A. CELL AND TISSUE CULTURE, AND MUTATION INDUCTION

1. Banana cell and tissue culture - review - Strosse, H., I. Van den Houwe, B. Panis

Laboratory of Tropical Crop Improvement
Catholic University of Leuven
Kasteelpark Arenberg 13
B-3001 Heverlee
Belgium

Abstract

The International *Musa* germplasm collection is sited at the INIBAP (International Network for the Improvement of Banana and Plantain) Transit Centre at K.U.Leuven. By now, more than 1000 different accessions of shoot-tip cultures have been initiated *in vitro*, multiplied and maintained at reduced temperature conditions (16 ± 1°C). Shoot cultures are grown on MS (Murashige and Skoog) medium, supplemented with 30 g/l sucrose, 2.25 mg/l BA (6-benzyladenine) and 0.175 mg/l IAA (indole-3-acetic acid). In comparison with the culture medium on which shoot-tips are maintained, a tenfold decrease in cytokinin content (0.225 mg/l BA) induces regeneration of rooted plants. In contrast, adding 22.5 mg/l BA to the culture medium results in suppression of the apical dominance in shoot-tip cultures and a reduction of corm and leaf tissue between meristematic tissue. Highly proliferating meristem cultures are obtained and used as starting material in the scalp methodology, the technique most commonly applied for the development of embryogenic cell suspensions at the Laboratory of Tropical Crop Improvement, K.U.Leuven. Initiation and maintenance of cell cultures is rather labour intensive and time consuming. However, since 1 ml of settled cells of a highly regenerable cell suspension can yield more than 100,000 plants, cell cultures are most suitable for mass clonal propagation. Moreover, embryogenic cell suspensions are highly preferred as target material for protoplast culture and genetic engineering since the risk of chimerism is circumvented because of the unicellular origin of regenerated plants.

1. INTRODUCTION

Plant tissue culture is the science of growing plant cells, tissues or organs isolated from the mother plant, on artificial media. It includes techniques and methods appropriate to research into many botanical disciplines and several practical objectives. Both organized and unorganized growth is possible *in vitro* [1].

Organized growth of banana tissue *in vitro* is limited to embryo culture and shoot tip culture. Embryo culture is an important aid for classical breeding in banana since the germination frequency of seed is extremely low. Through embryo rescue, this frequency can be 10 times increased [2]. Major applications of shoot tip culture are mass clonal propagation and germplasm conservation. In the former already existing shoot tips are stimulated to multiply rapidly, while in the latter, the multiplication rate is slowed down. This technique is applied for the conservation of The International *Musa* germplasm collection at the INIBAP (International Network for the Improvement of Banana and Plantain) Transit Centre at K.U. Leuven.

In vitro culture of unorganized tissue in banana is almost exclusively related to the establishment of embryogenic cell cultures. When an embryogenic callus is induced on solid media containing high auxin concentrations, it can be transferred to liquid medium where it gives rise to embryogenic cell suspensions. Such suspensions with high regeneration capacity can be used for mass clonal propagation and are the only source of regenerable protoplasts in banana. More importantly, they are the preferred target material for induced mutations and genetic engineering.

In this article, a brief overview is given on the most common techniques in banana cell and tissue culture and their applications.

2. SHOOT-TIP CULTURES

2.1. Micropropagation

2.1.1. 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 [3]. The apex of the inflorescence and axillary flower buds [4] are also suitable explants for tissue culture initiation. 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 cm^3 containing the apical meristem is excised. This block of tissue is dipped in 70% ethanol for 10 s, 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) [5], type of disinfectant (calcium hypochlorite instead of sodium hypochlorite) and its concentration and treatment duration [6]. 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 [7] which rapidly develop into shoots.

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 meristematic 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 micropropagation, MS-based media [8] are widely adopted. 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 [9].

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-3-acetic acid (IAA) to the initiation medium.

In most banana micropropagation 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 [10], but for maximum plant production and survival *ex vitro*, one culture cycle on semi-solid medium is also needed.

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 $\mu E/m^2s^1$.

2.1.2. 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 [11]. 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.

2.1.3. Stage 3: Regeneration of plants

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 rhizogenesis IAA, NAA (a-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. After rooting, plants are hardened *in vitro* for 2-4 extra weeks on the regeneration/rooting medium prior to transplantation to soil.

2.2. Conservation of shoot cultures

Shoot or meristem tip cultures are suitable not only for the large-scale production of uniform and vigorously growing propagules for field establishment, but also for germplasm conservation. For banana species which are predominately propagated vegetatively, *in vitro* techniques are thus complementary to field conservation.

Banana species can be stored under normal growth conditions at 28°C, but this involves transfer every 2-4 months. Hence there is the risk of losing material through microbial contamination or through human error (mislabelling of culture vessels). Nevertheless, conservation of germplasm under short-term storage conditions is most useful, for instance, to breeders or researchers who wish to maintain a small working collection of breeding material during evaluation, testing, selection and hybridisation, or to maintain products of *in vitro* manipulation.

Slow growth conservation has the benefit of reducing the number of subcultures, thus making significant savings in labour input. Germplasm still remains readily available for regeneration, multiplication and distribution. Minimal growth is clearly useful for conservation of genotypes, but is constrained by the risk of genetic changes (somaclonal variation) resulting in the loss of distinct genotypes.

Suppression of growth can be achieved by various modifications of the physical and/or chemical tissue-culture environment. The most common and widely applied growth-retarding factor in banana is low temperature. This technique has been used routinely for many years for the conservation of the International *Musa* Germplasm collection at the INIBAP (International Network for the Improvement of Banana and Plantain) Transit Centre at K.U.Leuven. Banana shoot cultures are stored at 16 ± 1°C. Each accession in the collection is represented by a set of 20 replicates of shoot cultures grown on MS medium, supplemented with 30 g/l sucrose, 2.25 mg/l BA and 0.175 mg/l IAA. Subculture intervals are extended to about 12 months, although large differences in storage potential have been observed among the different genotypes: some genotypes keep for about 20 months whereas others require subculturing every 2 months [12].

Alternatively, the use of osmotica in banana tissue cultures has been investigated [13]. The addition of 4% mannitol and 3-6% sucrose to the growth medium resulted in 50% growth reduction. Cultures grown on mannitol-enriched medium at 27°C could be kept for 6 months, whereas cultures on the control medium (3% sucrose) started to deteriorate after 4 months of incubation.

2.3. Contamination

Contamination in tissue cultures may be caused by endogenous bacteria that escape initial disinfection or by micro-organisms introduced during tissue-culture manipulations. Both types of contaminants may survive in the plant material for several subculture cycles and over extended periods of time without expressing symptoms in the tissue or visible signs in the medium.

'Internal' bacteria are a considerable source of concern in all aspects of plant cell, tissue and organ culture because they hinder the international exchange of germplasm or become a nuisance when contaminated tissues are used as explant material for cryopreservation or for the initiation of embryogenic cell suspensions. It is therefore important that control measures are taken at every tissue-culture step. At the INIBAP Transit Centre, plant material is tested for endophytic bacteria on a broad spectrum bacteriological medium at tissue culture initiation and during annual subculturing. Prior to placing each shoot-tip on culture medium, the base of the explant is streaked onto Difco Bacto nutrient agar, enriched with glucose (1%) and yeast extract (0.5%) in Petri dishes [14]. Testing reveals the presence of cryptic contaminants in 5% of the stored germplasm. In mass propagation systems, positive stock materials should immediately be destroyed. However, germplasm in collections or cultures that are not readily replaceable with fresh material (e.g. from field or from greenhouse stock plants) are 'cleaned up'.

Some antibiotics are successful in controlling bacterial contaminants in banana tissue cultures. Rifampicin (100 mg/l) added to liquid cultures for 10-30 days was found most suitable for controlling frequently occurring Gram-positive bacteria in banana shoot tips without affecting plant growth. However, culturing small meristem tips (1 mm) isolated from contaminated *in vitro* plants or from greenhouse plants obtained from contaminated *in vitro* plants was found to eliminate any bacterial contaminant [15].

2.4. Somaclonal variation

Shoot-tip culture preserves genetic stability much better than callus or cell suspension cultures, yet somaclonal variation appears to be widespread among plants regenerated from banana shoot-tip cultures. Off-type frequencies vary from 1% [16] to 74% [17]. The phenomenon of somaclonal variation in the plantain subgroup (*Musa* spp. AAB group) was extensively studied [17]. It revealed that the incidence of somaclonal variation is strongly influenced by the genetic stability of each cultivar, and that its frequency is amplified by culture-induced factors. There is no evidence that growth regulators routinely used in tissue culture directly affect the rate of variation, but it has been found that the rate of somaclonal variation is positively related to the generation number. For 'Williams' (AAA) *in vitro* plants obtained after one and five subculture cycles, dwarfism and leaf-off types counted for 3.7% and 0.7% respectively after one *in vitro* cycle and increased to 6.1% and 1.9% respectively after five *in vitro* cycles [18]. It is therefore recommended that the number of subculture cycles should be limited to ten [19] or that the number of plants produced from a primary

explant should be limited to no more than 1000 [20].

3. SOMATIC EMBRYOGENESIS

3.1. Procedures to produce embryogenic cell suspensions: an overview

In bananas and plantains, there are four main procedures for the development of embryogenic cell suspensions. They differ mainly by the explant in which embryogenesis is induced: zygotic embryos [21, 22], rhizome slices and leaf sheaths [23], immature (fe)male flowers [24-26], and proliferating meristem cultures [27, 28]. Each method has its own limitations, rendering the establishment of *Musa* embryogenic cell suspensions still far from routine [29]. Since most edible banana cultivars rarely set seeds, zygotic embryos are of limited value as starting material. Reports on the efficiency of embryogenesis induction in highly differentiated rhizome and leaf tissue are scarce. The scalp-methodology (described below) relies on proliferating meristem cultures as explants. This involves an extensive material preparation phase preceding induction of embryogenesis. In contrast, the starting material for the widely used male-flower technology can be collected directly from the flowering banana plants. A fast decline of the male flower embryogenic response soon after harvest, as well a seasonal dependence [24], requires direct field access and inoculation of explants quickly after harvest. In addition, this method cannot be applied to the highly preferred False Horn and Harton plantains, which do not produce male flowers. This can be overcome by the use of female flowers as alternative explants, but results in loss of the bunch and is therefore difficult for large-scale use.

3.2. Scalp-derived embryogenic cell suspensions

At the Laboratory of Tropical Crop Improvement, K.U.Leuven, research on somatic embryogenesis is focused on the scalp methodology [28]. The different *in vitro* phases involved, the behaviour of the plant material, and the duration of different steps are given in Table 1 and illustrated in Figure 1.

3.2.1. 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 (Figure 1A). 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 (Figure 2).

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 [30]

Phase	Behaviour of plant material	Duration (months*)
1. Preparation of embryogenesis competent explants (scalps)	Homogeneous proliferation; good quality scalps	5-14
2. Embryogenesis induction	Embryogenic complexes (globules, embryogenic cells, embryos)	4-7
3. Suspension initiation and upgrading	Embryogenic cell suspensions	3-6
	Subtotal	12-27
4. Regeneration	Rooted plantlets of test tube size	3-8
	Grand total	15-35

* Genotype and cultivar dependent

Figure 1 Behaviour of plant material at different *in vitro* phases involved in the establishment and regeneration of scalp-derived embryogenic cell suspensions: (A) scalp; (B) embryogenic complex; (C) embryogenic cell suspension; (D) plants regenerating through somatic embryogenesis.

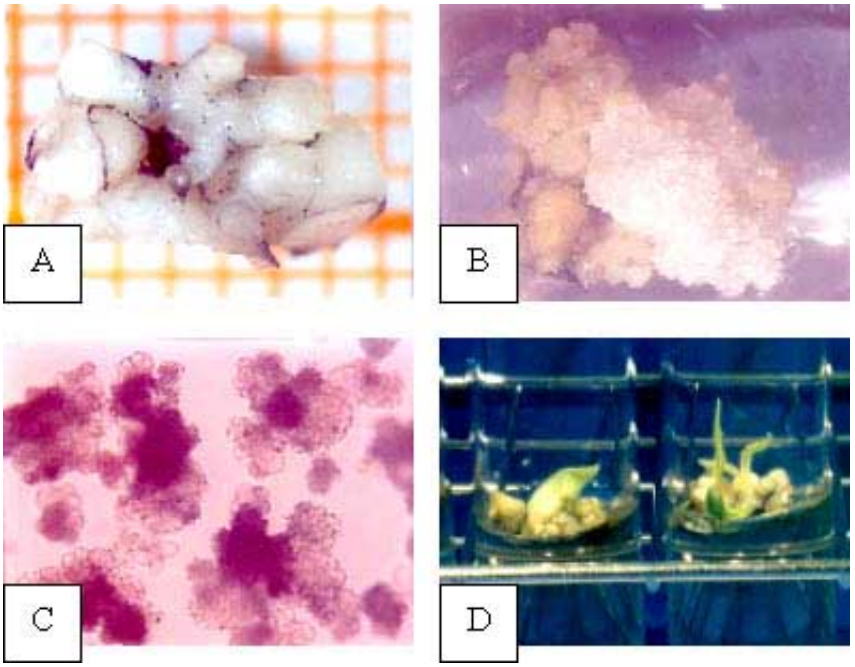

Figure 2 Improvement of *in vitro* proliferation behaviour in 'Grande Naine' (AAA group). From left to right: proliferating cultures after 1 and 2 cycles on standard proliferation medium p5 followed by respectively 3 and 7 cycles on proliferation medium p4.

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 meristematic tissue is dependent on the genomic constitution and even the cultivar [28]. 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 [BA, thidiazuron (TDZ), 6-g,g-(dimethylallylamino) purine, zeatin and kinetin] 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.

3.2.2. 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
AAA	Gran enano	Cavendish	0-11.7
AAA	Williams BSJ	Cavendish	0
AAA	Williams JD	Cavendish	0-22.2
E-AAA	Ingarama	Highland	0
E-AAA	Mbwazirume	Highland	0
E-AAA	Nyamwihogora	Highland	0
AAB	Agbagba	Plantain	0-0.5
AAB	Obino l'Ewai	Plantain	0-2
AAB	Orishele	Plantain	0-5.8
ABB	Burro Cemsa	Cooking banana	0

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

3.2.3. 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 non-embryogenic 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 labour-intensive. 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 [28]. To avoid differentiation and to stimulate multiplication of embryogenic cell 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 [31].

3.2.4. 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 10^4 to 10^5 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 [31]. The number of plants obtained from 1 ml settled cells of several scalp-derived cell suspensions is given in Table 3. Assuming (i) an initial 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 (× 10^4) per ml settled cells
AAA	Gran enano	Cavendish	0.81-5.61
AAA	Williams JD	Cavendish	4.12-10.15
AAB	Agbagba	Plantain	0-6.02
AAB	Orishele	Plantain	1.50-6.50

3.3. Bottlenecks

Unlike dicots [32, 33] and seedsetting monocots [34, 35], embryogenesis in bananas is very difficult. An extensive material preparation phase is required for preparing scalps for induction of somatic embryogenesis, and there are other problems and inconveniences that apply to all available methods. Low (often less than 5%) and variable embryogenic responses, combined with only 20-50% calluses resulting in highly regenerable cell suspensions, hamper the smooth establishment of *Musa* embryogenic cell cultures. Consequently, cell suspensions that do reach the phase of mass multiplication are maintained as long as possible. This implies that the health status and quality of the cell cultures has to be checked regularly. In order to visualise whether bacteria are present, a sample of cells is transferred onto bacteriological medium and incubated at 27°C for at least 6 weeks [14]. Since the regeneration potential of cell suspensions decreases with time, it is recommended that embryos should be germinated regularly and plants grown subsequently from the cell cultures. Flow cytometry analysis of embryogenic cell suspensions (in collaboration with IAEA, Seibersdorf) indicates that the loss of regeneration capacity could be associated with changes in DNA content [36]. True-to-typeness of cell suspension-derived plants varies widely, but with existing technologies field evaluation is the only reliable system. The rate of somaclonal variation can be very low (2% in the diploid acuminata 'IRFA 903') [37], zero in 'Kamaramasenge' (unpublished data) or extremely high (99% in 'Williams' line E4000, unpublished data). Considering the labour-intensive and time-consuming development of *Musa* embryogenic cell cultures, and taking into account their loss of embryogenic capacity,

part of the newly established cell suspensions need to be cryopreserved for backup purposes [38, 39].

3.4. Applications

3.4.1. Mass clonal propagation

Multiple shoot clumps cultured on media with high cytokinin content have a multiplication factor ranging between 2 and 5 per month. Embryogenic cell cultures have a similar multiplication rate (4-6 times per month). However, the high regeneration capacity of embryogenic cell suspensions and the faster manipulation of liquid cultures make mass clonal propagation through somatic embryogenesis more attractive. Indeed, 1 ml settled cells of a highly regenerable cell suspension can yield up to more than 100,000 plants.

3.4.2. Protoplast culture

Protoplast cultures are essential for transformation through electroporation [40] and somatic hybridisation. Like other monocot systems, embryogenic cell suspensions are the material of choice for the isolation of regenerable protoplasts in banana. Regeneration of whole banana plants derived from embryogenic cell suspensions was reported for the cv. Bluggoe (ABB group) [41, 42] and Grande Naine (AAA group) [43]. In all cases, a high protoplast yield is obtained after enzymatic digestion of the cell wall. Plant regeneration through somatic embryogenesis can be obtained after culture on feeder layers or using high plating densities [41].

3.4.3. Genetic engineering

Classical breeding in *Musa* has always been limited since most cultivated varieties have lost the ability to set seeds. For efficient generation of disease resistant/tolerant *Musa* spp., one often relies on genetic transformation techniques. Although very high transient expression frequencies can be obtained through protoplast electroporation (up to 1.8%) [40], currently, genetic engineering of banana mainly proceeds with cells using particle bombardment and *Agrobacterium*-mediated transformation [44].

Despite the rather labour-intensive and time-consuming establishment of embryogenic cell suspensions, cell cultures are still the most suitable target material [45-48]. Embryogenic cell suspensions are also suitable for gamma irradiation because of the unicellular origin of regenerated plants and thus the avoidance of chimaeras. For more information on *in vitro* mutagenesis in *Musa* embryogenic cell suspensions, the reader is referred to Roux et al. [49].

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