Highly efficient somatic embryogenesis and plant regeneration *via* suspension cultures of banana (*Musa* spp.)

(Received: 05.09.2003; Accepted: 07.10.2003)

Said M. Khalil*, and A.A.M. Elbanna ****
*Agricultural Genetic Engineering Research Institute (AGERI), ARC, 12619, Giza, Egypt
** Tissue Culture Lab., El Zoherya Garden, HSU, ARC, Zamalek, 11211, Cairo, Egypt

ABSTRACT

A protocol has been developed for the high efficient regeneration of the banana cultivar Dwarf Brazilian (Musa spp. AAB group) via cell suspension. Primary somatic embryos were produced when explants of immature male flower buds were cultured on Murashige and Skoog (MS) medium plus 1 mg/l biotin, 100 mg/l malt extract, 100 mg/l glutamine, 4 mg/l 2,4-dichlorophenoxyacetic acid, 1 mg/l indole-3-acetic acid (IAA), 1 mg/l . -naphthaleneacetic acid, 30 g/l sucrose and 2.6 g/l Phytagel, pH 5.8 (M1 medium) and then transferred to M1 medium plus 200 mg/l casein hydrolysate and 2 mg/l proline. Suspension cultures were initiated from embryogenic tissues placed in liquid medium supplemented with 2,4-D (1mg/l), biotin (1 mg/l), L-glutamate (100 mg/l), malt extract (100 mg/l), and sucrose (45 g/l), the pH of the medium was adjusted to 5.3. The packed cell volume (PCV) of the suspension increased 2-5 fold with each monthly cycle. The somatic embryos were developed when suspension culture aspirated on MS medium supplemented with biotin (1 mg/l), malt extract (100 mg/l), Glutamine (100mg/l), NAA (1mg/l), Kinetin (0.5 mg/l) Zeatin (0.2 mg/l), sucrose (45 g/l), and phytagel (2.6 g/l). Differentiated embryos were transferred to MS medium supplemented with 5 mg/l 6-benzylaminopurine (BA) for development of the mature somatic embryos, which were isolated and cultured on hormone-free MS medium for germination and development into plantlets. Approximately 90% of the somatic embryogenesis germinated and developed into plantlets, and these were subcultured onto MS medium plus 0.1% activated charcoal and 1 mg/l IAA. Approximately 900-1050 plants were obtained from initial starting material (regeneration 90%) of 0.5 ml PCV suspension culture in 4-5 months. Morphologically normal banana plants were developed from all regenerated plants. Somatic embryogenesis via cell suspension might be an excellent technique for mass production, developing a breeding strategy and genetic transformation of banana.

Key words: *In vitro, plant tissue culture, regeneration, somatic embryogenesis, cell suspension, banana.*

Abbreviations *MS*: Murashige and Skoog, medium (1962), *BA*: 6-benzylaminopurine, 2,4-D; dihlorophenoxyacetic acid , *IAA*: indole-3-acetic acid, *NAA*: -naphtaleneacetic acid, *SE*:somatic embryos, PCV: packed cell volume.

INTRODUCTION

Banana (*Musa* spp.) is one of the most important tropical fruits in the world trade. It is a staple food for nearly 400 million people (Haicour *et al.*, 1998). In many countries, banana and plantain represent the major fruit exports and are essential sources of income for national economies, with world production in 2000 estimated at 64.6 million metric tons (Anonymous, 2001). The production is limited primarily by black Sigatoka (*Mycosphaerella fugiensis*), Fusarium wilt (*Fusarium oxysporum* var. cubense), viruses, and nematodes (Radopholus similes) (Sasson, 1997). Banana bunchy top disease (BBTD) caused by banana bunchy top nanovirus (BBTV) and banana CMV disease caused by banana-cucumber mosaic cucumovirus (Banana-CMV), have enormous negative impacts on banana productivity Costs for control of this fungal disease alone average between $ 0.30 and $1.0 per plant in banana plantations in order to avoid yield losses of 30-50% (Sagi *et al.*, 1995). In the last 70 years the application of classical methods of breeding for disease resistance has resulted in only limited success, due primarily to long generation period of times for banana, high sterility, and triploidy of most cultivated bananas. The integration of genetic engineering into breeding programs may provide powerful tools to overcome these limitations by inducing specific genetic changes that can be utilized for banana improvement within a short period of time. However, these applications require reliable plant regeneration protocols for banana (Fitchen and Beachy, 1993 and Vuylsteke and Swennen, 1992).

In vitro culture of banana has been extensively used to quickly propagate vegetative clones of many genotypes (Vuylsteke and De Langhe, 1985; Das *et al.*, 1998). Many obstacles remain to be overcome before an efficient banana regeneration protocol suitable for genetic transformation is developed. Plants regenerated through organogenesis are not appropriate for genetic transformation since many chimeric plants are produced. Somatic embryogenesis, the process whereby either a single somatic cell or clusters of cells develop into embryos, is a useful approach for *in vitro* plant regeneration of many species. This technique in the genus *Musa* aimed to the following the development of high-performance micropropagation techniques and plant regeneration systems useful for genetic improvement.

Somatic embryogenesis in *Musa* using cell suspension, has been the subject of research since the 1960s (Novak 1992; Krikorian and Scott, 1995). On the basis of differences in explant types, four main methods have been developed. Novak *et al.*, (1989) used the bases of leafs heaths or rhizome fragments of plants produced *in vitro*. Dhed'a *et al.* (1991) started from thin sections of a highly proliferation and culture placed in a liquid medium. Marroquin *et al.* (1993) established embryogenic suspension from immature zygotic embryos; in this case, the nature of the explants limited the method. Escalant *et al.*, (1994), Cote *et al.* (1996), and Navarro *et al.*, (1997) used young male flowers as starting material for initiating suspension culture for Grand Nain. Somatic embryogenesis using embryogenic for cell suspension cultures have the potential to produce non-chimeric plants, but they are limited by low embryo germination [1.5–12%, Novak *et al.*, (1989); 10–23%, Dhed'a *et al.*, (1991); 3–20%, Côte *et al.* (1996)] and low plant regeneration rates [(13–25%) Navarro *et al.* (1997)] and long culture times from culture initiation to plant regeneration [16 months, Côte *et al.* (1996); 18 months, Becker *et al.* (2000); 10–11 months, Ganapathi *et al.*

(2001). Kosky *et al.* (2002) and Khalil *et al.* (2002). recently reported high germination rates (89.5%) by secondary somatic embryos for banana cv. Dwarf brazilian (AAB). Immature male inflorescences have been used to initiate cultures of several banana and plantain cultivars (Shii *et al.*, 1992; Escalant *et al* *et al.*, 1996; Navarro *et al.*, 1997; Sagi *et al.*, 1998; Becker *et al.*, 2000 and Khalil *et al.*, 2002, 2003).

We describe here an efficient and reproducible protocol with high frequency regeneration for somatic embryogenesis *via* cell suspension which mass production of plantlets and genetic transformation to improve *Musa* spp. AAB group cv. Dwarf Brazilian.

MATERIALS AND METHODS

Plant material and culture initiation

The initial plant material used was the "Dwarf Brazilian" cultivar of banana (*Musa* spp.) belonging to the AAB group. Male flower buds were collected from field grown one-month-old male flowers (about 1 foot in length). Cultures were initiated within 4 days after removing the male flowers from the mother plants. Tissues were sterilized by washing with 1 % (v/v) detergent solution for 5 min and the outer bracts were removed until inflorescence was 5 cm long which was surface sterilized by 100 % Clorox® for 15 min and finally rinsed 3 times with sterile water. In a sterile petri dish, the outer bracts were peeled with forceps until flower axis of 1.5 cm in length. The remaining flower bud was cut into 4 segments longitudinally (orthogonally) through the shoot tip, 2 mm each (transverse section). A total of 16-20 segments can easily be obtained from a single male flower.

Callus initiation

Immature male flowers explants were isolated and cultured on solid M1 medium consisting of macro- and micro-nutrients and vitamins of Murashige and Skoog, (1962) plus 1 mg/l biotin, 100 mg/l malt extract, 100 mg/l glutamine, 4 mg/l 2,4-D, 1 mg/l IAA, 1 mg/l NAA and 30 g/l sucrose with pH adjusted to 5.8. The cultures were incubated in the dark at 28 °C for two weeks. The flower bud segments became swollen 2 weeks after cultures were initiated in which they could be seen as whitish tissue protruding from the inflorescence tissues. They were carefully excised from the mother tissue, transferred to fresh M1 medium and kept in darkness. Primary somatic embryos were produced after tissues were transferred to MM1 modified medium (Khalil *et al.*, 2002), which composed (M1 medium with 200 mg/l casein hydrolyzate and 2 mg/l proline) for 2 months. Compact white calluses and friable embryogenic tissues with globular structures containing primary somatic embryos were formed.

Suspension cultures

Suspension culture was established from the friable embryogenic callus. Half gram from such callus was transferred to 10 ml of liquid medium designated M2 (Cote *et al.*, 1996) which is an MS based liquid medium supplemented with 2,4-D (1 mg/l), biotin (1 mg/l), L-glutamate (100 mg/l), malt extract (100 mg/l), and sucrose (45 g/l). The pH of the medium was adjusted to 5.3. The suspensions were cultured in 250-ml Erlenmeyer flasks on a reciprocal shaker at 100 rpm in darkness for 3 weeks until suspension cultures start to multiply. Culture medium was changed weekly during the first month of culture and subsequently biweekly. Pipetting 1 ml of mother stock to 20 ml of fresh M2 medium and changing medium every 7-10 days did regular maintenance of suspension cultures. Cultures were maintained at 28 °C under a photoperiod of 16 h.

Embryo development

The suspension cultures 180 µl after 7 days of subculture were saved to avoiding any cell aggregates. The 0.5 ml packed cell volume (PCV) of suspension culture was aspirated on sterile filter paper on four different media to determine the suitable medium for embryo development. The first medium SK4 designated (Khalil *et al.*, 2002) is MS micro and macro-elements, MS vitamins, supplemented with 10 % coconut water and 30 g/l sucrose. The second medium designated as SK12 contained MS salts, MS vitamins in addition to biotin (1 mg/l), L-glutamine (100 mg/l), malt extract (100 mg/l), BA (2.5 mg/l) gibberellic (GA3) (1mg/l), sucrose (45 g/l) and phytagel (2.6 g/l). The third medium designated as contained SK13, MS salts, MS vitamins, supplemented with biotin (1 mg/l), malt extract (100 mg/l), Glutamine (100mg/l), NAA (1mg/l), Kinetin (0.5 mg/l) Zeatin (0.2 mg/l), sucrose (45 g/l), and phytagel (2.6 g/l). The fourth medium designated M2 modified MS salts, MS vitamin, supplemented with biotin (1 mg/l), malt extract (100 mg/l), Glutamine (100mg/l), 2,4-D (1 mg/l), Zeatin (0.2 mg/l), sucrose (45 g/l), and phytagel (2.6 g/l). The pH of the four media was adjusted to 5.8 prior to autoclaving. Half of the cultures was incubated dark at 28 °C in the dark and the other half was incubated at 28 °C with photoperiod of 16 h light and 8 h to be study the effect of incubation conditions on embryo development.

Differentiation of embryos

Embryo development was achieved by culturing the embryonic suspension culture on SK4, SK12, SK13, and Modified M2 medium then transferred to either of the following two media to determine the best medium for embryo differentiation. The first medium designated SK8 (Khalil *et al.*, 2002), which contains MS salts, MS vitamin, BA (5 mg/l), sucrose (30 g/l), and phytagel (2.6 g/l). The second medium designated as MM4 is a modification of MM4 medium reported by Cote *et al.* (1996) containing half strength MS salts, MS vitamins, L-glutamin (100 mg/l), malt extract (100 mg/l), biotin (1 mg/l) BA (0.5 mg/l), gibberellic acid (GA3) (1 mg/l), sucrose (45 mg/l), and phytagel (2.6 g/l). The cultures were incubated at 28°C with a photoperiod of 16 h light and 8 h dark for 4 weeks.

Embryo germination and plantlet formation

Germination of embryos was performed on a medium designated as SK10, which consisted of MS salts with MS vitamins, 30g/l sucrose and no growth regulators. The embryos were germinated in petri dishes (diameter 9 cm). Embryos were maintained on SK10 medium for 15 days at 28 °C with a photoperiod of 16h light and 8 h dark. The plantlets were then transferred to SK11 medium (MS salts, MS vitamin, 1 g/l activated charcoal, 1 mg/l IAA and 30 g/l sucrose) for elongation at 28 °C with a photoperiod of 16 h light and 8 h dark. Mature plantlets 5-6 cm long were obtained after 2-3 weeks on SK11 and were ready for acclimatization.

RESULTS AND DISCUSSION

Culture initiation and callus development

Immature male flowers were isolated and cultured on MS medium supplemented with 1 mg/l biotin, 100 mg/l malt extract, 100 mg/l glutamine, 4 mg/l 2,4-D, 1 mg/l IAA, 1 mg/l NAA and 30g/l sucrose at pH 5.7. The flower bud segments became swollen 2 weeks after cultures were initiated. Flower buds could be seen as whitish tissue protruding from the inflorescence tissues. These were carefully excised from the mother tissue and transferred to fresh M1 medium and kept in darkness. Primary somatic embryos were

produced after tissues were transferred to MM1 modified medium (M1 medium with 200 mg/l casein hydrolyzate and 2 mg/l proline) for 2 months. Initially, the callus that formed was yellowish and compact, with white friable embryogenic tissues developing 10-13 weeks after initiation (Fig.1A). The white friable embryogenic tissues were used to initiate suspension cultures.

For initiation of suspension culture transfer about 0.5 g of callus was transferred in 10 ml of liquid M2 medium in 250 ml flasks. Calli from each independent embryogenic line were kept separately, these tissues released embryogenic cells and cell aggregates. The proembryos became necrotic during the first week of culture. The cell aggregates multiplied and formed many-lobed structures, from the peripheries of which new aggregates were released. The suspensions were subcultured once every week by adjusting to 3 % of the packed cell volume (PCV). A multiplication ratio of 1:2 to 1:3 was noted every two weeks. Multiplication rates in terms PCV of between 2 and 5 were observed at each monthly culture cycle in the liquid medium. The 2-5 fold monthly growth of the suspension is similar to that is commonly observed in embryogenic suspension of other monocotyledons (Redway *et al.*, 1990; De Touchet *et al.*, 1992 and Emons and Kieft 1995). The highest rates were obtained for suspensions maintained in liquid medium for three months (Figure 1B). After three months of culture, the suspension consisted mainly of aggregates varying in size. Some proembryos and nodular structures were also present in the suspension (data not shown). The suspension could be maintained in liquid medium for more than 13 months.

Effects of different media composition on the development of somatic embryos

The effects of SK12 and SK13 media modified in the present study, SK4 medium (Khalil *et al.*, 2002) and M2 medium (Cote *et al.*, 1996) on percentage of micro-embryonic calli and embryos from early stages (Globular, torpedo, and mature) was studied. The suspension culture of approximately 0.5 ml PCV were aspirated onto sterilized filter paper (Wanton No.1) on solidified four media (SK4, SK12, SK13 and M2) to determine the suitable medium for embryo development (Table 1). When SK13 was used the highest number of clones and percentage of embryos (Globular, torpedo and mature) were obtained to compared SK4, SK12 and M2 media under dark condition at 28 °C (Figure 3).

Somatic embryos initially appeared small and rapidly enlarged into distinct globular structure, which passing through recognizable torpedo structure (Figure 2). Somatic embryos initially globular and torpedo observed was noted within 18-21 days after aspirated on SK13 regeneration medium.

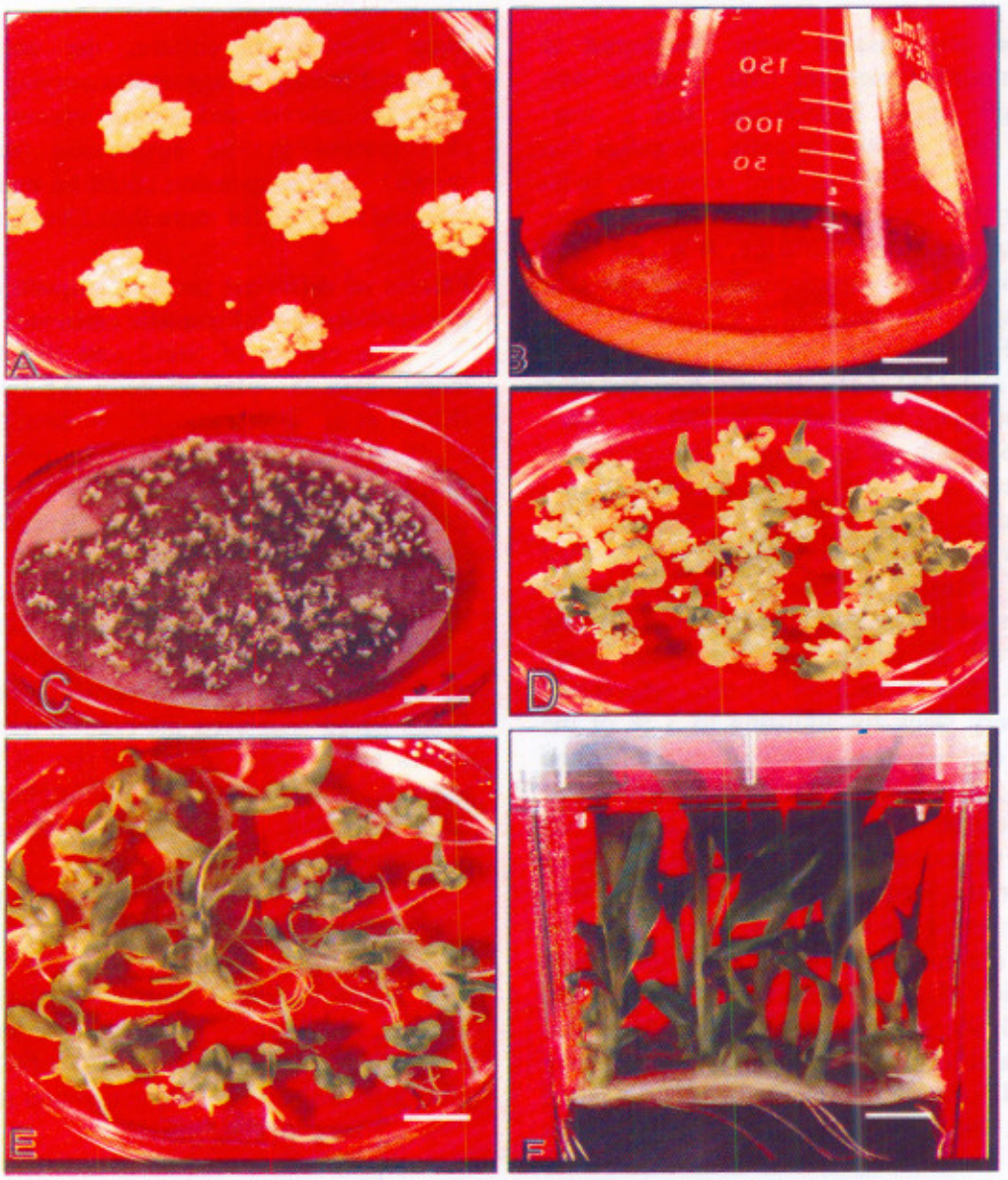

Fig. (1): Stages of regeneration via cell suspension and production of plantlets from immature male flower buds in Musa cv. Dwarf Brazilian cultivar . A, primary somatic embryogenesis from explants on modified M1 medium (Bar 0.5 cm); B, suspension culture was developed on M2 medium (Bar 0.5 cm); C, embryos induction on SK13 medium 4 weeks after plating on culture medium; D, differentiation of embryos and maturation on SK8 medium (bar 0.5 cm); E, germination of embryos and production of small plantlets on SK10 medium two weeks post cultured on germination medium (Bar 0.5 cm); F, elongation and development of mature regenerated plantlet of banana on SK11 3 weeks after subcultured on elongation medium (bar 0.5 cm).

Table (1): Effects of different media composition on development of embryos from 0.5 ml of cell suspension of banana after 40 days aspirated on media with different conditions of incubation.

Type of medium	Number of clones	Percentage of micro-calli	Percentage of Globular embryos	Percentage of torpedo embryo	Percentage of mature embryos
Dark					
SK4[a]	1070	66.3	18.1	15.6	0.0
SK12	712	10.2	37.2	41.6	11.0
SK13	1230	3.1	19.3	44.2	33.4
M2[b]	573	49.2	37.2	13.6	0.0
Light					
SK4	990	28.9	48.1	23.0	0.0
SK12	660	15.9	28.2	40.7	15.2
SK13	1120	9.8	38.3	32.1	19.8
M2	620	45.3	39.2	15.5	0.0

[a] Khalil *et al.* (2002)
[b] Cote *et al.* (1996)

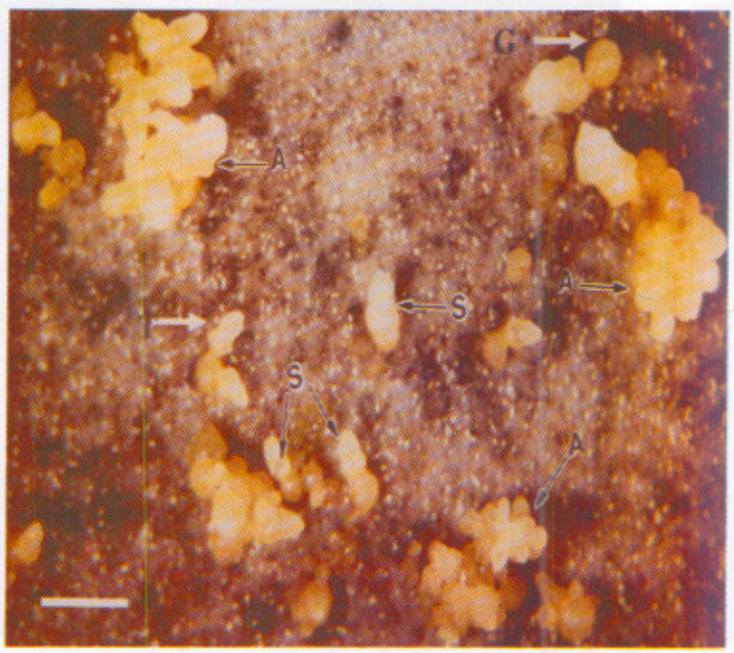

Fig. (2): Somatic embryogenesis from suspension culture using male flower buds in banana (Musa AAB cv. Dwarf Brazilian) Single (S) or cluster (A) of embryos were observed on SK13 medium after 18 days from plating suspension culture on sterilized filter paper on culture medium (bar 2 mm).

Effects of two media on the development of somatic embryogenesis and plant regeneration

The effects of SK8 developed by Khalil *et al.* (2002) and a differentiation medium (MM4) developed by Cote *et al.* (1996) on the maturation of embryos and development of regenerated banana plants were studied. Figure (4) illustrated that the somatic embryos, which developed on SK13 and transferred to differentiation SK8 produced the highest number of shoots (96%). Culture on MM4 medium makes these embryos to be differentiated with small shoot (Fig 1D). However, embryonic suspension originally cultured on SK13 produced 96 % embryos developed to shoots when transferred to SK8 differentiation medium (Fig. 4). The percentage of embryos developed to shoots from embryonic suspension originally cultured on SK12, SK4 and M2 was decreased to 58.2, 32.1 and 12.8% respectively, when transferred to SK8 differentiation medium (Fig. 4). On the other hand, the percentage of embryos developed to shoot from embryogenic suspension originally cultured on SK12, SK13, SK4 and M2 was decreased 57.8, 42.3, 32.9 and 6.5, respectively, when transferred to MM4 differentiation medium.

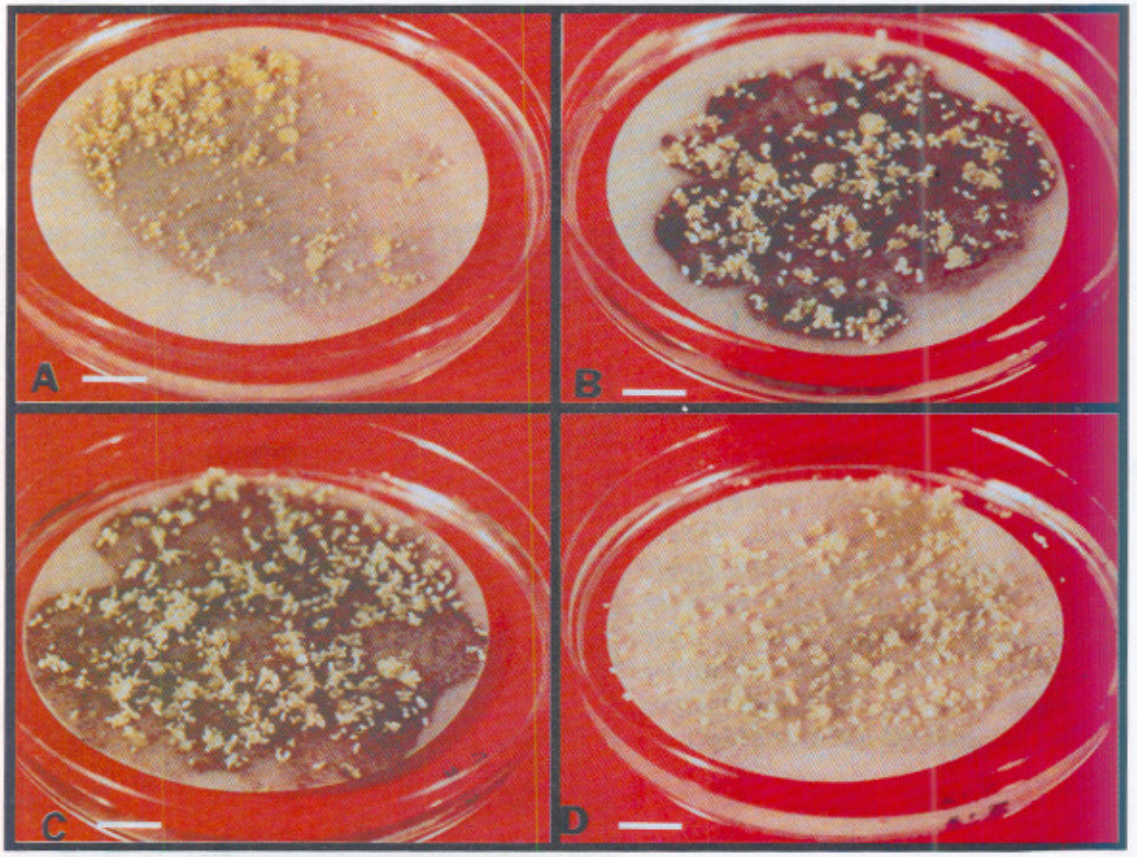

Fig. (3): Development of early stages (globular, torpedo and mature) of somatic embryos from embryonic cell suspension after 40 days from plating on solidified media. A, somatic embryos on M2 medium; B, Somatic embryos on SK4 medium; C, somatic embryos on SK13 medium; D, Somatic embryos on SK12 medium (bar 0.5 cm).

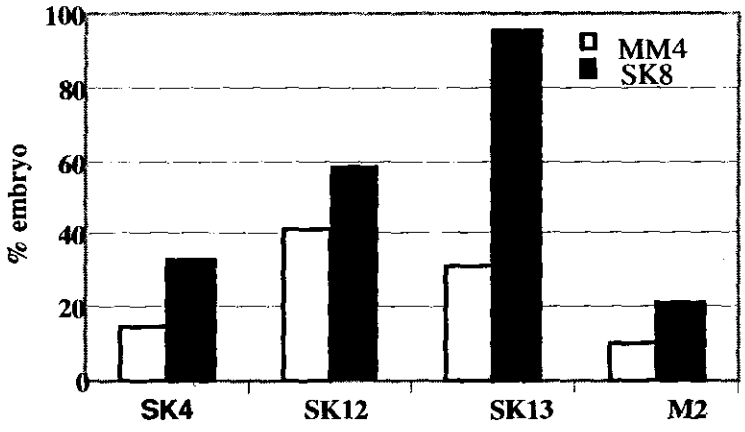

Fig. (4): Development of embryos to shoots on two-differentiation media. Bars represent the percentage of embryos developed to banana shoots on MM4 medium (white) and on SK8 medium (Black).

Germination of somatic embryos and development of regenerated banana plants

Mature somatic embryos, which differentiated on SK8 and MM4 media were separated from the culture mass and placed directly on MS free hormones medium (without growth regulators). Somatic embryos gave rise to small plantlets (shoots and roots) within 10 days (Fig. 1E). The small plantlets were subcultured onto MS medium supplemented with 1 mg/l IAA and 0.1% activated charcoal for elongation and development of new leaves (Fig. 1F). Figure (5) showed that the somatic embryos developed on SK13 and transferred to differentiation medium SK8 produced a higher number of germinated embryos (3.5) time than cultured on MM4 medium, when the small shoot were separating and cultured on MS medium without hormone. On the other hand, the percentage-germinated embryos were decreased when media like SK12, SK4 and M2 (Fig. 5). The percentage of germination and development of completely regenerated banana plants from embryogenic suspension reported in this study 90-96% (Fig. 5), were higher than those reported by Novak *et al.* (1989) (1.5-12%); Dhed'a *et al.* (1991) (10 - 23%)., Cote *et al.* (1996) 3-20% ; Grapin *et al.* (1996) 10-20 % and Navarro *et al.* (1997) (13-25 %) . Our results are even higher than to those of Kosky *et al.* (2002) who reported 89.3 % germination using TIS and cell suspension using a bioreacter and Khalil *et al.* (2002) who reported 89.5 % germination percentage using secondary somatic embryos. The established protocol in this paper for regeneration takes 4.5-5.5 months from initiation of suspension culture, but, longer time in other studies e.g. 16 months (Cote *et al.* 1996); 18 months, (Backer *et al.*, 2000) and 10-11 months (Ganapathi *et al.*, 2001). We have successfully regenerated plants using the system described in this paper *via* cell suspension culture. The high efficiency of regeneration reported in this paper is useful for plant mass propagation and for genetic transformation experiments, indicating that this protocol is reproducible and needs short time.

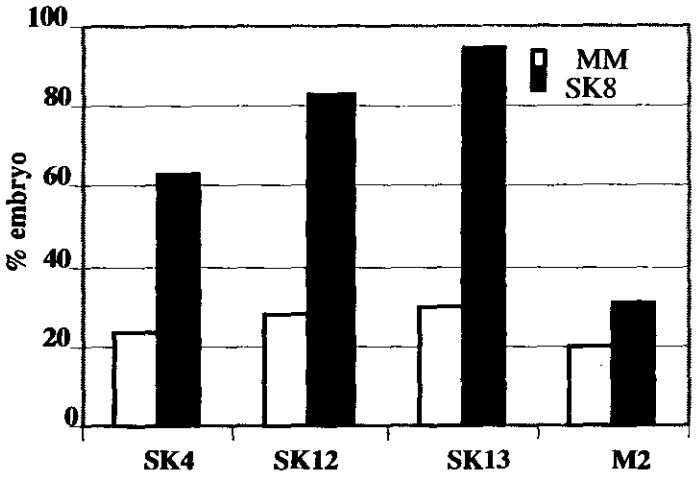

Fig. (5): Effects of differentiation SK8 and MM4 media on the embryo germination and production of plantlets.

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الملخص العربي

طريقة عالية الكفاءة لتكوين الأجنة الجسدية وإعادة توليد النباتات من خلال مزارع الخلايا المعلقة في الموز

سعيد محمد خليل *,** , عبد المنعم ألبنا **

* معهد بحوث الهندسة الوراثية الزراعية ــ مركز البحوث الزراعية ــ الجيزة ــ ١٢٦١٩

** معمل زراعة الأنسجة بالزهرية ــ وحدة الخدمات البستانية ــ مركز البحوث الزراعية ــ القاهرة ١١٢١١

تم تطوير تكنيك ذو كفاءة عالية لإعادة التوليد في صنف الموز دوارف برازليان ذو التركيب الوراثي AAB من خلال مزارع الخلايا المعلقة. الأجنة البدائية تم إنتاجها عند زراعة الأجزاء النباتية وهى البراعم الزهرية المذكرة غير الناضجة على بيئة موراشيج وسكوج مضاف لها ١ مليجرام/ اللتر بيوتين و ١٠٠ مليجرام/ اللتر مستخلص المولت و ١٠٠جلوتامين و ٤ ملليجرام/ اللتر داى كلوروفينوكسى استيك أسد و ١ مليجرام/ اللتر أندول استيك أسد و ١ مليجرام/ اللتر نفثا لين استيك أسد و ٣٠ جرام/ اللتر سكروز و ٢،٦جرام / اللتر فيتاجل وذلك لمدة شهر. ثم بعد ذلك يتم نقلها على نفس البيئة السابقة مضافا لها ٢٠٠ مليجرام/ اللتر كازين هيدروليزيت و ٢ مليجرام/ اللتر برولين وذلك لمدة ثلاث شهور. تم استحداث الخلايا المعلقة من الكالس الجنيني بعد نقلة على بيئة سائلة تحتوى على أملاح موراشج وسكوج مضاف لها ١ مليجرام/ اللتر داى كلوروفينوكسى استيك أسد و ١ مليجرام/ اللتر بيوتين و ١٠٠ مليجرام/ اللتر و جلوتامين و ١٠٠ مليجرام/ اللتر مستخلص المولت و ٤٥ جرام/ اللتر سكروز ونم ضبط الـ pH ٥,٣ . ازداد معدل نمو الخلايا المعلقة PCV بنسبة ٢،٧ مرة كل شهر على البيئة السائلة . وقد تم تطور الأجنة الجسدية من الخلايا المنفردة على بيئة موراشج وسكوج مضاف لها ١ ملليجرام/ اللتر بيوتين و ١٠٠ مليجرام/ اللتر مستخلص المولت و ١٠٠مليجرام/اللتر جلوتامين و ٥ مليجرام/اللتر كينيتين و ٢ ملليجرام/اللتر زياتين و ١ مليجرام/اللتر نفثالين استيك أسد و ٤٥ جرام/اللتر سكروز ٢،٦ جرام/اللتر فيتاجيل . وقد تم نقل الأجنة المتكشفة على بيئة موراشيج وسكوج مضاف لبها ٥ مليجرام/اللتر بنزيل ادانين وذلك لتطوير المراحل الناضجة من الأجنة . وقد تم عزل الأجنة الناضجة Mature Embryos وتم زراعتها على بيئة خالية من منظمات النمو وذلك للإنبات وتكوين النبيتات الصغيرة . وقد تم تكشف حوالي ٩٤ % من الخلايا الجنينية وأنتجت نبيتات تم نقلها على بيئة الاستطالة التي تحتوى على ٠,١ %/ فحم نباتي و ١ مليجرام/اللتر اندول استيك اسد وذلك لإنتاج النباتات الكاملة جاهزة لمرحلة الأقلمة . تم إنتاج حوالي ٩٠٠ إلى ١٠٥٠ نبات موز (٩٠%) من ٠،٥ ملي من PCV من الخلايا المعلقة خلال ٤-٥ شهور. وكان الشكل المرفولوجى والنمو الخضري للنباتات الناتجة من الخلايا الجنينية المعلقة طبيعيا ومطابقا لنبات الأصل ولم يلاحظ أي اختلافات على أي نبات على الإطلاق وذلك بعد الأقلمة بـ٥ شهور حتى مرحلة خروج الخلفات . هذا التكنيك لتكون الأجنة الجسدية من الخلايا المعلقة الذي طور في هذه الدراسة يعتبر ممتاز كوسيلة لإنتاج شتلات الموز الخالية من الأمراض والطفرات على نطاق تجارى واسع وأيضا ذو كفاءة عالية للاستخدام في مجال الهندسة الوراثية لتحسن الموز وتعتبر النتائج ونسبة الإنبات التي وصلت هنا ٩٠% للأجنة أول مرة على مستوى العالم في إعادة التوليد من الخلايا المعلقة في الموز .