea and Fiji. Panje and Babu (1960) also mapped the different cytotypes of *S. spontaneum*, showing clear geographical differences, lower chromosome numbers being concentrated in India and central Asia with higher chromosome numbers at the western and eastern ends of the range.

S. officinarum is thought to have originated in New Guinea, the Malayan Archipelago, or the Melanesian and Polynesian islands (Mukerjee 1957). Bhat et al. (1962) suggest a narrower distribution of New Guinea and a few adjacent islands. The origins of both *S. sinense* and *S. barberi* seem to be accepted as being from hybrids of locally cultivated *S. officinarum* and locally growing *S. spontaneum* in Southeast China and Northwest India, respectively (Daniels and Roach 1987) – a view supported by analysis of more recent diversity studies based on differences in DNA (D'Hont et al. 2008). *S. robustum* has been reported in the highlands of Mindanao, Philippines as well as New Guinea (Naidu and Sreenivasan 1987). Roach (1972) reports the range of *S. edule* to include Fiji, New Guinea, Indonesia, and Malaysia.

The geographical range of *Saccharum* has been extended (see Sect. 10.9) as a result of commercial production and the movement of non-commercial species to support breeding efforts.

10.1.3 Morphology

The grasses of the *Saccharum* genus are tall, up to 10 m, clumping grasses that are perennial. New culms develop from the base of the clump. The inflorescences of the genus have a characteristic arrow shape and is the organ providing the main morphological characteristics for taxonomy. The swollen, abortive inflorescence consisting of undeveloped floral primordia is used to separate *S. edule* from the other species (Whalen 1991). *S. robustum* and *S. officinarum* are separated from *S. spontaneum* and *S. sinense* (Whalen 1991) and *S. barberi* (Alfonse Amalraj and Balasundaram 2006) by (1) the lack of long hairs on the principal axis (rachis) of the inflorescence; (2) the sessile spikelet flowering before the pedicellate one; and (3) a reduced or lack of lemma of the fertile floret. The hairs on the lodicules of *S. spontaneum* separate it from the non-cillate lodicules of *S. sinense*. The three-nerve venation of the second glume of *S. robustum* separates it from *S. officinarum,* which only has one nerve (Whalen 1991).

The most distinctive vegetative feature of the genus is the rhizomes of *S. spontaneum*, which also has generally thinner stalks. Alfonse Amalraj and Balasundaram (2006) separate *S. berberi* with gray green/white or ivory canes and narrow leaves from *S. sinense* with green-bronze canes and broad leaves. Whilst many of the characters overlap, a feature of *S. officinarum* is the higher levels of sucrose in its culms. Whilst Whalen (1991) and Alfonse Amalraj and Balasundaram (2006)'s keys are based on Brandes (1958), Brandes' key only separated four species compared to five and six in the more recent keys, respectively.

10.1.4 Cytology and Karyotype

All of the genotypes of the *Saccharum* genus that have been characterized are polyploids (Table 10.2). The basal chromosome number of *S. spontaneum* is 8 and *S. officinarum* 10 (Piperidis et al. 2010). These basal numbers have been suggested by cytology and been confirmed by mapping the ribosomal RNA genes to particular chromosomes using fluorescent techniques (D'Hont et al. 1998). In addition, when the linkage groups within two of the homology groups of a map of a commercial cultivar are examined there are some large linkage groups as well as two sets of smaller linkage groups that line up to either end of the larger

Table 10.2 Karyotype of *Saccharum* species

Species	Range of chromosome number	Most common number	Reference
S. barberi	82–124	–	Bhat et al. (1962)
S. edule	60, 70, 80	70	Roach (1972)
	80	80	Bhat et al. (1962), Sreenivasan et al. (1987)
S. officinarum			
S. robustum	60–84[a]	60, 80, 84	Bhat et al. (1962)
S. sinense	116–120	–	D'Hont et al. (2008)
S. spontaneum	40, 48, 56, 64, 72, 80, 96, 104, 112, 120, 128	–	Panje and Babu (1960)

[a]Numbers up to 194 have been reported but these are considered hybrids (Sreenivasan et al. 1987)

ones (Aitken et al. 2005). The larger linkage groups are thought to derive from the *S. spontaneum* ancestry and smaller ones from *S. officinarum* linkage groups. Whilst the basal number would imply that a polyploidy series with increases in chromosome number incremented by 8 or 10, because of aneuploidy this is not always observed, particularly among the commercial hybrids. The number of chromosomes within any particular species varies; the numbers not divisible by the basal number of *S. barberi* (reviewed by Bhat et al. 1962) and *S. sinense* underlie the hybrid nature of these species and the frequent occurrence of aneuploidy. Roach (1972) tried to clarify earlier discrepancies in chromosome number in *S. edule* and among the nine clones he studied and found chromosome numbers of 60, 70, and 80. *S. spontaneum* has a very variable chromosome number and this variation has been mapped to geography by Panje and Babu (1960). *S. officinarum* is often the most dogmatically described species in relation to chromosome number. Deviation from $2n = 80$ is taken as aneuploidy when the chromosome number is less than 80 or as evidence of a hybrid, if chromosome number is greater than 80 (Bhat et al. 1962; Nair and Ratnambal 1978; Sreenivasan et al. 1987). There is more agreement that *S. robustum* has multiple cytotypes (Bhat et al. 1962) but individuals with very high chromosome number are assigned as hybrids (Sreenivasan et al. 1987).

10.1.5 Genome Size

Using flow cytometry, and various internal standards, the size of the genomes of several *Saccharum* species and hybrids have been estimated by Arumuganthan and Earle (1991) (Table 10.3) as part of a study of 100 plants. When arranged in ascending order of genome size *Arabidopsis* had the smallest genome (145 Mbp/1C) with the *Saccharum* species sampled amongst the 20 largest genomes in the study. If these 1C values are multiplied by the level of ploidy they move closer to the largest genomes. These figures are within the range of DNA content measured in a similar manner for a selection of individuals from interspecific crosses with 3.05–8.91 pg corresponding to a genome size between 1,474 and 4,298 Mbp (Edme et al. 2005). The estimates may be relatively close to the actual genome size, as the genome size of *Arabidopsis* listed on the *Arabidopsis* Information Resource web site (http://www.arabidopsis.org/portals/genAnnotation/gene_structural_annotation/agicomplete.jsp#status) is estimated at 134.6 Mbp of which 120 Mbp has been sequenced and assembled.

Table 10.3 Estimates of the genome size of *Saccharum* species and interspecific hybrids

	Arumuganathan and Earle (1991)	
Species	pg/2C	Mbp/1C[a]
S. barberi	6.54–8.54	3,156–4,121
S. edule	–	–
S. officinarum	5.28–7.47	2,547–3,605
S. robustum	6.53	3,151
S. sinense	8.67	4,183
S. spontaneum	–	–
S. berberi × *S. spontaneum*	6.12	2,953

[a]Unreplicated haploid genome of the species

The size of the genome of the most closely related relative that has been sequenced, sorghum, is 730 Mbp (Paterson et al. 2009).

10.1.6 Agricultural Status

The tribal use of *S. officinarum* as a food source is widely acknowledged (e.g., Warner and Grassl 1958). These authors also provide photographic evidence of *S. robustum* being used as fencing material and inflorescences of *S. edule* in markets in New Guinea. The weediness potential and actuality of *S. spontaneum* is addressed in Sect. 10.9.

10.2 Conservation Initiatives

10.2.1 Germplasm Banks

A description of the history of germplasm collection, maintenance, and use related to sugarcane has been given by Berding and Roach (1987). They describe the world collections and collecting expeditions undertaken to 1977 and describe the problems of germplasm maintenance. Naidu and Sreenivasan (1987) give a detailed account of more recent collecting expeditions. He et al. (1999) describe the collection of material from the *Saccharum* complex in China between 1985 and 1993. From 1929, seeds as well as clonal material were being collected (Naidu and Sreenivasan 1987). The numbers of *Saccharum* clones currently held in these collections at Miami, USA and Kannur, India are given in Table 10.4. Many other countries and breeding entities within countries also have collections of *Saccharum* germplasm and some of these are also given in Table 10.4.

The characterization of these collections continues. Subsets of the collection based in the USA have been characterized phenotypically for yellow leaf virus infection (Comstock et al. 2005) and sugar content (Tai and Miller 2002). The *S. spontaneum* accessions in the Chinese collection have been studied to determine the differences in flowering time, mode of reproduction, and Brix (He et al. 1999).

Some of the issues raised by Berding and Roach (1987) about the maintenance of clonal material were highlighted by the loss of clones from the world collection in the USA as a result of hurricane Andrew in 1992 (Comstock et al. 1996). These problems may be overcome, at least in part, by preserving the genetic material as seeds (Tai et al. 1994, 1999; Prasad and Balasundaram 2006). Although Tai et al. Tai et al. (1999) were successful in storing seeds generated from 66% of the *S. spontaneum* accessions in the collection: genetic variation will be lost if seed cannot be collected from all genotypes can be coaxed into producing seed genetic material could still be lost. This can in part be addressed by identification of a set of genotypes that encompass a significant proportion of the variation and concentrate conservation efforts on this core set. Tai and Miller (2001) using a combination of geographical origin and morphological characters identified a core set of 75 *S. spontaneum* clones.

Table 10.4 Numbers of accessions of each species in the *Saccharum* genus held in major collections of sugarcane germplasm around the world

	USA	India	China		Brazil		Australia
Species	Miami[a]	Kannur (ISSCT)[b]	Hainan[c]	Yunnan[c]	CTC	RIDESA[d]	BSES[e]
S. barberi	57	43	13	5	–	5	–
S. edule	22	[f]–	–	–	–	1	5
S. officinarum	733	764	38	15	568[g]	50	214
S. robustum	127	145	12	3	–	9	17
S. sinense	62	29	17	19	–	7	4
S. spontaneum	620	67[h]	572	610[i]	–	3	93

[a]Determined by searching species name at http://www.ars-grin.gov/cgi-bin/npgs/html/crop.pl?101 (28 April 2009)
[b]Listed at http://sugarcane-breeding.tn.nc.in/genresources.htm (28 April 2009)
[c]Document handed to the senior author 1999
[d] http://www.fapesp.br/pdf/bioen1903/Bioen_Vieira.pdf – presentation given by Prof Marcos A. Sanches Vierira
[e]Personal communication N. Berding on 24 June 2009
[f]None in the table but 16 accessions listed as part of a 1985 catalog on sugarcane genetic resources II by TC Ramana Rao et al. cited at http://sugarcane-breeding.tn.nic.in/genresources.htm
[g]Number of *S. officinarum* clones sent to Copersucra from US-based world germplasm collection (Comstock et al. 1995)
[h]Additional 398 *S. spontaneum* clones listed as part of a separate Indian collection and 598 listed at Coimbatore
[i]He et al. (1999) list the number of *S. spontaneum* clones maintained as 398

An alternative to storing seed would be to store pollen. In studies of pollen of *S. spontaneum*, pollen was stored frozen to allow crosses between non-synchronous parents (Tai 1988). Pollen stored after drying to reduce moisture content proved viable after being frozen for 129 days. Longer term cryopreservation would be required to provide a germplasm bank. Clones can potentially be stored vegetatively in tissue culture. In order to achieve this, changes induced by tissue culture (Vickers et al. 2005) would have to be overcome.

Further collection of more *S. barberi* and *S. sinense* in India, in particular, has been proposed because of the reduction of the habitat where they are growing (Naidu and Sreenivasan 1987).

10.3 Role in Elucidation of Origin and Evolution of Sugarcane

An analysis of the variation in DNA sequence conducted by many authors has been assessed by D'Hont et al. (2008). They conclude that *S. officinarum* and *S. edule* have evolved from *S. robustum* and that *S. sinense* and S. *berberi* were hybrids of *S. spontaneum* and *S. robustum*.

Traditionally villagers in New Guinea and surrounding islands grow *S. officinarum* as a source of chewing canes. The movement of *S. officinarum* and *S. robustum* has been charted from historical data and was reproduced by Daniels and Roach (1987). The movement of these canes with high sugar to India and China gave the opportunity for hybridization with local *S. spontaneum,* leading to the establishment of *S. berberi* and *S. sinense* (500–100 BC and 300–500 AD, respectively). This derivation is supported by genomic in situ hybridization (GISH) analysis (D'Hont et al. 2002). These species formed the basis of sugar production in cottage industries until in the sixteenth century they were displaced by selected clones of *S. officinarum* (Roach 1989).

Unlike crops such as wheat that have undergone domestication over thousands of years, modern sugarcane cultivars have only been developed in the last 120 years. Consequently, the development of the modern hybrids that cane sugar industries are based upon is well recorded.

The observation that stimulated breeding activities was the growth of seedlings from true seed of *S. officinarum*. This was first recorded in Barbados in 1858 (Parris 1954). However, the significance of the observation that sugarcane flowers could produce fertile seeds seems to have been lost until the independent rediscovery by Soltwedel in Java and Harrison and Bovell in Barbados in the 1880s (Bremer 1923). This then led to the use of *S. officinarum*, as a female parent for crossing with *S. spontaneum*. The hybrids produced have become the basis of the sugar industries around the world ever since, gradually replacing clones of *S. officinarum* by the 1960s (Arceneaux 1967). The analysis of Arceneaux (1967) showed that relatively few *S. officinarum* clones had given rise to most of the important cultivars. Roach (1989) took this analysis a step further, determining that only 19 gametes of *S. officinarum*, two each of *S. barberi*, *S. sinense,* and *S. robustum,* and only a few *S. spontaneum* clones had been used to generate the world's hybrid cultivars in use to that time.

Analysis of the chloroplast and mitochondrial genomes also supports a narrow derivation of cultivars (Al-Janabi et al. 1994). Takahashi et al. (2005) were able to separate the six species of *Saccharum* on the basis of the chloroplast genome but the differences were small indicating a separation of *S. officinarum* and *S. spontaneum* only 730–780,000 years ago.

It is interesting to note that *S. robustum* has given rise to cultivars used only in Hawaii (Arceneaux 1967). Since 1989 several cultivars have been released as a result of more recent introgressions. For example in Australia, an introgression process started by Roach in the 1960s has now yielded several cultivars with the *S. spontaneum* clone Mandalay in the background (Reffay et al. 2005). In India under a US PL480 program, several Indian clones of *S. spontaneum* were used in an introgression program between 1966 and 1973 that has led to the release of several cultivars (Naidu and Sreenivasan 1987). These authors also indicate an on-growing program of introgression.

D'Hont et al. (2008) summarized the available work on genome composition and concluded that 15–25% of the chromosomes of modern cultivars are derived from *S. spontaneum* via whole chromosomes and chromosomes that have recombined with *S. officinarum*.

10.4 Role in Development of Cytogenetic Stocks and Their Utility

Aneuploids are common, particularly in *Saccharum* clones that are hybrid in origin. However, the highly polyploid nature of the *Saccharum* genome reduces the utility of a clone with a missing chromosome for genetic studies because there are so many other chromosomes within the homology group still present. Consequently, cytogenetic stocks of any species of *Saccharum* have not been developed.

Haploids of *S. spontaneum* have been produced from anther culture (Fitch and Moore 1983). A doubled haploid produced this way has been used as a parent in a cross to produce a *S. spontaneum* mapping population (Da Silva et al. 1995).

10.5 Role in Classical and Molecular Genetic Studies

The high ploidy displayed in the *Saccharum* genus makes classical genetics more complex. Very few traits have been demonstrated to be controlled by a single allele of a single gene. The most studied exception is the *Bru1* gene that confers resistance to brown rust (Daugrois et al. 1996). However, the complexity of the genome means that the actual gene has yet to be cloned (Cunff et al. 2008). It is more usual for traits to be quantitative in nature and, where they have been linked to markers, each individual marker is associated to loci that contribute a small proportion to the trait.

Genetic maps have been constructed for several of the species of *Saccharum*. *S. spontaneum* maps were the first developed by Al-Janabi et al. (1993), Da Silva et al. (1993, 1995), Ming et al. (1998), and more recently by Alwala et al. (2008). This has been followed by maps of *S. robustum* (Guimaraes et al. 1999) and *S. officinarum* (Mudge et al. 1996; Ming et al. 1998; Guimaraes et al. 1999; Aitken et al. 2007a; Alwala et al. 2008).

These maps have been used to dissect traits influencing sucrose content (Ming et al. 2001, 2002; Hoarau et al. 2002; Aitken et al. 2006) and yield (Hoarau et al. 2002; Aitken et al. 2008). The consensus from these studies is that there are many quantitative trait loci (QTL) accounting for a small proportion of the variation for any particular trait. This makes it difficult to use these markers to introgress traits of importance from clones of basic germplasm. Because of the polyploid nature of the genomes and the restriction to use only simplex and duplex markers not all loci of the homology groups are tagged. Attempts to increase the number of alleles that can be mapped by cloning all alleles of a sucrose phosphate synthase gene and looking for unique primer sequence have been unsuccessful (McIntyre et al. 2006).

More effort recently has been made in attempting to map individual genes. It is hoped that coding regions will underlie QTL and that any polymorphisms in these coding regions may be closer to the polymorphism causing the variation or actually contain the polymorphism. Sequence related amplified polymorphisms (Alwala et al. 2008) and single nucleotide polymorphism (SNP) discovery in genes of interest using 454 sequencing in a cultivar (Bundock et al. 2009) are both attempts to place markers in gene-rich regions.

The complex genetics of *Saccharum* means that dissecting the contribution of all alleles involved in a cross is not yet possible. The first step in attempts to identify all alleles of particular genes through sequencing (McIntyre et al. 2006; Bundock et al. 2009) has followed the use of expressed sequence tag (EST) sequences to identify allelic variants in the alcohol dehydrogenase genes (Grivet et al. 2003). When dosage of particular alleles can be accurately measured, unraveling the contributions of alleles and the effect of their combination may be possible.

10.6 Role in Crop Improvement Through Traditional and Advanced Tools

As described earlier, the plants from the *Saccharum* genus that have formed the basis of the world's cane sugar production have changed over time. As well as the discovery of true sugarcane seedlings, the impetus behind the initial *S. officinarum* × *S. spontaneum* hybridizations was the susceptibility of the *S. officinarum* clones used for sugar production to Serah disease. The resistance to Serah and mosaic diseases as well as the strong root system of the hybrid Kassor was inherited by *S. spontaneum* (Naidu and

Sreenivasan 1987). Bremer (1923) describes much of the early hybridization work that led to the modern cultivars. Many characteristics have been claimed to have been introduced from *S. spontaneum* to the nobilized hybrids including ratooning ability, resistance to abiotic stress (drought and waterlogging) but the actual experimental data to prove these claims is much less frequently presented. The wide range of abiotic stress tolerance claimed for *S. spontaneum* is plausible given both the large geographical range and consequently environments it has evolved in together with the large genetic diversity identified (Panje and Babu 1960; Mary et al. 2006).

10.6.1 Interspecific and Intergeneric Crosses

The crosses between *S. officinarum* and hybrids grown for sucrose content with other species and genera have recently been reviewed by Bonnett et al. (2008). In addition to the fertility within the genus, wide crosses with *Sorghum bicolor* have been verified (Nair et al. 2006) as have other crosses with genera within the *Saccharum* complex; *Miscanthus* (Alix et al. 1999); *Erianthus arundinaceus* (D'Hont et al. 1995; Cai et al. 2005); and *E. rockii* (Aitken et al. 2007b). These studies were primarily conducted to demonstrate that these crosses are possible. The transfer of a specific trait from these genera into a commercial background has yet to be achieved and may be hindered by variation in the ability of clones with desirable traits to produce hybrids. The motivation for crosses with *Sorghum* has been to move the early maturing trait from *Sorghum* to *Saccharum* and resistance to shoot-fly from *Saccharum* to *Sorghum* (Gupta et al. 1978).

The generation of these hybrids has used the cytoplasm of *S. officinarum*. There are two notable exceptions. The first is the production of a few hybrids from *Sorghum bicolor* as a female receiving *S. officinarum* pollen (Nair 1999), though these individuals showed poor growth and have not been developed further. The second is the production of *S. spontaneum* × *S. officinarum* crosses to generate hybrids with the *S. spontaneum* cytoplasm that can be used in breeding (Pan et al. 2004, 2006). The benefits of this cytoplasm compared to the one derived from *S. officinarum,* if any, have yet to be determined.

10.6.2 Genetic Transformation

The development of sugarcane tissue culture, transformation of cells, and regeneration of transgenic sugarcane has recently been reviewed (Lakshmanan et al. 2005). Whilst stably transformed sugarcane plants that can pass on the introduced gene to the next generation (Butterfield et al. 2002) have been produced for a variety of traits (Lakshmanan et al. 2005) no commercial releases have been made. There is considerable research continuing in this area and nearly all of this involves the use of commercial hybrids to provide the explant. These are variable in their ability to proliferate in tissue culture and then regenerate into new plants. A notable exception has been the transformation of Badila, a *S. officinarum* clone, in China (Xu et al. 2008). The generation of agronomically superior hybrids for sugar production would suggest that even for non-sucrose applications, genetic modification of hybrids through transformation is more likely than development and deployment of clones based on *S. officinarum* or *S. spontaneum*.

10.7 Genomics Resources Developed

10.7.1 Genomic Resources Available

Whilst the most similar genome sequence to sugarcane available is sorghum (Paterson et al. 2009), more tools are becoming available, though most of these are based around commercial hybrids of sugarcane rather than wild relatives. Synteny between sorghum and sugarcane has been used in mapping studies (Dufour et al. 1996; Ming et al. 1998) and it is possible for gene identification and cloning to be assisted by reference to sorghum (Jannoo et al. 2007).

Microsatellite or simple sequence repeat (SSR) markers have been developed as a resource for the *Saccharum* species with support of the International Sugarcane Biotechnology Consortium (ICSB). SSRs developed in *Saccharum* were found to be transferable

to related genera such as *Erianthus* (Cordeiro et al. 2001). As these SSRs are mapped in sugarcane, they will form an increasingly dense bridge between the genetic maps developed in sugarcane and the physical sequence of the sorghum genome. This will allow exploration of the genes underlying QTL.

A very large number of *Saccharum* ESTs have been reported (Casu et al. 2010). Several groups around the world have contributed to the ESTs currently available for *Saccharum*. The Brazilian SUCEST project (Vettore et al. 2003) is the largest contributor providing over 265,000 ESTs. Other contributions have been made by Carson and Botha (2000), Casu et al. (2003, 2004), and Ma et al. (2004). In addition, some genomic sequences and sequences of bacterial artificial chromosomes (BACs) have been reported. The naming of these gene sequences in public databases has proven difficult because of different approaches to naming of *Saccharum* species (Casu 2010).

The variation in *Saccharum* gene sequences at the SNP level has been determined by deep sequencing of large numbers of amplicons using the 454 sequencing platform (Bundock et al. 2009). *S. officinarum* had one SNP every 58 bp while a modern cultivar (Q165) had an SNP every 35 bp probably due to the presence of chromosomes from the *S. spontaneum* genome.

The availability of these sequences has led to the development of tools for expression analysis. These experiments have been conducted on macroarrays (Carson and Botha 2002; Carson et al. 2002), spotted cDNA microarrays (Casu et al. 2003, 2004, 2005), and more recently on oligonucleotide arrays on the Affymetrix platform (Casu et al. 2007; McCormick et al. 2008). An alternative approach being developed to increase the coverage of particular classes of genes is under development (GM Souza personal communication). This is based on an array using longer features developed on the Agilent platform.

Whilst a complete genome analysis to develop more advanced tools such as whole transcriptome or genome tiling arrays is being considered (Paterson et al. 2010), progress towards the shotgun sequencing of the *S. officinarum* progenitor is underway (R Ming et al. unpublished), *S. spontaneum* (RJ Henry et al. unpublished) and for a commercial hybrid (GM Souza et al. unpublished) as part of an international sugarcane genome sequencing collaboration.

There have yet to be reports describing an analysis of the regulome of small RNAs or the proteome.

10.7.2 Metabolites

Attempts to describe the soluble metabolome of sugarcane and its relatives are underway and producing early results. This area has recently been reviewed by Watt et al. (2010) and in the area of measurement of multiple components in a single pass, 120 metabolites have been measured at different stages of development (Glassop et al. 2007).

10.8 Scope for Domestication and Commercialization

10.8.1 Biomass and Bioenergy

The most active area of domestication of the *Saccharum* complex for non-sucrose purposes is for biomass or "energycane" crops. In Europe and North America, much effort has been made to evaluate *Miscauthus gigantus* as a biomass crop (Anzoua et al. 2011). This is allied to the development of second-generation biofuels based on the conversion of lignocellulosic material to simple sugars and then to ethanol or other liquid fuels. The problem with improving *Miscanthus* is that the plant utilized is a sterile hybrid (where described) so alternative approaches have been evolving around current sugarcane systems. The domestication of sugarcane and the plant breeding since has focused strongly on selection for accumulation of high levels of sugar (sucrose) in the culms (Henry 2010). Initial interest in the use of sugarcane as an energy crop arose during the oil crisis of the 1970s (Grassl 1980). Renewed interest in bioenergy production more recently has reinforced a greater emphasis on selecting for total plant biomass (Wang et al. 2008). First-generation biofuels have been produced from sugar but second-generation biofuels are desirable since this will also include the conversion of the cell wall to additional fuel. The conversion of

soluble sugars to energy may always be more efficient but the importance of sucrose content will decline as the efficiency of second-generation fuel production increases. Sugar accumulation in the stem reaches an apparent limit as sucrose content has not been improved by recent breeding efforts (Jackson 2005), while carbon stored as cell walls or other insoluble polymers does not have such limitations (Botha 2009). Consequently, some of the attributes of *S. spontaneum* have been explored in early generation hybrids to provide material relatively high in soluble sugars (sucrose plus reducing sugars) but with much greater biomass yields (Wang et al. 2008). Crosses between *Miscanthus* and *Saccharum* "Miscane" are being suggested as potential energy crops (Jacob et al. 2009).

10.8.2 Other Uses

In addition to planting *S. spontaneum* for harvest in an agricultural setting, it has also been identified as having properties suitable for stabilization of slopes (Devkota et al. 2008). The characteristics of relatively high number of roots, root longevity, and resistance to pulling force led these authors to propose *S. spontaneum* as one of several species that could contribute to a bioengineering solution to landslides along roads in Nepal. In Australia, *S. spontaneum* has been established to stabilize river banks and drainage ditches (Bonnett et al. 2008). Whilst there are potential benefits here they would have to be weighed against potential weediness issues discussed below.

There are many uses of by-products from *Saccharum* around the world (Allen et al. 1997). Whilst utilizing the biomass for energy is receiving much attention it has also been demonstrated that new compounds can be made in sugarcane such as alternative sugars (Fong Chong et al. 2007; Wu and Birch 2007) or plastic precursors (Mcqualter et al. 2005). Whilst Other potential uses of *Saccharum* fibrr after chemical modification is an active area of research (Kaith et al. 2009). The production of furfural from sugarcane bagasse is an example of chemical production from *Saccharum* that is now commercial (Watson and Conners 2008). As the world turns to chemicals from biomass as well as energy as oil runs out, there will be more interest in utilizing biomass from plants such as *Saccharum* in different ways.

10.9 Some Dark Sides and Their Addressing

Commercial hybrids, *S. officinarum* and *S. edule*, the domesticated groups of species, are not reported to persist outside of cultivation. However, *S. spontaneum* is frequently rhizomatous and, even within countries it is native, is reported as a weed (Panje 1970). Naidu and Sreenivasan (1987) report the observation that, due to land changes in northern India, the diversity of *S. spontaneum* is reducing and only the forms that are weedy can survive in the rice fields of the area. *S. spontaneum* is listed as a weed in 33 countries (Holm et al. 1997). To prevent spread where it has been introduced for breeding and resides in germplasm gardens, it is often planted within an impenetrable cylinder. These conditions are among those imposed for the growing of *S. spontaneum* in the world collection in the USA because of the classification of *S. spontaneum* as a noxious weed in the USA (Tai and Miller 2001). However, it has established outside of cultivation in several countries such as Panama (Hammond 1999) and Australia (Bonnett et al. 2008).

In Panama, the extent of the invasion of land previously devoted to pasture is not documented but the problem is sufficiently great to have provoked studies into both the effect upon natural regeneration of rainforest tree species (Hooper et al. 2002) and on how to control *S. spontaneum* during establishment (Craven et al. 2009; Kim et al. 2008).

The weediness potential of *S. spontaneum* and the ability to hybridize with commercial canes means that its presence in the environment where sugarcane grows needs to be a consideration when genetically modified (GM) sugarcane is being evaluated for release (Bonnett et al. 2008).

10.10 Recommendations for Future Actions

The need for conservation seems to be a common plea and logic dictates that the genetic variation available in environments outside of agriculture and collections will decrease with time. However, this must be tempered by better exploitation of collections already made. Introgression using conventional breeding is

a time-consuming business before commercial results are obtained. The hope for the future is that with better molecular marker platforms and statistical approaches to detecting traits of economic importance that the speed of the introgression process will increase.

The increased interest in sugarcane as a valuable tropical crop has been demonstrated by multinational companies developing interests in sugarcane breeding. This is being driven by both a desire to deploy GM traits already developed in other crops into sugarcane and the increased interest in sugarcane and its relatives as an increasing part of the solution to generate liquid transport fuels and other biomaterials from sucrose and into the future, lignocellulosic material.

Whilst underway, the sequencing of *Saccharum* genomes must be completed. This would both build a platform for understanding how highly polyploidy genomes operate and provide a resource to encourage further study into how some genotypes accumulate high levels of sucrose and some high levels of biomass. The ultimate result of this more complete understanding would be better combinations of both these features than currently exists.

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Erratum to Chapter 2: *Corchorus*

M.K. Sinha, C.S. Kar, T. Ramasubramanian, A. Kundu, and B.S. Mahapatra

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In the caption to Fig. 2.2 and in the reference section, a citation was erroneously missed to be included. The corrected version is:

Fig. 2.2 Meiosis in *Corchorus* species ($2n = 2x = 14$) at diplotene stage. (**a**, **b**, **c**, **f**) Formation of seven bivalents (7II). (**d**) Formation of six bivalents (6II) and two univalent (2I, marked). (**e**) Formation of two quadrivalents (2IV, marked) and three bivalents (3II) (Maity and Datta 2009)

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Maity S, Datta AK (2009) Meiosis in nine species of Jute (Corchorus).
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