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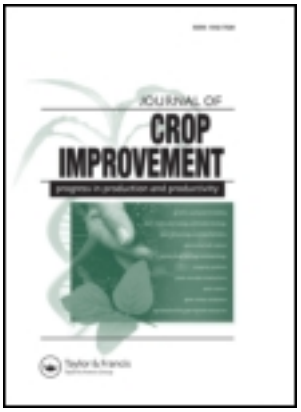

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Genetic Improvement of Banana Using Conventional and *In Vitro* Technologies

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*Banana (*Musa *spp.) is an important nutrient-rich fruit crop cultivated in the tropics and sub-tropics for local consumption and export. Targets for genetic improvement of banana range from improved fruit quality, yield, disease resistance, tolerance to biotic and abiotic stresses, and the biosynthesis of pharmaceutical compounds. Sterility has limited the success of generating new cultivars by conventional breeding. Tissue culture-based technologies that involve embryo rescue, the generation of somaclonal variation, and gene-transfer procedures are a useful adjunct to sexual hybridization, although considerable effort is required to establish robust protoplast-to-plant systems for somatic hybridization. Transformation involving Agrobacterium and biolistics-mediated gene transfer is feasible, underpinned by shoot regeneration from cultured cells and tissues. Molecular characterization of germplasm will facilitate the selection of material most relevant for incorporation into sexual and somatic genetic-improvement programs.*

*KEYWORDS banana (*Musa *spp.), conventional breeding, cryopreservation, genetic manipulation, tissue and cell culture, micropropagation, somatic hybridization/cybridization, transformation*

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INTRODUCTION

Banana (*Musa* spp.) is an evergreen perennial, monocotyledonous plant of the family Musaceae. The latter consists of seminiferous and cultivated species with broad biological diversity (Abadie et al. 2003). Currently, about 1,000 banana cultivars and landraces are recognized from 50 or so *Musa* species (Heslop-Harrison & Schwarzacher 2007). Bananas are grown in the tropics and subtropics at latitudes of 20 degrees above and below the equator, where there is a wide seasonal variation in rainfall and temperature (Pua 2007).

Two wild species, *M. acuminata* and *M. balbisiana*, are the progenitors of modern edible bananas. The main centers of diversity for *M. acuminata* and its derivative hybrids are Malaysia and Indonesia (Asif, Mak, & Othman 2001; Daniells et al. 2001), whereas *M. balbisiana* and its hybrids are presumed to be native to India (Robinson 1996). The distribution of bananas from their centers of origin is probably through planting of vegetative materials transported to other tropical and subtropical regions, such as Africa, the Caribbean, Latin America, Oceania, and the Middle East (Price 1995; Robinson 1996). Banana plants were introduced about 500 years ago into Central and Latin America, where the crop became of major economic importance (van den Houwe, Panis, & Swennen 2000). Distinct centers of secondary genetic diversification may have evolved in the Great Lakes region of East Africa and in the more humid forests of Central and West Africa (van den Houwe, Panis, & Swennen 2000). Currently, bananas are cultivated in 120 countries throughout the humid tropics and subtropics in the Americas, Asia, Africa, Australia (Queensland), and Europe (Canary Islands) (Heslop-Harrison & Schwarzacher 2007). The leading producers of bananas in 2007 were India, China, The Philippines, Brazil, and Ecuador (FAOSTAT 2009).

Bananas are multipurpose plants because most of their parts can be used in various ways, depending on the species. The most important part is the edible fruit, which can be eaten either ripe as a dessert, or unripe as boiled, fried or roasted food (Smith et al. 2005). Nutritionally, the fruit is rich in carbohydrates, vitamins A, B, and C, and potassium (Aurore, Parfait, & Fahrasmane 2009). The unripe fruit can be brewed to form beer and wine, or processed into sauce, flour, chips, crisps, smoked products, and confectionary. Unripe fruit is also a source of amylase and starch (van den Houwe, Panis, & Swennen 2000). Male floral buds can be eaten as a boiled vegetable, whereas pseudostems are a source of fiber for the manufacture of rope, paper, and textiles. Banana leaves are used for thatching, in the production of fabric and cordage, and as mulch and animal forage (Smith et al. 2005). Species such as *M. ornata* and *M. veluntina* are popular ornamental plants (Heslop-Harrison & Schwarzacher 2007).

Banana cultivation is focused mainly on parthenocarpic cultivars for the production of edible fleshy seedless fruit, an important food on a global scale. The world banana-fruit production in 2007 was estimated to be 86 million tons, harvested from an area of about 5 million hectares. In developing countries, bananas are the fourth most important crop after rice, wheat, and maize in production. Banana ranked second after citrus on the basis of the world fruit-crop production in 2007 (FAOSTAT 2009). Approximately 47% of the global banana-fruit production has been dominated by cultivars of the Cavendish type because of their high yields and short periods to maturity (Robinson 1996; Kulkarni et al. 2007). The leading producers of banana fruit in 2007 were India, China, the Philippines, Brazil, and Ecuador. Globally, the production of bananas is primarily for local consumption as a dietary supplement or staple food in the producing countries. An estimated 80% (68 million tons) of the world banana-fruit production in 2007 was consumed and traded locally in the producing countries. Banana fruit, essentially of the cooking type, is important as a staple food in the East African highlands. Consequently, fruit production is important to both food and income securities of the producing countries.

Bananas are popular as fresh fruit in temperate countries. In 2007, the world export of bananas, consisting mainly of Cavendish-type dessert bananas, was estimated to be 18 million tons (20% of world production), amounting to US$7.2 billion in economic terms (FAOSTAT 2009). The most important attributes that make the Cavendish subgroup the main bananas for export are related to their reliability during transport and their shelf life, rather than taste. In economic value, banana fruit ranked fifth in the world trade for agricultural crops (Aurore, Parfait, & Fahrasmane 2009), the leading banana exporters in 2007 being Colombia, Costa Rica, Ecuador, Guatemala, and the Philippines. The leading importers were Belgium, Germany, the United Kingdom (UK), and the United States.

Sexual hybridization and mutation have produced numerous banana varieties with different ploidy and genome combinations. Bananas are classified according to their ploidy, based on the number of sets of chromosomes, namely diploid ($2n = 2x = 22$), triploid ($2n = 3x = 33$), and tetraploid ($2n = 4x = 44$). The majority of cultivars are triploids, some are diploids and seedless, but very few are tetraploids. Bananas are also classified into genomic groups based on their genome constitutions, which are designated by the letters A, B, S, and T to represent *M. acuminata* (AA), *M. balbisiana* (BB), *M. schizocarpa* (SS), and *M. textilis* (TT), respectively. Additionally, they are classified into several main subgroups, including Sucrier, Gros Michel, Bluggoe, Ice Cream, Mysore, and Cavendish. The Cavendish subgroup is divided into Dwarf Cavendish, Giant Cavendish, Pisang Masak Hijau, Robusta, and Valery. Most commercial banana cultivars, with triploid AAA genomes, belong to the Cavendish subgroup (Robinson 1996).

TARGETS FOR GENETIC IMPROVEMENT OF BANANA

Genetic improvement of bananas is important to create new elite hybrids possessing traits of agronomic excellence, such as high yield, combined with resistance or tolerance to biotic and abiotic stresses. Other desirable characteristics include excellent fruit quality, early flowering/maturity, short stature, photosynthetic efficiency, minimum period between successive harvests, strong roots, cylindrical bunches of fruit, and fruits of uniform size (Pillay, Tenkouano, & Hartman 2002; Bakry et al. 2009). Initially, genetic improvement programs were conducted mainly to address some of the constraints of banana production, including the detrimental effects of pests, diseases, drought, and low yields (Pillay & Tripathi 2007). In general, progress in banana breeding has been slow because of very limited research on this crop in the past. Other inherent problems include polyploidy, parthenocarpic fruit development, low female fertility, and the generation of asexual progeny in sufficient numbers to recombine desirable characters. Additional difficulties include a prolonged life cycle, the low *in vivo* rate of propagation, narrow range of genetic variability, and retention of seedless cultivars by breeders related to the preference of consumers for seedless fruit (Kulkarni et al. 2007).

Initially, banana breeding programs aimed primarily to produce Gros Michel- and Cavendish-derived hybrids resistant to Panama and Sigatoka diseases. Panama disease, incited by *Fusarium oxysporum* f. sp. *cubense* Race 1, was the first disease to devastate the banana industry worldwide, because most commercial plantations cultivated the cultivar (cv.) Gros Michel that was highly susceptible to the disease. The Cavendish subgroups are prone to a more virulent form of Panama disease, namely *F. oxysporum* f. sp. *cubense* Race 4 (Heslop-Harrison & Schwarzacher 2007). The earliest attempts in banana breeding were to generate hybrids resistant to Panama disease by crossing the cv. Gros Michel with disease-resistant diploids (Smith et al. 2005) and Cavendish hybrids resistant to Black Sigatoka disease (Pillay & Tripathi 2007). According to Bakry et al. (2009), breeding of banana at the Brazilian Enterprise for Agricultural Research-National Center for Research on Cassava and Fruit Crops (EMBRAPA-CNPMF) has been targeted to the production of tetraploids with AAAB genomes resistant to *Fusarium* wilt (*F. oxysporum* f. sp. *cubense* Race 1), nematodes, Yellow Sigatoka disease caused by the fungus *Mycosphaerella musicola*, bacterial wilt (*Xanthomonas campestris* pv. *musacearum*), weevils, and black leaf-streak disease (*M. fijiensis*). Concurrently, starchy tetraploid bananas, such as the cvs. BITA 03 and PITA 16, were produced in genetic improvement programs at the International Institute of Tropical Agriculture (IITA), Uganda, with cvs. BITA 03 and PITA 16 being used for the production of wine and beer, respectively. In genetic improvement programs at the Banana Research Station (BRS), India, the tetraploid hybrid BRS-01 of the Pome subgroup

(*Musa* spp. AAA cv. Agniswar × *M. acuminata* AA cv. Pisang Lilin) and the hybrid BRS-02 (*Musa* spp. ABB cv. Vannan × *M. acuminata* AA cv. Pisang Lilin) of the Mysore subgroup exhibited immunity to *M. eumusae* and *M. musicola*. Two productive banana hybrids, FLHORBAN 920 and FLHORBAN 918, were developed at the French Agricultural Research Center (La Recherche Agronomique pour le Développement; CIRAD, France), these exhibiting resistance to black leaf spot disease (*M. fijiensis*), *Fusarium* wilt, and nematode attack (Bakry et al. 2009). Only a limited number of banana hybrids that have emerged from breeding programs have been released for field trials or for cultivation since the earlier breeding of bananas in the 1920s (Heslop-Harrison & Schwarzacher 2007). Smith et al. (2005) reported that one of the first hybrids released was Goldfinger AAAB (FHIA-01), 70 years after the first banana breeding program was initiated.

Other recent research has targeted the genetic transformation of bananas to produce human vaccines against infectious, autoimmune and tumor diseases, with potential applications in developing countries (Sala et al. 2003). Bananas, which are available year-round in the tropics and subtropics, are an ideal host because most of the edible bananas do not set seed and fruits develop parthenocarpically, preventing transgenes from being transferred to other plants, so-called gene escape. Success has also been reported in producing an antigen in banana, which can be used to make a vaccine against hepatitis B (Arntzen & Mason 1996; Sunil, Ganapathi, & Vapat 2004; Sunil et al. 2005). Arntzen and Mason (1996) reported that the accumulation of antigen in banana fruit at 1% of the total protein would allow 1 mg of vaccine to be provided by 10 g of edible banana fruit. Sunil et al. (2005) demonstrated that a recombinant hepatitis B surface antigen, HBsAg, could be expressed, albeit at low concentrations, in fruits of the transgenic banana cv. Rasthali (AAB). Expression of HBsAg could be increased in banana fruits by using the promoter of the abundant 31-kilodalton pulp protein (Clendennen et al. 1998). It is anticipated that recent biotechnological advances will facilitate the breeding and genetic improvement of bananas resistant to weevils, viruses, nematodes, and possibly other agronomically important traits (Swennen et al. 2004).

CONVENTIONAL TECHNIQUES FOR GENETIC IMPROVEMENT OF BANANA

Sexual Hybridization

Breeding of most cultivated bananas has relied upon conventional sexual hybridization, involving the crossing of triploid cultivars with wild or cultivated diploid parents. Generally, crossing triploid (3*x*) cultivars, which have residual fertility with diploid (2*x*) parents, generates tetraploid (4*x*) hybrids (Pillay, Tenkouano, & Hartman 2002). This strategy emphasized the

need to cross improve diploids, which have good agronomic qualities, with disease-resistant triploid accessions to generate diploid hybrids with agronomic excellence, such as pest and disease resistances (Pedraza et al. 2005). However, diploid bananas generally have unacceptable low yields. The $3x/2x$ procedure has generated triploid hybrids with low seed set (Smith et al. 2005). Further crossing of these triploid hybrids with wild disease-resistant diploids produced tetraploid hybrids, but the latter were unsuitable for cultivation because of undesirable features, such as premature senescence, fruit drop, short shelf-life, a weak pseudostem, and production of seeds (Smith et al. 2005). The main factor hampering progress in banana breeding using conventional genetic improvement methods is the sterility of most edible varieties because of their triploidy (Assani et al. 2005). However, the $3x/2x$ strategy enables the creation of AA diploid hybrids (Bakry et al. 2009).

Banana-breeding efforts have also focused on the improvement of selected wild, semi-parthenocarpic and parthenocarpic diploid male parents. Intensive breeding of fertile parthenocarpic edible diploids, which have large fruits of improved shape, resulted in the development of hybrid M53 showing resistance to Sigatoka leaf spot and *Fusarium* wilt (Bakry et al. 2009). A more recent strategy is the use of fertile diploid AA hybrids, resulting from $3x/2x$ crosses, as parents or as starting material for developing elite diploids, especially for plantains and East African highland bananas that are resistant to Sigatoka leaf spot.

Another breeding strategy is the generation of secondary triploids by crossing fertile tetraploid plants with diploid hybrids, a strategy that has been exploited to genetically improve some cooking bananas. Using this breeding approach, some of the banana cvs. obtained in this way have been AAB hybrids, such as IRFA909, IRFA910, and IRFA914, and AAA hybrids, including FLHORBAN 918 and FLHORBAN 920 (Bakry et al. 2009). A recent breeding strategy aimed at the synthesis of triploid hybrids directly from diploid germplasm, which is based on the specific combining ability between two diploids, one being the donor of diploid gametes, was developed by CIRAD. The production of diploid gametes has been achieved through chromosome doubling by treating selected mono-and inter-specific diploids with colchicine to generate auto- or allotetraploids. Clearly, while sexual hybridization will continue to be exploited for the genetic improvement of bananas, this approach has limitations, emphasizing the relevance of tissue culture-based technologies as important adjuncts to conventional breeding.

Induction of Mutations

Induced mutation by treatment of *in vitro* material with physical (e.g., gamma radiation) or chemical agents, such as ethyl methane-sulphonate, sodium azide, or diethylsulphate, has been applied to banana breeding

(Kulkarni et al. 2007) and has been exploited in attempts to compensate for agronomic weaknesses in existing cultivars (Heslop-Harrison & Schwarzacher 2007). Although the production of commercially interesting variants is possible by induced mutation, this approach has been of limited success. However, Novaria and Klue Hom Tong KU1 are two of the banana cultivars derived from gamma ray-induced mutation that have been released commercially (Smith et al. 2005). The important agronomic traits of these mutants include early flowering in Novaria and large bunches of fruit in Klue Hom Tong KU1 (Mak et al. 1996; Maluszynski 2001; Roux 2004; Smith et al. 2005). Most of the banana mutants released commercially have been induced by gamma irradiation.

A further breeding strategy is the triploid approach, which involves the induction of tetraploids from diploids by colchicine treatment of parental tissues, the subsequent selection of improved tetraploid lines, and hybridization of the selected tetraploids with diploids to produce triploids suitable for final evaluation (Smith et al. 2005). Both colchicine and oryzalin have been used as mutagens to induce tetraploids and autotetraploids in banana (Hamill, Smith, & Dodd 1992; van Duren et al. 1996), with the manipulation of ploidy by *in vitro* mutation technology being integrated into several *Musa* breeding programs. Escalant and Jain (2004) provided a useful discussion of the relevance of induced mutations to banana breeding.

TISSUE CULTURE-BASED TECHNOLOGIES FOR BANANA

In vitro techniques have been applied to banana, including embryo rescue, with shoot regeneration from cultured tissues by organogenesis and somatic embryogenesis as a basis for micropropagation, exposure of somaclonal variation, and gene transfer by somatic hybridization and transformation.

Embryo Rescue

Embryo rescue, involving the excision and culture of developing zygotes, represents the technology that most easily assists conventional breeding. This technique is considered crucial for breeding of bananas because only a few viable seeds normally result from sexual crosses involving edible cultivars. Banana seeds resulting from manual pollinations are often malformed and sometimes immature, with a low germination rate of zero to 25% in plant nurseries, depending on the sexual crosses (Bakry et al. 2009). Indeed, the culture of excised embryos under axenic conditions has provided an important addition to conventional sexual hybridization and has improved by three- to tenfold, under optimum conditions, the development of embryos into plants (Vuylsteke, Swennen, & De Langhe 1991; Pillay & Tripathi 2007). Embryo rescue is used to circumvent post-zygotic incompatibility and to

facilitate the recovery of progeny from new parental combinations following sexual hybridization. Importantly, embryo rescue has increased significantly the germination rate to as much as 95% under optimal conditions. In banana breeding, embryo rescue has been exploited to increase the size of progeny populations and to enable the evaluation of plant material arising from new parental combinations for seeds that fail to germinate following traditional sowing procedures (Bakry & Horry 1992). It is crucial to excise embryos before they reach maturity to recover the maximum number of hybrids in breeding programs (Uma et al. 2010). These latter authors reported that for embryos at 95% and 100% maturity in the wild banana *M. acuminata* cv. Pisang Jeje (AA), Murashige and Skoog (MS; 1962)-based medium lacking growth regulators was adequate for direct shoot regeneration, whereas less mature embryos of the same cultivar had to be excised from parental tissues and regenerated indirectly through a callus stage on MS-based medium supplemented with 4.4 μM benzyl adenine and 2.8 μM indole acetic acid.

Micropropagation

Tissue culture-based micropropagation systems are well developed for bananas and, consequently, can be exploited to multiply elite genotypes. Such procedures form a basis for germplasm conservation and genetic improvement of this crop using somatic-cell techniques. Tissue culture was first applied to shoot tips of *M. acuminata* AAA cv. Cavendish by Ma and Shii (1972), and subsequently extended to other cultivars and tissue explants, including meristems, rhizomes, inflorescences and immature male flowers, immature zygotic embryos, and leaf bases (Cronauer & Krikorian 1985; Vuylsteke 1989, 1998). Interestingly, plant material propagated *in vitro* has replaced completely the use of conventional vegetative suckers in many regions where there is intensive cultivation of bananas. In fact, bananas were one of the first fruit food crops to be micropropagated and are still multiplied in vitro more than any other fruit crop, with annual production figures estimated to exceed 2 million propagules (Swennen et al. 2004; Smith et al. 2005). Certainly, micropropagation has become a standard practice for the production of material for field planting of this seedless crop. Importantly, tissue culture enables mass production of elite clones with desirable agronomic qualities, in preference to the collection of more limited numbers of suckers from field-grown plants. Because only quality material is selected for micropropagation, the growth and yield of such propagules in the field are superior to traditionally produced plants. Thus, tissue culture-derived banana plants generally outperform plants derived from conventional planting materials with respect to their yield, finger size, cycle time, number of suckers, efficiency of nutrient uptake, emergence, and crop uniformity, even in ratoon crops.

Tissue culture enables plant material to be produced that is free of contaminating microorganisms, pests, and diseases, because only axenic explants are introduced into culture. Additionally, source materials may be virus-indexed prior to introduction into culture (Magnaye, Zamora, & Escobido 1995; Hamill 2000; Hwang & Su 2000). The culture of shoot tips, combined with virus indexing and quarantine procedures, guarantees the safe dissemination and conservation of certified *Musa* germplasm and prevents dissemination of serious diseases and pests from the native country (Crouch, Vuylsteke, & Ortiz 1998). Virus testing of germplasm is now recommended as a routine procedure to ensure safe international distribution, because many viruses that affect *Musa* remain difficult to eliminate even by the culture of meristems excised from stem apices (Drew, Moisander, & Smith 1989). The use of *in vitro*-derived planting materials can prolong the pest-free period of plants in the field, providing access to new banana cultivars across quarantine zones on a global scale and promoting the rapid introduction of elite selections (Vuylsteke 1998). The use of disease-free planting materials also ensures cost reduction and subsequent delay in the necessity for pest and disease management. Environmental issues, such as tolerance to drought, have been addressed using cultured shoot tips. For example, Ebrahim, Ibrahim, and Emara (2004) compared the drought tolerance of four *Musa* cultivars by exposing shoot tip-derived plants to culture medium containing polyethylene glycol (PEG) to simulate drought conditions. Similarly, Harb et al. (2005) included sea salt in the culture medium to evaluate the salt tolerance of bananas.

Cell-culture technology is exploited extensively to multiply elite germplasms. The ability to regenerate plants directly from cultured explants, explant-derived callus, cell suspensions, and isolated protoplasts through organogenesis and/or somatic embryogenesis also forms an essential basis for the generation of potential new cultivars by the induction of mutations in cultured cells, exposure of somaclonal variation, and genetic improvement through gene mobilization. The procedures involved include somatic hybridization/cybridization involving protoplast fusion and the introduction of specific genes by transformation. Cryopreservation to conserve rare germplasms also depends on robust cell- and tissue-culture procedures, with associated reproducible plant regeneration.

Plant Regeneration from Cultured Cells by Organogenesis and Somatic Embryogenesis

Plant regeneration in bananas can be achieved via organogenesis in the case of cultured shoot tips, and by somatic embryogenesis from callus and cell suspensions. In bananas, an efficient plant regeneration system via direct organogenesis and/or somatic embryogenesis is vital as a basis

for various biotechnological options. Shoot apices containing meristems produce multiple new shoots following the inhibition of apical dominance (Kulkarni et al. 2007). Suckers with sword-like leaves are normally excised from parent plants to provide source material for micropropagation. Micropropagated plants that originate from such "sword suckers" may act as a further source of shoot tips for multiplication. Regenerated shoots provide material for planting and research, whereas highly proliferating cauliflower (nodule)-like meristems may also be established from cultured shoots to provide scalps with which to establish embryogenic cell suspensions (Sadik et al. 2007). Scalps, the uppermost parts of highly proliferating nodule-like meristems, are rich in meristematic cells (Panis, Withers, & De Langhe 1990). Because plants can be regenerated from scalps, the latter have been exploited as target material for genetic transformation (Acereto-Escoffie et al. 2005) and cryopreservation (Strosse et al. 2006).

Effort has focused on the development of protocols to induce somatic embryogenesis as a pathway of plant regeneration in genotypes of dessert and cooking bananas, initially as a basis for micropropagation and, subsequently, as a basis for genetic manipulation (Strosse et al. 2003; Castellanos, Power, & Davey 2006). This procedure also underpins cryopreservation. Somatic embryogenesis involves the formation of embryo-like structures and their development into whole plants in a way analogous to zygotic embryos (Strosse et al. 2006). Such somatic embryos are produced either directly from somatic cells of cultured explants without an intervening callus stage or indirectly from callus generated from somatic tissues and from cell suspensions induced from callus. Cells develop into globular structures that progress to heart-shaped embryos and, subsequently, to torpedo-shaped embryos with hypocotyls and radicles in the case of dicotyledons, or globular, scutellar, and coleoptylar structures in monocotyledons. Both embryo induction and development depend on the culture conditions, including the composition of the culture medium, especially the concentration and type of plant-growth regulators, the carbohydrate source, and the osmotic potential of the medium (Jimenez 2005).

Plant regeneration via somatic embryogenesis in bananas has been reported from embryogenic cell suspensions established from embryogenic callus induced from apical meristems (Cronauer & Krikorian 1985), corm-like tissues (Novak et al. 1989; Navarro, Escobedo, & Mayo 1997), pseudostems, leaf bases and rhizome fragments (Novak et al. 1989), highly proliferating scalps (Dhed'a et al. 1991; Schoofs 1997; Ganapathi et al. 2001b; Sipen, Anthony, & Davey 2008), immature zygotic embryos (Escalant & Teisson 1989; Marroquin et al. 1993), immature male flowers (Ma 1991; Shii et al. 1992; Grapin et al. 1998; Chung et al. 2006; Sidha et al. 2007; Jalil et al. 2008), and immature female flowers (Navarro, Escobedo, & Mayo 1997; Grapin et al. 2000). In general, embryogenic cell suspensions of banana

are usually established from immature male flowers and scalps (Strosse et al. 2003).

Somatic embryogenesis in banana is constrained, however, by several factors, including the limited choice of explants, the restricted and often variable embryogenic response of cells and tissues *in vitro*, labor-intensive and time-consuming establishment of embryogenic cell suspensions, loss of embryogenic capability, and high incidence of somaclonal variation associated with long-term culture (Strosse et al. 2003, 2006; Strosse, Van Den Houwe, & Panis). Dhed'a et al. (1991) observed 5%–10% abnormal somatic embryos recovered from scalp-derived cell suspensions of the banana cv. Bluggoe (ABB). Morphological observations on plants regenerated from male flower-derived cell suspensions of the cv. French Sombre (AAB) revealed 16%–22% somaclonal variants (Grapin, Schwendiman, & Teisson 1996), whereas Schoofs et al. (1999) reported an extremely high number (>90%) of 'long narrow leaf' off-types for plants regenerated from scalp-derived cell suspensions of the cv. Williams (AAA). The same authors also noted that 9-year-old cell suspensions of the cv. Bluggoe (ABB) were aneuploid and lacked four to five chromosomes, as determined by flow cytometry. The latter technique is rapid for the quantification of euploidy and aneuploidy in plants, particularly those regenerated from cell suspensions, because only small numbers of cells are required for analysis (Schoofs et al. 1999).

Secondary embryogenesis is frequent in banana cultures (Escalant, Teisson, & Cote 1994; Kosky et al. 2002), this process involving the induction of new somatic embryos from similar pre-existing structures (Khalil et al. 2002). Consequently, secondary somatic embryogenesis has the potential for plant multiplication across an extended period of time, because new embryos are formed continuously from existing embryos. Plant regeneration rates in bananas via this process varied between 1.5%–20% (Cote et al. 1996) and 60%–89% (Escalant, Teisson, & Cote 1994; Kosky et al. 2002). The protocols of Escalant, Teisson, and Cote (1994) and Kosky et al. (2002) involved temporary immersion in liquid medium. Using such a procedure, Kosky et al. (2002) reported an improvement in mass propagation of the banana AAAB cv. FHIA-18 via somatic embryogenesis. Immature male flowers were induced to form embryogenic tissue, the latter being used to generate embryogenic cell suspensions in MS-based medium containing 1.0 mg l^{-1} biotin, 100 mg l^{-1} glutamine, 100 mg l^{-1} malt extract, 1.0 mg l^{-1} 2,4-dichlorophenoxyacetic acid, and 45 g l^{-1} sucrose. A temporary immersion system may not be available to all researchers and, indeed, may not be essential in some cases, for example, a plant regeneration rate of 90% was reported via somatic embrygenesis of the banana cv. Dwarf Brazilian (AAB) without the need for a temporary immersion system, cell suspensions as source material, or bioreactors as culture vessels (Khalil et al. 2002).

Generation of Somaclonal Variation

Variation may occur naturally during both conventional and *in vitro* propagation of bananas (Vroh-Bi et al. 2010). While the incidence of somatic mutations is low in bananas propagated conventionally, it is frequent in micropropagated material (Stover 1988; Robinson 1996) and often constrains regeneration by somatic embryogenesis (Strosse et al. 2006). Somatic mutations are limited to non-reproductive cells, with somaclonal variation often being exhibited by plants regenerated from cultured cells, particularly those regenerated via a callus phase. Mutations have been associated with somaclonal variation, including point mutations, gene duplication, chromosomal rearrangements, and changes in chromosome complements. Chromosome instability is among the most common causes of tissue culture-induced variations in bananas (Larkin 2004; Msogoya et al. 2008). The movement of transposable elements and changes in DNA methylation have also been implicated as possible mechanisms associated with such variation.

During micropropagation of elite clones, somaclonal variation can result in off-type plants of decreased commercial value. Indeed, the high incidence of off-types resulting from the culture of banana meristems is of major concern to commercial growers, with the incidence of morphological off-types being more frequent when the plants were propagated *in vitro* by meristem culture (Vuylsteke, Swennen, & De Langhe 1991). Even a low percentage of off-types is unacceptable in commercial production because the generation of off-types can be extremely costly (Larkin 2004). In contrast, several banana cultivars have originated from spontaneous somatic mutations (Robinson 1996; Sahijram, Soneji, & Bollamma 2003; Heslop-Harrison & Schwarzacher 2007) and, in this respect, somaclonal variation is important for the genetic improvement of banana (Khayat et al. 2004). Thus, exposure through culture of naturally occurring genetic variation in somatic cells has the potential to generate considerable novel and useful genetic variability not only in bananas but also for crops in general. Mutant and somaclonal variant banana plants, exhibiting traits such as tolerance to aluminum, dwarfism, and resistance to Panama and Sigatoka diseases, have been released for commercial production, or are still being evaluated for their growth potential and yield (Hwang 2001; Hwang & Ko 2004; Roux 2004). Tai-Chiao No.1, a variant from the banana cv. Pei-Chiao that resulted from multiplication of material *in vitro*, showed improved agronomic characteristics, including resistance to Tropical Race 4 of *Fusarium* wilt (Tang & Hwang 1994). Likewise, ShiChuan and Ko (2004) in Taiwan reported Cavendish banana cultivars resistant to *Fusarium* wilt.

Understanding natural and *in vitro* genomic variation and identifying such changes at an early stage of plant development are vital for breeding, mutagenesis, transgenic-plant characterization, and germplasm management (Vroh-Bi et al. 2010). Banana off-types can be detected by their morphology

and further characterized by genomic fingerprinting techniques. Although potentially extremely useful, somaclonal variation may be problematic in the genetic improvement of crops where individual transgenic plants need to be tested exhaustively so that only proven elite plants are selected for commercial release.

Somatic Hybridization

Somatic hybridization, involving the reproducible isolation, fusion, and culture of isolated protoplasts (Davey et al. 2010), is a procedure to circumvent naturally occurring pre- and post-zygotic incompatibility barriers that normally hamper sexual hybridization. Somatic hybridization can be exploited to manipulate polygenic traits without the requirement to isolate DNA, or to have knowledge of genes or their DNA-base sequences. Polygenic traits can be introgressed by nuclear and/or organelle transfer through symmetric and asymmetric protoplast fusion. The extensive genetic nuclear-cytoplasmic combinations generated by this procedure have been reviewed (Davey, Power, & Lowe 2000a, b; 2005a, b, c; 2010) and far exceed those combinations generated by sexual hybridization. The main constraint of somatic hybridization is that it is labor-intensive and relies upon the development of robust protoplast-to-plant systems. Consequently, to date, this method of gene introgression has been applied to banana-breeding programs by only a limited number of workers (Megia et al. 1993; Panis, Van Wauwe, & Swennen 1993; Matsumoto & Oka 1998; Assani et al. 2001, 2002, 2005). Although there exist a limited number of references relating to protoplast technology in banana, it is recognized that protoplast-fusion technology is a potential tool to overcome sterility and genetic variability in most edible banana varieties that are triploid (Assani et al. 2005). Somatic hybridization is the only way to generate banana hybrids between highly sterile cultivars, especially in the triploid Cavendish group; protoplast fusion can accelerate and facilitate the crossing of bananas that is difficult to achieve by conventional breeding methods (Bakry et al. 2009).

Procedures for protoplast fusion generally involve exposure of isolated protoplasts to chemical fusion agents, such as polyethylene glycol (PEG), exposure of mixtures of parental protoplasts to an alternating current, followed by high voltage direct current pulses (electrofusion), or a combination of these procedures (Davey et al. 2000b, 2005a). Generally, electrofusion is the preferred procedure to fuse banana protoplasts (Matsumoto, Vilarinhos, & Oka 2002) and is the most efficient procedure to generate somatic hybrid plants (Assani et al. 2005). Chen and Ku (1985) first attempted to fuse isolated banana protoplasts using leaves as a source of protoplasts. Subsequently, Matsumoto et al. (1992) isolated protoplasts from bracts. However, both research groups were unable to culture the material resulting from protoplast fusion, until Matsumoto, Vilarinhos, and

Oka (2002) generated pentaploid somatic hybrid cells resulted from the fusion of banana protoplasts. It is possible, by protoplast fusion, to generate somatic hybrid tetraploid parents for use in interploid crosses with other diploid lines, or for the direct release of triploid somatic hybrids by haploid/diploid protoplast fusion (Assani et al. 2003). In extensive investigations, Matsumoto, Vilarinhos, and Oka (2002) reported the generation of somatic hybrids following electrofusion of protoplasts of the cv. Maca (ABB) with protoplasts of the cv. Lidi (AA), and the use of nurse cultures to stimulate the growth of electrofusion-treated protoplasts. An interesting fact is that somatic-hybrid plants were generated only after embryogenic cell suspensions were initiated (Xu et al. 2005) and used as source material for the isolation of totipotent protoplasts (Matsumoto, Vilarinhos, & Oka 2002; Assani et al. 2005), with 85% of the regenerated plants being identified as somatic hybrids using random amplified polymorphic DNA (RAPD) analysis (Matsumoto, Vilarinhos, & Oka 2002). In a study that compared the two most frequently used fusion procedures, i.e., electrofusion and PEG, Assani et al. (2005) found that the former technique was superior with respect to the subsequent mitotic activity of treated protoplasts, somatic embryogenesis, and plant regeneration of protoplast-derived tissues. However, PEG-induced fusion was optimal with respect to the frequency of binary fusions. More recently, Matsumoto et al. (2010) summarized the literature relating to the source of cells, enzyme mixtures, and media, which they used to isolate and to culture banana protoplasts to plants. These workers also provided detailed laboratory notes relating to all stages of the procedures involved to develop a protoplast-to-plant system for banana. Interestingly, cells from suspension cultures in liquid medium have featured as source material in most of these reports.

Genetic Transformation

Genetic transformation, involving the introduction and stable integration of genes into the nuclear or plastid genomes with subsequent gene expression in transgenic or transplastomic plants, offers an additional approach for the genetic improvement of banana, particularly for those cultivars that are not amenable to sexual hybridization, e.g., those from the Cavendish subgroup (Jones 2000; Pillay & Tripathi 2007). Both particle bombardment (Becker et al. 2000) and *Agrobacterium*-mediated gene-transfer techniques have been used to introduce foreign genes into banana (Ganapathi et al. 2001; Khanna et al. 2004; Acereto-Escoffie et al. 2005). Particle bombardment utilizes accelerated metal microparticles, usually gold, coated with DNA to penetrate and deliver foreign genes into plant cells; transformed cells, recovered by their ability to grow in the presence of a selective agent, such as an antibiotic (e.g., kanamycin sulphate) or herbicide (e.g., glufosinate ammonium), are selected and regenerated into plants. Both of these

transformation methodologies have been reviewed extensively (Davey et al. 2005a, b), while Altpeter and Sandhu (2010) provided a detailed protocol for biolistics-mediated gene transfer and listed earlier references relevant to this procedure.

In banana, embryogenic cell suspensions (Becker et al. 2000) and scalps (Sagi et al. 1995) have been transformed by particle bombardment and meristems by *Agrobacterium*-mediated gene delivery (May et al. 1995), with variable rates of success. *Agrobacterium*-mediated DNA delivery resulted in low genetic transformation rates with the induction of chimeras. As a consequence, this technique is not commonly used for this crop (Smith et al. 2005) compared with biolistics-mediated gene delivery. Foreign genes, such as those for reporter and selectable markers, resistance to fungi (Sagi, Remy, & Swennen 1998, XinWu et al. 2005), nematodes, and viruses (Becker et al. 2000); delayed fruit ripening (Balint-Kurti et al. 2001), tolerance to salt stress (Ismail et al. 2005), and the synthesis of therapeutic proteins (e.g., hepatitis B surface antigen; Sunil et al. 2005), are some of the target genes for banana transformation. The synthesis of vaccines, antibodies, and other therapeutic proteins in transgenic bananas has several advantages because it eliminates costly and time-consuming processing, such as extraction and purification. Most importantly, this approach permits oral administration of vaccines to patients, including children, because of palatability and digestability without cooking, retaining heat-labile proteins that would otherwise be destroyed (Pua 2007). This is an important consideration in the tropics and subtropics where economical vaccines are required to immunize large human populations. Sunil et al. (2005) reported, for the first time, up to 38 ng per gram fresh weight of leaf tissue of hepatitis B surface antigen (HBsAg) in the cv. Rasthali (AAB). Although the expression level was low, this study demonstrated the feasibility of expressing HBsAG and possibly other novel therapeutic proteins and vaccines in banana (Pua 2007). The transformation of banana is influenced by several parameters, including the plant genotype, the physiology of explants, and the totipotency of cells in culture (Heslop-Harrison & Schwarzacher 2007). However, transformation has the potential to make a significant contribution to banana improvement. An important aspect of banana transformation is the fact that there is little chance of unintentional gene flow from transformed plants because of their sterility or extremely low fertility, making them particularly environmentally safe (Smith et al. 2005; Sunil et al. 2005; Pillay & Tripathi 2007).

GENOMIC STUDIES INVOLVING BANANA

Genomic technologies, such as analysis and sequencing of genomes, identification of genes and their expression, recombination and genetic diversity, may be exploited for the genetic improvement of bananas. Significant

progress has been made in the genetic mapping of bananas, with a number of important banana genomic resources becoming available or that are in the process of being developed, with particular emphasis on genes involved in disease resistance (Smith et al. 2005). As in other crops, the development of a genetic map for *Musa* will facilitate the identification of agronomically useful genes. Molecular markers are important tools for analysis of genetic composition, the detection of desirable traits, and somaclonal variation, whereas they also facilitate the preservation of novel germplasm (Pua 2007). Molecular markers have been used in banana breeding to identify cultivars as a basis for phylogenetic studies, analysis of recombination between genomes, and the identification of genes controlling specific traits. The latter is particularly important in assisting the selection and conservation of novel germplasm for breeding (Pillay, Nwakanma, & Tenkouana 2000; Pillay et al. 2001; Pillay & Tripathi 2007).

Several molecular-marker techniques have been applied to banana, including genomic *in situ* hybridization (GISH), fluorescence *in situ* hybridization (FISH), restriction fragment length polymorphism (RFLP), polymorphisms based on the variable number of tandem repeats (VNTR), microsatellite DNA, RAPD, amplified fragment length polymorphism (AFLP), and inter-simple sequence repeats (SSR) (D'Hont et al. 2000; Ude et al. 2002; Buhariwalla et al. 2005; D'Hont 2005; Ge et al. 2005; Ray et al. 2006). The polymerase chain reaction (PCR) has been used extensively to analyze *Musa* genomes because PCR-based markers can detect a high level of polymorphism within breeding populations (Pillay & Tripathi 2007). PCR has enabled genetic diversity studies and genetic mapping using non-radioactive technologies (Bakry et al. 2009). GISH has differentiated the chromosomes of four genotypes and interspecific cvs. of banana (D'Hont et al. 2000), whereas distribution of repeated sequences in the banana genome has been determined using FISH (D'Hont 2005).

RFLP analyses of diverse germplasm have been exploited to study the taxonomy and phylogeny of *Musa* spp. and variation in the chloroplast genome within the genus (Nwakanma et al. 2003). The use of RFLP and RAPD markers can increase the efficiency of identification of promising new banana genotypes by early detection of desirable genome combinations (Pillay & Tripathi 2007). RAPD and PCR-RFLP markers specific for the A and B genomes have been identified (Pillay, Nwakanma, & Tenkouana 2000; Nwakanma et al. 2003), whereas RAPD has been used to distinguish diverse *Musa* germplasm (Pillay et al. 2001). The advantage of RAPD analysis is that it does not require prior knowledge of the banana genome (Pillay & Tripathi 2007). AFLP is another powerful tool in the molecular breeding of bananas relative to its ability to identify large numbers of polymorphic bands within germplasm without any prior knowledge of genomes (Ude et al. 2003). Analyses that exploit AFLP and SSR have been used to identify

markers for fruit parthenocarpy, dwarfism, and apical dominance in bananas (Pillay & Tripathi 2007).

Sequence-tagged microsatellite site (STMS) markers based on VNTR polymorphism of microsatellites have also been developed (Pua 2007), a technique again based on PCR. STMS markers can be used to overcome time constraints when dealing with a large number of genotypes because they are SSRs (Pua 2007). The STMS and AFLP markers are more efficient than isozyme, RFLP, and RAPD markers for genetic mapping and marker-assisted breeding in bananas (Hautea et al. 2004; Pua 2007). Retrotransposon-derived markers have been used to identify and characterize banana cultivars and to classify banana genomes (Teo et al. 2005). Flow cytometry, which is rapid, precise and non-destructive, has been employed to determine the ploidy of banana to detect mixoploidy, particularly in segregating progeny populations (Pillay et al. 2001; Pillay & Tripathi 2007). All marker systems have different advantages and disadvantages with respect to specific applications. Consequently, it is important to develop the capacity to perform several assays on targeted germplasms to choose the systems most suitable for applying these procedures to the molecular breeding of bananas.

Genetic mapping of bananas is in progress and, to date, mapping populations remain limited, although several approaches are being exploited at research institutes aimed at developing segregating populations (Pillay & Tripathi 2007). Initially a low-density genetic map of *M. acuminata* was established using isozyme, RFLP, and RAPD markers based on a cross between two subspecies of *M. acuminata* AA, i.e., SF265 (AA) x *banksii* (AA), segregating for parthenocarpy (Faure et al. 1993). There are still no high-density linkage maps, despite projects being conducted at institutes such as CIRAD. A series of crosses have been established that segregate for traits that include Black Sigatoka resistance, bunch position, and chromosome rearrangements (Pillay & Tripathi 2007). However, genetic mapping of diploid species remains unsuccessful (Heslop-Harrison & Schwarzacher 2007). Several bacterial artificial chromosome (BAC) libraries of *M. acuminata* (A genome) and *M. balbisiana* (B genome) have been made that will facilitate the physical mapping of the banana genome (Pillay & Tripathi 2007). Approximately 100 useful microsatellite markers have been generated for *M. acuminata* (Pillay & Tripathi 2007), with a similar number being expected for *M. balbisiana*. Current microsatellite-isolation projects are expected to generate more than 500 markers for genetic analysis and molecular breeding in *Musa*. The first genetic map of banana was established on 90 loci, these being based on 58 RFLP**s**, four isozymes, and 28 RAPD markers (Bakry et al. 2009).

Complete genetic maps are essential for studying the genetics underlying quantitative traits and to locate accurately the genes or chromosomal regions that contribute to specific traits. A quantitative trait locus (QTL)

is a chromosomal region associated with both molecular markers and a quantitative trait (Xu 2002). The transfer of useful traits could be accelerated in banana breeding programs if QTLs are identified (Pillay & Tripathi 2007). However, studies to identify QTLs in bananas remain limited, primarily because of the absence of a high-density linkage map. Traits in banana are influenced considerably by the environment of the plants, necessitating consideration when identifying QTLs. Certainly the identification of QTL in *Musa* will be critical for genetic improvement of this fruit crop.

CONSERVATION OF BANANAS

Musa germplasm is gradually being depleted; possibly some wild, neglected, or underutilized cultivars are already extinct or are on the brink of extinction. Factors such as the dependency of the growers for specific cultivars, generally linked to consumer preference for specific fruit, and with other human activities, together with biotic and abiotic stresses, exacerbate these problems. Efforts have been made and continue to conserve existing banana germplasm to ensure the availability of genetic resources for future breeding programs and crop production. The conservation of banana germplasm is achieved by seeds and *in situ* and *ex situ* approaches in both the country of origin, as well as in other repositories.

Seed Conservation

Seed gene banks have long provided a safer storage alternative to field gene banks for plant genetic resources. Indeed, the majority of crop plant germplasm is stored in seed banks at temperatures of –15° to –20°C, although some seed stores, particularly those in developing countries, operate at 0° to 4°C for reasons of economy (Keller et al. 2006). Because most bananas are propagated vegetatively and fail to set seeds, seed gene banks are not applicable at present, except for seed-producing diploid wild *Musa* species.

In Situ and *Ex Situ* Conservation

In situ conservation of germplasm has some disadvantages because it requires large areas, with labor-intensive maintenance. Plants in field areas are also prone to biotic and abiotic stresses. Moreover, the distribution and exchange of materials for planting from field gene banks is problematic because of the nature of the vegetative growth of bananas and the high risk of disease being spread to other countries (Engelmann 1997). *Ex situ* field-conservation procedures also suffer from similar limitations as plant material retained under *in situ* conditions.

In vitro (tissue culture-based) conservation has been established and practiced worldwide to complement field and seed-bank conservation (Oliveira et al. 2000). Presently, culture-based technologies are the most suitable and preferred approaches to conserve and distribute banana germplasm. The methods consist of short- to medium-term storage (usually two to ten months) in defined culture media under controlled environmental conditions to reduce growth, or long-term storage by cryopreservation. In the former case, methods have been devised to limit growth by increasing the osmotic potential of the culture medium using sugars and sugar alcohols (Ko, Hwang, & Ku 1991; Bhat & Chandel 1993) combined with reduced temperature (Bhat & Chandel 1993). Medium-term storage of banana plantlets *in vitro* is best achieved at 16°C under low-intensity illumination (Banerjee & De Langhe 1995). Rao et al. (1993) encapsulated excised shoot tips in sodium alginate for short-term storage and transportation. Nevertheless, the maintenance of banana collections *in vitro* remains labor-intensive, even with reduced growth, and there remains the risk of losing collections because of latent contamination by microorganisms and human error. Cultured plant material may also exhibit somaclonal variation as discussed earlier. Such variation may be incited by the intrinsic nature and genetic stability of the plant material and by external factors, including the culture system, particularly growth regulators in the medium, the environmental conditions, and duration of storage.

Cryopreservation is the preferred option for long-term conservation of plant germplasm, particularly for vegetatively propagated, seedless, or recalcitrant (non-orthodox) seed species. Indeed, cryopreservation is considered to be the only valid alternative for long-term conservation of *Musa* germplasm. For many plants, cryopreservation is currently being exploited to overcome some of the serious limitations encountered using traditional germplasm-conservation strategies in field, seed, and *in vitro* collections. Cryopreservation is achieved in liquid nitrogen at –196°C where all metabolic processes are inactivated, but samples remain intact. In theory, materials frozen at this temperature are preserved safely without alteration for an indefinite period of time. Cryopreservation, therefore, complements other conservation approaches and offers an alternative or additional tool for plant-germplasm collections (Gonzalez-Arnao et al. 2008).

Storage at low temperatures is excellent for long-term conservation of banana germplasm because it requires minimum space, eliminates the requirement for frequent subculture, and reduces the probability of contamination by microorganisms and mislabeling. Currently, this technology is the only safe and cost-effective option for long-term preservation of germplasm of non-orthodox seed species, vegetatively propagated species, and many of the unique plant materials generated by biotechnological approaches (Engelmann 2004). Cryopreservation can reduce the maintenance cost of germplasm compared with field and other *in vitro* storage techniques. For

example, Hummer and Reed (2000) indicated that the annual maintenance cost at the National Clonal Germplasm Repository (NCGR) in the United States of one temperate fruit tree accession was US$77 in the field and US$23 under *in vitro* slow-growth storage, but only US$1 for cryopreservation of meristems or buds, to which US$50–$60 was added initially for cryopreserving the accession. The annual maintenance cost of the cassava collection at the International Center for Tropical Agriculture in Colombia (CIAT), which includes 5,000 accessions, was approximately US$5,000 with cryopreservation, compared with US$30,000 under *in vitro* slow-growth storage (Engelmann 2004).

The cryopreservation techniques that have been developed and that are currently exploited consist of both conventional and "new" approaches. In contrast to the earlier reports of plant cryopreservation, when slow freezing was generally a standard procedure, subsequent development has been dominated by rapid freezing methods (Keller et al. 2006). Such "new" techniques are based on vitrification, followed by rapid freezing in liquid nitrogen. Vitrification by cryoprotectants, such as glycerol, dimethyl sulfoxide, and ethylene glycol, followed by rapid freezing in liquid nitrogen, enables dehydrated plant cells to withstand more easily exposure to the temperature of liquid nitrogen (Sakai 2000). Eight cryopreservation procedures have been reported, including preculture, dehydration, preculture-dehydration, vitrification, preculture-vitrification, encapsulation-dehydration, encapsulation-vitrification, and droplet-freezing (droplet-vitrification) techniques. The main advantage of these "new" techniques is that a programmable freezer is unnecessary, with the protocols being applicable to most plant systems.

Cryopreservation is used for the reliable long-term storage of transformation-competent tissues, genetically transformed lines and cells synthesizing important secondary products (Ramon et al. 2002). Such materials are generally of elite clones, often with high value-added traits, that are usually difficult to establish. Consequently, they require reliable storage facilities at low temperature because their special attributes may be lost during *in vitro* slow- and medium-term storage. For example, the production and multiplication of embryogenic cell suspensions for use in micropropagation, the generation of transgenic plants and somatic hybrid/cybrid plants, all involve procedures that are labor-intensive and not readily transferable between laboratories. Cell suspensions, for example, also lose their totipotency and are prone to contamination during frequent handling under normal maintenance. Thus, reliable long-term storage can be achieved only by cryopreservation. Indeed, the morphogenetic potential of embryogenic cell suspensions is retained during their storage in liquid nitrogen (Panis & Lambardi 2006). The ability to minimize genetic changes in plant material for extended periods is important in relation to the longer-term biotechnological exploitation of such material.

Another application of cryopreservation is in cryotherapy for virus eradication, as reported initially for plum shoots (Brison et al. 1997). Cryotherapy is based on selective cell destruction by cold treatment. The differentiated cells of shoots that contain viruses have high water content; such cells are destroyed by the formation of ice crystals during freezing. In contrast, meristematic cells, which remain uninfected by viruses, have a more concentrated cytoplasm and can more readily withstand freezing (Engelmann 2004). Thus, Helliot et al. (2002) reported the successful eradication of cucumber mosaic virus (CMV) and banana streak virus (BSV), with 30% and 90% rates of success, respectively, from banana scalps derived from highly proliferative meristematic cultures of the cv. Williams (AAA) following cryopreservation by vitrification. The latter involved two weeks' preculture of scalps on semi-solid MS-based medium enriched with 10 μM benzyladenine and 0.4 M sucrose, followed by incubation in a loading solution based on the MS formulation and containing 2 M glycerol and 0.4 M sucrose. Vitrification was achieved by incubation in PVS2 solution (Sakai, Kobayashi, & Oiyama 1990), the latter being based on the formulation of MS medium with the addition of 3.26 M glycerol, 2.42 M ethylene glycol, 1.9 M dimethylsulfoxide, and 0.4 M sucrose, prior to cryopreservation in liquid nitrogen. The application of a short cryotherapy treatment of a few hours for virus eradication is an advantage compared with a few weeks to several years by traditional treatments.

GENERAL CONCLUSIONS

Sustainable banana production is vital to ensure a constant supply of fruit to meet world food demand. However, fruit production faces challenges from changing economic, social, and environmental conditions. The genetic improvement of banana is one of the strategies to ensure sustained production. Consequently, strategies that exploit both conventional and biotechnological approaches, particularly genomic analyses and transformation, have considerable potential to play a role in achieving sustainable fruit production. Genetic and physical mapping of the *Musa* genome will facilitate the isolation of genes that are potentially useful in genetic transformation, with significant progress being achieved in this area in recent years. Improved understanding of genomes will facilitate targeted breeding and more efficient use of existing *Musa* biodiversity. *In vitro*-based technologies, particularly genetic transformation, offer excellent opportunities to create novel cultivars with targeted traits through the manipulation of nuclear and cytoplasmic genomes. Exposure of somaclonal variation through basic tissue-culture procedures will continue to generate new cultivars, whereas somatic hybridization and cybridization by protoplast fusion will also enable the mobilization of genetic material without the requirement to isolate and

characterize DNA. Overall, the genetic improvement of bananas is crucial to generate new cultivars that are productive as well as adapted to different environmental conditions. It requires the availability of suitable germplasm combined with experimental procedures and the practical expertise and theoretical knowledge of biotechnologists and breeders to manipulate nuclear and cytoplasmic genomes using both conventional and biotechnological approaches. In the long term, genetically improved banana cultivars could ensure sustained fruit production for food security, with the additional advantage of guaranteed income for farmers in producing countries.

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