Chapter 7
Bananas and Plantains (*Musa* spp.)

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7.1 Introduction

With a production of 145 million metric tons worldwide (worth 26.5 billion Euro), banana (*Musa* spp.) is one of the world's most important staple food crops and arguably the world's most popular fruit in terms of international trade (FAO 2014). Banana and plantains (*Musa* spp.), collectively referred to here as bananas, are grown in more than 135 countries and found in most tropical and subtropical regions around the world. While industrialized nations view banana primarily as a dessert

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item, many regions of the developing world consider cooking bananas and plantains as essential staples that contribute significantly to the caloric intake of low-income subsistence farmers. Although sensitivity to photoperiod has been noted in certain cultivars (Fortescue et al. 2011), banana is an almost nonseasonal crop that reliably provides a carbohydrate source year-round which makes it vitally important to both nutrition and food security. Propagation by farmers is commonly through suckers or side shoots originating from lateral buds at the base of the main plant. Multiple fungal and bacterial pathogens present serious constraints to production of bananas, as does the occurrence of insects and nematodes (Jones 1999). Viral diseases caused by banana streak virus (BSV), cucumber mosaic virus (CMV), banana bract mosaic virus (BBMV), and the emerging banana bunchy top virus (BBTV) are also receiving increased attention (Kumar et al. 2015). The predominance of these biotic agents differs from region to region, but most are found throughout the banana production regions in Asia, Africa, and the Americas and represent common targets for plant improvement worldwide. As with all crops, abiotic factors associated with climate change such as drought and heat stress also present considerable challenges to production (van Asten et al. 2011; Wairegi et al. 2010), but arguably the single greatest constraint to genetic improvement is the narrow genetic basis of most cultivated bananas (Hippolyte et al. 2012) and the physiological and reproductive barriers of the plant itself (Ssesuliba et al. 2008; Fortescue and Turner 2004; Dumpe and Ortiz 1996). Reproductive barriers limit sexual recombination in banana and hinder plant improvement. While all of the seed-bearing progenitors of modern banana cultivars are diploid in nature, those that have been cultivated for consumption are primarily seedless triploids. Female fertility of triploids has been described, but seed set is generally extremely limited which complicates breeding efforts and intensives resources and time required to develop superior varieties with enhanced resistance to multiple biotic agents and abiotic agents. Banana improvement is further complicated by parthenocarpy, reduced male fertility in some cultivars, low seed viability, irregular meiotic behavior, long generation times, and diverse genomic configurations (Ortiz 2013, 2015; Ortiz and Swennen 2014). To date, the limited progress that has been achieved in banana breeding has occurred through crossbreeding approaches that involve hybridization followed by phenotypic selection among half sibs and/or full sib progenies.

7.2 Banana Classification

Banana is a monocotyledon herbaceous plant represented by three genera (*Musa*, *Ensete*, *and Musella*) within the family Musaceae of the order Zingiberales (De Langhe et al. 2009). The genus *Ensete* consists of monocarpic, unbranched herbaceous plants that rarely produce suckers and are used for food, fiber, and ornamental purposes. They resemble banana, but their oversized, edible corms and widespreading and immensely long, paddle-shaped leaves with crimson midribs make them very distinctive. Their fruits are similar in appearance to those of banana, but

are dry, seedy, and inedible (Deckers et al. 2001). The most recognizable member of this genus is perhaps the false or Abyssinian banana (*E. ventricosum*) that plays a significant role in Ethiopian agriculture and food security (Tsegaye and Struik 2002).

The genus *Musa* consists of cultivated triploid cultivars and clones propagated through vegetative methods with limited genetic variation beyond what could be expected through somaclonal variation (and perhaps epigenetics) and the diploid wild progenitors of these cultivars that are capable of sexual recombination. More than 60 species within four recognized sections of the genus *Musa* have been described, but the taxonomy of *Musa* and the relationship between wild and cultivated bananas are far from settled (De Langhe et al. 2009; Janssens et al. 2016). Almost all diploid species are native to Southeast Asia, from India and Thailand to New Guinea and Queensland, Australia (Simmonds 1987). Edible bananas, with the exception of the Fe'i group of the Australimusa section, are derived almost exclusively from two species, *Musa acuminata* and *Musa balbisiana* of the section *Musa* (Dodds 1945). *M. acuminata* and *M. balbisiana* are diploid ($2n = 2x = 22$) in their base genomic complements and designated as AA and BB, respectively (Simmonds and Shepherd 1955). In addition to monospecific cultivars (AA, BB), interspecific diploid clones (AB) are also recognized. Higher-order combinations of the AA and BB base genomes arose through chromosome restitution at meiosis, to produce distinct groupings at the triploid (AAA, AAB, ABB groups) and occasionally tetraploid levels (Simmonds 1962). Triploids, due to their optimal vigor and seedless characteristic, are the preferred configuration for most consumers throughout the world (Simmonds 1987). The rare tetraploid cultivars tend to be physically larger but have relatively small bunches, while most diploid cultivars tend to be weaker plants with smaller bunches (De Langhe 1986). Edible parthenocarpic diploids are however cultivated in certain regions such as Tanzania (Simmonds 1962) where the Mchare (or Mshale) diploids are preferred for the unique texture characteristics of the fruit.

Generally, modern classification systems of banana tend to follow Simmonds and others (Simmonds and Shepherd 1955; Stover and Simmonds 1987) and are based on ploidy status and the relative contribution of the two genomes. Simmonds (1962, 1966) suggested that the formal Latin nomenclature should be replaced by a ploidy-based nomenclature in which the cultivar is referred to by the genus and a genomic grouping (e.g., *Musa*, AAA Group, "Gros Michel"). Cultivars are placed in higher-level groupings based on the number of chromosomes and the species that contribute to their genetic makeup (AA, BB, AAA, AAB, and ABB) (Karamura et al. 2012). Simmonds and Shepherd (1955) utilized 15 taxonomic characters specific to *M. balbisiana* and *M. acuminata* to assign cultivars to groups, and this classification scheme has been periodically updated (Stover and Simmonds 1987).

Below the level of group, cultivars are assigned to clusters of subgroups that are characterized by a representative member. For example, "Cavendish" and "Gros Michel" are considered separate subgroups under the AAA grouping along with several mutants and variants derived from these economically important cultivars. The grouping (AAA) also includes all of the economically important East African Highland cooking bananas. While this classification system may be

convenient, it appears to lack hierarchical, biological, or economic significance, for example, Mysore, Pome, and Plantain are all recognized subgroup clusters within the AAB group, but they are utilized for different purposes, and subsequent results of molecular and morphological diversity studies suggest that they are genetically distinct and likely have arisen from dissimilar parentage (De Langhe et al. 2010; Christelová et al. 2017).

Likely, cultivars within the AAA, AAB, and ABB groupings arose from multiple hybridization events followed by subsequent backcrossing to various AA, BB, and AB progenitors which results in an unequal chromosomal allocation at meiosis (De Langhe et al. 2010). It has been suggested that this phenomenon could explain the unequal and nonadditive chromosomal complementation which has been observed among interspecific hybrids (d'Hont et al. 2000). If indeed cultivars within groupings arose from multiple hybridization events, it suggests that classification may be more dependent on specific diploid progenitors than on traditional groupings based on ploidy. Morphological characteristics and nuclear and cytoplasmic molecular markers have been used to differentiate the progenitor *M. acuminata* diploids into several subspecies that correspond to specific geographic ranges from mainland Asia to the archipelagoes of Indonesia, New Guinea, and the Philippines (Hippolyte et al. 2012; Carreel et al. 2002; Perrier et al. 2011). Currently, there are eight recognized diploid AA subspecies that include roughly from West to East: *M. acuminata* ssp. *burmannica*, *M. acuminata* ssp. *siamea*, *M. acuminata* ssp. *truncata*, *M. acuminata* ssp. *malaccensis*, *M. acuminata* ssp. *zebrina*, *M. acuminata* ssp. *microcarpa*, *M. acuminata* ssp. *errans*, and *Musa acuminata* ssp. *banksii*. The subspecies have contributed important diploid parents to modern breeding programs, but much work remains toward evaluating and preserving germplasm that has not been readily accessed due to logistic or political reasons in past collecting expeditions.

Considerable efforts have been made over the past few decades to preserve, characterize, and provide access to genetic resources of *Musa*. Banana germplasm for use in breeding is distributed through the Biodiversity International Musa Germplasm Transit Centre which oversees more than 1500 accessions. The center secures available banana germplasm for long-term conservation and holds the collection in trust for the benefit of future generations under the auspices of the Food and Agriculture Organization of the United Nations. The conserved germplasm is placed in the Multilateral System of Access and Benefit Sharing of the International Treaty on Plant Genetic Resources for Food and Agriculture. All accessions have been indexed, conserved in vitro (Van den houwe et al. 1995), and most stored under cryopreservation (Panis et al. 2005). Characterization of germplasm occurs in both field trials and at the molecular level. Passport and characterization data is freely available through the Musa Germplasm Information System (MGIS) (https://www.crop-diversity.org/mgis/).

7.3 Banana Breeding Objectives

The primary objective of most banana breeding programs is the uniform production of large bunches that meet the regional qualitative and quantitative demands of growers. These demands include superior fruit quality, high suckering ability, short stature, and enhanced root systems that provide effective soil anchorage and efficient uptake of water and minerals. Other agronomic traits such as photosynthetic efficiency and rapid cycling are also important breeding objectives for increased yield. The relative importance of these objectives varies across geographic regions, among subgroups of banana, and with the intended final use of the product. In recent years, the anticipated and realized threats of pests and diseases have resulted in increased emphasis placed on identifying and utilizing improved sources of host-plant resistance to pests and diseases, particularly in regard to the Sigatoka complex, multiple races of Fusarium wilt, bacterial wilt, bunchy top, nematodes, and weevils.

In Uganda, banana breeding focuses largely on the improvement of East African Highland (cooking) bananas (EAHBs) (AAA). The expected yield and plantation life of these bananas has significantly declined, in no small part due to pests (such as banana weevils and nematodes) and diseases (including black Sigatoka and bacterial wilt). Some of the key breeding objectives by the National Banana Research Program of the Uganda National Agricultural Research Organization (NARO), in partnership with the International Institute of Tropical Agriculture (IITA), have been to identify and integrate host-plant resistance to the Sigatoka complex, weevils, and nematodes from wild diploid progenitors into elite EAHB backgrounds. A generalized criterion for selection of EAHB based on agronomic traits is presented in Table 7.1.

Table 7.1 Characteristics of the ideotype of East African Highland cooking bananas

Trait	Description
Yield potential	>25 t/ha/year
Bunch weight	>15 kg
Plant height	<3 m
Time of flowering	210–270 days
Time of bunch maturity	90–120 days
Number of hands	8–12/bunch
Number of fingers	100–190/bunch
Fruit finger circumference	10–15 cm
Fruit finger length	13–20 cm
Suckering ability	75% follower sucker growth at harvest
Root system	Vigorous (fast growing, deep, and branched)
Bunch orientation	Pendent
Reaction to prevalent diseases	Resistant to the black Sigatoka complex and bacterial wilt
Reaction to prevalent pests	Resistant to weevils and nematodes
Reaction to drought stress	Resistant/tolerant

7.4 Constraints to Banana Breeding

As previously discussed, the greatest constraint to banana genetic improvement is the limited production of viable seeds due to polyploidy, female sterility, and other factors affecting seed production in triploid and diploid banana. Female sterility has been intensified as a consequence of human selection for parthenocarpy in banana. Simmonds (1962) first suggested that continuous clonal propagation of diploids has led to an accumulation of structural chromosomal changes (translocations, inversions, and other events) that restrict normal meiosis and pollen fertility and reduce expected recombination. Specific abnormalities such as translocations have been noted in the diploids "Pisang Lilin" and "Pisang Jari Buaya," but the extent to which this phenomenon occurs throughout *Musa* is still not well understood. Adeleke et al. (2004) observed that in general, a higher incidence of univalent formation was related to low pollen fertility in both diploids and triploids. Sterility in plantains and EAHB triploid bananas has been associated with meiotic irregularities and uneven number of chromosomes, as well as to environmental factors and to influences of individual genotypes (Swennen and Vuylsteke 1993; Ssebuliba et al. 2000). Seed yield is influenced by time of pollination, environmental conditions, genetic variation in female fertility, differences observed among pollinations made between the basal and distal hand, and variation associated with the relative contributions of the *acuminata* and *balbisiana* genomes (Simmonds 1962). Sathiamoorthy and Rao (1980) observed increased seed set with proportional contributions from the *balbisiana* genome and speculated that the factors for seed sterility have accumulated preferentially in the *M. acuminata* genome (Simmonds 1962). The use of embryo rescue has significantly improved seed germination with observations of up to 30% increases in viable embryos (Swennen and Vuylsteke 1993).

A further complication associated with improvement of cultivated bananas is that the highly heterozygous state of the parents results in extremely variable progeny that makes predictions of progeny performance on the basis of parental phenotypes unreliable (Ortiz 2000). The progeny from crosses can include mixtures of ploidy levels and sometimes aneuploids. Oselebe et al. (2006) reported that while progeny of 2×–2× crosses were almost exclusively diploid (99.7%), those of mixed ploidy crosses tended to include individuals that varied in their chromosomal compliment. The same study observed that the direction of the cross impacted results. When the diploid is used as the maternal parent in a 2×–4× cross, over 96% of progeny are diploids, but when the tetraploid is used as the maternal parent (4×–2×), the observed progeny is predominately triploid (94%) with varying degrees of other ploidy levels between the diploid and the pentaploid observed. While these mixed ploidy progenies provide a mechanism for enhancing genetic diversity and recombination, they also necessitate the use of early screening of ploidy levels.

7.5 Breeding Strategies

Plant breeding provides one of the highest returns of investment in agricultural research, and while banana production has benefited from these investments, few banana and plantain cultivars acceptable to farmers and consumers were produced prior to the 1980s (Roux 2001). Triploid bananas are preferred by growers as they commonly display the most advantageous combination of fruit and vegetative characters (De Langhe 1986; Stover and Simmonds 1987), but it was generally assumed that these triploid cultivars (such as "Cavendish") were effectively sterile (Stover and Buddenhagen 1986). Persistent efforts, however, from multiple breeding programs including IITA and the Honduran Foundation for Agricultural Research (FHIA) have demonstrated that viable embryos could be produced from what were previously considered recalcitrant triploids by making hybridizations with selected pollen of diploid banana (Aguilar-Moran 2013). FHIA successfully produced 40 viable tetraploid embryos utilizing "Cavendish" as a female, although this effort required an almost Herculean task that included the pollination of over 20,000 bunches. By the end of the twentieth century, efforts to improve banana focused primarily on the use of improved diploid and synthesized tetraploid gene pools to develop secondary triploids of bananas and plantains (Fig. 7.1).

Under this breeding scheme, the identification of improved diploids that provide donor traits of interest and cultivated triploids with superior quality characteristics takes on a vital role in synthesizing superior secondary triploids. Conventionally, it was assumed that when crosses were made between male diploids and female triploids to obtain tetraploid progeny, all three sets of maternal chromosomes were trans-

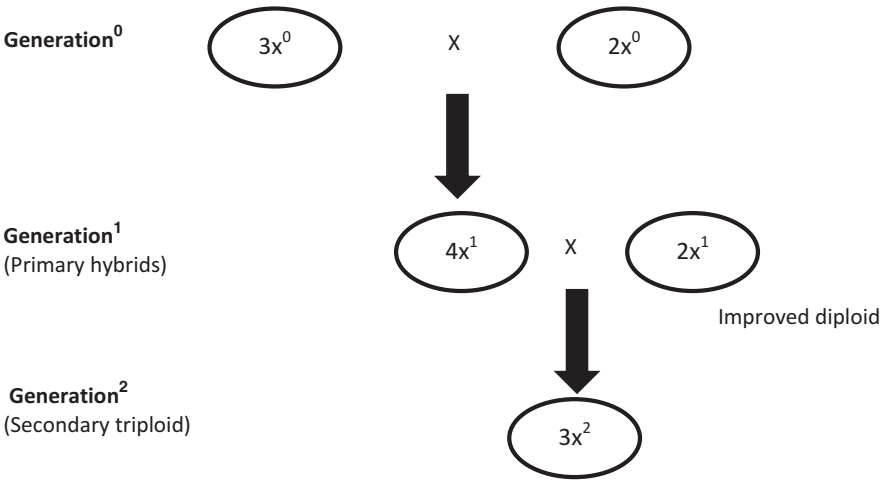

Fig. 7.1 The scheme of the banana breeding process whereby initial crosses are carried out between triploid landrace ($2n = 3x$) and diploid ($2n = 2x$)

ferred intact to the tetraploid offspring with recombination only occurring as a result of the contribution of diploid male parent (Dodds 1943). Vuylsteke et al. (1993), however, noted that tetraploid progeny from such crosses displayed variation in disease resistance, morphological traits, and growth and yield parameters that were inconsistent with this hypothesis. It was further suggested that segregation and recombination during modified megasporogenesis leading to the formation of 2n eggs in the triploid parent perhaps better explained the observed results. With the advent of new genomic resources and tools, this phenomenon needs to be further investigated in order to better understand the extent of sexual recombination in banana breeding.

This breeding strategy has been adopted by multiple programs including FHIA in Honduras which has produced a generation of acceptable tetraploids that are still currently being used in breeding (Rowe and Rosales 1993). An example includes FHIA 21, a tetraploid derived from AVP-67 French plantain that is still being utilized in plantain improvement. IITA has also utilized this approach successfully to introgress alleles for resistance/tolerance to key pest and diseases in high-yielding hybrids derived from preferred plantain cultivars (Ortiz et al. 1995; Tenkouano and Swennen 2004; Vuylsteke et al. 1993). Plantain hybrid releases include PITA 14 and PITA 17 (primary tetraploids) and more recently PITA 21, PITA 23, and PITA 24 (secondary triploids) all derived from three seed-fertile triploid French plantains Obino L'ewai, Bobby Tannap, and Mbi Egome. Their common attributes include BLS resistance/tolerance and good bunch characteristics (Tenkouano et al. 2011) and early suckering (Vuylsteke et al. 1993). These IITA hybrids have currently been distributed to ten countries in Africa and three countries in Central and South America for evaluation and adoption.

At NARO, tetraploids (AAAA) were synthesized from EAHBs (AAA) by crossing to the wild-seeded, fertile male parent Calcutta 4 (AA) that is used by many programs as a source of resistance to multiple pests and diseases. A number of these tetraploids developed such as 365K-1, 1201K-1, 917K-2, 660K-1, 1438K-1, and 222K-1 25 were fundamental in the development of 27 NARITA triploid banana hybrids by NARO and IITA (Tushemereirwe et al. 2015). These banana hybrids were selected from early evaluation trials based largely on resistance to black Sigatoka and bunch size and subsequently advanced to the preliminary yield trials in Uganda. NARITAs are currently under evaluation for agronomic, sensory, pest, and disease resistance traits in multi-environment trials in Uganda and Tanzania.

An alternative breeding scheme has been suggested by Vakili (1968) and involves the polyploidization of diploid hybrids or cultivars through the use of colchicine or oryzalin to obtain tetraploids for crossing with 2× lines to generate triploids. This approach is currently being pursued by multiple breeding programs and shows considerable promise (Bakry et al. 2009). According to Tenkouano et al. (2011), the two schemes conform to differences in breeding philosophies. The former can be viewed as evolutionary breeding as it attempts to mimic the developmental pathway of *Musa* by crossing female triploid landraces to diploid accessions of *M. acuminata* or *M. balbisiana*, while the latter can be considered reconstitutive breeding as it utilizes the most likely diploid ancestors or relatives of triploid landraces for chromosome doubling to create improved triploids.

7.5.1 Development of Improved Diploids

Regardless of the breeding scheme, the narrow genetic variability and limited fertility among cultivated triploid bananas make diploid bananas vital to genetic improvement. A number of fertile improved diploids with varying degrees of disease resistance have been released by IITA and FHIA (Tenkouano et al. 2003; Rowe and Rosales 1993). Diploid improvement has almost exclusively been through the use of *M. acuminata* cultivars such as "Calcutta 4" (*M. acuminata*), a source of resistance to the Sigatoka complex, yellow Sigatoka, fusarium wilt, banana weevil, and burrowing nematodes (Ortiz 2015). Decades of breeding utilizing this material have resulted in the production of improved diploid lines which combine disease/pest resistance, short stature, and interesting bunch characteristics (Tenkouano et al. 2003; Krishnamoorthy and Kumar 2005). Developing further improved diploids that possess multiple sources of resistance, while preserving the quality characteristics of preferred triploid would greatly increase the efficiency of breeding efforts that are hindered by the constraints previously described.

M. balbisiana diploid cultivars have made limited contributions to breeding due to the presence of endogenous banana streak virus (eBSV) sequences originating from the B genome that are activated under the appropriate conditions (Iskra-Caruana et al. 2014). Progenies of interspecific *acuminata/balbisiana* hybridizations have often been associated with the occurrence of banana streak disease, and this has resulted in the underutilization of the B genome which could be an important source of drought tolerance and resistance alleles not found in *M. acuminata* (Bakry et al. 2009). Recently, Noumbissie et al. (2016) reported segregation of the eBSV sequences in the progeny of crosses between the tetraploid hybrid CRBP 39 (+eBSV) and the AA male parent Pahang (−eBSV), from which resulted triploid eBSV-free offspring. Umber et al. (2016) also documented the successful creation of diploids free of eBSV alleles from *M. balbisiana* diploids suggesting that recombination between *M. acuminata* and *M. balbisiana* can be accomplished for improvement of both cultivated banana and plantain without concern of introducing banana leaf streak.

7.5.2 Breeding Methodology and Evaluation of Hybrids

Production of viable seed through hybridization is critical for the success of breeding and is dependent on residual fertility of triploid cultivars. Practical aspects of artificial hybridization have been described by Tenkouano et al. (2011). Hybridizations are made through manual pollinations in the early hours of the morning when pollen availability is not a limiting factor. It can take up to several months to obtain seeds from a desired cross, and production of seeds is generally poor and has been reported in the range of 0.3–21.7 seeds per bunch (Swennen and Vuylsteke 1993) (Fig. 7.2). Seeds obtained from crosses also germinate poorly, and it is standard practice by most programs to recover hybrids through in vitro culture (Bakry 2008) (Fig. 7.3).

Fig. 7.2 Male banana flowers open in the evening and are ready for pollination early in the morning

Fig. 7.3 Bract is pulled back and pollen applied directly to the receptive female flowers

Bunches are harvested prior to physiological maturity, generally when the first signs of yellow color are observed in the distal fingers. Bunches are left to ripen in protected sheds, and seeds are extracted and surface-sterilized for embryo culture to avoid seed/embryo desiccation. Embryos are extracted from dissected seeds under aseptic conditions and cultured on artificial culture media (Bakry et al. 2009; Uma et al. 2011). Longitudinal excisions are made on the seed to expose the embryo

beneath the micropyle. The embryo is placed on sterile culture medium and incubated in the dark for germination. Embryos typically germinate between 5 and 20 days after which time they are transferred to an environment with appropriate light and dark cycles for shoot and root development. Well-developed seedlings can then be cloned to replicate one to four rooted plantlets or transferred to a screenhouse for conditioning prior to field evaluations. More research on pollen production, pollen tube growth, and embryo viability is required to better understand issues associated with poor seed production and to optimize conditions that will lead to better seed yield (Uma et al. 2011). In particular, detailed knowledge of floral biology and seed development is crucial for recovery of progeny from crosses (Fortescue and Turner 2011).

In the field, new hybrids are subjected to early evaluation trials (EETs) with limited replication (one to five plants). EETs are observed generally for two cycles during which a few simply inherited traits such as bunch size and orientation, Sigatoka resistance, seed production, and ploidy level are evaluated. Plants that show promise in EETs are further evaluated in preliminary yield trials (PYTs) where replicated clones of selected hybrids are evaluated over two cycles. PYTs involve more detailed evaluation for additional, complex traits such as yield and disease resistance. Finally, superior-performing plants from PYTs are cloned in significant numbers to allow for multi-locational evaluation trials (MET) that often include direct input from farmers (Tenkouano et al. 2011). In theory, this process can take a minimum of 7 years to produce a superior banana hybrid, although in practice this time frame is often exceeded.

7.6 Applied Biotechnology

7.6.1 Molecular-Assisted Breeding

Due to the breeding constraints previously discussed, the use of molecular markers holds considerable promise in improving the efficiency of banana breeding but is currently not routinely used in most breeding programs. Efforts toward the development and use of molecular markers have been greatly facilitated by the recent release and refinement of the draft genomic sequence of the double haploid *M. acuminata* cultivar "Pahang" (A genome) (D'Hont et al. 2012; Martin et al. 2016) and a draft sequence of *M. balbisiana* "Pisang Klutuk Wulung" (B genome, Davey et al. 2013). Multiple transcriptome data sets have also been published (Li et al. 2013; Wang et al. 2012), and of these publications, over 45,000 expressed sequence tags, and 34,000 annotated genes associated with *Musa* are currently available through NCBI-EST database. These genomic resources have contributed to the availability of multiple classes of markers summarized in Tables 7.1 and 7.2. Molecular markers provide genetic "landmarks" for tagging important traits in plant breeding, conducting linkage analysis and estimates of genetic diversity, facilitating gene introgression through marker-assisted studies, providing validation of taxonomy and cultivar identification, and estimating evolutionary and speciation events.

Table 7.2 A summary of molecular markers utilized in banana research and their research applications

Marker application	Marker type	Reference
Molecular systematics	Isozymes, SSR, DArTs, RFLP and ITS	Perrier et al. (2011), Christelová et al. (2017), and Simmonds (1966)
Genetic diversity studies	RAPD, SSR, AFLP and MSAP	Karamura et al. (2016), Kitavi et al. (2016), Nyine and Pillay (2011), Opara et al. (2010) Wang et al. (2007), Noyer et al. (2005), Creste et al. (2004), Ude et al. (2003), Ude et al. (2002), Pillay et al. (2001), Crouch et al. (2000), and Crouch et al. (1999)
Genome characterization	RAPD, RFLP, ITS, dCAP and IRAP	
Cultivar identification and pedigree tracking	Isozymes, RFLP, SSR, RAPD, EST-SSR and ISSR	Mbanjo et al. (2012), Hippolyte et al. (2012), and Raboin et al. (2005)
Linkage analysis	Isozyme, RAPD, RFLP, AFLP, SSR AS-PCR and DArTs	Mbanjo et al. (2012), Hippolyte et al. (2010) and Fauré et al. (1993)
Genome-wide association studies and marker-assisted selection	Isozymes, dCAP and SNP	Sardos et al. (2016), Umber et al. (2016), and Noumbissié et al. (2016)

7.6.1.1 Association of Molecular Marker with Important Genes

The tagging of significant genes contributing to traits of interest with genic or linked markers allows for screening of plant germplasm at the earliest stages of development. The association of these markers with important traits can come through classical linkage or association studies or through candidate gene approaches that leverage recently available genomic resources. An example of the candidate gene approach is provided by Emediato et al. (2009) who amplified homologues of black leaf streak disease resistance genes in *Musa* through the use of degenerate primers based on genes from other crops. The study successfully amplified sequence differences between the diploid *M. acuminata* cultivars "Calcutta 4" (resistant) and "Pisang Berlin" (susceptible). This work followed the earlier identification of 50 distinct tagged nucleotide binding site-leucine-rich repeat (NBS-LRR) resistance gene analogs in cultivar "Calcutta 4" by Miller et al. (2008). Wang et al. (2012) used pooled DNA from *Fusarium oxysporum* f.sp. *cubensis* (Foc TR4)-resistant and susceptible cultivars to identify randomly amplified polymorphic DNA (RAPD) markers that could distinguish between resistant and susceptible cultivars. Two RAPD markers were converted to sequence characterized amplified region (SCAR) markers which could be amplified in Foc TR4-resistant banana genotypes ("Williams 8818-1" and Goldfinger), but not in five tested susceptible banana cultivars. Work on this continues at the national banana program in Brazil (EMBRAPA) and shows great promise in providing an early screen for resistance to Foc TR4 (Silva et al. 2016).

As previously discussed, endogenous banana streak virus (eBSV) limits the extensive use of the B genome in banana breeding, but the tagging of this sequence has opened possibilities of greater utilization in the future. Lheureux et al. (2003) mapped the eBSV sequence using amplified fragment length polymorphism (AFLP) markers, and Noumbissié et al. (2016) used simple sequence repeat (SSR) markers and eBSV-specific PCR markers to identify hybrids lacking the eBSV sequence. Umber et al. (2016) successfully identified infectious and noninfectious BSV alleles using derived cleaved amplified polymorphic sequences (dCAPS). These studies suggest that these markers can be used early in the breeding process as diagnostic markers for eBSV-free B genome hybrids that will greatly enhance breeding efforts. While the progress shown by these early efforts is promising, markers associated with traits of economic importance need to be validated in broader germplasm pools over multiple years to ensure that they will prove to be reliable and stable and that genotypic predictions at the early stages of screening will be highly correlated with plant phenotypes at full maturity under field conditions.

7.6.1.2 Linkage, Association Mapping, and Genomic Selection

Genetic linkage maps provide opportunities for gene identification and a mechanism for understanding the inheritance pattern of both qualitative and quantitative traits. Mapping requires appropriate plant populations of known structure derived from parents that differ significantly in traits of interest, a set of markers segregating in the given population that provides substantial coverage of all chromosomes, and the careful collection of phenotypic data from multiple years and preferably locations. Linkage mapping has not gained significant practical application in banana breeding. This could be attributed in part to limitations inherent in marker technologies and analysis (Foolad 2007; Pillay et al. 2012), to previously described chromosomal abnormalities in banana that inhibit recombination and to contribute ambiguous assignment of marker location. To date, principally F1 and F2 diploid populations have been utilized due to difficulties associated with developing double haploid or recombinant inbred lines in banana.

The first genetic mapping population in banana was reported in 1993 (Fauré et al. 1993) and consisted of 92 F_2 progeny (AA) derived from an F_1 hybrid (SFB5) of the cross "SF265" (CIRAD-IRFA II.04.20.004.020) × "banksii" (CIRAD-IRFA II.04.01.004.001). Seventy-seven loci consisting of RAPDs, isozymes, and restriction fragment length polymorphisms (RFLPs) were mapped onto 15 linkage groups spanning 606 cM. Segregation distortion was associated with 36% of the mapped loci and was biased toward the "banksii" parent. Hippolyte et al. (2010) published a more saturated map using an F_1 diploid "AA" population created from a cross between "Borneo" and "Pisang Lilin." The map was constructed using 426 markers (SSR and DArT). Separate maps were constructed for markers that segregated from each of the heterozygous parents, and a synthetic map was constructed that spanned 11 linkage groups and represented 1197 cM. Three regions of this synthetic map were inconsistent between the two parents and were attributed by the authors to structural rearrangements. Subsequent mapping projects have also noted such incongruities, and while these suggestions are supported by cytogenetic evidence such as multivalent pairing (Shepherd 1999), much work needs to be done to verify this hypothesis and determine the extent that such phenomenon occurs across *Musa* spp. Mbanjo et al. (2012) produced the most recent map utilizing an F_1 population consisting of crosses between 6142-1 × 8075-7 and 6142-1-S × 8075-7. Two maternal (6142-1 and 6142-1-S) and one paternal (8075-7) maps were generated using diversity array technology (DArT), SSR, and allele-specific PCR (AS-PCR) markers. As with other maps, considerable (41%) segregation distortion was observed at marker loci.

Association mapping has been proposed as an alternative to conventional linkage mapping. In this strategy, a panel of genotypes from unrelated population (or a population with known genetic substructure) is utilized to identify associations between molecular markers that are in linkage disequilibrium with genetic loci affecting phenotypes. Molecular markers that are distributed throughout the genome such as single nucleotide polymorphisms (SNP) are preferred for genome-wide association studies (GWAS). Sardos et al. (2016) demonstrated the technique by assembling a GWAS panel of 104 AA accessions using 5544 SNP markers

derived from genotyping by sequencing (GBS) and publicly available phenotypic data on parthenocarpy. The study identified 13 genomic regions associated with parthenocarpy, and multiple candidate genes in these regions corresponded with putative growth regulators and genes associated with gametophyte development and female sterility in other plant species.

Genomic selection (GS) is a form of marker-assisted selection that utilizes high-density molecular markers such as SNPs to provide coverage of the whole genome, ensuring that all quantitative trait loci (QTL) are in linkage disequilibrium with at least one marker (Hayes and Goddard 2010). GS estimates the genomic breeding value of individual genotypes in a large segregating population utilizing one of several GS models (Meuwissen et al. 2001). GS is less concerned with the identification of individual QTL as it is with developing appropriate models to enhance selection efficiency. As the cost of generating marker data becomes increasingly more affordable, GS has become an attractive alternative to many breeding programs (Lorenz et al. 2011; Crossa et al. 2010). Currently, efforts are underway to evaluate GS as a strategy to improve banana by generating appropriate breeding models for the improvement of EAHB (Nyine et al. 2016).

7.6.1.3 Estimating Genetic Diversity and Evolutionary Events

Estimates of genetic diversity determine to a large extent the potential of plant improvement that can be anticipated and can also provide guidance to breeders as to the appropriate parents to use in breeding schemes. Estimates based on phenotypic or morphological characters have long been used in banana (Karamura 1998), but often these estimates can be biased by environmental influences as well as the sometimes complimentary and polygenic nature of underlying genetic factors. Molecular markers avoid these issues as they are highly heritable and have supplemented or replaced the usage of such measurements in most plant species where they are available. In banana, several classes of molecular markers have been used to estimate diversity among populations of varying size representing regional collections and breeding program germplasm. These include RFLPs (Jarret et al. 1993; Bhat et al. 1995), RAPDs (Bhat et al. 1995; Crouch et al. 2000; Pillay et al. 2001; Ude et al. 2003; Nyine and Pillay 2011), AFLPs (Ude et al. 2002, 2003; Noyer et al. 2005; Wang et al. 2007; Opara et al. 2010), SSRs (Kaemmer et al. 1997; Crouch et al. 1999; Tenkouano et al. 1999; Noyer et al. 2005; Creste et al. 2004; Hippolyte et al. 2012; Kitavi et al. 2016; Karamura et al. 2016), sequence-related amplified polymorphisms (SRAPs) (Wei et al. 2011; Valdez-Ojeda et al. 2014), DArT (Risterucci et al. 2009), and methylation-sensitive amplification polymorphism (MASP) (Noyer et al. 2005).

Estimates of genetic diversity generated from these studies vary with the class and number of markers used and with the genotypes selected for inclusion in any given study, but a few generalized observations can be made: (1) Diversity estimates based on phenotypic measurements are often poorly correlated with molecular estimates (Crouch et al. 2000); (2) despite considerable phenotypic or morphological variation among regional *Musa* landraces, they tend to have limited genetic variation when assayed with molecular markers. For example, East African

Highland bananas (EAHBs) have been classified into five clades (clone sets) based on their end use and morphological distinctiveness (Karamura 1998). Studies focusing on EAHB using both RAPDs and SSR markers, however, found limited evidence to support the significant variation either within or between these clades (Pillay et al. 2001; Kitavi et al. 2016; Karamura et al. 2016). This led to the suggestion that EAHB arose from a single hybridization event that has subsequently been acted on by a series of somatic mutations and influenced by natural and directed selection leading to many distinct cultivars. Presumably, the finite numbers of markers used are unable to distinguish among the clades. Utilizing different classes of molecular markers can sometimes reveal variation in populations where it has not been previously noted. In plantain landraces of West Africa, RAPD, SSR, and amplified fragment length polymorphism (AFLP) markers displayed few polymorphisms (Crouch et al. 2000). However, when HpaII and MspI methylation-sensitive amplified markers were used, polymorphism (MSAP) profiles revealed three clusters (Noyer et al. 2005) and a genetically distinct subset of plantains from Cameroon (Ude et al. 2003).

Molecular markers have played important roles in determining the evolutionary history of cultivated banana and establishing links to diploid progenitors. Whether the breeder utilizes an evolutionary or reconstitutive approach to banana improvement (discussed in a previous section) plays a vital role in effectively combining novel resistance traits with quality characteristics desired by growers. Perrier et al. (2011) detail the available molecular, archaeology, genetic, and linguistic evidence for this important aspect of breeding. Of particular interest to the dessert banana industry has been the observation that East African diploid bananas appear to have played an important evolutionary role in the development of "Cavendish" and "Gros Michel," the most widely used cultivars that have dominated the banana export industry over the past century (Raboin et al. 2005; Risterucci et al. 2009).

7.7 Conclusions

While progress has been made toward genetic improvement since the first formal programs were established almost a hundred years ago, in some aspects the breeding of banana is still in its infancy when compared to the improvement of other important staple crops. In no small part, this is due to the physical and reproductive constraints of the plant itself, but there is room for optimism as these constraints appear to be neither absolute nor prohibitive. Molecular markers, the availability of additional genomic resources, and ongoing studies elucidating the floral and reproductive biology of banana hold great promise for the next hundred years of banana improvement.

Genetic engineering has not been discussed in this chapter, but the early work in this arena also suggests that it also has the potential to make an important contribution to *Musa* improvement through the introduction of genetic factors not found within cultivated or wild *Musa* germplasm (Tripathi et al. 2012).

Further work is needed on predicting the performance and combining ability of male and female parents in *Musa* improvement. Tenkouano et al. (2012) reported the significance of additive genetic effects on expression of bunch weight, fruit filling time, fruit length, plant height, and number of leaves and nonadditive effects for suckering behavior and fruit circumference in 3× hybrids obtained from plantain derived 4×–2× crosses. They further suggested that maternal general combining ability (GCA) accounted for the additive genetic variation for plant height and number of leaves, while paternal GCA effects accounted for fruit filling time, bunch weight, and fruit length. On the other hand, specific combining ability (SCA) effects were observed for all traits, except fruit filling time, suggesting that additional genetic gain could be achieved through recombinative heterosis for such traits. They concluded that increased bunch weight and faster cycling are inherited from the 2× male parent, while plant height, number of leaves, and suckering behavior are inherited from the 4× female parent which should guide parental selection for 4×–2× crossbreeding. More of this information is needed to efficiently guide the decision-making efforts of breeders and allow them to allocate limited resources.

Finally, in the popular press, there has been considerable alarm in recent years as to the future of banana in the face of an evolving pathogen (Foc TR4) that threatens the production of much of the world's dessert banana production. In some ways, the economic damage that this pathogen will likely cause can be viewed as self-inflicted in nature. The export industry has demonstrated an overdependence on monoculture and complacency in regard to breeding that has significantly contributed to creating an environment conducive to the selection and spread of novel pathogenic races. This is a lesson that should have been learned more than a half century before when a similar threat was encountered by a different race of the same pathogen. Banana breeding efforts to improve the industry standard "Gros Michel" were curtailed or sidelined when a suitable replacement ("Cavendish") was selected from existing stock. Hopefully, the current crises will provide an impetus and serve as a reminder to all that proactive breeding programs are the most efficient and cost-effective frontline defense against current and evolving threats to production.

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