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Full Length Research Paper

Influence of genotype and age of explant source on the capacity for somatic embryogenesis of two Cavendish banana cultivars (*Musa acuminata* Colla, AAA)

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The embryogenic capacities of flower explants from one- and two-week-old male inflorescence buds from *Musa acuminata* Cavendish, AAA, genotypes 'Williams' and 'Grand Naine' were investigated. Explants of hands with immature flowers were excised and induced for embryogenesis. Highly significant differences were found in the embryogenic response, depending on the genotype and the developmental stage of the buds from which the explants were excised. After four months of induction, the total percentage of callus formed ranged from 97.81% in explants of two-week-old 'Williams' buds to 52.11% in explants of two-week-old 'Grand Naine' buds. Embryogenic callus formation was, on average, higher in the two-week-old 'Williams' explants (10.01%) than in the one-week-old explants (0.78%). The opposite was true for 'Grand Naine', with 7.51% embryogenic callus produced in explants of one-week-old buds compared to 2.49% in two-week-old buds. Selected embryogenic calluses that were successfully established on proliferation medium led to embryogenic cell suspensions with good regeneration capacities. Fifteen to thirty-five percent of the embryos germinated, demonstrated high plant-conversion capacity (99%). The effect of the interaction between the developmental age of explant and the genotype on the embryogenesis response is discussed.

Key words: *Musa*, embryogenic callus, genotype, male bud age, Cavendish banana.

INTRODUCTION

Bananas and plantains (*Musa* sp.) are two of the world's major fruit crops. They are the staple food for millions of people in the tropics (Bakry et al., 2009). Cavendish bananas dominate the world market for dessert bananas. Classical breeding is difficult in edible *Musa* sp. due to differing levels of ploidy and sterility. Consequently, somatic embryogenesis is a valuable tool for use with *Musa* cultivars, for improvement by mass propagation of selected genotypes, mutagenesis, genetic transformation, protoplast fusion or other biotechnological applications (Strosse et al., 2003). In bananas and plantains, different types of explants have been used to induce embryogenic callus and regenerate plants. These include zygotic embryos (Cronauer-Mitra and Krikorian, 1988; Escalant and Teisson, 1989; Marroquin et al., 1993; Navarro et al., 1997), rhizome and leaf sheaths (Novak et al., 1989), proliferating meristems and scalps (Cronauer and Krikorian, 1983; Dhed'a et al., 1991) and immature male (Ma, 1991; Côte et al., 1996; Navarro et al., 1997; Grapin et al., 2000; Pérez-Hernández and Rosell-Gracía, 2008) and female (Grapin et al., 1996) flowers. Of these explants, immature male flowers appear to be the most responsive starting material for initiating embryogenic cultures of

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Abbreviations: MS, Murashige and Skoog; **IAA,** indole-3-acetic acid; **2, 4-D,** 2, 4-dichlorophenoxyacetic acid; **NAA,** 1-naphthaleneacetic acid; **SE,** somatic embryogenesis; **IMFs,** immature male flowers; **SCV**, settled cell volume; **ECS,** embryogenic cell suspension; **MM,** maturation medium; **GM,** germination media; **ABA,** abscisic acid.

Cavendish banana (Escalant et al., 1994; Côte et al., 1996; Navarro et al., 1997; Domergue et al., 2000; Becker et al., 2000) and for mass propagation (Doreswamy and Sahijram, 1989; Wirakarnain et al., 2008). However, recent findings show that the efficiency outcome of embryogenesis using the scalp method (Strosse et al., 2006) and technologies that use immature male flowers are comparable.

Several endogenous and exogenous factors exert influences on the growth, development and performance of plant cells in culture (Neumann et al., 2009). Among these, the genotype, the developmental status of the "mother plant" and the type and developmental status (or age) of the plant organ from which the primary explants are obtained are of critical importance in eliciting the desired response to somatic embryogenesis (Botti and Vasil, 1984; Parrot, 1993; Neumann et al., 2009). The effect of the genotype and explant age on somatic embryogenesis has been investigated in several plant species, e.g., in coffee (Molina et al., 2002; Simões-Costa et al., 2009), triticale (Atak et al., 2008), *Brassica napus* L. (Burbulis et al., 2007), *Solanum trilobatum* L. (Dhavala et al., 2009) and *Anacardium occidentale* L. (Martin, 2003). These studies demonstrate that these factors are of enormous significance for the outcome of the inducement of somatic embryogenesis. In banana, some data suggest that the parameters mentioned above are equally important for the development of somatic embryogenesis (Escalant et al., 1994; Chong et al., 2005).

To gain insights into the mechanisms of somatic embryogenesis in *Musa*, the present study assesses the effect of the genotype and the developmental age of the male inflorescence bud, the source of the explant and the interaction between these two factors on the somatic embryogenesis capacities of two *Musa acuminata*, AAA, Cavendish banana cultivars, namely, 'Williams' and ''Grand Naine''.

MATERIALS AND METHODS

Plant materials and explant preparation

To evaluate the influence of the developmental stage of the male inflorescence bud on somatic embryogenesis (SE), the buds were classified as age one or two week(s), according to their growth stage, defined as follows: After the bract subtending the most basal node or the first cluster of female flowers (fingers) opens, the flowers are at anthesis on day 1 to day 7; when the 7^{th} cluster is completely exposed, the bud age is classified as one week. After the 8^{th} bract has opened and has passed anthesis over the next 14-15 days and after the successive flowers have emerged, the bud age is classified as two weeks.

'Grand Naine' and 'Williams' (AAA) male inflorescence buds of one and two weeks of age were collected from field-grown plants in a commercial plantation at Teapa, Tabasco, Mexico and used for *in vitro* culture within two days of collection. The collected male buds were washed in tap water and shortened to 6 - 8 cm in length by successively removing the bracts and the hands of the male flowers subtended by them. The buds were disinfected with 70% (v/v) ethanol for five minutes, rinsed three times with sterile double-distilled water and further reduced in size to tips that were 3.0 cm longx1.0 cm wide, under a laminar flow hood. To prevent dehydration of the tissues, the reduced tips were kept in a jar with 500 µl of sterile double-distilled water during further processing.

Induction of somatic embryogenesis

The explants, that is, the hands of immature male flowers (IMFs), were isolated aseptically from the reduced buds by removing the bracts under a binocular stereomicroscope. The immature flower hands that were excised from positions 16 to 8 (1 being the hand closest to the meristematic dome of the male bud, Strosse et al., 2003) of each bud were inoculated and cultured in jars containing semi-solid callus induction medium (M1). The medium is composed of MS salts and vitamins (Murashige and Skoog, 1962) supplemented with 5.71 µM IAA, 18 µM 2, 4-D, 5.4 µM NAA, 4.1 µM biotin, 87 mM sucrose (Escalant et al., 1994), and 2 g/L Gelrite. The medium was adjusted to pH 5.7 before autoclaving at 120°C for 20 min. The cut surface of the explant hand base, with its two rows of IMFs, was placed in contact with 25 ml of M1 medium in 100 ml containers (baby food jars), and the jars were sealed with plastic film (Kleen-Pack). The cultures were kept in a growth room at 27 ± 2°C under total darkness and maintained without subculture for four months. The cultures were examined twice a week to record any visible changes in the appearance of the explants in culture. Following four months on M1, the percentages of embryogenic callus, yellow nodular callus, white compact callus and translucent callus formed were recorded after Strosse et al. (2003).

Embryogenic callus proliferation

After four months of culture, selected embryogenic calluses with expression of somatic embryos were placed on a modified proliferation medium based on Navarro et al. (1997), consisting of ½ strength MS salts supplemented with vitamins (Morel, 1950), 4.5 µM 2, 4-D, 200mg/L KH_2PO_4, 174 mM sucrose, and 2 g/L Gelrite. The medium was adjusted to pH 5.8. The culture containers were sealed and maintained under induction conditions, and the medium was refreshed each month.

Initiation and maintenance of the embryogenic cell suspension

For initiation of embryogenic suspension cultures, approximately 0.75 g fresh weight of embryogenic calluses with expression of early stage transparent embryos was transferred to 25 ml of proliferation medium (without the gelling agent and with full-strength MS salts and 87 mM sucrose) into 125 ml Erlenmeyer flasks. To allow for air exchange, the flasks were sealed with rubber stoppers with pre-drilled holes that were plugged with cotton. The cultures were agitated on an orbital shaker at 85 rpm in a growth room that was maintained at 27 ± 2°C in total darkness. The suspensions were filtered through a 380 µm pore size metallic mesh after the second week of culture, separating the primary calluses from the fine filtrate. Once the filtrate was recovered into a sterile 50 ml Falcon tube, after allowing the cells to sediment for 15 min, the settled cell volume (SCV) was measured. Two thirds of the liquid medium was refreshed, thereby maintaining ~ 3% SCV, as suggested by Strosse et al. (2006). The medium was changed every week during the first two months of establishment of the embryogenic suspension. Thereafter, the medium was changed every two weeks.

Embryo germination and plant regeneration

The somatic embryos were germinated after Navarro et al. (1997).

In addition, the regeneration capacities of two embryogenic suspension lines were tested by plating 0.2 ml of each embryogenic cell suspension (ECS) onto semi-solid maturation medium (MM) (25 ml in baby food jars), consisting of MS salts and vitamins supplemented with 0.43 µM kinetin, 0.23 µM zeatin, 87 mM sucrose, and 2 g/L Gelrite. Five replicates were plated for each line, and these were maintained for three to four weeks in darkness. The number of somatic embryos recovered per plated suspension was recorded by counting the embryos from each container before they were transferred to the germination medium. Next, the embryos were transferred to medium containing MS salts and vitamins supplemented with 87 mM sucrose, 2 g/L Gelrite and different combinations of 6-benzylaminopurine (BAP) and IAA, for one month. The combinations that were tested were GM1: 11.4 µM IAA and 2.22 µM BAP, GM2: 1.14 µM IAA and 0.22 µM BAP, GM3: 0.22 µM BAP and GM4: free growth regulator. The plantlets were rooted on medium that was composed of ½ strength MS supplemented with 87 mM sucrose, and 2 g/L Gelrite (pH 5.8) for one month. The germination and rooting cultures were incubated at 27 ± 2°C under total darkness for three days, afterwards using a 16-hour (60 $\mu E.m^{-2}s^{-1}$) photoperiod.

Experimental design and statistical analysis

To induce calluses, two independent experiments, each with three replicates were used. Each replicate consisted of 20 jars, and each jar contained 9 explants (IMF hands from positions 16-8). The germination experiment was carried out using somatic embryos from the 'Grand Naine' cultivar derived from two independent embryogenic cell suspension lines, using four replicates per treatment. Each replicate used ten somatic embryos per container. The experimental design for both experiments used a randomised complete block (RCBD). Analysis of variance (ANOVA) was carried out using the MSTAT-C statistical program (Nissen, 1984). The treatment means were compared using Duncan's multiple-range test.

Scanning electron and light microscopy

For scanning electron microscopy, the tissues, IMFs hands and embryogenic callus with well-developed embryoids were fixed overnight in FAA fixative solution (formalin: acetic acid: alcohol) at 4 ± 1°C. After this, the FAA fixative was exchanged twice with fresh FAA, using additional 4-h incubations at 4 ± 1°C. Then, the embryoids were dehydrated by washing in an ethanol/water series that progressed to absolute ethanol. The samples were dried using the critical-point method and coated with a thin layer of gold. The samples were observed and photographed using a Jeol JSM-6460 LC scanning electron microscope operating at 18 kV. Light microscopy micrographs were taken using a Carl Zeiss ICM-405 inverted microscope.

RESULTS

Effect of the explant source age on the response to somatic embryogenesis

Explants from IMF-hands (Figure 1a) of the *M. acuminata* AAA cultivars 'Williams' and 'Grand Naine' formed calluses on M1 medium after four months. This medium supported the growth and development of callus for the two cultivars and the two ages of buds. Four types of calluses were observed, that is, embryogenic callus, yellow nodular callus, white compact callus and translucent callus (Figures 1b-e, respectively). The percentage of explants that initiated the formation of callus depended more on the developmental stage of the male bud from which the explants were excised than on the cultivar. This is shown by the percentage of response estimated for each type of callus (Table 1). The total percentage of callus formed ranged from 97.81% in two-week-old explants of 'Williams' to 52.11% in two-week-old explants of 'Grand Naine'. The results showed that the age of explant source had the greatest effect on the percentage of embryogenic callus that was induced. That is, explants derived from two-week-old male buds of 'Williams' showed the highest percentage of embryogenic callus development (10.01 ± 0.19%), whereas explants derived from one-week-old buds of the same genotype yielded a significantly lower percentage of embryogenic callus development (0.78 ± 0.78%). The inverse relation was observed for 'Grand Naine'; explants from the one-week buds were associated with highest percentage of embryogenic callus growth (7.51 ± 0.19%), three times that of the two-week buds (2.49 ± 0.40%). Among the other callus types, the highest percentage was found in the yellow nodular callus that ranged from 51.39 to 42.08%, followed by translucent callus that ranges from 40.03 to 1.94% and white compact callus that ranged from 3.71 to 0.00% (Table 1). The analysis of variance showed highly significant differences ($p < 0.01$) between the genotypes for total callus and translucent callus formation, and between the two ages of the explant source in the development of embryogenic callus ($p < 0.01$) and white compact callus ($p < 0.05$). Moreover, the interaction between the genotype and age of the explant source was significantly different ($p < 0.01$) for the embryogenic callus, translucent callus and total callus, and significant ($p < 0.05$) for the yellow nodular callus produced.

Embryogenic callus proliferation

Cultures of 'Williams' and 'Grand Naine' were examined twice a week for further development; after four months on induction medium; the explants (Figure 1a) formed embryogenic and yellow nodular calluses that predominated in the cultures (Figures 1b - c). On transfer of the selected embryogenic callus onto proliferation medium, somatic embryos were produced. Some individual embryos separated from the embryogenic callus, cultured on proliferation medium, showed enhanced formation of secondary embryogenic callus after one month. White, compact and translucent calluses (Figures 1d, 1 left panel and 1e, respectively) were non-embryogenic and were eliminated from the cultures before the transfer to the proliferation medium. These calluses were not used during initiation of embryogenic suspension cultures.

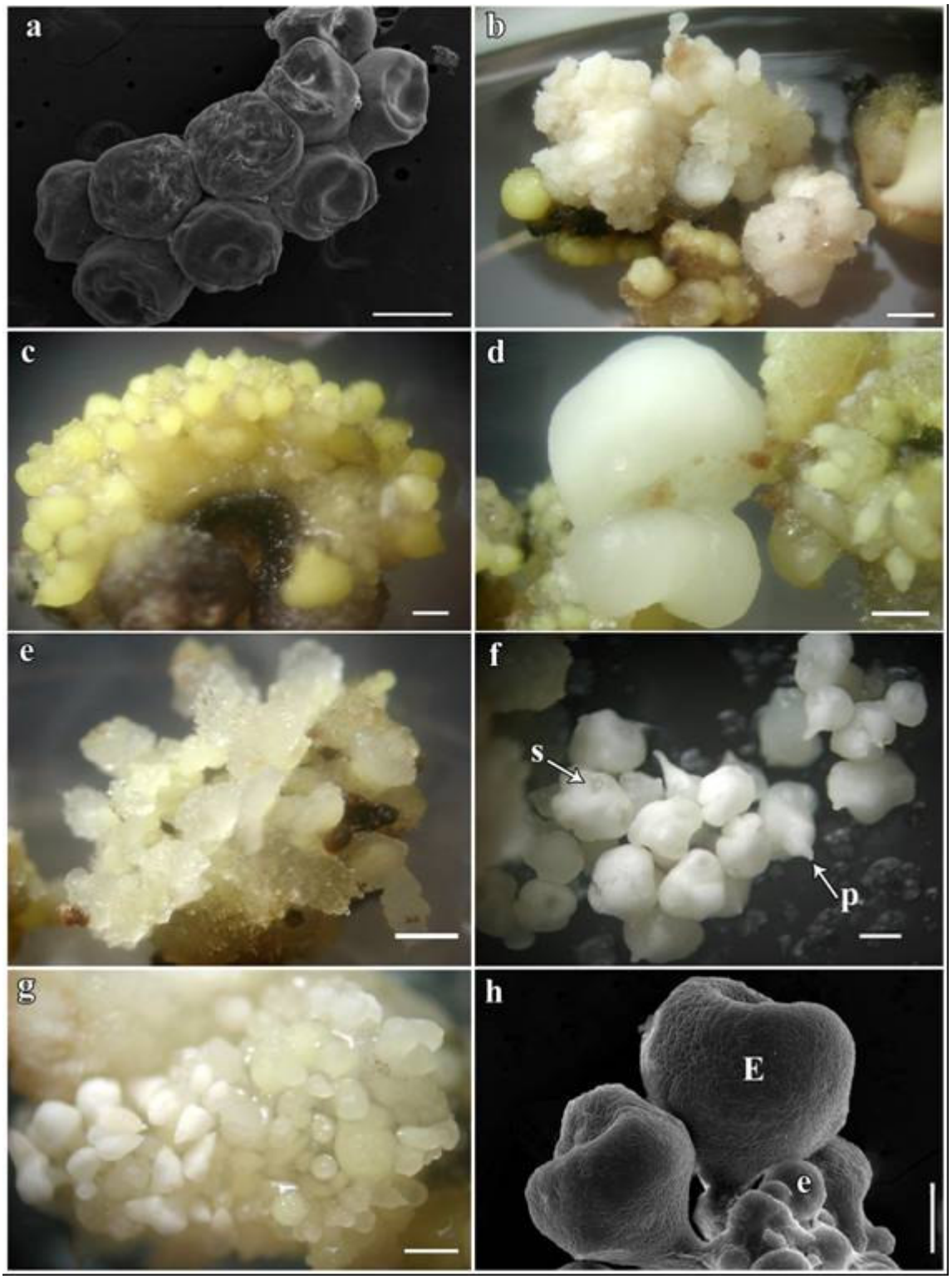

Figure 1. The somatic embryogenesis response of the initial explant (male flower primordia cultured on M1 medium) and differentiation of the somatic embryos on MM medium. (a) A scanning electron micrograph of the flower primordia. (b) an embryogenic callus with expression of embryos (c) yellow nodular callus (d) white compact callus (e) translucent callus (f) individual developed somatic embryos on MM; at this stage, the cotyledonary slit (s) and the plumule (p) which are emerging from some embryos, are visible (g) a cluster of developing somatic embryos at different stages (h) a scanning electron micrograph of well-developed somatic embryos (E) and numerous embryoids (e). Scale bars: (a, f and g) 500 µm, (b, c, d and e) 2.5 mm and (h) 200 µm.

Table 1. The effect of the genotype and age of the male bud floral explant (in weeks) on callus formation after four months in culture on induction medium.

Genotypes / age of MB	Percent of response as different callus types ± SE				Total callus
	Embryogenic	Yellow nodular	White compact	Translucent	
W* 1 week	$0.78^{D} \pm 0.78$	$51.39^{A} \pm 1.54$	$3.71^{A} \pm 0.71$	$21.83^{C} \pm 3.28$	$77.71^{B} \pm 0.73$
W 2 weeks	$10.01^{A} \pm 0.19$	$46.67^{AB} \pm 1.67$	$1.11^{AB} \pm 1.11$	$40.03^{A} \pm 0.78$	$97.81^{A} \pm 1.10$
GN* 1 week	$7.51^{B} \pm 0.19$	$42.08^{B} \pm 2.65$	$1.56^{AB} \pm 0.31$	$30.10^{B} \pm 2.11$	$81.25^{B} \pm 2.13$
GN 2 weeks	$2.49^{C} \pm 0.40$	$47.68^{AB} \pm 3.65$	$0.00^{B} \pm 0.00$	$1.94^{D} \pm 1.30$	$52.11^{C} \pm 5.96$

W* = 'Williams' cv.; GN* = 'Grand Naine' cv. Means within the columns that have the same letters are statistically similar (Duncan's multiple range test, $p < 0.05$). The values reported are the means ± standard error, SE (n = 3).

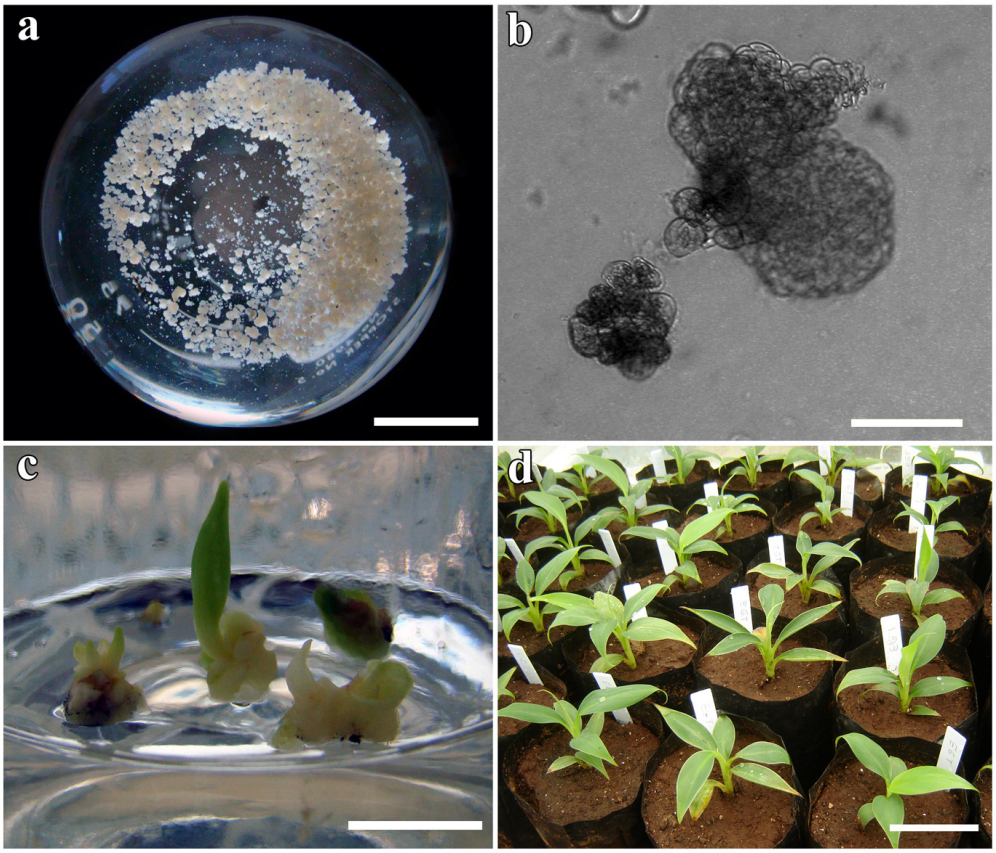

Figure 2. (a) The embryogenic cell suspension derived from 'Grand Naine' embryogenic calluses cultured in liquid medium, one month after the start of the culture. (b) ECS with cell aggregates. (c) the germination of somatic embryos on GM medium, showing shoot growth (*SG)* and root development (RD). (d) SE-regenerated banana plants. Scale bars: (a) 2 cm, (b) 100 µm, (c) 1 cm and (d) 6 cm.

Embryogenic cell suspension and plant regeneration

Cell suspensions, initiated with calluses like those depicted in Figure 1b and Figure 1g, were generated after about 6-8 weeks of culture. Fine ECS were recognised with high proportion (≥ 75 - 80%) of cell aggregates and pro-embryos (Figures 2a and b). The first sign of the formation of somatic embryos occurred approximately 3 - 4 weeks after the plating of ECS onto MM. This was revealed by an increase in size and development of the somatic embryos, and by the presence of the cotyledonary slit (Figure 1f, marked "s" in the figure). On the average, the suspensions yielded 1.2 x 10^5 embryos per ml of SCV. The emergence of the plumule in the embryos on MM (Figure 1f, marked "p" in the figure) was a clear early sign of the appropriate time for the transfer onto germination media (GM) (Figure 2c). On transfer of the embryos onto different GM media, the embryos germinated

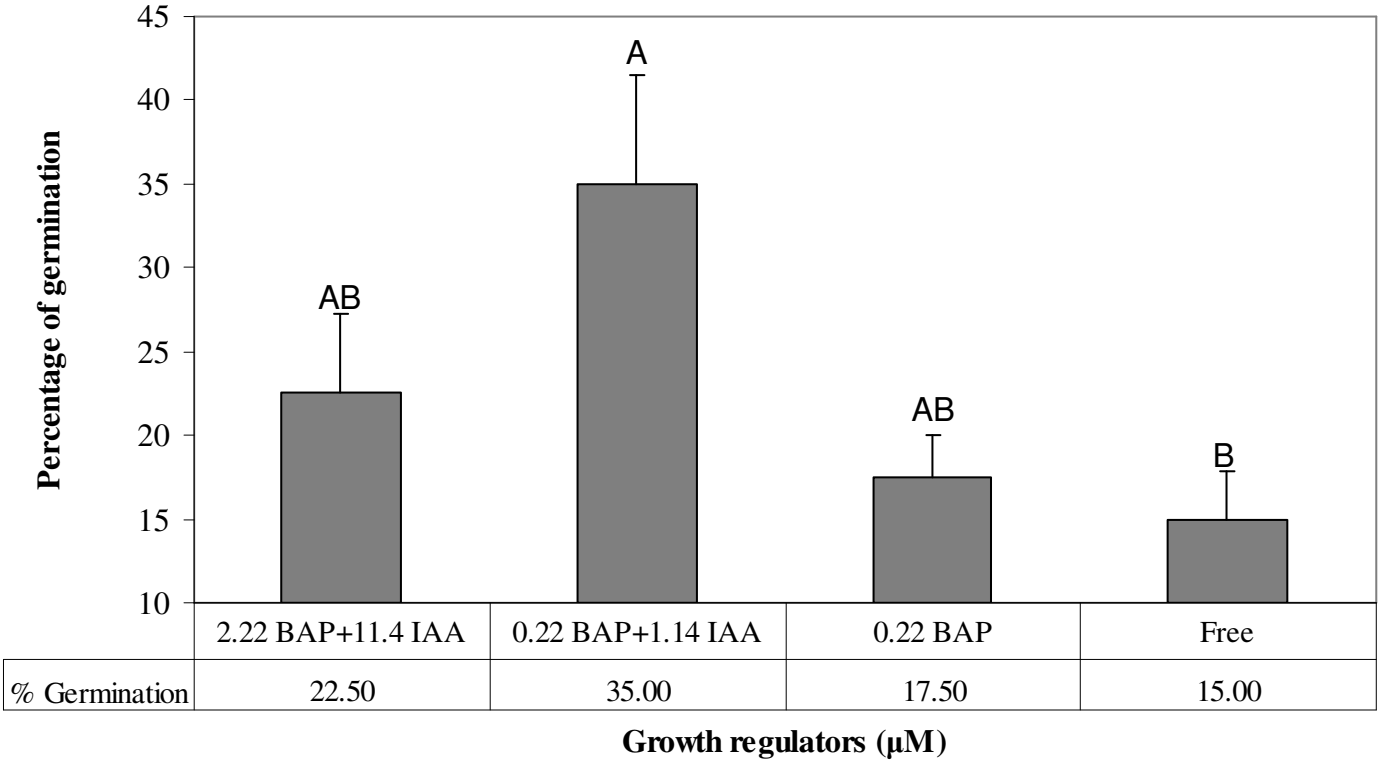

Figure 3. The average germination percentage of 'Grand Naine' somatic embryos on germination medium with different growth-regulator combinations. The bars with the same letters are statistically similar (using Duncan's multiple range test, $p < 0.01$). The columns and bars represent the mean values and the standard errors (n = 4), respectively.

after about one month. The percentage of germination ranged from 15 to 35%, depending on the combination of the growth regulators used (Figure 3). Thus, the average regeneration frequency for the 'Grand Naine' somatic embryos ranged from 1.67×10^4 to 3.9×10^4 plants per ml of the settled cells. These values were calculated for the germinated embryos on medium that was free of growth regulators (GM4) and GM2 (0.22 µM BAP and 1.14 µM IAA), respectively. There were highly significant differences ($p < 0.01$) in the percentage of germination between the growth regulator combinations. When the plant-growth regulators were omitted from the medium (GM4), the lowest percentage of embryo germination was observed (15%). This was followed by 17.5% for the GM3 medium (0.22 BAP). In contrast, GM1 medium (2.22 µM BAP and 11.4 µM IAA) showed an intermediate germination percentage (22.5%) and GM2 (0.22 µM BAP and 1.14 µM IAA) was the best (35%). Figure 2c illustrates the germination of somatic embryos on GM medium. In the figure, the more advanced embryo showed first the growth of a green shoot, then root development. After germination, the plantlets were transferred to rooting medium. Later rooted plants were transferred successfully (99%) to potting medium (peat moss). Figure 2d shows some of the regenerated *Musa* plants that were derived from somatic embryogenesis and ready for transfer to the field.

DISCUSSION

To our knowledge, this is the first report that addresses the effect of the age of the inflorescence male bud and its interaction with the genotype on the formation of embryogenic callus during the somatic embryogenesis induction phase in bananas. The evidence presented here supports the widely-held idea that these parameters play a critical role in the outcome and the progress of embryogenic competence that is triggered in the cells of the explant by the auxins that are present in the culture medium.

Using two *Musa* Cavendish AAA genotypes, we were able to demonstrate in this study that the maturation stage or age of the male bud, from which the explant was sourced, plays a key role during the earliest steps of the standardization of the somatic embryogenesis using the IMF-method in bananas. Of the two bud-ages investigated for the 'Williams' genotype, the explants derived from the two-week-old IMF-buds showed superior embryogenic callus formation to the one-week-old buds, indicating that matured explant sources formed more embryogenic callus than the younger ones in this genotype. The inverse relation was observed for 'Grand Naine'. These inverse responses to the age of the explant sources show that the effect is dependent on the genotype during initiation of the somatic embryogenesis process. The callus-induction responses found here for both Cavendish cultivars

are comparable to the levels reported using the IMF-method by others. For example, Escalant et al. (1994), found a variable 0-7% embryogenic response within five different banana and plantain genotypes, including *Musa* (AAA) 'Grande Naine'; Navarro et al. (1997), obtained a variable 2 - 6% embryogenic callus response for ''Grand Naine'' and Strosse et al. (2003) reported a mean percentage of ideal embryogenic callus of 8% for ''Grand Naine''. Moreover, our data is also within the range that has been described for the scalp-method for 'Williams' (10%, Xu et al., 2005) and other Cavendish-type bananas (0.1 to 6.2%). These observations support the opinion of Strosse et al. (2006) that both methods yield rather similar results once embryogenesis has been induced.

It is generally accepted that the successful culture of plant material *in vitro* is influenced greatly by the developmental age of the tissues or organs that are used as the source of the explants (George et al., 2008). Here, we show the effect of the ontogenetic age of the male bud of the inflorescence on the induction of somatic embryogenesis. It is reasonable to suggest that these results are attributable to the maturation age of the inflorescence bud, and not to effects arising from the position of the IMF-hands that were used for induction, as the experimental design minimised this possibility by using the same position (that is, flowers from the 8^{th} to 16^{th} position in each container). In addition, the environmental effects were minimised by using explants that had been collected simultaneously from both genotypes growing under similar cultural and field conditions. The IMF position effect on the formation of embryogenic callus in 'Grand Naine' has been established by Escalant et al. (1994) who reported that *ca*. 74% of the embryogenic clusters formed were on IMF positions 7 to 13, and that the embryogenic potential was affected not only by differences in the genotype, but also by seasonal effects. In agreement with this finding, Chong et al. (2005) reported that the predominant response occurred within flowers from the 8^{th} to the 15^{th} position. This positional effect is perhaps more related to the age of the explant tissue (in terms of its degree of differentiation, along a gradient in the organ) than to the maturation stage of the organ as judged by our results. Considering the outcome that has been discussed above, together with the significant ($p < 0.05$) difference that was found between the genotypes in terms of the total callus induction responses, this work suggests that the protocols that are used should be broad enough to capture the full range of possible genetic and developmental differences for each genotype. This is especially important because the availability of embryogenesis-competent explants is a common goal when establishing good quality *Musa* embryogenic suspension cultures. Therefore, to increase the initial number of good responsive explants, the first step in the protocol should be to identify the optimum window of competence for each explant-genotype and take advantage of factors that give the highest response. The regeneration competence of the embryogenic cell lines has been estimated from the average number of somatic embryos that are differentiated in the maturation media from the plated fixed volume and the average percentage of geminated embryos. The present data is comparable to that found previously in the literature. For example, Cote et al. (1996) reported an average of 3.7×10^5 embryos per ml of settled cell volume with a 3 - 20% germination percentage for *Musa* AAA, 'Grande Naine', Chong et al. (2005) reported an average of 3.0×10^5 embryos per ml with a 80.5% germination percentage for *Musa* AAA 'Grande Naine' and Xu et al. (2005) obtained an ECS yield of $0.92\text{-}2.17 \times 10^5$ embryos per ml with a 4.12 - 10.5% conversion frequency for *Musa* AAA 'Williams'.

The germination of somatic embryos and their conversion into vigorous plantlets depends on the history of the tissue in culture, the presence and type of growth regulators and the type of cell aggregates in the suspension. In this work, it was found that there was a twofold increase in germination when a proper combination of IAA and BAP was used. Comparable values of 13 to 25% embryo/plant conversion rates using GM1 have been reported by Navarro et al. (1997) for 'Grand Naine' (AAA), whereas, using the same genotype and a medium of the same composition as the GM2 combination, Cóte et al. (1996) reported a range of 3 to 20% germination percentage. In the latter work, the authors found that the rate of germination depended on the size of the embryos at the stage when they were transferred to the germination medium. Domergue et al. (2000) reported a range of 0 to 91.4% germination percentage, and this was dependent upon the type of cell aggregates from which the embryos were differentiated. Daniels et al. (2003), using a GM1 medium in combination with 0.01 mg/L Biobras-6 and the tetraploid cultivar FHIA-21 (AAAB), found a range from 36.25 to 81.25% germination percentage that depended on the cell density. More recently, the presence of abscisic acid (ABA) and BAP during the differentiation of the embryos in the maturation phase has been found to enhance germination up to 80% in a plantain (AAB) cultivar (Sholi et al., 2009). In summary, the precise role of different growth regulators such as IAA, BAP, brassinosteroids and ABA (together with other osmotic stressors) remains to be determined during the maturation of somatic embryos in different *Musa* genotypes.

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REFERENCES

Atak M, Kaya M, Khaear KM, Saglam S, Özcan S, Ciftci CY (2008). Effect of age on somatic embryogenesis from immature zygotic embryos of 5 Turkish triticale genotypes. Afr. J. Biotechnol. 7: 1765-1768.

Bakry F, Carreel F, Jenny C, Horry JP (2009). Genetic improvement of banana, In: Jain SM, Priyadarshan PM (eds) Breeding plantation tree crops: Tropical Species. Springer Science & Business Media, pp. 3-50.

Becker DK, Dugdale B, Smith MK, Harding RM, Dale JL (2000). Genetic transformation of Cavendish banana (*Musa* sp. AAA group) cv 'Grand Nain' via microprojectile bombrament. Plant Cell Rep. 19: 229-234.

Botti C, Vasil IK (1984). Ontogeny of somatic embryos of *Pennisetum americanum*. II. In cultured immature inflorescences. Can. J. Bot. 62: 1629-1635.

Burbulis N, Kupriene R, Liakas V (2007). Somatic embryogenesis and plant regeneration in immature zygotic embryos of *Brassica napus*. Acta Universitais Latviensis, 723: 27-35.

Chong B, Gómez R, Reyes M, Bermúdez I, Gallardo J, Freire M, Posada L, Herrera I, Swennen R (2005). New methodology for the establishment of cell suspensions of Grand Naine (AAA). InfoMusa, 14: 13-17.

Côte FX, Domergue R, Monmarson S, Schwendiman J, Teisson C, Escalant JV (1996). Embryogenic cell suspensions from male flower of *Musa* AAA cv. Grand nain. Physiol. Plant, 97: 285-290. doi: 10.1111/j.1399-3054.1996.tb08858.x.

Cronauer SS, Krikorian AD (1983). Somatic embryos from cultured tissues of triploid plantains (*Musa* 'ABB'). Plant Cell Rep. 2: 289-291

Cronauer-Mitra SS, Krikorian AD (1988). Plant regeneration via somatic embryogenesis in the seeded diploid banana *Musa ornata* Roxb. Plant Cell Rep. 7: 23-25.

Daniels D, Gomez R, Reyes M (2003). Plant regeneration system via somatic embryogenesis in the hybrid cultivar FHIA-21 (*Musa* sp. AAAB group). In vitro cell Dev. Biol. Plant, 38: 330-333.

Dhavala VN, Tejeswara RD, Yechuri VR, Prabavathi K (2009). Effect of explant age, hormones on somatic embryogenesis and production of multiple shoot from cotyledonary leaf explants of *Solanum trilobatum* L. Afr. J. Biotechnol. 8: 630-634.

Dhed'a D, Dumortier F, Panis B, Vuylsteke D, De Langhe E (1991). Plant regeneration in cell suspension cultures of the cooking banana Bluggoe cultivar (*Musa* sp. ABB group). Fruits, 46: 125-135.

Domergue F, Ferriére N, Côte FX (2000). Morphohistological study of the different constituents of a banana (*Musa* AAA, cv. Grande naine) embryogenesis cell suspension. Plant Cell Rep. 19: 748-754.

Doreswamy SR, Sahijram L (1989). Micropropagation of banana from male floral apices cultured *in vitro*. Sci. Hortic. 40: 181-188.

Escalant JV, Teisson C (1989). Somatic embryogenesis and plants from immature zygotic embryos of the species *Musa acuminata* and *Musa balbisiana*. Plant Cell Rep. 7: 665-668.

Escalant JV, Teisson C, Côte FX (1994). Amplified somatic embryogenesis from male flowers of triploid banana and plantain cultivars (*Musa* Sp.). In Vitro Cell Dev. Biol. Plant, 30: 181-186.

George EF, Hall MA, De Klerk G-J (2008). Plant propagation by tissue culture. 3th Edition, Volume 1. The Background. Springer. The Netherlands, p. 551.

Grapin A, Ortiz JL, Lescot T, Ferriére N, Côte FX (2000). Recovery and regeneration of embryogenic cultures from female flowers of False Horn Plantain. Plant Cell Tissue Organ Cult. 8: 237-244.

Grapin A, Schwendiman J, Teisson C (1996). Somatic embryogenesis in plantain banana. In Vitro Cell Dev. Biol. Plant 32: 66-71.

Ma SS (1991). Somatic embryogenesis and plant regeneration from cell suspension culture of banana. In: Department of Agriculture, National Taiwan University (eds) Proc. Symp. Tissue Cult. Hortic. Crops, pp. 181-188. National Taiwan University, Taipei, Taiwan.

Marroquin CG, Paduscheck C, Escalant JV, Teisson C (1993). Somatic embryogenesis and plant regeneration through cell suspensions in *Musa acuminata*. In Vitro Cell Dev. Biol. 29: 43-46.

Martin KP (2003). Plant regeneration through direct somatic embryogenesis on seed coat explants of cashew (*Anacardium occidentale* L.). Sci. Hortic. 98: 299-304. doi: 10.1016/S0304-4238(03)00005-0.

Molina DM, Aponte ME, Cortina H, Moreno G (2002). The effect of genotype and explant age on somatic embryogenesis of coffee. Plant Cell Tissue Organ Cult. 71: 117-123.

Morel G (1950). Sur la culture des tissus de deux monocotylédones. CR Acad Sci. Paris, 230: 1099-1101.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15: 473-497.

Navarro C, Escobedo RM, Mayo A (1997). *In vitro* plant regeneration from embryogenic cultures of a diploid and a triploid, Cavendish banana. Plant Cell Tissue Organ Cult. 51: 17-25.

Neumann KH, Kumar A, Imani J (2009). Plant cell and tissue culture- A tool in Biotechnology: Basics and Application. Springer-Verlag, Berlin Heidelberg.

Nissen O (1984). MSTAT. A microcomputer program for statistical analyses of experiments and surveys. In: Riley H, Skjelvag AO (eds). The impact of climate on grass production and quality. Proc. General Meet Eur. Grassl Fed, 10th, As, Norway. 26-30 June 1984. Norwegian State Agric. Res. Stations, As. pp. 555-559.

Novak FJ, Afza R, Van Duren M, Parea-Dallos M, Conger BV, Xiaolang T (1989). Somatic embryogenesis and plant regeneration in suspension cultures of dessert (AA and AAA) and cooking (ABB) bananas (*Musa* sp.). Biotechnology, 7: 154-159.

Parrot W (1993). Cell-Culture techniques. In: International network for the improvement of banana and plantain (ed.). Proceedings of the workshop on Biotechnology applications for banana and plantain improvement. San Jose, Costa Rica, INIBAP 1993. pp. 183-191.

Pérez-Hernández JB, Rosell-Gracía P (2008). Inflorescence proliferation for somatic embryogenesis induction and suspension-derived plant regeneration from banana (*Musa* AAA, cv. Dwarf Cavendish) male flowers. Plant Cell Rep. 27: 965-971. doi: 10.1007/s00299-008-0509-x.

Sholi NJY, Chaurasia A, Agrawal A, Bhalla SN (2009). ABA enhances plant regeneration of somatic embryos derived from cell suspension cultures of plantain cv. Spambia (*Musa* sp.). Plant Cell Tissue Organ Cult. Online first, doi:10.1007/s11240-009-9585-z.

Simões-Costa MC, Carapuca E, Moura IR (2009). Somatic embryogenesis induction in different genotypes of *Coffea* sp. Acta Hortic. 812: 295-299.

Strosse H, Domergue R, Pains B, Escalant JV, Côte F (2003). Banana and plantain embryogenic cell suspensions. INIBAP Technical Guideline 8, In: Vézina A, Picq C (Eds). International Network for the Improvement of Banana and Plantain, Montpellier, France.

Strosse H, Schoofs H, Panis B, Andre E, Reyniers K, Swennen R (2006). Development of embryogenic cell suspensions from shoot meristematic tissue in bananas and plantains (*Musa* sp.). Plant Sci. 170: 104-112. doi: 10.1016/j.plantsci. 2005.08.007.

Wirakarnain S, Hossain ABMS, Chandran S (2008). Plantlet production through development of competent multiple meristem cultures form male inflorescence of banana, *Musa acuminata* cv. 'Pisang Mas' (AA). Am. J. Biochem. Biotech. 4: 325-328**.**

Xu CX, Pains B, Strosse H, Li HP, Xiao HG, Fan H, Swennen R (2005). Establishment of embryogenic cell suspensions and plant regeneration of the dessert banana 'Williams' (*Musa* AAA group). J. Hortic. Sci. Biotechnol. 80: 523-528.