Chapter Two

Literature Review

2.0 Literature Review

2.1 Origin and distribution of banana

Bananas constitute a distinctive group within the order Zingiberales, which is defined under the family Musaceae. The Musaceae is further divided into the genus *Musa* and *Ensete*. The genus *Musa* has wide geographical distribution ranging approximately in longitude from 75^{O} E to 150^{O} W and in latitude from 30^{O} N to the Tropic of Capricorn.

Within the genus *Musa,* four sections have been well documented that include Callimusa, Australimusa, Rhodochlamys and Eumusa plus a group of uncertain affinity *Incertae sedis*. Of the four named sections, *Eumusa* is the best known partly because the plants are hardy and fairly easy to grow whereas the Callimusa is the least known section.

Eumusa is the most important as the progenitor of all cultivated bananas. *Musa acuminata* (AA) and *Musa balbisiana* (BB) belong to this section. The cultivated edible bananas were produced as a result of natural, inter and intraspecific hybridization of two diploid species i.e. *Musa acuminata* (AA) and *Musa balbisiana* (BB). *Musa acuminata* is a diverse species, and at least nine subspecies have been described or suggested. *Musa balbisiana* is less diverse and no subspecies for it are recognized. Most of the edible types that are derived from these species are triploid, although diploid and tetraploid cultivars are also known. According to the Simmonds and Shepherd (1955) the ploidy and relative genomic contribution of the two species to a given banana cultivar are specified. For example, cultivars of the Cavendish subgroup, which are triploids of pure *Musa acuminata* are, designated 'AAA' whereas the cultivar Bluggoe, which is a triploid hybrid of *Musa acuminata* (AA) and *Musa balbisiana* (BB) is 'ABB'. However two other species has also been reported to be the progenitors of the different cultivated bananas. Carreell (1994)

reported *Musa schizocarpa* (S) and one or two species of Australimusa (*Musa textilis* = T) contributed to the genome of some of the cultivars found in Papua New Guinea.

In Peninsular Malaysia, the distribution of several known *M. acuminata ssp.* family members (*M. acuminata ssp. Malaccensis, M.acuminata ssp. Microcarpa and M. acuminata ssp.siamea*) overlaps in different geographical regions. It is concluded by Simmonds (1962) that Peninsular Malaysia was the center of diversity and the primary center of origin for the cultivated banana, all of which sprang from *M. acuminata.*

M. acuminata is the most important species and wild seeded diploid forms have their center of diversity in the Malaysian region where six of the nine subspecies overlap namely, *malaccensis*, *siamea*, *truncata*, *microcarpa*, *burmannica*, and *burmannicoides*. Four of these subspecies are reported from Malaysia only, i.e. *malaccensis*, *siamea* and *truncata* from Peninsular Malaysia and *microcarpa* mainly from East Malaysia (Borneo) (Simmonds, 1962). Therefore, Malaysia is the most important centre of diversity for wild *Musa acuminata* forms. Among the four subspecific forms, *malaccensis* is the most diverse and is the progenitor of the local 'AA' cultivated bananas (Simmonds, 1962).

2.2 Taxonomic classification of *Musa*

Plantae
Anthophyta
Monocotyledons
Zingiberales
Musaceae
Musa

Each family member of *Musaceae* has typical characteristics, which include androecium not petaloid, 5-6 stamen, spirally arranged leaves and bracts, male and female (or hermaphrodite) flowers separated within one inflorescence and many-seeded berry fruit (Simmonds, 1962).

Linnaeus made the earliest scientific classification in 1783. He gave the name *M.sapientium* to all desert bananas and *M.paradisica* for cooked varieties. Hutchinson (1959) and Simmonds (1962) classified the family Musaceae in the order Zingiberales. Musaceae is restricted to two genera *Musa* and *Ensete*, with about 30 -40 species. They are confined to the Old World, from West Africa to the Pacific, but with the greatest number of species in southeastern Asia.

The Musaceae consists of large to gigantic herbs, with pseudostem of leaf sheaths; leaves spirally arranged; new leaves formed from meristem near ground level push up through pseudostem in a tightly rolled condition; lamina large, usually oblong with stout midrib and numerous pinnately parallel veins extending to margin; inflorescence terminal from meristem pushing up on peduncle in the center of pseudostem and emerging in center of leaf crown; flowers in nodal clusters; each cluster in axil of large spataceous bract; perianth of one compound lobed tepal and one narrow inner tepal; ovary inferior, 3-locular, each loculus with numerous ovules in axile placentation; fruit a berry; seeds with thick hard testa, straight embryo and copious endosperm.

2.2.1 *Ensete*

The genus *Ensete* is distributed in a wild state in Africa from the Cameroon throughout the East Africa down to Transvaal in South Africa. A few species are also found from northeast India to the Philippines and New Guinea (Purseglove, 1972). The genus differs from *Musa* by being monocarpic, non - suckering with a distinctly swollen base, and

having large-sized seeds while *Musa* produces suckers and has small seeds (Cobley and Steele, 1976; Samson, 1992).

2.2.2 *Musa*

The genus *Musa* has 40 species, perennial, stooling or rhizomatous herbs in southeastern Asia and the Pacific, with their center of diversity in the Assam-Burma-Thailand region (Simmonds, 1962; Purseglove, 1972). Usually the plants grow into clumps. Leaves are large, long and spirally arranged. Fruits are berries; some are dehiscent and some are not. Pseudostem composed of tightly clasping leaf sheaths, slightly swollen at base; suckers freely produced; bracts and flowers inserted independently on peduncle, commonly deciduous by abscission, except for functionally female ovaries in basal hands; basal flowers generally female only; male flowers on distal hands; bracts usually reddish, purple or violet due to anthocyanins; pollen grains with finely granular surface; seeds 7 mm or less in diameter. The genus *Musa* is divided into the following sections:

1. **Eumusa**

   With 13-15 species the largest section is *Eumusa*, it is most diversified, most widely distributed geographically, and the oldest section probably, extending from southern India to Japan and Samoa. Pseudostem usually exceeds 3 m in height; inflorescence pendent or semi-pendent; bracts usually dull in color; flowers many, in two series in each bract. *M. acuminata*, *M. balbisiana* and their interspecific crosses derive edible parthenocarpic bananas .

2. **Rhodochlamys**

   With 5-7 species form India to Indonesia. They appear to be connected to the Eumusa section via *Musa flaviflora* Simmonds. Pseudostems are less than 3 m

high; inflorescence erect; few flowers to a bract, usually in a single series; parthenocarpy absent. For example *Musa ornata Rob.* and *Musa velutina* Wendl. & Drude are grown as ornamentals.

3. Callimusa

In Indochina, Malaya and Borneo there are 5-6 species; plants of small stature; usually with erect inflorescence and purplish bracts; parthenocarpy absent; for example *Musa coccinea* Andr. Is grown as an ornamental.

4. Australimusa

This group contains 5-7 species from Queensland to the Philippines. It is probably the most recently differentiated of the sections and includes the easterly species, mainly in Malaysian islands. The inflorescence is pendent, semi-pendent or erect. The section includes *Musa textilis,* (abaca or Manila hemp) and the Fe'i banana. Their fruits are parthenocarpic and predominantly female sterile; the fruiting bunch is erect and contains a red sap; the skin is orange in color when ripe and the flesh is yellow.

5. Incertae sedis

There are few species of doubtful affinity. These include *Musa ingens* Simmonds (x = 7; 2n = 14), which was discovered in New Guinea growing to a height of over 10 m and is the largest herb known to science, and *Musa beccarii* Simmonds (x = 9; 2n = 18) from north Borneo.

2.3 *Musa* Species in Malaysia

In Malaysia *Musa acuminata* is very variable containing 8-9 subspecies (De Langhe, 1969). Four of the subspecies overlap in the Malaysian center of diversity while others form disjunct populations on islands far removed from the main area of distribution. Four subspecies whose distributions overlap are *malaccensis*, *siamea*, *microcarpa* and *burmannica* (Simmonds, 1956). Other known species of series of *Musa* are listed in bellow.

Table 2.1:Classification of the genus *Musa*.

	Species	Chromosome number (2n)
Eumusa	*Musa acuminata* Colla	22
	Ssp. malaccensis Simmonds	22
	Ssp. microcarpa Simmonds	22
	Ssp. burmannica Simmonds	22
	Ssp. burmanniccoides De Langhe and Devreux	22
	Ssp. siamea Simmonds	22
	Ssp. banksii (F. Muell) Simmonds	22
	Ssp. errans Allen	22
	Ssp. zebrina nom. nud.	22
	Ssp. truncata (Ridl.) Shepherd	22
	M. balbisiana Colla	22
	M. itinerans Cheesman	22
	M. basjoo Siebold	22
	M. schizocarpa Simmonds	22
	M. nagensium Prain	22
	M. sikkimensis Kurz	22
	M. cheesmani Simmonds	22
	M. ochracea Shepherd	22
	M. flaviflora Simmonds	22
Rhodochlamys	*M. ornata* Roxb.	22
	M. laterita Cheesman	22
	M. velutina Wendl & Drude	22
	M. sanguinea Hook. f.	22
Australimusa	*M. textilis* Nees	20
	M. maclayi F. Muell	20
	M. peekelii Lauterb	20
	M. lolodensis Cheesman	20
	M. angustigemna Simmonds	20
Callimusa	*M. coccinea* Andr	20
	M. violascens Ridl.	20
	M. gracilis Holttum	20
	M. bornensis Beccari	20
Incertae sedis	*M. ingens* Simmonds	14
	M. beccarii ,Simmonds	18

Source: De Langhe (1969) ,

Malaysia is the most important center of diversity for wild *Musa acuminata* De Langhe (1969) forms. *Musa acuminata* is the most important species and wild seeded diploid forms have their center of diversity in the Malaysian region where six of the nine subspecies overlap namely, *malaccensis*, *siamea*, *truncata*, *microcarpa*, *burmannica*, and *burmannicoides*. Four of these subspecies are reported from Malaysia only, i.e. *malaccensis*, *siamea* and *truncata* from Peninsular Malaysia and *microcarpa* mainly from East Malaysia (Borneo) (Simmonds, 1962). Among the four subspecific forms, *malaccensis* is the most diverse and is the progenitor of the local 'AA' cultivated bananas (Simmonds, 1962).

Musa species and subspecies were classified by Simmonds (1962) based on morphological characteristics, and chromosome number. Morphological descriptions can easily be studied and large numbers of characters are used for variability studies. Chromosome numbers have been found to be variable among the wild species as well as among cultivated bananas. A wide range of variability has been described in bract color (red, purple, yellowish green, or violet) in the wild bananas. This variability is due to the presence of different anthocyanin pigments (Simmonds, 1954 b, c; Horry and Jay 1988 a,b). These pigments were found to be very useful in characterizing both wild and cultivated banana species. Recently genomic DNA contents were found to be very distinct at the species level and hence can be used for the identification of hybrids and cultivated bananas (Dolezel, 1994; Lysak *et al.,* 1999).

Simmonds described a key based on 25 morphological characteristics for the successful identification of species and subspecies, which belongs to five sections of the genus *Musa* (Pascua and Espino, 1986) and able to differentiate 79 Philippine banana clones encompassing members of six genomic and two ploidy levels using morphological characteristics only. Recently, based on sixty-one morphological characters, Karamura

(1999) successfully classified 238 accessions of the African highland banana clones grown in Uganda.

Although morpho - cytotaxonomic characters proved very effective in the classification of wild bananas, they are less effective for clonal identification. This was attributed to narrow genetic base of the clones (Pascua and Espino, 1986; Swennen and Vuylsteke, 1986). It has been recommended that the morphological scoring method suggested by Simmonds and Shepherd, (1955) should be modified because of the variation encountered in different plant's characters which were observed as a result of variation in the environmental conditions in which the banana plants were cultivated under (Silayoi and Chamchalow, 1985). The situation is more complicated as postulated by De Langhe (1961), who stated that groups of bananas were homogenous (derived from a single clonal source) but greatly diversified by somatic mutations to give a complex reticulate pattern of variability. With the addition of environmentally induced variation, one can end up with a virtual continuum of variation for some characteristics. However Valsalakumari and Sivaraman (1991) were able to classify Indian cultivars based on a composite of morphological characters, taxonomic scoring and chromosome numbers. In addition to the use of whole - plant or floral morphology to identify specific clones, or to characterize and quantify genetic diversity, various chemotaxonomic techniques have been employed. These techniques can supply additional information not available from the examination of basic morphological characteristics. Chemotaxonomy is the elucidation of differences and relationships between related organisms based on biochemical and/or molecular characteristics. These differences may be visually or mathematically analyzed. As a result, deduction of phylogenies, identification of mutants, and estimates of diversity can be accessed.

2.4 Numerical Taxonomy

Actual numerical - taxonomic studies of plant data were very scarce before the 1960's (Sokal and Sneath, 1963) because computers were primitive, making the inclusion of large number of characters difficult. The numerical taxonomy treatment adapted then for *Musa* was also primitive amounting only to a more or less qualitative study of mean difference scores between pre-chosen groups of entities (Simmonds and Weatherup, 1990 a, b). However currently a number of numerical - taxonomic methods are available and computer aided analysis of data made possible the use of large number of both qualitative and quantitative data sets. Simmonds (1962) described the taxonomy of wild bananas in numerical terms many years ago, well before 'numerical taxonomic' became popular. Those descriptions have been slightly supplemented and then subjected to analysis by modern methods (Simmonds and Weatherup, 1990 a, b). Principal co-ordinate analysis and clustering techniques gives essentially similar results. Five groupings, rather than the four sections traditionally recognized, can be discerned. The fifth group contains five taxa previously included in section *Musa* (informally Eumusa) or of uncertain affinity. This group is only somewhat weakly distinguishable from sections Australimusa whereas several species previously regarded as of uncertain affinity seen to find 'natural places'. Thus the results are by no means revolutionary but do encourage modest alteration of traditional views. Similarly, Perrier (1992) described genetic diversity in banana through numerical taxonomy. He concluded that genetic diversity in wild and cultivated bananas is the result of inter and intraspecific crosses, vegetative multiplication, and a long domestication process. Recently, Karamura (1999) described the classification of the African Highland bananas grown in Uganda. Different techniques of numerical taxonomy were employed successfully to determine the variation pattern and these included were two

different coefficients, three different clustering methods, principal component analysis and classificatory discriminant analysis. Sixty-one morphological characters were employed to determine differences among the 238 accessions. Phenetic classifications resulting from different analyses were compared with an independent subjective classification. The phenetic classification agreed with the subjective classification with regard to the positions of the majority of the accessions. Accessions, which were inconsistently placed in the cluster analyses, were classified by classificatory discriminant analysis and 84 clones were identified. He further suggested that it was advantageous to compare different methods because they often gave complementary results. For example the comparison of cluster analysis versus principal component analysis revealed similar clusters of accessions in the phenograms and along the first four principal components.

Jarret *et al.,* (1992) presented an example of the application of phylogenetic methods to RFLP analysis for detecting genetic diversity in bananas. Phylogenetic methods have proved their importance when applied to molecular markers.

2.5 Morphological studies in *Musa*

Morphological characters have proven very useful for clonal identification and taxonomic studies in *Musa*. Simmonds (1962) described a key based on 25 morphological characteristics for the successful identification of species and subspecies belonging to five sections of the genus *Musa*. Pascua and Espino (1986) was able to differentiate 79 Philippine banana clones encompassing members of six genomic and two ploidy levels using morphological characteristics only. Recently, Karamura (1999) successfully classified 238 accessions of the African highland banana clones grown in Uganda based on sixty-one morphological characters alone. Although morpho - cytotaxonomic characters

proved very effective in the classification of wild bananas, they are less effective for clonal identification as the genetic base of the clones is narrow (Pascua and Espino, 1986; Swennen and Vuylsteke, 1986). It has been recommended that the morphological scoring method suggested by Simmonds and Shepherd, (1955) should be modified because of the variation encountered in different characters planted under different environmental conditions (Silayoi and Chamchalow, 1985). The situation is more complicated as postulated by De Langhe (1961), who stated that groups of bananas are homogenous (derived from a single clonal source) but greatly diversified by somatic mutations to give a complex reticulate pattern of variability. With the addition of environmentally induced variation, one can end up with a virtual continuum of variation for some characteristics. However Valsalakumari and Sivaraman (1991) were able to classify Indian cultivars based on morphological characters, taxonomic scoring and chromosome numbers.

In addition to the use of whole - plant or floral morphology to identify specific clones, or to characterize and quantify genetic diversity, various chemotaxonomic techniques have been employed. These techniques can supply additional information not available from the examination of morphological characteristics alone. Chemotaxonomy is the elucidation of differences and relationships between organisms based on biochemical and/or molecular characteristics. These differences may be analyzed visually or mathematically, resulting in the deduction of phylogenies, identification of mutants, estimates of diversity.

2.6 Biochemical Markers

There are numerous indicators that morphological variation within the seed fertile *Musa acuminata* subspecies are much more extensive than suggested by previous observations. The term population might better describe the nature of these species and subspecies. In the areas of transition where one form or subspecies overlaps with a second form or subspecies, the range of variation often exceeds that of the two combined.

It is inconceivable that such a range of diversity could be accurately defined by descriptors. The great variation in bract color illustrates the complexity of this situation. When these populations are systematically studied, it will be necessary to complement the morphological descriptors with data on more subtle differences, such as anthocyanin analyses. Hence, the variability existing in the wild forms cannot be determined by the use of descriptors alone.

The process of adaptation and selection reflects the variation in metabolites and metabolic pathways. Flavonoids are mainly the products of secondary metabolism and the processes by which they are synthesized are then susceptible to selective/evolutionary pressures. Flavonoids due to hydroxylations, methylations and glycosylations of the precursor molecule exist in many different forms. This situation in turn reflects both the plant's response to current environmental stresses and to the availability of various biosynthetic pathways. This diversity has been utilized in studies of plant species diversification (Young and Sterner, 1981; Ardouvin *et al.,* 1985; Harborne, 1986; Glucchoff - Fiasson and Jay, 1987). Different forms of flavonoids can be characterized as being either advanced or primitive types (Simmonds, 1954 b,c; Gluchoff-Fiasson and Jay; 1987). Simmonds (1954 c) described that bracts of wild banana species are pigmented by glycosides (probably 3-diglucosides) of four combinations of anthocyanidins: cyanidin–

pelargonidin; cyanidin–delphinidin; delphinidin–petunidin–cyanidin– malvidin–peonidin; and malvidin–peonidin. The proportions of the various components are slightly altered between clones of one species and even between samples, but the general pattern is species-specific and making it a considerable taxonomic value.

Horry and Jay (1988 a) examined bract anthocyanins in 59 banana varieties and separated them into five main chemotypes based on the presence and absence of methylated compounds and / or the ratio between delphinidin and cyanidin derivatives. Similarly, they described that flavonoid analysis has thrown a new light on the evolution of bananas. They concluded that the various members of this subspecies complex developed adaptive metabolism in response to different geographical habitats. The relationship revealed by these studies confirmed the need to distinguish between the *acuminata* subspecies genomes. Hence, recognition of the principal species involved in cultivar development is of great importance in banana breeding programs (Horry and Jay (1988 a,b).

Isozymes are enzymes that have different molecular forms but catalyze identical reactions (Gottlieb, 1971). Genetic tests are frequently performed to determine bands representing gene products coded for by allelic genes (allozymes) as opposed to gene products coded for by gene at different loci (isozymes). Bonner *et al.,* (1974) found a significant number of polymorphic markers in peroxidase enzyme polymorphism in *Musa* species but detected only a slight variation among the clones. Rivera and Coronell (1983) described that polymorphism for peroxidases and polyphenoloxidases could differentiate between members of the Saba (BBB) and Bluggoe (ABB) genome groups. Analyses of a number of self-pollinated *Musa acuminata* diploids, *Musa balbisiana* and the interspecific hybrids between the two species showed highly species specific banding patterns for enzymes such as peroxidase (Jarret and Litz, 1986a). It was reported that differentiation of individual *Musa acuminata* subspecies could be accomplished by analysis of as few as six

enzyme systems. Apparent subspecies specific alleles have been identified in *Musa acuminata* subspecies *microcarpa*, *burmannica*, *errans* and *zebrina* (Jarret and Litz, 1986b). Espino and Pimentel (1990) have demonstrated the species-specific nature of isozymes of shikimate dehydrogenase (SKDH) and malate dehydrogenase (MDH). By the utilization of these they have also detected the interspecific hybrids between *Musa acuminata* and *Musa balbisiana* but they were less effective in differentiating the BB/BBB cultivars and clones of other genomic groups.Although isozyme polymorphism has successfully been used in banana taxonomy, isozymes are often developmentally and environmentally regulated. The degree of polymorphism detected in isozymes is also lower compared to DNA based markers such as RFLP, RAPDs and STMS markers.

2.7 Molecular Methods For Detecting Genetic Diversity

Considerable attempts have been made to distinguish and classify *Musa* accessions based on morphological characteristics (Simmonds and Shepherd, 1955). However the classification of certain accessions on this basis has been disputed (Gawel and Jarret, 1991a,b). This is mainly due to the narrow genetic base of the cultivated bananas. This resulted in the introduction of more sensitive techniques either independently or in combination with morphological description.

Based on difference in the nucleotide sequences of the DNA in different individuals, DNA markers relate directly to the plant's genotype rather than its phenotype. The use of DNA markers involves the examination, using sophisticated biochemical techniques of variations in cellular molecules. DNA markers offer the advantage of being unaffected by the environment. They are also stable and detectable in all tissues regardless of growth,

differentiation, development, or status of the plant cell and generally lack pleiotropic and epistatic effects.

The methods of DNA fingerprinting can be used to know the amount of variation in germplasm of crop plants, and to make comparisons between different accessions or groups of accessions within collections to assist in the management and future use of the conserved material.

2.7.1 Molecular Markers

There are two kinds of molecular markers:

- Non-PCR based markers
- PCR based markers

2.7.1.1 Non-PCR based Markers

The non-PCR based includes those techniques that use labeled nucleic acid molecules as hybridization probes. One such technique Restriction Fragment Length Polymorphism (RFLP) is used as a DNA probes to detect an altered fragment size at a locus. Thus, the analysis of a number of RLFPs gives a genomic profile of identified loci of the individual being studied.

2.7.1.1.1 Restriction Fragment Length Polymorphism (RFLP)

This technique uses cDNA or other cloned single-copy DNA elements as radioactivity labeled probes in hybridization with restricted genomic DNA. Usually, several endonucleases and different genotypes are screened. The combination of DNA probe and genotype-specific restriction enzyme pattern reveal a "restriction fragment length polymorphism". RFLP is a reliable polymorphism, which can be used for accurate scoring

of genotypes. RFLPs are co-dominant and identify a unique locus and, therefore, are very informative. In plants, RFLP remains the most widely used DNA marker assay, and is the basis for detailed genetic maps of major crops.

Restriction fragment length polymorphism (RFLP) of diverse germplasm, have been used to study the taxonomy and phylogeny of *Musa* species (Jarret *et al.*, 1992; Gawel *et al.*, 1992; Lanaud *et al.*, 1992; Carreel *et al.*, 1994; Jenny *et al.*, 1997), and variation in the chloroplast genome within the *Musa* genus (Gawel and Jarret 1991b; Baurens *et al.*, 1997).

Although it remains widely-used, tow basis limitations of the RFLP technique have motivated the development of several alternative technologies. The first limitation is the quantity of DNA required. Fifty to two hundred micrograms of DNA per individual are necessary to generate a DNA fingerprint or RFLP analysis of the entire genome. In contrast to RFLPs, PCR-based techniques developed during the last ten years require only approximately 10% of this amount, as template for PCR amplification of large quantities of the target sequence. The second limitation is that closely-related species usually contain the same alleles.

2.7.1.2 PCR-based Markers

All PCR-based molecular markers appear to detect a high level of polymorphism within a range of *Musa* breeding populations. In addition, PCR-based assays are amenable to large-scale screening of breeding populations. The PCR based markers can be divided into two groups. The first group produces multi-locus DNA fingerprints that can be used as dominant inherited genetic markers (e.g.: RAPD) and the second group produces single-locus, co-dominant inherited genetic markers (e.g.: Sequenced Tagged Microsatellite (STMS)) (Caetano-Anolles, 1996).

2.7.1.2.1 Random Amplified Polymorphic DNA (RAPD)

The Random Amplified Polymorphic DNA (RAPD) technique, which uses short segments of DNA (primers) of arbitrary nucleotide sequence, allows random amplification of DNA sequences throughout the entire genome. This is because RAPD polymorphisms result from either a nucleotide base change that alters the primer-binding site, or from an insertion or deletion within the amplified region (Williams *et al.*, 1993), polymorphisms usually result in the presence or absence of an amplification product from a single locus. In *Musa* therefore, there have been a number of applications of the RAPD technique.

The (RAPD) technique has been successfully used to distinguish *Musa* germplasm's diverse (Kaemmer *et al.*, 1992; Howell *et al.*, 1994; Bhat and Jarret 1995). In addition, a molecular linkage map is being developed using a variety of marker systems including RAPD (Faure *et al.*, 1993). RAPD assays are particularly useful, as they require no prior knowledge of the organism. RAPD analysis has been used to differentiate *Musa* genome groups (Howell *et al.*, 1994), from more closely related *Musa* germplasm (Bhat and Jarret 1995) and full-sib hybrids in plantain breeding populations (Crouch *et al.*, 1998a, b). These reports clearly demonstrated the potential value of this technique for germplasm characterization and cultivar's identification but give little insight into the value of the assay for molecular breeding.

However, RAPD analysis has several disadvantages including the dominant nature of the marker system and reproducibility problems, which may limit their application in marker assisted selection (MAS). This has led to a focus on the development and utilization of primers for *Musa* microsatellites (Jarret *et al.*, 1994; Kaemmer *et al.*, 1997), which were considered optimum markers in other systems due to their abundance, polymorphism and reliability.

2.7.1.2 Amplified Fragment Length Polymorphism (AFLP)

This PCR-based technique requires no sequencing or cloning. It is similar to RAPD, but the primer consists of a longer fixed portion and a short random portion. The fixed portion gives the primer stability and hence repeatability and the random portion allow it to detect many loci. This method generates a large number of restriction fragments (50-100) facilitating the detection of polymorphisms. The number of DNA fragments, which are amplified, can be controlled by choosing different base numbers and composition of nucleotides in adapters. Although not many maps have been developed so far using AFLPs, this method is now widely used for developing polymorphic markers. The approach is very useful in saturation mapping and for discrimination between varieties. High reproducibility, rapid generation and high frequency of identifiable polymorphisms make AFLP analysis an attractive technique for determining linkages by analyzing from segregating populations. However, AFLPs are predominantly not co-dominant and still expensive to generate because the fragments are detected by silver staining, fluorescent dye or radioactivity.

2.7.1.3 Sequenced Tagged Microsatellite Sites (STMS)

If short sequence repeats (SSR) loci are cloned and Sequenced, primers to the flanking region can be designed to produce a Sequence-Tagged Macrosatellite Site (STMS).

Microsatellites are short sequence head to tail repeats of approximately one to five nucleotides in length that are present in genomes of all higher eukaryotes (Lagercrantz *et al.*, 1993). STMSs are detected by using a locus specific PCR, where a pair of primers is used under high stringency conditions i.e. the optimization must be done first to select a suitable denaturing temperature to get a specific banding patterns.

Because microsatellite markers are generated by highly specific PCR amplification, they do not have the problems experienced with RAPD analysis. Simple sequence repeats (SSR) are regions of short tandemly repeated DNA motifs (generally less than or equal to 4 bp) with an overall length in the order of tens of pairs. SSR have been reported to be highly abundant and randomly dispersed throughout the genomes of many plant species. Variation in the number of times the motif is repeated is thought to arise through slippage errors during DNA replication. Thus, SSRLP may occur even between closely related individuals. 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.*, 1998b). However the isolation of microsatellite, is time consuming and expensive. Nevertheless, several hundred SSRLP markers have been generated in *Musa* (Jarret *et al.*, 1994; Lagoda *et al.*, 1995; Kaemmer *et al.*, 1997; Crouch *et al.*, 1998a). Furthermore, the isolation of SSR is becoming increasingly routine with the availability of automated DNA sequencing facilities; improved techniques for the construction of genomic libraries enriched for SSR and improved techniques for the screening of appropriate clones.

STMS reported to be very useful for genome analysis (Kaemmer *et al.*, 1997). They are randomly interspersed in eukaryotic genomes; highly variable in the number of repeats they contain and are co-dominantly inherited. They have been widely used rice (Zhang *et al.*, 1995), maize (Senior and Heun, 1993), wheat (Plaschke *et al.*, 1995) and citrus (Kijas *et al.*, 1995). STMS markers have been shown to detect more polymorphic alleles per locus than allozymes (Terauchi and Konuma, 1994) or RFLPs (Röder *et al.*, 1994) and they are increasingly being used for genome analysis and mapping in plants.

STMS markers though proved to be very useful however; the cost of production of these markers is relatively higher. This can be justified by information gain per locus

compared to other marker systems. Large PAGE gels can easily be used to screen 100 equivalent locus-individuals by multiplexing, in 3 hours. Therefore mapping microsatellites without radioactivity, using very simple protocols, can be accomplished in a very unsophisticated laboratory environment, once STMS are well defined and tested (Kaemmer *et al.,* 1997). It seems that STMS markers are presently the systems of great interest for linkage mapping, gene cloning and marker assisted selection breeding in bananas. Although application of microsatellites in the plants is currently limited due to the amount of published sequence data for most species (Winter and Kahl, 1995). This is particularly true in case of bananas. However, an increasing emphasis on the molecular characterization of bananas without doubt will provide more DNA sequence data.

All markers systems have different advantages and disadvantages in specific applications. Thus, it is important for molecular breeding programs to develop capacity in several assays in order that the most suitable system can be chosen and rapidly applied for any particular application (Bhat *et al.,* 1997).