

Chapter 8

Genetic Diversity in Banana



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Abstract Banana and plantain are one of the globally important commercial crops supporting livelihood and food security of millions across the globe. In view of environmental degradation, climate change, loss of diversity of crop plants and thereafter the need for diversity-based farming for the sustainable food system, the importance of conservation and utilization of genetic diversity of *Musa* are globally signified. The edible banana fruit is available entirely from section Eumusa and rarely from section Australimusa of the genus *Musa* under family Musaceae and developed from two ancestor species *M. acuminata* and *M. balbisiana* in the South East Asian centre of origin. The diversity and distribution of species, subspecies and groups are important aspects in evolutionary and conservation studies. There are so far three major geographical regions of distributions of cultivated bananas, viz. Asia and the Pacific (29%), Africa (35%) and Latin America and the Caribbean (36%). A large proportion (70–85%) of the gene pool of the domesticated banana is available within Asia and the Pacific regions. The conservation of *Musa* germplasm is the priority objective of programmes and activities related to *Musa* (Banana and Plantain) diversity. The strategy for *Musa* conservation has been developed by the International Network for the Improvement of Banana and Plantain (INIBAP). The international banana germplasm collection is managed by the International Transit Centre (ITC) with INIBAP/Bioversity International, having the world's largest *Musa* germplasm collection and conservation with around 1500 accessions. Morphological and molecular characterization is useful in the classification of cultivars and newly discovered wild *Musa* species. There are two major utilizations of the collected and evaluated *Musa*

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germplasm, viz. use of accession in breeding and crop improvement programmes and secondly, boosting production through direct distribution of better accession to the small-scale banana growers. The International *Musa* Testing Programme (IMTP), coordinated by INIBAP with the NARS and breeding programmes, resulted in several promising banana hybrids. Direct use of *Musa* accessions by the farmers from the ITC also showed encouraging impact on small banana growers in Cuba, Northern Tanzania and Nicaragua for their food security and better income. There is a need for more intensification of *Musa* germplasm collection, conservation and utilization, so as to achieve the call of the ‘Delhi Declaration on Agrobiodiversity Management 2016’.

Keywords Agrobiodiversity · Genetic diversity · *Musa* · Germplasm · Species · Wild · Banana · Plantain · Cultivar · Variety · Collection · Characterization · Utilization

8.1 Introduction

Banana and plantain are one of the globally important commercial crops, belonging to the genus *Musa* and supporting a significant contribution to the global food commodity sources (in terms of gross value of production) after rice, wheat and milk (Horry 2000). It is grown in more than 300 countries in the world (Singh 2008) with an annual production of 106.84 million tons from 5.03 million hectares of the plantation (Anon 2015). Livelihood and food security of millions across the globe are dependent on this crop. The sustainable banana production system is supposed to be one of the main pillars of the sustainable food system with minimal impact on the environment, for the much needed focus on addressing world hunger and malnutrition especially under the changing scenario of climate. But the real challenges are multidimensional and interconnected in respect of sustainability, as recognized by the Sustainable Development Goals, signed by 193 world leaders in 2015. In the past, it was needed to increase food production but could not reimburse due attention for the environment, water and biodiversity, and hence, contributed to considerable environmental degradation and loss of crop diversity. For a sustainable solution, agricultural diversity and diversity-based farming have been advocated as the major backbones of sustainable agricultural intensification and sustainable food system (Tutwiler et al. 2017). This line of sustainable food system signifies the importance of genetic diversity of *Musa* and its conservation and utilization. The genetic diversity is the sum of the gene pool in wild relatives, native landraces, local selections, elite cultivars and released and introduced varieties ranging from its centre of origin, diversification and commercial cultivation. The primary responsibility is the collection and conservation of the existing diversity in a systematic manner (Dinesh and Veena 2015), followed by its ultimate objectives of characterization, evaluation and utilization of the gene pool for a sustainable banana production system. Our effort was to compile

the constructive information selectively into a simplified and summarized article on genetic diversity in banana.

8.2 Family (Taxonomic) Background of Banana

The commercially available edible banana and plantain are the finest representative members of the plant family Musaceae and genus *Musa* that resulted from historical evolution and domestication processes. The order Zingiberales (previously called Scitaminae) accommodates the Musaceae family along with other seven related families. Altogether of these eight families have over 2000 species, of which there are about 50 species of the banana family, Musaceae. However, when genetic diversity of edible banana is concerned, interest is directed towards Musaceae family only, with special emphasis on genus *Musa* and its sections Eumusa and Australimusa. Under the family Musaceae, there are two genera, viz. *Ensete* and *Musa*. The genus *Musa* includes four sections—Callimusa, Rhodochlamys, Australimusa and Eumusa, of which edible fruit is available only from Eumusa and rarely from Australimusa (Table 8.1). The economic importance of the sections Callimusa and Rhodochlamys is limited to commercial exploitation of natural fibre and ornamental plant (Valmayor 2000).

Table 8.1 Genus and species of the banana family Musaceae^a

Genus ^b	Section	Species	Distribution	Uses	Chromosome
Ensete	–	7–8	West Africa to Papua New Guinea	Vegetable, fibre	9
Musa	Callimusa	5–6	Indo-China, Thailand, Malaysia and Indonesia	Ornamental	10
	Rhodochlamys	5–6	India to Indo-China	Ornamental	11
	Australimusa	5–6	Queensland, Australia to the Philippines	Fibre, fruit	10
	Eumusa	9–10	India, South East Asia, Papua New Guinea, to South Pacific and Japan	Fruit, fibre, vegetable	11

^aSource Valmayor (2000)

^bInclusion of the third genera, *Musella* was controversial, while *Insertae sedis* was a new section added to genus *Musa* (Uma et al. 2005)

8.3 Origin and Evolution

Earlier it was considered that banana is originated in India, owing to its antique evidence in Indian culture, epics and wide diversity and adoption of banana (Valmayor 2000). Later, based on a systematic study by Simmonds and Shepherd (1955) with expertise in genetics and cytotaxonomy, it was concluded that South East Asia is the centre of origin of the ancestors of edible banana. Those ancestors of the present day seedless edible banana were seedy, non-pulpy and non-edible (Uma et al. 2005). There were two most widespread ancestor species under the genus *Musa*, viz. *M. acuminata* and *M. balbisiana*, of which *M. acuminata* types had its primary centre of origin in the Malayan region while *M. balbisiana* was of Indian origin (Horry 2000; Chandel and Agarwal 2000). The natural distribution of the genus *Musa* stretches in the north from Nepal and southern mountainous China and in the south to the southern islands of Indonesia and New Guinea, with an outlier in the wet tropical rainforests of Queensland. The western limit is India, with an outlier on Pemba Island near the East African coast. To the east, wild *Musa* were recorded in Melanesia, with an outlier on Samoa. No wild *Musa* have been recorded on the African continent or in the Americas. These boundaries define the area of primary (natural) diversity of *Musa* (Langhe et al. 2009). Evolution of edible banana was initiated with the *M. acuminata* types. Due to the gradual development of parthenocarpic traits, early human settlers discovered the edible types of banana fruit, leading to the most striking human interventions in the form of selection and perpetuation of edible types of banana across geographical locations. As a result, the *M. acuminata* types come across the Indian subcontinent where it introgressed with *M. balbisiana*, and hence, the earliest bispecific types, i.e. the natural hybrids of banana were evolved. Differential combinations of these two wild progenitor species resulted in the development of different genomic constituents and groups, ranging from diploid to tetraploids. Considering 'A' genome contribution from *M. acuminata* colla and 'B' genome contribution from *M. balbisiana* colla, the bispecific genomic groups of diploid edible bananas are AA, AB and BB, triploid bananas are AAA, AAB and ABB and the tetraploid bananas are AABB and ABBB (Uma et al. 2005). As the present day edible banana is the outcome of the initial natural hybrids and carrying genome contributions of two species (*M. acuminata* and *M. balbisiana*), hence giving a species name to edible banana is inappropriate. The name of edible banana is to be decided as per genomic nomenclature based on the proportionate genomic contribution of the progenitor species (Valmayor 2000), for example, 'Musa (AAB) Martaman' is the genomic nomenclature of the banana cultivar 'Martaman'. However, the recent molecular genetic studies suggested that the cultivated bananas might have been derived through intra- and inter-specific hybridizations of four wild *Musa* species, namely *M. acuminata* (A-genome), *M. balbisiana* (B-genome), *M. schizocarpa* (S-genome) and *M. textilis* (T-genome) with identification of two more genotypes (AS and AT) and the S- and T-genome cultivars being occurring in New Guinea (Li and Ge 2017). A few, mostly tetraploid edible banana varieties in the Melanesia-Philippines region show some characteristics of the species *Musa schizocarpa* Simmonds (Section Eumusa) and

Musa textilis Née (Section Australimusa) besides those of the major contributing species, *M. acuminata* and *M. balbisiana*. However, the contribution of *M. schizocarpa* and *M. textilis* to the generation of edible bananas is very minor and probably relatively recent (Langhe et al. 2009).

8.4 Diversity and Distribution of Different Sections, Species and Subspecies of the Genus *Musa*

8.4.1 Diversity and Distribution of Different Sections of the Genus *Musa*

There are four sections of the genus *Musa*, viz. Australimusa, Callimusa, Rhodochlamys and Eumusa, and they have major diversity and distribution in South East Asia, except Australimusa. Champion (1967) presented the geographical distribution of these sections and that had been accepted traditionally and widely. However, a need was felt for revision of these traditionally accepted boundaries of distribution of sections to develop more accurate ones and could reasonably justify the presence of Callimusa in China and Australimusa in Borneo (Fig. 8.1) (Pollefeys et al. 2004).

The Philippines, Queensland and Australia are the major regions of diversity, distribution and commercial utilization of the species of section Australimusa that comprises of 5–6 species. The commercial production of textile fibres in the Philippines, called ‘Abacca’ or ‘Manila hemp’ is done from *Musa textilis* which is a species under the section Australimusa. Another species under this section is *Musa fehi*, grown in the South Pacific (locally known as ‘Fe’i banana’) and Indonesia (locally known as ‘Pisang Tongat Langit’) for edible fruits that are borne on upward-erect bunch with its male axis pointed to the sky (in contrast to the species of Eumusa section which bear fruit on hanging bunches). The species (about 5–6) under the section Callimusa are mostly non-domesticated and wild, with major distribution in Indo-China and Indonesia, and utilization is limited to its ornamental values. The section Rhodochlamys comprises the species which are distributed in India, Indo-China, the Philippines, Thailand and Malaysia and mostly ornamental with small plant size and bright colour, erect to semi-erect inflorescences (Horry 2000; Valmayor 2000). Uma et al. (2006) redefined the distribution of the section Rhodochlamys in India, based on their explorations and earlier literature and reported the distribution of six species across 11 states of the country. There are at least ten species under the section Eumusa, and they are distributed in India, South East Asia, Papua New Guinea, to South Pacific and Japan (Table 8.2).

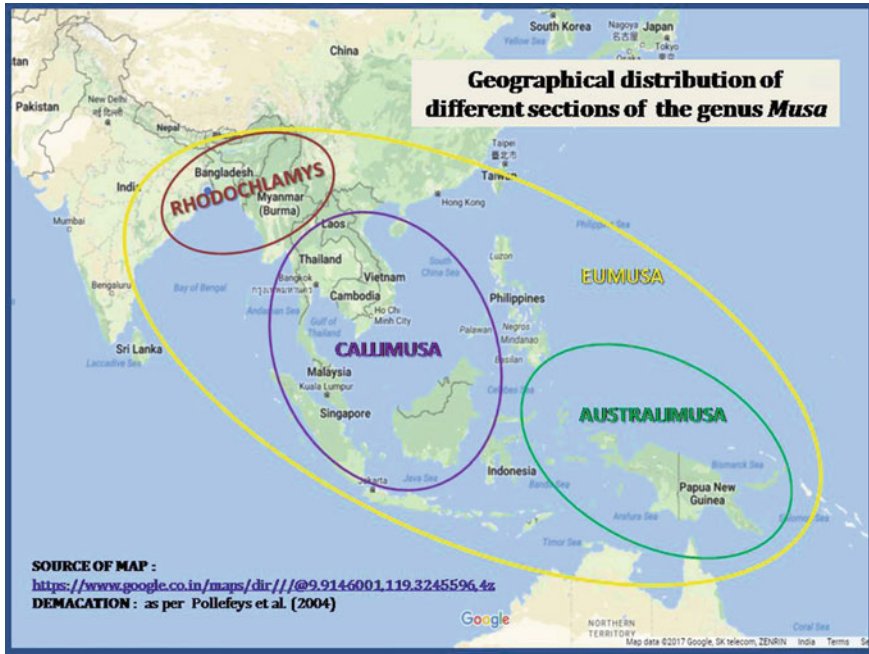


Fig. 8.1 Geographical distribution of different sections of the genus *Musa*. Figure construction is based on a map source (Google maps 2018) of <https://www.google.co.in/maps/dir///@9.9146001,119.3245596,4z> and information source of Pollefeys et al. (2004)

8.4.2 Diversity and Distribution of Different Species and Subspecies of the Genus *Musa*

Among the species of *Eumusa*, *M. acuminata* and *M. balbisiana* are two very wide spread species across the centre of origin and diversity. However, the distribution of *M. balbisiana* was supposed to be less extensive than *M. acuminata*. The southern India, northern Myanmar (Burma) and the Philippines were considered as the regions of the original population of this species, from where they were introduced to other locations (Cheesman 1948; Argent 1976). In the studies for clarification on the issue of origin of ‘Butuhan’ clone (an ancient hybrid of *M. balbisiana* and *M. textilis*), it was indicated that *M. balbisiana* was an introduced species also in the Philippines, and hence the origin of *M. balbisiana* was limited to India and Myanmar only (Carreel 1994; Horry 2000). Shepherd (1990) presented the diversity and geographical distribution of the subdivisions of *M. acuminata* (Table 8.3, Fig. 8.2). Based on molecular studies, some of the subspecies were similar to others (ssp. *banksii* vs. *microcarpa*; *zebrine* vs. *sumatrana*) and were tentatively summarized by Horry (2000) into six subspecies, distributed in six regions (Table 8.4). However, Perrier et al. (2009) differentiated *M. acuminata* seeded diploids into four basic clusters on the basis of molecular markers (RFLP and SSR), viz. (i) *M. acuminata* spp. *banksii*

Table 8.2 Diversity and distribution of species under different sections of the genus *Musa*

Section	Selected species	Distribution	Uses
<i>Australimusa</i> (Basic chromosome No. 10)	<i>M. textilis</i> <i>M. maclayi</i> <i>M. lolodensis</i> <i>M. peekelii</i> <i>M. fehi</i>	Queensland New Caledonia Philippines Australia	Fibre, fruit and vegetable
<i>Callimusa</i> (Basic chromosome No. 10)	<i>M. coccinea</i> <i>M. violascens</i> <i>M. gracilis</i>	Indo-China Indonesia	Ornamental
<i>Eumusa</i> (Basic chromosome No. 11)	<i>M. acuminata</i> <i>M. balbisiana</i> <i>M. schizocarpa</i> <i>M. itinerans</i> <i>M. flaviflora</i> <i>M. sikkimensis</i> <i>M. cheesmani</i> <i>M. nagensium</i> <i>M. halabanensis</i> <i>M. ochracea</i>	India	Fruit, vegetable, fibre and medicinal
<i>Rhodochlamys</i> (Basic chromosome No. 11)	<i>M. ornate</i> <i>M. velutina</i> <i>M. laterita</i> <i>M. sanguinea</i> <i>M. mannii</i> <i>M. aurantiaca</i> <i>M. rosea</i> <i>M. rubra</i>	India Indo-China Philippines Thailand Malaysia	Ornamental
<i>Incertaesedis</i> (Basic chromosome No. 7, 9)	<i>M. boman</i> <i>M. ingens</i> <i>M. lasiocarpa</i>	–	Medicinal

Source Uma et al. (2005)

cluster from New Guinea, (ii) *M. acuminata* spp. *malaccensis* cluster from Malayan Peninsula, (iii) *M. acuminata* spp. *burmanica*, *burmanicoides*, *siamea* from north-east India, Burma, southern China and Thailand and (iv) *M. acuminata* ssp. *zebrina* cluster from Java.

8.4.3 Diversity and Distribution of Different Groups and Subgroups of Edible Bananas

The diversity and distribution of different groups and subgroups of cultivated bananas and plantains have been the consequences of the long history of continuous evolutionary and domestication pathways through its wild, cultiwild and neutralized forms, towards its basic cultivars (clones) and derived cultivars. The genome assemblies

Table 8.3 Diversity and geographical distribution of the subdivisions of *M. acuminata*^a

Subspecies of <i>M. acuminata</i>	Geographical distribution
<i>M. acuminata</i> ssp. <i>urmannica/burmannicoides</i>	Sri Lanka, Eastern India (including Assam), Myanmar (Burma), northwest Thailand
<i>M. acuminata</i> ssp. <i>siamea</i>	Thailand (central, northern, eastern-?), Indo-China
<i>M. acuminata</i> ssp. <i>malaccensis</i>	Thailand (southern), Malaysia (northern), Sumatra
<i>M. acuminata</i> ssp. <i>truncata</i>	Malaysia (upland areas), Thailand (southern-?)
<i>M. acuminata</i> ssp. <i>microcarpa</i>	Only at Sabah and Sarawak
<i>M. acuminata</i> ssp. <i>banksii</i>	Irian Jaya, Papua New Guinea and neighbouring islands, Australia (northern)
<i>M. acuminata</i> ssp. <i>errans</i>	The Philippines
Non-characterized Indonesian forms (<i>zebrina</i> , <i>M. sumtrana</i> ?)	Sumatra, Java, Kalimantan, Sulawesi
'Pemba' forms	Zanzibar (?)

^aSource Shepherd (1990)

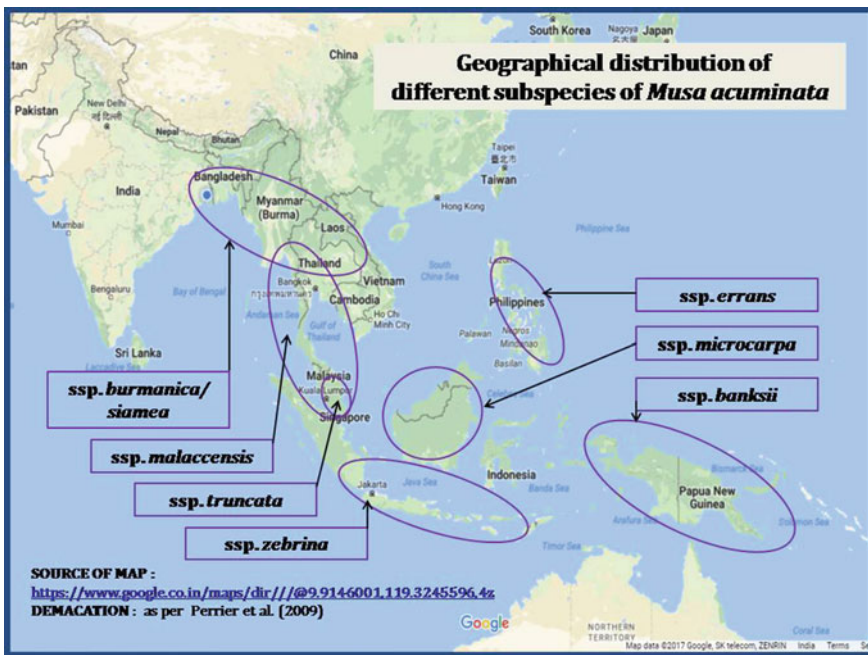


Fig. 8.2 Geographical distribution of different subspecies of *Musa acuminata*. Figure construction is based on a map source (Google maps 2018) of <https://www.google.co.in/maps/dir///@9.9146001,119.3245596,4z> and information source of Perrier et al. (2009)

Table 8.4 Revised diversity and geographical distribution of the subspecies of *M. acuminata*^a

Subspecies of <i>M. acuminata</i>	Geographical zones of distribution
<i>M. acuminata</i> ssp. <i>urmannica/burmannicoides/siamea</i>	Northern zone —India, Myanmar, Thailand (central, northern)
<i>M. acuminata</i> ssp. <i>truncata</i>	Malaysian mountains
<i>M. acuminata</i> ssp. <i>malaccensis</i>	Central-western zone —Thailand (southern), Malaysia (northern), Sumatra
<i>M. acuminata</i> ssp. <i>microcarpa</i>	Central-eastern zone —Borneo
<i>M. acuminata</i> ssp. <i>banksii/errans</i>	Eastern zone —The Philippines, Papua New Guinea, Australia (northern)
<i>M. acuminata</i> ssp. <i>zebrina</i> (<i>M. acuminata</i> ?)	Southern zone —Indonesian archipelago

^aSource Horry (2000)

that constitute the entire spectrum of edible banana and plantains, such as AA, AAA, AAB or ABB, are designated as the ‘Groups’, while the total set of a basic cultivar and its derived clones form a ‘Subgroup’. Development of subgroups appeared to occur in regions distant from the primary (natural) distribution of *Musa*. These distant regions are usually known as the centres of secondary and tertiary diversity. Derived cultivars can undergo somatic mutation, thus leading to new derived cultivars. Hence, continued diversifications at secondary and tertiary centres of diversity are supposed to result in distributions of the high density of specific banana cultivar groups at notable geographical areas, as given in Table 8.5 (Langhe et al. 2009). There are so far three major geographical regions of distributions of the cultivated bananas and plantains, with a somewhat one-third contribution by each to the total global production, viz. Asia and the Pacific (29%), Africa (35%) and Latin America and the Caribbean (36%). Each of these three geographical regions has a separate set of diversity with respect to cultivated bananas. Among the groups of cultivated

Table 8.5 Geographical distribution of the main banana cultivar groups^a

	Group	Geographical distribution
1	The AA and AAA cultivars	The ‘Indonesia-Philippines-Melanesian’ region, with exceptional AA density in New Guinea and around
2	The Highland AAA bananas (East African Highland bananas, EA-AAA)	The Great Lakes region in East Africa
3	The AAB-Plantains	The rainforest zone in Africa
4	The AAB Maia Maoli-Popoulu-Iholena cultivars	Oceania
5	The AB and other AAB	South India
6	The Eastern ABB cultivars	Philippines and Vietnam
7	The Western ABB subgroup	Northeast India and South India

^aSource Langhe et al. (2009)

banana in South East Asia, the dessert and cooking banana belonging to AAB and ABB groups showed the highest diversities in that regions. In Africa, the highland banana (EA-AAA) of AAA group is predominantly cultivated in East Africa, while the AAB clones of Plantain subgroup are grown in the Central and West Africa. The cultivated bananas of the Latin America and the Caribbean belong to three major subgroups, viz. Cavendish subgroup of AAA, Plantain subgroup of AAB and Pome and Silk subgroups of AAB group. The banana varieties exported throughout the world are only from the Cavendish subgroup of the AAA group (Table 8.6) (Horry 2000; INIBAP-IBPGR 1990).

Table 8.6 Diversity and distribution of triploid subgroups of cultivated banana and plantains^a

Subgroup	Distribution
AAA—Cavendish	It is a main export of banana and grown for local consumption in many countries. Humid tropical regions between 20°N and 20°S also extended to 20° and 30° latitude of both hemispheres
AAB—Plantain	Mainly found in hillsides of humid tropics to the lowland humid tropical forest of Americas, West Africa and South India. It can grow in very poor to fertile alluvial soil
AAB—Silk, Mysore and Pome	These types of bananas are slightly acidic in taste. They are very popular in the southern states of India. Other than India, they can be found in Brazil, few restricted areas of Mexico and Venezuela. Pome type can be found in Australia; whereas, Silk type is grown in the Caribbean and South East Asia
ABB—Bluggoe and Pisang Awak	These are hardy cooking-type banana but also used as a dual purpose (dessert type). Bluggoe and Pisang Awak can grow even well in marginal land and harsh environment, as well as higher altitudes in tropical regions. Bananas from this group used a staple food in South America, especially indigenous people of Savannahs and Amazon basin. Pisang Awak is popular in the backyard garden of Asia and grown on small to medium scale
AAB—Maia Maoli/Popoulu	Maia Maoli/Popoulu cultivars are mainly grown as a backyard banana in Pacific Island. They are very important cooking-type banana in this area. They are also found in the west coast of South America ('Maqueno' cv. In Ecuador)
AAA—Mutika/Lujugira	These types are also known as East African Highland banana, grown between higher altitudes of 1000–2000 m. Major food crop of Uganda, cultivated in 40% of total arable land

^aSource Horry (2000)

8.5 Collection and Conservation of *Musa* Germplasm

The major proportion (70–85%) of the gene pool of domesticated dessert banana is constituted from the Asia and the Pacific regions. Whereas the gene pool of the cooking type banana and plantain predominate in the African continent. More than 60 cooking banana types are available in the East African Highlands, and more than 120 plantain types are available in the West and Central Africa. Introduction of banana to the African continent dated back to about 3000 years ago and its remarkable diversification resulted in a source of staple food for the people of many countries of this continent based on those cooking-type banana and plantains. Another edible banana group, known as Fe'I bananas, is confined to the Pacific. However, the threats posed by habitat destruction and the replacement or loss of traditional cultivars intensify the urgency for collection and conservation efforts. Besides, the crop improvement strategy needs a well collected and conserved gene pool (INIBAP 2006). Widespread diseases and pests of *Musa* such as banana bunchy top virus, weevil borer, Sigatoka leaf spot and Fusarium wilt are major problems for the conservation of *Musa* germplasm at ex situ field gene bank. Many uncommon and rare species are on the verge of extinction even in in situ conditions. Various species of *Musa* face challenges due to the destruction of their wild habitat by deforestation, shifting cultivation and wild animals. Therefore, there is utmost importance for conserving natural habitats of different rare and uncommon species (Menon 2016). According to the approach of the diversity-based production system, productivity and sustainability may be enhanced by integrating inter- and/or intracrop diversity within the production system and the losses from epidemic diseases can be mitigated by planting mixed genotypes in place of extensive monocrops of a single variety of banana. In view of the 'Global Conservation Strategy for *Musa* (Banana and Plantain)' has been developed by INIBAP to provide a framework for the efficient ex-situ conservation of the globally important collections of *Musa species* (INIBAP 2006).

The planning for conservation of *Musa* germplasm was laid out at an international level at a seminar in 1989 in Belgium under the aegis of INIBAP and IBPGR. Conservation of *Musa* genotypes in their natural habitat requires the protection of their centre of origin and secondary centre of diversification. It is very challenging to implement any recommendation due to the vast land area. For supporting regional research and development initiatives and conservation efforts, there are four regional banana research networks, viz. BARNESA (for Southern and Eastern Africa), MUSACO (for West and Central Africa), BAPNET (for Asia and the Pacific) and MUSALAC (for Latin America and the Caribbean) (Fig. 8.3). These networks are integrated with the national research organizations of respective banana-producing countries, coordinated by a regionally posted INIBAP scientist. Several countries having high *Musa* diversity have been supported by INIBAP for collection, conservation and characterization of *Musa* germplasm. The banana germplasm collections and conservations of major gene banks in Asia and the Pacific Region have been presented in Table 8.7 (Valmayor 2000).

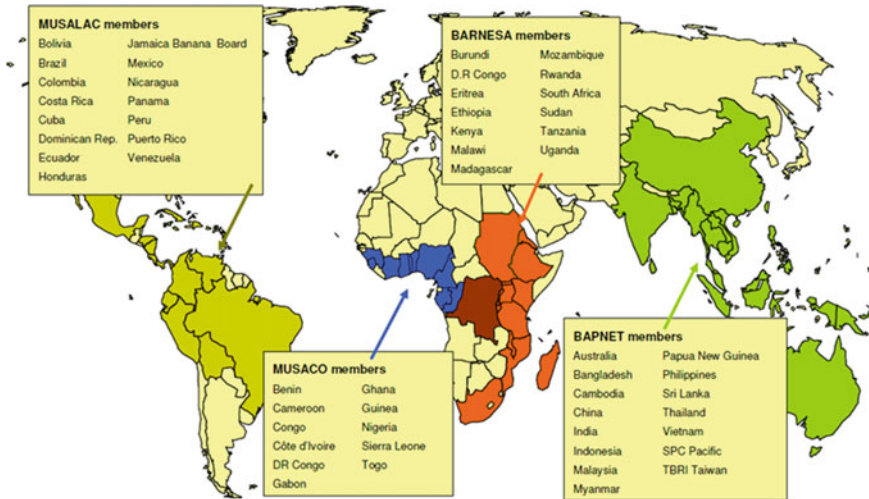


Fig. 8.3 Regional banana research networks—BARNESA, MUSACO, BAPNET and MUSALAC

8.5.1 Methods for Conservation of *Musa* Germplasm

The conservation of *Musa* germplasm is the priority objective of programmes and activities related to *Musa* diversity. The methods generally followed for the conservation of *Musa* germplasm are broadly categorized as in situ and ex situ conservation, depending on the site of conservation with respect to its natural habitat.

- (1) **In situ conservation:** It refers to the conservation efforts of *Musa* germplasm on-site under natural habitat where those were originated and evolved. Conservation of traditional local cultivars in the farmer's field under the observation of farmer with continuous cultivation and improvement of cultivars may also be considered under this type of conservation.
- (2) **Ex situ conservation:** The ex situ conservation refers to the conservation and maintenance of samples of living organisms outside their natural habitat. The plant parts used for ex situ conservation are whole plant, seed, pollen, vegetative propagule, tissue or cell culture, and accordingly the ex situ conservation techniques are also different. For example, short to medium period conservation of the whole plant can be done in field gene bank, while medium to long-term conservation is possible in cryopreservation using vegetative propagule or tissue culture materials.
 - (a) **Seed conservation:** Long-term conservation of genetic diversity of the natural population of *Musa* species by seed can be possible through different technological intervention (Darjo and Bakry 1990). The feasibility of conserving the wider *Musa* wild diversity needs to be explored, and with available knowledge of seed biology, seed conservation needs to be researched

Table 8.7 Banana germplasm collections of major gene banks in Asia and Pacific Region^a

Country	Germplasm collection	Institute
Australia	5 AA, 23 AAA, 7AAAA, 1 AB, 9 AAB, 4 ABB, 3 wild <i>Musa</i> species: <i>M. acuminata</i> , <i>M. balbisiana</i> , <i>M. zebrina</i>	Maroochy Horticultural Research Station, Department of Primary Industries, Nambour, Queensland 4560, Australia
	81 AA, 13 AAA, 24 AAB, 9 ABB, 1 ABBB, 3 BBB, 1 Fe'i banana, 5 AS, 3 AAT, 27 wild <i>Musa</i> species	South Johnstone Research Station, Department of Primary Industries, South Johnstone, Queensland 4859, Australia
Indonesia	9 AA, 9 AAA, 9 ABB, 2 BBB, 1 each <i>Musa acuminata</i> and <i>M. balbisiana</i>	Centre for Research and Development in Biotechnology, Cibinong, Java, Indonesia
	10 wild <i>Musa</i> species and 3 subspecies of <i>Musa acuminata</i>	Bogor Botanical Garden, Bogor, Java, Indonesia
Malaysia	16 AA, 16 AAA, 12 AAB, 6 ABB, 2 ABBB, 3 BBB, 6 wild <i>Musa</i> species and 6 <i>Musa acuminata</i> subspecies and 1 <i>Ensete</i>	Malaysian Agricultural Research and Development Institute, Serdang, Selangor, Malaysia
Philippines	18 AA, 25 AAA, 16 AAB, 9 ABB, 1 ABBB, 1 BB, 9 BBB, 9 wild <i>Musa</i> species, 1 <i>Ensete</i>	Bureau of Plant Industry, Department of Agriculture, Davao National Crop Research and Development Centre, Bago Oshiro, Davao City, Philippine
Thailand	9 AA, 8 AAA, 10 AAB, 10 ABB, 1 ABBB, 1 BBB, 3 wild species of <i>Musa</i> species, 4 <i>Musa acuminata</i> subspecies and 2 <i>Ensete</i>	Kesetsart University, Department of Horticulture, Pakchong Research Station, Nakhon Rachasim, Thailand
Vietnam	10 AA, 18 AAA, 12 AB, 9 AAB, 14 ABB, 9 wild <i>Musa</i> species, 1 <i>Ensete</i>	Phu Ho Fruit Research Centre, VinhPhu Province, Vietnam
Taiwan	15 AA, 86 AAA, 8 AAAAA, 4 AB, 36 AAB, 20 ABB, 4 BBB, wild and ornamental bananas	Taiwan Banana Research Institute, Chiuju, Pingtung, Taiwan
Papua New Guinea	106 AA, 71 AAA, 60 AAB, 38 ABB, 1 AB, 6 AAAB/AABB, 1 ABBB, 2 AS, 3 AAS, 3 ABBS, 1 AAAT, 1 Fe'i, and 103 unclassified, 6 wild <i>Musa</i> species and 1 <i>Ensete</i>	Department of Agriculture and Livestock Laloki, Konedobu, Port Moresby, Papua New Guinea

(continued)

Table 8.7 (continued)

Country	Germplasm collection	Institute
India	8 AA, 48 AAA, 1 AAAA, 12 AB, 84 AAB, 78 ABB, 3 AB BB, 52 unidentified, 6 wild <i>Musa</i> species	Consolidated data from field gene banks at National Research Centre for Banana (Trichy, 540 accession), Indian Institute of Horticultural Research (Bangalore, 241), Tamil Nadu Agricultural University (Coimbatore, 125), Banana Research Station (Kannara, 121), Banana Research Station (Hajipur, 115) and others

^aSource Valmayor (2000)

more widely. Seed and embryo cryopreservation have very good prospects but seed storage behaviour and seed germination after a long period of storage are very unpredictable; therefore, embryo rescue protocols are necessary with expertise for successful regeneration of new plantlets (MusaNet 2016). Although properly dried banana seeds can live for a few months to two years, but the germination requirement for different species varies from each other depending upon the germination temperature (Chin 1996). Seed conservation of banana is limited to wild species only, and this option cannot be used to local varieties and cultivars since female sterility is observed.

- (b) Field collections and on-farm conservation: Small number of accession to several hundreds of accession is distributed as field collection throughout the whole inter-tropical zone, and this type of field conservation is very essential for assessing and characterization of existing *Musa* germplasm. Establishment of regional field collection in each geographic region made it possible to conserve the diversity on-site in each continent (INIBAP-IBPGR 1990), e.g. for Asia, the Davao in the Philippines (Department of Agriculture, Bureau of Plant Industry-BPI); for East Africa, the Gitega in Burundi (Institut de Recherches Agronomique et Zootechnique-IRAZ); for Central and West Africa, one in Nigeria (IITA) and for Latin America and the Caribbean, another at La Lima in Honduras (FHIA). But, maintaining this large on-site field germplasm centre is very challenging due to natural hazard, and disease–pest problems. A significant amount of *Musa* diversity continues to be maintained in farmers' fields. Many farmers are already practicing de facto on-farm conservation through the continued cultivation of landraces or traditional cultivars or landraces of bananas and plantains. Hence, the traditional local cultivars and local landraces with cultural significance and nutritional value are conserved.
- (c) In vitro collections and cryopreservation: In 1989, setting up of an international in vitro collection centre at INIBAP transit centre (INIBAP TC) at Leuven, Belgium energized the collection of various banana germplasm in vitro and duplicating the material in other collection centre around

the different region. Cryopreservation or cryo-conservation is a process where plant parts are preserved by cooling to very low temperatures (typically at -80°C using solid carbon dioxide or at -196°C using liquid nitrogen). Satisfactory conservation in liquid nitrogen, followed by explants generation and plantlet formation was reported in banana (Villalobos and Abdelnour 1991; Agrawal et al. 2004). Improved protocol for cryopreservation of *Musa* species has been developed at ITC (KU, Leuven) which involves the use cryoprotectants, followed by slow freezing and plunging into liquid nitrogen. Recovery of banana cell suspensions after five years of storage in liquid nitrogen has been reported and those stored materials recorded with 95% regeneration (Panis et al. 2004, 2007). For conservation of the elite and rare banana germplasm, the ITC took up the programme of cryopreservation of *Musa* germplasm at the global level.

In 1984, the International Network for the Improvement of Banana and Plantain (INIBAP) was established at Leuven, Belgium. Collection of germplasm, its conservation and distribution of diseases-free germplasm were the major activities under *Musa* germplasm management programmes of INIBAP. As per the decision of the CGIAR, the INIBAP was brought under the governance and administration of the International Plant Genetic Resources Institute (IPGRI) in 1993 and henceforth, the international banana germplasm collection is managed by the IPGRI/INIBAP Transit Centre (ITC). For the benefit of the international community, the collections were placed under the auspices of FAO and are held in trust by INIBAP in 1994. With the contribution from 44 countries across the world, including the accessions of wild, cultivated and improved bananas, the collection of INIBAP consisted of approximately 1200 accessions (Van Den Houwe et al. 2003). The genetic diversity of genus *Musa* was largely covered in the collections located at INIBAP Transit Centre (ITC) in Catholic University in Leuven, Belgium, which was the largest assemblage of *Musa* collections and conservation with around 1500 accessions (Table 8.8) and represented by about 15% wild relatives, 75% landraces and 10% advanced cultivars of the genus. The accessions are maintained permanently by in vitro conservation techniques using proliferating shoot culture and slow growth conditions at low temperature (16°C) and reduced light intensity ($25\ \mu\text{mol}/\text{m}^2/\text{s}$). Standardized indexing processes against five viral diseases are followed for the incoming germplasm in collaboration with three indexing centres located at Australia, France and South Africa. Only the pathogen-free accessions are made freely available for international distribution (Van Den Houwe et al. 2003).

Major objectives of collection and conservation efforts by the ITC are:

- (i) Long-term conservation of *Musa* genetic resources,
- (ii) Maintaining a diversity in public domain,
- (iii) Contributing to understand *Musa* diversity through characterization,
- (iv) Services for safe movement of germplasm and related information,
- (v) Developing and transferring ex situ conservation.

Table 8.8 *Musa* germplasm collection and conservation at ITC^a

Type of source	Donor	Accessions	Genotypes
Major Field collections	FHIA, Honduras (1988)	97	Wild/cultivated forms
	IITA, Nigeria (1986–1987)	85	AAB-plantain
	IRAZ, Burundi (1987)	54	EA-Highland bananas
	CIRAD, Guadeloupe (1987–1990)	267	Wild/cultivated forms
	CARBAP, Cameroon (2010)	41	AAB-plantain
	NRCB, India (2010–2011)	57	AB, AAB and ABBs
Collecting missions	Papua New Guinea (1989–1990)	278	Diploid wild/cultivated
	Vietnam (1996)	43	Wild/cultivated forms
	Tanzania (2002–2005)	56	EA-highland bananas
	DR Congo (2005)	38	Semi-dwarf AAB-plantains
Breeding programmes	CARBAP, CIRAD, EMBRAPA, FHIA, IAEA, IITA, INIVIT, TBRI	126	Improved high-yielding and disease-resistant cultivars
Others	Other collections, botanical gardens, private persons	337	Wild/cultivated forms
Total		1479	

^aSource Musanet (2016)

8.6 Characterization and Evaluation of *Musa* Germplasm

The collected *Musa* germplasm essentially need proper characterization and evaluation to ascertain its genetic relatedness, diversity, conservation strategy and utilization. The characterization is done on the basis of morphological characters, essentially supported by cytological, biochemical and molecular studies.

8.6.1 Morphological Characterization

The most widely used technique of morphological characterization for tentative genomic classification of *Musa* germplasm was given by Simmonds and Shepherd (1955), and it was on the basis of 15 morphological characters contributing to the development of score card for classification. Despite its classical nature and useful-

Table 8.9 Modified score card for assigning tentative genomic groups to collected *Musa* germplasm^a

Genome	Score card		
	Simmonds and Shepherd (1955)	Silayoi and ChomChalow (1987)	Singh and Uma (1996)
AA/AAA	15–23	15–25	15–25
AAB	24–46	26–46	26–45
AB	49	–	46–49
ABB	59–63	59–63	59–65
ABBB	67	–	66–69
BB/BBB	–	70–75	70–75

^aSource Singh and Uma (2000)

ness, this score card technique has had some lacunae like discontinuity and ambiguity with respect to score ranges. In efforts to overcome the lacunae, modified score cards have been the development by Silayoi and Chomchalow (1987) and Singh and Uma (1996) for assigning tentative genomic groups to collected *Musa* germplasm (Table 8.9). The modifications of score cards were useful for addressing the collected germplasm belonging to AB, ABBB and BB/BBB genomes for morphological characterization. However, a major limitation of score card technique had not been resolved and contributed to the difficulty in distinct differentiation of genomes like AAB and AB or ABB and ABBB, due to overlapping values of scoring. For African plantain cultivars (AAB group), Swennen (1990) developed a morphology-based key and discussed the limitations of morpho-taxonomy for clonal identification. He noted that the incidence of somaclonal variation during *in vitro* propagation or germplasm maintenance could further complicate the correct identification of clones and confound efforts to use morphological variation to estimate genetic diversity. The ability of (CMT) classical morpho-taxonomy to discriminate various clones weakens as the genetic base of the clones under examination narrows (Jarret and Gawel 1995). The ‘Descriptor for banana’ developed by IPGRI-INIBAP/CIRAD is a more comprehensive in nature and available since 1996 for use of *Musa* germplasm collectors. A minimum set of descriptors was agreed upon by the Taxonomy Advisory Group (TAG) to field verified accessions from ITC, with the aims to establish a standardized procedure for a routine morphological characterization of banana plants and providing instructions on how to document with photographs, etc. (Gueco et al. 2017).

Another major limitation of morphological character-based score card technique is the unreliability and authenticity of those plant morphological characters which are supposed to be readily influenced by biotic and abiotic factors, resulting in misleading score estimates. The difficulty arises due to the presence of several duplicates and synonyms in a large gene pool that of like *Musa* is also not easy for handling. Hence, apart from morphological characters, cytological, biochemical and molecular studies are also encouraged for fine-tuned characterization and authentic identity of *Musa* germplasm (Singh and Uma 2000).

It is required to using stable and reproducible characters for the classification of recent cultivars and newly discovered wild *Musa* species. From the view point of cytology, one of the basic and stable characteristics of a species is its nuclear genome size, which has been estimated on many edible bananas and their wild ancestors. A genome size of 600–650 Mbp was determined in *M. acuminata* and 550 Mbp in *M. balbisiana*, clearly discriminating both species (Doležel et al. 1994; Lysák et al. 1999). Another important characteristic of a species is karyotype, i.e. the number and morphology of chromosomes. The chromosome number had been shown to determine the sectional classification of individual species of the genus *Musa*. There were some difficulty and complication in cytogenetic studies for detailing and supporting the classification, because of the small size of chromosomes, similarity in the morphology of chromosomes and absence of landmark specific to chromosome. Determination of the ploidy level of collected *Musa* germplasm is very important and that can be done by ‘flow cytometry’ technique. The INIBAP Transit Centre (ITC) collaborated with the Institute of Experimental Botany, Olomouc and the Czech Republic for determination of ploidy of every ITC accession using flow cytometry (ProMusa 2011).

8.6.2 Molecular Characterization

The biochemical and molecular approaches have been successfully utilized for reliable characterization of *Musa* germplasm by overcoming or supplementing to overcome many known lacunae or limitations those encountered in sole dependence on morphological character-based characterization. There is always a possibility of variations in morphological characters or altered expression of the genetic makeup of the germplasm under the influence of climatic factors and management practices. Whereas, the DNA and its genetic makeup is stable and specific to the germplasm and provides dependable and distinguished characterizations. To identify the germplasm of the *Musa* cultivars, biotechnologists used different markers such as isozymes (MDH, EST, PRX, PGM, GOT, ME, ADH, GDH, SUDH, SDH, GUDH, etc.), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), simple sequence repeats (SSR), diversity arrays technology (DArT), etc. (Uma et al. 2005; Pillay et al. 2012). Microsatellite-based molecular characterization was adopted more widely for *Musa* germplasm and resulted in dependable genotyping and strengthening the knowledge of phylogenetic relationships (Čížková et al. 2015).

8.7 Utilization of *Musa* Germplasm

The Delhi Declaration on Agrobiodiversity Management, adopted at the first International Agrobiodiversity Congress, held in November 2016 in India, called for ‘An agrobiodiversity index to help monitor conservation and use of agrobiodiversity’. The Prime Minister of India (the host country), Sri Narendra Modi addressed the Congress and pointed that we a treasure of valuable agrobiodiversity that need to be explored scientifically (Bioversity International 2017). It appeared to be a message of thrust area for widening the utilization of agrobiodiversity. It is equally applicable to the utilization of *Musa* germplasm, like other crops. The thrust for utilization of *Musa* germplasm is an integral part of the strategic framework of the INIBAP (a network of Bioversity International) in their ‘Global Conservation Strategy for *Musa* (Banana and Plantain)’, where the use of diversity and the services of collections had been targeted to promote worldwide by upgrading collections, serving users’ needs and providing easy access to key information about the use of accessions. In this strategy, high priority was given to partnership of germplasm collections with multiplication, demonstration and dissemination facilities. Web-based portal has been initiated, and it provides a comprehensive one-stop reference system on *Musa* taxonomy, accession availability, characterization, evaluation and practical experiences in using diversity for improving livelihoods, along with other initiatives and programmes like *Musa* Germplasm Information System (MGIS) Database, International *Musa* Testing Programme (IMPT), Regional networks (LACNET, ASPNET), the HarvestPlus and Generation Challenge Programmes, etc. (INIBAP 2006; Horry 2000).

8.7.1 Utilization in Banana Breeding and Improvement Programmes

There are two major utilizations of the collected and evaluated *Musa* germplasm, viz. use of accession in breeding and crop improvement programmes and secondly, boosting production through distribution of better accession to the small-scale banana growers (Van Den Houwe et al. 2003). The important research centres involved in banana breeding programmes are—(i) Honduran Agricultural Research Foundation (FHIA: Fundaci’ on Hondure’na de Investigaci’ on Agrícola) in Honduras, (ii) Brazilian Agricultural Research Centre (EMBRAPA-CNPMPF: Empresa Brasileira de Pesquisa Agropecu’aria Centro) in Brazil, (iii) International Institute of Tropical Agriculture (IITA) in Nigeria, (iv) The African Centre for Research on Banana and Plantain (CARBAP) in Cameroon, (v) ICAR-National Research Centre for Banana (ICAR-NRCB) in India, (vi) Banana Research Station (BRS) of Kerala Agricultural University in India, (vii) Tamil Nadu Agricultural University (TNAU) in India and (viii) French Agricultural Research Centre for International Development (CIRAD) in France (Bakry et al. 2009). The National Agriculture Research System (NARS) of each banana growing country in the world has research activities related to *Musa*

germplasm collection, evaluation and utilization and regional strategy of cropping and breeding objectives (Table 8.10).

In conjunction with NARS, breeding programmes and researchers, INIBAP coordinates the International *Musa* Testing Programme (IMTP) (Van Den Houwe et al. 2003). The establishment of the IMTP phase I began in 1989 as a programme to evaluate germplasm from the FHIA breeding programme in Honduras for resistance to banana leaf spot diseases (BLSD) and released three varieties, of which two

Table 8.10 Breeding objectives for bananas^a

Subgroup	Regions of production	Cropping strategy	Breeding objectives
Cavendish (AAA) Dessert type	Latin America, Caribbean, Philippines, India, West Africa, Mediterranean countries	Export banana intensive system, Local markets	Resistance to diseases, (Black Leaf Streak, Sigatoka, <i>Fusarium</i> wilt race 4), nematodes, weevils. Slow fruit ripening. Tolerance to drought and cold temperature
Silk and Pome (AAB) Dessert type	Brazil, India, Australia, South East Asia	Local and regional markets, Food crop system Extensive system. Intensification in progress	Resistance to diseases (Black Leaf Streak, Sigatoka, <i>Fusarium</i> wilt), nematodes, weevils. Fruit quality (fragility). Adaptation to cold temperature
Bananas of East Africa (AAA) Dessert and beer types	East Africa	Local market, Food crop system	Resistance to diseases (Black Leaf Streak, Sigatoka, <i>Fusarium</i> wilt), nematodes, weevils
Plantains (ABA) Cooking type	West Africa, India, Latin America	Local and regional markets. Food crop system. Intensification in progress	Resistance to diseases (Black Leaf Streak), nematodes, weevils. Productivity, Sucker production
Popoulou/Maia Maoli (ABB) Cooking type	Pacific	Local market, Food crop system	Resistance to diseases (Black Leaf Streak, <i>Fusarium</i> wilt)
Saba, Bluggoe (ABB) Cooking type	South East Asia, All marginal zones, Latin America, Caribbean	Local market, Food crop system. Processing industry	Resistance to <i>Fusarium</i> wilt, Moko disease and nematodes

^aSource Bakry et al. (2009)

dessert banana varieties (viz. FHIA-01 and FHIA-02) and one cooking banana variety (FHIA-03). The second phase of the IMTP started in 1996 involving 37 testing sites for developing hybrids resistant or tolerant to BLSD, Sigatoka and *Fusarium* wilt, and the results suggested that FHIA-23 and SH-3436-9 were the most tolerant to BLSD and GCTCV-119 (an improved cultivar) had good yield under good management (Bakry et al. 2009).

Under phase III of IMTP, twenty-one varieties were introduced and were evaluated and tested against black and yellow Sigatoka, *Fusarium* wilt and nematodes. Information on pathogen populations, host-pathogen relationships and adaptability and productivity were obtained through the evaluation trials (Table 8.11) (Molina et al. 2005).

8.7.2 *Direct Use of Genetic Resources by the Farmers*

Regarding direct use of genetic resources by the farmers from the International Transit Centre (ITC) for impacting on small-scale banana growers and local consumption, the accessions (that do not contain virus particles) are freely available on request to bonafide users, provided that should be maintained in public domain (Van Den Houwe et al. 2003). The ITC provided 42 *Musa* accessions of different genomes and subgroups, which were characterized at Lapanday Foods Corporation, Davao City, Philippines, and published a catalogue including 25 ITC *Musa* accessions to assist in distinguishing highly heritable characters with supplemental information on the disease reaction of the accessions against *Fusarium* Wilt (Foc TR4) and Banana Bunchy Top Virus (Gueco et al. 2017). Similarly, 70 ITC accessions were introduced to India through the ICAR-NBPGR, New Delhi, of which 44 accessions are being evaluated at the ICAR-NRC for Banana, Trichy (ICAR-NRCB 2017). Selected accessions through evaluation and framework of IMTP are demonstrated at research stations and extension fields so that small-scale banana growers can observe the better characteristics (more productive and disease resistance) and taste fruit qualities of the new introductions. The superior variety selected by farmers is then multiplied and distributed for direct use by farmers for boosting production and consumption. These efforts recorded an encouraging impact on small banana growers in Cuba, Northern Tanzania and Nicaragua for their food security and better income. Feedbacks by the small banana growers also indicated the need for more intensification of these activities for *Musa* germplasm conservation and utilization (Van Den Houwe et al. 2003; Garming et al. 2010).

Table 8.11 List of hybrids evaluated in IMTP Phase III^a

Hybrid	Type	Characteristic
FHIA-01	Dessert/cooking	Resistant to BS ^b and FW ^c
FHIA-02	Dessert/cooking	Resistant to BS
FHIA-03	Dessert/cooking	Resistant to BS and FW, drought tolerant
FHIA-17	Dessert/cooking	Tolerant to BS and resistant to FW race 1
FHIA-18	Dessert	Resistant to BS
FHIA-21	Plantain	Resistant to BS
FHIA-23	Dessert/cooking	Tolerant to BS and FW
FHIA-25	Cooking	Resistant to BS
SH-3640	Dessert/cooking	Resistant to BS
BITA-2	Cooking	Resistant to BS, Susceptible FW
BITA-3	Cooking	Resistant to BS
CRBP-39	Plantain	Resistant to BS
SH-3436-9	Dessert	Tolerant to BS
IRFA-911	Plantain	Resistant to BS
GCTCV-119	Dessert	Resistant to FW race 1
GCTCV-106	Dessert	Resistant to FW race 1
GCTCV-247	Dessert	Resistant to FW race 1
‘Yangambi km 5’	Dessert/cooking	Reference clone (Sigatoka)
‘PisangCeylan’	Dessert	Reference clone (Sigatoka)
‘Gros Michel’	Dessert	Reference clone (Fusarium)
‘Williams’	Dessert	Reference clone (Fusarium)
‘Cultivar Rose’	Dessert	Reference clone (Fusarium)
‘Cachaco’	Cooking/dessert	Reference clone (Fusarium)
‘PisangJariBuaya’	Dessert	Reference clone

^aSource Molina et al. (2005)^bBS black Sigatoka^cFW Fusarium wilt

8.8 Conclusion

Remarkable progress has been made in *Musa* germplasm collection, conservation and utilization with intensive efforts at regional levels and by INIBAP and Bioversity International at international level. Scientific clarification and technical feasibility in these processes have been increased by adopting advancement in cytology, molecular biology and cryopreservation. The diversity and distribution of species, subspecies, groups and subgroups of *Musa* across growing regions and hotspots have been studied by the banana workers. Present collection and conservation of *Musa* germplasm at ITC are the world's largest with around 1500 accessions conserved. Banana breeding and improvement programmes coordinated by INIBAP with the NARS have yielded several promising banana hybrids through IMTP. Evaluated *Musa* accessions being directly utilized by farmers have impacted positively on food security and better income of small banana growers in Cuba, Northern Tanzania and Nicaragua for their food security and better income. These mankind services of *Musa* diversity conservation and utilization are the vivid proposer of the need for more intensification of *Musa* germplasm collection, conservation and utilization, so as to achieve the call of the 'Delhi Declaration on Agrobiodiversity Management 2016'.

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