CHAPTER TWO

REVIEW OF LITERATURES
2.1 Broad Taxonomic Positioning of The Genus *Musa* L.

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 family Strelitziaceae, whose members were formerly and are still sometimes included in the Musaceae consists of four genera with about 100 species are confined mainly to the American tropics alternatively these members are also sometimes included within the family Heliconiaceae: *Ravenala* with one species, in Madagascar, *Strelitzia* with five species in South Africa and one species in southeastern South America. *Orchidantha*, which occurs in Malaysia, is the only genus in the family Lowiaceae, and is also sometimes included in Musaceae.

In the above families the androecium is not petaloid and consists of 5-6 stamens. In the other three families of the order Zingiberales, namely, Zingeberaceae, Marantaceae, and Cannaceae, the androecium is petaloid and there is a solitary stamen.

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.
2.1.1 *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).

2.1.2 *Musa* L. (x = 10, 11; rarely 7 or 9)

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* (x = 11; 2n = 22 in wild species; 22,33,44 in cultivars)

Eumusa is the largest section with 13-15 species, is the most diversified, geographically most widely distributed, and probably the oldest section, 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. The edible parthenocarpic bananas are derived from *M. acuminata*, *M. balbisiana* and their interspecific crosses.

2- Rhodochlamys (x = 11; 2n = 22)

It contains 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 (x = 10; 2n = 20).

There are 5-6 species in Indochina, Malaya and Borneo; 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 (x = 10; 2n = 20).

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.
2.2 Wild *Musa* Species In Malaysia

*Musa acuminata* originates in Malaysia and is very variable containing 8-9 subspecies (Table 3) (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 Table 3.

<table>
<thead>
<tr>
<th>Series</th>
<th>Species</th>
<th>Chromosome number (2n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eumusa</td>
<td><em>Musa acuminata</em> Colla</td>
<td>22</td>
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<tr>
<td></td>
<td>ssp. <em>malaccensis</em> Simmonds</td>
<td>22</td>
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<td></td>
<td>ssp. <em>microcarpa</em> Simmonds</td>
<td>22</td>
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<td></td>
<td>ssp. <em>burmannica</em> Simmonds</td>
<td>22</td>
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<td></td>
<td>ssp. <em>burmannicoides</em> De Langhe and Devreux</td>
<td>22</td>
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<td></td>
<td>ssp. <em>siamea</em> Simmonds</td>
<td>22</td>
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<tr>
<td></td>
<td>ssp. <em>banksii</em> (F. Muell) Simmonds</td>
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<td></td>
<td>ssp. <em>errans</em> Allen</td>
<td>22</td>
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<td></td>
<td>ssp. <em>zebrina</em> nom. nud.</td>
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<tr>
<td></td>
<td>ssp. <em>truncata</em> (Ridl.) Shepherd</td>
<td>22</td>
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<tr>
<td></td>
<td><em>M. balbisiana</em> Colla</td>
<td>22</td>
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<td></td>
<td><em>M. itinerans</em> Cheesman</td>
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<td></td>
<td><em>M. basjoo</em> Siebold</td>
<td>22</td>
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<td></td>
<td><em>M. schizocarpa</em> Simmonds</td>
<td>22</td>
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<td></td>
<td><em>M. nagensium</em> Prain</td>
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<td><em>M. sikkimensis</em> Kurz</td>
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<td></td>
<td><em>M. cheesmani</em> Simmonds</td>
<td>22</td>
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<tr>
<td></td>
<td><em>M. ochracea</em> Shepherd</td>
<td>22</td>
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<tr>
<td></td>
<td><em>M. flaviflora</em> Simmonds</td>
<td>22</td>
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<td>Rhodochlamys</td>
<td><em>M. ornata</em> Roxb.</td>
<td>22</td>
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<tr>
<td></td>
<td><em>M. laterita</em> Cheesman</td>
<td>22</td>
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<td></td>
<td><em>M. velutina</em> Wendell &amp; Drude</td>
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<td></td>
<td><em>M. sanguinea</em> Hook. f.</td>
<td>22</td>
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<tr>
<td>Australinusa</td>
<td><em>M. textilis</em> Nees</td>
<td>20</td>
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<td></td>
<td><em>M. maclayi</em> F. Muell</td>
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<td></td>
<td><em>M. peekelii</em> Lauterb</td>
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<td></td>
<td><em>M. lolodensis</em> Cheesman</td>
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<td></td>
<td><em>M. angustigemna</em> Simmonds</td>
<td>20</td>
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<tr>
<td>Callimusa</td>
<td><em>M. coccinea</em> Andr</td>
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<td></td>
<td><em>M. violascens</em> Ridl.</td>
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<td></td>
<td><em>M. gracilis</em> Holtum</td>
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<td></td>
<td><em>M. bornensis</em> Beccari</td>
<td>20</td>
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<tr>
<td>Incertae sedis</td>
<td><em>M. ingens</em> Simmonds</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td><em>M. beccarii</em> Simmonds</td>
<td>18</td>
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</table>
Simmonds (1955) reported four species from Peninsular Malaysia namely; *M. acuminata, M. balbisiana, M. gracilis* and *M. violascens*. *M. acuminata* and *M. balbisiana* are the tallest and their inflorescence is similar to cultivated bananas, being pendent and having brownish-red bracts. *M. gracilis* and *M. violascens* are ornamental bananas having erect inflorescence with polished bracts and seeds are barrel-shaped compared to sub-globose angular ones in *M. acuminata* and *M. balbisiana*. *M. gracilis* and *M. violascens* are ornamental bananas.

The distribution of *Musa gracilis* does not overlap with that of *Musa violascens* (Kiew, 1987). On the west coast of Peninsular Malaysia *Musa gracilis* has been reported in Batang Melaka, Malacca and *Musa violascens* in Negri Sembilan. In Peninsular Malaysia, *Musa acuminata* has been collected from all states except Johor (Simmonds, 1955). *Musa balbisiana* is locally called Pisang Batu or Pisang Gala and are reported from Malacca, Terengganu, and Johor. The species *Musa acuminata* is the most variable locally and three different forms are reported by Simmonds (1955) based on the following morphological descriptions;

1. The “Selangor form” It has intensely waxy green leaves; sheaths usually with much black smudging, midribs usually pink, male buds bright solid red, bracts quickly deciduous, not imbricate, bright red within, male flowers with the tepal tips bright yellow.

   The distribution of Selangor form ranges at low altitudes in Malacca, Negri Sembilan, Selangor, and Perak north to Grik, ascending to Genting Sempak on the Pahang side.

2. The “Kedah form” It is considered as nearly allied to the Selangor form but differing in the yellowish cast of foliage, slighter waxiness and especially in the male bud with bracts imbricate and yellowish at the tips. Commonly, slightly variegated on the back and often persistent for a day before falling.
Kedah form has distribution at low elevations ranging from Kedah, Perlis, Kelantan and to probably also in Southern Thailand.

(3) The "Cameron form" It has nonwaxy yellow green leaves, sheath and midribs deep chocolate-brown (probably more intense at higher altitude), male bud dull purple plumper than in the preceding forms, bracts not imbricate, quickly deciduous, pale within, tepal tips pale yellow.

This is the highland form whose distribution ranges from Selangor to North Perak and Pahang where it descends to 100m at Genting Sempak, to 1000m above Tapah, and to low elevations between Grik and Kuala Kangsar and near Taiping. It ascends to 2000m or more in the Cameron highlands.

The following keys were suggested for the identification of Malaysian wild banana species and subspecies (Simmonds, 1955).

1- a, Stems 8 or more tall, inflorescences horizontal or pendulous, seed angular or subglobose ------- 2.
   b, Stems usually less than 8 feet tall, inflorescences erect, seed barrel shaped --------- 3.

2- a, Petiole canal open; fruits slender, rounded, glabrous; male bracts not or at most slightly imbricate, rolled back lifting; ovules biseriate; seed compressed - angular ------- Musa acuminata.
   b, Petiole canal closed, fruits plump, angular, waxy; male bracts strongly imbricate, not rolled back at lifting; ovules quadriseriate; seed subglobose----- Musa balbisiana

3- a, Fruits biseriate ---------- (3) Musa violascens.
   b, Fruits uniseriate ------- (4) Musa gracilis.

Key for Musa acuminata subspecies;

1. a, Leaves waxy ------------------------ Selangor
b, Leaves glabrous  ------------------ Cameron form

2. a, Bracts of male bud imbricated  -------------- Kedah

b, Bracts of male bud convolute  --------------- Selangor

Simmonds (1956a) described three Malaysian forms of *Musa acuminata* as subspecies based on morphological variation. Simmonds then described five different subspecies of *Musa acuminata*. These species were classified based on the following key;

1, Sheaths waxless except in young suckers  --------- (2)

Sheaths waxy-------- (3)

2, Pseudostem intensely brown - pigmented; male bracts pale red within,

not imbricate  --------- ssp. *microcarpa*

Pseudostem slightly pigmented; male bracts crimson within, imbricate -- ssp. *burmannica*

3, Sheaths lightly waxy; female flowers at the upper ones, male fertile; fruits subobtuse;

ovules more than 200 per ovary; bracts yellow or dirtily dull red  --------- ssp. *banksii*

Sheaths strongly waxy; female flowers male sterile; fruits acuminate; ovules fewer than 200 per ovary; bracts bright red or purplish red  --------- 4

4, Bracts bright red without, quickly deciduous; non - imbricate  --------- ssp. *malaccensis*

Bracts purplish red without, with yellowish tips, often slightly persistent, usually imbricate in the young bud  --------- ssp. *siamea*

Simmonds (1956a) placed much confidence in bract imbrication and used this feature as a key character to differentiate the known subspecies of *Musa acuminata*, ssp. *burmannica* and *siamea* that had imbricate bracts and subspecies *microcarpa*, *banksii* and *malaccensis* with non-imbricate bracts.

Bract disposition of the male bud in *M. acuminata* and its subspecies is subject to morphological change from convolute to imbricate during bud development. Therefore
this character should be considered transient and unreliable for classification purposes (Hari, 1968). Shepherd (1988) further suggested that limitation of the range of the ssp. siamea omits the North Malaysian Kedah form included by Simmonds (1956a). When seen in collection alongside other subspecies, rather than in isolation, the Kedah form is clearly ssp. malaccensis. This judgment is further reinforced by its chromosomes structure. It would now seem, in fact that ssp. malaccensis extends into South Thailand rather than ssp. siamea into Malaysia. He further stated that Simmonds (1956a) also erred in including the highland banana M. truncata Ridley under ssp. microcarpa. The strongest evidence for its separation is the translocated structure of its chromosomes (three changes relative to ssp. microcarpa). Therefore the highland banana from Malaya is now considered as ssp. truncata.

2.3 Classification of Cultivated Bananas

The edible bananas are usually seedless, while the wild ones are seeded. Edible bananas are commonly triploid (sometimes diploid and tetraploid), while the wild bananas are diploid. Edible bananas can be classified into two groups; (1) those derived from members of the Eumusa and (2) Fei bananas, derived from the members of Australimusa. The first are those commonly cultivated globally while the second are confined only to Papua New Guinea and Polynesia. Edible seedless bananas, in the section Eumusa had their origin from two wild species, Musa acuminata Colla and Musa balbisiana Colla. Simmonds and Shepherd (1955) indicated that wild bananas have arisen in five main stages in their evolutions from these two species: (1) through the evolution of parthenocarpy and sterility in diploid Musa acuminata Colla, (2) through outcrossing of edible diploid of Musa acuminata Colla with Musa balbisiana Colla, followed by human selection, (3) through the occurrence of triploidy in Musa acuminata Colla with further outcross with Musa acuminata Colla and Musa balbisiana Colla; (4) through the
occurrence of tetraploid hybrids, and (5) through somatic mutations, as in other vegetatively reproduced crops. This shows that edible bananas may be derived from *Musa acuminata* Colla as diploid, triploid or as hybrids among *Musa acuminata* Colla and *Musa balbisiana* Colla that is either diploid, triploid or even tetraploid.

These hybrids have developed independently in several places in Southeast Asia, but the center of origin is probably Peninsular Malaysia (formerly Malaya) and its immediate neighbourhood as the probable primary center, with a secondary center of diversity dispersing from India to Indonesia, Philippines and Papua New Guinea (Moore, 1957).

Simmonds and Shepherd (1955) suggested that the name *Musa paradysica* L. and *Musa sapientum* L. adapted by Linnaeus (1783) for the cultivated bananas should be rejected from the nomenclature of bananas. They introduced a scoring method based on 15 morphological characters derived from *M. acuminata* and *M. balbisiana*. The edible bananas were then named as *Musa* (AAA Group) Pisang Ambon for cultivar entirely derived from *M. acuminata* Colla whereas *Musa* (ABB Group) Pisang Tanduk for a hybrid of *M. acuminata* (AA) and *M. balbisiana* (BB) as indicated by the presence of letters A and B. Such taxonomic description of cultivars by Simmonds and Shepherd (1955), has the advantage of being more precise than the use of broad botanical categories and make possible the distinction of such an enormous number of cultivars. In addition, it indicates their evolution as well (Moore, 1957).

By using 15 different morphological characters each of which was diagnostic of differences between *M. acuminata* and *M. balbisiana*, Simmonds and Shepherd (1955) showed that the contributions of the two species could be clearly distinguished. For each character in which a cultivar agreed completely with *acuminata*, a score of 1 was given, and for each character in which the cultivar agreed with *balbisiana*, a score of 5 was
given. Intermediate expressions of the character were assigned a score of 2, 3 or 4, according to its intensity. As far as the ploidy is concerned edible bananas belonging to the section Eumusa have either 22, 33 or 44 chromosomes. The basic haploid number is 11, thus cultivars can only be diploid, triploid or tetraploid. Of the 200 to 300 clones, which are thought to exist, more than half are triploids, with the remainder being mostly diploids. The tetraploids clones are very rare. It is necessary to know the ploidy of a clone before it can be correctly classified. This can only be done cytologically by making a quantitative chromosome count. Morphologically, triploids and tetraploids are larger and more robust than diploids. Scoring technique comprises of 15 morphological characters, allowing a range of total scores from 15 (pure M. acuminata) to 75 (pure M. balbisiana). Scores in between would be based on the relative contribution of the two species plus the level of ploidy in the interspecific hybrid. However it is very difficult to characterize hybrids like AAB, ABB based on their morphological characters only. Whereas the ploidy estimation based on chromosome counting could be a difficult task due to the small size of the chromosomes. Recently, Horry et al., (1998) reported that the clone ‘Klue Teparod’, well known throughout the world as a tetraploid turned out to be a triploid by using flow cytometry and chromosomal analysis. Similarly, Silayoi and Chomchaloow (1985) classified 137 accessions in the Thai banana gene bank on the basis of 15 morphological characters suggested by Simmonds and Shepherd (1955), and recognized some deficiencies in the original classification.

2.4 Studies on the Systematics of Musa

2.4.1 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 analysed visually
or mathematically, resulting in the deduction of phylogenies, identification of mutants, estimates of diversity.

2.4.2 Biochemical Markers

There are numerous indicators that morphological variation within the seed fertile *Musa acuminata* subspecies is much more extensive than suggested by previous observations of a limited number of samples. 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.

Variation in metabolites and metabolic pathways reflect the process of adaptation and selection. Flavonoids are the products of secondary metabolism and the processes by which they are synthesized are susceptible to selective /evolutionary pressures. Flavonoids exist in many different forms (due to hydroxylation's, methylations and glycosylations of the molecule), which reflect both a plant 's response to environmental conditions 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 (Simmonds, 1954 b,c; Glucchoff - 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; malvidin – peonidin. The proportions of the various components are slightly variable between clones of one species and even between samples, but the general pattern is characteristics of a species and is therefore of 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 and RAPD’s.

### 2.4.3 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.

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;
Burens et al., 1997). However, there is only one report of their use to distinguish more closely related material (Bhat et al., 1994). More importantly perhaps, the relatively high cost and technically demanding nature of these techniques are not appropriate for routine breeding applications. Thus, researchers have concentrated mainly on the application of the polymerase chain reaction (PCR) for Musa genome analysis.

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 random amplified polymorphic DNA (RAPD) technique has been successfully used to distinguish diverse Musa germplasm (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 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.
Microsatellite markers, otherwise known as simple sequence repeats length polymorphism (SSRLP) are generated by highly specific PCR amplification and, therefore, should not suffer from the reproducibility 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. Similarly, Engelborghs et al., (1998) reported the potential of amplified fragment length polymorphism (AFLP) to detect genetic variability in somaclonal variants in Musa species. AFLP has the ability to identify a large number of polymorphic bands without any prior knowledge of the organism. Unfortunately the information content of these banding patterns is restricted, as they must initially be treated as dominant markers. However, when AFLP analysis is applied to large populations, circumstantial allelic relationship may be sufficient for practical purposes. AFLP assays are technically demanding and expensive, as they require a number of DNA
manipulations and a complex visualization procedure. In addition, they require relatively large amounts of reasonably high quality DNA. The use of poor quality DNA may lead to incomplete digestion, which can result in artificial polymorphism.

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).

2.4.4 Analysis of *Musa* Genome Using Flow Cytometry and Cytogenetics

The cytogenetics of *Musa* has been poorly developed mainly due to difficulties with the analysis of small chromosomes. Until now, ploidy of many accessions is not known and identification of individual chromosomes within the karyotype is not possible. This situation contrasts with the rapid progress made in the analysis of *Musa* genome at molecular level.

Flow cytometry has been shown suitable for analysis of nuclear DNA content in *Musa* (Dolezel et al., 1997). The method is based on preparation of suspensions of intact nuclei from small amounts of fresh tissue. DNA of nuclei in suspensions is then stained by a suitable fluorochrome and the fluorescence of individual nuclei is analysed using flow cytometry. The analysis can be made at high speed and thousands of nuclei can be measured within several minutes. Because the nuclei are analysed individually, subpopulations differing in DNA content (i.e., mixoploidy) may be detected. The method is rapid, non-destructive, requires only small amounts of leaf tissue, and thus finds extensive number of applications both in taxonomy and breeding.

Flow cytometry has been used to reclassify the ploidy of several well-known clones previously believed to be tetraploid. It turned out that three of them were triploid (Horry et al., 1998). The method is being increasingly used also in breeding programs to
screen ploidy in progenies obtained in various crosses as well as for mass production of non-chimeric polyploids (Van Duren et al., 1996).

It has also been shown to be suitable for the estimation of genome size. The results of DNA content analysis demonstrated that Musa genomes are small (537-615 Mbp) and that A and B genomes of Musa differ in size, the B genome being smaller by 12% on average (Dolezel et al., 1994; Lysak et al., 1999). No variation in genome size was observed among the accession of Musa balbisiana. Small but statistically significant variation was found among the subspecies and clones of Musa acuminata. This difference may relate to geographical origin of individual accessions. Larger variation in genome size was found among the triploid Musa clones. The variation may be due to different genomic constitution as well due to the differences in the size of A genome. Statistical analysis of data on genome size resulted in grouping that agreed fairly well with generally accepted taxonomic classification of Musa (Lysak et al., 1999). Based on these results it has been proposed that a comparative analysis of genome size in diploids and triploids might be useful in identifying putative diploid progenitors of cultivated triploid Musa clones. In some plant species flow cytometry has been shown useful for sorting individual chromosome-specific DNA (Dolezel et al., 1995). Once developed for Musa, the technique would simplify gene isolation and mapping.

Early work on genomic size established the concept of DNA constancy for a species given a constant basic chromosome number and (i.e. with no variation due to aneuploidy, sex chromosomes, supernumerary chromosomes or polyploidy etc).

Cytophotometric evidence in several plant species indicated unexpected commonness of intraspecific genome size variability. Genome size variation is currently being used for taxonomic classification both at inter and intraspecific level. Variation of more than 12% has been reported at interspecific level being statistically significant
(Lysak et al., 1999). Bennett (1985) listed 24 angiosperm species in which intraspecific variation in DNA C value was reported. There have been numerous reports of intraspecific variation in nuclear DNA amount in angiosperms (Cavallini and Natali, 1990). At several instances, striking intraspecific variation of a size previously thought to occur only between species was reported. The examples of intraspecific variation in nuclear DNA content are rapidly increasing (Bennett and Leitch, 1995). This variation, in spite of a constant chromosome number was reported to be as high as 255%.

Iannelli et al., (1998) used flow cytometry to identify lemon genotypes. Eight genotypes out of seventeen showed characteristic DNA content and could be differentiated based on this. While the evidence of the intraspecific quantitative nuclear DNA variation is broadening, the function of this variation remained elusive. Orgel and Crick (1980) argued that the variation in nuclear genome size was the result of repeated sequences (satellite DNA and transposable DNA elements) maintained in their genome by their ability to replicate, but without adaptive value. On the other hand it is difficult to imagine that this widespread phenomenon (Bennett and Leitch, 1995) is always without significance for the host (Bennett, 1972).

Although flow cytometry has been shown to be extremely useful, detailed genome mapping is impossible without the analysis of the structure and behavior of chromosomes during mitosis and meiosis. Until recently, this has been difficult due to small size of Musa chromosomes. New methods have been developed for preparation of metaphase spreads suitable for high-resolution chromosome analysis (Dolezel et al., 1998). The method allows reliable chromosomes counting and analysis of basic chromosome morphology. Furthermore, metaphase spreads were shown to be suitable for the localization of DNA sequences on mitotic chromosomes using methods of molecular cytogenetics.
While genomic in situ - hybridization (GISH) has been used to determine parental origin of chromosomes in hybrids, fluorescent in situ hybridization (FISH) permits physical mapping of DNA sequences to Musa chromosomes. FISH with probes for rRNA genes in wild seeded species of Musa acuminata and Musa balbisiana showed that the 18S-25S rDNA sequences are localized on one chromosome pair, and that the 5S rDNA sequences are localized on two or three chromosome pairs. Interestingly, only five sites of 5S rDNA were observed in vegetatively propagated diploid edible varieties indicated their hybrid origin (Dolezelova et al., 1998). Identification of individual chromosomes using physically mapped DNA sequences will allow analysis of their behavior and segregation during evolution and breeding programs. Recently, Balint - Kurti et al., (2000) identified and localized retrotransposons on Musa chromosomes in both A and B genomes of Musa species. The cultivar Grand Nain contained 200 - 500 copies per haploid genome. In the same study, chromosomal localization by fluorescent in situ hybridization showed that copies of monkey retrotransposons are concentrated in the nucleolar organizer regions and colocalize with rRNA genes. Other copies of monkey appear to be dispersed throughout the genome.

2.4.5 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 Seed Germination and In Vitro Studies

The wild progenitors of the edible bananas produce seeds freely. The use of seedling banana plants as research tools and the increased emphasis on banana breeding programs has necessitated elucidation of factors affecting germination of these seeds. Efforts in banana improvement via conventional breeding rely upon the fact that "seedless" clones will produce a miniscule number of seeds when pollinated by male diploids. The seed yield in hybrid banana clones is usually low and for a successful breeding program it is imperative that a maximum number of these seeds be germinated (Shepherd et al., 1987). Several research programs require a continuous supply of small and uniform plants, which can be obtained most readily from seeds. Furthermore seedlings are more easily handled and transported in greenhouse and laboratory than are vegetatively propagated plants, and space requirements are considerably lower. Certain rhizosphere studies and related studies (Stotzky et al., 1962) require the use of aseptic banana plants, which because of the difficulty encountered in freeing vegetative propagating material from micro-organisms, can best be obtained from true seeds. Vakili (1962) used Musa balbisiana seeds for ploidy induction by using colchicine treatment. Several other studies reported the use of seed progenies in the screening of various pathogens (Stover, 1962; Vakili, 1965). Recently, excised embryos have been utilized for cryopreservation (Bhatt et al., 1994) in efforts to preserve the banana germplasm resources due to accelerated loss of germplasm both in the wild and cultivated tropical
species. Hence knowledge of factors influencing the germination of banana seeds is needed for the effective execution and understanding of \textit{Musa} seed germination.

\textbf{2.5.1 Breeding System of \textit{Musa}}

No direct studies of the breeding systems of wild bananas have been made but various inferences suggest that plants are intermediate between two extremes of inbreeding and out-breeding. First the flowers are predominantly unisexual and abundantly nectariferous. They are visited by many animal pollinators, of which the bats are probably the most important. In any inflorescence, the female precedes the male phase without any overlap in times, it follows that the first bunch thrown by a stool is invariably out-pollinated. The bananas however are clump-forming plants and since temporarily, it is possible that second and later bunches thrown by a stool can be pollinated from the male flowers of the preceding bunch, selfing can happen. Since wild bananas tend to be gregarious plants sib – pollination could have taken place. However cross-pollination had been shown by the occurrence of occasional interspecific hybrids in batches of open – pollinated seeds (Simmonds, 1962).

As the majority of bananas have unisexual male and female flowers, a few have hermaphrodite and male., presumably self-pollination can also take place. Hermaphrodite flowers occur in \textit{Musa acuminata ssp. Indent.} in South India; in \textit{Musa acuminata ssp. banksii} in the Pacific, Queensland and New Guinea; in \textit{Musa acuminata} in the Philippines; in \textit{Musa ingens} and \textit{Musa schizocarpa} in New Guinea; in \textit{Musa velutina} and \textit{Musa sanguinea} in Assam; and \textit{Musa jackeyi} in Queensland.

Experiments in which seed yield of various clones were compared in respect of pollen parent showed that cross-pollination yielded more seeds than selfing, suggesting the early elimination of inviable zygotes or endosperm (Simmonds, 1952 a, b).
The evidence reviewed suggested that collectively the bananas are moderately out-bred (though self – pollination may be frequent) and that they tolerate an occasional generation of close inbreeding without significant harm. This sort of mating system seems well adapted to their ecological status as “jungle weeds”.

2.5.2 Banana Seed Structure and Germination

Seed morphology of *Musa* seed, was first described by McGahan (1961a,b). Seeds of *Musa balbisiana* averages 3-5mm in diameter and are irregularly globose in shape and frequently have somewhat flattened sides. The seeds are grayish - brown and a scarios membrane covers the rugose seed coat. The seeds of the species *Musa acuminata* from Malaya are almost similar to *Musa balbisiana* and reported to be black to brownish black whose diameter ranges from 4-6 mm and globose in shape (Chin, 1995). The cuticular hilum is actually the upper surface of the truncate conical micropylar plug, the tapering outside face of separation from the body of the testa being smooth and polished and evidently a line of predetermined breakage, which closes the only opening in an otherwise intact seed coat.

The seed of *Musa balbisiana* is derived from a bitegmic anatropous ovule and is divided into two chambers, the large contains the embryo and endosperm, and the smaller enclose the chalazal mass.

The basic morphology of the *Musa balbisiana* embryo is similar to *Musa acuminata* species. The embryo, ranging in length form 1-2mm, has the mushroom shape characteristic of the Musaceae (Humphery, 1896). The enlarged cap shape structure is termed as the ‘haustorium’ (Gatin, 1908) and is the principal part of the cotyledon. The stalk like portion of the embryo represents the epicotyl - hypocotyl - radicle axis as well as part of the cotyledon. This stalk like portion is located within the micropylar collar and the remnant of its suspensor lies against the nucellar pad. All the cells of the embryo are
densely packed with a lipo-protein storage material and no starch is present (McGahan, 1961b). Recently, Graven et al., (1996) studied the structure and macromolecular composition of the seed coat of the Musaceae. They used different mass spectrophotometric techniques compared with scanning electron microscopy and light microscopy in combination with histochemical techniques. The macromolecular 'fingerprint' of the seed coats of the Musaceae showed unique components of aromatic phenols. Very remarkable are the separation of the outer cell walls from the exotestal layer, exposing a secondary surface with silica crystals, and relatively thick mesotesta which protects the seed e.g. against the biting forces and passage through the digestive tracts of dispersing agents. Germination takes place with an operculum and is facilitated by a predetermined rupture layer in the micropylar collar. The musaceaous seed presents a good example of resolving the conflicting demands of protection and germination.

The first visible sign of germination is the exudation from the micropyle of a drop of brownish fluid. The micropylar lid is then extruded by the elongating radicle - hypocotyle axis. The first conspicuous organ of seedling is the extrusion of the primary root, which may be several millimeters long. The primary roots springs from an irregularly swollen hypocotyl on which adventitious roots and the aerial shoots are differentiated (Simmonds, 1959; McGahan, 1961b).

A week after germination, the adventitious roots have outgrown the primary roots and the plumule then become prominent; in two to three weeks the primary root is hardly identifiable as such, the plant consisting in effect of a prominent shoot which bears at its base an adventitious root system and the remains of the seed. The first two leaves are represented by bladeless sheath; the third leaf, which is produced about two or three weeks after germination, bears an expanded blade. In about four weeks seedlings grown in sand show evident signs of malnutrition and are by then presumably dependent on the
roots for their mineral nutrients. All visible traces of endosperm disappear from the seed within two weeks of germination but the testa and haustorium persists for at least five weeks before they are detached or rot. The root system of the young seedlings is composed of slender, mostly descending, somewhat branched, adventitious roots. At about one month of age a new type of root suddenly appears and swiftly replaces the juvenile system the new type of root is markedly thicker, longer and less branched than the juvenile type. The new type of root appears without transition and is the first indication of the form that the mature root system is later to take (Simmonds, 1959).

2.5.3 Problems of Seed Viability

Simmonds (1952 a, b; 1959) elaborated the factors affecting the *Musa* seeds viability and germination. Seeds should be extracted from mature and ripe fruits, ripeness being particularly important if the fruits are at all immature. Seeds from the mature fruits should be sown at once or dried before sowing those from immature fruits are probably better not dried. If storage is necessary the sun - dried seeds can be kept in the laboratory for up to six months without any special treatment; for longer storage they must be kept in a desiccator. Storage of wet seeds or of seeds from which the damp pulp has not been removed is to be avoided. Pre-sowing treatments such as soaking in sulphuric acid, chipping of the testa, soaking in water, scorching and application of temperature shocks are either without effect or are deleterious and never improve the germination. In the greenhouse the most important factor seems to be freedom from excessive water hence light soils, well drained boxes and light watering are indicated. Depth of planting the seeds (from 0 to 3 cm) does not matter. Lime and artificial manures should be avoided.

Simmonds (1959) described different ecological aspects of *Musa* seed germination. He described these effects as conditions necessary for maintenance of viability in soil and the implication of experimental sterilization.
Seeds stored in the open air lost nearly all viability in a year; the same seeds stored in the soil retained a significant degree of viability during the same period of time and the same seeds sown in a seed box soon after harvest gave a high germination rate after about a month.

Evidently, there is some feature of the soil environment that tends to preserve viability of moist banana seeds. It was observed that carbon dioxide concentration is concerned in the problem. Crocker and Barton (1953) noted that moderate CO₂ concentrations inhibited the respiration of certain seeds and that various studies showed that carbon dioxide - oxygen relationships were probably responsible for the preservation of viability of various seeds in moist storage, conspicuously in soil. Therefore it seems reasonable to suppose that the preservation of viability of banana seeds in soil is at least partly dependent upon the composition of the soil atmosphere.

Sterilization of the seeds showed a variable effect on germination of different batches of seeds. Presumably three factors are involved; firstly, a direct adverse toxicity effect of the sterilizing agents on the viability; secondly, the killing of micro - organisms responsible for the encouragement of germination; and thirdly the killing of pathogenic micro - organisms. The first and second effects cannot yet be formally distinguished. However the fact that some sterilized samples gave high germination rates suggested that the direct toxicity effect is not serious, at least at moderate concentrations of mercuric chloride (Simmonds, 1959).

The normal fate of banana seeds are to be eaten by animals; in the field one very rarely sees a ripe or fallen bunch because the fingers are consumed as soon as they start to turn yellow. They are rarely attacked in the hard green condition and it may be that the astringency of the unripe fruit makes them unpalatable enough to deter animals from attacking them. However the banana seeds can remain dormant in soil for fairly long
periods certainly for one year and probably for longer. Presumably, majority of banana seeds dropped by animals remain in the soil surface, germinate and die. Seeds that survived in the soil in a viable state germinate when the site is disturbed i.e. newly disturbed soils followed by burning. Another example of a disturbance that provides buried seeds with suitable conditions for germination is landslip. The final result of any disturbance is commonly a more or less gregarious germination that reflects an intense seed rain in the past and the localized occurrence of conditions suitable for the preservation of viability (Simmonds, 1959).

Escalant and Teisson, (1987) observed that seed germination of diploids is highly dependent on the maturity of the fruits when harvested and the conditions to which the seeds are subjected before germination. They also reported that germination rate is significantly different between batches of seeds.

Vuylsteke and Swennen (1993), reported different causes of low seed germination in Musa. Firstly, the embryos were malformed, shriveled, and brownish. This was often observed in conjunction with the absence of endosperm and the seed coat being somewhat softer than normal. Secondly, embryos were missing, although the endosperm and chalazal mass were fully developed and the testa was of normal hardness.

Average seed set in crosses of plantain cultivars with "Calcutta 4" range between 0.3 to 21.7 seeds per bunch, depending on the cultivars (Vuylsteke and Swennen, 1993). However the variation in seed set is considerable, the cultivar 'Bobby Tannap' produced up to 219 seeds from a bunch whereas other pollinated 'Bobby Tannap' had produced no seeds (Swennen and Vuylsteke, 1991). This large fluctuation in seed number appears to be influenced by climate. Maximum seed set occurs in the dry season, characterized by high temperature, low relative humidity and the highest hours of sunshine. Seed set declines to a minimum level during the mid - rainy season, but then increases again to

35
second lower peak at the end of the rainy season. This complex interaction between seed set and climate is not clearly understood.

Bhat et al., (1994) reported that hard seed coat is the major barrier to water permeation thereby preventing the germination of whole seeds of *Musa balbisiana*. The adverse effect of chalazal mass and the favorable role of microbes in seed germination were also indicated. Graven et al., (1996) studied the structure and macromolecular composition of the seed coat of Musaceae. The macromolecular ‘fingerprint’ of the seed coats of Musaceae showed unique components of aromatic phenols, silica crystals, and relatively thick mesotesta which protects the seeds, e.g. against the binding forces and passage through the digestive tracts of dispersing agents. The seed coat could be the major barrier to water permeability.

2.5.3.1 Methods Used To Improve Seed Germination

2.5.3.2 Physical Factors

Several studies have been carried out to improve seed germination in *Musa*.

Stotzky et al., (1962), reported that scarification was required for germination and mechanical treatment appeared to be superior to chemical scarification in *Musa balbisiana* seeds. Various methods of mechanical scarification and cultural procedures were developed which facilitated the operation when large numbers of aseptic seedlings were required. Removing a chip from the lateral portion of the seed coat to expose the endosperm was the most effective way of scarification, germination percentages average 80% and the time required for germination in sterile culture was shortened from 3-6 weeks required for germination in soil to 6-10 days. However, scarification did not shorten the time required for germination in soil, and seeds treated with some methods of mechanical scarification failed to germinate, as a result of their decomposition by microorganisms. The effectiveness of scarification in causing germination in aseptic
culture is not presently understood but as the excised embryos exhibits no dormancy, the factors delaying germination apparently reside in other portion of the seed. Stotzky and Cox (1962) stated that alternating temperature treatment was required for the germination of *Musa balbisiana* seeds. The temperature differentials optimal for germination in soil are dependent upon both high and low temperatures, and ranges from 8 - 23°C. Alternating temperatures also required for germination of mechanically scarified seeds, although the temperature differentials are less than those necessary for intact seeds, indicating that the action of alternating temperatures is not on the permeability of integument.

### 2.5.3.3 Embryo Rescue Culture

The erratic and generally low germination levels of seeds from many *Musa* clones have rendered use of embryo culture methodologies very valuable. *In vitro* techniques have been in use for a long time (Cox *et al.*, 1960). *Musa balbisiana* embryos were cultured on a modified Knudson's medium or Randolph and Cox (1943) medium containing 0.12M sucrose (but without growth regulators) solidified with agar (0.5 - 0.7%) used to rear young plantlets form the tiny embryos excised from seeds.

The first sign of embryo germination was a change in coloration of the embryos from white to yellowish (Afele and De Langhe, 1991) or blackening of embryo tissues and medium within 4-14 days of culture (Vuylsteke *et al.*, 1990a). Embryos that remained creamy white during a month in culture never germinated. Escalant and Teisson (1987) made similar observations. By the fifth day of culture, the stalk like meristematic regions had become swollen, nearly globular in shape and larger than the hitherto larger haustorium. Greenish shoots and roots emerged after 7-11 days. Shoot emergence preceded by root emergence. Several studies indicated the effect of different factors affecting the embryo germination *in vitro*.
Vuylsteke and Swennen (1993) reported that low germination percentages were partly due to the fact that 30 - 70 % of mature plantain and cooking banana seeds showed one of the two following irregularities:

1. Embryo was malformed, shrivelled and brownish. This was often observed in conjunction with the absence of endosperm and the seed coat being somewhat softer than normal.

2. Embryo was missing although the endosperm and chalazal mass were fully developed and the testa was of normal hardness.

They also evaluated the effects of different sterilizing agents and time of exposure on the germination of embryos. Seeds were obtained from a cross between *Musa balbisiana* and *Musa acuminata ssp. burmannicoides*. Preliminary results indicated that decreasing NaOCl sterilization times did not improve the germination rate. Less infection occurred when HgCl₂ was used as the sterilizing agent, but this did not improve overall germination. Presoaking seeds in tap water for 3 days, followed by a soaking in either NaOCl or HgCl₂ treatment, resulted in 100% germination. Apparently, the low germination rates are not a consequence of embryo injury by hypochlorite.

Analysis of seed germination revealed a seasonal influence on embryo germination *in vitro* and thus on seed viability (Vuylsteke *et al.*, 1990a). Seeds of Bobby Tannap showed germination rates of 0.9% and 4.6% when harvested from bunches, pollinated during the dry season and the wet season respectively. In Obino I' Ewai, pollinated during the drier months resulted in 1.2% germination against 6.6% for pollination in months with high rainfall. Similarly in Bluggoe, the germination rate increased from 1.6% to 14.5%. There are 5 to 9 fold increases for bunches pollinated during August - October as compared to January - March. Embryo viability was above average in seeds harvested during November - January, i.e. bunches pollinated during
August - October (second half of the rainy season). Seed germination was low during April - July i.e. when pollination occurred in January - March (dry season). A seasonal fluctuation in the number of seeds per bunch was also established, (Swennen and Vuylsteke, 1989) with maximum seed set in bunches pollinated during February.

Afele and De Langhe (1991) studied the effects of different in vitro conditions on Musa balbisiana embryo germination and growth. Seeds were soaked in water for five days prior to the excision of the embryos. Soaking seeds for 5 days was reported to be better than 3 or 9 days. Embryos derived from soaked seeds germinated faster than the unsoaked ones. Similarly, embryos from soaked seeds produced shoots and roots earlier than the unsoaked seeds. Embryos from unsoaked seeds produced the shortest shoot length, average length of roots and the total root length, followed by embryos from seeds soaked for 3, 9 and 5 days. They have also studied the effect of different embryo orientations on the germination of M. balbisiana embryos. Embryos with their longitudinal axis laid flat and halfway embedded on agar - solidified medium produced the highest germination rate and the most desirable plantlet characteristics. Germination in vitro was 94% within 7 days compared to 50% after 54 days for greenhouse - sown seeds.

2.6 Fusarium Wilt Disease

2.6.1 History, Origin and Distribution of Fusarium

Panama disease or Fusarium vascular wilt disease is widespread and highly destructive. The disease is determined by three major interacting variables, namely the genetical constitution of the host plant, the pathogenic properties of the different strains of the fungus, and the effects of environmental factors both on the host and pathogen. The fungal pathogen Fusarium oxysporum f. sp. cubense (FOC) was first described by
Schlechtendahl in 1824 (Booth, 1982). In 1913 when Wollenweber established the section Elegans as one of the six sections described for the genus, *Fusarium oxysporum* f. sp. *cubense* was one of nine species described in that section (Booth, 1971). Snyder and Hansen in 1940 reduced the section Elegans to only one species, namely *Fusarium oxysporum*. The first report of the disease caused by *Fusarium oxysporum* f. sp. *cubense* in banana plants came from Bancroft in Australia in 1876 (Pegg et al., 1996). It was next reported in banana plantations in Central America in 1890. From 1890 to the mid of 1950's some 40000 h of the banana cultivar Gros Michel in Central and South America were destroyed or abandoned because of the disease (Pegg et al., 1996). The first formal description of the pathogen by Erwin F. Smith in 1910 who used the name *Fusarium oxysporum* f. sp. *cubense* (Subramanian, 1971) after he discovered the pathogen from host tissue sent from Cuba (Pegg et al., 1996). Brandes (1919) demonstrated the first detailed and conclusive descriptions of pathogenicity. Snyder and Hansen (1940) proposed the name *Fusarium oxysporum* Schelcht f. sp. *cubense*.

The disease became known as Panama disease because it was first epidemic in the Central American country of Panama. The name, Panama disease was initially used in the literature and the more descriptive term, banana wilt was first used in Jamaica in 1915 (Stover, 1962). Since *Fusarium oxysporum* f. sp. *cubense* consists of many strains and a wide range of banana clones are affected, the name Fusarium wilt was more appropriate and in agreement with the use of Fusarium wilt for other crop diseases caused by *Fusarium oxysporum* (Pegg et al., 1996).

The different races of *Fusarium oxysporum* f. sp.cubense gradually appeared in banana growing regions of the world. Gros Michel the major dessert banana cultivar grown in Central America, was devastated by Race 1 and this had lead to the introduction of Cavendish clones resistant to Race 1. Race 2 attacks Bluggoe (ABB) while Race 3 was
pathogenic to the related species of Heliconia (Musaceae) (Thomas et al., 1994).
Recently, a Race 4 has been found to attack clones of the Cavendish subgroup (Ploetz,
1990).

Some authors believed that the Fusarium wilt pathogen has probably evolved in
antiquity on several susceptible varieties of edible bananas in the Indo-Malayan area
(Stover, 1962). Recent studies on the genetic diversity of the fungus, indicated that it
most likely originated in South East Asia (Ploetz and Pegg, 1997). Others believe that it
may have evolved independently in the Western hemisphere (Stover, 1962).

The wilt disease has been reported in all banana growing regions of the world
except in Papua New Guinea, the South Pacific Islands and countries bordering the
Mediterranean (Bentley et al., 1995). In areas where bananas are not grown, there is some
evidence of the Fusarium oxysporum f.sp. cubense which could be isolated from the roots
of the weeds. By growing selected weed species in a soil inoculated with the pathogen, it
has been showed that Fusarium oxysporum f.sp. cubense could survive via the roots of
weeds externally in most and systemically in others (Epp, 1987). The distribution of the
race attacking the Cavendish cultivars, though more aggressive, is less than that of Race
1. Originally Race 4 has been reported from subtropical countries of South Asia,
Australia and Taiwan (Stover, 1990). Subsequently, it was found attacking Cavendish in
areas in the equatorial climate such as Mindanao of the Philippines (Buddenhagen, 1987),
Indonesia and Malaysia (Pegg et al., 1996) Although Cavendish clones growing in the
tropical areas of Latin America - Caribbean region succumbs only occasionally, the world
export industries may once again be threatened by Fusarium wilt. In the Philippines, there
are some indications of the existence of a race attacking Cavendish, slightly different
from Race 4. When Fusarium oxysporum f. sp. cubense from Philippine were grown on
modified Komada medium (Sun et al., 1978) no laciniated colonies developed. All
isolates grew slowly with appressed mycelia with very sparse sporulation. This would indicate that the Philippine isolate was not Race 4. However by the definition of Race 4 as suggested by Stover and Simmonds (1987), the Philippine race would need to be considered as Race 4 because it infects Cavendish (Epp, 1987).

In Malaysia, a commercial farm planted with cv. Grand Naine derived from meristem culture on agricultural land with no prior report of banana cultivation. Fusarium wilt reported initial infection within its first year of cultivation and was devastated 4-5 years after its establishment. Isolates cultured on K2 medium formed laciniate colonies similar to Race 4 isolates from Taiwan (Ong, 1995).

2.6.2 Pathogen Variability

*Fusarium oxysporum* is normally a soil-living fungus with both saprophytic and plant pathogenic strains. Although no perfect sexual stage has yet been discovered for this species, it is highly variable both phenotypically and by its ability to cause plant diseases. The phytopathogenic forms are principally responsible for host-specific vascular wilts that are designated as *formae specialis*, of which more than 100 can be differentiated (Gerlach & Nirenberg, 1982). The specialized forms that attack bananas (and the *Heliconia* spp. from the plant order Zingiberales) is identified as *Fusarium oxysporum* f. sp. *cubense* (Stover, 1962). However within the banana *formae specialis*, there exists another specialized subgroup called pathogenic races differentiated by their ability to infect a selected range of differential banana clones. Four races are currently recognized (Ploetz, 1990). Race 1 has a worldwide distribution and caused the displacement of Gros Michel with the resistant Cavendish group of bananas, which predominates the international trade. Race 2 attacks Bluggoe and other cooking bananas while Race 3 infects *Heliconia* spp., which is botanically related to the banana. Sun *et al.*, (1978)
reported that Cavendish clones are attacked by *Fusarium* in Taiwan and was designated by them as Race 4.

Genetic variation within a non-sexually reproducing fungal population can be studied using suitable genetic markers such as vegetative compatibility. The grouping depends on the ability of fungal hyphae from strain with similar compatibility genes to anastomose and fuse together to form heterokaryons. Using complementation matings between nitrate non-utilizing mutants, isolates can be categorized into similar genetic classes called Vegetative Compatibility Groups or VCG's. (Puhalla, 1985; Correll *et al.*, 1987). Until recently 21 VCG's or VCG Complexes have been established (Ploetz and Pegg, 1997; Ploetz *et al.*, 1997).

Vegetative compatibility analysis of isolates from Malaysia confirmed the existence of VCG's 0123, 0125, 01216, 01217 01218 and VCG complex 0120 - 01215, (Pegg *et al.*, 1993; Ploetz *et al.*, 1997).

**2.6.3 Screening of *Musa* For *Fusarium* Wilt**

The pathogen produces abundant resistant chlamydomospores in root tissues of infected plants. Soil populations are unevenly distributed. A normally root-inhabiting facultative parasite, the fungus can invade roots of other plants and thus the pathogen can survive in infested soils for several years. Infected suckers used to be the primary means of dispersal, but the inoculum is easily moved about by transfer of adhering soil by shoes and machinery, contaminated desuckering and harvesting knives and surface irrigation and drainage water (Stover, 1962; Jeger *et al.*, 1995). Screening for resistance required procedure capable of screening large populations efficiently and cheaply. There are two basic systems of screening:

(1) Rhizomes planted in the field with or without artificial inoculation.
(2) Seedlings, small rhizomes, or in vitro meristem plants planted in containers with artificial inoculations.

Field evaluation for banana plants for disease tolerance in soil infested with *Fusarium oxysporum* f. sp. *cubense* had been found to be effective and reliable. Field evaluation although, the most reliable method for disease resistance screening, is demanding in terms of cost, manpower and space requirement (Pegg *et al*., 1996). There is also need to maintain strict quarantine control to avoid pathogen spread. In addition plants tend to show symptoms only after 4-5 months (Morpurgo *et al*., 1994). The uneven distribution of pathogen in the field can also lead to "disease escape" with many variables that can affect infection and symptom expression. Simmonds (1966) reported that in Jamaica, tetraploids were screened, by planting at sites where diseased Gros Michel plants were removed. Whereas in Honduras, rhizomes of the tetraploid clones to be tested are planted in holes in which chopped tissue from plants infected with Races 1 and 2 has been added. It requires 18-24 months to evaluate disease reaction (Rowe and Richardson, 1975).

A mass screening of the wild banana seed progenies in flats was reported by Vakili (1965) and Shepherd and Lacy (1968). They found great genetic variability in differential wilt resistance to Races 1 and 2 in *Musa acuminata* subspecies *errans*, *banksii*, and *microcarpa* and among hybrids.

Sun and Su (1984) reported a rapid method for the determination of differential pathogenicity of *Fusarium oxysporum* f. sp. *cubense* using tissue culture banana plantlets. They described that when Cavendish and Cocos plantlets derived from tissue culture were inoculated with Race 1 and Race 4 of FOC, by immersing roots in a spore suspension for 1 minute, susceptible plantlets showed external symptoms of leaf yellowing within two
weeks and wilted within four weeks of inoculation. Results of pathogenecity tests with banana plantlets agree with those of mature banana plants, which take an average of nine months for the expression of external symptoms.

Buddenhagen (1987) and Pegg et al., (1996) expressed the need for improved methods with small plant tests not only screening for resistance but also for comparative virulence and pathogenecity studies.

Epp (1987) experimentally determined that two month old seedlings of *Musa balbisiana* and meriplants with 6-8 leaves gave reproducible results that were very similar to field response. Brake et al., (1995) studied the effect of a number of factors on disease reaction of banana cv. Cavendish. Cavendish banana plants grown in the soil infested with different levels of microconidia of Races 1 and 4 of FOC were maintained at temperature 20°C and 28°C in growth cabinets. Race 1, which does not normally infect Cavendish in the field, infected the plants at both temperatures, as did Race 4. Results indicated that temperature was primarily affecting plant growth rather than influencing the aggressiveness of the pathogen. Different inoculum levels were also found to influence disease severity with a minimum of $4.5 \times 10^4$ microconidia/g dry weight of soil required before macro-symptoms were observed.

Double cup sand - culture containment method was developed earlier for laboratory testing of pathogen virulence (Liew, 1996). Recently, a "double compartment" apparatus made up of two plastic trays, one fitting inside other, replaced the double cup. This double tray technique has the capacity for pathogen containment so as to eliminate cross contamination. It is also amenable to modifications to allow investigations into the effects of variable inoculum concentrations and environment variables on infection and disease expression. Bigger size of the tray also allows to more plants to be screened.
Hence this is a method of choice for large-scale banana screening (Mohamed et al., 1999).

Morpurgo et al., (1994) described the in vitro screening of plantlets derived from somaclonal variation, in the presence of non-host-specific toxin i.e. fusaric acid and fungal crude filtrate. However no clear linkage between in vivo and in vitro behavior was observed and their results suggested that the use of crude filtrate or non-host specific toxin (Fusaric acid) in a screening program for selecting a novel resistant genotype of Musa to FOC is not feasible. Hence the toxin in vitro screening procedure was found to be not reliable, and pathogenecity testing and screening has to depend on greenhouse testing of tissue culture derived plantlets that require at least several weeks for confirmation (Sun and Su, 1984).

2.6.4 Fusarium Resistance Mechanism

For Fusarium wilt on bananas, the interaction between the pathogen and host is complex. Wardlaw (1930a) began the work to define ways in which FOC and banana interact and to show the formation of tyloses in banana in response to vascular colonization by this fungus. Although much remains to be learned about these processes, a good basic understanding of the steps leading from root colonization to death of susceptible cultivars, or defense of a resistant plant, is now available (Beckman 1987,1989,1990). Resistance and susceptibility are ultimately determined by a series of chemical and physical events that occur in the xylem (Pegg, 1985; Beckman, 1987). Resistant hosts effectively wall off the invading parasite by forming gels, gums, tyloses and other xylem-containing products (Vander Molen et al., 1977a, b). In turn, a successful pathogen breaches host defenses by virtue of, among other things, an array of hydrolytic enzymes that degrade host cell walls and reaction products (Pegg, 1985).
Phenolic compounds that impregnate host reaction products are thought to play an important role in the resistance process, either making physical barriers stronger or chemically impervious to the previously mentioned hydrolytic enzymes of the pathogen (MacHardy and Beckman, 1981; Pegg, 1985; Beckman, 1987). Enzymes that are instrumental in the formation of these compounds had been studied in many different hosts, including banana (Mace and Wilson, 1964; Mace et al., 1972; Mueller and Beckman, 1978). Phenol-oxidizing enzymes such as peroxidase, tyrosinase and laccase, are associated with many different vascular diseases (Pegg, 1985). In general, these enzymes are inducible. Their role in resistance may be a timing phenomenon; that is hosts quickly produce sufficient levels of the important products in response to the pathogen thereby conferring resistance to disease development.

Peroxidase and polyphenol oxidases are stored, preformed, in various, localized sites in banana (Mace et al., 1972; Mueller and Beckman, 1978). Different isoforms of the enzymes (isozymes) and the quantities produced are known to differ among different banana genotypes (Jarret and Litz, 1986 a, b) and since, the levels and number of peroxidase isoymes produced are greatest in roots of banana and other hosts, it has been postulated that they may help to protect plant against infection by root pathogens (Bonner et al., 1974; Lagrini and Rothstein, 1978). The involvement of these enzymes in the resistance process and the considerable enzymatic variation that exists among different banana genotypes indicate that enzyme assays could help to characterize and identify Fusarium wilt resistance. If specific forms or relatively high quantities of these enzymes are correlated with resistance in the host, it is conceivable that products of breeding programs or pre-existing clones could be quickly and effectively evaluated via enzyme analyses, without resort to conventional disease screening trials. Recently, biochemical basis of Fusarium wilt tolerance in banana reported by Ana and Dubery (2000). For
tolerant cv. Goldfinger and susceptible cv. Williams, defense responses were induced by the treatment of the plants with an elicitor preparation from the mycelial cell walls of the pathogen. Root tissue of the tolerant cv. Goldfinger responded to the fungal elicitor through the strong deposition of lignin then preceded by the induction or activation of enzymatic activities involved in the synthesis and polymerization thereof, whereas only slight increases were observed for the susceptible cv. Williams. Their studies indicated the important role for cell wall strengthening due to the deposition of lignin as an inducible defense mechanism of banana roots against *Fusarium oxysporum* f. sp. *cubense* Race 4.

Larter (1947) suggested that immunity to Panama wilt was under the control of a dominant gene in a tetraploid offspring obtained by crossing the susceptible “Gros Michel” with a diploid accession. However segregation in progenies derived from crosses between three susceptible *Musa* species and the resistant cultivated diploid banana “Pisang Lilin” suggested the presence of a single dominant factor for resistance to Race 1 (Vakili, 1965), and the resistance to Race 4 seems to be under polygenic control (Rowe and Rosales, 1993). If we assume that resistance to different races of Fusarium wilt, in other banana genotypes is controlled by a single gene, it should be possible to identify markers for resistance in future (Gonzales de Leon and Faure, 1992).

2.7 Exploitation of Wild *Musa* Species For Genetic Improvement and Disease Resistance

2.7.1 Genetic Studies on Disease Resistance

Genetic studies in the genus have lagged behind those conducted with far less important crops. The paucity of the available data can be attributed to the sterile nature of the triploid clones, the inaccessibility and lack of interest in fertile wild diploid bananas.
A number of studies had been reported where wild banana species were used for screening to viruses, bacterial and fungal pathogens. Magee (1927) reported \textit{M. textilis}, \textit{M. acuminata ssp. banksii} and \textit{M. ensete} as the host for banana bunchy top virus (BBTV) whereas \textit{M. acuminata} and \textit{M. balbisiana} showed a very high resistance to BBTV (Vakili, 1969a). Waite (1960) based on his field observation did not find CMV virus symptoms on mature \textit{M. balbisiana}. However \textit{M. balbisiana} seedlings showed whitish chlorotic mottling characteristics of CMV virus infection even though virus symptoms are not seen on mature plants. Buddenhagen (1962) and Vakili (1965) studied different wild \textit{Musa} species for reaction to bacterial wilt. \textit{M. schizocarpa}, \textit{M. angustigemma}, \textit{M. acuminata ssp. microcarpa} and \textit{M. acuminata ssp. errans} were found to be attacked by tomato bacterial strain of \textit{P. solanacearum}. Vakili (1965) reported that the resistance was dominant in \textit{M. acuminata ssp. banksii} whereas susceptibility was dominant in \textit{M. acuminata ssp. microcarpa}. Vakili (1965) observed Fusarium wilt resistance against Races 1 and 2 in seedlings of \textit{M. acuminata} subspecies \textit{errans, banksii, and microcarpa}. \textit{M. balbisiana} was reported to be the only species tested that displayed seedling susceptibility and mature plant resistance to Fusarium wilt. Stover (1963) reported the damping-off (\textit{Deightonella torulosa}) of seedlings of \textit{M. balbisiana}, \textit{M. acuminata ssp. banksii}, \textit{M. schizocarpa}, \textit{M. textilis} and hybrids from crosses of \textit{M. acuminata} and edible banana varieties. Kaiser and Lukezic (1965) found selections of \textit{M. acuminata ssp. malaccensis} and \textit{microcarpa} with brown spots on the fruits caused by \textit{Cercospora hayi} whereas \textit{M. balbisiana} was resistant to the pathogen. Vakili (1968) studied modes of inheritance of resistance to Sigatoka (\textit{Mycosphaerella musicola}). Segregation of F1 progenies from crosses between susceptible \textit{M. acuminata ssp. banksii} and partially resistant \textit{M. acuminata ssp. errans} or resistant \textit{M. acuminata ssp. microcarpa} indicated that multiple genes conditioned resistance to the pathogen. Resistance was readily
incorporated into tetraploid varieties produced by crossing resistant male diploid pollen parents with susceptible dwarf mutant of Gros Michel. Resistance was however variable, with various levels of spotting develop on the older leaves when conditions were highly favorable for infection.

Wild diploid fully seeded *Musa acuminata* ssp. *malaccensis* from Malaya were the principal sources of resistance to Sigatoka leaf spot used in the breeding efforts. With the advent of the more virulent form of black Sigatoka pathogen (*Mycosphaerella fijiensis var. difformis*) in Central and South America, the *M. acuminata* ssp. *malaccensis* accessions are still highly resistant to the black Sigatoka fungus. Several dominant genes control the resistance in *M. acuminata* ssp. *malaccensis*. Similarly seed progenies derived from *M. acuminata* ssp. *burmannica* accessions showed high levels of resistance to black Sigatoka (Rowe, 1984).

These studies reported that there is a great amount of variability present in the wild bananas resistant to different pathogens. This provides opportunity for hybridization to generate different triploid subgroups as well as other hybrids between diploid (*M. acuminata*) A - genome and (*M. balbisiana*) B-genome.

Selection and evaluation of naturally occurring variants from the centers of diversity is an important approach for banana improvement. Field and molecular characterization of 200 diploid bananas for their potential as parents in breeding were initiated by CIRAD (Center of International Development of Cooperation Agronomic Research) in Guadeloupe.

The importance of the diploid cultivars as a basic source in breeding program was recognized by the International Network for the Improvement of Banana and Plantains (INIBAP) which organized and co-ordinated global research efforts on banana and plantains. Their main activities include collecting, conservation, characterization, and
evaluation of *M. germplasm*, studies on banana diseases and development of a *Musa* germplasm Information System (MGIS) (Suzanne and Jan, 1996).

The Malaysian Agriculture Research and Development Institute (MARDI), had documented over 200 accessions of cultivated bananas from all possible types in *M. acuminata* and *M. balbisiana* groups using the recommended IBPGR descriptors for banana. However only 5% are commercially utilized (Siti Hawa, 1998).

As it is necessary to attempt many crosses to get one with good characteristics introgression through conventional breeding, DNA marker assisted selection and genetic engineering should be used to develop improved cultivars.

### 2.7.2 Molecular Markers

Modern crop improvement utilizes molecular marker assisted selection and introgression of agronomic traits of interest such as pest resistance or quality. *Musa* improvement has been slow due to problems associated with polyploidy, sterility and parthenocarpy. The identification of molecular markers linked to genes enhancing yield, particularly those with large effects will allow *Musa* breeders to select parents based on their genotype rather than phenotype.

However marker assisted selection demands the linkage information. There is no conclusive linkage map currently available in *Musa*. The generation of highly relevant and precise linkage map is not routinely achievable in the current breeding populations due to the triploid nature of the crop. The other problems are related to the variability of the chromosome structure such as translocations at the diploid *acuminata* level are responsible for irregularities during meiosis. Retrotransposon activity and/or methylation may be responsible for the creation of off-types during *in vitro* propagation.

Recently, a partial linkage map of *Musa* was made available (Gonzales and Faure 1992; Lagoda *et al.*, 1999). For the production of more dense linkage maps a number of
segregating populations are needed. The two classical schemes adapted for the construction of linkage maps are:

(1) Crossing a diploid usually with a fertile wild banana showing some desirable traits with a good triploid recipient cultivar having good female restitution. However this approach has intrinsic genetic constraints; insufficient knowledge about the genomic origin and compositions of the best triploid cultivars complicate the choice of best diploid materials for desirable introgressions.

(2) The second scheme of crossing between diploids of both cultivated clones and wild banana accessions has become increasingly important (Bakry et al., 1990; Rowe, 1987).

The wild *Musa acuminata* species, being fertile, seed bearing with normal meiosis and many of them are resistant to several pathogens (Vakili, 1965), are good choices for the production of improved diploids. The linkage map currently available uses different wild *Musa* species namely, *M. acuminata* ssp. *malaccensis* (Kedah type), and *banksii* (Samoa type), (Lagoda et al., 1999).

However the current genetic map lacks co-dominant locus specific information and polymorphic markers. For a better understanding of different genetic traits, it is imperative to look for sources with desirable traits, and then produce segregating populations. The construction of chromosomal maps in combination with genetic maps will result in integrated maps, which are highly desirable to study the genome structures and to clone genes of interest. An essential step in the development of such integrated maps is the accumulation of sufficient numbers of molecular and cytogenetic markers.

2.7.3 Ploidy Breeding

The absence of improved hybrids has been attributed to difficulties associated with breeding the *Musa* crop at the genetic and practical levels. Genetic improvement was severely hampered by a lack of useful genetic variability and low levels of female fertility
in cultivars. The commercial *Musa* cultivars are mainly triploid and triploidy leads to problems during meiosis due to uneven segregation of the number of chromosomes. Consequently, *Musa* species give low seed set and poor seed germination. Plant multiplication is mainly by vegetative means, which is inherently slow. At least 2 years are required to complete a seed-to-seed crop cycle. Very few viable seedlings are obtained from extensive number of crosses, and large amount of space is needed for the evaluation of seed progenies.

In view of these constraints, the production of tetraploid hybrids is considered an important breakthrough. Genetic improvement and heterogeneity could be introduced in the breeding programs of triploid population by increasing the ploidy to tetraploid level and then returning crop to a non-seed bearing triploid for production (Gowen 1995; Stover and Simmonds, 1987).

A breeding scheme involving mass production of tetraploids from improved diploids through seed treatment with colchicine was proposed by Vakili (1967). Until recently, little or no work was done on the production of autotetraploids from vegetative material. Hamill *et al.*, (1992) recovered tetraploids from a diploid banana clone SH – 3362 after treating the shoot tips with colchicine. Although these polyploids were weak, produced few suckers and seemed to be even more cold susceptible than the diploid clone SH – 3362, however they maintained their level of resistance to Race 4 Fusarium wilt.

In addition to an effective system for induction of polyploidy, mass production of autotetraploids requires an effective method for ploidy screening. Classically, this is done by chromosome counting (Hamill *et al.*, 1992). However, this procedure is not suitable for mass screening. Ploidy estimation based on stomata number, length and density (Speckmann *et al.*, 1965; Sreenivasan *et al.*, 1992; Blanke *et al.*, 1994), and recently Tenkouano *et al.*, (1998) reported easier ploidy determination based on pollen and
chloroplast characteristics are more amenable. However, the method is not always reliable due to environmental effects. Flow cytometric analysis of nuclear DNA content is being increasingly used for large-scale ploidy screening (De Laat et al., 1987; Dolezel et al., 1989; Dolezel, 1991). Van Duren et al. (1996) reported the screening of ploidy levels in a large population of in vitro induced autotetraploids of Musa acuminata through flow cytometry and stomata characteristics. They found that tetraploids identified by flow cytometry remained solid and non-chimeric during two more cycles when compared to a rough pre-selection of regenerated plants based on their stomata characteristics, which resulted in a population where only 56.2% of the plants were solid tetraploids. Azhar (2000) reported successful induction of tetraploids in Pisang Mas (AA). He reported a high tetraploid frequency at 0.5% colchicine concentration compared to 0.25%, 0.75% and 1.0% colchicine concentrations. The solid tetraploids were screened through flow cytometry and further confirmation was made through chromosomal counting.