15 Banana

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15.1 Introduction

Genomics is a rapidly emerging field of research, which came into existence at the end of the last century and promises to become the dominant theme of intellectual activity in the coming decades, revolutionizing our understanding of biology as never before (Maheshwari et al. 2001). It took approximately 100 years from the rediscovery of Mendel's work to the complete sequencing of a higher plant (*Arabidopsis*). The advent of genomics is the direct result of the development DNA sequencing by Sanger et al. (1977) and Maxam and Gilbert (1977). However, genome-wide sequencing could not have proceeded without extensive automation such as high throughput sequencing and the innovation of methods such as fluorescent primers, laser excitation of DNA bands, and the detection of these bands with photomultipliers (Smith et al. 1985, 1986). DNA sequencing makes it possible to decipher the entire blueprint of the development of an organism, its genes and their functions. Genomics can play a vital role in agriculture. The world's population depends to a very large extent on a few crops like rice, wheat, maize, bananas, beans and potato for their food. Understanding the genomics of these crops can lead to enhanced yields and survival under adverse conditions. In the next 20 years the world faces the tremendous challenge of feeding the global population from rapidly diminishing resources. The study of plant genomics helps reveal the alleles and biochemical pathways linked to many processes such as flowering, nutrition, disease and pest resistance, as well as their tolerance to abiotic stresses (Zandstra 2005).

Despite the importance of *Musa* as a food crop for over half a billion people worldwide, genomic research currently undertaken can best be described as a patchwork of initiatives undertaken by a few advanced laboratories. Genetic and physical maps of *Musa* are being developed by CIRAD (Centre de Cooperation Internationale en Recherché Agronomique pour le Developpement), France, and the Institute of Experimental Botany, Czech Republic. The BAC (bacterial artificial chromosomes), BIBAC (binary bacterial artificial chromosomes) libraries and ESTs (expressed sequence tags) have been developed by laboratories in Mexico, France and Europe (Vilarinhos et al. 2003; Ortiz-Vaquez et al. 2005). Segregating populations are being developed by the International Institute of Tropical Agriculture (IITA), Nigeria and Centre Africain de Recherches sur Bananiers et Plantains (CARBAP), Cameroon and CIRAD. The Queensland University of Technology (QUT) in Australia is identifying genes for resistance to *Fusarium* wilt. Genetic transformation of bananas is routinely being done at the Katholieke Universiteit Leuven (KUL), QUT and IITA. Sequencing of BAC clones is being conducted at KUL and The Institute for Genomics Research (TIGR). Activities for discovery of functional genes in *Musa* are being undertaken in Israel (Khayat 2004). This chapter provides an overview of banana breeding and genome analysis of *Musa*.

15.1.1 Botanical Origin and Distribution of Banana

Bananas and plantains (*Musa* spp. L), hereafter referred to as bananas are perennial monocotyledonous herbs that grow well in humid tropical and subtropical regions. There is a wide variety of historic references to bananas and the crop is mentioned in ancient Hindu, Chinese, Greek and Roman texts. The earliest reference to banana dates back to about 500 BC. Some horticulturists suspect that banana was the earth's first fruit. Nevertheless, the origin of bananas is traced back to Southeast Asia in the jungles of Malaysia, Indonesia or Philippines (Simmonds 1966, 1987). Banana originated from two wild diploid ($2n = 22$) species namely, *Musa acuminata* Colla and *M. balbisiana* Colla, with genomic compositions of AA and

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BB, respectively (Cheesman 1948). *Musa accuminata* is a native of the Malay Peninsula and adjacent regions while *M. balbisiana* is found in India eastwards to the tropical Pacific (Simmonds 1966). Many wild varieties still exist in the natural vegetation of Southeast Asia, the center of origin. Although South East Asia and the Western Pacific are believed to be the primary center of origin and domestication of edible bananas (Simmonds 1962; Robinson 1996; Jones 2000), they are also widely distributed in the humid tropical and subtropical world. From Asia, bananas and plantains are believed to have spread throughout the humid tropics (Swennen and Ortiz 1997; Valmayor 2000) solely by humans through suckers (Simmonds 1962). The history of banana cultivation is therefore closely linked to the early movement of human populations. Movement eastwards resulted in the development of a distinct group of AAB bananas, which are cultivated throughout the Pacific Islands. In Africa, banana is thought to have been introduced by Arab traders from India, through Madagascar on the Eastern Coast during the 15th century (Simmonds 1962). The crop was then moved inwards by local migrants and later, from Africa it spread to other parts of the tropical and subtropical world. A great diversity of bananas and plantains now exist in sub-Saharan Africa with various types cultivated in different eco-regions (Swennen and Vuylsteke 1991). The AAB plantains dominate the humid lowlands of West and Central Africa while the AAA cooking and beer bananas prevail in the East African Highlands. The latter two eco-regions harbor the world's greatest diversity of plantains and highland bananas, respectively, and are thus considered the secondary centers of diversification of plantain and bananas (Swennen 1990). The secondary centers of diversification are believed to have enriched the diversity of *Musa* with about 100 clones each (Lescot 2000).

15.1.2 Taxonomy of *Musa*

Banana belongs to the genus *Musa* in the family *Musaceae*, order Zingiberales. It belongs to the subclass *Zingiberidae*, Class *Liliopsida* and Division *Magnoliophyta*. The family *Musaceae* comprises two genera viz., *Musa* and *Ensete* (Fig. 1). The genus *Ensete* consists of monocarpic herbs none of which bears edible fruit. The genus was formally recognized by Cheesman in 1948 comprising 25 species. However, only eight species are now recognized in the genus *Ensete* (Novak 1992). *Ensete* is cultivated in Southern Ethiopia as a major source of food that is obtained from the pseudostem and rhizome (Novak 1992). Only two species, *E. ventricosum* and *E. edule,* are of economic importance as food and fiber crops (Bezuneh and Feleke 1966). Genetic relationships between *Musa* species and *Ensete* clones based on genome size, number of chromosomes and number of 45S rDNA loci showed that *Ensete* is related to *M. beccarii* of section *Callimusa* (Bartos et al. 2005).

The genus *Musa* comprises all the edible bananas and plantains with over 50 species. Five sections namely, *Australimusa, Callimusa, Rhodochlamys, Eumusa* and *Ingentimusa* exist in the genus *Musa* (Stover and Simmonds 1987; Purseglove 1988). These sections vary in the basic chromosomes, i.e. species of *Callimusa* and *Australimusa* have a basic chromosome number of $x = 10$, while species in *Eumusa* and *Rhodochlamys* have a basic chromosome number of $x = 11$. *Ingentimusa* has a single species *M. ingens* with a chromosome number of $2n = 14$. *Musa ingens,* a highland banana in Papua New Guinea, is the largest known herb (Argent 1976). Sections *Callimusa* and *Rhodochlamys* consist of non-parthenocarpic species with no nutritional value and are only important as ornamental crops. *Australimusa* consists of parthenocarpic edible types, collectively known as Fe'i cultivars. The Fe'i bananas have erect fruit bunches and red sap which differentiates this section from other cultivated bananas. Bananas in this section are not only important for food and fiber but their pseudostems also produce a valuable dark red dye which is used in various ways. The origin of Fe'i is controversial among banana authors. Simmonds (1966) suggested that *M. maclayi* is the most likely ancestor, while Cheesman (1950) suggested that Fe'i is closely related to *M. lolodensis*. RFLP analysis by Jarret et al. (1992) revealed that Fe'i was indeed closest to *M. lolodensis* and thus concurred with Cheesman's (1950) findings. *Eumusa* is the largest, most widely distributed, highly diversified and the most important section to which all edible bananas belong. Most cultivars in this section are derived from two species, *Musa acuminata* (A genome) and *M. balbisiana* (B genome). *Musa acuminata* is the most widespread of the *Eumusa* species being found throughout the range of the section with Malaysia (Simmonds 1962) or Indonesia (Nasution 1991; Horry et al. 1997) as the center of diversity.

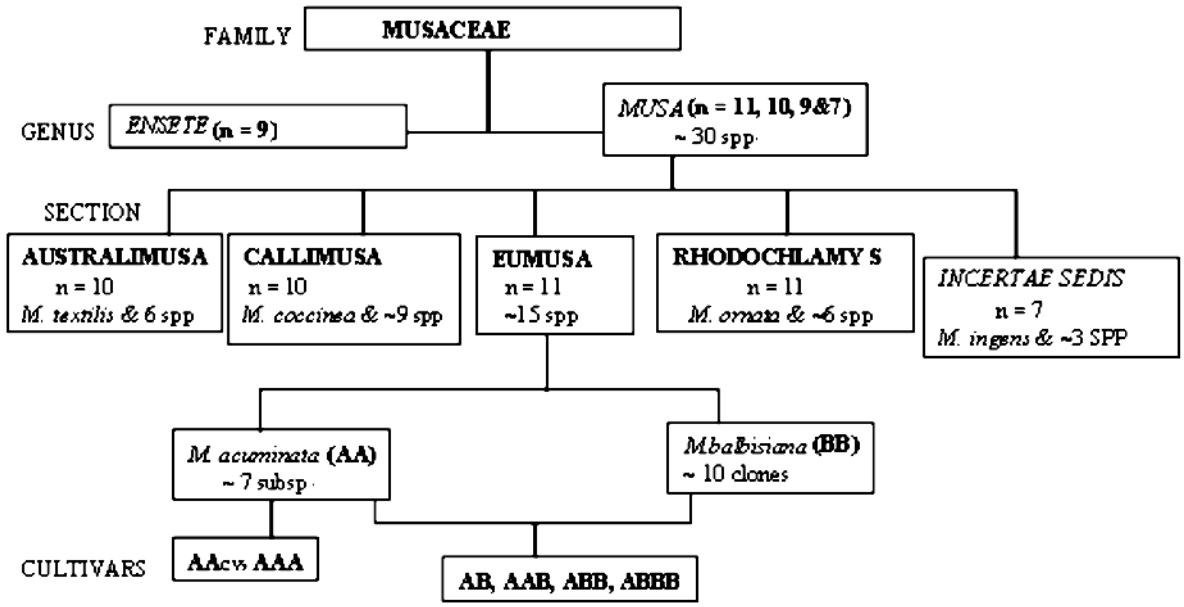

Fig. 1. Classification of Family *Musaceae* showing sectional treatment of the genus *Musa*. Based on Cheesman (1948); Simmonds (1962); Simmonds and Shepherd (1955)

Edibility and subsequent domestication of diploid *M. acuminata* (AA) came about as a result of female sterility and parthenocarpy. Triploid AAA cultivars arose from diploids, perhaps, following crosses between edible diploids and wild *M. acuminata* subspecies, giving rise to a wide range of diverse AAA genotypes such as the AAA dessert bananas and the AAA East African Highland bananas. These two AAA groups of bananas differ markedly in their fruit characteristics with regard to starch content and taste. This suggests that the A genomes of the different subspecies of *Musa* are different from each other. In most parts of South East Asia triploids have replaced the original AA diploids due to their larger fruit and vigorous growth. However in Papua New Guinea, AA diploids remain agriculturally significant and a wide diversity is still found in cultivation.

15.1.3 Botanical Description and Morphology of Banana

East African Highland beer and cooking bananas grow best at altitudes between 1,200 and 1,800 meters above sea level, while dessert bananas and plantains thrive well in the lowlands. Bananas grow well in deep loamy and well-drained soils. The optimum temperature for most cultivated bananas is 26–30 °C (Stover and Simmonds 1987). Temperatures lower than the optimum result in low leaf production, thus limiting the supply of food from the limited photosynthetic leaf area. Banana growth stops at temperatures beyond 38 °C and dies at temperatures below 0 °C. A relative humidity of 60–100% is necessary for proper banana growth, and depending on the evapotranspiration, 25–75 mm of water is required by a banana plant per week which is equivalent to 100 mm rainfall per month. Bananas are prone to wind damage because of weak pseudostems, large leaves that trap wind and shallow root system.

A banana plant is a giant perennial herb with a height of 1.5 to 9 m. It consists of a true stem called corm with roots and a false stem (pseudostem) consisting of leaf sheaths. At maturity, the leaves surround the "heart" that carries the bunch with fruits. The stem (corm) is usually underground and its shape is cultivar dependent. However, in most cultivars the corm is round with the apical meristem at its tip. The meristem remains below the soil level until flowering when it develops into the flower inflorescence axis that bears the bunch. Roots develop from the corm from the region between the inner zone (central cylinder) and the outer zone (cortex) into the soil. Leaves also develop from the meristem of the corm and consist of a sheath, a petiole and lamina or blade. The leaf sheaths' of successive leaves overlap and closely

encircle each other forming the pseudostem or the false stem. The pseudostems of Highland and dessert bananas are green to dark green with many black blotches, while those of plantains are yellowish green with few brown-black blotches. As new leaves develop at the meristem, older leaves are pushed outwards, die and dry out (Simmonds 1962). Most bananas produce approximently 30–40 leaves in its lifetime. After a fixed number of leaves are produced, the meristem gives rise to the flowering stem, which begins to grow upwards through the pseudostem. The flowering stem emerges in the middle of the leaf crown and a complex inflorescence of flower clusters develops. The female flowers appear first and have large ovaries that develop into fruits. As the inflorescence develops, a bulb shaped male bud containing small flowers develops at the end. However, in most cultivated bananas, the fruit develops by parthenocarpy preventing formation of seeds that would otherwise make the fruit unsuitable for human consumption. Three types of flowers are produced on the banana inflorescence. The female (pistillate) flowers develop into the fruit, while the male (staminate) flowers found in the male bud may produce pollen that may or may not be fertile. The third type of flowers called hermaphrodite or neuters are found on the inflorescence axis or rachis between the female flowers and the male bud. They are usually sterile. The female flowers of most cultivated bananas are almost always sterile and the fruits develop by parthenocarpy. In all bananas the growing shoot dies after fruiting once (Simmonds 1962) and its life is perpetuated by means of suckers, which develop from adventitious buds produced on the corm. The suckers are the major form of vegetative planting material and form the subsequent vegetative generation. When the first plant fruits and dies, the maiden sucker (large but non fruiting ratoon with foliage leaves) continues the growth cycle. Bananas are propagated vegetatively through suckers, although wild species can also be propagated by seed (Stover and Simmonds 1987). Sucker development consists of three distinct stages; peeper (young sucker bearing scale leaves only), sword sucker (sucker bearing narrow sword leaves) and maiden sucker (large but non fruiting ratoon with foliage leaves) (Simmonds 1966; Swennen et al. 1984). The cluster formed by the mother plant and the surrounding suckers is referred to as a 'mat'. The number of suckers produced by a plant is very important to farmers in banana production since the crop is vegetatively propagated.

15.1.4 Importance of Bananas and Major Areas of Production

Bananas are the 4th world's most important food crop after rice, wheat and maize, with vast majority of the crop grown and consumed in the tropical and subtropical zones (FAO 2002). The annual world banana production is estimated at 98 million tons of which only 7 million tons enter the world market, suggesting that the crop is more important as food for local consumption than for export. Bananas supply more than 25% of the carbohydrate requirements for over 70 million people in humid forest and mid altitude region of Africa (Robinson 1996), with per capita consumption of approximately 250 kg. Its ability to produce fruits all the year round makes it an important food security crop and cash crop in the tropics (Jones 2000). Bananas are prepared and consumed in a number of ways with each country that produces the crop having its own traditional dishes and methods of processing (Frison and Sharrock 1998). For example, mature unripe bananas are eaten as a starchy food while ripe bananas are consumed raw as a dessert fruit. They can also be consumed boiled, roasted, or fried in ripe or unripe state. Nutritionally, fresh bananas contain 35% carbohydrates, 6–7% fiber, 1–2% protein and fat, besides the major elements such as potassium, magnesium, phosphorus, calcium, iron, and vitamins A, B6 and C (Robinson 1996). Bananas are also used in the manufacture of beer, wine and other products and form an important part of the cultural life of many people (Stover and Simmonds 1987). Other products produced from banana include jam, juice and squashes, banana chips or crisps, sweet banana figs, banana flour, banana powder and starch.

Although, a small proportion of banana production enters the world market, the banana fruit is extremely important as an export commodity especially in Latin America and Caribbean which contribute over 83% of the total banana in the international market (FAO 2002). In Africa, only five countries namely, Ivory Coast, Cameroon, Somalia, Ghana and Cape Verde export approximately 427,000 tons banana and plantain (FAO 2002). The introduction of refrigerated shipment has greatly accelerated the growth of the export trade from Central America and the Caribbean to other parts of the world. Most of the bananas exported are the dessert type from triploid cultivars of *M. acuminata*.

Table 1. Largest producers of banana/plantain in 2004 (FAOSTAT 2004)

Country	Production (metric tons)	Country	Production (metric tons)
India	16,820,000	Nigeria	2,103,000
Uganda	10,515,000	Mexico	2,026,610
Brazil	6,602,750	Thailand	1,900,000
Ecuador	6,552,000	Cameroon	1,830,000
China	6,420,000	Peru	1,660,310
Philippines	5,638,060	Côte d'Ivoire	1,602,423
Colombia	4,400,000	Burundi	1,600,000
Indonesia	4,393,685	Democratic Republic of Congo	1,412,000
Rwanda	2,469,741	Vietnam	1,353,800
Ghana	2,390,858	Guatemala	1,268,000
Costa Rica	2,230,000	Honduras	1,225,066

Because of their high vitamin A and B6 content, bananas are beneficial in the prevention of cancer and heart diseases in humans. Bananas are used to treat diseases such as gastric ulcer and diarrhoea. Vitamin rich nectar sap from banana flower buds is fed to babies and children to strengthen their growth, while potassium helps in boosting brain functioning.

Besides the food and income, banana plays many important roles. For example banana leaves can be used as thatching materials for houses, as plates, tablecloths, umbrellas, sleeping mats, animal feed and in food preparation. Non-fruit parts of the banana plant, including the corm, shoots, pseudostem and male buds are eaten as vegetables in Africa and parts of Asia (Simmonds 1962). The banana pseudostems can also be used as animal feed. Banana leaves and pseudostems contain a high quality fiber which is used for making ropes, handcraft, baskets, carpets and manufacturing of banana paper. In mixed cropping systems, banana plants provide shade for crops that grow better in shade conditions such as cocoa, black pepper, coffee and vanilla. At the system level, bananas maintain the soil structure and cover throughout the year, protecting it from wind and rain erosion. Further more, if the biomass is used as mulch, soil fertility and organic matter remains stable.

Between 1970 and 1997 the annual world banana production increased, from 51 million tons to 88 million tons, an increase of seventy percent (Sharrock and Frisson 1998). At that time banana production was estimated to be growing faster than the production of any other starchy crop in the world. The world's current banana/plantain production is estimated at about 104 million metric tones, grown on about 10 million acres of land in over 100 countries (FAOSTAT 2004). Africa produces 35% of banana and plantains, Asia and Pacific 29%, and Latin America and the Caribbean 35%. India is the world's leading producer of banana and plantain with a production of about 16 million tons followed by Uganda with 10.5 million tons (FAOSTAT 2004). Most bananas produced in Africa are used for local consumption and for local markets than for international trade. The major world banana producing countries are summarized in Table 1.

15.1.5 Genome Groups and Genome Size of *Musa*

Cultivated bananas are grouped on the basis of their genomic origins in relation to *M. acuminata* and *Musa balbisiana* and their ploidy level (Simmonds 1966). Currently, the known cultivars are the diploids (AA), triploids (AAA), and tetraploids (AAAA) forms of *M. acuminata* and diploids (BB), triploids and tetraploids (BBBB) forms of *M. balbisiana* or their hybrids (AB, AAB or ABB) (Simmonds and Shepherd 1955). However, other genomic groups including AAAB, AABB and ABBB from either natural or artificial hybridization are also known to exist (Pillay et al. 2004). The Indian subcontinent is thought to have been the major center of hybridization of 'acuminata' types with the indigenous *M. balbisiana*. The region is

known for its wide variety of AAB, and ABB cultivars (Price 1995). Triploid *M. acuminata* derived (AAA) cultivars are the most common and the most important grown cultivars, and include 'Gros Michel' and 'Cavendish' types (dessert bananas), which constitute most of the world's banana trade. Cooking and beer bananas (AAA) are indigenous to East Africa while plantains (AAB) are very important staple crop in West Africa and some parts of central Africa (Simmonds 1976). The B genome from *M. balbisiana* confers hardiness and resistance to drought observed in the diploid AB and triploid AAB and ABB hybrids (Purseglove 1988). *Musa balbisiana* derivatives show greater variability and produce fruits with more starch and acid, higher dry matter content and more vitamin C. They also give textures and flavors that are not characteristic in *M. acuminata* derived genotypes. *Musa acuminata* has traces of wax on fruits while *M. balbisiana* is often strongly waxy (Stover and Simmonds 1987).

Banana has a small haploid genome of 552–607 Mbp divided among 11 chromosomes. This is only 25% larger than rice, a crop that has been used as a model species in monocotyledon plant genomics studies. Due to its relatively small size, the *Musa* genome is highly amenable to complete functional and sequence analysis and extensive characterization of genes. Banana being one of the few plants with biparental cytoplasmic inheritance namely, mitochondrial paternal inheritance and maternal inheritance of chloroplasts, can act as a good genomic model.

15.1.6 Banana Breeding Objectives

Breeding programs of crops are designed/initiated to address production constraints. Banana production is affected by a wide range of pests and diseases, drought and low yielding cultivars. Therefore, the primary objective of banana breeding programs worldwide is to address these constraints and develop cultivars that are acceptable by farmers. The earliest focus in banana breeding programs was to develop disease-resistant dessert cultivars for export (Rowe and Rosales 1993) following the outbreak of *Fusarium* wilt (Panama disease) in 'Gros Michel'. Shortly thereafter the Cavendish variety that took over as the number one dessert banana replacing 'Gros Michel' was attacked by another fungus, *Mycosphaerella fijiensis*, which caused the Black Leaf Streak (BLS) disease or black Sigatoka. Consequently, breeding efforts for the genetic improvement of Cavendish by developing hybrids resistant to BLS were also initiated. Since then breeders especially in the banana exporting regions have been mainly aiming at developing banana cultivars similar to 'Gros Michel' but with resistance to Panama and leaf diseases (Sathiamoorthy and Balamohan 1993). Besides the diseases, other traits of concern in breeding include high yield, fruit quality (finger length, finger curvature and finger pedicel length), flavor, ripening, plant height (stature) and production efficiency (Stover and Simmonds 1987). Indeed several authors (Simmond 1987; Eckstein et al. 1995; Pillay et al. 2002) described and emphasized the ideotype cultivar as one which is disease and pest resistant, high yielding, photosynthetically efficient, early maturing, display minimum delay between consecutive harvests, short stature, strong roots for optimal nutrient uptake and greater resistance to wind damage.

Breeding for pest resistance especially against the banana weevil has, however, not featured prominently in any breeding program. This is probably because of the absence of good sources of resistance, and lack of a simple screening method for weevil resistance, to enable breeders to rapidly pinpoint resistant line across the available germplasm (Kiggundu et al. 1999). Nevertheless with the advancement of biotechnology, breeding objectives such as resistance to the banana borer, viruses, nematodes, and modification of the fruit ripening patterns are expected to be vigorously pursued.

15.1.7 Limitations of Classical Breeding of Bananas

The overall strategy in banana breeding is to incorporate the desired traits often harbored in wild and cultivated diploids (2×) such as resistance to diseases and pests to the existing cultivars rather than aiming at genetic materials that are completely different from the existing cultivars. However, most of the cultivated clones are triploids (3×) characterized by low male and female fertility (Vuylsteke et al. 1993). The high level of sterility is attributed to uneven chromosome distribution during meiosis that is characteristic of triploids (Adeleke et al. 2004). Other mechanisms of sterility result from morphological errors in post meiotic stages and physiological dysfunction during pollination and fertilization (Simmonds 1962; Pillay et al.

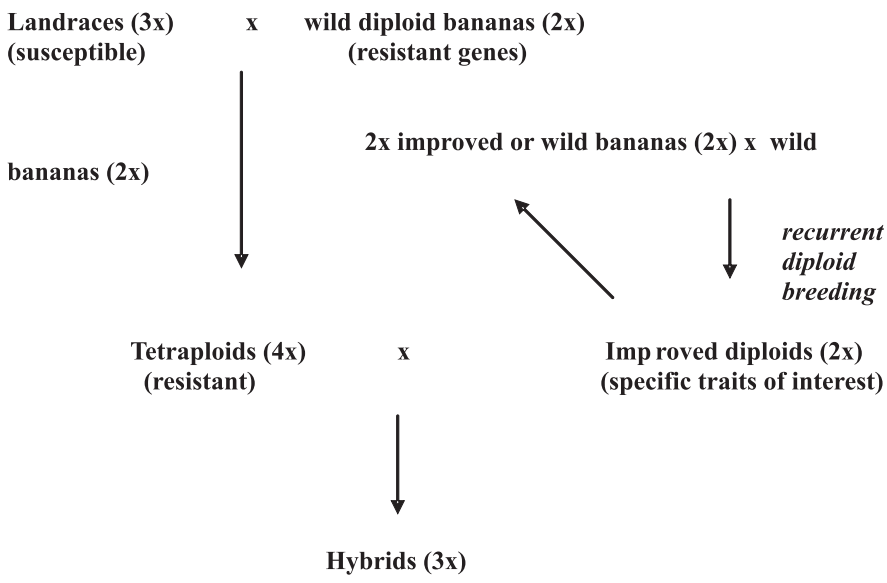

Fig. 2. Simplified *Musa* breeding scheme (adapted from Pillay et al. 2002)

2002). Seed set per bunch in many clones is less than one seed, and germination in soil is less than one percent (Ortiz and Vuylsteke 1996). Bananas also take up to 18 months to mature, which prolongs breeding efforts compared to annual crops. Consequently, breeders currently devolve much of their resources for obtaining, rather than evaluating progeny from crosses. Compounded to this is the fact that the initial steps in genetic improvement of most cultivated bananas involve crossing triploid (3×) accessions to diploid (2×) to produce 4× hybrids as described in Fig. 2 (Pillay et al. 2002). Although the process is conceptually straight forward, complex ploidy and genome arrays which complicate selection can arise. The effect of multiploidy and autopolyploidy chromosome behavior results in unpredictable frequency of aneuploids and undesirable hyperpolyploids (>5×) in addition to 2×, 3×, and 4× euploids (Simmonds 1966). Diploid bananas generally have unacceptably low yield potential, while tetraploid bananas often suffer from premature senescence, fruit drop, short shelf life, weak pseudostem and are prone to undesirable production of seed (Pillay et al. 2002). In addition, banana seeds are large and hard and are not acceptable to most banana consumers (Pillay et al. 2002).

15.1.8 Overcoming *Musa* Breeding Difficulties

Low seed set and germination rates are the major hindrances to hybrid plant production in most triploid *Musa*. This makes it difficult to obtain adequate population sizes to select disease resistant cultivars through crossing. The application of aseptic embryo culture techniques has improved seed germination rates by a factor of 3 to 10 (Vuylsteke et al. 1990). The discovery of seed-fertile landraces capable of producing true seed upon hand pollination (Vuylsteke et al. 1993) and use of advanced breeding populations with improved fertility (Pillay et al. 2000) are sought to further improve the efficiency of banana breeding. Breeders are using in vitro techniques such as shoot-tip culture for multiplication of newly bred genotypes. Micropropagated plants establish faster than conventional suckers and have almost uniform production cycle, which further facilitates establishment and evaluation of hybrids (Robinson 1996). In vitro culture also guarantees safe collection, exchange and conservation of germplasm required for identification of breeding traits and facilitates dissemination and propagation of newly selected cultivars or hybrids. The high in vitro multiplication rates also enable rapid production of clean or disease-free planting material for establishment of large pollination blocks.

In addition, the problems of fertility could be bypassed by using an array of available biotechnological techniques (Pillay et al. 2002). Recombinant DNA technology for instance has been beneficial in the improvement of *Musa* cultivars that are difficult to breed and remains the most promising solution for those varieties that are totally sterile. Biotechnology makes it possible to incorporate genes coding for characters that are not available in the *Musa* gene

pool. Using molecular techniques, novel genes encoding agronomically important traits can be identified, isolated, characterized and introduced into cultivars via a combination of genetic transformation and in vitro regeneration (Sagi 2000; Tripathi 2003). Genetic transformation, i.e. the introduction and stable integration of genes into the nuclear genome and their expression in a transgenic plant, offers a better alternative for the genetic improvement of cultivars not amenable to conventional cross breeding such as Cavendish bananas or Horn plantains (Jones 2000). Some success has been registered in genetic engineering of bananas and plantains, which enabled the transfer of foreign genes into plant cells. Protocols for electroporation of protoplasts derived from embryogenic cell suspensions (Sagi et al. 1994), particle bombardment of embryogenic cells (Sagi et al. 1995; Remy et al. 1998), and co-cultivation of wounded meristems (May et al. 1995; Tripathi et al. 2005) or cell suspension cultures with *Agrobacterium tumefaciens* (Ganapathi et al. 2001; Khanna et al. 2004) are available for bananas and plantains. Particle bombardment (or biolistic transformation) uses accelerated heavy-metal microparticles coated with DNA to penetrate and deliver foreign genes into plant cells, which are then selected and regenerated into plants while *A. tumefaciens*, a soil bacterium, transforms its plant hosts by integrating a segment (T-DNA) of its tumor-inducing plasmid into the plant nuclear genome allowing introduction of virtually any novel gene. Both of these systems have been used successfully on banana. *Agrobacterium*-mediated transformation has been applied to a range of plantain and banana cultivars and synthetic hybrids. The status of research on genetic engineering of banana for disease resistance and future possibilities has been reviewed (Sagi 2000; Tripathi 2003). Since the cultivated banana is largely sterile, its genetically manipulated plants are environmentally safe because the modified genes would not be able to easily escape from the transformed crop.

On the other hand the application of molecular techniques such as RFLP and RAPD in banana breeding can increase the efficiency of identification of promising new genotypes. Early detection of desirable genome combinations significantly improves breeding efficiency and saves field evaluation costs. Faster, precise, none destructive methods for ploidy determination based on the use of flow cytometry (Pillay et al. 2001) have made it easier to detect mixoploidy of especially segregating progeny populations. RAPD and PCR-RFLP markers that are specific for the A and B genomes have been identified (Pillay et al. 2000; Nwakanma et al. 2003a). These molecular methods can be used at any developmental stage of the plant and therefore provide an objective and reliable way for genome classification in bananas and plantains. Indeed the role played by molecular markers in banana and plantain breeding is crucial and inexhaustive.

15.1.9 Banana Breeding Achievements

Though no new banana hybrid has reached the fruit quality of the natural varieties to replace the current Cavendish varieties, plantains, East African Highland and other banana groups in regard to their eating qualities, the recent advances in several breeding programs hold a lot of hope in eventually achieving manbred acceptable cultivars for commercial production and local consumption through conventional breeding. The initial major challenge that faced banana breeders was developing diploids with combinations of disease resistance and desirable agronomic qualities and identification of seed-fertile triploids for use in breeding desired types of banana. The quest for bred diploids came from the realization that most traits of economic importance are more predictably inherited from the diploid parents than from parents with higher ploidy level (Tenkouano et al. 1999a, b). It is also easier to carry genetic analysis in a diploid background due to disomic inheritance, which facilitates and accelerates breeding. Furthermore, population improvement at the 2× level is effective for eliminating deleterious recessive alleles in selected 2× progenitors for further crosses with cultivated bananas and plantains (Ortiz and Vuylsteke 1996). Hence, the major breeding programs worldwide initially invested in the development of diploid breeding stocks (Ortiz and Vuylsteke 1996) to permit the development of the desired hybrids. Since then several diploid hybrids have been successfully developed by the various breeding programs and registered in the public domain and/or distributed to breeders and geneticists for use in germplasm enhancement and genetic analysis of *Musa* genomes (Rowe 1984; Rowe and Rosales 1993; Vuylsteke 1993; Ortiz et al. 1994; Vuylsteke and Ortiz 1995; Tenkouano et al. 2003). The development of these diploids is undoubtedly a breakthrough which was needed for more rapid progress towards the development of new cultivars. For instance, improved

diploid banana germplasm developed by the Fundacion Hondurenea de Investigacion Agricola (FHIA Honduras) has been successfully utilized as parents of internationally released tetraploid hybrids such as the dessert banana 'FHIA-1' (Goldfinger), 'FHIA-3', 'FHIA 17, 23, 25' (Rowe and Rosales 1993). These improved diploids have also been used to produce East African Highland banana type hybrids (Pillay, unpublished).

Although there have been arguments against tetraploids as potential cultivars because of their weak leaf petioles and theoretical possibility of spontaneous seed setting, several tetraploid hybrids of plantains and other banana types have been developed, some of them having striking resistance to black Sigatoka and good plant and bunch characteristics (Vuylsteke et al. 1993, 1995; Ortiz and Vuylsteke 1998a, b; Jones 2000) and have been globally tested for possible adoption as new cultivars. The yield of most of the tetraploid hybrids is relatively higher than that of their parent landraces. This has been attributed to characteristics such as shorter and robust plant stature, better suckering behavior and at times early maturity, which are all linked with yield. The high yield in plantain tetraploids is particularly attributed to improve ratooning as compared to their parents, which have generally low suckering behavior.

Nevertheless, further ploidy manipulation to reduce the chromosome number to the triploid level to develop male sterile hybrids has been pursued (Ortiz 1997). Major gains in fruit quality have been achieved by restoration of the seedless character in resulting 3× offspring (Tenkouano et al. 1998). For instance, Tropical *Musa* secondary triploid hybrids (here after TM3×) resistant to black Sigatoka have been obtained from tetraploid-diploid crosses and made available to breeders and geneticists interested in germplasm enhancement or for further testing and cultivar release in accordance with the countries specific variety release regulations (Ortiz et al. 1998).

On the other hand, although some important *Musa* subgroups (Cavendish, False Horn plantain) remain recalcitrant to conventional breeding, seed set rates have tremendously improved in many *Musa* hybrids and the germination percentage drastically enhanced using established tissue culture techniques. Consequently, a number of improved genotypes have been widely evaluated and knowledge on genotype-by-environment interaction and stability of the important traits gained. Insight into combining abilities, heterotic groups, and the genetics of qualitative and quantitative traits has been gained and is being applied to make breeding more efficient. A wide array of breeding schemes has been explored, combining conventional and innovative approaches, and producing potential cultivars from primary tetraploids, secondary triploids and other populations (Tenkouano et al. 2003).

15.2 Gene Mapping in *Musa*

Basically, mapping aims at identifying molecular markers genetically (genetic maps) or physically (physical maps) linked to major or minor genes (generally loci) contributing to the expression of a particular trait or continuously varying character (e.g. a QTL). Linked markers can then be exploited to isolate the gene(s) underlying the trait. The isolated genes in turn are used to improve selected genotypes via direct or *Agrobacterium*-mediated gene transfer or, alternatively, the linked markers may serve to select segregants of a cross that carry a desirable trait (marker-assisted selection, MAS). For these reasons genetic and (in a more advanced state) physical maps have now been established for almost all the important crop plants. Most of these maps are integrated maps, i.e. they contain a series of different molecular markers, preferably in a framework of STMS (sequence-tagged microsatellite sites). Genetic mapping in *Musa* is not very far advanced, though a first low-density map of *M. acuminata* was established using isozyme, RFLP, and RAPD markers based on a cross between SF265 (AA) × a *banksii* (AA) segregating for parthenocarpy (Faure et al. 1993). Although a series of crosses segregating for other traits like black Sigatoka resistance, bunch position, chromosome rearrangements have been developed, and mapping projects have been undertaken at CIRAD, no high-density linkage map is yet available. Till now, mapping populations are limited in number, despite the fact that several activities are aimed at developing suitable segregating populations at various Research Institutes. The International Institute of Tropical Agriculture is developing several populations based on the A genome and B genomes. Segregating populations of *M. acuminata* (Calcutta 4) × *M. acuminata* (Calcutta 4), *M. balbisiana* × *M. balbisiana, M. acuminata* × *M. balbisiana* have been developed. Field evaluations of populations

show that the BB segregating populations show very little variation among the progeny while the 'Calcutta 4' × 'Calcutta 4' cross shows a high degree of variation, suggesting that *M. acuminata* (Calcutta 4) may not be homozygous as was previously suggested.

Although genetic mapping of the *Musa* genome obviously lags behind that of other crop plants of comparable market value, several bacterial artificial chromosome (BAC) libraries of the A and B genomes, which will allow the physical mapping of the banana genome, have been established. Genes known only by their phenotypes are best cloned by positional or map-based cloning. This requires the development of large-insert genomic libraries and ordering them into contigs that span the genome region carrying the gene(s) of interest. The BAC system of cloning large DNA fragments is the preferred method for constructing large insert-libraries of genomes (Tomkins et al. 1999). Physical mapping aims at defining the location of a particular gene (or DNA sequence) on a cloned genomic sequence of a size of 100–200 kb. It also allows one to relate genetic distances (cM) between two (or more) markers to physical distances (kb), to align syntenic (and also non-syntenic) regions of two or more genomes from related or non-related organisms to search for homologous, orthologous or paralogous sequences, and to build contigs of specific genomic regions to pinpoint a target gene and to isolate it using map-based cloning approaches (Budiman et al. 2000). The physical mapping methodology involves FISH (fluorescent in situ hybridization) or fiber-FISH to locate sequences on chromosomal preparations (Jackson et al. 1998), or the production of yeast artificial chromosomes, or more efficiently, BAC libraries with large inserts, or transformation-competent artificial chromosomes (TACs). Global physical mapping comprising the entire genome has been achieved for only a few plant species like *Arabidopsis thaliana* and *Oryza sativa* (Kurata et al. 1997; Mozo et al. 1999), but is in progress for many other crop plants.

Although several BAC libraries of different *Musa* species (*M. acuminata*, A library; *M. balbisiana,* B library) have been produced, the clones have not generally been ordered, nor has a tiling path been constructed around interesting regions. However, it is to be expected that the growing awareness of the scientific banana community will catalyse the process of physical mapping, which is the path to the isolation of agronomically interesting genes.

Genomic libraries from *M. acuminata* and *M. balbisiana* accessions have been screened with a variety of repetitive oligonucleotides including $(GA)_{11}$, $(AT)_{11}$, $(CT)_{11}$, $(ATT)_{10}$ and $(CTT)_{10}$ (Jarret et al. 1994). The sequence of selected fragments was then determined and PCR primers designed from sequences flanking the SSR. More than half of the SSR isolated from *M. acuminata* had simple dinucleotide (GA) or (CT) core motifs (Crouch et al. 1997). No simple (AT) repeats were isolated despite their reported abundance in plant species. This is likely to be due to self-annealing of the $(AT)_{11}$ probe. However, several complex SSR which included $(AT)_n$ motifs were isolated by virtue of their association with (GA), (AG) or (CT) motifs. In common with other genera, trinucleotide and tetranucleotide repeats appear to be less abundant in *Musa* than dinucleotide repeats. In this way, approximately 100 useful microsatellite markers have been generated from *M. acuminata* while a similar number is expected to result from parallel work on *M. balbisiana*. Similar microsatellite isolation projects are also ongoing at CIRAD (Lagoda et al. 1995), the University of Frankfurt (Weising et al. 1996) and the University of Saskatchewan while smaller projects have been initiated elsewhere. This is likely to result in the availability of more than 500 microsatellite markers for genetic analysis and molecular breeding in *Musa*.

15.3 Identification of Quantitative Trait Loci (QTL) in *Musa*

Plant characters are often referred to as qualitative or quantitative depending on the number of genes that control them (Fehr 1987). Qualitative characters such as flower color are controlled by one or a few major genes. On the other hand quantitative traits show continuous variation and are controlled by a number of minor genes (polygenes) that are greatly affected by the environment. It is known that genetic and the environmental factors interact to make up the phenotype of a plant. Traditional genetic studies have quantified these factors by using statistical models such as:

$$Y_{ij} = \mu + G_i + E_j + I_{ij} + e$$

Where Y_{ij} is the observed phenotype, μ is the mean phenotype in the population, G_i and E_j are the net effects due to an individual having genotype i and j, I_{ij} is the interaction effect between i and j, and e is the random contribution to the phenotype. These

models can estimate the statistical effects as means variances or covariances of a group of genes, but give very little information about the nature of the polygenes that underlies the trait (Kearsey 2002). In addition, while these models described the effects of genetic and environmental factors, it was not possible to locate the exact genes or chromosomal regions of a plant that contributed to the trait. Locating genes on a chromosome cannot be achieved without gene maps. In the past, classical markers such as pigmentation, morphological traits and isozyme loci were used to generate linkage maps. The general lack of abundance of these types of markers meant that most linkage maps had large intervals between the markers. The first linkage map to be established was that of maize (Sturtevant 1965). Complete genetic maps are essential for studying the genetics underlying quantitative traits. Genetic maps show the ordering of loci along a chromosome and the relative distances between them (Lynch and Walsh 1998). Early 'genetic' linkage maps provided a rough road map to order some genes. Thereafter 'physical' maps provided with landmarks along the whole length of the chromosomes. The physical map was superseded by the concept of molecular maps which rely on molecular markers. Molecular markers such as restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) have now made it possible to dissect traits into their genetic components. A chromosomal region that is associated with molecular markers and with a quantitative trait is defined as quantitative trait loci (QTL, Xu 2002). Plant breeders are interested in knowing how many genes are involved in a given trait, the nature of the dominance and epistatic properties of these genes and how they interact with the environment, and whether these genes are structural or regulatory. These questions can only be answered if we have an accurate location of gene(s) on a chromosome. If QTLs could be identified the transfer of traits such as yield, drought tolerance etc. could be accelerated in breeding programs.

Plant populations with different genetic structures have been created for genetic mapping, including F_2/F_3, backcross, doubled haploids, recombinant inbred lines, near isogenic lines, back cross inbred lines and various mutants (Xu 2002). Several advanced statistical methods have been proposed for mapping quantitative trait loci (Lander and Botstein 1989). Much work has also been covered on the theoretical aspects of mapping quantitative trait loci. This is beyond the scope of this chapter.

Several traits in banana such as fruit filling period, bunch weight, and fruit parameters are considered to be quantitative in nature (Ortiz 1995). Studies to identify QTLs in banana are limited. The primary reason for this is the absence of a high density linkage map for *Musa*. A partial linkage map for diploid bananas based on 58 RFLP, four isozyme and 28 RAPD markers was published in 1993 (Faure et al. 1993). A composite linkage map has been constructed by CIRAD from two mapping populations at a LOD score of 4.75. This map covers 1,227 cM and links 373 isozyme, microsatellite, RFLP, RAPD and AFLP markers in 11 linkage groups (unpublished). A QTL for Sigatoka resistance has been placed on the map developed by CIRAD. The identification of QTLs in *Musa* will be of great value for genetic improvement of bananas for polygenic traits such as yield, drought tolerance and others. Published reports and our own unpublished data show that bananas are greatly influenced by the environment. Researchers identifying QTLs in *Musa* must consider environment as a factor. The level of QTLs across environments is trait specific. For example, in soybean out of 11 RFLP markers associated with plant height and eight with lodging, only two markers for plant height and one for lodging were detected in all four locations where the experiments were conducted (Lee et al. 1996).

15.4 Marker-Assisted Breeding in *Musa*

The success of crop improvement programs is highly reliant on the power and efficiency with which the genetic variability can be manipulated. For thousands of years, breeders have been relying on morphological characters to select and cross plants carrying desired traits to finally yield superior cultivars. However, the practice is extremely slow and highly unpredictable often limited by the low number of morphological characters available to them for crop improvement programs. Besides, the expression of morphological characters is affected by environmental conditions and sometimes altered by epistatic and pleiotropic interactions resulting in the difficulty to obtain reliable data. Where breeding goals cannot be achieved using traditional approaches, there is now considerable scope for using molecular or genetic markers to

develop new varieties. Genetic markers offer plant breeders the potential of making genetic progress more precisely and more rapidly than through phenotypic selection. The potential benefits of using markers linked to genes of interest in breeding programs have been obvious for many decades. However, the realization of this potential has been limited by the lack of useful markers. With the advent of DNA-based genetic markers in the late 1970s, the situation changed and researchers could, for the first time, begin to identify large numbers of markers dispersed throughout the genetic material of any species of interest and use the markers to detect associations with traits of interest, thus allowing MAS to finally become a reality. Molecular markers can be conveniently used in plant breeding programs for the characterization of germplasm, assessment of genetic diversity and identification of crop varieties via DNA fingerprinting.

Marker-assisted breeding provides a dramatic improvement in the efficiency with which breeders can select plants with desirable combinations of genes. To date, many markers linked to useful traits have been identified and being utilized in many breeding programs. However, before initiating marker-assisted breeding, it is imperative to define the specific objectives to be achieved through the use of markers, the specific problem and the cost of the technologies to be used, especially in the developmental phase. The success of MAS depends upon several critical factors, including the number of target genes to be transferred, the distance between the flanking markers and the target gene, the number of genotypes selected in each breeding generation, the nature of germplasm and the technical options available at the marker level.

The use of molecular markers in banana breeding is reported for many purposes, such as cultivar identification (Pillay et al. 2001), phylogenetic studies (Kardolus et al. 1998), analysis of recombination between genomes (Osuji et al. 1997a, b), identification of genes controlling traits (Damasco et al. 1996), and assisted selection (Shanmugavelu et al. 1992). Marker-assisted breeding is a powerful tool in many breeding programs and it is being utilized by many breeders to transfer useful genes among species. Thus, MAS offers clear advantages in genetic terms over traditional selection in many circumstances (Crouch et al. 1998). RFLPs of diverse germplasm have been used to study the taxonomy and phylogeny of *Musa* species (Gawel et al. 1992; Jarret et al. 1992; Nwakanma et al. 2003a), and variation in the chloroplast genome within the *Musa* genus (Gawel and Jarret 1991a, b). 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 this technique is not appropriate to routine breeding applications. Thus, researchers have concentrated on applications 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. PCR-based assays are amenable to the large-scale throughput demands of screening breeding populations. The RAPD technique has been successfully used to distinguish diverse *Musa* germplasm (Howell et al. 1994; Bhat and Jarret 1995; Pillay et al. 2001). In addition, a molecular linkage map has also been 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 genome of an organism. RAPD analysis has been used to differentiate *Musa* genome groups (Howell et al. 1994; Pillay et al. 2000), more closely related *Musa* germplasm (Bhat and Jarret 1995) and full-sib hybrids in plantain breeding populations (Crouch et al. 1998, 2000). Teo et al. (2005) used retrotransposon derived markers for identification and characterization of banana cultivars and classification of *Musa* genome constitutions. These reports clearly demonstrate 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.

Recently the technique of AFLP has been successfully applied in *Musa*. AFLP analysis of *Musa* breeding populations suggests that this technique may be a powerful tool in the molecular breeding of plantain and banana (Ude et al. 2002a, b; Ude et al. 2003). Using AFLP markers, Lheureux et al. (2003) found that 10 markers were co-segregating with the presence and/or absence of BSV infection in *Musa* hybrids. AFLP analysis is clearly a powerful technique in terms of its 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 relationships may be sufficient for practical purposes. Software has been developed to distinguish homozygotes and heterozygotes on the basis of band intensity. Yet, such an approach may be frequently confounded by the presence of bands of intermediate intensity.

AFLP assays are also technically demanding and expensive in that 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 polymorphisms. Nevertheless, AFLP and SSR markers are now being used to identify markers for fruit parthenocarpy, dwarfism and apical dominance in banana and plantain.

Microsatellite markers and AFLP analysis appear to be the most appropriate technologies for marker-assisted breeding in *Musa* (Crouch et al. 1999; Hautea et al. 2005). DNA markers have provided powerful tools for genetic analysis in *Musa* (Crouch et al. 1998, 2000) but may not provide an effective means of predicting progeny performance (Tenkouano et al. 1999b). All marker 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.

15.5 Marker-Assisted Introgression

Marker-assisted selection and aided introgression are being employed by plant breeders mostly to locate and select genes controlling important quality and disease or pest resistant traits (Crouch and Ortiz 2004). The first step however, is the identification of one or more markers linked to the gene(s) to be introgressed and their localization on the molecular map. Though mapping of the *Musa* genome is still in its infancy, the results of functional genomics of model plants will increase the understanding of the basic *Musa* biology as well as the exploitation of genomic information for its improvement. Crouch et al. (1998, 2000) emphasized the fact that introgression of genes for pest and disease resistance from wild germplasm has been and is likely to continue to be a crucial aspect in *Musa* improvement. DNA markers are now being sought for several characters of importance including parthenocarpy, apical dominance, resistance to black Sigatoka, nematodes, and other pests and diseases. Fruit quality parameters (color, texture, ripening) are other candidate traits for DNA markers. RAPD assays have proven to be powerful and efficient means of assisting introgression and backcross breeding. In fact, RAPD markers that are specific for the A and B genomes of *Musa* have been identified and are routinely used. 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. Consquently, this led to a focus on the development and utilization of primers for *Musa* microsatellites (Jarret et al. 1994; Kaemmer et al. 1997; Creste et al. 2004), which have been considered optimum markers in other systems due to their abundance, polymorphism and reliability. 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 base 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. 1998, 2000). However, the isolation of microsatellites is time consuming and expensive. Nevertheless, several hundred SSRLP markers have been generated in *Musa* (Jarret et al. 1994; Kaemmer et al. 1997; Crouch et al. 1998). Furthermore, the isolation of SSR is becoming 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. This has recently allowed the rapid isolation of several hundred microsatellites from the *Musa* B genome (Buhariwalla et al. 2005).

Marker-assisted gene introgression offers an extremely efficient means of precisely identifying rare segregants with the required genome compositions and it is routinely being applied in many breeding programs. While selection theory is the most important tool for the design of breeding programs for improvement of quantitative characters, no general selection theory is available for marker-assisted backcrossing. Its efficiency depends mostly on marker density and position, population size, and selection strategy. Adopting a selection theory approach to predict response to marker-assisted selection for the genetic background of the recurrent parent promises to com-

bine several of the factors determining the efficiency of a gene introgression program into one criterion.

15.6 Map-Based Cloning

The isolation of agriculturally important genes is an important goal in plant molecular biology. Since most agriculturally important genes are known only by phenotype, techniques have been developed to isolate such genes. Currently, map-based cloning (or positional cloning), insertional mutagenesis and subtraction cloning are three of the best-developed strategies. Map-based cloning has been successfully used to isolate plant genes based solely its position on a genetic map. The strategy of map-based cloning is to find molecular markers that are very closely linked to the gene of interest. Those molecular markers can serve as the starting point for chromosome walking or jumping to the gene.

Map-based gene cloning includes four steps:

(a) Target gene mapping: The first step of map-based or positional cloning is to identify a molecular marker that lies close to the gene of interest (5 cM). This procedure typically is done by first finding a marker in the vicinity of the gene. For the initial screening smaller population sizes are used (60–150 individuals).

(b) Physical mapping: The next step is to saturate the region around that original molecular marker with other markers. At this point you are looking for a one that rarely shows recombination with your gene. At this stage, the population size could increase to 300–600 individuals.

The next step is to screen a large insert genomic library (BAC or YAC) with the marker to isolate clones that hybridize to the molecular marker. Once the initial markers that are flanking the target gene have been identified and hybridized to a clone, the position of the gene can be determined. Distance is measured in base pairs other than genetic recombination (in cM). Methods for physical mapping involve FISH, YACs, BACs, STSs (sequence tagged sites).

(c) Chromosome walking or landing:

1. Chromosome walking relies on isolation of a DNA fragment at or near an end of a cloned insert for use as a probe to screen the library and identify more clones. Chromosomal walking was the major map-based cloning method in the past.
2. Chromosomal landing starts with identifying tightly linked molecular markers. The DNA markers are then used to screen a library and isolate (or land on) the clone containing the gene.

Chromosome walking involves creating new markers (usually sequences at the end of the clone) and screening of a segregating population with these new markers. Often this population is large (1,000–3,000 individuals). The goal is to find a set of markers that co-segregate (no recombination) with the gene of interest. Co-segregation means that whenever one allele of the target gene is expressed, the markers associated with that allele are also present. In other words, recombination is not seen between the gene and the markers. If these markers do not co-segregate, new large insert clones should be selected and the process is repeated until the finding of a clone whose markers co-segregate with the gene. To speed the cloning process, it is best to begin with a marker that is tightly linked to the target gene.

(d) Gene identification: Genetic complementation through transformation. DNA fragments between the flanking markers are cloned and introduced into a genotype mutant for your gene by a genetic engineering technique called plant transformation. If the transgenic plant expresses the wild type phenotype, it confirms the presence of the gene of interest on that fragment. At this point the fragment must be sequenced to find a potential open reading frame (ORF), sequences that most likely will encode a gene product. In the best situation, only a single ORF is found, but often this is not the case. Usually several possible ORFs are found and new transgenic plants are created by transforming with a single ORF. Once this ORF is shown to rescue the mutant phenotype, an in-depth molecular and biochemical analysis of the newly cloned gene could then be performed.

RFLP or other molecular genetic markers can be used in chromosome walking procedures. High density genetic maps have been developed or are being prepared in a number of crops. Using RFLPs, the chromosomal location of a particular probe can be determined and a map of various RFLP probe positions

can be constructed. Genes can then be located genetically by their co-segregation with a particular RFLP. The starting gene can be cloned using a closely linked RFLP probe and isolating genomic clones that it corresponds with, then walking from these genomic clones to the gene of interest.

The cloning of genes underlying important agronomic characters offers to revolutionize progress in plant research and breeding, particularly in the area of pest and disease resistance. In addition, map-based cloning of gene(s) responsible for parthenocarpy, dwarfism and albinism in plantains and bananas, would be of great value to both fundamental and applied researchers of many crops.

In fact, only in *Arabidopsis thaliana* have map-based approaches been widely applied (Giraudat et al. 1992; Busch et al. 1996; Lukowitz et al. 1996). In other plants there are only few reports of genes that have been cloned by a map-based approach (Martin et al. 1993; Dixon et al. 1996; BuK Schges et al. 1997).

15.7 Genes and Gene Expression in *Musa*

Although several expressed sequence tag (EST) and cDNA libraries have been established (e.g. for *M. acuminata* ssp. *malaccensis*), no *Musa* EST database can yet be tapped for information about expressed genes (Caetano et al. 2005; Carlos et al. 2005). In addition, the depth of these libraries is not known. Yet full-length cDNA libraries, normalized and representative, would be needed from a whole series of tissues and states (e.g. normal vs. diseased; susceptible vs. resistant; different developmental states). A series of resistance gene analogs have been isolated, using degenerate PCR primers targeting highly conserved regions in proven plant resistance genes (e.g. kinase or transmembrane-encoding domains, or leucine-rich repeat sequences, to name only few). Plant disease resistance genes involved in signal transduction contain domains that are conserved throughout mono- and dicotyledons. Primers have been designed to those domains in the *RPS2* gene of *Arabidopsis thaliana* and the N gene of tobacco. Using these primers for PCR, candidate resistance genes have already been cloned from soybean, potato, rice, barley and *Arabidopsis*. A similar strategy has been applied to clone candidate resistance genes from banana (Wiame et al. 2000). A series of disease resistant genes were isolated from the somaclonal mutant CIEN-BTA-03 (resistant to both *M. fijiensis* and *M. musicola*) and the parent 'Williams' that fall into two classes: nucleotide-binding site-leucine-rich repeat-containing kinases, and serine-threonine protein kinases of the *pto* type (Kahl 2004). All the resistance genes were fully sequenced, and eight of them are also transcribed in the mutant, its parental genotype, 'Pisang Mas' and a tetraploid *M. acuminata*. The researchers at QUT have isolated the complete gene sequence of R gene candidate (RGC-2) from *Musa acuminata* ssp. *malaccensis,* a wild diploid banana segregating for resistance to *Fusarium oxysporum* fsp. *Cubense (*FOC) Race 4. The development of *Fusarium* wilt resistant transgenic banana using this gene is in progress (Dale et al. 2004).

Few genes are targeted, some sequences are known, fewer publications have appeared, but no banana gene has been applied in any way (e.g. for transformation). Also, no attempt has yet been made to design expression chips with families of genes whose sequences are derived from either cDNAs (cDNA microarray), or oligonucleotides, or from clones obtained from related or unrelated plants. Relative success in genetic engineering of bananas and plantains has been achieved recently to enable the transfer of foreign genes into plant cells.

Genetic transformation using microprojectile bombardment of embryogenic cell suspension is now a routine procedure (Sagi et al. 1995; Becker et al. 2000). An efficient method for direct gene transfer via particle bombardment of embryogenic cell suspension has been reported in the cooking banana cultivar 'Bluggoe' and the plantain 'Three Hand Planty' (Sagi et al. 1995). Becker et al. (2000) reported the genetic transformation of the Cavendish banana cv. 'Grand Naine'. *Agrobacterium*-mediated transformation offers several advantages over direct gene transfer methodologies (particle bombardment, electroporation, etc), such as the possibility to transfer only one or few copies of DNA fragments carrying the genes of interest at higher efficiencies with lower cost and the transfer of very large DNA fragments with minimal rearrangement (Gheysen et al. 1998; Hansen and Wright 1999; Shibata and Liu 2000).

Musa was generally regarded as recalcitrant for *Agrobacterium* mediated transformation. Hernandez (1999) has reported that *A. tumefaciens* is compati-

ble with banana indicating the potential for genetic transformation. The recovery of transgenic plants of banana obtained by means of *A. tumefaciens* mediated transformation has been reported. The protocol has been developed for *Agrobacterium* mediated transformation of embryogenic cell suspensions of the banana (Ganapathi et al. 2001; Khanna et al. 2004). At present most of the transformation protocol use cell suspension, however establishing cell suspension is lengthy process and cultivar dependent. The protocol has also been established using shoot tips from various cultivars of *Musa* (May et al. 1995; Tripathi et al. 2002, 2005a). This technique is applicable to a wide range of *Musa* cultivars irrespective of ploidy or genotype (Tripathi et al. 2003, 2005a). This process does not incorporate steps using disorganized cell cultures but uses micropropagation which has the important advantage that it allows regeneration of homogeneous populations of plants in a short period of time. This procedure offers several potential advantages over the use of embryogenic cell suspensions (ECS) as it allows for rapid transformation of *Musa* species.

Currently, the transgenes used for banana improvement have been exclusively isolated from heterologous sources like other plant species, insects, microbes and animals (Tripathi 2003; Tripathi et al. 2004, 2005b). For example the most attractive strategy for serious fungal disease like black Sigatoka control in *Musa* is the production of disease resistant plants through the transgenic approach including the expression of antifungal peptide genes from radish, onion and dahlia. Similarly, research is in progress at IITA for producing bacterial wilt disease (caused by *Xanthomonas campestris* pv. *musacearum*) resistant banana varieties using genes encoding for plant ferredoxin like protein *(pflp)* and hypersensitive response assisting protein *(hrap)* isolated from sweet pepper.

Since most cultivated varieties of banana are sterile and therefore do not set seed, traditional breeding by hybridization is difficult making genetic transformation a viable tool for improving bananas. Although attempts to produce transgenic bananas and plantains are proceeding slowly, public acceptance of these novel plants and their products should be encouraged through sound information and risk assessment studies. The chances of transfer of transgenes from field material to wild species (the major public concern) are expected to be negligible in *Musa* in view of the sterility of many cultivars.

15.8 Future Scope of Works

Bananas are staple food crops for over half a 100 million people in sub-Saharan Africa and over half a billion worldwide. Bananas are the developing world's fourth most important food crop after rice, wheat and corn. Despite these statistics, *Musa* is not included in international genome analysis initiatives. A Global *Musa* Genomics Consortium was established in 2001 with the goal of assuring the sustainability of banana as a staple food crop by developing an integrated genetic and genomic understanding, allowing targeted breeding, transformation and more efficient use of *Musa* biodiversity. Basically, the Consortium aims to apply genomics to the sustainable improvement of bananas. The consortium believes that genomic technologies such as analysis and sequencing of the banana genome, identification of its genes and their expression, recombination and diversity can be applied for the genetic improvement of the crop (Frison et al. 2004). However, large scale funding for this initiative has not been realized as yet.

Banana breeding is a complex procedure that is fraught with constraints such as female and male sterility and long generation times. *Musa* genomics can open up new avenues for more efficient breeding of the crop. It is important to investigate the possibilities via which the primary production and other uses of *Musa* can be promoted for the benefit of the growing world's population.

Strategies for future genomics research in *Musa* include the development molecular markers, construction of genetic and physical maps, identification of genes and gene expression and whole genome sequencing. Sequencing of other plant genomes such as *A. thaliana* and *O. sativa* has provided an enormous amount of data that could reveal unknown features of their genomes. Such data could also be generated for *Musa*. These include sequence composition of various genomic regions, an inventory of the various genic and non-genic sequences (genes and repetitive DNA such as satellites, mini- and macrosatellites, pseudogenes, retropseudogenes, retrotransposons, LINES, SINES, DNA transposons and many others), the distribution of various elements along the chromosomes, potential duplications, translocations, inversions, macro- and microsynteny, structure of centromeres and telomeres, the exact genome size, and number of open reading frames (Kahl 2004). Together with genome

sequencing, the handling of such data must be considered since bioinformatics for banana genomics has not been developed.

Although a number of marker techniques are now available for genomic research, they have not been as widely used in *Musa* as in some of the other crop plants. Markers that co-segregate with a trait can be exploited to accelerate the selection of that trait. This will be especially useful in *Musa* because of its long life cycle. New markers such as SNPs have not yet been applied to banana research and promise to have an impact on protein function. Genetic and physical mapping of the *Musa* genome will make it possible to isolate genes that can be used in genetic transformation. Although a map of a diploid banana is available a much greater effort in developing high density maps to identify QTLs is necessary for *Musa*. The development of ESTs and cDNA libraries are crucial areas of research in *Musa* that needs greater emphasis. In addition, no attempt has been made to design expression chips. Expression chips are being used in many laboratories for other crops. There is enormous potential for genetic manipulation of *Musa* species for disease and pest resistance using the existing transformation systems with the genes isolated from *Musa* genome. The use of appropriate gene constructs may allow the production of nematode, fungus, bacterial and virus-resistant plants in a significantly shorter period of time than using conventional breeding, especially if several traits can be introduced at the same time. It may also be possible to incorporate other characteristics such as drought tolerance, thus extending the geographic spread of banana and plantain production, and thus contributing significantly to food security and poverty alleviation in developing countries. Long-term and multiple disease resistance can be achieved by integrating several genes with different targets or modes of action into the plant genome. Technically, this can be done either in several consecutive steps or simultaneously.

Banana and plantain are regarded as 'orphan crops' or the 'poor man's fruit' with regards to research and the amount of funding devoted to the crop. Yet they are important plants in the subsistence diet of the poor millions. New diseases such as banana bacterial wilt threaten to wipe out the crop in many countries. A whole repertoire of techniques is now available to study the genomes of plants, including *Musa*. Such research will have a tremendous impact in *Musa* breeding and genomics.

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