# REGENERATION OF BANANA (*Musa* spp. AAA Group) cv. WILLIAMS *via* SECONDARY SOMATIC EMBRYOGENESIS AND CELL SUSPENSION FROM IMMATURE MALE FLOWER BUDS

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Banana (*Musa* sp. L.) is the most important of the cultivated tropical fruits and is produced in the tropical and subtropical regions. Banana is grown in more than 130 countries throughout the world on an area 8.25 million ha with a production of 97.38 million tons (Singh, 2007). Banana and plantain in many countries represent major fruit exports and are essential sources of income for national economies, (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. Average costs for control of this fungal disease alone ranges between $ 0.30 and $1.0 per plant in banana plantations in order to avoid yield losses of 30-50% (Sagi *et al.,* 1995). Secondary somatic embryogenesis ($SE_2$) is a phenomenon whereby new somatic embryos are initiated from other somatic embryos. Some cultures are able to retain their competence for secondary $SE_2$ for many years, and thus provide useful material for various studies, as described for *Vitis rupestris* (Martinelli *et al*., 2001), cork oak (*Quercus suber*) (Hernández *et al.*, 2003) banana (Khalil *et al*., 2002). Recently, the development of different methods for somatic embryogenesis has considerably assisted *in vitro* technologies for these species. Diverse procedures have been originally described for somatic embryo induction in banana and differ depending on the type of explants employed, to include zygotic embryos, foliar bases and corm slices (Novak *et al*., 1989), *in vitro* cultured meristems (Dhed'a *et al*., 1991), and immature male (Escalant *et al.*, 1994) and female (Grapin *et al*., 2000) flowers. Despite the many options, the most widely applicable methodologies for the establishment of regenerable embryogenic cell suspensions from valuable seedless cultivars have been restricted to the use of either in vitro multiple meristems (Xu *et al*., 2005; Kulkarni *et al*., 2006; Strosse *et al*., 2006) or immature flowers (Côte *et al*., 1996; Grapin *et al*., 1996; Ganapathi *et al.*, 2001; Khalil *et al*., 2002; Suprasanna *et al*., 2006) as starting

material. Nevertheless, somatic embryogenesis in banana is still far from being considered a routine technology and has not even been successfully applied to some cultivars.

The present study reports an efficient and reproducible protocol with high regeneration frequency for secondary somatic embryogenesis and cell suspension culture for mass propagation of banana plantlets to improve *Musa* spp. AAA group cv. Williams.

## MATERIALS AND METHODS

### *Plant material and culture initiation*

The initial plant material used was the cultivar cv. "Williams" belong to the AAA genotype group. One month-old male flower buds about 30 cm in length were collected from the field. Cultures were initiated within two 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, surface sterilized by 100% Clorox® (5.25% sodium hypochlorite) 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 was 1.5 cm in length. The remaining flower bud was cut into 4 segments longitudinally (orthogonally) through the shoot tip, into 2 mm segments (transverse section). A total of 16-20 such segments can easily be obtained from a single inflorescence male flower bud.

### *Establishment of primary somatic embryogenic cultures*

Immature male flower explants were isolated and cultured on solid M1 medium (Table 1). The cultures were incubated in the dark at 28°C for two weeks. The flower bud segments became swollen two 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, transferred to fresh M1 medium and kept in darkness. Primary somatic embryos were produced after tissues were transferred to modified MM1 medium which was composed of (M1 medium with 200 mg/l casein hydrozylate and 2 mg/l proline) for 2 months. Compact white calli and friable embryogenic tissues with globular structures containing primary somatic embryos were formed.

### *Induction of secondary somatic embryogenesis*

After the production of primary embryogenesis in the first three months, the compact calli were transferred to seven modified different media (SK1 to SK7) illustrated in Table (1) to developed secondary somatic embryogenesis ($SE_2$). These cultures, and all subsequent transfers, were incubated at 28°C under a 16/8 h (light/dark) photoperiod with light supplied by 40 W cool-white fluorescent lamps. At this stage, $SE_2$ were produced on SK4 medium and then sub-cultured on both SK8 and GTR media (Table 1) for

embryo development. The response of $SE_2$ to several different concentrations of the cytokinin 6-benzylaminopurine (BA) in the differentiation media SK8 was investigated. Differentiation media were prepared with 0.0, 2.5, 5.0, 7.5 or 10.0 mg/l BA, and the production of mature embryos and plantlets from each medium was recorded. After two weeks, cultures were transferred from SK8 to MS (Murashige and Skoog, 1962) free hormone medium for further differentiation into mature embryos with leaf primordial.

***Production of cell suspension cultures from $SE_2$***

Suspension cultures were established from friable embryogenic calli containing $SE_2$ embryos. One gram of callus with $SE_2$ was transferred to 10 ml of liquid medium designated M2 (Table 1). 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 two weeks until suspension cultures start to multiply. Culture medium was changed weekly during the first month of culture and subsequently biweekly. Regular maintenance of suspension cultures was done by pipetting 1 ml of mother stock to 20 ml of fresh M2 medium. The medium was changed every 7-10 days. Cultures were maintained at 28°C under a photoperiod of 16 h/8h (light/dark).

***Proliferation of embryonic cells***

Cell suspension cultures after four subcultures were collected through sieve (180 μl) to avoid any cell aggregates. The 0.5 ml packed cell volume (PCV) of suspension culture was aspirated on sterile filter paper on the four different media, SK4, SK12, SK13 and SK14 to determine the suitable medium for embryo development. The compositions of four media were illustrated in (Table 1). The pH of these media was adjusted to 5.8 prior to autoclaving. Half of the cultures were incubated under dark conditions at 28°C and the other half was incubated at photoperiod 16 h light and 8 h dark at 28°C to 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 SK14 then transferred to either of the following two media to determine the best medium for embryo differentiation. The first medium designated SK8 medium and MM4 medium were illustrated in Table (1). The cultures were incubated at 28°C with a photoperiod of 16 h light and 8 h dark for four weeks

***Embryo germination and plantlet formation***

Germination of embryos was performed on a medium designated SK10 medium which consisted of MS medium with MS vitamins and 30 g/l sucrose without 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 16-h light and 8 h dark. The plantlets were then transferred to SK11 medium (Table 1) 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

### *Callus formation with embryogenic structures in cv.Williams*

Callus formation with embryogenic structures was characterized explants type immature male flowers using as starting material in the embryogenic process. When cultured explants into M1 media and incubated in the dark for one month and re-subculture in the same for another one month. The white tissues were excised from the mother explants and subculture into MM1 medium (Table 1) for developmental callus, non-friable embryogenic tissue with early stages of primary embryos were formed (Fig. 1A). About (62.5%) explants producing embryogenic callus primary embryos. When added proline and casein hydrolysate into the MM1 medium to increase callus proliferation and production of primary embryos and the embryogenic potential in other banana cultivars. The similar results were obtained when used the M1 and MM1 media for developmental primary embryos from immature male flower explants in banana (*Musa* AAB cv. Dwarf Brazilian) (Khalil *et al.*, 2002).

### *Induction of secondary somatic embryos ($SE_2$)*

After the production of primary embryogenesis in the first six months, the compact calli (Fig. 1A) were transferred to seven modified different media (SK1 to SK7) to develop secondary somatic embryogenesis ($SE_2$). After 28 days from subculture, results in Table (2) showed that significant percentages of $SE_2$ were produced on SK4 and SK5 media, 55% and 44.12%, respectively, while the lowest percentage of $SE_2$ (15.71%) was produced on SK7 medium. Similar results were reported by Khalil *et al*. (2002) on banana (*Musa* spp. AAB Group) cv. Dwarf Brazilian. Secondary somatic embryos typically entered the globular stage directly from primary somatic embryos However, in other embryogenic systems, the early developmental embryo stages (e.g. globular and heart stages) showed greater potential for secondary embryogenesis compared with later stages (Neves *et al.*, 1999; Nair and Gupta, 2006).

### *Secondary somatic embryogenesis stages and development of mature embryos*

The all stages of developmental $SE_2$ were demonstrated in Fig. (2). Secondary somatic embryogenesis typically entered the globular stage directly from primary somatic embryos and occurred in clusters (Fig. 2A), initially appearing as small, on the surface of the clusters. The embryos passed through a recognizable

heart-shaped stage, a primary torpedo stage (Fig. 2B), a later torpedo stage (Fig. 2C) and finally green plumules emerged from these embryos (Fig. 2D). The embryos passed through different developmental stages and finally green plumules emerged from these embryos. Concomitant with morphological development there was a change in opacity from hyaline to translucent to opaque with a simultaneous change in color from pale white to yellow to green. The green-plumule stage developed to germination stag with root initiation (Fig. 2E) after two weeks of subculture on SK8 medium with elongation of root and leaf primordial initiation (Fig. 2F). Khalil *et al*. (2002) and Pérez and Rosell (2008) reported the same trend in development of $SE_2$ from banana AAB group cv. Dwarf Bazilian.

***Embryo differentiation of secondary somatic embryogenesis***

Differentiation, maturation of embryos and development of plantlets were compared using two types of media SK8 medium and GTR medium. Results in Table (3) showed that SK8 medium was superior to GTR medium as it produced a higher number of globular. Vuylsteke (1989), reported that high BA concentrations induced shoot formation; however, our results showed a lower formation of mature embryos and plantlets at high (>5 mg/l) BA concentrations. The same results were reported by Khalil *et al.* (2002) on Dwarf Brazilian cultivars (AAB Group). The transfer of embryos to MS free hormone medium resulted in a threefold increase in the number of $SE_2$ that developed, irrespective of whether they were originally cultured on SK8 media. However, embryonic tissue originally cultured on SK8 produced three times more mature $SE_2$ when transferred to SK10 medium than calli originally cultured on GTR medium. Furthermore, transfer from SK8 to MS free hormone medium dramatically increased the number of germinated embryos and plantlets when compared to those that were formed on SK8 medium directly.

***Plant regeneration via cell suspension culture of banana Musa spp. cv. Williams.***

Suspension culture was established from the friable embryogenic callus with secondary somatic embryogenesis by transferring one gram from such callus of secondary somatic embryogenesis calli to 10 ml of M2 liquid medium according to Côte *et al*. (1996). The suspensions were cultured in 250-ml Erlenmeyer flasks on a reciprocal shaker at 100 rpm in darkness for three weeks until suspension cultures start to multiply. Culture medium was changed weekly during the first month of culture and subsequently biweekly with 20 ml of liquid M2 medium. 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 16/8) h (light/dark). Kosky *et al*. (2002) developed embryogenic cell suspension (ECS) for large scale growth in temporary im-

mersion systems and showed that different cell densities gave the best results of somatic embryo development with an initial inoculums of 100 mg and embryo diameter from 0.5 to 1.2 mm. Ma (1991), Escalant *et al*. (1994) and Jalil *et al*. (2003) obtained ECS cultures and plant regeneration of *Musa acuminata* by culture of male flowers. Data in Table (4) shows that SK13 medium has the highest number of clones and the highest number of embryos (Globular, torpedo and mature) under dark condition at 28°C (Fig. 3C). Somatic embryos initially appeared small and rapidly enlarged into distinct globular structure, which passing through recognizable torpedo structure previously illustrated in (Fig. 2). Somatic embryos initially globular and torpedo were observed within 23-28 days after aspiration on SK13 regeneration medium. On the other hand, the SK12, SK14 and SK4 media have the lowest percentage of mature embryo in dark condition. The results in Table (4) indicated that the aspiration of embryogenic cell suspension on SK13 medium and incubation in dark at 28°C the best media compensation and incubation for developed embryogenic cell suspension. Pinto *et al*. (2008) reported the same conditions were used for developed embryogenic calli.

***Maturation of embryos***

Embryo development was achieved by culturing the embryonic suspension culture on SK4, SK12, SK13 and SK14 medium then transferred two media SK8 (Khalil *et al*., 2002) and MM4 medium (Côte *et al*., 1996), to determine the best medium for embryo maturation and development regenerated banana plants. The cultures were incubated at 28°C with a photoperiod of 16/8 h (light/dark) for four weeks. The data in Table (5) reported that the embryos were sub-cultured on the SK8 medium with the highest number of globular stage, later of torpedo, green-plumula stages and the total number of germination, embryos. Date in Fig. (3) indicated that the embryos initially on SK13 medium and subculture on SK8 medium development the highest number of regenerated banana plants. Data in Table (5) illustrate that the somatic embryos, which developed on SK13 medium and transferred to differentiation SK8 medium produced the highest number of germinated embryos compared with MM4 medium. Considering the effect of light on repetitive ECS, in general, and until the transition to mature stage, dark had a positive or neutral effect on embryo proliferation and total number of embryos in torpedo on stage. These quantitative and qualitative results strongly suggest that, in this species, cell suspension must be kept in the dark until they reach the globular stage, and should then be transferred to light on differentiation medium, the same data was reported by Pinto *et al*. (2008).

Mature somatic embryos, which differentiated on SK8 and MM4 media were separated from the culture mass and placed directly on MS free hormones medium (SK10, Table 1). Somatic embryos gave rise to small plantlets (shoots and

roots) within 10 days. The small plantlets were sub-cultured onto SK11 for elongation and development of new leaves. Figure (3) shows that the somatic embryos developed on SK13 and transferred to differentiation medium SK8 produced a higher number of germinated embryos (91%), but decreased the percentage to 30.5% when sub-cultured on MM4 medium. On the other hand, the percentage-germinated embryos were decreased when sub-cultured on SK12, SK4 and SK14 medium. However, embryonic suspension originally cultured on SK13 produced 96% embryos developed to shoots when transferred to SK8 differentiation medium. The date recorded on the regenerated banana plantlets from those of Escalant *et al*. (1994), who reported 60-70% germination using a temporary immersion system, Kosky *et al*. (2002), who reported an 89.3% regeneration using and cell suspension using a bioreactor.

***Embryo germination and plantlet formation***

To develop the mature true to type of regeneration cv. Williams banana germination of embryos was performed on a medium designated as SK10. Embryos germinated were maintained on SK10 medium for 15 days at 28°C with a photoperiod of 16/8h (light/dark). The small plantlets were then transferred to SK11 medium for elongation. Mature plantlets (5-6 cm long) were obtained after 2-3 weeks on SK11 and were ready for acclimatization (Khalil *et al*., 2002)

The stages of regeneration *via* cell suspension were demonstrated in Fig. (4) and production of plantlets from immature male flower buds in *Musa* cv. Williams, primary somatic embryogenesis from explants on modified M1 medium (Fig. 4A), suspension culture was developed on liquid M2 medium for multiply of embryonic cells (Fig. 4B), embryogenic cell suspension (ECS) aspiration and embryos induction on SK13 after four weeks from plating on culture medium (Fig. 4C), differentiation of embryos and maturation on SK8 medium (Fig. 4D), germination of embryos and production of small plantlets on SK10 medium two weeks post cultured on germination medium (Fig. 4E). Plantlets obtained were transferred to hormone-free MS medium and placed in light condition for formation of chloroplasts and further growth of the shoots and root (Fig. 4F). The successfully regenerated plantlets of banana cv. Williams acclimatized with suitable soil mixture were illustrated in Fig. (4 G and H).

Finally, the established protocol in this work for regeneration takes 12.5 months from initiation of suspension culture. We have successfully regenerated plants using the system described in this report *via* secondary somatic embryogenesis and cell suspension culture.

***Plantlet formation and acclimatization of banana plants via $SE_2$ and cell suspension.***

Mature embryos from $SE_2$ and cell suspension were sub-cultured on SK10 medium for germination, while the elongation was achieved by transferring the

small plantlets to SK11 medium were obtained. After 2-3 weeks mature plantlets (5 to 6 cm in length) were obtained. Plants (10 plantlets/Fitakone or Jar) suitable for acclimatization were placed in a green-house maintained at 28°C for 3-5 days. Medium was rinsed from the plantlets, and the latter were transplanted to 5-cm-diameter pots containing peat-moss, sand and vermiculite (1:1:1; v:v). The plantlets were acclimatized for three weeks in a green-house at 28°C, shown this stage in Fig. (4 G and H). To date, over 120 plants have been maintained in the greenhouse for more than two months. No abnormal morphological variations have been observed.

## CONCLUSION

The development of an *in vitro* regeneration system is essential for genetic manipulation of banana. This is the first report using secondary somatic embryogenesis and cell suspension to produce a high percentage of germinated embryos and a high number of regenerated banana plants. The culture system described in this paper is a high regeneration capacity system and demonstrates the potential of using primary somatic embryos derived from immature male flower bud explants to raise highly proliferate secondary somatic embryo cultures of banana cultivar ‘Williams’, and this is the first report of tissue culture of this variety in Egypt. The use of secondary somatic embryos could provide an efficient solution to the problems limiting plant regeneration in other banana cultivars, and may result in a system for production of transgenic plants engineered for disease resistance or other purposes.

## SUMMARY

Development of embryogenic cultures having high regeneration efficiency from important, commercial varieties of banana is a prerequisite for genetic manipulation and for *in vitro* propagation. In the present study, we have studied the induction of somatic embryogenesis from young immature male inflorescences of the banana cultivar "Williams" (*Musa* spp. AAA group) *via* secondary somatic embryogenesis and 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 α - 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 (MM1). Secondary somatic embryogenesis ($SE_2$) was developed when primary embryos were subculture on SK4 medium (MS medium supplemented with 10 ml of coconut milk). Embryos differentiated when sub-cultured on SK8 medium (MS medium supplemented with 5 mg/l BA and the embryos germinated when subculture on MS free hormone medium. Suspension cultures were initiated from $SE_2$ embryogenic tissues from when 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), 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 were 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) (SK13). Differentiated embryos were transferred to MS medium supplemented with 5 mg/l 6-benzylaminopurine (BA) for development of mature somatic embryos, which were isolated and cultured on hormone-free MS medium for germination and development into plantlets. Approximately 55% of the somatic embryos germinated and developed into plantlets. Somatic embryogenesis *via* $SE_2$ and cell suspension might be an excellent technique for mass propagation, developing a breeding strategy and genetic transformation of banana.

## ACKNOWLEDGEMENTS

This work was supported in part by a grant from Science & Technology Development Fund (STDF) of Agriculture for project ID 957

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Table (1): Media used for plant regeneration from immature male flower buds of banana cv. Williams (*Musa* spp., AAA group), through secondary somatic embryogenesis and cell suspension.

| Media[a] | Sucrose (g $l^{-1}$) | Malt extract (mg $l^{-1}$) | 2,4-D (mg $l^{-1}$) | IAA (mg $l^{-1}$) | Biotin (mg $l^{-1}$) | Glutamine (mg $l^{-1}$) | NAA (mg $l^{-1}$) | Casein hydrolysate (mg $l^{-1}$) | Proline (mg $l^{-1}$) | Coconut water (%) | Zeatin[b] (mg $l^{-1}$) | 6-BA (mg $l^{-1}$) | Activated Charcoal | Kinetin Mg/l | GA3 Mg/l |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M1 | 30 | 100 | 4 | 1.0 | 1.0 | 100 | 1 | – | – | – | – | – | – | - | - |
| M2 | 45 | 100 | 1 | – | 1 | 100 | – | – | – | – | – | – | – | - | - |
| MM1 | 30 | 100 | 4 | 1 | 1 | 100 | 1 | 200 | 2 | – | – | – | – | - | - |
| SK1 | 45 | – | – | – | – | – | – | – | – | 5 | – | – | – | - | - |
| SK2 | 45 | – | – | – | – | – | – | – | – | – | 0.2 | – | – | - | - |
| SK3 | 45 | – | – | 1 | – | – | – | – | – | – | – | 0.2 | – | - | - |
| SK4 | 30 | – | – | – | – | – | – | – | – | 10 | – | – | – | - | - |
| SK5 | 30 | – | 1 | – | – | – | – | – | – | 10 | – | – | – | - | - |
| SK6 | 60 | – | – | 0.2 | – | – | – | – | – | – | – | 2 | – | - | - |
| SK7c | 40 | – | – | – | – | – | – | – | – | – | 0.5 | – | – | - | - |
| SK8c | 30 | – | – | – | – | – | – | – | – | – | – | 5 | – | - | - |
| GTR | 30 | 500 | – | – | – | – | – | – | – | – | – | – | 0.1 | - | |
| SK10 | 30 | – | – | – | – | – | – | – | – | – | – | – | – | - | - |
| SK11 | 30 | – | – | 1 | – | – | – | – | – | – | – | 1 | 0.1 | - | - |
| SK12 | 45 | 100 | - | - | 1 | 100 | - | - | - | - | - | 2.5 | - | 1 | 1 |
| SK13 | 45 | 100 | - | - | 1 | 100 | 1 | - | - | - | 0.2 | - | - | 0.5 | |
| SK14 | 45 | 100 | 1.0 | 1.0 | | 100 | - | - | - | - | 0.2 | - | - | - | - |
| MM4 | 45 | 100 | 1.0 | – | 1.0 | 100 | - | – | – | – | – | 0.5 | – | - | 1.0 |

[a]All media used in this study contained MS salts, myo-inositol (100 mg $l^{-1}$), nicotinic acid (1 mg $l^{-1}$), thiamine HCl (1 mg $l^{-1}$) and pyridoxine HCl (10 mg $l^{-1}$), solidified with Phytagel (2.6 g $l^{-1}$).

Table (2): The effect of different media for production of primary embryos and secondary somatic embryo.

| Type of media | No. of starting explants | No. of calli containing PE[a] | No. of explants producing $SE_2$ | Percentage of $SE_2$ |
|---|---|---|---|---|
| SK1 | 50 | 121 | 23 | 19.01[a] |
| SK2 | 50 | 125 | 36 | 28.80[b] |
| SK3 | 50 | 142 | 31 | 21.83[a] |
| SK4 | 50 | 120 | 66 | 55.00[d] |
| SK5 | 50 | 136 | 60 | 44.12[c] |
| SK6 | 50 | 127 | 23 | 18.11[a] |
| SK7 | 50 | 140 | 22 | 15.71[a] |
| [a]Primary embryos | | | Mean | 28.24 |

Table (3): Effect of two differentiation media developmental stages of $SE_2$ and the regeneration of banana plantlets cv. Williams.

| Type of media | No. of calli | No. of globolur embryos | No. of Terpedo ebmbryos | No. of green-plumules | No. of germination Embryos | No. of regenerated plantlets |
|---|---|---|---|---|---|---|
| SK8 | 120 | 2230 | 510 | 430 | 230 | 210 |
| GTR | 120 | 2110 | 320 | 280 | 110 | 40 |

Table (4): Effects of different media compositions and light condition on development of embryos from 0.5 ml of cell suspension of banana William cultivar after 40 days aspirated on media with different conditions of incubation.

| Type of media | No. of Micro clones | Percentage of micro-calli | Percentage of Globular embryos | Percentage of torpedo embryos | Percentage of mature embryos |
|---|---|---|---|---|---|
| Dark condition | | | | | |
| SK4 | 1080 | 68.30$^{d}$ | 30.40$^{a}$ | 14.80$^{a}$ | 0.10$^{a}$ |
| SK12 | 760 | 24.80$^{b}$ | 29.20$^{a}$ | 43.60$^{d}$ | 15.00$^{b}$ |
| SK13 | 1630 | 12.10$^{a}$ | 39.30$^{b}$ | 45.20$^{d}$ | 34.40$^{c}$ |
| SK14 | 473 | 59.20$^{d}$ | 47.20$^{c}$ | 16.60$^{a}$ | 0.00$^{a}$ |
| Light condition | | | | | |
| SK4 | 990 | 28.90$^{b}$ | 48.10$^{c}$ | 23.00$^{b}$ | 0.00$^{a}$ |
| SK12 | 660 | 15.90$^{a}$ | 28.20$^{a}$ | 40.70$^{d}$ | 15.20$^{b}$ |
| SK13 | 1120 | 10.20$^{a}$ | 41.60$^{b}$ | 33.00$^{c}$ | 17.20$^{b}$ |
| SK14 | 620 | 50.30$^{c}$ | 39.40$^{b}$ | 13.10$^{a}$ | 0.00$^{a}$ |
| Mean | | 33.32 | 37.27 | 28.58 | 10.55 |

Table (5): Effect of two types of media on maturation of embryos.

| Type of medium | No. of calli | No. of Globular | No. of later of torpedo | No. of green-plumules embryos | No. of mature embryos | No. germi-nation em-bryos |
|---|---|---|---|---|---|---|
| SK8 | 120 | 2910 | 815 | 545 | 168 | 121 |
| MM4 | 120 | 1898 | 410 | 169 | 92 | 19 |

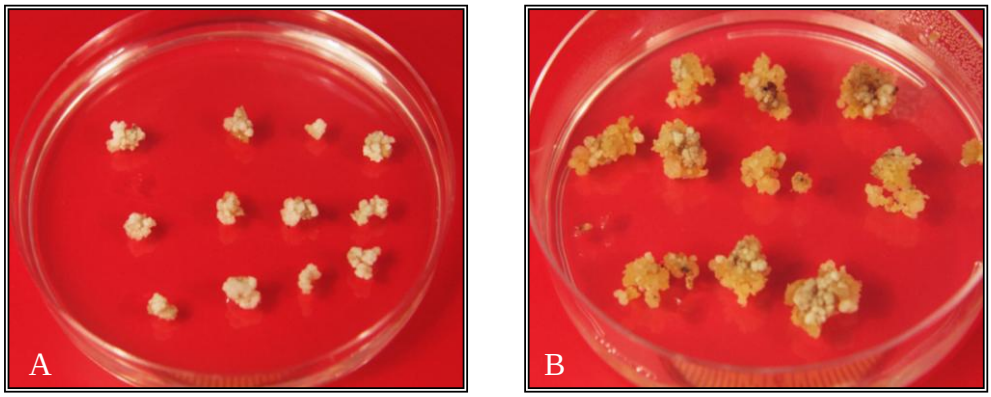



Fig. (1): Production of primary embryo on M2 medium (A) and development of secondary somatic Embryogenesis on SK4 medium using immature male flower buds explants. (B), development of secondary somatic embryogenesis from primary embryos after four weeks on SK4 medium.

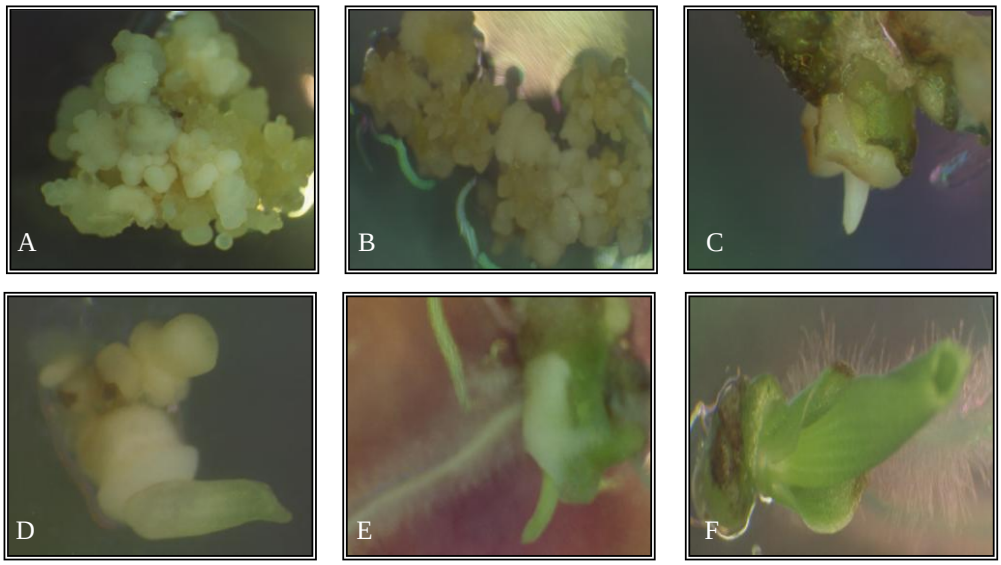
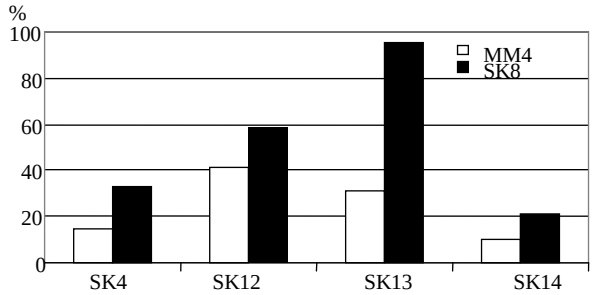



Fig. (2): Stages of embryos developmental via secondary somatic embryogenesis. A, globular stag on SK4 medium. B, hart stag on the same medium. C, later of torpedo stag. D, green-plamual stag. E, germination stag with root initiation. F, elongation of root with development of leaf primordial.

Fig. (3): Development of embryos to shoots on two differentiation media. Bars represent the percentage of embryos developed to banana shoots.



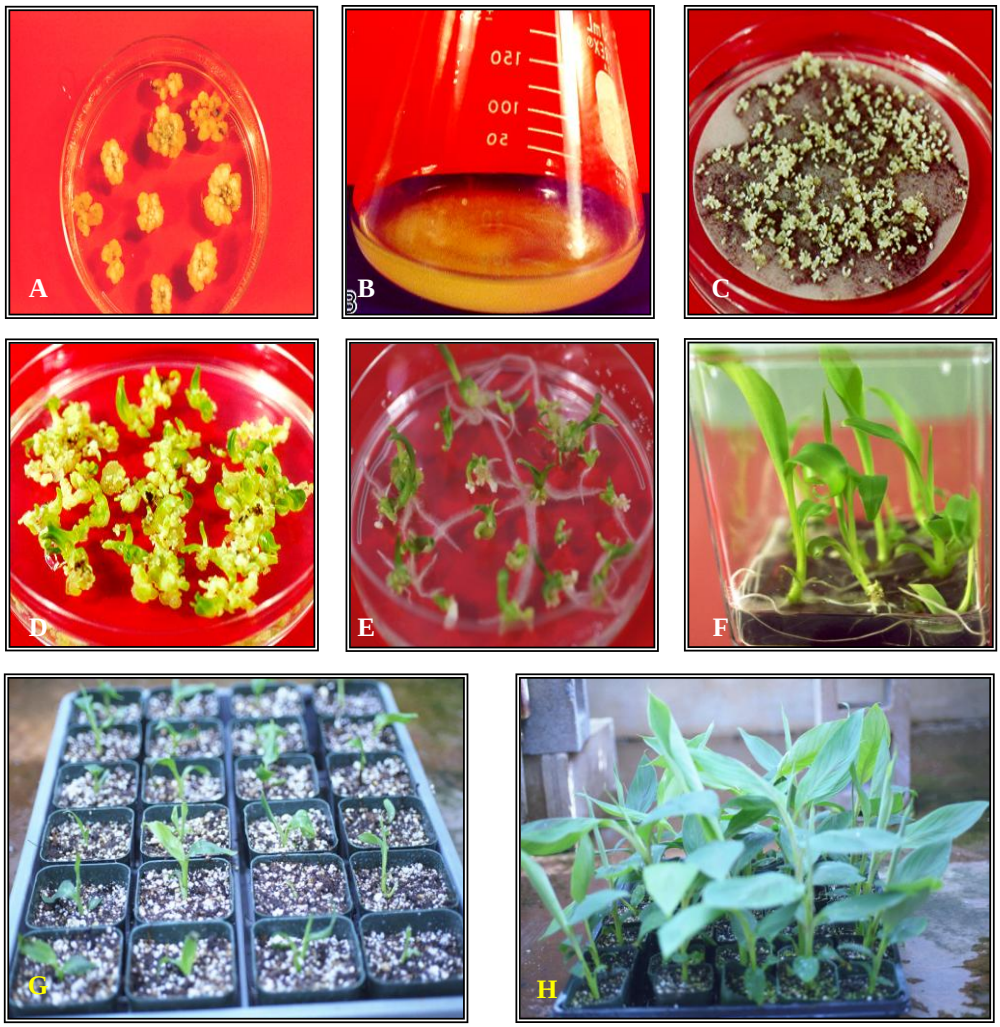



Fig. (4): Stages of regeneration via cell suspension and production of plantlets from immature male flower buds in *Musa* cv. Williams cultivar. A, primary somatic embryogenesis from explants on modified M1 medium (Bar 0.5 cm); B, suspension culture was developed on M2 medium (Bar 0.5 cm); C, embryos induction on SK13 medium four 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 three weeks after subcultured on elongation medium (Bar 0.5 cm G., acclimatization of banana plantlets after 10 days of plantation with suitable soil mixture. H., regenerated banana plant with normal morphological growth and leaves, the all regenerated banana true to type.