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Micro propagation in Banana: A review

Vangapandu Thriveni, M Viswanath and Anindita Roy

Abstract
A number of biotechnological tools have been developed which could help breeders to evolve new plant types to meet the demand of the food industry in the next century. Available techniques for the transfer of genes could significantly shorten the breeding procedures and overcome some of the agronomic and environmental problems which would otherwise not be possible through conventional methods. *In vitro* protocols have been standardized to allow commercially viable propagation of desired clones of *Musa*. An overview of the regeneration of banana by direct and indirect organogenesis, and somatic embryogenesis is presented in this article. In addition, the use of several other biotechnological techniques to enrich the genome of banana, such as selection of somaclonal variants, screening for various useful characteristics, cryopreservation, genetic transformation and molecular genetics are reviewed. In conclusion, the improvement of banana through modern biotechnology should help ensure food security by stabilizing production levels in sustainable cropping systems geared towards meeting domestic and export market demands.

Keywords: Tissue culture, genetic transformation, *in vitro*, *Musa* spp., molecular markers

Introduction

Banana is an important and widely grown fruit crop, cultivated in more than 130 countries both in tropical and subtropical regions of the world. India ranks first in banana production with a production of 28.5 mt and it contributes to lion's share of 34.4% of the total fruit production from an area of 8.58 lakh ha with a productivity of 34.72 t/ha. Andhra Pradesh is one of the leading producer of banana in India and is grown in an area of 86,320 ha with a production and productivity of 414,355 t and 4.8 t/ha respectively (National Horticulture Board, 2018). Unfortunately, their production is hampered by several diseases and pests, largely due to poor quality clones (Novak,1992) [29].Today, yield losses up to 50% are caused by pathogenic fungi (especially *Mycosphaerella fijiensis* and *Fusarium oxysporum f.sp. cubense*, the causal agents of Black Sigatoka and Panama disease, respectively), recorded in areas where banana and plantain are cultivated for local consumption The genetic make-up of Musa is extremely complicated, sterility caused by different factors, interspecific hybridity, heterozygosity and polyploidy are common in most of the cultivated clones (Simmonds, 1976) [59]. The asexual nature of propagation in Musa clones is often an unsurmountable barrier to cross hybridization. These are also the major obstacles in the successful breeding for resistance to the major diseases and pests caused by fungi, viruses and nematodes. Hence, there is a need to complement conventional breeding programmes with additional technology via transformation systems using Agrobacterium-based gene vectors and particle bombardment. Breeding for disease-resistant banana cultivars using classical breeding methods remains a difficult and time-consuming endeavour because of the high sterility, polyploidy and long generation times of most edible cultivars.

Biotechnology involving modern tissue culture, cell biology and molecular biology provides an opportunity to develop new germplasm better adapted to changing demands. Extensive studies have been carried out with banana on various aspects of its biotechnology, such as micro propagation, plant regeneration via somatic embryogenesis, synthetic seed formation, cryopreservation and genetic transformation. A wide set of target genes is currently available which may confer resistance to insect pests or fungal pathogens, and nematodes. Embryogenic cell suspensions appear, as is the case with many monocotyledons, to be the material of choice for non-conventional Musa breeding. In this communication, we provide an overview of the different biotechnological applications available for micro propagation, regeneration via somatic embryogenesis and improvement of banana, a major starchy staple food crop of the tropics.

Shoot-Tip Cultures

Micro propagation

Stage 1: Initiation of shoot cultures

Shoot cultures of banana start conventionally from any plant part that contains a shoot meristem, i.e. the parental pseudostem, small suckers, peepers and lateral buds (Vuylsteke, 1989) [73]. The apex of the inflorescence and axillary flower budsare also suitable explants for tissue culture initiation (Cronauer, 1985) [12]. Overall, it is important to select explant material from preferably mature individuals whose response to environmental factors is known, and whose quality traits governed by genotypic and environmental effects have been identified. For rapid in vitro multiplication of banana, shoot tips from young suckers of 40–100 cm height are most commonly used as explants. From the selected sucker a cube of tissue of about 1–2 cm³ containing the apical meristem is excised. This block of tissue is dipped in 70% ethanol for 10 seconds, surface sterilized in a 2% sodium hypochlorite solution, and after 20 min rinsed three times for 10 min in sterile water. Variants of this decontamination protocol exist. They differ in explant type and size, disinfection procedure (single or double sterilisation) (Hamill, 1933) [25]. The type of disinfectants (calcium hypochlorite instead of sodium hypochlorite) used and its concentration and treatment duration (Wong, 1986) [75]. Subsequently a shoot tip of about 3 × 5 mm, consisting of the apical dome covered with several leaf primordia and a thin layer of corm tissue is aseptically dissected. Larger explants have the merit of consisting of a shoot apex bearing more lateral buds which rapidly develop into shoots (Lee, 1933).

The optimal size of the explant depends on the purpose. For rapid multiplication, a relatively larger explant (3–10 mm) is desirable despite its higher susceptibility to blackening and contamination. When virus or bacteria elimination is needed, meristem-tip culture is the preferred option. The explant is then further reduced in size (0.5–1 mm length), leaving a meristimatic dome with one or two leaf initials. Meristem cultures have the disadvantage that they may have a higher mortality rate and an initial slower growth. The explant is placed directly on a multiplication-inducing culture medium. For banana micro propagation, MS-based media are widely adopted (Murashige & Skoog, 1962) [42]. Generally, they are supplemented with sucrose as a carbon source at a concentration of 30–40 g/l. Banana tissue cultures often suffer from excessive blackening caused by oxidation of polyphenolic compounds released from wounded tissues. These undesirable exudates form a barrier round the tissue, preventing nutrient uptake and hindering growth. Therefore, during the first 4–6 weeks, fresh shoot-tips are transferred to new medium every 1–2 weeks. Alternatively, freshly initiated cultures can be kept in complete darkness for one week. Antioxidants, such as ascorbic acid or citric acid in concentrations ranging from 10–150 mg/l, are added to the growth medium to reduce blackening, or the explants are dipped in antioxidant solution (cysteine 50 mg/l) prior to their transfer to culture medium (Jarret. 1985) [28].

Usually two types of growth regulators, a cytokinin and an auxin, are added to the banana growth medium. Their concentration and ratio determines the growth and morphogenesis of the banana tissue. We routinely add 2.25 mg/l 6-benzyladenine (BA) and 0.175 mg/l indole-3acetic acid (IAA) to the initiation medium. In most banana micro propagation systems, semi-solid media are used. As a gelling agent agar (5–8 g/l) is frequently added to the culture medium but our preference is for Gelrite (2–4 g/l) because of its higher transparency, allowing much earlier detection of microbial contamination. Liquid media are superior for shoot multiplication, but for maximum plant production and survival ex vitro, one culture cycle on semi-solid medium is also needed (Bhagyalakshmi & Singh, 1995) [6]. Banana shoot-tip cultures are incubated at an optimal growth temperature of 28 ± 2 °C in a light cycle of 12 -16 h with a photosynthetic photon flux (PPF) of about 60 $\mu E/m^2s^1$.

Stage 2: Multiplication of shoot-tip cultures

The formation of multiple shoots and buds is promoted by supplementing the medium with relatively high concentrations of cytokinins. In banana, BA is the preferred cytokinin and is usually added in a concentration of 0.1–20 mg/l (Banerjee and Langhe, 1985) [5]. For the multiplication of propagules, we use the same medium as for the initiation of shoot cultures (p5 medium containing 2.25 mg/l BA and 0.175 mg/l IAA). If the production of highly proliferating meristem cultures is required (Section 3.2.1.), a tenfold higher concentration of BA is added to the culture medium (p4 medium containing 22.5 mg/l BA and 0.175 mg/l IAA). Higher concentrations of the cytokinin BA tend to have an adverse effect on the multiplication rate and morphology of the culture and should therefore be avoided. The rate of multiplication depends both on the cytokinin concentration and the genotype. In general, shoot tips of cultivars having only A genomes produce 2–4 new shoots, whereas cultivars having one or two B genomes produce a cluster of many shoots and buds at each subculture cycle. Approximately 6–12 weeks after culture initiation, depending on the initial explant size, new axillary and adventitious shoots may arise directly from the shoot-tip explant. Clusters can be separated, trimmed and repeatedly subcultured at 4–6 week intervals.

Stage 3: Rooting of regenerated plantlets

Individual shoot or shoot clumps are transferred to a nutrient medium which does not promote further shoot proliferation but stimulates root formation. The cytokinin in the regeneration medium is greatly reduced or even completely omitted. Within 2 weeks, shoot tips develop into unrooted shoots. To initiate rhizogensis IAA, NAA (-naphthalene acetic acid) or IBA (indole-3-butyric acid) are commonly included in the medium at between 0.1 and 2 mg/l. We use the same auxin concentration as in the proliferation medium (0.175 mg/l IAA), but a tenfold lower BA concentration (0.225 mg/l). For some genotypes (Musa spp. ABB and BB group) that produce compact proliferating masses of buds, activated charcoal (0.1–0.25%) is added to the regeneration/rooting medium to enhance shoot elongation and rooting.

Stage 4: Hardening

During hardening, the plantlets undergo physiological adaptation to changing external factors like water, temperature, relative humidity and nutrient supply. Primary hardening should be done in a controlled environment of 24-26 °C temperature and more than 80% humidity. Planting media for primary hardening range from sieved sand augmented with nutrients to mixtures of cocopeat and Soilrite with fine sand in equal proportions. Cocopeat + vermiculite (1:1) showed the optimum growth and development in cultivar Udhayam-ABB26. The reason might be that cocopeat + vermiculite would have improved the water retention ability

and aeration to the growing plant as well as altered anchorage and nutrient content of the medium thereby promoting the growth and development of nursery plants. Whereas, in secondary hardening NPK is provided in liquid form on weekly basis.

Somatic embryogenesis

In vitro somatic embryogenesis offers opportunities for largescale production of plant material and establishment of new technologies for improvement of banana. Somatic embryogenesis in Musa spp. was achieved using thin meristimatic tissues with successful regeneration of plants (Banerjee *et al*., 1987). Subsequently, regeneration via somatic embryogenesis in diploid and triploid genotypes of banana was reported by several researchers using meristems, rhizome tissues, leaf bases, immature zygotic embryos and young male flowers as explants (Escalant and Teisson, 1988) [19]. Lee *et al*. (1997) [34] reported development of embryogeniccalli from rhizome explants of triploid Musa cv. Grande Nain and regeneration of plantlets from somatic embryos on MS medium supplemented with 5µM 2,4-dichlorophenoxyacetic acid (2,4-D), 1 µM proline, 100mg/l casein hydrolysate, 40 mg/l cystein-HCl, 10µM ascorbic acid and 40g/l sucrose after 8 weeks of culture. They showed histological evidence of induction of somatic embryogenesis. Navarro *et al*. 1997 [44] reported complete plant regeneration via somatic embryogenesis from a diploid (*Musa acuminata* spp. *Malaccensis*) and triploid (Grande Naine) bananas using immature zygotic embryos and male flower bud primordia on MS medium supplemented with 2,4-D and NAA. They also observed that zeatin and kinetin were necessary for embryo maturation and BA and IAA for germination of somatic embryos. Schoofs *et al*. (1998) [57] indicated that the 18 cultivars, including diploid and triploid genome groups, showed embryogenic response from scalp explants. Embryogenic frequencies of responding accessions varied between 15 and 80%. Further, Ganapathi *et al*. (1999) [23] reported complete plant regeneration via somatic embryogenesis from young male flowers on MS medium supplemented with 0.22 µM BA and 1.14µM IAA. They also observed that the plumule and radicles were observed within a span of 6–8 weeks upon transfer to 1/2 MS supplemented with 0.5 mg/l malt extract and 0.1% activated charcoal.

Scalp-derived Embryogenic cell suspensions

Preparation of embryogenesis competent explants (scalps)

Embryogenesis competent scalps are 3–5 mm explants containing a high number of tiny white meristems with a small amount of corm or leaf tissue. The time needed to prepare highly proliferating meristem cultures from which scalps of good quality can be excised ranges from a few months to more than one year. For cultivars like ‘Bluggoe’ (ABB group) with a high initial in vitro proliferation rate, suitable starting material can be obtained on standard proliferation medium (p5 medium with 2.25 mg/l BA). For other types like Plantains (AAB group), Cavendish (AAA group) and East African Highland bananas (E-AAA group), several cultures on medium enriched in cytokinin (p4 medium with 22.5 mg/l BA) are needed.

Table 1: Different in vitro phases involved, behaviour of plant material and duration of different steps in the establishment of scalp-derived embryogenic cell suspensions [19]

Phase	Behaviour of plant material	Duration
1.Preparation of embryogenesis competent explants (scalps)	Homogeneous proliferation; good quality scalps	5-7
2. Embryogenesis induction	Embryogenic complexes (globules, embryogenic cells, embryos)	4-7
3.Suspension initiation and upgrading	Embryogenic cell suspensions	3-6
4. Regeneration	Rooted plantlets of test tube size	3-8

Proliferating meristem cultures that allow scalp preparation can be obtained in theory for any landrace. However, the extent that corm and leaf tissue can be reduced between the meristimatictissue is dependent on the genomic constitution and even the cultivar (Schoofs, 1997) [56]. The minimum number of cycles on p4 proliferation medium was negatively correlated with the percentage of B chromosome sets in the genome. Recently a broad range of cytokinins was explored for their influence on shoot tips freshly excised from in vitro rooted plantlets. Based on percentages of outgrowth and multiplication, and the amount and length of developing shoots, the cytokinins can be ranged as follows (from strongest to weakest activity in triggering multiplication): TDZ > BA > kinetin >zeatin> 2iP. The lengthy material preparation phase for highly proliferating meristem cultures could be reduced up to threefold by (i) inoculation of freshly excised 5 mm explants (instead of shoot tip cultures) and (ii) the use of TDZ (instead of BAP) as cytokinin. Investigations are currently being conducted to find out whether these highly proliferating TDZ cultures are also competent for embryogenesis.

Induction of somatic embryogenesis:

Embryogenesis is induced by scalp inoculation onto semi-solid medium containing 1 mg/l 2,4-D (2,4-dichlorophenoxyacetic acid) and 0.22 mg/l zeatin as plant growth regulators (ZZ medium). According to the embryogenic response (no embryogenic response, occurrence of individual embryos, presence of embryogenic callus), three main patterns of development are found. The formation of fast-growing, yellowish white callus during the first weeks after embryogenesis induction is not wanted since such calluses eventually become necrotic, most often without any embryogenic structure. A positive embryogenic response occurs generally on 3–8-month-old induced explants. The appearance of individual embryos is a promising indication of the embryogenic capacity of the starting material. More interesting is a white callus, consisting of only early-stage somatic embryos and non-organised embryogenic cell clusters. Only these friable embryogenic complexes are suitable for the initiation of embryogenic cell suspensions. The nature of the embryogenic response depends not only on the genotype but also on the selected line and even the experiment (Table 2).

Table 2: Genomic constitution, variety, type and corresponding frequency of embryogenic callus encountered on scalps induced for embryogenesis

Genomic constitution	Variety	Type	Successful induction of embryogenesis (%)
AA	Calcutta4	Wild diploid	0
AAA	GN FHIA	Cavendish	0–2.9
AAA	GN JD	Cavendish	0–4.2
AAB	Agbagba	Plantain	0–0.5
AAB	Obino l Ewai	Plantain	0–2
ABB	Orishele	Plantain	0–5.8
ABB	Burro Cemsa	Cooking banana	0

Lowest and highest frequency (%) of embryogenic callus encountered in a single experiment

Initiation and maintenance of embryogenic cell suspensions

Success rates for the initiation of embryogenic cell suspensions depend largely on the quality and volume of available embryogenic complexes. In our experience, it is not worthwhile to transfer distinct embryos to liquid ZZ medium. Overdeveloped embryos either turn black due to oxidation of phenolic compounds or dedifferentiate into globules which only release nonembryogenic cells. In contrast, homogeneous complexes consisting of a high proportion of embryogenic callus and early-stage transparent embryos are suitable as inoculum. The first few months following the initiation of embryogenic cell suspensions are labourintensive. This is mainly due to the heterogeneity of the freshly initiated cultures. Components of young cell suspensions and their evolution in time are discussed by Schoofs, 1997 [56]. To avoid differentiation and to stimulate multiplication of embryogeniccell clusters, the maintenance medium must be refreshed weekly. In addition, to maintain the regeneration capacity of the cell suspension, globules which release only starchy dense or empty cells have to be discarded. On average six months after initiation, embryogenic cell suspensions reach the phase of mass multiplication. The maintenance medium of the cell cultures is then refreshed every 2 weeks with an optimal initial inoculum density ranging from 1.5 to 3%. A two- to threefold increase in settled cell volume is reached at the end of each subculture period. Even at this level, embryogenic cell suspensions remain more or less heterogeneous Cronauer & Krikorian, 1988 [13].

Plant regeneration from embryogenic cell suspensions

Regeneration from banana cell suspensions is not really a problem when the suspension consists entirely of embryogenic cell clusters. Medium composition, light conditions and inoculum age only slightly affect the regeneration capacity. Based on weight measurements and counting of germinating embryos and plants, the regeneration capacity of cell suspensions established using the scalp methodology ranges between 104 to 105 somatic embryos per millilitre of settled cell volume. The conversion rate of germinating embryos into rooted plantlets is 90–100%. These results are in accordance with data obtained for male flower derived embryogenic cell suspensions. The number of plants obtained from 1 ml settled cells of several scalp-derived cell suspensions is given in Table 3. Assuming (i) aninitial inoculum density of 1.5% settled cell volume in 60 ml ZZ maintenance medium, and (ii) a twofold increase of cell volume at the end of one subculture period (two weeks), a ‘Gran enano’ cell suspension can give rise to between 14,580 and 100,980 plants, while between 27,000 and 117,000 plants can be regenerated from an ‘Orishele’ suspension

Table 3: Genomic constitution, cultivar, type and number of plants obtained per millilitre of settled cells from scalp-derived embryogenic cell suspensions

Genomic constitution	Cultivar	Type	Number of plants (× 104) per ml settled cells
AAA	Gran enano	Cavendish	0.81–5.61
AAB	Williams JD	Cavendish	4.12–10.15
AAB	Agbagba	Plantain	0–6.02
AAB	Orishele	Plantain	1.50–6.50

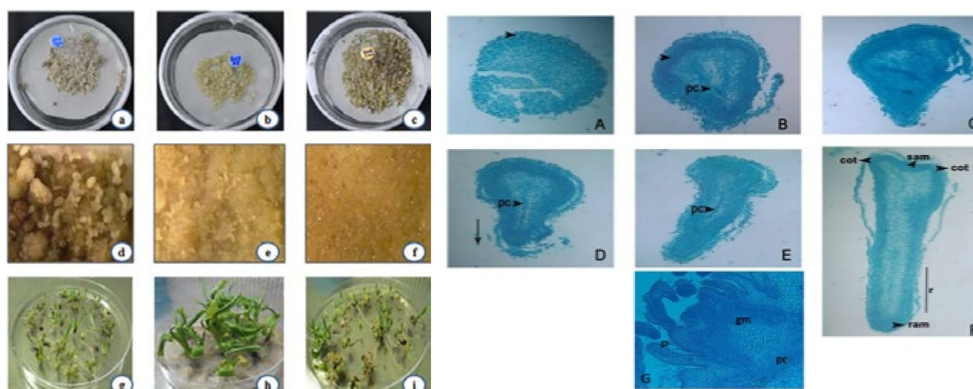

Fig 1: Apical and basal differentiation patterns in somatic embryo development. A: Globular stage somatic embryos; B–C: Gradual establishment of polarity associated with differentiation of procambial cell layer; D: Oblong stage somatic embryo with procambial cell layer; E: Advanced globular embryo with established polarity. The two meristems are separated by a linear array of juvenile cells; F: Torpedo stage somatic embryos with visible three embryogenic cell domains (arrow heads). The apical domain, derived from the upper tier, has been partitioned into cotyledon (cot), primordial and shoots apical meristem (sam). The basal domain derived from the tier, formed the root (r)

Induction of Variability in *In Vitro* Culture

Genetic improvement in cultivated bananas has been difficult due to the absence of sexual reproduction mechanisms. The tissue variability, a curse to the propagator, could be of much interest to the banana breeder (Skirvin, 1978 [60]; Micke *et al*., 1987) [38] and, with tissue culture, it might be possible to isolate improved forms of standard cultivars having resistance to pests and diseases. Variability has been reported in different banana cultivars through physical and chemical mutagenesis Azzam and Linden, 1965. Novak *et al*. (1990) [3, 46] described the responsiveness of tissue-cultured shoot tips to different doses of gamma irradiation (15, 30, 45 and 60 GY) at a dose of 8 GY/min in seven clones of dessert banana (AA, AAA and AAAA), plantain (AAB) and cooking banana. They also observed considerable phenotypic variation among plants regenerated from in vitro shoot tips after mutagenic treatment. They selected early-flowering putative mutant plants of Cavendish banana (Grande Naine) (GN-60 GY/A). This mutant was reported to grow vigorously and flower after 9 months, in comparison to non-irradiated control plants which took 15 months to flower. The mutant also showed differences in soluble proteins, esterase and DNA molecular markers (Kaemmer *et al*., 1992) [29]. Smith *et al*. (1993) [62] and Tan *et al*. (1993) [66] developed a technique to develop tetraploids from a micropropagated diploid clone, SH-3362 using 0.5% colchicine and 2% DMSO. They found SH-3362 to be very susceptible to cold damage but resistant to Fusarium wilt. The latter type was introduced to Malaysia and cultivated for several years.

Novak *et al*. (1993) [47] reported the development of in vitro plants by application of chemical mutagens. The optimal response of cultured shoot tips to the chemical mutagen ethyl methanesulphonate (EMS) in both diploid (SH-3362) and triploid (Grande Naine) clones was achieved after 3 h incubation with 24.67 mM (0.2%) mutagen. Two percent DMSO enhanced the uptake of EMS into the apical meristematic dome, leaf primordia and corm tissue. Morpurgo *et al*. (1994) [40] described the selection procedures to induce resistance/susceptibility of banana to *Fusariumoxysporum* f. sp. Cubense through shoot tip culture of two diploid (AA) clones, namely SH-3362 and Pisang Mas. They used peroxidase activity as a marker to discriminate susceptibility and tolerance to Fusarium disease.

Somaclonal Variation and Clonal Stability

In recent years it has become evident that in vitro tissue culture induces genetic variation (Larkin and Scowcroft, 1981) [33]. The genetic behaviour of these variants generally appears to be similar to that of naturally occurring mutants. D'Amato (1975) and Skirvin and Janick (1976) [16, 61] were the first toemphasize the importance of clonal variation in genotype improvement of horticultural crops. Somaclonal variations in in vitro culture shoots were common in many plant species, including banana. Vuylsteke *et al*., 1991 [71]; Cote *et al*., 1993) [11]. In banana, variants were obtained from meristem tip cultures ranging from 3% inTaiwan to 25% in Jamaica. Inplantains, Vuylsteke *et al*. (1988) [72] observed a frequency of 6% off-types and described five different forms of phenotypic variations including variegated leaf, leaf drooping, distorted lamina and delay in flowering. Reuveni *et al*. (1986) [52] reported the occurrence of dwarf plants and plants with curled leaves with a reddish petiole and leaf blade. The occurrence of high level somaclonal variation was used as a breeding tool to improve banana resistance against *Fusarium oxysporum* f. sp. Cubense in Cavendish. The level of variation obtained by subculturing the meristems was around 3%. Out of 18000 meristematic tissue-derived plants, 45 were reported to be disease resistant. Morpurgo *et al*. (1994) [41] reported that neither fusaric acid nor crude filtrate could be used as selective agents. Peroxidase activity was used as a marker to discriminate between susceptible and the tolerant clones of banana. Several factors could contribute to the appearance of variants: strictly genetic alteration of the plant genome, as well as epigenetic modification. A molecular-based approach (AFLP technique) to somaclonal variation analysis in banana was also reported (Crouch *et al*., 1999) [14].

Genetic Transformation

Genetic transformation studies have led to further development of plant breeding techniques and a better understanding of the basic mechanisms involved in plant gene regulation (Wising *et al*., 1988) [74]. In the past 70 years, the application of classical methods to breeding for disease resistance has shown limited success due to long generation times, high sterility and triploidy in most cultivated bananas. During thelast decade a wide range of methods and different approaches to gene transfer into plant cells have been explored (protoplast transformation, direct gene transfer across the cell wall without carrier particles, biolistic gene transferand Agro bacterium mediated gene transfer in numerous variations with limited success (Potrykus, 1990) [51]. Genetic transformation as a tool for genetic improvement in Musa species has been implemented in genetic programmes designed for creation of resistance to major diseases, such as black sigatoka and Fusarium wilt (fungal diseases) and banana bunchy top virus (Harding *et al*., 1993 [26], which have been identified as the primary agronomic problems for banana and plantain production on a worldwide basis (Huggan, 1993) [27]. Direct DNA introduction by electroporations (Fromm *et al*., 1985) [22] into viable and highly regenerative protoplasts provided opportunities for efficient genetic transformation of banana. Embryogenic cell suspensions have been used for particle bombardment (Bakry *et al*., 1993) [4]. Agrobacterium-mediated transformations have been reported for regeneration of transgenic bananaplants (Arntzen and Lam, 1992) [2]. The integration of genetic engineering into breeding programmes may help to overcome these limitations by inducing specific genetic changes within a short span of time. Such genetic techniques have been successfully applied to banana (Vuylsteke and Swennen, 1992; Sagi *et al*., 1994 [70, 54], using Agrobacterium tumefaciens or a biolistic approach. May *et al*. (1995) [36] demonstrated banana transformation by Agrobacterium-mediated gene introduction and scored the regenerants by both phenotypic observation and molecular characterization. The system provided an opportunity for the recovery of putative transformants within 4 weeks of co-cultivation of the tissue samples with Agrobacterium. They developed procedures for the recovery of genetically transformed banana (var. Grande Naine) using kanamycin as the selective agent for the npt-II gene. Sagi *et al.* (1995) [53] developed a simple protocol to allow production of transgenic banana plants. Foreign genes were delivered into embryogenic suspension cells using accelerated particles coated with DNA. Bombardment parameters were optimised for a modified particle gun resulting in high level of transient expression of the β-glucoronidase gene both in banana and plantain cells. Bombarded banana cells were selected with

hygromycin and regenerated into plants. They successfully demonstrated stable integration of the transferred genes into the banana genome, which was confirmed by molecular and histochemical characterization of the transform ants. Currently, micro projectile bombardment experiment using genes for novel types of antifungal proteins (AFPs) are being carried out with banana and plantain cultivars, aimed at regeneration of fungus-resistant transgenic plants. Table 4 lists the heterologous promoters applied in various cultivars of Musa and in different tissues (Panis *et al*., 1996) [48]. Sagi*et al.* (1994) established electroporation conditions for transient expression of DNA introduced to banana (*Musa* spp. cv. Bluggoe) protoplasts isolated from regenerableembryogenic cell suspensions. The maximum frequency of DNA introduction,as detected by an in situ assay for transient expression of the uidA gene, accounted for 1.8% of the total protoplasts. After 3 months, actively growing cell aggregates were selected and regenerated into plants. Plants regenerated from banana protoplasts at a high frequency (Panis *et al*., 1993) [50]. The embryogenic cell suspensions could be stored using cryopreservation techniques without any loss of the regeneration ability (Panis *et al*., 1992) [49]. May *et al*. (1995) [36] reported a case of meristem transformation, after a first cycle of plant selection with 100mg/l kanamycin sulphate, chimeric regenerants were obtained. Shoot tips of selected putative transformants were excised and subjected to a second cycle of plant selection. Only 10% of the putative transformants survived this second selection. Further, Swennen *et al*. (1998) [57] developed transgenic cultures of Cavendish banana Williams and subsequently regenerated plantlets. The expression of foreign genes (Ace – AMP1) has been achieved in the bunch of a flowering banana, as well as in the leaves of the mother plant, daughter and granddaughter suckers.

Table 4: Different type of tissues and cultivars utilized in genetic transformation of banana

Tissue (origin)	Cultivar/Variety	Transformation system	Selection after transformation	Transgenic plants
SE (ECS)	Grande Naine (AAA)	PB	×	–
ECS (male flowers)	Grande Naine (AAA)	PB	×	–
ECS (male flowers)	French Sombre (AAB*)	PB	×	–
ECS (zygotic embryos)	M. accuminata ssp. Malaccensis	PB	–	–
ECS (scalps)	Bluggoe (ABB)	PB	×	×
ECS (scalps)	Three Hand Planty (AAB*)	PB	×	–
ECS (scalps)	Williams (AAA)	PB	×	–
ECS (scalps)	M. balbisiana Tan	PB	–	–
ECS (scalps)	Monthan (ABB)	PB	–	–
ECS (scalps)	Cardaba (ABB)	PB	–	–
SE (ECS)	Monthan (ABB)	PB	–	–
SE (ECS)	Bluggoe (ABB)	PB	–	–
Scalps	Bluggoe (ABB)	PB	–	–
Protoplasts (ECS)	Bluggoe (ABB)	Electroporation	–	–
meristems/corm	Grande Naine (AAA)	Agrobacterium	×	×
ECS (zygotic embryos)	M.accuminata ssp. Burmannica Long Tavoy	PB	–	–
ECS (zygotic embryos)	M. accuminata ssp. Banksii	PB	–	–
ECS (zygotic embryos)	M. accuminata ssp. Malaccensis	PB	–	–
ECS (scalps)	Matavia (ABB)	PB	–	–

SE: somatic embryos, ECS: embryogenic cell suspensions, PB: Particle bombardment. AAB*: AAB group Plantain subgroup

Table 5: Plasmids and promoters of the gusA gene utilized for transient gene expression in bananaurce: Panis *et al*., 1996b [48]

Tissue (origin)	Cultivar/Variety	GUS assay	Plasmid	GUS promoter
SE (ECS)	Grande Naine (AAA)	hist	pCaMV2 GUS pUGC1 p0021	35S-35S Ubi Act1
ECS (male flowers)	French Sombre (AAB*)	hist/fluor	pEmuGN pUGGC1 pAct1D pBI221 pCaMV2GUS pJB4	Emu Ubi Act1 35S 35S-35S 35S
ECS (scalps)	Bluggoe (ABB)	hist/fluor	pBI221 PBI-364 PBI-505 PEmuGN PWRG1525 PAHC27	35S 35S-35S 35S-35S-AMV Emu 35S-AMV Ubi
Protoplasts (ECS)	Bluggoe (ABB)	hist/fluor	PBI221 PAHC27 PBI-505	35S Ubi 35S-35S-AMV

Molecular Genetics of Banana

Conventional breeding of Musa spp. and plantain remains a difficult endeavour because of high sterility levels and polyploidy. Molecular genetic techniques have great potential to overcome some of the factors limiting traditional approaches to banana and plantain improvement (Krikorian and Cronauer, 1984a [32]; Murfett and Clarke, 1987 [43]). A large number of genes of economic importance, such as genes

for disease and insect resistance, are quite difficult to transfer together into a crop. Sometimes the screening procedures are cumbersome and expensive and require large field space. If such genes can be tagged by tight linkage with DNA or isozyme markers, time and money can be saved in moving these genes from one varietal background to another. The presence or absence of the associated molecular marker (desired genes) would be apparent at a veryearly stage of the crop. The genetics of banana and the application of breeding procedures for its improvement have been reported by Dodds (1947) [17]. Simmonds (1954) [58] classified the Cavendish group of bananas for plant breeding. Chromosomes of Musa are small and difficult to work with (Vakili, 1967) [68]. The potential advantages of a breeding scheme to overcome the undesirable characters of tetraploids and to incorporate a second diploid male into the recombination process have been reported Buddenhagen, 1986) [63]. Molecular markers have shown tremendous potential for analyzing problems in plant genetics and breeding (Tanksley, 1983 [67]; Bhat *et al*., 1994 [9]; Kaemmer *et al*., 1997 [29]).

DNA based marker techniques (DNA oligonucleotide and amplifications fingerprinting) were successfully used to detect genetic polymorphisms in 15 representative species and cultivars of the genus Musa, comprising AA, AAA, AAAA, AAB, ABB and BB genotypes by Kaemmer *et al*. (1992) [29]. They developed fingerprinting techniques which helped in the detection of bands with characteristic A and B genomes. Carrel *et al*. (1993) [10] used RFLPs (restriction fragment length polymorphisms) to study genetic diversity in Musa ssp. This analysis provided evidence for a strong bias towards maternal transmission of chloroplast DNA and paternal transmission of mitochondrial DNA in Musa acuminata (Faure *et al*., 1994) [20]. These results suggest the existence of two separate mechanisms of organelle transmission and selection. Knowledge of the organelle mode of inheritance constitutes an important point for phylogeny analyses in banana and may offer a powerful tool to confirm hybrid origins. Faure *et al*. (1994) [20] have developedthe genetic map of the diploid genome of banana (*Musa acuminata*). Afza *et al*. (1992) [1] reported the variability and individual identity of different genotypes of Musa by DNA fingerprinting. They also indicated the variability in DNA samples isolated from different somatic tissues (rhizome, pseudostem, leaf, male inflorescence, fruits) of one Grande Naine banana plant. There were no differences between the individuals of the same clone. Variable numbers of tandem repetitions were quite differentin diploid *Musa acuminata* (AA) and *Musa balbisiana* (BB) accessions. Bhat *et al*. (1995a) [8] reported cultivar identification and overall genome analysis to establish relationships among the various accessions of the *Musa*germplasm originating from different geographical regions, using oligo-deoxyribo-nucleotide probes. Subsequently, Bhat and Jarret (1995) [7] noted 57 accessions of Musa, including cultivated clones of 6 genomic groups (AA, AB, AAA, ABB, ABBB), *Musa balbisiana*Colla (BB), *Musa accuminata*Colla ssp. Banksii F. Muell (AA), *Musa accuminata*Colla ssp. MalaccensisRidl (AA) and *Musa velutina*Wendl. andDrude, using RAPD genetic markers. Random amplified polymorphic DNA (RAPD) and DNA amplification fingerprinting (DAF) provided high specificity and reproducibility of banding patterns and potential application in several areas of Musa improvement (Bhat *et al*., 1995b). Grapin and Lanaud (1998) [24] reported the powerful marker system (microsatellite and locus-specific PCR: STMS) for a breeding programme of improvement of banana and plantain. This method allows for identification of A and B genomespecific bands and the classification of Musa (sub) species and cultivars. Sequence tagged microsatellite site (STMS) discrimination potential was explored using 9 microsatellite primer pairs. Genetic relationships were examined among 59 genotypes of wild or cultivated accessions of diploid *Musa acuminata*. Crouch *et al*. (1999) [15] reported that molecular markers assisted breeding potential to dramatically enhanced the pace and efficiency of genetic improvement of Musa. They studied the PCR-based marker systems (RAPD, VNTR, AFLP) for analysis of breeding populations generated from two diverse Musa breeding schemes. All three assays detected a high level of polymorphism between parental genotypes and within progeny populations. They also noted that both VNTR and RAPD analysis have high frequency homologous recombination during n (2 ×) gamete formation by tetraploid hybrids.

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