Plant regeneration in cell suspension cultures of the cooking banana cv. 'Bluggoe' (*Musa* spp. ABB group).

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PLANT REGENERATION IN CELL SUSPENSION CULTURES OF THE COOKING BANANA CV. 'BLUGGOE' (*MUSA* spp. ABB GROUP).

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ABSTRACT - Embryogenic cell suspension cultures of the widespread cooking banana clone 'Bluggoe' (*Musa* spp., ABB group) were established by culturing meristematic «scalps», taken from proliferating shoot-tip cultures, in a modified liquid MS medium containing 5 μM 2,4-D and 1 μM zeatin. Plant regeneration from suspension cultures was achieved through a sequence of four steps involving different defined media. Embryogenic globules formed when a sieved suspension was cultured in liquid medium without growth regulators. The addition of cytokinin was essential for the subsequent maturation and germination of these globules and resulted in plant recovery frequencies of 10-14.5%. Plant regeneration proceeded through the developmental pathway of somatic embryogeny, which at all stages showed conspicious morphological and histological resemblance with zygotic embryogenesis in a wild *Musa* species. Somatic embryos were produced directly from cells in suspension and not via callus. Germinated banana somatic embryos were successfully established in soil.

The relative simplicity of this cell culture protocol may enhance the feasibility of integrating biotechnological approaches in conventional schemes of banana and plantain improvement.

REGENERATION DE PLANTES EN CULTURES DE SUSPENSIONS CELLULAIRES CHEZ LE BANANIER A CUIRE CV. 'BLUGGOE' (*MUSA* SPP., GROUPE ABB).

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RESUME - Des cultures de suspensions cellulaires chez un clone bien connu du bananier à cuire 'Bluggoe' (*Musa* spp., groupe ABB) ont été établies dans un milieu MS modifié contenant 5 μM 2,4-D et 1 μM zéatine en cultivant les «scalps» méristématiques prélevés sur les cultures des bourgeons apicales en prolifération. La régénération en plantes à partir des cultures de suspensions a été réalisée en passant par une séquence à quatre étapes impliquant des milieux déterminés différents. Des globules embryogéniques se sont formés lorsqu'une suspension filtrée a été cultivée dans un milieu liquide sans régulateurs de croissance. L'addition de cytokinine a été essentielle par la suite pour la maturation et la régénération de ces globules et a permis la régénération de plantes à une fréquence de 10-14,5 p. 100. La régénération en plantes a suivi la voie de l'embryogénèse somatique, laquelle a montré, à tous les stades, une ressemblance morphologique et histologique remarquable avec l'embryogénèse zygotique chez une espèce de bananier sauvage, Les embryons somatiques ont été produits directement à partir de cellules en suspensions et non à travers un cal. Les embryons somatiques germés se sont établis sur sol avec succès.

La simplicité relative de ce protocole de culture cellulaire peut accroître la faisabilité des approches intégrées en biotechnologie dans les schémas de l'amélioration du bananier et bananier plantain.

INTRODUCTION

Banana and plantain (*Musa* spp) are important staple food crops in the humid and subhumid tropical regions of the world. Recent concerns over declining yields, due to the spread of virulent diseases such as black sigatoka, fusarial wilt, and the banana bunchy top virus, have resulted in increasing efforts to genetically improve the crop (Persley and De Langhe, 1987). But conventional breeding of cultivated *Musa* remains a difficult endeavour because of high sterility levels and polyploidy. Hence a number of plant tissue culture and molecular genetic techniques have great potential to overcome some of the factors limiting traditional approaches to banana and plantain improvement (Krikorian and Cronauer, 1984 ; Murfett and Clarke, 1987). Such procedures largely depend on successful regeneration of plants from cells or protoplasts. Prospective benefits that might accrue from the integration of biotechnologies into banana and plantain breeding programmes therefore require access to reliable cell culture protocols (Krikorian, 1987).

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Abbreviations : 2,4-D : 2,4-dichlorophenoxyacetic acid.
BAP : 6-benzylaminopurine.
MS : Murashige and Skoog (1962).
IAA : Indole-3-Acetic Acid.

Several authors described efforts to obtain somatic embryos of *Musa* either in vegetative tissue (Cronauer and Krikorian, 1983, 1986 ; Bakry and Rossignol, 1985 ; Jarret *et al.*, 1985 ; Banerjee *et al.*, 1987 ; Sannasgala *et al.*, 1987 a, b ; Sannasgala, 1989) or in floral and inflorescence parts (Srinivasa Rao *et al.*, 1982 ; Bakry *et al.*, 1985). Spherical callus masses and embryo-like structures were formed, but plant regerenation by typical somatic embryogenesis was not observed. In constrast, plants have been recovered from somatic embryogenesis that emerged on callus of immature zygotic embryos (Cronauer-Mitra and Krikorian, 1988 ; Escalant and Teisson, 1988, 1989). However, such procedure has pratical limitations in breeding schemes as it is amenable only to wild species of *Musa* and not to the economically important cultivars, in which botanical seed production is erratic.

Reports on cell suspension cultures of *Musa* are few. Moham Ram and Steward (1964) initiated slow growing cell suspensions from callus of immature banana fruit. Cultures of palisade cells, isolated enzymatically from mature banana leaves, were obtained by Cronauer and Krikorian (1986). In these two cases, cell cultures proved to be non-morphogenic. More recently, the establishment of morphogenetically competent cell cultures has been reported by two groups. Teisson (1989) observed proembryos in suspensions initiated from embryogenic callus of immature zygotic embryos of wild *Musa* species, but plants were not yet recovered ; Novak *et al.*, (1989) produced cell suspensions from embryogenic callus of explanted leaf and rhizome tissue in diploid and triploid banana cultivars. Plants were recovered from somatic embryos at rates of 1.5-12%, but their protocol entails the passage on seven different culture media.

A common feature of procedures for cell suspensions reported so far is that these were initiated from callus, which has the obvious disadvantages in applications that require clonal uniformity. This illustrates the need for the development of culture procedures in which somatic embryogenesis is achieved in a way that is as direct and simple as possible. To further increase the efficiency of plant recovery from somatic cells in suspension, the embryogenic pathway of regeneration needs detailed morphological and histological examination and comparison with zygotic embryogenesis. This will eventually result in an enhanced understanding of the parameters that mark the occurrence of genuine somatic embryogenesis.

This report aims to contribute to the knowledge of cell suspension culture of cultivated *Musa*, and the regeneration of plants via somatic embryogenesis therein. A relatively simple protocol for the initiation of embryogenic suspensions and subsequent plant recovery is described. Detailed morphological and histological evidence of somatic embryogenesis is presented and substantiated by a comparison with some typical stages of zygotic embriogeny in a wild *Musa* species.

MATERIALS AND METHODS

Plant material.

The cooking banana cultivar 'Bluggoe' (*Musa* spp, ABB group) was chosen as experimental genotype. We obtained this cultivar from the collection of the Philippine Council for Agriculture and Resources Research and Development (PCARRD), Los Baños, The Philippines, under the name 'Matavia' (Valmayor *et al.*, 1981). 'Matavia' is identical to the widespread and well-known cultivar 'Bluggoe', which has been described by Simmonds (1966).

Explants.

The cultivar 'Bluggoe' is a genotype that demonstrates continuous, very high proliferation in shoot-tip culture, with cultures appearing as compact clumps of meristematic buds (Banerjee and De Langhe, 1985 ; Vuylsteke and De Langhe, 1985 ; Banerjee *et al.*, 1986). Explants (4-5 mm in diameter), which we termed as «scalps» were carefully excised from the uppermost part of these clumps using a dissecting microscope.

Initiation and maintenance of cell suspension cultures.

Five «scalps» were transferred into 20 ml of liquid MS medium (Murashige and Skoog, 1962), but with the following modifications : half strength macroelements and iron, 0.4 mg. l^{-1} thiamine, 10 mg.l^{-1} ascorbic acid, 5 μM 2,4-D, 1 μM zeatin, and without myo-inositol. Cultures were kept in 100 ml erlenmeyer flasks on a rotary shaker at about 70 rpm and at 26 ± 2°C under continuous light of 2,57 W.m^{-2} (± 40%) irradiance provided by 36 W Osram cool-white fluorescent tubes.

Cultures were refreshed with the same medium after 8 weeks. Subsequently, subcultures were done every 2-3 weeks by decanting the old medium and diluting the suspension two to three times with the same maintenance medium (Figure 1). As soon small embryogenic cell clusters appeared in the suspension, scalps were removed by sieving (0.5 mm). The embryogenic suspensions were maintained in the medium used for their initiation. The medium was routinely refreshed every 3-4 weeks (dilution by two or three times). The viability of cells in suspension was estimated by fluorescein diacetate (FDA) test (Widholm, 1972).

Plant regeneration from suspensions.

Plant regeneration from cell suspensions proceeded in four steps (Figure 1).

Step 1 : Mass formation of embryogenic globules. An established cell suspension culture was passed through a 100 μm sieve. Aliquots (1 ml) of cell suspension were inoculated into a liquid medium of the same basic salt composition as the initiation and maintenance medium, but with the addition of 100 mg.l^{-1} myo-inositol and devoid of plant growth regulators. These cultures were maintained for four weeks.

Step 2 : Maturation of globules.
Subsequently, the globules produced in step 1 were transferred into a maturation medium, consisting of the previous medium, but supplemented with cytokinin. BAP as well as zeatin at concentrations of 1 μM and 10 μM were tested. This stage lasted two weeks.

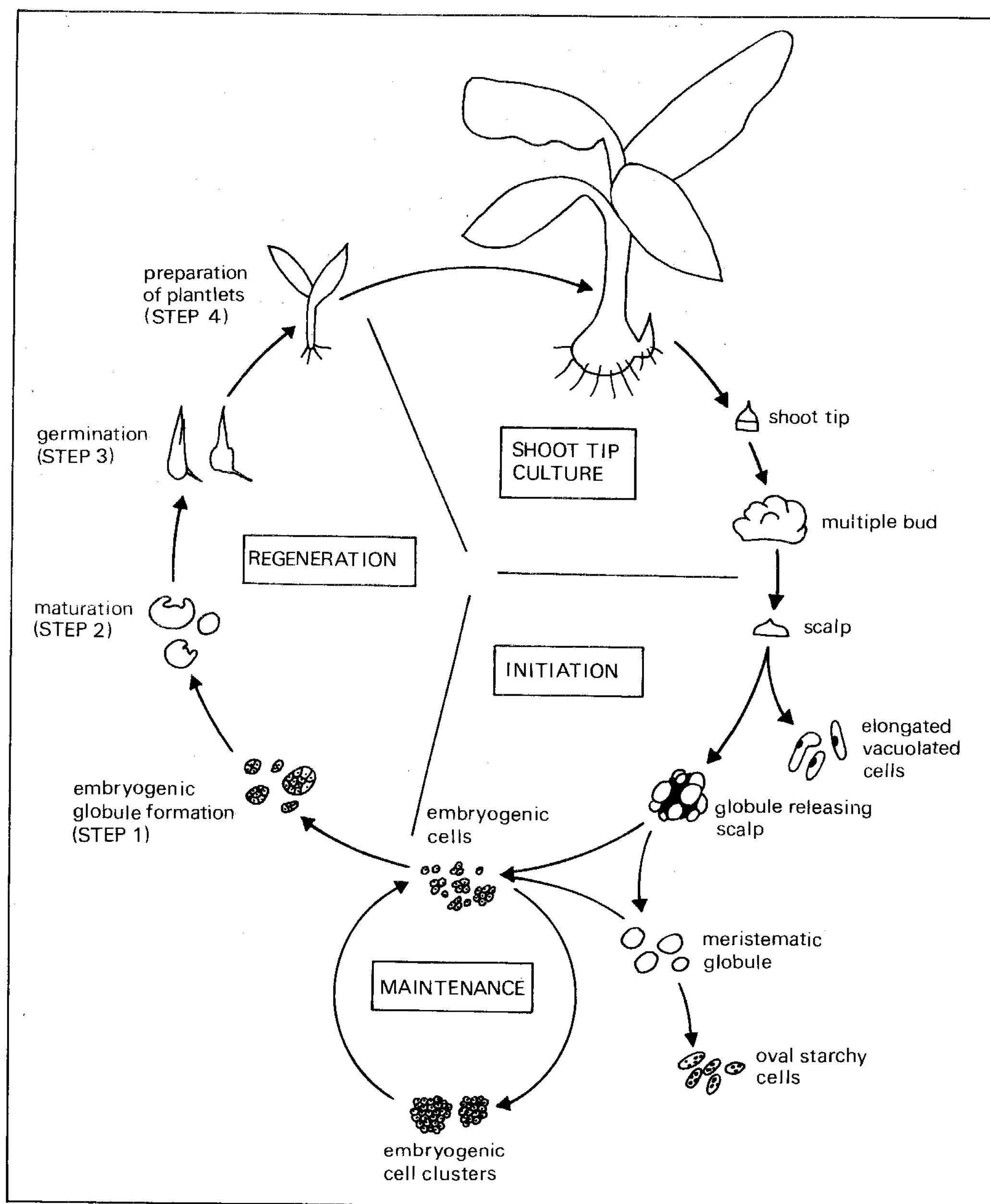

FIGURE 1 - Diagrammatic representation of *Musa* cell suspension cultures and the regeneration of plants therefrom.

Step 3 : Germination of globules.
Germination of the embryogenic globules was achieved both in liquid and in semi-solid medium, the latter, following sieving (1 mm) of the suspension from step 2. These germination media were both identical in composition to the maturation medium, but the type (BAP or zeatin) and concentration (1 μM or 10 μM) of the cytokinin varied. The semi-solid medium included the gelling agent 'gelrite' at 2 $g.l^{-1}$.

Step 4 : Preparation of plantlets for the nursery.
The plantlets developing in step 3 were transferred to semi-solid medium without growth regulators for further growth of roots and shoots. After about 2 months, plantlets were moved to a medium with 1% sucrose one month before transplantation to soil.

Regenerating cultures were always maintained under light and temperature conditions identical to those for the initiation of suspensions. Liquid cultures were kept on a rotary shaker.

Microscopy.

Morphological and histological studies of morphogenetic events were performed by sampling all stages of culture. Suspension cultures were observed either directly under the microscope or specimens were fixed in FAA (formaldehyde-glacial acetic acid - 70% alcohol - 5:5:90 v/v) embedded in paraffin, serially sectioned at 9 μm, and stained with safranin and fast green (Berlyn and Mikscche, 1976). For detailed histological observations,

TABLE 1 - Type of medium and time requirements for *Musa* embryogenic cell suspension culture and plant regeneration therefrom using shoot-tip as explant.

Steps		Medium	Time
Shoot tip culture		MS (semi-solid) + 1 μM IAA + 10 μM BAP	2-3 months (3-4 transfers)
Initiation		1/2 MS (liquid) + 5 μM 2,4-D + 1 μM zeatin	4-6 months (2-3 week interval refreshments)
Maintenance		1/2 MS (liquid) + 5 μM 2,4-D + 1 μM zeatin	not relevant * (3-4 week interval refreshments)
Regeneration	step 1	1/2 MS (liquid) + 100 mg.l^{-1} myoinositol	3-4 weeks
	step 2	1/2 MS (liquid) + 100 mg.l^{-1} myoinositol + 1-10 μM BAP or zeatin	2 weeks
	step 3	1/2 MS (liquid or semi-solid) + 100 mg.l^{-1} myoinositol + 10 μM BAP or zeatin	4 weeks
	step 4	1/2 MS (semi-solid)	2 months

* - The duration of the maintenance phase has, until now (2 years), no influence on plant regeneration potential.

specimens were fixed in 3% glutaraldehyde in 0.06 M phosphate buffer (pH 6.8), embedded in LKB 2218-500 historesin, serially sectioned at 3 μm with glass knives, and stained with PAS (Periodic Acid Schiff base), for staining of starch and cell wall material, and Regaud's hematoxylin for protein staining (McManus, 1946 ; Fisher, 1968). Sections were viewed under a Leitz Dialux 20 microscope. Material for scanning electron microscopy was prepared by fixing in 50% glutaraldehyde in 0.14 M cacodylate buffer (pH 6.8). Fixed specimens were dehydrated in a graded ethanol series, critical point dried, coated with gold, and viewed on a Philips PSEM 500 scanning electron microscope at 25KV.

RESULTS AND DISCUSSION

A scheme of the protocol for the initiation of cell suspensions, and the regeneration of banana plants therefrom, is given in Figure 1.

Initiation and maintenance of cell suspensions.

Explants inoculated responded by loosing their organization as multiple buds, as well as their epidermis (Sannasgala, 1989). Four to five weeks later, globular structures appeared on the surface of the explants and were released into the medium together with loose, single cells. Explanted scalps consist of non meristematic and meristematic tissue (Banerjee *et al.*, 1986 ; Sannasgala *et al.*, 1987 a ; Sannasgala, 1989). The elongated, highly vacuolated cells which appeared first in the medium apparently originated from the non-meristematic tissue. However, the oval cells, containing large starch granules, originated from the meristematic globules which were released earlier by the scalps (Figure 1). Microscopic examination of sections through these meristematic globules showed that their surface consisted of loose cells filled with starch granules.

After 16-24 weeks on initiation medium, clusters of small, tightly packed cells with a dense cytoplasm appeared in the medium (Figure 2A). These cells (Figure 2B) are round and characterized by a relatively large nucleus, very dense nucleolus, small multiple vacuoles, and tiny starch and protein grains. They fluoresced brightly with FDA under an ultraviolet microscope. These are qualities generally associated with morphogenetic competence and, in addition to their resemblance to embryogenic cell suspensions of other plant species, suggests that these cell aggregates are embryogenic masses (McWilliam *et al.*, 1974 ; Vasil and Vasil, 1981, 1982 ; Williams and Maheswaran, 1986 ; De Vries *et al.*, 1988). The actively dividing, embryogenic cells probably derived from meristematic cells in the perivascular region of the scalps or in the meristematic globules (Figure 1). Dense cytoplasm cells in division were also observed by Bakry and Rossignol (1985) in the perivascular parenchyma during the callogenesis of banana leaves. Cells with embryogenic characteristics were also observed in the perivascular tissue of scalps by Sannasgala (1989). The embryogenic cell aggregates sometimes developed into large masses necessitating sieving (1 mm) of the suspension before further subculturing.

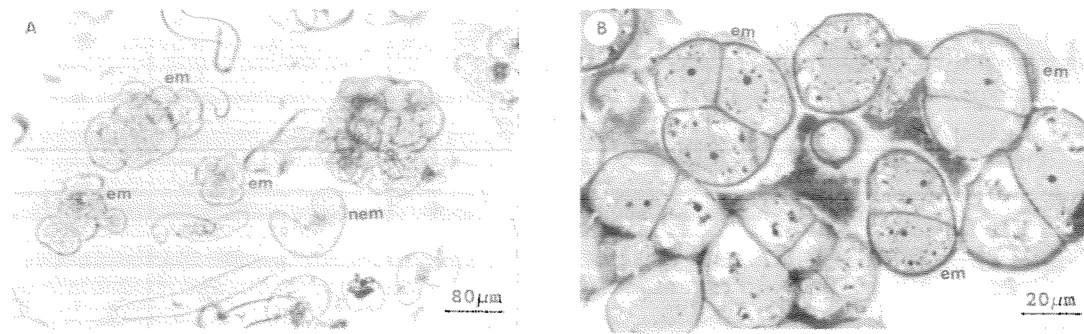

FIGURE 2 - Embryogenic cell suspension culture of *Musa* (ABB) cv. 'Bluggoe'.
A - Suspension of embryogenic cell clusters (em) and non-embryogenic cells (nem).
B - Close-up of a section of embryogenic cell clusters (em).

Plant regeneration from cell suspensions.

In the first step of plant regeneration, globular structures formed in great numbers after 3-4 weeks. The events leading to the formation of embryogenic globules from single cells in suspension were investigated microscopically in fine histological sections of a sieved (1 mm) globular suspension and are illustrated in Figure 3 (A and B). Consecutive divisions in the somatic cells result in the formation of small globules, which resemble the early proembryonal stage of zygotic embryogenesis in *Musa velutina* (Figure 3C). Then, an epidermal layer, completely surrounding the spherical proembryo, is formed both in somatic (Figure 3B) and zygotic (Figure 3D) embryogenesis. The formation of a continuous epidermal layer is considered as an important step in embryogenesis (Escalant and Teisson, 1988 ; Schwendiman *et al.*, 1988). Individual cells in the globules at these stages (Figure 3) still maintain the embryogenic qualities described above.

In the second step (maturation), and within one week of transfer to liquid medium now supplemented with BAP or zeatin (1 or 10 μM), the embryogenic globules proliferated, became transluscent with an opaque centre, and invaginated in a circular fashion at one end of the globular structure. Such whitish, invaginated globules (Figure 4A) closely match the somatic embryos of *M. acuminata* and *M. balbisiana* as described by Escalant and Teisson (1988).

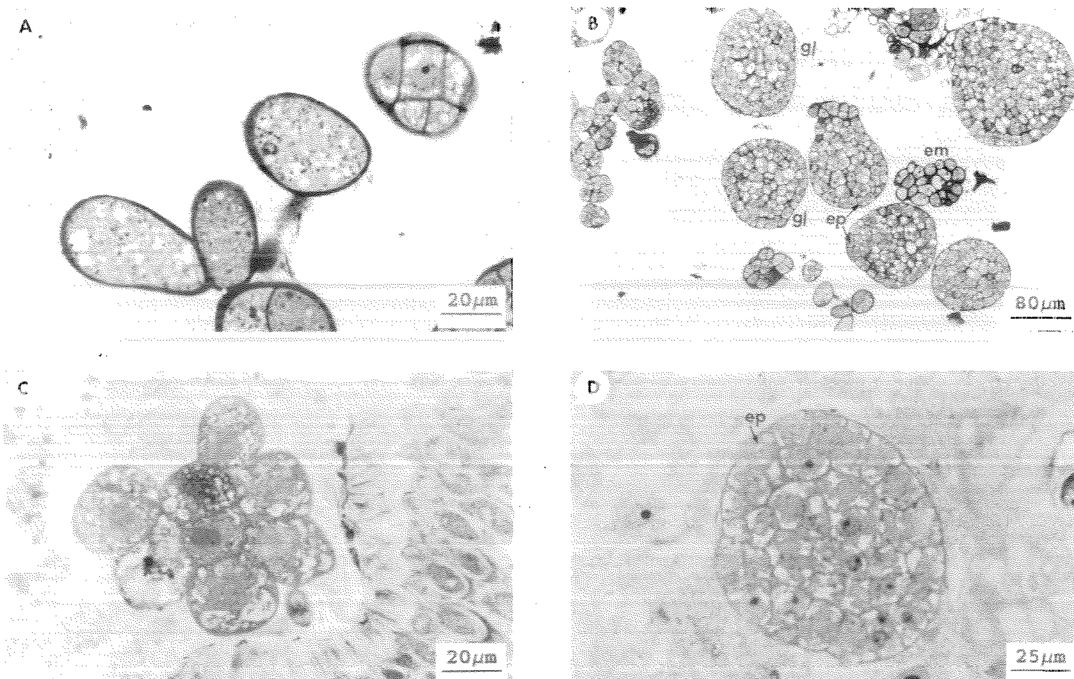

FIGURE 3 - Globule formation during somatic embryogenesis in *Musa* (ABB) cv. 'Bluggoe' (A-B) and zygotic embryogenesis in *Musa velutina* (C-D).
A - Dividing single cells and B - their subsequent development into embryogenic globules.
C - early proembryonal stage (2 weeks old) and D - globular stage (4 weeks old) during zygotic embryogenesis in *Musa velutina.*
gl : globule ; ep : epidermis ; em : embryogenic cluster.

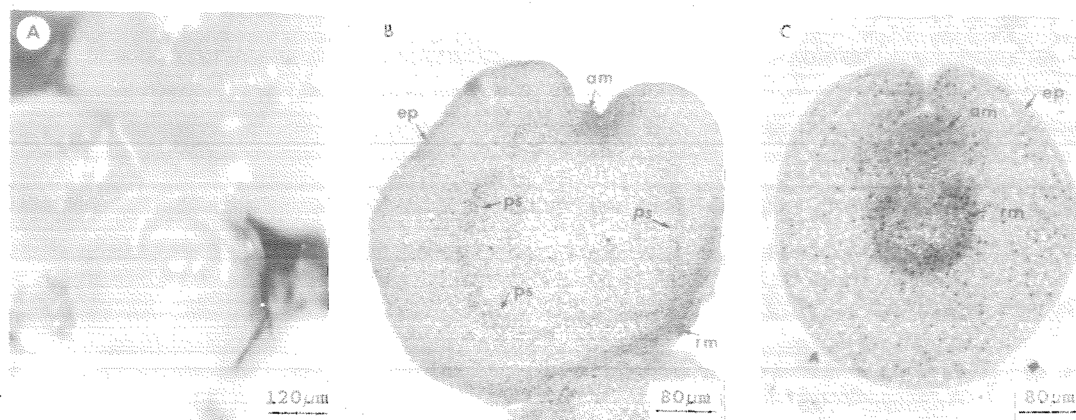

FIGURE 4 - Maturation of somatic embryos in cell suspension cultures of *Musa* (ABB) cv. 'Bluggoe' (compared with a zygotic embryo of *Musa velutina*.

A - Globules manifesting a circular invagination to form a cotyledonary slit (viewed under stereomicroscope).

B - section through an invaginated globule.

C - section of a zygotic embryo of *M. velutina* at a similar stage (5 weeks old).

am : apical meristem ; rm : root meristem ; ps : procambium strands ; ep : epidermis.

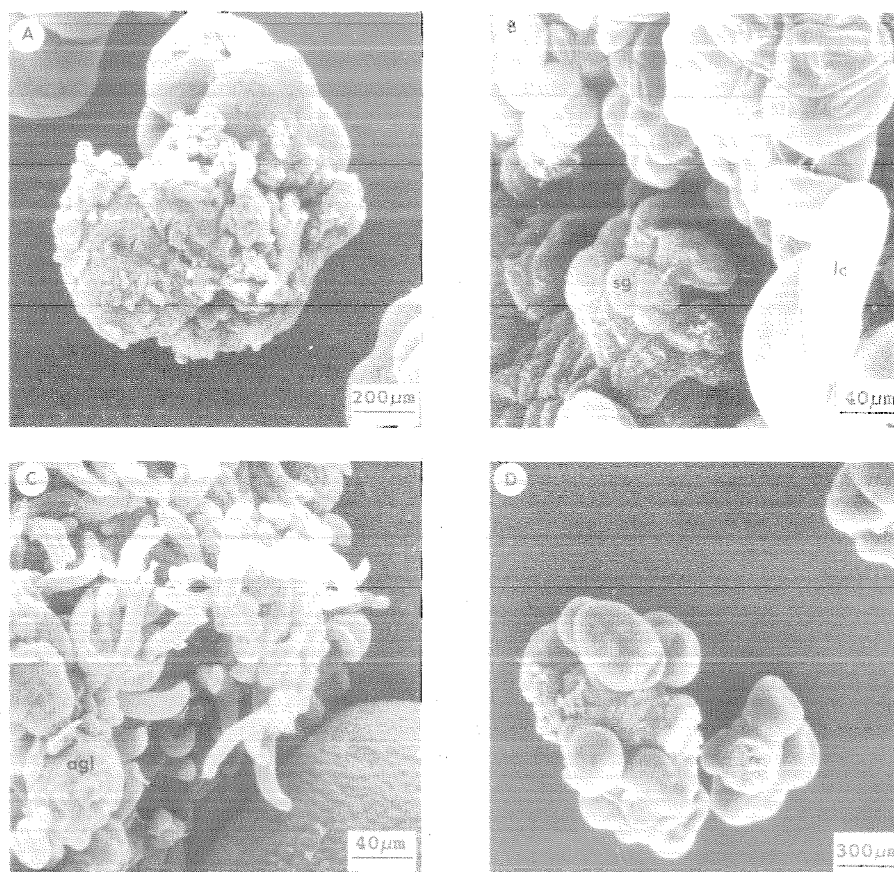

FIGURE 5 - Scanning electron micrograph (SEM) of adventitious somatic embryo development from the surface of a globule during maturation.

A - disorganization of an embryogenic globule with B - close-up of disorganized surface exhibiting small groups of cells (eg) and loose cells (lc).

C - adventitious globule formation (agl) from small groups of cells.

D - cluster of adventitious somatic embryos on dedifferentiated embryogenic globule.

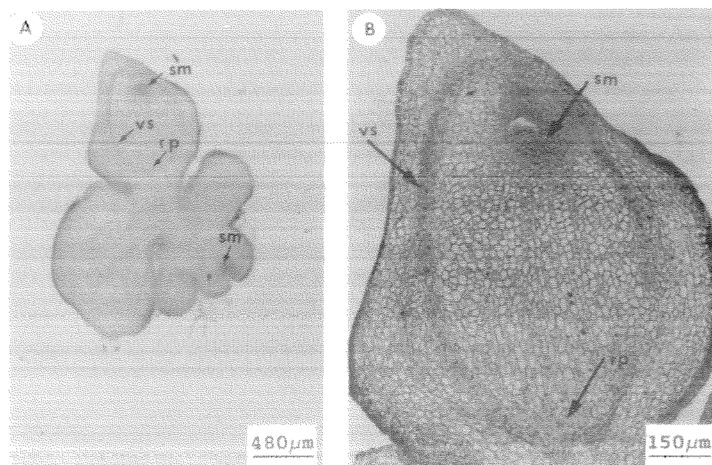

FIGURE 6 - Longitudinal section through a cluster of adventitious somatic embryos of *Musa* (ABB) cv. 'Bluggoe'.
A - cluster of 5 adventitious somatic embryos and B - close up of a section of an adventitious somatic embryo.
sm : shoot meristem ; rp : root pole ; vs : vascular strands.

Thin sections through an invaginated globule show the cotyledonary slit with the apical meristematic zone, corresponding to the invaginated side, and the closed procambium strands connecting this apical zone with the root meristem (Figure 4B). The cotyledonary stage of zygotic embryogenesis in *M. velutina*, in which invagination and two mersitematic zones are readily discernible, is shown in Figure 4C. The definite histological similarities of somatic and zygotic specimens (Figures 3-4) indicate that the globular masses originating from cell suspensions are the product of somatic embryogenesis. Most importantly, somatic embryos were produced directly from cells in suspension, i.e. without an transitional callus phase.

The formation of adventive somatic embryos by budding on isolated globules was frequently observed in suspensions undergoing steps 1 and 2 of regeneration. Scanning electron micrographs (Figure 5A-D) illustrate this process. Initially, the epidermis become disorganized (Figure 5A,B). Subsequently, cell clumps sprout from the surface, resulting in the formation of multiple, adventive globules (Figure 5C,D) which also show the characteristic invagination at one pole. Sections of such clusters of somatic embryos (Figure 6 A,B) show the involving epidermis and bipolar structure of single units, with the shoot and root pole connected by vascular elements. Haccius (1978) considered such closed vascular connection between the shoot and root apex as the most distinctive character of an embryo.

If step 2 is prolonged, then germination in liquid medium is observed after 3-4 weeks (step 3). Alternatively, germination on semi-solid medium may also be obtained providing globules remained for 2 weeks in the liquid maturation medium before sieving (Figure 7A,B). After somatic embryos have developed into plantlets, the cytokinin-containing medium was replaced by half strength MS semi-solid medium without myo-inositol and devoid of growth regulators to stimulate further plantlet development (step 4 ; Figure 7C). Figure 8 (A,B) highlights the development of the plumule which is the main morphogenetic event ocurring during somatic embryo germination. Histological sections illustrate the presence of the shoot and root apex and the central vascularization (Figure 8 C, D). The regenerated plantlets developed and grew normally after field transplantation.

The cytokinin was essential for the maturation and germination of banana somatic embryos (Figure 7A). This was demonstrated by the effect of two concentrations (1 μM and 10 μM) of cytokinin (BAP and zeatin) (detailed results not shown). Globules that matured in the control medium without cytokinin gave none to poor regeneration. The addition of 1 μM zeatin to the maturation medium resulted in the highest frequency of subsequent recovery. In contrast, zeatin at 10 μM gave rise to many abnormalities, such as plants with a calloid root pole or multiple shoots on a calloid base. Again, in the germination medium, zeatin was slightly superior to BAP. Of all combinations tested, the best results in normal plant recovery from somatic embryos (10-11%) were obtained with 1 μM zeatin for the maturation medium followed by 10 μM BAP or zeatin in the semi-solid germination medium.

Whenever a liquid medium is used for the germination (step 3), higher cytokinin levels also stimulated the transformation of somatic embryos into plantlets (Figure 9). Cytokinin at 10 μM produced 7-14.5% normal plants against 2-3% at 1 μM. At 10 μM concentration, the response to BAP was better than to zeatin. Abnormal plantlets were always produced, but particularly so in medium with high cytokinin levels. Nevertheless, the ratio of normal/abnormal plantlets with 10 M BAP was the best.

Novak *et al.* (1989) similarly reported that the passage through a zeatin-containing medium was an essential step for the production of somatic embryos from cell suspension of *Musa*. The role of cytokinin in the development of cotyledons and somatic embryo maturation has been discussed by Rashid (1988). BAP has been listed as one of the major factors that promote somatic embryo formation, especially in direct somatic embryogenesis (Williams and

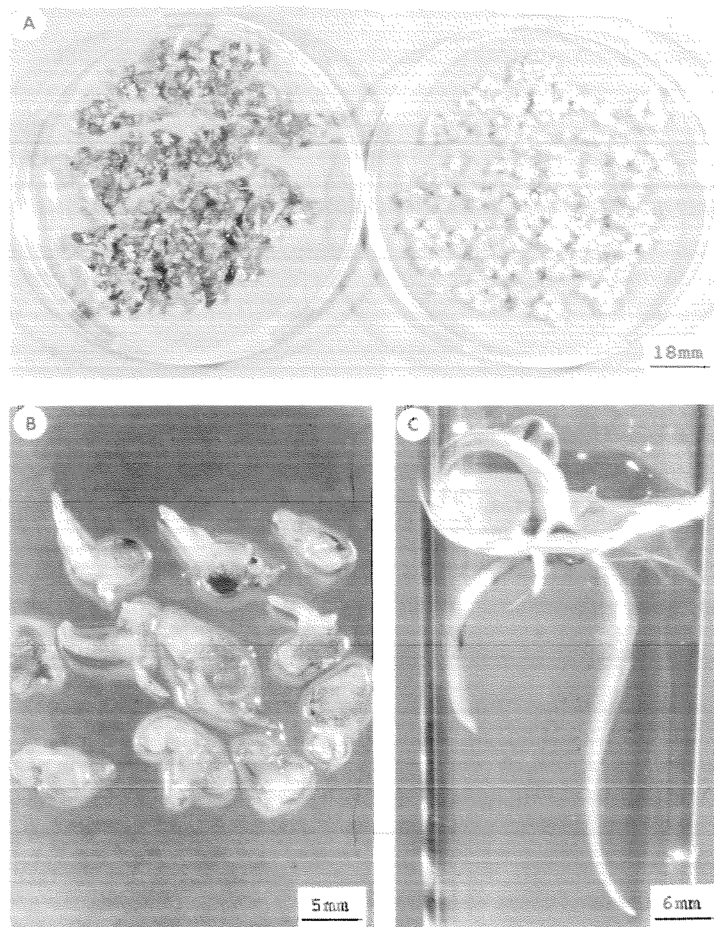

FIGURE 7 - Plant production from cell suspensions of *Musa* (ABB) cv. 'Bluggoe'.
A - effect of cytokinin on somatic embryo germination on semi-solid medium with (left) and without (right) cytokinin.
B - plantlet development clearly exhibiting shoot and root primordia.
C - plantlet development on semi-solid medium without growth regulators.

Maheswaran, 1986).

The regeneration frequencies of normal plantlets reported here, i.e., 10-14% in the best treatments, are higher than those (1.5-12%) reported by Novak *et al.* (1989). It is important to stress that, in our case, plantlets from abnormal somatic embryos could also grow into normal plants, so that the total plant recovery frequency could reach the maximum of 23.1%.

CONCLUSION

Embryogenically competent cell suspensions of banana were readily established by culturing meristematic scalps in liquid medium containing 2,4-D and zeatin. Scalps seem to be an excellent explant source for this purpose as they are easily available from *in vitro* proliferating shoot-tip cultures. Besides, a culture procedure starting from vegetative explant material, unlike one based on zygotic embryos, should be reproducible on many *Musa* genotypes, which is presently under investigation.

Plant regeneration from suspension cultures occurred by somatic embryogenesis, the pattern of which showed striking resemblance with zygotic embryogenesis in a wild banana. Somatic embryos were produced directly from cells in suspension and not via callus. Such procedure is likely to be of great benefit in applications requiring high levels of genetic stability, such as *in vitro* germplasm management and enhancement. The clonal uniformity of plants recovered from cell suspensions is currently investigated by monitoring a population of field established plants.

A sequence of five defined media, one for suspension culture initiation and four for complete plant recovery, was necessary to achieve a high rate of regeneration from cell suspensions. This protocol is simpler than that described by Novak *et al.* (1989), in which the alternation between Murashige and Skoog (1962) and Schenk and Hildebrandt (1972) media, the use of a complex culture support system, and a sequence of seven media are required for regeneration.

Cytokinin was essential for the successful maturation

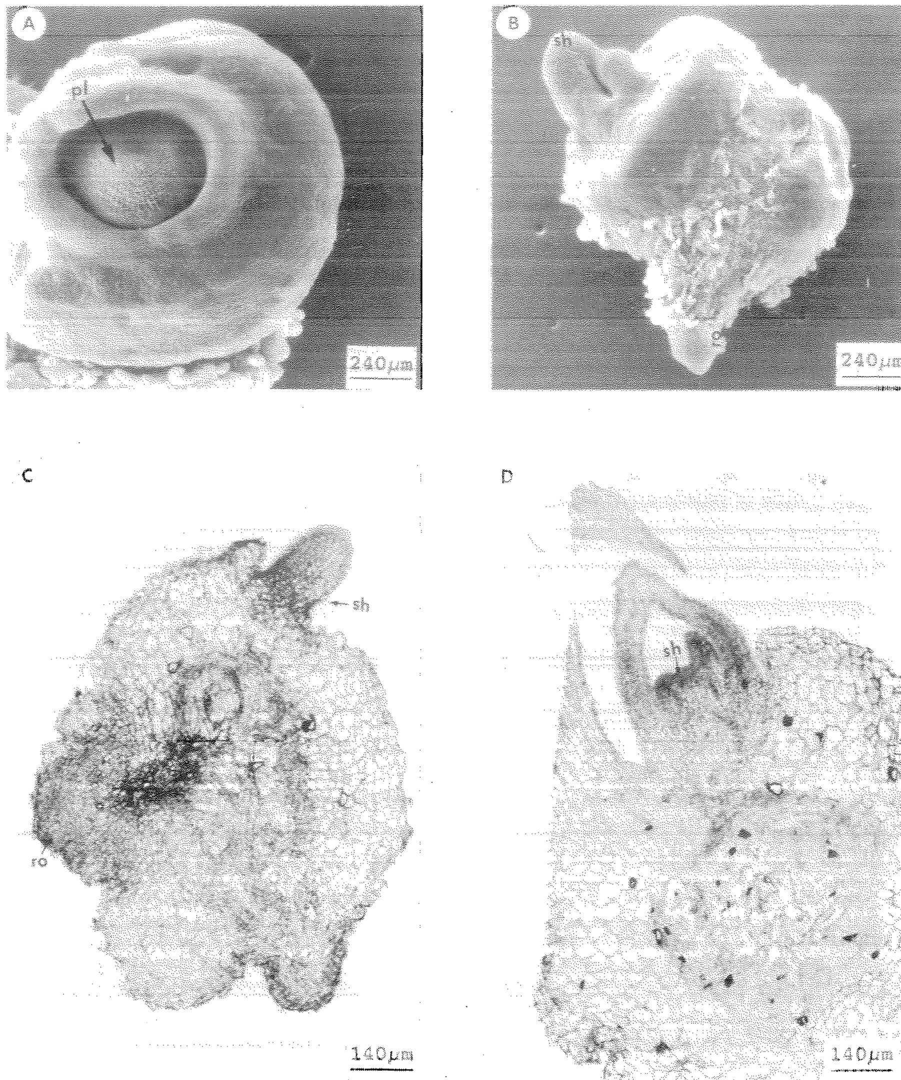

FIGURE 8 - Germination of somatic embryos in suspension cultures of *Musa* (ABB) cv. 'Bluggoe'.
A - scanning electron micrograph (SEM) showing the plumule (pl) at an early stage of germination and B - shoot (sh) and root (ro) outgrowth at a later stage.
C and D - longitudinal section through a germinating somatic embryo showing shoot and root pole and the central vascularization.

and germination of somatic embryos. This resulted in 10-14.5% plant recovery from embryogenic suspensions, which is the highest frequency so far reported in the genus *Musa*.

The simple methodology of recovering of somatic embryos from cell suspensions makes this culture procedure attractive for mass cloning, for *in vitro* selection schemes to evaluate with pathogens or elicitors, and for plant transformation studies. Moreover, embryogenic suspensions seem to be the material of choice in the cryopreservation of *Musa* germplasm (Panis *et al.*, 1990).

ACKNOWLEDGEMENT

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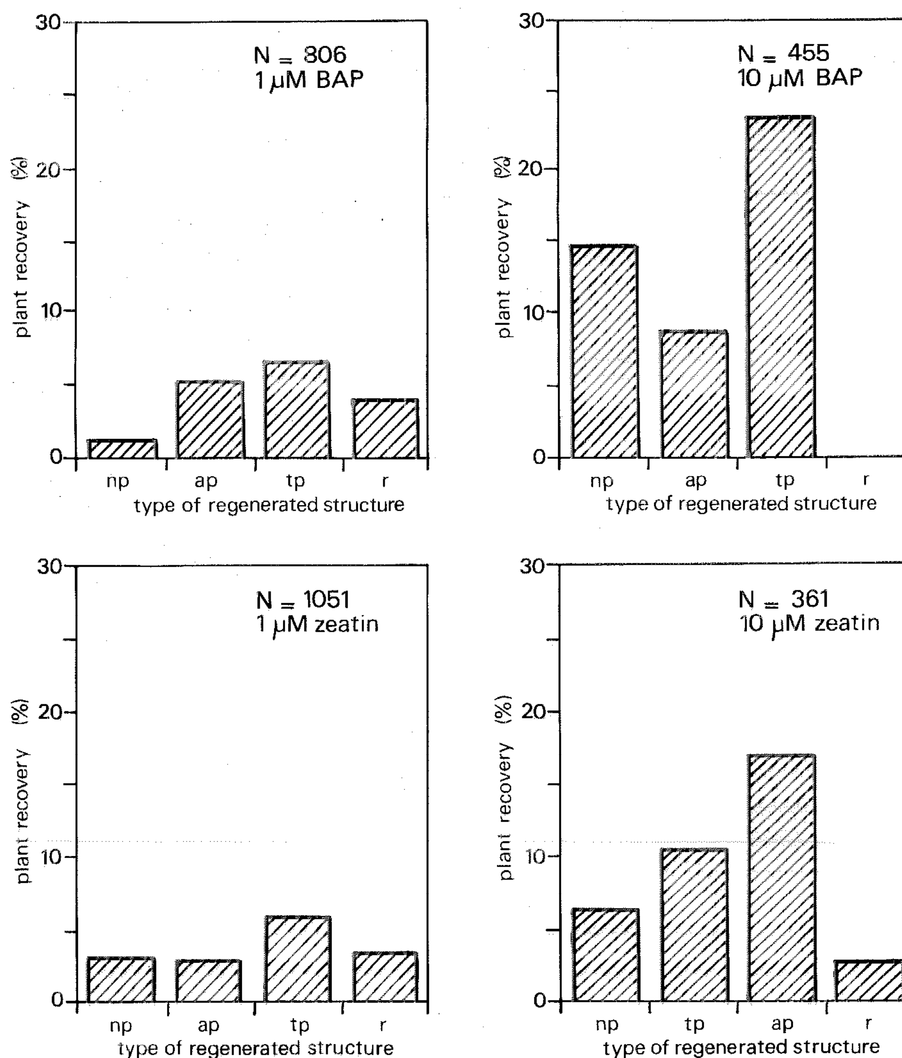

FIGURE 9 - Frequency of plant recovery from somatic embryos of *Musa* (ABB) cv. 'Bluggoe' cultured in liquid germination medium supplemented with cytokinin.
np . normal plantlets ; ap : abnormal plantlets ; tp : total plantlets (tp=np+ ap) ; r : root ; N : total number of structures in 40 ml of medium. Observations at 3 weeks.

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REGENERACION DE PLANTAS EN CULTIVO DE SUSPENSIONES CELULARES EN EL PLATANO CV. «BLUGGOE» (*MUSA* SPP. GRUPO ABB).

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RESUMEN - Cultivos de suspensiones celulares en un clon bastante conocido del plátano «Bluggoe» (*Musa* spp., grupo ABB) fueron establecidos en un medio MS modificado conteniendo 5 µM 2,4-D y 1 µM zeatina cultivando los «scalps» meristemáticos tomados de los cultivos de yemas apicales en proliferación. La regeneración en plantas a partir de cultivos de suspensiones ha sido realizada pasando por una secuencia de cuatro etapas que implica medios específicos diferentes. Glóbulos embriogénicos son formados luego que una suspensión filtrada ha sido cultivada en un medio líquido sin reguladores de crecimiento. La adición de citroquinina fué esencial para continuar con la maduración y regeneración de esos glóbulos, y dió una regeneración de plantas con una frecuencia de 10-14,5 p. 100. La regeneración en plantas ha seguido la vía de la embriogénesis somática. la cual mostró, en todos los estados, una similitud morfológica e histológica notable con la embriogénesis zigótica en una especie de banano salvaje. Los embriones somáticos fueron producidos directamente a partir de células en suspensión y no a través de un callo. Los embriones somáticos germinados se establecieron en suelo con éxito.
La simplicidad relativa de este protocole de cultivo celular puede ampliar la factibilidad de los enfoques integrados en biotecnología dentro de los esquemas de mejoramiento del banano y del plátano.