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A Case for Molecular Breeding in *Musa*

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ABSTRACT

Conventional breeding of *Musa* that is based mainly on mass phenotypic recurrent selection is handicapped by a number of factors. The rapid development of molecular biology techniques and their application to plant breeding has resulted in significant genetic gains in agricultural crops. Marker assisted breeding will be very useful for a crop like banana that has a relatively long life cycle. DNA markers are being sought for several characters of importance in *Musa* including resistance to pests and diseases. Achievements and prospects of molecular breeding for black Sigatoka, Fusarium, Banana bunchy top virus (BBTV), nematodes and Xanthomonas wilt resistance are discussed. In addition gains made in nutritional enhancement of banana are described. The development of modern plant molecular and quantitative genetics in the last two decades has the potential to revolutionize what has mostly been experienced-based empirical plant breeding. This chapter outlines the value of modern molecular tools for molecular breeding of banana.

Keywords: breeding challenges, molecular markers, molecular breeding achievements

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List of abbreviations after the text.

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15.1 Introduction

Currently the production of improved banana and plantain cultivars that are nutritionally acceptable to consumers, with resistance or tolerance to biotic and abiotic stresses, and reduced post-harvest losses, has been met largely through conventional breeding that has made steady progress over the years producing a large number of hybrids (Rowe 1984; Vuylsteke et al. 1995). The pressure of an increasing population and consequent increase in demand for food on the one hand and the depletion of arable land on the other have placed new emphases on conventional plant breeding (Pillay et al. 2011). However, conventional breeding of *Musa* is handicapped by sterility and a number of other factors that are discussed in Pillay et al. (2002), and Pillay and Tripathi (2006, 2007).

The rapid development of molecular techniques and their application to plant breeding has resulted in significant genetic gains in agricultural crops, some of which have already entered the market (Newell-McGloughlin 2008). Molecular and biotechnological tools such as marker-assisted breeding, tissue culture, *in vitro* mutagenesis and genetic transformation can contribute to solving or reducing some of the constraints of conventional banana breeding. This chapter examines some aspects of molecular breeding in *Musa*.

15.1.1 Breeding Challenges in Musa

Musa is a polyploid crop with ploidy ranging from diploid ($2n = 2x = 22$) to tetraploids ($2n = 4x = 44$). Most cultivated bananas are triploids ($2n = 3x = 33$) and sterile harboring various combinations of either one, two or three A, B, S, or T genomes. New banana cultivars are exceptionally cumbersome to develop. Selection for desirable characters is time consuming and it may take up to 12 years to develop a new cultivar. *Musa* breeding is based mainly on phenotypic mass recurrent selection. The high levels of heterozygosity make identification of ideal parental material difficult and very large populations are required for selection of individual clones with good agronomic traits. This is virtually impossible to attain due to the low seed set in crosses. Generally, few seeds are obtained (an average of 1 to 1.5) and acquiring large numbers of seeds is a labor intensive and tedious process (Ortiz and Vuylsteke 1995; Ssebuliba et al. 2006a, b, 2009). The genes for resistance to diseases and pests are introgressed from wild diploid species. Wild species also carry many undesirable traits, e.g., low yield and non-parthenocary. The process of eliminating the unwanted traits requires several backcrosses that lengthen the breeding process. The multigenic nature and low heritability

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of some traits also slow down the breeding process. *Musa* breeding is also problematic due to the narrow genetic diversity of the germplasm (Pillay et al. 2001; Nyine and Pillay 2011) and the lack of information about wild species that carry useful agronomic traits. Only a few wild diploids have been used so far and mostly as male parents by majority of the breeding programs. As cultivated banana is propagated asexually, its genetic base is narrow with diversity dependent on somatic mutation. Limited genetic variation has resulted in a crop lacking resistance to fungal, bacterial and viral pathogens and numerous pests (Miller et al. 2009). Very little knowledge exists on the genetics of important agronomic traits in *Musa* and precise genetic control is known for relatively few traits (see Chapters 6, 7).

15.1.2 Production Constraints

The production constraints of *Musa* have been well documented (Pillay et al. 2002; Pillay and Tripathi 2006, 2007; Tenkouano et al. 2011). Briefly the production of bananas worldwide is threatened by a complex of foliar diseases, nematodes, viruses and pests. The use of resistant varieties is considered to be the most effective, economical and environmentally friendly approach to controlling diseases and pests. Two of the most important fungal diseases include black Sigatoka (*Mycosphaerella fijiensis* Morelet) and fusarium wilt (*Fusarium oxysporum* Schlect. f.sp. *cubense* (E.F. Smith). The main pests include a complex of nematodes (*Radopholus similis*, *Pratylenchus* spp. *Helicotylenchus*) and the banana weevil (*Cosmopolites sordidus* Germar). New diseases such as banana *Xanthomonas* wilt (BXW) have been recently identified in East Africa.

15.1.3 Breeding Objectives in Musa

The most important objectives of *Musa* breeding include:

- increased bunch size and yield
- host plant resistance against the major pathogens including those causing Sigatoka, Fusarium and Xanthomonas wilts, and viruses
- host plant resistance against nematodes and insect pests
- fruit quality traits, e.g., increased vitamin A, iron and zinc levels
- better adaptation to abiotic stresses such as drought, heat and other stresses that may be enforced by predictions in climate change.

Breeding for yield is a major target followed by breeding for host plant resistance to pathogens and pests that impact on yield.

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15.2 Molecular Breeding

Molecular breeding (MB) is the generic term used to describe several modern breeding methods including, (i) marker-assisted selection (MAS)—the selection of specific alleles for traits conditioned by a few loci, (ii) marker-assisted backcrossing (MABC)—the transfer of a limited number of loci from one genetic background to another, including transgenes, (iii) marker-assisted recurrent selection (MARS)—the identification and selection of several genomic regions involved in the expression of complex traits to "assemble" the best-performing genotype within a single, or across related, populations, and (iv) genome wide selection (GWS)—selection based on markers without significant testing and without identifying *a priori* a subset of markers associated with the trait (Ribaut et al. 2010). MABC is one of the most anticipated and frequently cited benefits of molecular markers as indirect selection tools in breeding programs (Semagn et al. 2006).

Routine use of MAS in ongoing plant breeding programs has not been achieved as yet. The implementation of MAS has been slow due to the high relative cost compared to conventional phenotypic selection. To be useful to plant breeders, gains made from MAS must be more cost-effective than gains through traditional breeding or MABC must generate significant time savings to justify the additional cost involved (Semagn et al. 2006).

Since a large number of traits in plants are polygenic, MABC of traits controlled by single genes is the most effective way of using DNA markers effectively. The improvement of quantitative trait loci (QTL) through MABC has produced variable results ranging from limited success and/or even a failure to a few highly successful stories (Semagn et al. 2006). Marker-assisted breeding will be very useful for a crop like banana that has a relatively long life cycle. The use of molecular markers for the indirect selection of improved cultivars speeds up the selection process by alleviating time-consuming approaches of direct screening under greenhouse or field conditions. Some of the most important characters to the *Musa* breeder have been reported to have an oligogenic epistatic basis (Ortiz 1995).

15.2.1 Molecular Markers in Musa

It is now generally accepted that molecular markers represent the most significant advance in breeding technology in the last few decades and are currently the most important application of molecular biology to plant breeding. There appears to be no resistance to the use of molecular marker technology in breeding as there is for genetically-modified organisms (Pillay et al. 2011). DNA markers are being sought for several characters of importance in *Musa* including resistance to pests and diseases. Fruit quality (color, texture, ripening) are other candidate traits for selecting with

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DNA markers. Most of these traits are expressed only late in the life cycle of the plants or are difficult to screen. Identification of markers linked to loci governing important traits will facilitate gene introgression and other MAS applications. Accessing genes from various genomes, including the S (*M. schizocarpa*) and T (*M. textilis*) genomes will increasingly become important for *Musa* breeding. To date very few markers have been linked to traits of interest in *Musa* and are limited to markers for disease resistance and the main genomes.

Methylation-sensitive amplification polymorphism (MSAP) markers were used to identify molecular markers associated with resistance to *Mycosphaerella fijiensis* toxins (black Sigatoka) with a set of reference cultivars and somaclonal variants (Gimenez et al. 2006). The study identified four MSAP markers that were associated with resistance to *M. fijiensis* toxins. The MSAP markers showed a high degree of sequence similarity with resistance gene analogs and with retrotransposon sequences. These markers were cited as being useful as molecular indicators of tolerance to *M. fijiensis* toxins and resistance to black Sigatoka.

A reliable molecular method to detect *Fusarium oxysporum* f.sp. *cubense* (*Foc*) race 4 isolates in Taiwan was developed by (Lin et al. 2010). By PCR amplification, the primer set *Foc*-1/*Foc*-2 derived from the sequence of a random primer OP-A02 amplified fragment produced a 242 bp size DNA fragment, which was specific to *Foc* race 4. With the optimized PCR parameters, the molecular method was sensitive and could detect small quantities of *Foc* DNA as low as 10 pg in 50 to 2,000 ng host genomic DNA with high efficiency.

A putative RAPD marker for Sigatoka resistance has been identified at the National Research Center for Banana (NRCB), India. The marker has been cloned, sequenced and converted into a sequence characterized amplified region (SCAR) marker and is being validated using contrasting parents for expression of Sigatoka (*M. musicola*) disease resistance and their progenies. Parallel studies have led to the identification of a putative random amplified polymorphic DNA (RAPD) marker for nematode resistance (S. Uma, pers. comm.) An RAPD marker has been identified for salt tolerance among clones of cv. "Dwarf Cavendish" that were obtained through induced mutagenesis (Miri et al. 2009).

A banana somatic embryogenesis receptor-like kinase (*SERK*) gene, designated as *MaSERK1*, isolated from *Musa acuminata* cv. "Mas" (AA) was associated with somatic embryogenic competence and disease resistance response in *Musa* (Xia et al. 2010). The gene encoded a protein of 628 amino acids with identities of above 82% to *SERK* genes in coconut, rice, maize, *Arabidopsis*, carrot, and *Medicago truncatula*. *MaSERK1* was expressed weakly in male flower clusters, but not in male flower-derived non-embryogenic calli. It was highly expressed in male flower-derived embryogenic calli

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and embryogenic cell suspensions (ECS). The frequency of somatic embryogenesis of ECS positively correlated with *MaSERK1* transcript levels. *MaSERK1* expression in leaves of cultivar "Dongguan Dajiao" (ABB), known to be resistant to *FOC* race 4, was induced by exogenous salicylic acid (SA) or inoculation with *FOC* race 4. However, *MaSERK1* expression levels in leaves of "Pisang awak" (ABB), known to be susceptible to *FOC* race 4, did not change following either treatment (Xia et al. 2010). It was suggested that *MaSERK1* gene expression not only could serve as a molecular marker for banana somatic embryogenesis, but could also play a role in host plant resistance response to banana pathogens.

15.2.2 Marker-Assisted Introgression

Marker-assisted breeding takes advantage of the association between agronomic traits and allelic variants of genetic markers, mostly molecular markers (Stam 2003). Generally these associations are the result of genetic linkages between markers and gene loci underlying the trait(s) of interest. These associations are also known as linkage disequilibrium. Linkage disequilibrium arise in experimental populations used for linkage mapping, e.g., backcross generations (BC), F_2 segregating populations, recombinant inbred lines (RILs) or doubled haploids (DHs) (Stam 2003). In cross-fertilizing plant species such as *Musa* a mapping population usually consists of a large full-sib family resulting from a cross between single plants of divergent genotypes. Before a plant breeder can utilize linkage-based associations between traits and markers, the associations have to be assessed with a certain degree of accuracy, such that it can be safely relied on, and thus marker genotypes can be used as indicators or predictors of trait genotypes and phenotypes (Stam 2003). For monogenic traits with a clear qualitative contrast between genotypes, such as a single gene-based host plant resistance to pathogens, the assessment of association is straightforward: mapping a monogenic trait goes along with the mapping of markers. For quantitative, multigenic traits, however, a reliable assessment of trait-marker association requires large-scale field experiments as well as statistical techniques, known as QTL mapping (Stam 2003). Progress in the breeding of plantain and banana has been restricted by the complex genetic structure and behavior of cultivated polyploid *Musa*. Mapping in *Musa* has been hampered by the low levels of male and female fertility and seed viability and the absence of large segregating populations. The key to successfully integrating marker-aided breeding into breeding programs will lie in identifying applications in which markers offer real advantages over conventional breeding methods or complement them in novel ways (Semagn et al. 2006). Marker-aided breeding offers significant advantages in the following cases.

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1) When phenotypic screening is expensive, difficult or impossible.
2) When the trait is of low heritability (incorporating genes that are highly affected by environment).
3) When the selected trait is expressed late in plant development, like fruit and flower features or adult characters in species with a juvenile period.
4) For incorporating genes for host plant resistance to pathogens or pests that cannot be easily screened for due to special requirement for the gene to be expressed.
5) When the expression of the target gene is recessive.
6) To accumulate multiple genes for one or more traits within the same cultivar, a process used is called gene pyramiding (Sharma et al. 2004; Barone et al. 2005; Yang et al. 2005).

Highly precise MAS approaches require the development of high density linkage maps. Improved molecular markers systems are required to enhance the adoption of MAS. Several factors are important when considering MAS including ease of use, robustness, cost and linkage to trait of interest (de Koeyer et al. 2010). The ideal marker systems for polyploid crops should be dosage sensitive and have the ability to distinguish heterozygous genotypes with multiple haplotypes within the target genomic region by the marker (de Koeyer et al. 2010).

Currently, several molecular marker methods have been used in *Musa* and these differ from each other in their technical requirements, sensitivity and reliability (see Chapter 4). New markers systems are available and have not been exploited in *Musa* as yet.

15.2.3 Gene Pyramiding

Gene pyramiding is the accumulation of multiple genes for one or more traits within the same cultivar (Barone et al. 2005; XiangYan et al. 2005). Genetic stocks produced from gene pyramiding can be used in breeding programs. Gene pyramiding is a very useful approach for the introgression of genes controlling different agronomic traits into one cultivar to ensure that the cultivar has acquired several traits simultaneously (Semagn et al. 2006). For example, genes leading to host plant resistance to different races or biotypes of a pathogen or an insect pest can be pyramided together to make a line with multi-race or multi-biotype resistances, which could be more durable than any single-race or single-biotype resistance (Jiang et al. 2004). The joint expression of pyramided genes was found to provide numerical increases or a broader spectrum of host plant resistance over that conferred by single genes through gene interaction and quantitative complementation (Yoshimura et al. 1995; Singh et al. 2001). Gene pyramiding

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has been successfully applied in several crop breeding programs, and many cultivars and lines possessing multiple attributes have been produced (Porter et al. 2000; Wang et al. 2001; Samis et al. 2002; Jiang et al. 2004).

Traits which are traditionally regarded as quantitative and not targeted by gene pyramiding programs can be improved using gene pyramiding if major genes affecting the trait are identified (Ashikari and Matsuoka 2006). Gene pyramiding is, however, difficult using conventional breeding methods due to the dominance and epistasis effects of genes governing disease resistance (the stronger resistance genes will always mask the less strong, which cannot be revealed without screening using a virulent strain on the former—in itself undesirable) (Semagn et al. 2006). Moreover, genes with similar reactions to two or more races—so called race-non specific or partial resistance—are difficult to identify and transfer through conventional approaches (Singh et al. 2001), and virtually impossible if stronger race-specific genes are present.

Gene pyramiding programs are also thought to be highly cost intensive. While the breeder's work will be made easier by using a single donor, phenotyping is still required to select the desired segregants in field experiments. It is also highly likely that whatever genes are being stacked into one cultivar might lose their usefulness by the time they are pyramided and subsequently by the time they are used by the breeder.

15.2.4 Marker Systems and Germplasm Characterization

The most frequent use of molecular marker methods in *Musa* has been limited to germplasm characterization and diversity analysis. There are about 1,500 to 3,000 *Musa* accessions with a wide range of morphological variation and genome constitutions (Heslop-Harrison and Schwarzacher 2007) within the germplasm. About 1,000 *Musa* cultivars and 180 wild species, are maintained in tissue culture at the International Transit Centre (ITC) in the Catholic University of Leuven (KULeuven) in Belgium, and these provide a valuable reference collection that is mostly in the public domain and freely accessible for research and breeding. Numerous banana researchers in Asia have developed field-based germplasm collections and well-curated internet databases are now disseminating information about these collections (Pollefeys et al. 2004). Although diversity can be assessed by morphology and flow cytometry, these analyses have limitations and there remain questions about the presence of multiple genotypes with a single name or a single genotype with multiple names (Heslop-Harrison and Schwarzacher 2007). Therefore, DNA-based molecular diversity studies

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will help to direct plant breeders towards appropriate germplasm to test and select, and to focus germplasm collections towards representing the full range of diversity present in the genus at all ploidy levels (Heslop-Harrison and Schwarzacher 2007). The various techniques used to assess *Musa* diversity and phylogenetic relationships have been addressed in Chapter 3.

15.2.5 Transgenic Breeding

Conventional breeding of bananas is hindered by a number of factors including the long-generation time, triploidy and sterility of most edible cultivars (Pillay et al. 2002). Sources of resistance to many of the major pests and diseases have been identified in a few wild diploid species. However, most landraces are often sterile and cannot be used in breeding, while crosses involving wild species result in the transfer of many unwanted traits together with the desired resistance genes. Furthermore, there are certain diseases such as banana bunchy top virus (BBTV) for which sources of resistance are not known (Sagi et al. 1998).

Although conventional breeding of *Musa* is faced with difficulties, currently available transformation methods may not solve all these difficulties. Increased understanding of responses in *Musa* to biotic and abiotic stresses may provide new opportunities for genetic improvement.

Genetic transformation provides an opportunity for single genes or gene combinations, such as those associated with host plant resistance to pathogens, to be extracted from the genome of the source organism and transferred directly into the desired cultivar, which allows to retain all the original characteristics of the cultivar and adding the desired trait. Furthermore, since most banana cultivars do not produce seeds under natural conditions, crosses with other cultivars or species will seldom occur. In these cases, the introduced gene remains confined to the cultivar in which it has been introduced (Sagi et al. 1998).

Relative success in genetic engineering of bananas and plantains has been achieved enabling the transfer of foreign genes into some cultivars (Sagi et al. 2007). But the scarcity of useful genes, factors that affect transgene expression such as RNA interference, interactions between transgenes and those already present in the plant and the quantitative nature of some traits are still problems that must be considered before accepting that genetic transformation is the only choice for *Musa* improvement. Protocols for the introduction of genes, including the efficient regeneration of shoots in tissue cultures, and transformation methods still remain as major bottlenecks in genetic engineering (Sharma et al. 2005).

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15.3 Achievements and Prospects of Transgenic Breeding in *Musa*

15.3.1 Resistance to Black Sigatoka and Fusarium Wilt Disease

Transformation of banana and plantains for fungal diseases started in the 1990s (Sagi et al. 1995). Various transformation techniques have been used to produce transgenic bananas for the cultivars "Williams" (AAA) export banana, "Gros Michel" (AAA) fruit banana, "Bluggoe" (ABB) cooking banana and "Three Hand Planty" (AAB) plantain with antifungal peptides which are highly active *in vitro* against major pathogenic fungi such as black Sigatoka and Fusarium wilt of bananas (Remy et al. 2000). The transgenics showed resistance to black Sigatoka under laboratory conditions. Antimicrobial proteins (AMPs) which are stable, cysteine-rich small peptides isolated from seeds of diverse plant species were also used in developing transgenics for fungal diseases (Sagi et al. 1998).

Improved resistance to Sigatoka was obtained when banana was transformed with the endochitinase gene *ThEn-42* from *Trichoderma harzianum* and the grape stilbene synthase (*StSy*) gene (Vishnevetsky et al. 2010). The superoxide dismutase gene *Cu,Zn-SOD* from tomato, under control of the ubiquitin promoter, was also added to this cassette to improve scavenging of free radicals generated during fungal attack. A 4-year field trial demonstrated several transgenic banana lines with improved tolerance to Sigatoka. Since the genes conferring Sigatoka tolerance may have a wide range of antifungal activities the regenerated banana plants were also inoculated with the fungus *Botrytis cinerea*. The best transgenic lines exhibiting Sigatoka tolerance were also found to have tolerance to *B. cinerea* in laboratory assays (Vishnevetsky et al. 2010). Gene discovery via analysis of EST data from cDNA libraries produced from *Mycosphaerella fijiensis*-infected leaf material from *M. acuminata* ssp. *burmannicoides* "Calcutta 4" (resistant) and "Grande Naine" (AAA genome, susceptible) is ongoing in Brazil (Miller et al. 2009)

15.3.2 Resistance to Banana Bunchy Top Virus (BBTV)

Transgenic research to develop resistance to BBTV has been in progress in Australia and Hawaii. The replication initiation protein (Rep) of nanoviruses is the only viral protein essential for viral replication and represents an ideal target for pathogen derived resistance. In Australia, a Rep-encoded protein (DNA-S1) was identified that suppressed the replication of BBTV. Different constructs of the *Rep* gene were shown to significantly suppress

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replication of BBTV in banana embryogenic cell suspensions (Tsao and Tsun-Hui 2008). Using such constructs, transgenic bananas with resistance to BBTV have been developed in Australia and Hawaii. The resistant lines have been field tested in Hawaii.

15.3.3 Resistance to Nematodes

The approach adopted for nematode resistance in *Musa* relies on introducing an additional plant gene coding for a protein called cystatin that prevents the digestion in parasitic nematodes. The cystatin suppresses the nematode's ability to grow, lay eggs and build to population levels that damage crops. The advantage of using cystatins is that they are part of the human diet (e.g., present in cereal seeds or eggs) and have no effect on our digestion or health. This approach has been already been used in developing a transgenic Cavendish bananas (AAA) that showed resistance to *Radopholous similis* one of the major nematodes of *Musa* (Atkinson et al. 2004).

15.3.4 Resistance to Banana Xanthomonas Wilt

Banana Xanthomonas or bacterial wilt disease caused by infection with *Xanthomonas campestris* pv. *musacearum* (BXW) has reached epidemic proportions in the Great Lakes region of East and Central Africa (Biruma et al. 2007). The lack of banana germplasm exhibiting resistance to the disease makes it an ideal target for transformation. Transgenic technologies may hold the key for developing bananas that are resistant to the BXW pandemic. The ferredoxin-like amphipathic protein (*pflp*) and hypersensitive response assisting protein (*hrap*), isolated from sweet pepper (*Capsicum annuum*) are novel proteins that can intensify the harpinPSS-mediated hypersensitive response (Chen et al. 2000). Transgenic rice carrying the *pflp* gene showed enhanced resistance to *Xanthomonas oryzae* pv. *oryzae* (Tang et al. 2001). The *pflp* has also been shown to enhance resistance in transgenic orchids against *E. carotovora* (Liau et al. 2003). The elicitor-induced resistance is not specific against particular pathogens, so it could be a very useful strategy for developing broad spectrum resistance. This strategy has been used for developing transgenic banana with resistance to Xanthomonas wilt. Transgenic lines with *pflp* or *hrap* genes have been developed using a protocol based on the *Agrobacterium tumefaciens* technology (Tripathi et al. 2009). These transformed lines of various cultivars have been validated via PCR assay and Southern blot analysis. They have been tested for disease resistance under laboratory conditions and transgenic bananas showed complete resistance. The transgenic lines are now under field trials in Uganda (Tripathi et al. 2010).

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15.3.5 Nutritional Enhancement

Banana is rich in natural antioxidants such as vitamin C and vitamin E (Someya et al. 2002; Amorim et al. 2009a, b). High levels of vitamin A deficiency leads to serious health problems, especially in children in low-income regions of the world, such as parts of Asia, Africa and Latin America (Bloem et al. 2005). Micronutrient deficiencies of iron and zinc also results in serious health problems such as mental and physical retardation, reduced resistance to infections and hypogonadism (Whittaker 1998). The genetic enhancement of micronutrient content (i.e., biofortification) of banana by conventional breeding combined with the use of biotechnological tools has the potential to increase the concentrations of micronutrients (Fe, Zn) and vitamin A in new cultivars (Amorim et al. 2011). Improving the nutritional content of *Musa* would have a significant impact on vitamin and nutrient intake for millions of people who depend on the crop for food.

Researchers in Australia are transforming bananas for increased vitamin A, vitamin E or iron. A large suite of both fruit-specific and constitutive promoters that drive pro-vitamin A, vitamin E, or iron accumulation genes, have been cloned into vectors. Four cultivars "Nakinyika", "Mpologoma", "Nakasabira", and "Sukalindizi" have been selected for this study (Dale and Tushemeirewe 2008). A study by Fungo and Pillay (2011) showed that cultivars "Nakitembe", "Entukura" and "Nakhaki" had the highest levels of vitamin A among 10 East African Highland bananas in Uganda and these cultivars may be suited for transformation studies for micronutrients.

15.4 Limitations and Prospects of MAS

One of the major limitations in the use of MAS is the high costs associated with the identification and verification of genetic markers, development of genetic maps, etc. Economics is the key determinant for the application of molecular markers in genetic improvement programs (Dekkers and Hospital 2002). Other factors that influence the cost of utilizing marker-aided breeding include inheritance of the trait, method of phenotypic evaluation, and high costs. The main factors that slow down using molecular breeding technologies in most developing countries include poor infrastructure; inadequate capacity and operational support; and lack of an enabling policy, statutory and regulatory framework at country level, which in turn affects research institutions. Despite these difficulties some developing countries are making progress in using biotechnology for *Musa* improvement.

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15.5 Conclusion

Although conventional breeding programs have their limitations, they have shown over time that they can be highly successful. Genome manipulations and interspecific crosses have allowed considerable genetic progress in *Musa* breeding but much remains to be done in the identification of parental combinations that are likely to produce progenies with both high mean and genetic variability (Tenkouano 2001). The development of modern plant molecular and quantitative genetics in the last two decades has the potential to revolutionize what has mostly been experience-based empirical plant breeding (Ye and Smith 2008). Molecular breeding is expected to improve the efficiency of crop breeding by selecting and stacking favorable alleles at target loci (Ribaut et al. 2010). New developments and improvements in marker technology, the integration of functional genomics with QTL mapping, and the availability of more high-density maps are the other factors that will greatly affect the efficiency and effectiveness of QTL mapping and marker-aided breeding in the future (Collard et al. 2005). The development of high-density maps that incorporate new marker types, such as single nucleotide polymorphisms (SNPs) and expressed sequence tags (EST) will provide researchers with a greater arsenal of tools for QTL mapping and marker-aided breeding (Semagn et al. 2010). The number of EST and genomic sequences available in databases is growing rapidly (especially from genome sequencing projects), and the accumulation of these sequences will be extremely useful for the discovery of SNPs and data mining for new markers in the future (Gupta et al. 2001). The potential genetic and economic benefits of using molecular breeding need to be critically compared to those achieved or expected from any existing conventional breeding programs.

Abbreviations

AMPs	:	antimicrobial proteins
BBTV	:	banana bunchy top virus
GWS	:	genome wide selection
MABC	:	marker-assisted backcrossing
MARS	:	marker assisted recurrent selection
MAS	:	marker-assisted selection
MB	:	molecular breeding
MSAP	:	methylation-sensitive amplification polymorphism

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PCR	:	polymerase chain reaction
pflp	:	ferredoxin-like amphipathic protein
QTL	:	quantitative trait loci
RAPD	:	random amplified polymorphic DNA

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