Sukhada Mohandas
Kundapura V. Ravishankar *Editors*

Banana: Genomics and Transgenic Approaches for Genetic Improvement

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Foreword

Bananas and plantains represent a staple food crop for 400 million people. Its world production amounts to more than 145 million tonnes, of which close to 20 % are grown in India. This is because bananas are so popular but also because they are an important part of the culture. For example, it is a tradition to tie banana plants at the entrance of a house where a marriage is taking place to bring the couple good luck. Bananas continue to be vulnerable to pests and diseases but also to abiotic stresses that are becoming increasingly problematic due to climate change. India, with its enormous diversity of bananas, including wild relatives, is well placed to face these challenges.

The field of genomics is picking up speed. After the whole genome sequence was published in *Nature* in 2012, we noticed the doubling of germplasm requests from the Bioversity International *Musa* Germplasm International Transit Centre (ITC). Recent efforts to sequence the banana genome and studies on cloning genes of important traits using next generation techniques, proteomics, transcriptomics and metabolomics have immensely helped to understand the response of banana at the molecular and cellular level.

In this context, the present book, edited by **Dr. Sukhada Mohandas** and **Dr. K.V. Ravishankar** entitled *Banana: Genomics and Transgenic Approaches for Crop Improvement*, is a unique blend of information on banana genomics and transgenic approach for crop improvement. The overview of progress in this area of research has been put together by leading experts who have organized the book into two parts. The first part deals with evolution, taxonomy, classical breeding and understanding of the banana genome through next generation sequencing and molecular markers. Metabolomics and molecular aspects of fruit ripening are also discussed. The second part covers all aspects of transgenic development starting with genes, and gene transfer techniques, regeneration protocol and strategies used for the development of trait-specific transgenic bananas. The latest advances are included, such as the successful field trials conducted on bacterial blight, wilt resistance and insect resistant plants. The review articles included in the book draw from a vast bibliography which is a valuable source for scientists and

students. Overall, the book gives the reader comprehensive information and discussion on the banana genome, and its improvement through classical breeding and genetic engineering.

The book poses the two following main challenges in banana research:

First and foremost, *Musa* research relies on the availability of a broad genetic base. Only by continually increasing the diversity conserved in *ex situ* as well as in *in situ* collections can we maximize our progress in selecting and improving the right varieties.

As banana pests and diseases continue to spread across the globe, and with the effects of climatic change becoming evident, new approaches that unlock potential resistance are urgently needed. With the recent great strides in the field of genomics, there is a movement to link novel methods and technologies to ongoing breeding efforts. Promising genomics-based approaches for containing/eradicating threats to production are therefore discussed at length in this book.

The editors and scientists who have contributed to the book have made significant advances in their research. I congratulate the editors for bringing out this compilation, which will have a great impact on *Musa* research, teaching and the transfer of new technologies. This book will be read by a wide range of scientists and donors, not only from India but also from around the world. It is of interest to the public and private sectors from both developed and developing countries and will promote a rapid uptake of the latest technologies, contributing to improving food security and better livelihoods in countries where it is most needed.

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Preface

Banana and plantain are the important crops of the world with an annual production of 144 million tonnes (FAOSTAT, 2013). Of late, banana production has been severely threatened by both biotic and abiotic stress factors due to rapid change in climate and the evolution of new races of pathogens. Recent advancements in science and technology have helped generate large amount of information on banana genome sequence and transgenic technologies, which have helped evolve strategies to improve banana for various agronomical situations.

Keeping this in view, we have designed this book to collate holistic genomic and biotechnological information on banana crop. We have organized this book into two parts. The first eight chapters deal with evolution, taxonomy, classical breeding, understanding banana genome through next generation sequencing and markers available for this crop. We have also discussed metablomics and molecular aspects of fruit ripening in detail. In the other twelve chapters of the book, we present plant tissue culture, various techniques used for transformation and strategies used for the development of trait specific transgenic. On the whole, we have attempted to provide up-to-date information available on the banana genome and novel transgenic technologies, which are being adapted in banana improvement.

The majority of the cultivated banana are evolved from two wild species *Musa acuminata* and *M. balbisiana* through natural inter-species hybridization. Cultivated banana are sterile and polyploidy in nature, and hence improvement through classical breeding is a herculean task. However, breeders have adopted different strategies to overcome many of the difficulties. The traditional breeding methods involving diploids and the use of molecular markers have helped to develop the high-density linkage map. Further, this has helped assembly of whole genome sequence data in banana. Next-generation sequencing techniques proteomics and metablomics have immensely helped to understand the response of banana at molecular and cellular level. The chapter on abiotic stress studies deals in detail on banana crop response to various abiotic stresses. Similarly, we present molecular and genomic aspects of biotic stress like fungal, bacterial, virus and insect pests in a chapter. The chapter on molecular aspects of ripening deals mainly with gene expression studies and biochemical changes during ripening. Following this chapter, we present a chapter on metablomics, where many biochemical and phytochemical aspects of banana have been discussed. Phytochemical

aspects of various parts of banana and their use in traditional medicines are presented.

For genetic improvement of banana and plantains, biotechnological approaches have been widely used without compromising basic characters of the plant. A lot of concerted effort over the years from different groups has helped in obtaining good transformation protocol for banana. In the early years of banana research, regeneration of the crop was mainly through asexual means, and hence gene transfer using apical meristems and its stable integration was found difficult as transformation using meristems was found to result in chimeras. Therefore, methods using embryogenic cell suspensions (ECS) were attempted. Electroporation or *Agrobacterium* co-cultivation of gene carrying vector with ECS was successfully used to transfer different foreign genes into banana. Particle bombardment and co-cultivation of wounded meristems with *Agrobacterium* were also used to get transformants. The introduction of a centrifugation step during co-cultivation was found to improve transformation efficiency significantly. The chapter on somatic embryogenesis and novel tools for banana transformation provides an insight into the latest development in the field of banana transformation.

Identification of organ specific promoters is essential for successful expression of genes in different locations. Keeping this in view, the isolation of promoters using insertional mutagenesis is widely employed method. Advances in genomics led to the genome sequencing and identification of candidate promoters for specific expression patterns. Actually, promoter analysis for activity characterization has been confirmed through experimentation with different techniques, including reporter genes, bioinformatics analysis for candidate *cis*-acting element and promoter prediction and expression analysis of related genes. A chapter devoted to banana promoter analysis reviews characterization of different banana promoters and the analysis of promoter sequences available in GenBank using available bioinformatics tools and a novel method to identify motif sequences. A list of promoters used in the development of genetically modified banana is presented.

Abiotic and biotic factors adversely affect plant growth and development. Plants react to adverse conditions by producing specialized signals and modulate the transcription factors which regulate the genes coding for synthesis proteins and metabolites which are involved in stress tolerance. Several transcription factors involved in abiotic stress resistance and genes like dehydrins and aquaporins upregulted during stress-induced conditions have been identified and their role elucidated. Pathogenicity-related proteins, stress-associated proteins, vegetative storage proteins and several transcriptional factors have been discussed in the genomic chapter. Overexpression of some of these genes and transcription factors in banana and their effectiveness in combating stress have been examined later in the book. Factors involved in regulating gene expression, including microRNAs or miRNAs which are non-coding RNAs and are involved in post-transcriptional regulation of gene expression, have been identified from banana cultivars and their role validated in banana by overexpressing them in the crop. Recent developments in genomics, high-throughput sequencing and phenotyping platforms have given way to molecular breeding. Ecotilling and genome editing are also used to induce new

variations and to incorporate new traits. Enhancement of abiotic stress tolerance in banana through transgenic means is discussed in a seperate chapter which gives an account of the recent developments in this area.

Several diseases affecting banana are debilitating and reducing the yield drastically. Genetic modification of banana has been a widely accepted tool due to the limited success of conventional breeding. Panama disease caused by *Fusarium oxysporum* f sp *cubense* (Foc) is the most devastating and causes 100 % yield loss in many cultivars of banana. Foc is known to exist as four important races (race 1, 2, 3 and 4) of which race 1 and 4 are of serious concern as they attack the commercially acceptable banana cultivars across the globe. A review on transgenic banana for Fusarium wilt resistance highlights the application of genetic engineering for imparting resistance against Fusarium wilt and discusses various strategies that have been employed involving PR-related genes (Ace-AMP1 gene and defensin gene), antimicrobial genes, anti-apoptosis gene, RNAi-mediated approach and host-induced gene silencing (HIGS) that confer certain level of tolerance towards pathogen infection. Further, the cisgenic approach utilizing R genes and native cell death genes from Musa sp. have also proven promising. The understanding of host pathogen interaction in terms of defense and signalling related pathways and the study of pathogenecity mechanism which help in identifying critical genes for targeting pathogen are discussed.

Overexpression of antimicrobial peptides, like defensin, antimicrobial peptide MSI-99, magainin, endochitinase gene (TnEn-42) and chitinases stilbene synthase, have been found beneficial in producing fungal resistant crops. The resulted transgenic banana plants were phenotypically normal. RNA interference (RNAi) is another emerging strategy for control of pathogens, through silencing of a vital gene associated with pathogens. The chapter on the development of Sigatoka resistance using transgenic means discusses the merits of such an approach.

Several R genes and AMPs like lysozymes, magainins, cecropins, attacins, thionins and defensins have been identified to control bacterial pathogens. Transgenic bananas expressing either sweet pepper *Pflp* or *Hrap* gene have been developed and are under evaluation for resistance to Xanthomonas wilt disease in field trials in Uganda. The chapter on transgenics for bacterial wilt resistance discusses in detail the recent developments in the area and the management practices adopted and through cultural practices to check the spread of diseases.

Viruses are great limiting factors for banana production. Banana bunchy top caused by *Banana bunchy top virus* (BBTV) is one of the most devastating diseases of bananas in Hawaii and many areas of Asia, Africa and the Pacific. Several groups have investigated this possibility and utilized post-transcriptional gene silencing (PTGS) or RNA interference (RNAi) approaches to generate BBTV resistance in several cultivars. Recent efforts and approaches to develop BBTV-resistant transgenic banana is reviewed in the chapter on viral resistance. Future potentials of using transgenic banana as alternatives for the management of banana virus diseases are also discussed. Another strategy that utilizes a process termed "virus-activated cell death" has been developed by James Dale at the Queensland University of

Technology (QUT) in Australia. Transgenic plants using this strategy have been developed and are under evaluation. Different strategies used for developing virus resistance are discussed in this chapter.

A chapter on molecular farming provides an overview of different plant-derived products currently in the market or are in different stages of development including phases of clinical trials. Special emphasis has been given on banana being used as an expression host, advantages and limitations of using banana in plant molecular farming and the different approaches which can be utilized to overcome those limitations. Iron deficiency anemia (IDA) is a global problem, affecting women and children of the lower strata of society. Banana is considered as a potential fruit crop to become "micronutrient-enriched". Fortification of banana is more advantageous over other plants owing to its ploidy, parthenocarpic fruit development, its reach to the masses at large and availability throughout the year. Studies on the physiology of iron uptake in plants, translocation, storage and redistribution in plants and recent advances made in the understanding of the mechanisms of iron uptake in humans, homeostasis and transgenic approaches for increasing the iron content in bananas are described in a chapter on biofortification for iron deficiency anemia.

Vitamin A is an essential micronutrient required for several physiological functions. Hence, the biofortification of banana to develop a rich source of pro-vitamin A through genetic engineering tool could be an ideal approach. Retinyl esters and pre-vitamin A are the two dietary sources for the body. Retinyl esters are obtained from the meat and dairy products, whereas PVA is obtained from plant sources in the form of carotenoids. Only certain forms of carotenoids such as α-carotene, β-carotene and β-cryptoxanthin known as PVA are converted to vitamin A in the body. Genome engineering tools used to enhance the PVA content and lycopene are discussed in the chapter on pro-vitamin A-enriched banana for tackling malnutrition.

Overall, the present book gives a comprehensive information and discussion on banana genome, its improvement through classical breeding and genetic engineering. We are highly obliged to the experts who contributed to this book. We acknowledge continuous support from Indian Council of Agricultural Research, New Delhi, through project on "Network Project on Transgenic in Crops" for more than a decade to our banana research work. The expertise gained through these projects have made us to embark on editing this book. Finally, we hope the readers will enjoy reading this book and it would be useful to get comprehensive information on banana.

Bangalore, Karnataka, India — Sukhada Mohandas
May 2, 2016 — Kundapura V. Ravishankar

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Part I

Genomics

History, Origin, Domestication, and Evolution

1

A. Rekha

Abstract

Banana, a popular and ancient fruit, has a complex state of evolution in plant systematics. Being one of the highly evolved crops, it has attracted many researchers to study various aspects of its origin, evolution, and domestication. In the present chapter, an account of history of domestication, spread of the species to various continents, and studies on ethnobotanical and linguistic evidence of crop origin and distribution has been presented. Further, their taxonomic status as evidenced by various research works, including numerical taxonomy, cytological studies, and molecular markers to understand the involvement of genomes other than *M. acuminata* and *M. balbisiana* in the evolution of the cultivated bananas, is given.

Keywords

Musa • Taxonomy • Evolution • Domestication • History • Origin

1.1 Introduction

Banana and plantains are the important sources of starch for the millions of people in the developing world; it is a major commercially cultivated fruit crop of India which is as old as the Indian civilization. Bananas are also considered as the poor man's apple and said to be the fruit of heaven (Amalraj et al. 1993). It appears to be one of the earliest fruit crops cultivated by mankind at the beginning of civilization. In India and Africa, bananas are very predominant and popular among people, and they are liked by both poor and rich alike. Unlike other fruits, banana is available throughout the year, and it is the cheapest among all other fruits in the country. In any Indian household, it is an inevitable necessity for all social and cultural occasions as it is considered as a symbol of good omen, fertility, and prosperity. Bananas are put into a variety of uses in India, especially in South India. Almost every part of the plant is used in some way or another; hence it is popularly known as "Kalpatharu." The fruit is easily digestible, a good food for people suffering from gastritis and other stomach ail-

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ments (Rao 1984). Apart from its fruit which is eaten either raw as a vegetable or cooked, the leaves are used as plates. The male flower bud and the central axis of the pseudostem are used as vegetable. The juice of the central axis is said to be useful in kidney stone treatment, and the leaf sheaths find their use in fiber and paper industry some Eumusa cultivars are listed in Table 1.1.

1.2 History and Domestication

There is a clear reference to banana in Sanskrit literature like the "Ramayana," Kautilya's "Arthashastra," and the Tamil classic "Shilappadikaram" (Krishnamurthi and V.S.Seshadri 1958). Descriptions of bananas are given in Greek writings on 327 BC in Indus

Table 1.1 Eumusa cultivars

Genomic group	Name	Country
AA	Pisang Mas, Pisang Lilin, Pisang Keladi, Pisang Boyan, Pisang Kapas	Malaysia, Indonesia
	Lady Finger	Hawaii
	Klue Khai, Kulai Lai, Kulai Hom, Kulai Sa	Thailand
	Kadali, Matti, Anaikomban, Namari, Sanna Chen Kadali	India
	Bata-Bata, Lakatan, Pogpogan, Amas	Philippines
	Banana Ouro	Brazil
AAA	Pisang Ambon, Pisang Serendah, Pisnag Thai, Pisang Tualang, Pisang Masak Hijau	Malaysia, Indonesia
	Bluefields, Chinese, Hamakua, Red	Hawaii
	Kulai Nak, Kulai Nang Nuan, Kulai Nam, Kulai Khom, Kulai Hom Thong	Thailand
	Harichal, Jahaji, Pacha Vazhai, Basrai, Lal Kela	India
	Bangan, Inabaca, Manang	Philippines
	Nanicao, Mestica	Brazil
AB	Ney Poovan, Safed Velchi, Kunnan, Vannetu Kunnan	India
	Lady Finger	Hawaii
AAB	Pisang Raja, Pisang Tandok, Pisang Rasthali, Pisang Kelat	Malaysia, Indonesia
	Brazilian, Apple, Dwarf Plantain, Eslesno, Father Leonore	Hawaii
	Klue Nagar Chang	Thailand
	Rasthali, Rajapuri, Virupakshi, Nendran, Thiruvananthapuram	India
	Tundoc, Letondal, Ternate, Canara	Philippines
	Banana Pacova	Brazil
ABB	Pisang Awak, Pisang Kelat Siam, Pisan Abu, Pisang Batu	Malaysia, Indonesia
	Largo, Ice Cream, Chamaluco	Hawaii
	Klue Namawa, Klue Maliong, Klue Hak Muk, Klue Ong	Thailand
	Peyan, Monthan, Boothi Bale, Nalla Bontha Batheesa, Kach Kola	India
	Pitogo, Garango, Bisco, Inabaniko, Pelipita, Sabang Iloco	Philippines

Source: Stover and Simmonds (1987)

Valley, during the expedition of Alexander the Great in India (Reynolds 1927; Kervegant 1935). Most botanists believe that bananas were introduced from India to the Middle East and across North America by Arabs. The Portuguese along with the Spanish were instrumental in the worldwide spread of bananas and plantains especially to America (Price 1995). These evidences suggest the early existence of banana in India. The wild *Musa acuminata* occurs in Assam, Burma, Siam, Indo-China, the Malayan peninsula and archipelago, and the Philippines. The center of diversity of *M. acuminata* lies in the Malayan area where four out of five subspecies were found and is thus considered as the primary center of origin of cultivated bananas. Historical evidences show that the Arabs have introduced the banana from India to Palestine and Egypt, perhaps in the seventh century AD. It soon became popular in those areas and later spread to the east coast of Africa at a very early date and subsequently throughout the African continent. Bananas were reported to have been introduced in Central America in 1516 AD, where it spread rapidly and attained commercial significance. The spread of bananas to the West Indian Islands was through Christian missionaries, and wherever banana reached, it assumed economic importance due to its greater adaptability and commercial value.

Bananas are the source of starch for a sizable population in the tropics and subtropics for millions of years. The studies involving archaeology, genetics, and linguistics have provided an understanding regarding the history of banana domestication (Perrier et al. 2011). Historically, *Musa* species and genotypes have been created by migration of human populations and interaction between the groups which helped in the exchange of genotypes. These interactions created the introduction of species and helped in further generation of natural hybrids, which are parthenocarpic diploids or triploids. Some of these hybrid cultivars were widely adopted and dispersed either by preference or by chance. A group of triploids, maybe because of the environmental adaptability and triploid nature, might have been dispersed by clonal propagation across vast areas. In addition, the dispersal of bananas through humid tropics and subtropical regions, across the Indian Ocean, proves interlinkages, predominantly local, social networks extending from New Guinea to West Africa; these networks may be about 2500 years old.

There are some hints that the banana cultivation was prevalent in Harappan civilization (2500–1900 BC). Such studies would provide exciting proofs about the prehistorical dispersal of banana in Southeast Asia. However, the other evidences, especially that of historical and linguistics, suggested that the main introduction of edible bananas was about 2000 years later. This paved way to hypothesis that the "Kot Diji" *Musa*-like phytoliths might have been in cultivation for fiber or as ornamental plants. However, evolution of local names in relation to different *Musa* genome groups (AAA, ABB, and AAB), involving starchy or sweet varieties, is not well understood. New linguistic and ethnobotanical data from India is lacking, to fill the gap in the understanding of *Musa* names.

It appears that there is close similarity between the proposed history and botanical classification with observed history and linguistic terminologies. As far as bananas in cultures are concerned, introduction of bananas in Africa and the ancient terms in Papuan languages strongly suggest a pre-Austronesian dispersal across the islands of Southeast Asia. The linguistic evidence is a proof to archaeobotanical record, which helped in approximately tracing the dispersal of bananas from New Guinea.

Musa spp. domestication was a highly complex process; it has taken over thousands of years and involved multiple steps, separated by time and place (Carreel et al. 2002; De Langhe and de Maret 1999). Banana also has a unique testimony to the early, long-term, and deep impacts of people in rainforests. The long-term management and manipulation of specific plant resources within rainforests might have influenced the evolution of these plants. The archaeobotanical evidence of *Musa* bananas in areas other than the natural ecological regions of the genus, like Africa, indicates the fact that there would have been introduction, adoption, and dispersal by the people during very early or later years (Neumann

and Hildebrand 2009; Vrydaghs and De Langhe 2003; De Langhe et al. 2009; Vrydaghs et al. 2003). Dispersal of bananas from New Guinea to Eastern Indonesia during mid-Holocene was inferred by studies of Denham and Donohue (2009), Donohue and Denham (2009), and Kennedy (2009). *Ensete*, the closely related genus of *Musa*, would have contributed in the evolution of the cultivars in the initial stages of banana domestication which needs further understanding. The domestication of Ethiopian *Ensete ventricosum* (Welw.) Cheesman, as a source of diverse products and as a staple source of starch, is well documented (Brandt et al. 1997; Purseglove 1975). There may also be a possibility that a parallel selection for starch production in the corm and pseudostem of Malaysian *Musa* species would have occurred. The classic example is selection for enhanced starch storage in the rhizome of a New Caledonian *Musa* plant, which is described as having a "glaucous, violet stem and a turnip-like rhizome when cooked, it resembled a yam in taste" (Simmonds 1959). The cultivation of *M. textilis* and *M. balbisiana* for fiber indicates that seedy fruit is not necessarily an indication of "wildness." While parthenocarpy is the key to the edibility of fruit, its reproduction and transmission depended upon vegetative propagation (Simmonds 1962; Daniells et al. 2001).

The evolution of seedless edible bananas from seeded wild species is complex. Recent research on genetic studies revealed that the process involved a long hybridization period including different taxa (Perrier et al. 2009). This could happen with human interventions who took a major role in carrying different taxa to new zones helping in hybridizations. It has been proved by the ancient events where human interventions were observed. The mode of dispersal was verified by phytoliths from archeological sites.

Process of evolution involved thousands of *Musa* species with high genetic diversity which indicates that it may be having multiple origins. Knowledge on functional structural genomics and genes; reproductive physiology; comparative genomics with rice, *Arabidopsis*, and other model species; and cytogenetics has helped in understanding the *Musa* diversity (Heslop-Harrison and Schwarzacher 2007).

The complicate genome constitution and ploidy of banana accessions were determined by the plant and fruit morphological studies since the 1940s; methods of numerical taxonomy were adapted which gave better understanding (Simmonds and Weatherup 1990a, b; Ortiz 1997; Ortiz et al. 1998; Pollefeys et al. 2004). Flow cytometric analysis (Doležel et al. 1999; Doležel and Bartos 2005) provided accurate and rapid surveys at the juvenile stage of the plants to know the ploidy status of the collections and new hybrids. Chromosome preparations with in situ hybridization techniques using DNA probes which can distinctly label the A and B genomes have shown that the full sets of $x=11$ chromosomes of A genome are present (Osuji et al. 1997), and most cultivars have 11 chromosomes with complete genomes. The classic example is the work of d'Hont et al. (2000) who used in situ hybridization technique to show that the variety "Pelipita" ($2n = 3x = 33$) included 8 A genome chromosomes and 25 B genome chromosomes instead of 11 A and 22 B genome chromosomes which is normally expected in the case of ABB type, whereas two other AAB plantain types consisting of 33 chromosomes had more than 11 B genome chromosomes. Their studies also confirmed the presence of complete "S" and "T" genomes, from *Musa schizocarpa* and *M. textilis*, respectively. All these studies indicate probability of backcrossing, or chromosome elimination would have occurred during the process of evolution of some varieties. Molecular analyses have proved the existence of chromosome markers from *Musa* species other than the A and B genome. The diploid variety "Wompa" had AS genome, whereas other genotypes were found to be consisting of AAT and ABBT genome composition.

Cultivar Pelipita (ABB genomic group): 2n = 3x = 33 chromosomes

Expected – 11A chromosomes + 22 B chromosomes

Observed – 8 A chromosomes + 25 B chromosomes

Other plantain group cultivars (AAB genomic group)

Expected – 22A chromosomes + 11B chromosomes

Observed – 22 A chromosomes + > 11 B chromosomes and chromosomes of S and T genomes (i.e., *M. schizocarpa* and *M. textilis*)

1.3 Taxonomy

The bananas are indigenous to warm, humid areas of South Asia, and they are giant herbs of monocotyledons, under the order Zingiberales as classified by Huchinson, but was classified under the order Scitamineae by Bentham and Hooker in *Genera Plantarum. Musa* is placed under the family Musaceae, consisting of two genera *Musa* and *Ensete*. The genus *Musa* is further divided into four sections, two of which contain species with chromosome number of 2n = 20; they are Callimusa and Australimusa. The other two sections Eumusa and Rhodochlymus have a basic chromosome number of 2n = 22. The Rhodochlymus and Callimusa consist of plants with ornamental importance which do not produce edible fruits, whereas Australimusa contains *M. textilis* from which Manila hemp is produced (Figs. 1.1 and 1.2).

Earlier edible bananas were placed under *M. paradisiaca* L. for plantain and *M. sapientum* L. for banana by Linnaeus, which were later considered to be hybrids of two main species *M. acuminata* and *M. balbisiana* based on 15 important morphological characters (Dodds and Simmonds 1948). All banana and plantain cultivars evolved from the section Eumusa and its group of species. This is the biggest section in the genus and geographically widespread. The section contains 11 species which was divided into two subsections Eumusa (1) and Eumusa (2) by Simmonds and Weatherup (1990a, b) based on numerical taxonomy. Shepherd and Ferreira (1982) identified cultivars which may be the result of hybridization involving *M. schizocarpa* among *Musa* germplasm collections of Papua New Guinea. A Philippine clone was found to be the result of an early hybridization between *M. balbisiana* and *M. textilis* and landraces consisting of three

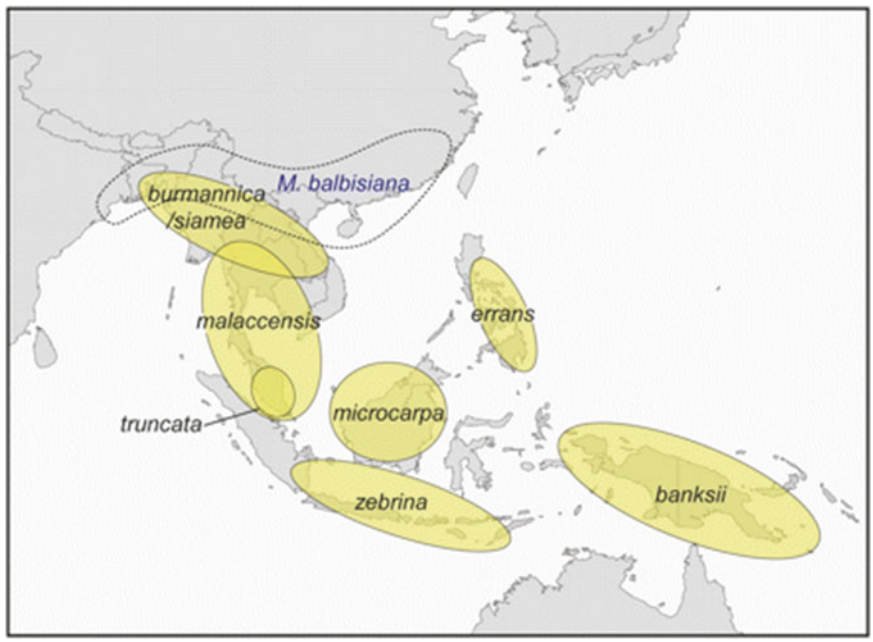

Fig. 1.1 Distribution of subspecies of *Musa acuminata* in Southeast Asia (Source Perrier et al. 2011. From PROMUSA website)

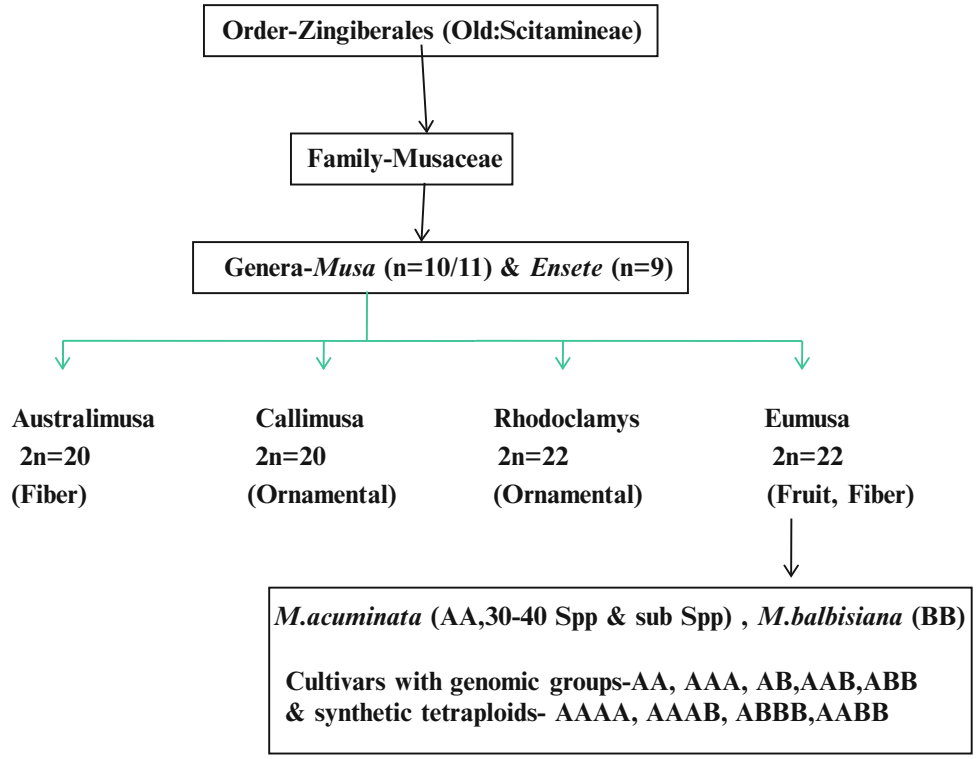

Fig. 1.2 Classification of Musaceae (Simmonds 1962)

genomes, *acuminata*, *balbisiana*, and *textilis*, which was in Papua New Guinea (Carreel et al. 1994). These evidences prove the complexity of origin of banana cultivars and their taxonomy.

The edibility of fruits of diploid *M. acuminata* (AA) originated as a result of two mutation events, involving induction of female sterility and parthenocarpy. Triploid AAA cultivars were derived from these diploids which might be the result of crosses between edible diploids and wild *M. acuminata* subspecies, which resulted in a wide variation among AAA cultivars. These triploids are highly vigorous and have larger fruits, which replaced the AA diploids of Asian countries. The diploid and triploid *acuminata* cultivars were carried by people to the regions where *balbisiana* was found which might have resulted in natural hybridization and formation of new hybrid progenies with the different genome composition like AB, AAB, and ABB. It was thought that subsequent dispersal of these edible bananas from Asia was brought about again by human interventions. Secondary diversification within the groups of cultivated bananas is the result of somatic mutations. There are allo- and autopolyploids in banana.

Eumusa and Rhodochlymus are found in Assam (India) and Thailand area, whereas Callimusa and Rhodochlymus groups are seen in Borneo and its surrounding islands and Indonesia. Australimusa is largely found in Malayan islands. The subspecies of *Musa acuminata* are largely found in Assam, Indo-China, Malayan islands, and Papua New Guinea which is also the primary center of cultivated AA types. *M. balbisiana* occurred in Ceylon, India, Burma, Siam, and Malayan islands where the A × B hybrids have evolved.

1.4 Molecular Evidences of Evolution

The *Musa* genome sequence provided an invaluable source for studies on plant gene and genome evolution studies (D'Hont et al. 2012). It also gave new insight in studies related to evolution of monocotyledons and their relations with each other. Characters specific to the family Poaceae could be highlighted, which helped in analyzing the emergence of this family. Identification of several deeply conserved regions within monocotyledons and between monocotyledons and eudicotyledons was possible with the available *Musa* genome sequence. This paved a way for detecting novel motifs with a functional gene regulation which provides valuable information on conserved genes. It was observed that there could have been three steps of polyploidization in the *Musa* lineage, followed by gene loss (deletion) and chromosome rearrangements (translocations or inversions); such changes might have resulted in little synteny conservation between lineages retaining some gene or group of genes; thus, the groups would have had an opportunity for independent diversification.

Several molecular methods were adapted to analyze diversity and evolution; in the early 1980s, analysis of isozyme and anthocyanins confirmed that *Musa* germplasm was genetically diverse (Jarret and Litz 1986; Horry and Jay 1988). As soon as DNA markers along with PCR-based techniques were available, several markers like RFLP, AFLP, RAPD, IARP, and SSR microsatellite markers are being used to analyze diversity across different countries and research groups with their germplasm collections.

De Jesus et al., in 2013, used flow cytometry and PCR-RFLP to characterize the Brazilian accessions. They studied 221 accessions by flow cytometry and confirmed the correct ploidy for 212 (95.9%); however, genomic constitution could not be identified with flow cytometry, whereas the genomic constitution of 209 (94.6%) accessions could be confirmed by digestion of the ITS region. Neighbor-joining cluster analysis from SSR binary data helped in detection of two major groups, which was distinguished by the presence or absence of the B genome, and subgroups were found to be as per the genomic composition and commercial classification of the cultivars.

Controlled reciprocal crosses were made among *Musa* species by Faure et al. (1994) to demonstrate maternal/cytoplasmic transmission of chloroplast DNA but showed an unusual phenomenon of paternal transmission of mitochondrial DNA in *Musa acuminata*. The study was further confirmed by Carreel et al. (2002) who analyzed the origins of more than 300 *Musa* genotypes and concluded that most cultivars show mitochondrial genome linkage to two subspecies of *M. acuminata*, *M. acuminata* ssp. *banksii* and *M. acuminata* ssp. *errans*. It was found that some cultivars of AB, AAB, and ABB need not be simple allopolyploids, but most cultivars have different proportions of A and B genome chromosomes and/or possess different doses of recombinant chromosomes. All the research results published so far involving studies on cytoplasmic and nuclear DNA and at chromosomal, as well as protein, levels proved this concept. The hypothesis that hybrid banana cultivars would have evolved through backcrossing of interspecific hybrids with parental species, which would have led to formation of a complex spectra of genotypes/cultivars, seems to be true. De Langhe et al. (2010) debated that AAB accessions could have arisen as a result of either AB × AA or AA × AB cross, as contribution of A or B genome in this group does not agree with simple allopolyploid genome formulae of Simmonds and Shepherd (1955). Deviations were observed in the required total score of 35–37 based on morphological characterization, in "Pome" and "Silk" group cultivars. This was further strengthened by the study of Carreel et al. (2002) where inheritance of organelle (chloroplast and mitochondria) DNA was used. They also described the probable evolution of interspecific hybrids through (BB × AA) × AA or (AA × BB) × AA cross combinations to get BAA- or AAB-type cultivars where the cultivars have "B-type" or "A-type" cytoplasm and hence dosage of A or B genome varies.

1.5 Conclusions

Banana is one of the fruits available throughout the year in tropical and subtropical humid regions of the world. It is the most difficult crop to the researchers as the evolution of the present-day cultivars is still a mystery. Various researchers have studied the evidences through ethonobotanical and linguistic observations to derive its place of origin and spread. Further evidences are required to know the origin and speciation with reference to an ample number of cultivars available at present with special reference to Indian varieties. The cytological and molecular studies involving mitochondrial and chloroplast-specific markers have indicated the complexity of the evolution of banana cultivars. It appears that further research is necessary to understand the correct genomic status of the many cultivars.

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Banana Breeding

2

Rema Menon

Abstract

The first attempt at breeding bananas through hybridization, in the 1920s was triggered by the devastation of the export banana 'Gros Michel' by *Fusarium* wilt. Conventional breeding despite being hindered by several plant based constraints has been responsible for the production of a whole range of hybrids resistant to diseases and pests. The approach commonly adopted aims at developing new tetraploid varieties by crossing triploids with wild or improved diploid clones with resistance, or secondary triploids derived from crosses between the developed tetraploids and the diploid clones. The limitations of the 3x/2x strategy are low gametic fertility of the triploid variety to be improved. This has led to the development of an alternate pathway, which targeted the development of triploid hybrids directly from crosses involving diploid and doubled diploid varieties. The method exploited the male and female fertility status of doubled diploids which otherwise are sterile at the diploid level. Following the 3x/2x method, hybrids with yield advantage and resistance to biotic stresses developed by major breeding programmes have been adopted in many countries, which was facilitated by the International *Musa* Testing Programme. The new insight gained in *Musa* genetic diversity through molecular tools provided key inputs, useful for the selection of parental combinations. Development of molecular markers linked to major traits is expected to speed up the improvement process. Mutation breeding is offering a unique, alternative approach for the improvement of banana has also been employed to develop new cultivars.

Keywords

Musa • Breeding strategies • Sterility • Parthenocarpy • Fertility

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2.1 Introduction

Bananas originated in South East Asia and are one of the most widely cultivated crops in over 130 countries throughout the tropical and subtropical regions of the world. The annual world production of banana is around 103 million metric tons from an area of 5.14 million ha (FAO 2014). Bananas and plantains are popular and cheap multipurpose crops by virtue of their ability to grow under a wide range of environments, producing fruits year-round and nutritional and have therapeutic values. A valuable source of nutrition for more than 400 million people in the tropics, bananas are rated as the developing world's fourth most important crop after rice, wheat and maize. In the tropics, where most of the population depends on carbohydrate foods as dietary staples, plantains and bananas along with root crops play a significant role as additional source of dietary energy and protein. The fact that global production of bananas and plantains increased by 70 % in the last 40 years representing the fastest growth among starchy staples in developing countries testifies their potential in strengthening food security and the alleviation of poverty in rural areas where 75 % of the population is concentrated.

Bananas and plantains are highly remunerative and play a pivotal role in the livelihood security of farmers. Around 85 % of global banana production is managed by small-scale farmers and most of it is being consumed locally. The livelihood of these farmers depends on the availability of a wide range of cultivars that are adapted to local environmental conditions. The existing diversity is mainly composed of farmer-selected cultivars. The vast gene pool provides immense possibility to use the material in response to environmental changes and continuous emergence of biotic threats. Increasing population pressure and consequent increase in the demand for food on the one hand and depletion of arable land on the other have placed new emphasis on conventional plant breeding. Conventional or classical plant breeding has been responsible for improvement in numerous cultivated plant species in the twentieth century (Johnson 2000). An important component of classical breeding is the extensive testing of the material in various environments in comparison with existing cultivars. Banana production is hampered by a wide range of pests and diseases (Jones 2000), and introducing host plant resistance is the most economical and sustainable way to manage them (Lorensen et al. 2010). A considerable progress has been made in the introgression of resistance to many of these biotic stresses and hybrids evolved (Tomekpe et al. 2004; Lorensen et al. 2010).

2.2 Taxonomy

The family Musaceae comprises of two genera, *Ensete* Haran and *Musa* L. The genus *Musa* has four sections, namely, *Callimusa* and *Australimusa* which have a basic chromosome number of 10 and *Eumusa* and *Rhodochlamys* having the chromosome number 11. *Callimusa* has many non-domesticated members with a lot of ornamental value. Multiplication is fast with their rhizomatous stems and has erect inflorescence with bright-coloured bracts. *Australimusa* has five to six species, but the most important are fibre-yielding *Musa textilis* and fruit-yielding *Musa fe'i*. The distribution of *M. textilis* is mostly in S.E. Asia with reports of occurrence even in Indo-Burma border, while Fe'i bananas have a commercial distribution in the Pacific Islands. *Rhodochlamys* consists of members which are ornamental in nature. They are distinguished by their slender stature and erect inflorescence with brightly coloured bracts. They are female fertile and cross freely with *Eumusa* members with F1 generation exhibiting a dominance of *Rhodochlamys* traits.

Eumusa is the only source of present-day edible bananas except for Fe'i bananas of *Australimusa*. They are characterized by robust pseudostem; horizontal, angular or pendulous bunches; and in most cases parthenocarpic fruits. All edible bananas are believed to have originated from two species, *Musa acuminata* designated by A genome and *Musa balbisiana* designated as B genome. The relative contribution of each genome has resulted in various combinations, viz. AA, AAA, AB, AAB, ABB, BB, BBB, ABBB, etc. (Stover and Simmonds 1987).

2.3 Origin, Distribution and Diversity in Bananas and Plantains

Virtually all banana varieties of today have evolved from intra- and interspecific hybridization of the two diploid ($2n = 2x = 22$) wild species *Musa acuminata* Colla and *Musa balbisiana* Colla. These diploid *Musa* species have seeded fruit with a little starch and a small amount of pulp and of no value as a crop. Evolution of edibility in the *Eumusa* series of cultivars started with wild *Musa acuminata* subspecies which are predominant in South East Asia. The appearance of vegetative parthenocarpy and female sterility in wild *acuminata* facilitated the production of seedless diploid *Musa acuminata*. Another key event was hybridization with *Musa balbisiana*, a hardier species. Both *Musa acuminata* and *Musa balbisiana* are diploids and represented, respectively, as AAw and BBw. Further, triploids evolved through fertilization of viable diploid cells formed due to breakdown of meiosis at the second division with haploid pollen (Simmonds 1962; Jones 2000).

India harbours a rich diversity of wild *balbisiana* types and few subspecies of *acuminata*. Even though the present-day bananas are the result of natural introgression between the two diploid wild species, the presence of *M. schizocarpa* and *M. textilis* represented by S and T genomes in cultivated and wild types has opened new avenues to the understanding of the evolution theory (Jones 2000). Molecular analyses have also confirmed the presence of S and T genomes (D'Hont et al. 2000).

Of 11 wild species of *Musa* reported across the globe, six are present in India. *M. acuminata and M. balbisiana*, the progenitors of cultivated banana, are widely distributed in the banana-growing regions, viz. South Indian states, north-eastern states and Andaman and Nicobar Islands. *M. itinerens*, *M. nagensium*, *M. sikkimensis* and *M. cheesmani* are the other wild species, mainly concentrated in the north-eastern region (Simmonds 1962).

Of the eight subspecies under *Musa acuminata* reported from Asian countries, only three have been reported in India – *banksii*, *burmanica* and *burmannicoides*.

Musa balbisiana originated in the drier parts of India and is widely distributed from this region to the Philippines and New Guinea, but is absent in Central Malaysia. It is hardier, drought and disease tolerant than *M. acuminata*. No subspecies are recognized.

Banana has been subjected to a rigorous course of evolution to transform itself from seedy non-pulpy wild progenitors to present-day parthenocarpic edible and high-yielding banana (Simmonds 1962). The four major factors, viz. parthenocarpy, sterility, polyploidy and vegetative propagation for perpetuation of useful traits, have contributed alone or in combination to the evolution of present-day bananas. Apart from the nuclear genome, the significance of plastid and mitochondrial genomes contributing to important agronomic traits has been suggested. Studies in this direction by Faure et al. (1994) not only demonstrated maternal transmission of chloroplast DNA but also showed the occurrence of paternal transmission of mitochondrial DNA in *Musa acuminata*. Carreel et al. (2002) concluded, based on genetic analyses of 300 *Musa* genotypes, that most cultivars are linked through their mitochondrial genomes to *banksii* and *errans*, two subspecies of *Musa acuminata*.

Most cultivated bananas and plantains are highly female sterile and cannot reproduce sexually. Clonal propagation is only possible and survival in nature and geographical dispersal cannot happen without human intervention. Consequently, secondary diversification in areas devoid of wild *Musa* plants has been attributed to somatic mutations of introduced materials (Purseglove 1975).

Diversity in the *Eumusa* series of edible banana is made up of varieties with AA, AB, AAA, AAB, ABB, AAAA and ABBB genomes. There are over a thousand types of bananas in existence, subdivided into 50 groups of varieties. The most important bananas are categorized, commonly as the AAA dessert bananas, the AAA highland cooking bananas and beer bananas of East Africa, the AAB plantains and the ABB cooking bananas (Simmonds 1962; Jones 2000).

The taxonomic method followed to determine the genome of banana cultivars based on morphological characters (pseudostem colour, shape of petiolar canal and bract features) gives a good

estimate of the genetic composition and ploidy of cultivars (Simmonds and Shepherd 1955). Numerical taxonomy has refined the approach (Ortiz et al. 1998). Flow cytometry provides accurate estimates without growing mature plants (Dolezel and Bartos 2005). A good correlation with results of molecular method is also obtained. Clones/cultivars within each genomic group are identified based on additional morphological characters. Many clones have given rise to morphotypes that differ in fruit and bunch morphology, pigmentation and height. The same clone/cultivar can have different names in different locations and synonyms, adding to taxonomic complexity (Menon 2000a; Uma et al. 2005a). For a better identification and classification of a cultivar or a landrace, a basic knowledge about their evolution, taxonomic status and description of traits is essential. For this purpose, the banana descriptor published (IPGRI/INIBAP/CIRAD 1996) is utilized for distinguishing the important traits.

Cultivars and landraces within a genome are referred to as 'group 'and 'subgroups'. Wild accessions are denoted as 'types'. Diploids AA or AB are characterized by their more slender pseudostems and more upright leaves. Triploid cultivars are classified under three genomic groups, viz. AAA, AAB and ABB. Triploids are bigger, sturdier plants than diploids with increased fruit size. Tetraploid cultivars are few in number and belong to the AAAA, AABB, AAAB and ABBB genomic groups. They possess robust pseudostem and leaves that tend to droop. Tetraploids have formed from fertilization of triploid egg cells by haploid pollen (Jones 2000).

Fruit development in banana is characterized by vegetative parthenocarpy, the development of pulp without pollination. In the case of wild seeded bananas, pollination precedes normal fruit development without which fruits remain unfilled and shrivelled. A normally mature fruit contains a mass of hard black seeds surrounded by a scanty sweetish pulp which develops from the ovary walls and septa. If ovaries of seeded bananas are protected against pollination, they do not develop. In contrast, edible bananas are vegetatively parthenocarpic, i.e. development of pulp without pollination. The ovules which shrivel early can be seen embedded in the edible pulp. The physiology of parthenocarpic development in banana is apparently mediated by an autonomous production of auxin in the mature ovary (Purseglove 1975; Stover and Simmonds 1987). Seeds vary with respect to size, shape and colour. The size varies from 4 to 20 mm and shape can be spherical, triangular or ovoid. Brown to pitch black-coloured seed coat may be warty or smooth.

2.3.1 Major Cultivar Groups

2.3.1.1 Cavendish Subgroup

Cavendish cultivars may have originated in the South China-Vietnam area and Malaysian region. They are high yielding than all other natural clones (Robinson 1996). The varieties in the Cavendish subgroup are separated mainly by differences in height and bunch and finger characteristics. There is a gradation in height from the shortest (Dwarf Cavendish) to the tallest. The Cavendish subgroup is responsible for 30 % of the world's production of banana fruit. Stover and Simmonds (1987) recognized four major clone sets distinguished on height: 'Dwarf Cavendish' types are the shortest in stature; 'Grand Nain' types are medium dwarfs; and intermediate in height are between 'Giant Cavendish' and 'Dwarf Cavendish' groups. 'Giant Cavendish' types are taller and 'Pisang Masak Hijau' types, the tallest. Apart from plant height, clones in the Cavendish subgroup also differ in other morphological characters such as petiole length, bract persistence, bunch grade and pseudostem colour.

2.3.1.2 Plantain Subgroup

The plantain subgroup is very important as plantain cultivars provide food for millions of people in the West and Central Africa and Latin America-Caribbean regions. Cultivars are also found in East Africa and South and South East Asia. A total of 23 % of the world's production of banana fruit comes from plantain. The term plantain has

been used in the past to describe all cooking banana types, but now it refers only to clones belonging specifically to the plantain subgroup within the AAB genomic group of banana where fruit remains starchy at ripeness. They are characterized by the orange yellow colour of the compound tepal in the flower and of pulp at ripeness. Fruits are long and slender, angular, pointed and unpalatable when raw (Stover and Simmonds 1987).

Cultivars in the plantain subgroup are placed in four main clone sets, which are distinguished on bunch and inflorescence characteristics (Tezenas du Montcel and Davos 1978). 'French' plantain types have many hands with comparatively small fingers and an inflorescence axis covered with persistent hermaphrodite and male flowers. The large male bud is also persistent. 'Horn' plantain types have few hands of very large fingers, no hermaphrodite flowers and no male axis. 'French Horn' and 'False Horn' plantain types are intermediate classification categories between 'French' and 'Horn' plantains. The male bud is absent at maturity in both of these types, but there are many hermaphrodite flowers on 'French Horn' cultivars and a few on 'False Horn'.

Though South India is believed to be the centre of origin of French plantain group (Simmonds 1966), wide diversity has been reported from Central Africa (De Langhe 1964). Nendran, the French plantain cultivar, is the predominant variety in India and is represented by several clones recognizable through variation in plant stature, pseudostem colour, bunch morphology and development of the male phase (Jacob 1952). Menon et al. (2002b) collected, characterized and catalogued the variability of Nendran in South India and based on morphological characterization recognized ten morphotypes. A key was developed for their identification.

2.3.1.3 Mysore Subgroup

Mysore is grown on a large scale in South Asia. However, outside India and Sri Lanka, it is only occasionally encountered. Exceptions are Trinidad, where it is used to shade cocoa, and Western Samoa. 'Poovan' is a popular cultivar of this group grown all over the India in a perennial cropping system and is the leading commercial cultivar of southern and north-eastern states and easily available all through the year. It is known by different names viz. Mysore Poovan, Palayankodan, Champa, Alpan, Karpurachakkarakeli in India (Singh et al. 2001). It is a large and extremely vigorous cultivar, easily recognized by its pinkish-purple midribs and large cylindrical bunches of tightly packed short, plump bottlenecked fruits which ripen to an attractive bright yellow, and the flesh is agreeably sweet acid (Stover and Simmonds 1987).

2.3.1.4 Silk Subgroup

Silk is a very popular dessert cultivar in South and South East Asia, East Africa and Latin American-Caribbean regions characterized by fruit with a sweet-acid taste (Stover and Simmonds 1987).

The ripe fruits are thin skinned and fall of easily from the bunches (highly deciduous) weighing 15–18 kg and it takes 13–15 months to come to harvest. Hard lumps in fruits occur, especially when grown in acidic soils. *Fusarium* wilt is a major threat to this cultivar resulting in restricted cultivation. It is tolerant to leaf spot.

2.3.1.5 Pome Subgroup

Cultivars in the Pome subgroup are important dessert banana types in India and Brazil, where their subacid flavour is much appreciated. The taste is also popular in Australia and Hawaii (Stover and Simmonds (1987)). However, cultivars are generally not very productive. The Pome subgroup has several cultivars and mutants. In general the bunch is semi-horizontal and fruits are slightly angular and the peel is thick (Stover and Simmonds 1987).

2.3.1.6 Pisang Awak Subgroup

Pisang Awak is a widely disseminated, high-yielding, cooking/dessert cultivar group, which is also used as a beer banana in East Africa. It is very common in Thailand, Vietnam and elsewhere in the Indo-China region. This triploid group has high inherent fertility which yields seedless edible fruits if un-pollinated, but bears seeds when pollinated by pollen from fertile dip-

loids growing in the neighbourhood or by wild bananas. This group is represented by many cultivars in India, viz. 'Karpooravalli', 'Pey Kunnan', 'Vella Palayankodan', 'Dakshinsagar' and 'Paloor' (Uma and Sathiamoorthy 2007).

2.3.2 Conservation of Genetic Resources

Banana improvement programmes depend on the full range of diversity and therefore warrant effective conservation and documentation of existing cultivars and wild relatives. Banana biodiversity is conserved as full-size plants in field gene banks (Amalraj et al. 1993; Menon et al. 2000a; Uma et al. 2005a) or as in vitro plantlets (Van den Houwe et al. 1995) or cryopreserved (Panis et al. 2007). The diversity of *Musa* germplasm is conserved in about 60 national collections. An in vitro collection comprising 1,200 *Musa* accessions are held at the global banana collection managed by Bioversity International and hosted by the Belgian university KU Leuven at the International Transit Centre (ITC). Characterization and evaluation data from 22 collections are available in the Musa Germplasm Information System (MGIS) (Channeliere et al. 2011). Widespread diseases such as banana bunchy top virus, banana bract mosaic virus, weevil borers, Sigatoka leaf spot and *Fusarium* wilt are serious threats to germplasm, small-scale production systems and field repositories. Several of these uncommon or rare homestead/backyard clones are on the verge of extinction. The various species of *Musa* that occur wild in the forests (Western Ghats and north-eastern region) also face a threat from deforestation, wild animals and shifting cultivation. Hence there is an urgent need to conserve the threatened wild species and rare genetic resources for their utilization in the future. In vitro conservation methods are routinely applied for banana germplasm conservation and exchange at the NBPGR, New Delhi (Agrawal et al. 2007). Field collections are maintained at NRCB, Trichy and some State Agricultural Universities. Germplasm conservation activity is financially supported by Indian Council of Agricultural Research through the All India Coordinated Research Project on Tropical Fruits.

2.3.3 Breeding Targets

Major threats to banana and plantain production include fungal, bacterial and viral pathogen, several insect pests and a complex of plant parasitic nematodes (Table 2.1). Among fungal diseases, black leaf streak disease (BLSD) or black Sigatoka caused by *Mycosphaerella fijiensis* is the most important in the world. Sigatoka leaf spot or yellow Sigatoka caused by *M. musicola* is a related disease causing similar damage. *Fusarium* wilt caused by the fungus *Fusarium oxysporum* f.sp. *cubense* is widely regarded as the most destructive plant disease in the recorded history (Moore et al. 1995). Being soilborne, it is also described as a disease that refuses to go away (Ploetz and Churchill 2011). The disease was responsible for the destruction of Gros Michel-based export trade and its replacement by the resistant Cavendish (AAA) group cultivars. Tropical race 4 attacking Cavendish is an extremely virulent form of the pathogen reported more recently (Hwang and Ko 2004). Since fungicidal sprays or cultural practices cannot contain the disease, long-term option of developing resistant varieties to replace susceptible one is suggested (Hwang and Ko 2004). Viruses also constitute major production constraints besides being an impediment to germplasm enhancement and movement (Jones 2000). Four major viruses encountered in banana include banana bunchy top virus (BBTV), banana bract mosaic virus (BBMV), cucumber mosaic virus (CMV) and banana streak virus (BSV). BSV-related sequences are integrated within the nuclear genome and can cause a viral infection (Harper et al. 1999).

Among insect pests, banana corm weevil (*Cosmopolites sordidus*) has attracted worldwide attention resistance to this pest that has been a breeding target. Banana stem weevil (*Odoiporus longicollis*) is a devastating pest of plantains in India (Padmanabhan and Sathiamoorthy 2007).

Table 2.1 Major diseases and pests of banana/plantain

I. *Diseases*	
i. *Fungal diseases*	
Sigatoka leaf spot	*Mycosphaerella musicola*
Black leaf streak	*Mycosphaerella fijiensis*
Eumusae leaf spot	*Mycosphaerella eumusae*
Panama wilt	*Fusarium oxysporum* f.sp. *cubense*
ii. *Bacterial diseases*	
Rhizome rot	*Erwinia* sp.
iii. *Viral diseases*	
Banana bunchy top virus	BBTV
Banana bract mosaic virus	BBMV
Cucumber mosaic virus	CMV
Banana streak virus	BSV
II. *Insect pests and nematodes*	
Rhizome weevil	*Cosmopolites sordidus*
Pseudostem weevil	*Odoiporus longicollis*
Banana aphid	*Pentalonia nigronervosa*
Nematodes	*Radopholus similis*, *Meloidogyne incognita*, *Pratylenchus* sp.

Plant parasitic nematodes are also a major constraint to *Musa* production (Stover and Simmonds 1987) and include *Radopholus similis*, *Meloidogyne incognita*, *Pratylenchus coffeae* and *Helicotylenchus multicinctus*.

The development of cultivars with high yield and resistance/tolerance to these production constraints is the primary focus of the banana improvement programme. The overall strategy has been to incorporate resistance to *Fusarium* wilt, Sigatoka and other pests in existing cultivars rather than aiming for genetic materials that are drastically different. Even though initial focus was on breeding for resistance to Sigatoka leaf spot, objectives have gradually widened to include resistance to nematodes and several other pests as well as modifying plant architecture, growth habit and fruit quality (Tomekpe et al. 2004).

2.3.4 Breeding Challenges in *Musa*

Musa is a polyploid crop with ploidy ranging from diploid ($2n = 2x = 22$) to tetraploids ($2n = 4x = 44$). Most cultivated bananas are triploids ($2n = 3x = 33$) and sterile harbouring various combinations of either one, two or three A, B, S or T genomes. New banana cultivars are exceptionally cumbersome to develop. Selection for desirable characters is time consuming and it may take up to 12 years to develop a new cultivar. *Musa* breeding is based mainly on the phenotypic mass recurrent selection. The high levels of heterozygosity make identification of ideal parental material difficult, and very large populations are required for the selection of individual clones with good agronomic traits. This is virtually impossible to attain due to the low seed set in crosses. Generally, few seeds are obtained (an average of 1–1.5) and acquiring large numbers of seeds is a labour-intensive and tedious process (Ortiz and Vuylsteke 1995a; Ssebuliba et al. 2006, 2009). The genes for resistance to diseases and pests are introgressed from wild diploid species which also carry many undesirable traits, e.g. low yield and non-parthenocarpy. The process of eliminating the unwanted traits requires several backcrosses that lengthen the breeding process (Rowe and Rosales 1992). The multigenic nature and low heritability of some traits also slow down the breeding process. *Musa* breeding is also problematic due to the narrow genetic diversity of the germplasm (Pillay et al. 2004) and the lack of information about wild species that carry useful agronomic traits. Only a few wild diploids have been used as male parents