Genes and Markers: Application in Banana Crop Improvement

3

Manosh Kumar Biswas and Ganjun Yi

Abstract

Banana is one of the most economically important horticultural crops. Its improvement is a big challenge to breeders due to complex genomic nature and lack of advanced knowledge of its genetics. In the recent years, banana scientists around the globe have made considerable progress in its improvement through gene identification and subsequently its transformation into cultivars and have developed trait-linked markers to speed up the selection process in the cross-breeding program. Two genomes of the diploid banana *Musa acuminata* (A genome) and *Musa balbisiana* (B genome) have been sequenced and made publicly available. This facilitated the identification of agronomically important trait-linked genes and studies on their function, mode of inheritance, evolution and development of gene-linked markers, the landmark of the gene on the chromosome. Identification and characterization of important genes is the key to any breeding program. In bananas, several genes associated with disease resistance and fruit quality have been isolated, and their molecular characterization reported. Several molecular markers have been developed, including RAPD, ISSR, AFLP, DArT, and SSR, for the genomic group identification, estimation of genetic stability of somaclonal variation, disease-resistant cultivar identification, etc,. This chapter summarizes the progress of the application of agronomically important gene and molecular markers in banana breeding programs around the world.

Keywords

Genetic markers • Diversity • *Musa*

M.K. Biswas (✉) • G. Yi
Institution of Fruit Tree Research, Guangdong Academy of Agricultural Sciences, Guangzhou, Guangdong, China

The College of Life Science, South China Agricultural University, Guangzhou, China
e-mail: mkbcit@ymail.com

3.1 Introduction

Banana is one of the commercially important crops that belongs to the family Musaceae and essential for food security in many tropical and

S. Mohandas, K.V. Ravishankar (eds.), *Banana: Genomics and Transgenic Approaches for Genetic Improvement*, DOI 10.1007/978-981-10-1585-4_3

subtropical countries (D'Hont et al. 2012). Due to its nutritional value, it has become one of the vital foodstuffs in our daily life (Biswas et al. 2015). As a consequence, the banana-producing area has increased, the processing industry has strengthened, and globally banana trade has expanded. A special emphasis has now been given to its improvements in many countries according to the consumer demand. In recent years, several research groups around the globe have initiated banana improvement projects using conventional and advance breeding technology such as cross-breeding, marker-assisted selection, transgenic breeding, biotechnological approach (micropropagation, somaclonal variation, protoplast culture), radiation breeding, etc. There are lots of obstacles in banana breeding due to its complex taxonomy, ploidy nature, low level of seed fertility and seed viability, and genome complexity. Recently, two genomes of banana have been sequenced which has facilitated the understanding of its genomics. A deep insight into banana genetics provides an enormous opportunity to elucidate the function of genes of interest and also to detect variable (or polymorphic) regions in the genome that are associated with agronomic traits (Liu 1997). Therefore, the genome sequence information of banana is the valuable resource to identify genes of interest and develop markers for the tagging of important agronomical traits. Banana scientists around the world are concentrating their view on genomics of banana to pull out pieces of vital information for the development of a marker and to identify genes responsible for disease, stress, yield, quality of fruits, etc.

In this chapter, we illustrate the present scenario in banana breeding programs as well as banana research around the world and also try to address how to overcome the difficulties in banana breeding.

3.2 Gene

Gene is the basic molecular unit of heredity that contains a series of nucleotides in which instructions are contained for the synthesis of RNA that translates into protein. Understanding its gene structure, function, evolution, and mode of inheritance is the key for improvement of any crop species. Crop improvement generally involves the insertion of important genes from cultivated or wild species to a cultivar. Most of the wild banana cultivars rich with disease-resistant, stress-tolerant, and pest- and nematode-resistant genes carry many other undesirable traits such as low yield, seedy fruits, nonedible fruits, etc., while edible banana cultivars carry only good fruit qualities. Therefore, important traits such as disease resistance need to be transferred to the cultivars for its improvement. There are two different strategies commonly used in banana crop improvements viz., conventional cross-breeding and transgenic breeding approach.

3.2.1 Identification and Characterization of Agronomical Important Genes in Banana

Genes are the functional parts of the genome and are directly linked to the agronomical traits of the crops. Identification and functional characterization of agronomically important genes is really a big challenge. It is essential to know the information on its molecular structure, position on the genome, evolution, segregation patterns, etc., for understanding how genes control traits. Recently, two banana genomes (A and B genomes) have been sequenced that speeds up the identification of important genes for subsequent application in the improvement of traits of the banana. Annotation of these genomes identified 36542 and 36638 protein-coding gene models in A and B genomes, respectively (D'Hont et al. 2012; Davey et al. 2013). This protein-coding gene model data set is the valuable resource for further identification and characterization of agronomically important genes in banana. In banana, disease-resistant genes are considered to be more important agronomical traits because several diseases such as black sigatoka, *Fusarium* wilt and bunchy top greatly hamper banana production around the world. Furthermore, most of the culti-

vated bananas are highly susceptible to the disease and they are vegetatively propagated. So, there is a high possibility of destruction of banana plantation in any particular region once the disease breaks out. Banana breeders have hence given more importance to disease resistance breeding. Several disease-resistant genes have been identified and characterized in banana; among them, NBS-RGCs, *Pto*-RGCs, and NPR1 are notable. Zhao et al. (2009) isolated and characterized *NPR1*-like gene from a local banana cultivar Dongguan Dajiao (*Musa* spp. ABB); this cultivar is well known to be resistant to *Fusarium oxysporum* f.sp. *cubense* (FOC) race 4. In this study, the author noted that this gene is induced by exogenous application of salicylic acid in cv. Dongguan Dajiao but not in cv. Fenjiao which is an FOC race 4 susceptible variety (Zhao et al. 2009). Therefore, this gene has a significant role in the combat FOC race 4 in banana, and it might be a promising candidate for a resistant variety breeding in banana through a transgenic approach. Carotenoids' biosynthesis gene (*PSY*) was isolated from the banana cv. Asupina and cv. Cavendish for understanding the mechanism of pro-vitamin A carotenoid (pVAC) accumulation in banana cultivars. This study showed that banana *PSY* gene is encoded by two paralogs (*PSY1* and *PSY2*) where in *PSY2* is highly expressed in fruit pulp than in the leaf. The enzymatic activity of Asupina PSYs is double to that of Cavendish PSYs; that is why Asupina and Cavendish accumulate different levels of fruit pVAC content (Mlalazi et al. 2012). Consequently, *A-PSY2a* is a good candidate for the genetic improvement of fruit pVAC content in bananas. In addition, gene discovery in banana is in progress using cDNA library sequencing and functional annotation.

3.2.2 Introduce or Transfer Important Genes for Improvement of Banana

In order to introduce/transfer new genes (or traits) into cultivars from their wild relatives of banana, two different strategies have been widely used, conventional breeding and transgenic breeding. Improvement of commercial variety of banana with important agronomic traits through cross-breeding approach is inefficient due to its triploidy and sterility. So, transgenic breeding approach overcomes the limitation of commercial banana variety improvement. A well-characterized gene could be cloned from the wild species of banana or other plant species and transferred to commercial cultivars. Furthermore, this approach is significantly effective only for the traits controlled by a single gene. Genetic transformation and transgenic regeneration method are well established for the banana plant. But to date, there is no significant progress of transgenic breeding in banana compared to other crop plants. In order to improve black sigatoka disease resistance in banana, several genes from different organisms (gene *ThEn*-42 from *Trichoderma harzianum*, *StSy* from grape, *Cu*, *Zn-SOD* from tomato) have been cloned and transferred to banana. After a 4-year field trial, some of the transgenic lines showed improved tolerance to black sigatoka disease (Vishnevetsky et al. 2011). The replication initiation protein-encoded gene (*Rep*) was transferred to the banana to develop a banana bunchy top virus-resistant cultivar (Tsao 2008). A plant gene that encoded the protein called cystatin is effective against nematodes. This gene which has been transferred to the Cavendish banana improved its tolerance to the nematode (Atkinson et al. 2004). Genes *pflp* and *hrap* were cloned from sweet potato and transferred to banana cultivars. *In vitro* assays showed that the transgenic lines were completely resistant to banana *Xanthomonas* wilt disease (Tripathi et al. 2009). A gene *AMP1* encoding antimicrobial peptide was cloned from onion seeds and transformed in banana cultivar Rasthali (AAB, Silk gp). In this study, embryogenic cells of cv. "Rasthali" were transformed with *Agrobacterium* strain LB4404 harboring binary vector pCAMBIA2301 containing gene *Ace*-AMP1. Transgenic plants were then tested with their performance against *Fusarium oxysporum* f.sp. *cubense* race1 (Foc), and results suggest that *Ace*-AMP1 gene is able to improve resistance of banana against Fusarium wilt disease (Mohandas

et al. 2013) under glass house conditions. To facilitate the improvement in nutritional content of banana with vitamin A, vitamin E, or iron, an Austrian scientist transferred vitamin A, vitamin E, or iron accumulation genes in banana cv. "Nakinyika," "Mpologoma," "Nakasabira," and "Sukalindizi" (Pillay et al. 2012b).

3.3 Genetic Markers

A genetic marker is a visible phenotype or biochemical compound or gene or a segment of DNA that can be used to identify individuals or species. Genetic markers are inherited and segregated in Mendelian manner. Based on the principle or methodology, genetic markers are of three types: morphological markers, biochemical markers, and molecular markers. These genetic markers further classified into several subgroups according to the technology and principle.

3.3.1 Overview of Different Marker Technology Applied in Banana Research

3.3.1.1 Morphological Markers

A morphological marker is the visible, prominent phenotype that can be used to distinguish one or more individuals from a population. Agronomical traits such as plant height, leaf architecture, seed coat color, flower color, fruit flavor, leaf aroma, etc., are used as morphological markers to select individual in plant breeding. In banana leaf, architecture and texture are commonly used as morphological markers. For example, the leaf arrangement of diploid banana genotypes is upright (around 45° angle), triploid genotype is horizontal, and tetraploid genotype is downward. Parthenocarpy is one of the most important agronomic traits of banana. Most of the edible banana cultivars are parthenocarpic, they don't produce seeds, and they are triploids. Consequently, this trait was also used to identify breeding materials in the banana breeding program. In 1955, Simmonds and Shepherd discovered banana hybrid identification system using 15 prominent traits, that are highly polymorphic between *M. acuminata* and *M. balbisiana* (Simmonds and Shepherd 1955). Among these, 13 traits were related to the reproductive organ, such as peduncle of inflorescence, pedicels of flower, male flower color, free tepal, stigma color, and arrangement of ovules, and the other seven were related to the bract of the male bud (shape, apex, shoulder, curling, color, color fading, and scars), while the other two were pseudostem color and petiolar canal of the leaves. Based on these phenotypic scoring systems, the *M. acuminata* × *M. balbisiana* hybrids could be characterized into different ploidy levels (diploid, triploid, and tetraploid) and five genetic groups (AA, AB, AAA, AAB, and ABB). Most of the morphological markers are polygenic and highly influenced by the environment. That is why the utility of morphological markers in plant breeding is limited. However, this type of marker is still used in banana breeding, only where other markers' assay facilities are not available.

3.3.1.2 Biochemical Markers

Biochemical markers are the molecules or the products of genes such as proteins/enzymes which are polymorphic in their structure as well as their amino acid composition. For example, isozymes are encoded by the homologous gene located in the different loci in the genome, while allozymes are allelic variants of the same gene. Allozymes and isozymes move at different speeds through a gel because they differ from each other in size, structure, and charge. So, allelic variation can be detected among the individual in a population by using this type of markers. Bonner et al. (1974) first used peroxidase isoenzyme to distinguish banana clones and species. Unfortunately, they failed to distinguish banana clones and species. Thereafter, Rivera (1983) successfully distinguished ABB/BBB (Saba) and ABB (Bluggoe) genomic group using peroxidase and polyphenoloxiydase. After that several researchers around the globe used bio-

chemical markers to characterize banana clones, hybrids, and species, and it was popular until the molecular markers were employed in banana (Rivera 1983; Jarret and Litz 1986a, b; Bhat et al. 1992a, b; Mandal et al. 2001; Megia et al. 2001; Dhanya et al. 2006). The biochemical markers are sensitive to the environment and have a reproducibility problem.

3.3.1.3 Molecular Markers

A molecular marker is a fragment of DNA that is located in a particular region of the genome and variable between two individuals. In the past decade, advances have been made in the molecular marker technology, and several marker technologies have been invented by a molecular biologist. The use of these marker technologies for crop improvement is now a routine work. Based on the principle of molecular marker technology, it can be categorized into several subgroups. The most useful and popular marker technologies that are commonly used in the banana crop improvement program are discussed herewith.

3.3.1.3.1 Restriction Fragment Length Polymorphism (RFLP)

RFLP marker is non-PCR-based molecular marker technique. The variation among the genotypes obtained from this technique is based on restriction site variation of the studied individuals. Restriction site is frequent in the genome, and it is variable between two individuals because the presence of restriction enzyme cleavage site in the genome of two different individuals may not be the same. The presence of restriction site variation between two sister individuals might be due to mutation, crossing over, genome duplication, insertion, or deletion of the DNA fragments. Restriction fragment length polymorphism (RFLP) is a robust, codominant in nature, highly reproducible, and able to characterize heterozygote and homozygote condition of the individual. RFLP markers successfully applied in banana genetics and breeding are useful in estimating variation in the chloroplast genome of the banana (Gawel and Jarret 1991; Baurens et al. 1997), phylogenetic relationship of the banana and its relatives (Gawel et al. 1992; Jarret et al. 1992; Wong et al. 2001), mapping (Miller et al. 2008), etc,. RFLP markers are often used in combination with other types of markers, such as RAPD, SSR, etc., in banana breeding (Bhat et al. 1995), although RFLP markers are not suitable for large-scale utilization in the breeding program because of their complicated assay technique, their expensive nature, and their requirement of sophisticated equipments.

3.3.1.3.2 Variable Number of Tandem Repeats (VNTR)

The VNTR marker also known as non-PCR-based marker technology is used in plant genetics and breeding research. This technique involves restriction digestion followed by the hybridization with the probe containing minisatellite sequences. In principle, the VNTR marker is the fragment of the genome composed of tandem repeat units of a 10–50-base motif (known as minisatellite) and flanked by the conserved DNA restriction site. Many reports showed that tandem repeats are highly abundant and randomly distributed throughout the plant genome (Biswas et al. 2015; Manzo-Sánchez 2008). Therefore tandem repeat containing fragments of the genome could be the vital source for development of a large number of VNTR markers. This marker is highly polymorphic and reproducible, but its assay technique is complex and costly. So this marker is not popular with banana researchers. Several VNTR markers have been developed in *Musa* as reported by Crouch et al. (1998, 1999a) and Kaemmer et al. (1997), and used in genotyping and characterization of *Musa* breeding populations. Recent advances in the genome sequence technology and availability of the whole genome sequence of *Musa* spp. provided the opportunity to develop *Musa* genome-specific VNTR marker. This marker can be useful in banana research for DNA fingerprinting, identification of varieties, cultivars, and individuals, and also at population-level study.

3.3.1.3.3 Random Amplified Polymorphic DNA (RAPD)

This marker is well known for its simple assay system. But a major drawback of this marker is reproducibility. Evidences showed that different laboratories achieved different results when tested under identical PCR parameters and conditions (Pillay et al. 2012a). The common uses of RAPD marker in banana research are to:

Detect diverse genotypes (Kaemmer et al. 1992; Howell et al. 1994; Bhat and Jarret 1995; Uma et al. 2006; Jain et al. 2007).

Identify duplicate accessions from the *in vitro* and *ex vitro* germplasm collection (Ray et al. 2006).

Identify somaclonal variation (Dhanapal et al. 2014).

Differentiate *Musa* genomic group (Howell et al. 1994; Rekha et al. 2001), genetic diversity study, etc. (Bhat and Jarret 1995).

The first RAPD marker was applied for fingerprinting of wild and cultivated species of banana in 1992 (Kaemmer et al. 1992). A and B genome-specific RAPD marker has been identified for *Musa*, and it is very useful to estimate genomic composition of banana cultivars (Pillay et al. 2000; Oselebe et al. 2006; Pillay et al. 2006). Uma et al. (2006) applied 80 RAPD primers to estimate intraspecific variation and relation of Indian wild *Musa balbisiana* Colla collection. In this study, only 4 primes produced 31 polymorphic bands, and all the 16 accessions were clustered into four as against seven clusters obtained through morphotaxonomic characterization. Some of the accessions failed to group in the same cluster as revealed by morphotaxonomy data. This result can be further improved using more RAPD markers and also using other types of markers such as ISSR, ITS, AFLP, etc. In recent years, RAPD has been used with other markers for the *Musa* genetics and breeding study (Howell et al. 1994; Lamare and Rao 2015). Bhat et al. (1995) reported that RAPD primer OPC-15 (5′-GACGGATCAG-3′) had the potential for distinguishing 55 of the cultivars by producing 24 bands but failed to characterize the clones of Gros Michel and Venkel.

3.3.1.3.4 Intersimple Sequence Repeats (ISSR)

ISSR is a PCR-based marker technique, and its assay system is similar to RAPD. This technique does not require any prior knowledge of genome sequences of the organism. Like RAPD, a versatile set of primers is used in the ISSR assay. Furthermore this marker technique is simple, fast, and cost effective. The main drawbacks of this technology are the dominant nature of the marker, that the homology of the bands is uncertain, and that they do not allow the identification of heterozygous from homozygous dominance. This marker involves amplification of the DNA fragments nearby at an amplifiable distance between two identical microsatellite repeat regions oriented in reverse orders (Spooner et al. 2005). ISSR has been widely used for varietal identification, genetic diversity analysis, genetic stability of the tissue culture-derived clone, etc. The use of ISSR in banana research has not received wide attention so far. Several studies using ISSR marker in banana research were mainly limited in small-scale germplasm characterization (Venkatachalam et al. 2007; HaiFei et al. 2010; Poerba and Ahmad 2010; QianJie et al. 2010; Khatri et al. 2011; Yao et al. 2012; Dhanapal et al. 2014; Lamare and Rao 2015) and in genetic fidelity testing of cultivated banana (Rout et al. 2009; Dhanapal et al. 2014). Choudhary et al. (2014) used 40 ISSRs with 60 RAPD markers to estimate molecular variability of 12 plantain ecotypes and found that ISSR is a better tool than RAPD for assessment of genetic diversity in plantain ecotypes. Another study reported the use of ISSR with RAPD to test genetic stability of three micropropagated banana (*Musa* spp.) cultivars and found that ISSR detected more polymorphism than RAPD (Ray et al. 2006). Similarly, ISSR were used for finding genetic uniformity of micropropagated banana plantlets (Rout et al. 2009), *in vitro* mutagenesis, and variance (Khatri et al. 2011). ISSRs were also employed to evaluate the genetic diver-

sity and classification of 27 wild banana accessions collected in Guangxi, China (Qin et al. 2011). Padmesh et al. (2012) explored the pattern of genetic variation of 32 wild *M. acuminata* Colla collected from the Southwestern Ghats in the peninsula of India using ISSR markers.

3.3.1.3.5 Internal Transcribed Spacers (ITS)

The nuclear ribosomal genes (rDNA) encoded three rRNA subunits, viz., 18S, 5.8S, and 26S. These subunits are present in the genome as clusters of tandemly repeated units of 250–20,000 copies (Rogers and Bendich 1987). rDNA is transcribed as a single unit along with the two spacers known as the internal transcribed spacers (ITS) that separate the 5.8S subunit from the 18S and the 26S subunits. The sequences of these ITS regions are variable among closely related clones or species. Therefore, this variation could be used as a marker, and it is a powerful tool for assessing phylogenetic relationships at the lower taxonomic levels. ITS markers are used for genetic diversity, population structure and clone identification. In *Musa*, ITS marker was used to distinguish A and B genomes. Nwakanma et al. (2003) amplified ITS region of seven *M. acuminata* (A genome) and five *M. balbisiana* (B genome) accessions. The result revealed that all the accessions produced 700-bp fragments; subsequently this fragment was digested with ten different restriction enzymes (*Alu*I, *Cfo*I, *Dde*I, *Hae*III, *Hin*fI, *Hpa*II, *Msp*I, *Rsa*I, *Sau*3AI, and *Taq*I). Only *Rsa*I produced a consistent polymorphic banding pattern between *M. acuminata* and *M. balbisiana*. The *Rsa*I produces four fragments (350 bp, 180 bp, 120 bp, and 50 bp) in the *M. balbisiana* accessions and three fragments (530 bp, 120 bp, and 50 bp) in *M. acuminata* accessions. The fragment of 530 bp was unique to the A genome, while two fragments of 350 bp and 180 bp were specific to the B genome. Further 56 accessions from different genomic combinations of *Musa* spp. (viz., AA, AAA, AAB, AB, and ABB) were amplified with ITS primer and subsequently digested with *Rsa*I. Results showed that only a 530-bp fragment was present in A genome-containing individuals but absent in B genome-containing individuals. On the other hand, a B genome-containing individual produced 350-bp and 180-bp fragments that were absent in A genome-containing individuals. This finding was a breakthrough for characterization of *Musa* spp. according to their genomic combination using the ITS marker.

3.3.1.3.6 Inter-retrotransposon Amplified Polymorphism (IRAP)

IRAP is a PCR-based dominant marker. This marker detects retrotransposon (RT) insertion-based polymorphism among the individuals. RTs are abundant in the plant genome, and they compose over 50 % of the total nuclear genome. RT elements act as a mutagenic agent by insertion or deletion in the genome thereby making them a good source of genomic variation (Heslop-Harrison 2000); thus RT can be used as a genetic marker for various purposes in plant genetics research. It has been used in fingerprinting, genetic diversity, and population structure studies in plants (Biswas et al. 2010a, b; Khadivi-Khub et al. 2015; Singh et al. 2015). IRAP markers are also used in *Musa* for classifying the genome constitution (Nair et al. 2005; Teo et al. 2005; Häkkinen et al. 2007; Pachuau et al. 2014), genetic diversity (Saraswathi et al. 2011), and identification of wild *Musa* spp. (Häkkinen et al. 2007; Häkkinen and Teo 2008).

Ty3-gypsy-like LTR sequence of banana (*Musa acuminata* monkey retrotransposon, AF 143332) was used to design IRAP primer, and it was used to identify the B genome in the banana cultivars. Nair et al. (2005) used this marker to classify 36 banana cultivars and observed multiple polymorphic bands. Among these bands, a specific band of 350 bp was observed in all the cultivars with the B genome. In ABB genomes, the band intensity was highly observed in AAB genomes. But there was an exceptional result found in the cultivar "Manoranjitham" (AAA), in which B-specific band was present with similar intensity observed in ABB ("Kosta bontha"). The author suggested that "Manoranjitham" was misidentified. Teo et al. (2005) applied IRAP methods to generate molecular markers for estimation of diversity, genome constitution, and

relationships of Malaysian banana cultivars. IRAP is used with an RAPD marker to study intragroup diversity among Cavendish (AAA) clones of banana (Saraswathi et al. 2011). Singh et al. (2015) used IRAP and morphological markers to characterize *Musa* germplasm from Northeast India.

3.3.1.3.7 Amplified Fragment Length Polymorphism (AFLP)

AFLP is a PCR-based multilocus marker technology employed in many plant studies relating to genetic identity, phylogenetic relation, parentage identification of clones and cultivars, etc. It is a robust, highly reproducible, and dominant marker, but in a segregating population, AFLP can be detected as codominant. The AFLP marker is capable of generating 80–500-bp fragments from different genomic sites at the same time; therefore this marker detects high polymorphisms in different genomic regions simultaneously. There are many advantages of AFLP compared with other markers such as RAPD, RFLP, SSR, etc. AFLP has become particularly useful in the study of plant taxa, where genomic information is not available.

Thirty-nine accessions from four main sections of the genus *Musa* were evaluated using eight AFLP markers (Ude et al. 2002a). A wide range of variability was observed among the species within the sections of the genus *Musa*. This study proved that AFLP is a very useful tool in determining taxonomic relationships in the genus *Musa* and potentially useful to resolve some of the complicated taxonomic questions in the genus *Musa*. According to morphological data, Simmonds and Weatherup (1990) place *M. peekeli ssp. peekeli* as a close relative of *M. peekeli ssp. angustigemma*, while Argent (1976) suggests that *M. angustigemma* should rank under *M. peekeli* as a subspecies. AFLP data suggest that *M. peekeli ssp. angustigemma* is clearly distinct from *M. peekeli ssp. peekeli*, and therefore the specific rank of *M. angustigemma* should be retained.

Several studies suggested that the AFLP marker is effective for genetic diversity analysis in *Musa* and the level of polymorphism comparatively higher than other markers used in the *Musa* diversity analysis (Crouch et al. 1999; Loh et al. 2000; Wong et al. 2001, 2002). The relationship of cultivated banana with *M. acuminata* and *M. balbisiana* is precisely classified by AFLP markers (Wongniam et al. 2010). Youssef et al. (2011a) reported that AFLP is able to discriminate A, B, S, and T genomes within *Musa* species. In order to detect genetic stability of somaclonal variation in somatic embryo-derived plants of two banana cultivars, namely, "Grand Naine" and "Williams" (*Musa acuminata* Colla, AAA), AFLP marker technology was applied (Youssef et al. 2011b). Thirty primer combinations were used to detect polymorphism among somaclonal variants, and the result showed that 1.4 % and 1.6 % bands were polymorphic in "Grand Naine" and "Williams," respectively. In addition, 8 and 16 bands were specific to the observed regenerated plants of "Grand Naine" and "Williams," respectively, which were absent in their parents, while ten and five bands are completely absent in the regenerated plant of "Grand Naine" and "Williams," respectively, which were exclusively present in their parents. AFLP markers were also used for the genetic analysis of *Musa* species in combination with other molecular markers (Opara et al. 2010; Youssef et al. 2011a) and also to identify somaclonal variation (James et al. 2004; Vroh-Bi et al. 2011; Youssef et al. 2011b).

3.3.1.3.8 Simple Sequence Repeat (SSR) or Microsatellite Marker

An SSR marker is well known for its genome-wide distribution, codominant inheritance, reproducibility, multiallelic nature, and easy assay technique. Recent progress in DNA sequencing technology has provided an opportunity to routinely develop large sets of SSR markers. Transferability of a genic SSR marker is higher than genomic SSRs. This feature helps to design anchor markers for comparative mapping studies. Since they are often more conserved, genic SSRs may provide an insufficient degree of polymorphism to discriminate between closely related germplasm. Therefore genomic SSRs may be valuable complements. In the recent past decade, SSR markers are extensively used in a wide range

of breeding applications. Several hundred EST-SSR markers were developed by in silico EST sequence mining of several *Musa* spp. (Li et al. 2012; Passos et al. 2012, 2013; Backiyarani et al. 2013; Ravishankar et al. 2015). SSR markers were developed from B genome of *Musa* (Buhariwalla et al. 2005; Ravishankar et al. 2013); 226 were developed from *Musa* GSS (survey of the genomic sequence) data (Ravishankar et al. 2012), and 41 were from Calcutta 4 using BAC sequences (Miller et al. 2010). Most of these markers are not freely accessible; some are redundant with alternate IDs or names, while their physical positions and functional natures are unknown. Consequently, the use of these markers in *Musa* spp. improvement is limited. In the whole genome sequences of two banana varieties, more than 0.1 million EST and several thousand GSS sequences were mined for SSR marker discovery, and 119,540 non-redundant SSR markers were developed (Biswas et al. 2015). Subsequently these markers were characterized, classified, and stored in a searchable database known as Musa marker database and it can be access through the following link http://www.agrogene.ac.cn:8088/mumdb/mumdb_home.html. A part of these markers was validated by wet lab assay and their potentiality was estimated for a genetic diversity study in *Musa* population.

3.3.1.3.9 DArT

Diversity arrays technology (DArT) is DNA/DNA hybridization-based molecular marker technology, extensively used for a quick assessment of the structure of germplasm collections. This is the cost-effective genotyping technology that can detect all types of DNA variation including SNP, indel, CNV, and methylation. It also can detect simultaneously variation at numerous genomic loci without any sequence information of the organism. In recent years, this technology become popular and is regularly used in the crop improvement including *Musa* (Risterucci et al. 2009). A total of 836 DArT markers were developed and used for *Musa* spp. genotyping. Ten percent of these markers were A genome specific and able to target this genome portion in a related analysis, among diverse ploidy constitutions (Risterucci et al. 2009). Risterucci et al. (2009) in their study clearly demonstrated the usefulness of DArT markers for *Musa* spp. genotyping and genetic diversity analysis. They used four complexity reduction methods of DArT for *Musa* spp. and tested their performance on 48 *Musa* genotypes and finally invented two methods that produced more polymorphic information content than others. Subsequently, selected methods were used for large-scale *Musa* spp. genotyping.*Musa* accessions, around 168 in number that were collected from CIRAD (Neufchateau, Guadeloupe) and IITA (Ibadan, Nigeria) were used. All these accessions were derived from *M. acuminata* (A genome) and *M. balbisiana* (B genome). And the result reveals that DArT markers classify them according to their origin and genomic combinations. Kilian (2007) developed 1,500 DArT markers using a wide array of *Musa* accessions, which were used for *Musa* framework map. Further these markers with additional 380 markers have been used to construct a map at CIRAD.

3.3.1.3.10 Ecotilling

Ecotilling is a new class of molecular marker technology that was developed as a high-throughput and low-cost platform for the SNPs discovery and small indels. This technology was first used in *Arabidopsis* ecotypes and then adopted for many species including humans, switchgrass, poplar, melon, banana, etc. (Till et al. 2010). Since its discovery, ecotilling has been used for more than 20 plant species for genetic diversity, population structure, mapping, and QTL analysis. In principle, ecotilling is an enzymatic mismatch cleavage-based DNA hybridization technology. Around 700–1,600-bp genic regions are amplified by PCR using fluorescent labeled gene-specific primers. Subsequently, samples are denatured and annealed; then heteroduplexed molecules are produced through the hybridization of polymorphic amplicons. Mismatched regions or double-stranded duplexes are then cleaved using crude extract of celery juice containing the single-strand specific nuclease CEL I. Cleaved products are then resolved in denaturing polyacrylamide

gel electrophoresis (PAGE) for observing banding pattern.

In *Musa*, ecotilling method was used for the discovery and characterization of nucleotide polymorphisms of diploid and polyploid accessions. Over 800 novel alleles in 80 accessions were identified as polymorphic using 14 gene-specific primers by Till et al. (2010). In this study, more than 6,000 polymorphisms were detected in over 800 alleles in 80 *Musa* accessions. Further, sequencing-based validation was performed for the detection of SNPs variation among the accessions. Consequently, sequencing and banding patterns reveal that ecotilling is the perfect platform for discovery of polymorphisms in homologous gene targets in *Musa* accessions.

3.3.2 Utility of Marker Technology in Banana Breeding

3.3.2.1 Genotype Identification

Genotype identification is known as genotyping. It is one of the routine works for plant breeders prior to selection of breeding material. Environment has great impact on phenotypic expression on gene or traits. Selection of breeding material based on the phenotypic traits from the population or germplasm collection maintained in a different environment may be misleading. Therefore, genotyping is important in a breeding programme. Genotyping is a DNA-based technology commonly used to characterize or identify any living organism. It is also known as fingerprinting or DNA fingerprinting. This methodology is extensively used in plant breeding for identification of individuals in a population, for discrimination between individuals in an inbred line, or for determination of genetic distance between genotypes in general. Different types of markers such as biochemical, RAPD, ISSR, RFLP, AFLP, SSR, etc., are commonly used for *Musa* spp. genotyping. In the early 1980s, biochemical markers are used for *Musa* spp. genotyping, for example, peroxidase and polyphenoloxidase used to identify "Saba" (ABB/BBB) and "Bluggoe" (ABB) types of banana (Rivera 1983). Dhanya et al. (2006) and Dhanya et al. (2006) used isozymes to identify banana cultivars resistant to *banana bract mosiac virus* (BBrMV). An RAPD marker has been developed for genotyping dwarf off-type Cavendish banana (*Musa* spp. AAA) cultivar. The primer OPJ-04 (5′-CCGAACACGG-3′) was found to amplify an approximately 1.5-kb band which is consistently present in all normal Cavendish cultivars but absent in dwarf Cavendish cultivars (Damasco et al. 1996). Bhat et al. (1995) and Bhat and Jarret (1995) used 60 RAPD markers for identification of 57 *Musa* cultivars; among these RAPD markers, 49 produced consistent results and were able to identify 55 cultivars but failed to characterize Gros Michel and Venkel clones. A total of 33 SSR markers were used to characterize 35 cultivated banana (*Musa* spp.) genotypes, including triploid cultivars and tetraploid hybrids (Creste et al. 2003). Christelova et al. (2011) used 19 sets of fluorescently labeled SSR primers for genotyping 70 diploid and 38 triploid banana accessions.

3.3.2.2 Germplasm Managements

Germplasm collection and its systematic management are essential for quick access of a right individual for the breeding program. In general, genebank collection consists of multiple copies of same genotypes; these duplicates may occur for various regions, for example, documentation error, the sampling of multiple individuals from genetically homogeneous collections, exchange of identical accessions between genebanks, etc. (Spooner et al. 2005). The redundant accessions of the genebank have no significant impact, but maintaining them in the genebank requires time, space, and resource. So the elimination of redundant accessions from a large collection of genebank is a big challenge. Correct classification and identification of unique accessions allows solving germplasm management problems. There is a high possibility of inclusion of redundant accessions in the vegetative propagated crop plant genebank such as banana, because the germplasms of this crop are collected based on its morphological traits and a similar clone is maintained in a different geographical region with a different name. Further, cultivars of the banana

are evolved through human selection. Therefore efficient germplasm management is an important factor in banana breeding. In the last few decades, a significant number of studies have been conducted for the germplasm characterization of *Musa* spp. around the world using different kinds of molecular markers including RAPD, AFLP, SSR, etc. (Bhat and Jarret 1995; Creste et al. 2003; Christelová et al. 2011).

3.3.2.3 Genome Composition Determination

Most of the banana cultivars are triploid and derived from interspecies hybridization between *M. acuminata* (A genome) and *M. balbisiana* (B genome), while other banana and plantain genomes are derived from the combination of A, B, T, and S species genomes. The knowledge of genomic combination of the cultivars or a clone of banana and plantain is essential for its improvement. Several molecular markers have already been developed to identify genomic combination of banana and plantain cultivars. Three RAPD primers (A17, A18, D10) from OPERON Technologies (Alameda, CA, USA) were able to identify A and B genome of *Musa* spp. Primer A17 generated two fragments (600 bp, 100 bp) and primer D10 one fragment (320 bp) that was unique to *M. acuminata* (A genome), while primer A18 produced three fragments (200 bp, 250 bp, 300 bp) in *M. balbisiana* (B genome). The fragments B18250 and B18300 were always present in genotypes with at least one B genome; on the other hand, fragment A18200 was present in clones with two B genomes. These three RAPD primers are extensively used for the determination of banana genomic composition (Jones 2000; Pillay et al. 2000). A PCR-RFLP-based ITS marker is also applicable for banana A and B genome identification (Nwakanma et al. 2003). The ITS marker produced one fragment of 530 bp that is A genome specific and two B genome-specific fragments of 350 bp and 180 bp. An interspecific hybrid of A and B genome possessed all three fragments.

3.3.2.4 Genetic Diversity and Population Structure Estimation

Molecular marker technology is widely used for study genetic diversity, population structure, and phylogenetic relationships of the crop plants. Many studies have been attempted to estimate genetic diversity using different types of markers in banana and plantain wild and cultivar collections. For example, the genetic diversity of 100 Indonesian *Musa* cultivars from the different genomic groups of Musa (AA, AAA, AAB, ABB, and BB) was estimated using isozymes of *malate dehydrogenase* (*MDH*), *peroxidase* (*PRX*), and *glutamate oxaloacetate transaminase* (*GOT*) (Megia et al. 2001). The result reveals that *MDH* and *PRX* were more useful than *GOT* for genetic diversity study of Indonesian *Musa* cultivars.

AFLP marker technology was applied to estimate the genetic diversity of banana and plantain population collected from different geographical regions and also different cultivars and wild accessions (Ude et al. 2002a, b; Wong et al. 2002; Wang et al. 2007; Opara et al. 2010; Wongniam et al. 2010; Youssef et al. 2011a). Wong et al. (2001) used an AFPL marker to assess the genetic diversity of 32 Malaysian wild bananas *Musa acuminata* Colla, and results showed that AFLP efficiently classified Malaysian wild bananas based on genetic distance. Ude et al. (2002b) assessed the genetic diversity and phylogenetic relationships of *M. acuminata* and *M. balbisiana* and their natural hybrids and noted that AFLP markers were able to produce enough information about the genetic diversity of *M. balbisiana* accessions to further classify them into two subgroups.

Seven SSR markers were used to estimate genetic diversity and population structure of six *M. ornate* populations (Burgos-Hernández et al. 2013). A low level of genetic diversity was observed in the *M. ornate* populations. This low diversity may occur due to recent fragmentation of events, which meant that there was not enough time gap between populations to detect differ-

ences. It may also be the cause of self-pollination, clonal reproduction, bottleneck selection, or the decline in pollinator in population. This study was proposed to conserve and maintain all the remaining *M. ornate* population to maintain gene flow and increase the genetic diversity. The ex situ collection of 224 *Musa* spp. at Embrapa, Brazil, was analyzed by 16 SSR markers for the estimation of genetic diversity and population structure. The findings of this study showed that structure analysis might be useful in identification of ancestry of recently developed tetraploid hybrids and triploid cultivars by breeding programs.

3.3.2.5 Mapping and Marker-Assisted Breeding

Genome mapping sets up the roadmap of a genome that helps to locate important genes, manipulate them, identify the molecular environment of both coding and noncoding DNA sequences, etc. The advancements in the area of sequencing technology and molecular marker developments open the door for tagging agronomically important traits via mapping and marker-assisted breeding. Although several linkage maps have been developed in *Musa* spp., their quality and marker saturation remain poor; that is why their utility in banana breeding is still limited (Faure et al. 1993; Baurens et al. 1997; Vilarhinos 2004; Hippolyte et al. 2010). Faure et al. (1993) developed first partial molecular linkage map based on 77 markers, in which a significant number (36%) of markers were deviated from Mendelian segregation. The second map was developed for *M. acuminata* cv "M53," in which it exhibited 11 linkage groups with a significant number of markers distorted from the Mendelian segregation ratio. The linkage map developed by Vilarhinos (2004) is composed of 14 linkage groups; 59% of markers are skewed from the Mendelian segregation ratio; the mapping population is derived from F1 and pseudotestcross strategy used to construct the linkage group. The oversized linkage groups of this map may be the cause of distorted markers that are involved in the structural rearrangements of chromosomes. The high-density linkage map was developed in *Musa* sp. using DArT and SSR markers (Hippolyte et al. 2010). In this study, two parental maps and one reference map were constructed. The female parent map consists of 11 linkage groups with 261 markers (125 SSRs and 136 DArTs). The map spanned 920 cM, with one marker per 3.8 cM.; 59 and 9 markers were comprised in the largest and smallest linkage group, respectively. The male parent map is obtained at LOD 5; a total of 359 markers consisted the 9 linkage groups with a total map length of 1,081 cM., in which one marker is distributed per 2.9 cM., but the marker distribution in each linkage group was not uniform. Further a synthetic linkage reference map was developed using 489 markers (167 SSRs, 322 DArTs), among which 132 were anchored markers. This map contains 11 linkage groups covering 1,197 cM., at an average of 38 markers per linkage group and one marker per 2.8 cM.

Marker-assisted breeding is useful for identifying markers linked to important traits; these markers can be used for the direct screening under greenhouse condition at early stage of growth. This approach reduces time, cost, and gaps in the breeding program that dramatically speeds up the selection process. There are a few markers identified in *Musa* linked with some important agronomical trait that is related to disease resistance. Four methylation-sensitive amplification polymorphism (MSAP) markers were identified as linked markers for black sigatoka disease-resistant gene of banana (Gimenez et al. 2006). There are some RAPD markers used for selecting black sigatoka disease-resistant, nematode-resistant, and salt-tolerant banana clones (Miri et al. 2009). A banana *SERK*-related marker is associated with somatic embryogenic competence and disease-resistant response in *Musa sp*. (Huang et al. 2010; Cunha et al. 2015).

3.4 Conclusions

In the last few decades, gene discovery and marker technology have revolutionized plant breeding. Genes and their related functions were identified; they were cloned and utilized in bred

crop improvement. Scientists around the globe are identifying agronomically important genes for banana varietal improvement. Molecular marker technology has made a great impact on banana breeding, including cultivar identification, parent selection for cross-breeding, identifying genomic group of wild species, germplasm management, characterizing somaclonal variation, etc.

Acknowledgments The author wishes to thank the National Natural Science Foundation of China for the research grant Category: International (Regional) Cooperation and Exchange Program. Program: The Research Fund for International Young Scientist, FY2015. The research grants No: 31550110217 and Dr. Yi Ganjun for his valuable suggestion and modification of MS.

References

Argent G (1976) The wild bananas of Papua New Guinea. Note Roy Bot Gard Edinb 35:77–114

Atkinson HJ, Grimwood S, Johnston K et al (2004) Prototype demonstration of transgenic resistance to the nematode Radopholus similis conferred on banana by a cystatin. Transgenic Res 13(2):135–142

Backiyarani S, Uma S, Varatharj P et al (2013) Mining of EST-SSR markers of Musa and their transferability studies among the members of order the Zingiberales. Appl Biochem Biotechnol 169(1):228–238. doi:10.1007/s12010-012-9975-2

Baurens F, Noyer J, Lanaud C et al (1997) Sequence tagged site markers to draft the genomic structure of the banana chloroplast. Fruits 52(4):247–259

Bhat K, Jarret R (1995) Random amplified polymorphic DNA and genetic diversity in Indian Musa germplasm. Genet Resour Crop Evol 42(2):107–118

Bhat K, Bhat S, Chandel K (1992a) Survey of isozyme polymorphism for clonal identification in Musa. I. Esterase, acid phosphatase and catalase. J Hortic Sci 67(4):501–507

Bhat K, Bhat S, Chandel K (1992b) Survey of isozyme polymorphism for clonal identification in Musa. II. Peroxidase, superoxide dismutase, shikimate dehydrogenase and malate dehydrogenase. J Hortic Sci (UK) 67:737–743

Bhat KV, Jarret RL, Rana RS (1995) DNA profiling of banana and plantain cultivars using random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers. Electrophoresis 16(1):1736–1745

Biswas MK, Baig M, Cheng Y-J et al (2010a) Retrotransposon based genetic similarity within the genus Citrus and its relatives. Genet Resour Crop Evol 57(7):963–972

Biswas MK, Xu Q, Deng X-X (2010b) Utility of RAPD, ISSR, IRAP and REMAP markers for the genetic analysis of Citrus spp. Sci Hortic 124(2):254–261

Biswas MK, Liu Y, Li C et al (2015) Genome-wide computational analysis of Musa microsatellites: classification, cross-taxon transferability, functional annotation, association with transposons & miRNAs, and genetic marker potential. PLoS ONE 10(6), e0131312. doi:10.1371/journal.pone.0131312, PONE-D-14-57877 [pii]

Bonner JW, Warner RM, Brewbaker JL (1974) A chemosystematic study of Musa cultivars. Hortic Sci 9:325–328

Buhariwalla HK, Jarret RL, Jayashree B et al (2005) Isolation and characterization of microsatellite markers from Musa balbisiana. Mol Ecol Notes 5(2):327–330. doi:10.1111/j.1471-8286.2005.00916.x

Burgos-Hernández M, Hernández D, Castillo-Campos G (2013) Genetic diversity and population genetic structure of wild banana Musa ornata (Musaceae) in Mexico. Plant Syst Evol 299(10):1899–1910. doi:10.1007/s00606-013-0846-

Choudhary R, Keshavachandran R, Menon R et al (2014) Molecular variability of plantain ecotypes from the genus Musa (Musaceae). Turk J Bot 38(5):827–834

Christelová P, Valárik M, Hřibová E et al (2011) A platform for efficient genotyping in Musa using microsatellite markers. AoB Plants. doi:10.1093/aobpla/plr024

Creste S, Tulmann Neto A, de Oliveira Silva S et al (2003) Genetic characterization of banana cultivars (Musa spp.) from Brazil using microsatellite markers. Euphytica 132(3):259–268. doi:10.1023/a:1025047421843

Crouch HK, Crouch JH, Jarret RL et al (1998) Segregation at microsatellite loci in haploid and diploid gametes of Musa. Crop Sci 38(1):211–217

Crouch J, Crouch H, Constandt H et al (1999) Comparison of PCR-based molecular marker analyses of Musa breeding populations. Mol Breed 5(3):233–244

Cunha CM, Hinz RH, Pereira A et al (2015) A SCAR marker for identifying susceptibility to Fusarium oxysporum f. sp. cubense in banana. Sci Hortic 191:108–112

Damasco O, Graham G, Henry R et al (1996) Random amplified polymorphic DNA (RAPD) detection of dwarf off-types in micropropagated Cavendish (Musa spp. AAA) bananas. Plant Cell Rep 16(1–2):118–123. doi:10.1007/bf01275464

Davey MW, Gudimella R, Harikrishna JA et al (2013) A draft Musa balbisiana genome sequence for molecular genetics in polyploid, inter-and intra-specific Musa hybrids. BMC Genomics 14(1):683

Dhanapal S, Sekar DS, Satheesh PM (2014) Efficiency of RAPD, SSR and ISSR markers in evaluating the genetic fidelity for micropropagated Musa accuminata plant exposed to coal extracted humic acid and commercially available products. Int J Agric Sci Res (IJASR) 4(4):77–86

Dhanya MK, Rajagopalan B, Umamaheswaran K et al (2006) Isozyme variation in banana (Musa sp.) in

response to bract mosaic virus infection. Indian J Crop Sci 1(1–2):1
D'Hont A, Denoeud F, Aury J-M et al (2012) The banana (Musa acuminata) genome and the evolution of monocotyledonous plants. Nature 488(7410):213–217, doi http://www.nature.com/nature/journal/v488/n7410/abs/nature11241.html#supplementary-information
Faure S, Noyer J, Horry J et al (1993) A molecular marker-based linkage map of diploid bananas (Musa acuminata). Theor Appl Genet 87(4):517–526
Gawel N, Jarret R (1991) Chloroplast DNA restriction fragment length polymorphisms (RFLPs) in Musa species. Theor Appl Genet 81(6):783–786
Gawel NJ, Jarret RL, Whittemore AP (1992) Restriction fragment length polymorphism (RFLP)-based phylogenetic analysis of Musa. Theor Appl Genet 84(3–4):286–290. doi:10.1007/bf00229484
Gimenez C, Palacios G, Colmenares M (2006) Musa methylated DNA sequences associated with tolerance to Mycosphaerella fijiensis toxins. Plant Mol Biol Report 24(1):33–43
HaiFei M, GuiMei L, Yu Z et al (2010) Genetic diversity analysis of banana (Musa spp.) based on ISSR molecular marker. Southwest China J Agric Sci 23(4):1206–1210
Häkkinen M, Teo C (2008) Musa rubinea, a new Musa species (Musaceae) from Yunnan, China. Folia Malaysiana 9.1:23–33
Häkkinen M, Teo CH, Othman YR (2007) Genome constitution for Musa beccarii (Musaceae) varieties. Acta Phytotaxonomica Sin 45(1):69–74
Heslop-Harrison J (2000) Comparative genome organization in plants: from sequence and markers to chromatin and chromosomes. Plant Cell 12(5):617–635
Hippolyte I, Bakry F, Seguin M et al (2010) A saturated SSR/DArT linkage map of Musa acuminata addressing genome rearrangements among bananas. BMC Plant Biol 10(1):65
Howell EC, Newbury HJ, Swennen RL et al (1994) The use of RAPD for identifying and classifying Musa germplasm. Genome 37(2):328–332
Huang X, Lu X-Y, Zhao J-T et al (2010) MaSERK1 gene expression associated with somatic embryogenic competence and disease resistance response in banana (Musa spp.). Plant Mol Biol Report 28(2):309–316
Jain PK, Saini ML, Pathak H et al (2007) Analysis of genetic variation in different banana (Musa species) variety using random amplified polymorphic DNAs (RAPDs). Afr J Biotechnol 6(17):1987–1989
James A, Peraza-Echeverria S, Herrera-Valencia V et al (2004) Application of the amplified fragment length polymorphism (AFLP) and the methylation-sensitive amplification polymorphism (MSAP) techniques for the detection of DNA polymorphisms and changes in DNA methylation in micropropagated bananasBanana improvement: cellular, molecular biology, and induced mutations Proceedings of a meeting held in Leuven, Belgium, 24–28 September 2001. Science Publishers, Inc., pp 287–305
Jarret RL, Litz RE (1986a) Enzyme polymorphism in Musa acuminata Colla. J Hered 77(3):183–188
Jarret RL, Litz RE (1986b) Isozymes as genetic markers in bananas and plantains. Euphytica 35(2):539–549. doi:10.1007/bf00021863
Jarret RL, Gawel N, Whittemore A et al (1992) RFLP-based phylogeny of Musa species in Papua New Guinea. Theor Appl Genet 84(5–6):579–584
Jones DR (2000) Introduction to banana, abaca and enset. Diseases of banana, abaca and enset. CAB International, Wallingford, pp 1–36
Kaemmer D, Afza R, Weising K et al (1992) Oligonucleotide and amplification fingerprinting of wild species and cultivars of banana (Musa spp.). Nat Biotechnol 10(9):1030–1035
Kaemmer D, Fischer D, Jarret R et al (1997) Molecular breeding in the genus Musa: a strong case for STMS marker technology. Euphytica 96(1):49–63
Khadivi-Khub A, Shabanian N, Alikhani L et al (2015) Genotypic analysis and population structure of Lebanon oak (Quercus libani G. Olivier) with molecular markers. Tree Genet Genome 11(5):1–10
Khatri A, Bibi S, Dahot MU et al (2011) In vitro mutagenesis in banana and variant screening through ISSR. Pak J Bot 43(2011):2427–2431
Kilian A (2007) Towards effective deployment of diversity arrays technology (dart) in banana genomics and sequencing
Lamare A, Rao S (2015) Efficacy of RAPD, ISSR and DAMD markers in assessment of genetic variability and population structure of wild Musa acuminata colla. Physiol Mol Biol Plants 21(3):349–358. doi:10.1007/s12298-015-0295-1
Li WJ, Ma H, Li ZH et al (2012) Thirty-four Musa (Musaceae) expressed sequence tag-derived microsatellite markers transferred to Musella lasiocarpa. Genet Mol Res 11(3):2094–2098. doi:gmr1898 [pii]10.4238/2012.August.6.13
Liu BH (1997) Statistical genomics: linkage, mapping, and QTL analysis. CRC Press, Boca Raton
Loh JP, Kiew R, Set O et al (2000) Amplified fragment length polymorphism fingerprinting of 16 banana cultivars (Musa cvs.). Mol Phylogenet Evol 17(3):360–366
Mandal AB, Maiti A, Chowdhury B et al (2001) Isoenzyme markers in varietal identification of banana. Vitro Cell Dev Biol-Plant 37(5):599–604. doi:10.1007/s11627-001-0105-z
Manzo-Sánchez G (2008) Construction of a genetic linkage map of the fungal pathogen of banana Mycosphaerella fijiensis, causal agent of black leaf streak disease. Curr Genet 53(5):299–311
Megia R, Caecelia Y, Sulistyaningsih N et al (2001) Isozyme polymorphisms for cultivar identification in Indonesia bananas Hayati 8:81–85
Miller RN, Passos MA, Menezes NN et al (2010) Characterization of novel microsatellite markers in Musa acuminata subsp. burmannicoides, var. Calcutta 4. BMC Res Notes 3:148. doi:1756-0500-3-148 [pii]10.1186/1756-0500-3-148
Miri SM, Mousavi A, Naghavi MR et al (2009) Analysis of induced mutants of salinity resistant banana (Musa

acuminata cv. Dwarf Cavendish) using morphological and molecular markers. Iran J Biotechnol 7(2):86–92

Mlalazi B, Welsch R, Namanya P et al (2012) Isolation and functional characterisation of banana phytoene synthase genes as potential cisgenes. Planta 236(5):1585–1598. doi:10.1007/s00425-012-1717-8

Mohandas S, Sowmya H, Saxena A et al (2013) Transgenic banana cv. Rasthali (AAB, Silk gp) harboring Ace-AMP1 gene imparts enhanced resistance to Fusarium oxysporum f. sp. cubense race 1. Sci Hortic 164:392–399

Nair AS, Teo CH, Schwarzacher T et al (2005) Genome classification of banana cultivars from South India using IRAP markers. Euphytica 144(3):285–290

Miller RN, Bertioli DJ, Baurens FC, Santos CM, Alves PC et al (2008) n-TIR NBS-LRR resistance gene analogs in Musa acuminata Colla: isolation, RFLP marker development, and physical mapping. BMC Plant Biol 8(1):15

Nwakanma D, Pillay M, Okoli B et al (2003) PCR-RFLP of the ribosomal DNA internal transcribed spacers (ITS) provides markers for the A and B genomes in Musa L. Theor Appl Genet 108(1):154–159

Opara UL, Jacobson D, Al-Saady NA (2010) Analysis of genetic diversity in banana cultivars (Musa cvs.) from the South of Oman using AFLP markers and classification by phylogenetic, hierarchical clustering and principal component analyses. J Zhejiang Univ Sci B 11(5):332–341. doi:10.1631/jzus.B0900310

Oselebe H, Tenkouano A, Pillay M, Obi I, Uguru M (2006) Ploidy and genome segregation in Musa breeding populations assessed by flow cytometry and randomly amplified polymorphic DNA markers. J Am Soc Hortic Sci 131:780–786

Pachuau L, Atom A, Thangjam R (2014) Genome classification of Musa cultivars from Northeast India as revealed by ITS and IRAP markers. Appl Biochem Biotechnol 172(8):3939–3948. doi:10.1007/s12010-014-0827-0

Padmesh P, Mukunthakumar S, Vineesh P et al (2012) Exploring wild genetic resources of Musa acuminata Colla distributed in the humid forests of southern Western Ghats of peninsular India using ISSR markers. Plant Cell Rep 31(9):1591–1601

Passos MA, de Oliveira Cruz V, Emediato FL et al (2012) Development of expressed sequence tag and expressed sequence tag-simple sequence repeat marker resources for Musa acuminata. AoB Plants 2012:pls030. doi:10.1093/aobpla/pls030pls030 [pii]

Passos MA, de Cruz VO, Emediato FL et al (2013) Analysis of the leaf transcriptome of Musa acuminata during interaction with Mycosphaerella musicola: gene assembly, annotation and marker development. BMC Genom 14:78. doi:1471-2164-14-78 [pii]10.1186/1471-2164-14-78

Pillay M, Nwakanma D, Tenkouano A (2000) Identification of RAPD markers linked to A and B genome sequences in Musa L. Genome 43(5):763–767

Pillay M, Ashokkumar K, James A et al (2012a) Molecular marker techniques in Musa genomic research. In: Genetics, genomics, and breeding of bananas. CRC Press, Boca Raton, pp 70–90

Pillay M, Ashokkumar K, Shunmugam ASK et al (2012b) A case for molecular breeding in Musa. In: Genetics, Genomics, and Breeding of Bananas 281. CRC Press

Pillay M, Ogundiwin E, Tenkouano A, Dolezel J (2006) Ploidy and genome composition of Musa germplasm at the International Institute of Tropical Agriculture (IITA). Afr J Biotechnol 5:1224–1232

Poerba YS, Ahmad F (2010) Genetic variability among 18 cultivars of cooking bananas and plantains by RAPD and ISSR markers. Biodiversitas 11(3):118–123

QianJie W, YongHua Q, HouBin C et al (2010) Establishment of an inter-simple sequence repeats reaction system (ISSR) for banana (Musa AAA). J South China Agric Univ 31(1):13–16

Qin X, Peng H, Long X et al (2011) Preliminary study on ISSR analysis and classification of wild Musa germplasm in Guangxi, China. Acta Hortic 897:259

Ravishankar KV, Vidhya L, Cyriac A, Rekha A, Goel R, Singh NK, Sharma TR (2012) Development of SSR markers based on a survey of genomic sequences and their molecular analysis in banana (*Musa* spp.). J Hortic Sci Biotechnol 87:84–88

Ravishankar KV, Raghavendra KP, Athani V, Rekha A, Sudeepa K, Bhavya D, Srinivas V, Ananad L (2013) Development and characterisation of microsatellite markers for wild banana (*Musa balbisiana*). J Hortic Sci Biotechnol 88(5):605–609

Ravishankar KV, Megha HS, Rekha A, Khadke GN, Veerraju CH (2015) Insights into Musa balbisiana and Musa acuminata species divergence and development of genic microsatellites by transcriptomics approach. Plant Genet 4:78–82

Ray T, Dutta I, Saha P et al (2006) Genetic stability of three economically important micropropagated banana (Musa spp.) cultivars of lower Indo-Gangetic plains, as assessed by RAPD and ISSR markers. Plant Cell Tissue Org Cult 85(1):11–21

Rekha A, Ravishankar K, Anand L et al (2001) Genetic and genomic diversity in banana (Musa spp. and cultivars) based on D2 analysis and RAPD markers. Infomusa 10:29–34

Risterucci AM, Hippolyte I, Perrier X et al (2009) Development and assessment of diversity arrays technology for high-throughput DNA analyses in Musa. Theor Appl Genet 119(6):1093–1103. doi:10.1007/s00122-009-1111–5

Rivera F (1983) Protein and isoenzyme banding patterns among Philippine cooking bananas and their wild parents (Musa species). Paradisiaca 6:7–12

Rogers SO, Bendich AJ (1987) Ribosomal RNA genes in plants: variability in copy number and in the intergenic spacer. Plant Mol Biol 9(5):509–520

Rout G, Senapati S, Aparajita S et al (2009) Studies on genetic identification and genetic fidelity of cultivated banana using ISSR markers. Plant Omics 2.6:250

Saraswathi M, Uma S, Prasanya Selvam K et al (2011) Assessing the robustness of IRAP and RAPD marker systems to study intra-group diversity among

Cavendish (AAA) clones of banana. J Hortic Sci Biotechnol 86(1):7

Simmonds NW, Shepherd K (1955) The taxonomy and origins of the cultivated bananas. J Linn Soc Lond Bot 55(359):302–312. doi:10.1111/j.1095-8339.1955.tb00015.x

Simmonds N, Weatherup S (1990) Numerical taxonomy of the wild bananas (Musa). New Phytol 115:567–571

Singh W, Singh N, Handique P et al (2015) Morpho-taxonomical and molecular assessment of Musa genotypes from north-east India by morphological and inter-retrotransposon amplified polymorphism markers. Plant Syst Evol 301(2):563–575. doi:10.1007/s00606-014-1094-9

Spooner DM, Rob van Treuren M, van Treuren R et al (2005) Molecular markers for genebank management. Bioversity Int. No. 10. Bioversity International

Teo C, Tan S, Ho C et al (2005) Genome constitution and classification using retrotransposon-based markers in the orphan crop banana. J Plant Biol 48(1):96–105. doi:10.1007/bf03030568

Till BJ, Jankowicz-Cieslak J, Sági L et al (2010) Discovery of nucleotide polymorphisms in the Musa gene pool by Ecotilling. Theor Appl Genet 121(7):1381–1389

Tripathi L, Mwangi M, Abele S et al (2009) Xanthomonas wilt: a threat to banana production in East and Central Africa. Plant Dis 93(5):440–451

Tsao TT-H (2008) Towards the development of transgenic banana bunchy top virus (BBTV)-resistant banana plants: interference with replication. Phd Thesis. Queens land University of technology, Australia

Ude G, Pillay M, Nwakanma D et al (2002a) Analysis of genetic diversity and sectional relationships in Musa using AFLP markers. Theor Appl Genet 104(8):1239–1245. doi:10.1007/s00122-001-0802-3

Ude G, Pillay M, Nwakanma D et al (2002b) Genetic diversity in Musa acuminata Colla and Musa balbisiana Colla and some of their natural hybrids using AFLP markers. Theor Appl Genet 104(8):1246–1252. doi:10.1007/s00122-002-0914-4

Uma S, Siva SA, Saraswathi MS et al (2006) Variation and intraspecific relationships in Indian Wild Musa balbisiana (BB) population as evidenced by random amplified polymorphic DNA. Genet Resour Crop Evol 53(2):349–355. doi:10.1007/s10722-004-0576-y

Venkatachalam L, Sreedhar R, Bhagyalakshmi N (2007) Genetic analyses of micropropagated and regenerated plantlets of banana as assessed by RAPD and ISSR markers. Vitro Cell Dev Biol-Plant 43(3):267–274

Vilarhinos A (2004) Cartographie genetique et cytogenetique chez le bananier: caracterisation des translocations. Montpellier: Ecole Nationale Supérieure Agronomique

Vishnevetsky J, White TL Jr, Palmateer AJ et al (2011) Improved tolerance toward fungal diseases in transgenic Cavendish banana (Musa spp. AAA group) cv. Grand Nain. Transgenic Res 20(1):61–72

Vroh-Bi I, Anagbogu C, Nnadi S et al (2011) Genomic characterization of natural and somaclonal variations in bananas (Musa spp.). Plant Mol Biol Rep 29(2):440–448

Wang XL, Chiang TY, Roux N et al (2007) Genetic diversity of wild banana (Musa balbisiana Colla) in China as revealed by AFLP markers. Genet Resour Crop Ev 54(5):1125–1132. doi:10.1007/s10722-006-9004-9

Wong C, Kiew R, Loh JP et al (2001) Genetic diversity of the wild banana Musa acuminata Colla in Malaysia as evidenced by AFLP. Ann Bot Lond 88(6):1017–1025

Wong C, Kiew R, Argent G et al (2002) Assessment of the validity of the sections in Musa (musaceae) using AFLP. Ann Bot 90(2):231–238

Wongniam S, Somana J, Swangpol S et al (2010) Genetic diversity and species-specific PCR-based markers from AFLP analyses of Thai bananas. Biochem Syst Ecol 38(3):416–427

Yao J-A, Cai H-J, Shi N-N et al (2012) Genetic relationship among banana (Musa spp.) germplasms revealed by ISSR analysis. Fujian J Agric Sci 1:008

Youssef M, James AC, Rivera-Madrid R et al (2011a) Musa genetic diversity revealed by SRAP and AFLP. Mol Biotechnol 47(3):189–199. doi:10.1007/s12033-010-9328-8

Youssef M, Ku-Cauich R, James A et al (2011b) Genetic analysis of somatic embryogenesis derived plants in banana. Assiut Jout Agric Sci 42:287–300

Zhao J-T, Huang X, Chen Y-P et al (2009) Molecular cloning and characterization of an ortholog of NPR1 gene from Dongguan Dajiao (Musa spp. ABB). Plant Mol Biol Report 27(3):243–249. doi:10.1007/s11105-008-0074-z