Chapter II

Review of literature

Plants are the basis of life on earth, its health and diversity is a primary indicator of the environment. Man’s necessities like food, fodder, fiber, shelter and medicine are derived out of the plant wealth. All the crops have developed from their wild species and relatives through evolution. The genetic diversity needed to sustain healthy food crops to the future is contained in the wild relatives as useful gene sources. But these wild progenitors are under severe threat due to several natural and created disasters for which banana and plantains have also been victimised. Presently a lot of emphasis is on the conservation of wild *Musa* genetic resources (Uma, 2006).

2.1. Need for collection

Collection and conservation of germplasm is gaining importance all over the world with an aim to utilize these genetic resources in plant breeding programmes. Germplasm collection, characterization and classification also provide the basic information necessary for the genetic improvement of crops (Ortiz, 1997). Genetic resources are the backbone of plant breeding programme. During exploration, various types of a crop inclusive of wild and related species are collected from diverse geographical regions. Genetic relationships have been estimated based on pedigree, morphological characteristics, sexual compatibility and taxonomic classification (Barrett *et al.*, 1998 and Renganayaki *et al.*, 2001). The genetic diversity collected and assembled in field gene banks need to be evaluated in a single location for their agronomic traits, biotic and abiotic stresses.

Like in other crop plants, wild *Musa* species and their relatives are under the threat of extinction due to several factors. Reports of the recent survey by
Uma et al. (2001 and 2003) revealed the disappearance of three wild species viz., *M. ochracia*, *M. flaviflora* and *M. ac.ssp.burmanicoides* from the Indian continent, reported earlier by Simmonds (1962). In this context, collection and conservation is gaining importance to reduce the process of genetic erosion.

### 2.2. Exploration

A number of exploration missions were conducted since early 1900, to collect and document the wild *Musa* diversity occurring in India.

The earliest reference of collection and their assemblage for taxonomic studies were made by Roxburg (1824) at Botanical garden, Calcutta, whose exploration extended from Chittagong forest areas of North Eastern India to Madras province in South India. After about seven decades, Baker (1893) also undertook exploration to collect 52 species resulting in the compilation of Monograph including Eumusa, Rhodochlamys and Physocaulis (Present Ensete).

Chakravarthy (1948a, 1948b and 1951) concentrated exploring mostly in Assam region and reported the non-stoloniferous banana (*Physocaulis*) named as *M. enset* presently known as *Ensete glaucum*. His exploration also resulted in the identification of *M. velutina* and *M. ornata*, ornamental wild bananas with bright colored bracts and erect bunches. Dutta (1952) added description of *Musa* diversity in cultivated and wild species of Assam, while Shukla and Roy (1956) described the banana cultivars of Bihar. Most of the explorations of South India for *Musa* species have been reported by Venkatramani (1946, 1948 and 1949), Jacob (1934, 1942a, 1942b and 1952), Sundararaj and Balasubramanian (1952), Nayer (1952 and 1958), Sundararaj (1955), Amalraj (1993) and Uma et al. (2001). National Bureau of Plant Genetic Resources, Thrissur and Indian Institute of Horticultural Research, Bangalore under took explorations in the Western Ghats of Maharashtra, Karnataka, Kerala and Tamil Nadu in 1990’s. Recent collections made through the explorations have been maintained in various field gene banks across the country of which, Banana Research Station,
Kannara, Kerala; Banana Research Station, Kovvur, Andhra Pradesh; GAU, Gandevi, Gujarat; AAU, Jorhat, Assam; RAU, Pusa, Bihar; TNAU, Coimbatore, Tamil Nadu etc. These *ex-situ* collections have been threatened by a number of biotic stresses like Banana Bunchy Top Virus, Banana Streak Virus, Fusarium wilt and Sigatoka leaf spot disease etc., which led to their slow deterioration in the last two decades. They also lacked systematic passport data, which is very essential in the present IPR (Intellectual property Rights) regime. These issues have reiterated the importance of systematic collection, conservation and documentation. The present study was undertaken to explore the areas of natural *Musa* diversity in India and document them for posterity in a systematic way. Emphasis was given to the less exploited section, Rhodochlamys, which consists of beautiful ornamental bananas and harboring valuable disease and pest resistant genes.

2.3. **Classification and description**

Classification is placing of individuals into a category or group based on their characteristics. Early classification systems were based on phenotypic appearance. Increased knowledge on the cytogenetics and biochemistry of organism necessitated the revision of earlier classifications, to reflect phylogenic classifications or genetic relationships. Natural classification of biological organism is based on the concept of the species, which are grouped into genera, families, orders, classes and phyla. At each stage of the hierarchy, similarity between the members of the group gets narrow.

Linnaeus was the first person to give scientific nomenclature for banana as *Musa paradisiaca* based on morphotaxonomic traits in 1783. He referred this to the plantains with long slender fruits, which are starchy. *Musa sapientum* was coined in 1789 for the sweet, dessert bananas, which are eaten fresh upon ripening. Considering many overlapping characters between these two, monospecific nomenclature as *M. sapientum* ssp. *paradisiaca* by Baker (1893).
M. paradisiaca ssp. sapientum by Schumann (1900) and M. sapidisiaca by Jacob (1952) were suggested, which were not accepted by taxonomists.

These classifications and descriptions were revised by Cheesman (1947) and his taxonomic analysis was based on chromosome numbers, classified the genus Musa into four sections viz. Eumusa (X=11), Rhodochlamys (X=11), Australimusa, (X=10) and Callimusa (X=10). Though this has been widely accepted, there are subsequent addition of one more section i.e. Ingentimusa with basic chromosome numbers 7 (n=7) for M. ingens and 9 (n=9) for M. beccari (Shepherd 1959; Argent 1976; Simmonds and Weatherup, 1990b.).

In fact, several issues remain unresolved including the evolutionary relationships among the four sections of the genus Musa, the separation of sections Eumusa and Rhodochlamys with apparently little taxonomic support and the relationship between the wild progenitors and the cultivated clones (Simmonds 1954, 1960; and Shepherd, 1999).

The classification in banana cultivars was simply based on the purpose for which it is used. Based on its diverse uses, banana and plantains are classified as dessert bananas, cooking bananas and beer bananas. Dessert bananas are those, which are consumed raw when they are fully ripe and are characterized by soft texture, high sugar and a pleasant aroma, while cooking bananas are starchy and invariably cooked to make them palatable. Beer bananas are those which are consumable only after processing into beer.

2.3.1 Morphotaxonomic characterization

Character is a feature that can be measured, counted or otherwise assessed (Heywood, 1967). Characters form the basis of any study concerned with identification, classification and characterization of plants. Good characters are those, which are not easily modified by the environment, highly inherited and referred as qualitative characters. Though majority of the morphological
characters are not important to the breeder but they are essential for the taxonomic classification and identification (Pickersgill, 1994).

In polyploidy crop like banana, the genomic classifications has a vital role in the classification of cultivars, since the majority of the banana cultivars originated from the two wild progenitors (M. acuminata and M. balbisiana) and their natural ploidy levels vary from 2x, 3x and 4x with different combinations like AA, AAA, AB, AAB, ABB, ABBB etc. To determine the genomic status, Simmonds and Shepherd (1955) developed a 15 character score card system to indicate the relative contributions of the two wild species. In this system, all the traits mentioned under M. acuminata are given the score 1 and those coinciding with M. balbisiana are scored 5. The intermediary characters are scored from 1.5 to 4.5 depending on the extent of deviation from the extremities. The total score ranged form 15-75 depending on the genome contribution, the total scores thus obtained were compared with score card and corresponding genomic status was assigned. Due to some of the lacunae like discontinuity and ambiguity with respect to score ranges in this score card, the modified score was developed by Silayoi and Chomchalow (1987). But it lacked the probable score for AB and ABBB genomes, which led to the development of a new score card by Singh and Uma (1996) to encompass Indian Musa diversity.

**Modified score card**

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<td>AA/AAA</td>
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<td>AAB</td>
<td>24-46</td>
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As the center of diversity is believed to be in South East Asia, large numbers of cultivars are present in Thailand and its neighboring countries. A total of 324 accessions were collected of which 64 were morphotaxanomically characterized and assigned their genomic group by using Simmonds and Shepherd’s 15 character score card by Silayoi and Babprasert (1983). Indian bananas were scored by Bhakthavatsalu and Sathiamoorthy (1979) and classified the bananas using the 15 character score card system.

The Cavendish clones are thought to have been derived from a single clone, which later got diversified by somatic mutation over time (Daniells, 1990). Stover and Simmonds (1987) grouped three mutants into Dwarf Cavendish, Giant Cavendish, Grand Naine and Lacatan. Depending on the crop diversity within AAB genomic group, De Langhe and Valmayor (1980) and Lebot et al., (1994), established the classification of triploid (AAB) bananas into subgroups like Silk, Mysore, Pome, Plantain etc.

Characterization and description have an important role in morphotaxonomy and hence many crop specific descriptors have been developed by IPGRI. For banana, International Network for the Improvement of Banana and Plantains (INIBAP), France and International Plant Genetic Resources (IPGRI), Rome (1996) developed “Banana Descriptor” which, contains 121 morphological traits and is being widely used for crop characterization.

Baker and Simmonds (1951 and 1952), made preliminary morphological investigations on cultivated and wild bananas in the East Africa. While De Langhe (1961) made detailed descriptions 56 banana clones grown Zaire River basin. Classification and preliminary understanding of the variation of banana cultivars grown in Eastern Africa and Banana germplasm of Institute de Research Agronomique et Zootchnique (IRAZ) was morphotaxanomically characterized by Sebasigari (1987). The study mainly concentrated on genomic
constitution and general characteristics of clones within the four types of bananas viz., beer, boiling, flour and sweet bananas based on their end utility.

Plantains are special subgroup of the AAB banana, although the centre of origin of these unique AAB types is supposed to be in South India (Simmonds 1966). But a remarkable diversity exists in Central Africa and it was suggested that this was the oldest cultivated bananas in Africa (De Langhe 1961, 1964a and 1964b). West African plantains were systematically studied for 30 morphological characters (Swennen and Vuylsteke 1987) and the results suggest that most of the characters showed no variation between the accessions however dissimilarities were found for bunch type (French, French Horn, False Horn and Horn), plant stature, bunch and fruit orientation, fruit apex, fruit shape and fruit curvature.

Bananas and plantains exhibit great diversity in the Philippines, which also is one of the centres of origin, and diversity. More than 80 distinct cultivars were reported in Philippines (Pascua et al. 1984), but their identification was more ambiguous. To overcome this, Pascua (1990) developed a tentative key for identification for Philippine bananas thorough morphological descriptions, which included free tepal corrugation, seediness apart from other morphotaxonomic traits. *M. balbisiana* is a distinct species in the genus *Musa* and is reported to be native of Philippines where it has widespread occurrence, the genetic diversity and morpho taxonomic variation of *M. balbisiana* in the Philippines was studied by Sotto and Rabara (2000) which included 105 balbisiana accessions and observed more variations among them.

Amalraj et al. (1993) studied the variability of 191 banana germplasm by morphological characters like pseudostem blotching, pigmentation, leaf length and breadth ratio, number of hands and fingers, peduncle nature, pedicel length and fruit size, taste and nature of ripe fruit flesh etc., in the germplasm collection. Based on this study a general key to morphological characters of
various genomic groups was developed. Sathiamoorthy (1994) characterized the Indian banana germplasm maintained at TNAU, Coimbatore through morphotaxonomy and identified 81 distinct clones and 23 mutants in the gene pool of 240 accessions.

Menon and Premalatha (2000) attempted morphotaxonomic characterization for 212 accessions using IPGRI descriptor; this has enabled the identification of synonyms in each genomic group. Out of the 212 accessions, 96 accessions were distinct clones and the rest of them accounting for synonyms in each genomic group.

Uma et al. (2005a) have also characterized 583 *Musa* germplasm accessions using IPGRI descriptor for 133 traits, which has resulted in the creation of ‘Banana - Indian Genetic Resources and Catalogue’.

Numerical taxonomy studies was conducted by Karamura (1999) to determine the pattern of variation that exists in the East African Highland bananas grown in Uganda, estimate levels of dissimilarity caused by different growing conditions and establish a flexible provisional classification and identification system. Sixty-one morphological characters were employed to study the differences among 238 accessions. Comparison of cluster analysis and PCA revealed similar clusters of accessions in the phenogram and along the first four principal components. These data can be used to develop hypothesis concerning the evolutionary background of the East African Highland bananas and to exploit vigour, pest and disease resistance in the representative clone of the different clone sets proposed.

### 2.4. Genetic diversity analysis

The variability and genetic divergence studies among Indian bananas were conducted Valsalakumari *et al.* (1984, 1993). Simmonds *et al.* (1990a & 1990b) reported relationship among cultivated bananas based on numerical
taxonomy. The results of the above studies revealed the relationships and helped to eliminate some synonyms.

2.5. **Disadvantages of morphotaxonomic characterization**

Morphological traits are influenced by genotype x environmental interactions making them unstable and variable over time and locations, which limits their use in taxonomy (Ortiz, 1995 and 1997). Moreover morphological characters are usually determined by a small number of genes that may not represent the total genetic diversity within the genomes (Brown-Gudira et al., 2000). Further Williams (1976) stated that numerical methods lack any reliable significant test to prove their accuracy. Another drawback of numerical taxonomy is that every time a new clone is incorporated, the whole numerical procedure has to be repeated. The unreliability of some parameters suggested characterization using molecular techniques for authentic identity.

2.6. **Breeding potential of wild *Musa* species (section Rhodochlamys and Eumusa)**

Banana improvement is very difficult due to inherent problems like parthenocarpy, male and female sterility and ployploidy. But most of its wild relatives are male and female fertile and more importantly parthenocarpic nature, male and female sterility. But its wild relatives are both male and female fertile and more importantly they harbour resistant genes for many biotic and abiotic stresses. So, from the crop improvement point of view wild species and wild relatives are greater important.

During a collection expedition, Cheesman (1948) raised plants from natural banana seeds and studied them for morphological characters, the intermediary characters of hybrids and their plant stature made him to conclude that seeds were natural hybrids of *M.nagensium* and *M.balbisiana*.
Shepherd (1999) was successful in developing Rhodochlamys hybrids following the crosses between *M. ornata* x *M. velutina*, *M. velutina* x *M. ornata*, *M. laterita* x *M. velutina* and *M. laterita* x *M. ornata* and confirmed their cross compatibility. He also developed intersectional hybrids of *M. itinerans* (Eumusa) x *M. velutina* (Rhodochlamys). In early 1990’s he developed hybrids from wild species involving BB x AA and AA x BB. AB hybrids also developed from BB (Bhutan) x *M. acuminata*. Of the Rhodochlamys x Eumusa combinations, Rhodochlamys x *M. acuminata* was successful in establishment while Rhodochlamys x *M. balbisiana* failed to survive. Thus the cross compatibility between Rhodochlamys x Eumusa and vice versa was established.

Simmonds (1954) noticed a natural hybrid of *M. flaviflora* x *M. velutina* in the areas of Assam and Arunachal Pradesh and stated that an intersectional hybrid of *M. laterita* x *M. acuminata* was vigorous. In the year 1962, he had also observed that *M. balbisiana* has frequently crossing naturally with other species like *M. acuminata*, *M. nagensium*, *M. sikkimensis* etc.

Shepherd *et al.* (1985 and 1987) developed new tetraploid hybrid by using *M.ac.ssp.burmannica* as male parent, for its high potency in seed setting and of the possible inheritance of resistance to *Mycosphaerella*. Shepherd *et al.* (1985) have also developed successful hybrids by the crosses of *M. balbisiana* x Prata (AAB).

Rowe (1987) developed a synthetic AA diploid with parthenocarpic bunch and Sigatoka leaf spot disease resistance by using *M.ac.ssp.burmannica* as one of the parents. Tezenas (1987) also used *M. balbisiana* as both male and female and developed successful hybrids with black Sigatoka leaf spot resistance.
Daniells and Bryde (2001) noted that most of the members belonging to the section Rhodochlamys including Musa velutina, Musa ornata were resistant to both Fusarium wilt and Sigatoka leaf spot diseases.

2.7. Molecular markers and their importance

Molecular techniques may help to promote germplasm use by providing exact data about the genotypic attributes of plants. Germplasm characterization offers information about individual genomic composition and as such allows breeders to select promising materials based on genotype as well as phenotype. The construction of molecular linkage map have opened up the possibility of locating important agronomic traits in crop genomes and consequently of selecting germplasm based on the presence of a particular gene of interest. Introgression of genes from donor germplasm can thus be followed in subsequent generations using so called marker assisted selection (MAS). The latest tools for molecular genetics expected to speed up breeding procedures through activities such as permitting the quick discovery of useful genes in the germplasm collections or correlating genotype with phenotype. Molecular markers may be used in gene bank management such as accurately identifying the germplasm. Screening of germplasm for the use of breeders and other researchers conduct routine maintenance of the gene bank, which will be streamlined by identifying duplicates, assessing stability through different rounds of regeneration or multiplication and measuring genetic erosion. Identifying the gaps in the existing collection at their DNA level by molecular markers will be useful to develop a core collection for specific crop.

Musa breeding is complicated by a number of factors. (Pillay et al., 2002, Vuylsteke et al., 1997; Ortiz and Vuylsteke, 1996). The development and use of new tools such as flowcytometry for ploidy selection, molecular markers for genome identification has widened the knowledge of behaviors of genome in Musa crosses. The value of the S and T genome in breeding has not been studied in detail. More efforts are stressed to examine these genomes, which
could harbour useful resistant genes for *Musa* improvement programme. (Pillay et al., 2002). Lanaud (1999) investigated the relationship of diploid and triploid cultivars with wild relatives using nuclear and cytoplasmic markers.

Due to significant barriers inherent in conventional breeding of banana, molecular breeding is seen as a potential benefit for the improvement programme. (Devaries and Toenniseesan, 2001). Molecular markers based on PCR are the most appropriate assays for molecular breeding application due to their relatively simple protocol and ease of automation (Rafalski and Tingey 1993).

2.8. Non-DNA molecular markers for genetic diversity studies

2.8.1. Isozymes

Considerable efforts have been made by various workers to classify and study the genetic diversity in *Musa* using isozyme markers.

Polyphenol diversification and isozyme polymorphism (Horry, 1993; Jarret and Litz 1986 a) were the first ventures into the realm of molecular markers. The results supported the model of hierarchical organization reflecting different evolutionary levels in the Musaceae. It also suggested that domestication occurred at many evolutionary stages during the separation of *M. acuminata* into sub-species, while isozyme polymorphisms corroborated the suspected reproductive isolation between *Musa* species.

Bonner et al. (1974) examined the isozyme peroxidase in various *Musa* clones and species and noted that there was no polymorphism while Rivera (1983) was able to distinguish between the Saba and Bluggoe group using the peroxidase and polyphenol oxidase polymorphism. Similarly high degree of polymorphism was observed for peroxidase and super oxide dismutase in 44 cultivars of Indian bananas and plantains (Bhat et al. 1992a).
Bhat et al., (1992b) carried out simultaneous study on isozymes like esterases, acid phosphatases and catalases for the same set of 44 Indian *Musa* cultivars belonging to AB, AAB, ABB and AAA genomic groups. They registered high degree of polymorphism for esterases and acid phosphatases and least degree of polymorphism for catalases. Results revealed only cultivar specific isozymes but not genome specific isozyme pattern.

Twenty four clones of banana and plantains representing various ploidy levels (AA, AAA, AAAA, AB, AAB, ABB, ABBB and BB) exhibited polymorphism when they were analyzed by Jarret and Litz (1986a) for enzymes Malate dehydrogenase (MDH), Phospho glucomutase (PGM), Glutamate oxaloacetate transaminase (GOT), Shikimate dehydrogenase (SKDH) and peroxidase. It was also shown that the Cavendish clones Robusta, Giant Cavendish, Dwarf Cavendish and Pisang Mask hijau were monomorphic for isozymes. But subsequent studies by Jarret and Litz (1986 b) in 35 clones of *M. acuminata* representing eight putative subspecies and *M. balbisiana* concluded that isozyme markers could be used for the identification of commercial cultivars, species and subspecies. Espino and Pimentel (1990) analyzed 16 cultivars using three enzyme systems namely SKDH, GOT and MDH. The zymogram results showed that it was possible to distinguish the presence and absence of *acuminata* genome. Of late, Lebot et al. (1993) determined the genetic relationships among cultivated bananas and open-pollinated seedlings using three enzyme systems viz., MDH, PGI and PGM. The isozyme data suggests that the genes contributed by *M. acuminata* to the Pacific plantains are similar to those of *acuminata / banksii* complex of Papua New Guinea. Therefore, the Pacific plantain groups might have probably originated in Papua New Guinea rather than in Asia or Malay Archipelago.

Uma et al. (2002) analyzed the isozyme polymorphism using four enzyme systems namely MDH, GDH, SKDH and Phospho-glucoisomerase (PGI) in ten *acuminata* clones of different origins. The resultant dendrogram
showed that the exotic introductions were distinctly different from indigenous diploids. However an indigenous red diploid (Sanna Chenkadali) paired with Pisang Berlin – an exotic introduction, which needs further confirmation about their evolutionary pattern. Using the same four enzyme systems, Selvarajan et al. (2002) analyzed the Sigatoka leaf spot resistant and susceptible varieties. SKDH was found to be an efficient tool in differentiating resistant and susceptible varieties. Though the resistant and susceptible varieties clustered separately in the dendrogram, Thiruvananthapuram and Chinali, which are highly resistant, paired with less susceptible Pisang Nangka, which needs further confirmation using DNA based markers like RAPD and AFLP.

Though isozymes and other biochemical markers have been used to study the *Musa* diversity, they are not adequate to form a comprehensive basis for diversity studies (Horry and Jay, 1988).

### 2.9. DNA markers for genetic diversity studies

Many of the problems associated with phenotypic evaluation for agronomic traits such as large environmental effects or quantitative inheritance can be overcome with the use of wide range of DNA markers (Annexure II), further morphological evaluation though essential for germplasm characterization are difficult to perform due to inherent problems associated with some of the perennial plants. In banana considerable works have been made to distinguish classify *Musa* accessions based on morphological characteristics. However, the classification of certain accessions on this basis has been disputed (Gawel and Jarret 1991a, b).

### 2.9.1. Restriction Fragment Length Polymorphism (RFLP)

RFLP markers are co-dominant markers used to confirm the *Musa* classification, to amend the genome formula and sub-species / sub-group classification of some varieties. It provides a powerful tool for phylogenetic studies in *Musa* (Carreel.1994). Further, these are the first anchor markers
developed for gene mapping and QTL identification (Faure et al., 1993). Yet, high cost and technical expertise required for this technique makes it unsuitable for routine applications.

2.9.2. RFLP in genetic diversity studies

The cladogram obtained as a result of examining 20 Musa spp. including sub-species using total DNA. RFLP s delineated them into two clear groupings, one containing species from the sections Eumusa and Rhodochlamys and the other with the species from Australimusa and Callimusa (Gawel et al., 1992). In a similar study, Jarret et al. (1992) used forty genome probes of M.ac.ssp.malaccensis and M. balbisiana. The resultant cladogram showed that M. schizocarpa is very close to M. acuminata. The analysis did not separate M.ac.ssp.malaccensis and M.ac.ssp.banksii. Fei bananas are distinct from the five species of Australimusa including M. maclayi, the presumed ancestor. M. lododensis is the closest to Fei species. M. textilis is the most remote indicating that the origin of Fei bananas is thus still not clear. Further, the parthenocarpic diploids of Papua New Guinea are not linked to one or other of the subspecies of M. acuminata.

Gawel and Jarret (1991) used chloroplast DNA RFLP to detect the cytoplasmic differences in 14 Musa species and cultivars. Variation was observed among the M. acuminata derived clones. The resultant cladogram showed clear clustering of the M. acuminata subspecies and differentiated M.acuminata and M.balbisiana cytoplasm. Generally, beer bananas could not be distinguished from similar types used for cooking (Simmonds, 1966) but apparently, their chloroplast genome is quite distinct for several cleavage sites when compared to dessert bananas.
Bhat et al. (1994) examined both the nuclear and chloroplast DNA variability among 57 Musa germplasm collections using 19 heterologous Vigna chloroplast DNA probes and observed polymorphism. The results confirmed the ability of RFLP’s to identify and classify Indian bananas.

In another study conducted at CIRAD, the genetic diversity of 160 diploid bananas and 150 polyploid clones were analysed using 30 mapped single copy nuclear probes and 10 chloroplast and mitochondrial probes. Alleles specific to the three species namely M.acuminata, M-balbisiana and M.schizocarpa were identified. All the diploid parthenocarpic bananas contained M.acuminata alleles and this confirmed the involvement of M.acuminata in the origin of parthenocarpy. Some cultivars were proven to be hybrids between M.acuminata and M.schizocarpa. Starchy cultivars were found to be closely associated with M.ac.ssp.banksii whereas sweet cultivars were close to M.ac. ssp. malaccensis. Based on cytosolic RFLP probes, the triploid Cavendish group was found to be related to M.ac. ssp. errans and M.ac.ssp.malaccensis whereas RFLP data from the nuclear genome did not allow for any association with either subspecies. In spite of the large number of nuclear probes used in this study, it was not possible to relate this triploid clone to any diploid wild type subspecies. Carreel et al. (2002) at CIRAD carried out another study for characterizing 71 wild accessions, 131 diploid and 103 triploid cultivated clones using RFLP in combination with heterologous mitochondrial and chloroplast probes. In this study, besides M.acuminata and M.balbisiana, other species from the four Musa sections were included to investigate their contribution to the origin of cultivated bananas. It was found that parthenocarpic varieties are linked to M.ac.ssp. banksii and M.ac. ssp. errans.

Nwakanma et al. (2002 b) were able to define two lines of evolution in Musa using six chloroplast and two mitochondrial DNA probes. One lineage comprised of the sections Australimusa and Callimusa (with basic chromosome number x = 10) while the species of section Rhodochlamys were in the other
lineage. Species of Eumusa were distributed in both lineages. Section Rhodochlamys appeared as a sister group of section Eumusa with *M. laterita* having genome identical to some sub-species of the *M. acuminata* complex. The progenitors of the present day bananas were evolutionarily distant from each other. *M. balbisiana* occupied a basal position in the cladogram indicating an evolutionary primitive status. This is the first molecular marker system, which was conceived and developed by Botstein *et al.* (1980).

2.9.3. Random Amplified Polymorphic DNA (RAPD) in genetic diversity studies

RAPD is a novel technique developed by William *et al.* (1990). Howell *et al.* (1994) were able to identify 116 amplification products in *Musa* germplasm using nine primers. This enabled them to identify markers specific to nine genotypes of each of the genomic groups AA, AAA, AAB, ABB and BB genomes. Further it was found that the multivariate analysis of the RAPD banding pattern was identical to the pattern of variation defined using morphological characters. In a similar study, Bhat and Jarret (1995) examined 57 *Musa* accessions including cultivated clones of six genomic groups (AA, AB, AAA, AAB, ABB and ABBB), *M. balbisiana*, *M. ac.ssp. banksii*, *M. ac.ssp. malaccensis* and *M. velutina* using 60 10-mer random primers. Phenetic analysis separated clones into distinct groupings that were in agreement with clusters analysed by Principal Co-ordinate analysis (PCO). In both the phenetic and PCO analysis, unclassified cultivars grouped with the cultivars already classified for their genomic group based on morphological keys.

RAPD is a rapid, precise and sensitive technique for varietals identification and their classification Thu *et al.* (2002). Attempts were made by Grajal-Martin *et al.* (1998) to assess the genetic variability among three wild species of *Musa* and cultivars of dessert and cooking bananas using RAPD markers. The polymorphism obtained in the study helped in the identification of different species and genomic groups.
Pillay et al. (2000) used 80 decamer Operon primers to amplify DNA from *M. ac. ssp. burmannicoides* and *M. balbisiana* clone Honduras. Three 10-mer RAPD primers (A-17, A-18 and D-10) produced unique banding profiles for the differentiation of *M. acuminata* and *M. balbisiana* genome. Primer A-17 amplified two fragments (600 and 100 bp) and D-10 another one fragment (320 bp) that were specific to *M. acuminata*. Similarly A18 produced three fragments (200, 250 and 300 bp) that were unique to *M. balbisiana*. Likewise, Rekha et al. (2001) conducted genomic relationship studies among 28 genotypes using RAPD markers. The dendrogram showed two main clusters differentiating AA from BB. The BB group in turn had two nodes with wild *M. balbisiana* types in one group and ABB types in another group.

Visser (2000) while assessing the genetic diversity among 37 different banana and plantain cultivars found that it is possible to distinguish AAB from ABB types with OPA-13. In the study by Jagannath et al. (2004), 22 *Musa* diploids belonging to AA and AB groups were subjected for RAPD analysis using 25 random primers. The accessions showed a high degree of polymorphism for primers OPF-4, 8,9,12 and 17. Dendrogram revealed that the members of AA and AB were distinctly separated from each other. The results were in agreement with those of earlier classification based on morphological characters. During the same year, Rekha et al. attempted to study the variability among 17 AB cultivars using RAPD markers. Of the 80 primers tested, 16 showed polymorphism. The results of the data analysis showed that there were two distinct clusters separating the Ney Poovan and Kunnan types.

Kayat et al. (2004) demonstrated the capability of RAPD markers to differentiate variants of Rasthali. Uma et al. (2004) analysed the genetic variation and phylogenetic relationships among 25 Silk group representatives of both indigenous and exotic origins using RAPD markers. The extent of variability was found to range from 0 to 30 percent indicating that the spectrum of variability is very narrow irrespective of the wider distribution of Silk group
members across the globe. In the recent studies by Uma et al. (2006), 16 wild *M. balbisiana* clones collected from different regions of India were examined for their intraspecific relationships using 80 RAPD primers. The extent of polymorphism (74.6%) has indicated the existence of considerable variation at the DNA level within the collections.

Kahangi et al. (2002) examined the 17 *Musa* cultivars in Kenya using ten decamer arbitrary primers. The test cultivars included five reference cultivars of different genomic groups namely AA, AB, AAA, AAB and ABB. The dissimilarity analysis showed that there was no duplication and each cultivar was genotypically different although some were closely related. In a similar study, Onguso et al. (2004) estimated the genetic relationships among 20 selected banana cultivars from different regions of Kenya using 19 random primers. When the polymorphisms in PCR amplification products were subjected to UPGMA and plotted in a phenogram, it was found that all the 20 cultivars analyzed were related.

In a simultaneous study by Sales et al. (2002), the genetic diversities and relationship were investigated among 19 Philippines *Musa* cultivars using 118 random primers. A study was conducted by Uma et al. (2002) using RAPD to understand the genetic diversity and phylogenetic relationship between 10 wild *M. balbisiana* and 40 ABB clones and the results suggested that 10 *M. balbisiana* clones were grouped into two distinct clusters, which correlated with their geographic locations of maximum diversity. Almost all the accessions distinctly gravitated towards either of the major *M. balbisiana* clusters suggesting their probable evolutionary ancestors. This also points out the varying degree of introgression than those observed phenotypically. Morphotaxonomic studies coupled with molecular characterization suggest that there is a lot of diversity in *M. balbisiana*, which needs further classification up to subspecies level as available with *M. acuminata*. Uma et al. (2004) evaluated 100 random primers to compare the *M. balbisiana* accessions collected from the Indian mainland and
Andaman and Nicobar Islands. Wild types from the Indian mainland were distinct from those collected from Andaman and Nicobar islands.

2.9.4. Amplified Fragment Length Polymorphism (AFLP) in genetic diversity studies

AFLP (Vos et al., 1995) is a robust and reliable molecular technique recently employed in many plant systematics. It is clearly a powerful technique in terms of its ability to identify a large number of polymorphic bands.

Wong et al. (2001) employed eight AFLP primer pairs to distinguish among the three subspecies of *M.acuminata* (namely ssp. *truncata*, *malaccensis* and *microcarpa*). UPGMA cluster analysis showed that the three taxa were distinct. In an identical study, Ude et al. (2002c) assessed the genetic diversity and relationships among 28 accessions of *M.acuminata*, *M.balbisiana* and some of their natural hybrids using 15 AFLP primer pairs. Neighbour joining tree and PCO analysis using Jaccard’s similarity coefficient produced four major clusters that closely corresponded with the genomic composition of the accessions (AA, BB, AAB and ABB). *M.ac.ssp.microcarpa*, *M.ac.ssp.truncata* and *M.ac.ssp.banksii* clustered very closely with Gros Michel and Yangambi-KM 5 indicating that more than one *M. ac.* ssp. may be involved in the origin of triploid AAA bananas. Calcutta 4 (*M.ac.ssp.burmannicoides*) and Long Tavoy (*M.ac.ssp.burmannica*) were closely related and could be together in the same subspecies. There is much more genetic diversity within *M.balbisiana*.

Ude et al. (2002a) used AFLP to determine the degree of sectional and inter / intra specific genetic variation in *Musa*. Thirty nine accessions of *Musa* were analysed with 10 different primer combinations. Cluster and PCO analysis were used to assess the genetic and evolutionary relationships. AFLP was useful in separating the different sections of the genus *Musa*. It also separated the different accessions according to their genomic groups. The study also showed that there is much more genetic diversity within *M.balbisiana* than those
suggested using morphological descriptors. PCO analysis of the AFLP data showed that Rhodochlamys whose identity was questioned in previous proposals was confirmed to be a distinct entity. Callimusa and Australimusa were genetically distinct though they were closer to each other (Ude et al., 2002b). Similarly Wong et al. (2002) used eight AFLP primer pairs to evaluate whether the genetic differences among the four sections of *Musa* justify distinction into separate groups. The results revealed the separation of the 11 chromosome species from the 10 chromosome species. It further suggests that Rhodochlamys could be combined with Eumusa and likewise Australimusa to be merged with those of Callimusa.

Ude et al. (2001, 2003) assessed the genetic diversity among 25 plantain accessions using 16 AFLP primer pairs and 60 decamer RAPD primers. Cluster analysis identified three major groups. False horn plantains showed more variation than the French and Horn plantains and appeared in all clusters. PIC was greater in AFLP than that of RAPD indicating greater discriminatory power of AFLP. Greater diversity was observed among accessions from Cameroon and hence future collecting missions should aim at capturing genetic variation available in Cameroon. The results further indicated closer relationships among similar inflorescence types and the cultivars of Cameroon were separated from the bulk of other plantains suggesting that Cameroon may harbour accessions with useful genes for widening the genetic base of breeding populations derived from plantains.

Tugume et al. (2002) used AFLP to define genetic relationships among 115 East African Highland bananas and found that the variation was minimum indicating the narrow genetic base of these genotypes.

### 2.9.5. Microsatellites

Microsatellites or simple sequence repeats are highly polymorphic, multi allelic, co-dominant, reproducible, easy to interpret and amplified via
Polymerase chain reaction (PCR). Microsatellites are abundant in nuclear genome and consist of a succession of a small repeat-unit, usually less than four nucleotides long (Wang et al., 1994). Microsatellites are ubiquitous components of eukaryotic genomes (Delseny et al., 1983, Tautz and Renz, 1984) and are generally inserted in regions of single copies (Tautz, 1989). However, first evidence of their abundance in plant genomes and their polymorphic character was reported only few years ago (Condit and Hubbell, 1990, Lagercrants et al., 1993; Morgante and Olivier, 1993). The polymorphism between genotypes is due to the variation in the number of repeats units. Simple sequence repeats (SSR) markers are routinely used for screening the germplasm for diversity analysis and molecular breeding in many crops because of their high level of polymorphism, co-dominant nature, efficiency and cost effectiveness (Terauchi and Konjuma, 1994).

Weising et al. (1996) were the first to attempt on the molecular characterization of banana and plantains using microsatellite based markers. Later, Grapin et al. (1998) using nine microsatellite primer pairs explored discrimination potential of STMS markers. The STMS polymorphism was assayed by non-radioactive urea polyacrylamide gel electrophoresis. The genetic relationships were examined among 59 genotypes of wild and cultivated accessions of diploid M. acuminata. 72 alleles were identified with a mean of 8 alleles per locus. AGMI 103/104 revealed the lowest polymorphism with four alleles and the highest by AGMI 24/25 and AGMI 93/94 with 12 alleles. This confirmed the sub-species organization and clarified some clonal relationships.

Assessment of functional diversity, the genomic SSRs are useful however because of higher polymorphism, genome SSRs is superior for fingerprinting (or) varietal identification studies (Varsheney et al., 2005).

Microsatellite markers have been used in plants for fingerprinting, mapping and genetic analysis. SSRLP analysis has been shown to detect a high
level of polymorphism between individuals of *Musa* breeding populations (Crouch *et al*., 1998). However the isolation of microsatellites is time consuming and expensive, several hundred SSRLP markers have been generated in *Musa* (Jarret *et al*., 1994, Kaemmer *et al*., 1997 and Crouch *et al*., 1998).

Forty-five STMS markers have been proposed by Lagoda *et al*. (1998 b) using the size fractionated Pst I genomic DNA library constructed from cultivar ‘Gobusik’. When all the 45 STMS markers were tested for polymorphism in two mapping populations, a total of 23 sites were mapped.

The frequency of microsatellites expected in banana by Lagoda *et al*. (1998 a) is one STMS per 30 kbp as that of humans. Therefore, approximately, 100 useful markers have been generated so far from *M. acuminata* while a similar number are expected to result from a parallel work on *M. balbisiana*. Similar microsatellite isolation projects are in progress at CIRAD, University of Frankfurt and University of Saskatchewan. These on-going projects along with other smaller projects are likely to result in the availability of more than 500 microsatellite markers for the genetic analysis and molecular breeding in *Musa*.

Subsequently, twelve new *M. acuminata* microsatellite markers have been identified by Ciampi *et al*. (2004) from the complete sequencing of five Calcutta-4 BAC clones and partial sequencing of full length enriched cDNA libraries from leaves of Calcutta-4 and this markers have proven useful for banana germplasm characterization and marker assisted selection, linkage map saturation in banana breeding programmes.

Buhariwalla *et al*. (2005) used the enriched library of *Musa balbisiana* cultivar Tani for the isolation and characterization of 44 microsatellite loci of which 25 were found to be polymorphic when tested on diverse population of diploid and triploid *Musa* accessions. These new microsatellite markers these
were used for diversity assessment and linkage mapping studies in Plantain (AAB) and cooking bananas (ABB).

Creste et al. (2003) used microsatellite markers to characterize 35 banana genotypes cultivated in Brazil including triploid cultivars and tetraploid hybrids. A total of 33 Musa specific primers were tested, of which 11 produced clear, reproducible and discrete bands. Phenetic analysis based on Jaccard’s similarity index derived from presence or absence of the alleles agreed with the morphological classification. Bootstrap analysis divided the genotypes into four clusters according to the genomic group and sub-group classification. The microsatellite loci were highly informative with some pair of primers generating an unique fingerprinting for each genomic group and discriminating a genotype of doubtful classification although some somatic mutants from a sub-group were seldom distinguished from their original clone.

Microsatellites were used by Creste et al. (2004) to investigate the genetic variability and relationships between 58 Musa genotypes including 49 diploids and 9 triploid cultivars maintained at the Musa germplasm collection of the Brazilian dessert banana breeding program. Thirty three Musa specific primers were tested of which nine produced reproducible and discrete fragments producing at total of 115 alleles. It was not possible to separate the wild diploid genotypes from the cultivated ones indicating a common origin of these genotypes. The information gathered about the similarity between diploid and triploid accessions would help to define potential crosses to maximize the recovery of the typical fruit qualities required in Brazil.

Simultaneously, Creste et al. (2005) were able to isolate and characterize 23 microsatellite loci from the genomic library of the commercial diploid cultivar ‘Ouro’. They were tested on 10 Musa genotypes representing various Musa genomic groups with distinct ploidy level. Twenty loci were found to be highly informative. Four loci were found to amplify B-genome specific alleles
while three loci were seemed to be absent in the B-genome. The polymorphism revealed by these loci will be extremely useful for genetic mapping, MAS, germplasm characterization and evolutionary studies in *Musa*.

Sotto and Volkaert (2004) used a set of microsatellite derived from *M. acuminata* for the diversity studies of wild *M. balbisiana* accessions from Thailand and Philippines, results confirmed that the *M. acuminata* microsatellites were less polymorphic in *M. balbisiana* than in *M. acuminata*.

Samarasinghe *et al.* (2002) determined the phylogenetic relationships and estimated genetic diversity among the 14 AA, AAA, AAB, ABB cultivars and one AA wild species using seven SSR primers. The study proved that A-genome in the genotypes might have evolved from long-term mutation. However, B-genome did not exhibit much variation. The overall results were in agreement with those of the morphological classification.

Noyer *et al.* (2005) assessed the genetic diversity of 30 plantains constituting a representative sample of the phenotypic diversity using SSR and AFLP markers. The results confirmed a very narrow genetic base of the plantain group and diversity data supports the hypothesis these cultivars may have arisen from vegetative multiplication of a single seed.

Ge *et al.* (2005) investigated the Phylogeography and population structure of wild bananas and *M. balbisiana* through analysis of genetic diversity of SSR markers.

Oriero *et al.* (2006) investigated the genetic variability between 40 *Musa* genotypes maintained at the *Musa* germplasm collection of the IITA, Ibadan using 9 B-genome derived SSR markers. The hierarchical cluster analysis separated diploid cultivars from those of triploids and the results revealed that there are several limitations in breeding and genetic improvement in banana.
therefore selecting the most suitable parents that would result in higher diversity is crucial for significant genetic progress.

SSR markers have already been developed and applied for soybean (Rongwen et al., 1995, Maughan et al., 1995 and Akkaya et al., 1995), rice (Wu and Tanksley, 1993, Yang et al., 1994 and Zhang et al., 1995), maize (Senior and Heun, 1993), Wheat (Rodger et al., 1994 and Plaschke et al., 1995), barley (Saghai Maroof et al., 1994; Becker and Heun, 1995), rapeseed (Kresovich et al., 1995), Citrullus lanatus (Jarret et al., 1996) and Arabidopsis thaliana (Depeiges et al., 1995). Microsatellite markers have also be employed in tropical plants such as trees (Condit and Hubbell, 1990), avocado (Lavi et al., 1994), Citrus (Kijas et al., 1995) and Kiwifruit (Weising et al., 1996).

From the above review, it is quite evident that microsatellite markers could be used as a potential tool for identification, classification and characterization of Musa germplasm. It can very well replace morphotaxonomic characterization that relies on subjective scoring of morphological traits. This greatly facilitates the selection of parents and assists in the development of new breeding strategies based on marker assisted selection.