

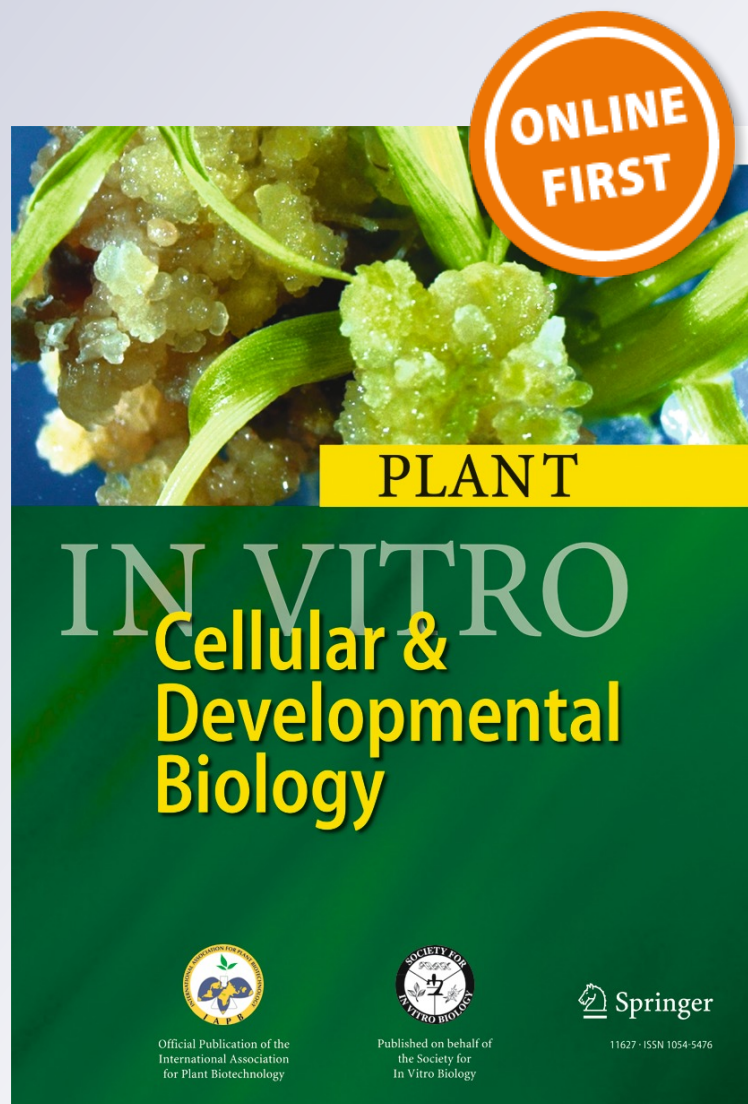
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**Priver Namanya, G. Mutumba,
S. M. Magambo & W. Tushemereirwe**

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Developing a cell suspension system for *Musa*-AAA-EA cv. ‘Nakyetengu’: a critical step for genetic improvement of Matooke East African Highland bananas

Priver Namanya · G. Mutumba · S. M. Magambo · W. Tushemereirwe

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Abstract Embryogenic cell suspensions of triploid East African Highland bananas (*Musa* AAA-EA) were initiated and generated using cooking cultivar ‘Nakyetengu’ belonging to the Nakabululu clone set. Immature male flowers produced embryogenic calli consisting of embryos and friable tissue after 4 mo culture on a modified MA1 callus induction medium. Friable calli were initiated and maintained in liquid MA2 medium. A cell growth rate of 1.5–2.0 sedimented cell volume (SCV) per month was observed. Embryo development was observed at 2.18×10^3 embryos per mL SCV. Germination of these embryos was observed at 2.8% and 6.2% for two cell suspension lines. Plant regeneration efficiency was 60–100%, all producing normal plants with a shoot and roots at weaning. In the field, somatic cell-derived plants were all normal morphology and comparable to control plants during vegetative and reproductive stages. This study is a breakthrough for recalcitrant East African Highland banana and offers a system that can provide essential raw materials for associated germplasm improvement through genetic engineering approaches.

Keywords Embryogenic cell suspensions · East African Highland bananas · Somatic embryogenesis

Introduction

Bananas (*Musa* spp.) are believed to have originated in South-East Asia where the best known wild parents of cultivated varieties are found. The cultivated edible varieties were derived from natural hybridization between the two seeded wild diploid parents *Musa acuminata* (A genome) and *Musa balbisiana* (B genome). Domesticated varieties are mainly triploids whose genomic make up is commonly AAA, AAB, or ABB (Simmonds and Shepherd 1995). Genotype AAB form the majority of plantains, ABB are cooking bananas (Bluggoe type), and AAA are mainly dessert bananas (Cavendish group). Outside South-East Asia, cultivation of bananas spread to different parts of the world in the tropical and subtropical regions resulting in two major secondary centers of banana diversity. One center is located in West and Central Africa where farmers predominantly cultivate plantains. The other is found in East and Central Africa where a unique type of cooking banana the East African Highland banana (EAHB; *Musa* spp. AAA-EA) is cultivated (Karamura 1998; INIBAP 2004). EAHB are mainly used as a staple food in Uganda, Rwanda, Burundi, regions of the Democratic Republic of Congo, Kenya, and Tanzania, with almost all the bananas produced being consumed domestically.

All the domesticated edible banana cultivars such as EAHB are triploid selections by farmers from natural hybrids that have been vegetatively propagated over many years. These are essentially sterile (seedless) or have extremely low fertility (Ssebuliba *et al.* 2006). This implies that, other than somatic mutations, they are clones identical to original collections and have undergone limited genetic variation from their ancestors (Karamura 1998). Their limited genetic diversity contributes to susceptibility to both biotic and abiotic stresses such as devastating pest and disease pressures and drought. In addition, due to near clonal uniformity, EAHB cultivars have very low micronutrient content, in particular vitamin A and

P. Namanya (✉) · W. Tushemereirwe
National Agricultural Research Laboratories, NARL-NARO,
P.O. Box 7065, Kampala, Uganda
e-mail: priver@kari.go.ug

G. Mutumba
School of Biosciences, College of Natural Sciences, Makerere
University, P.O. Box 7062, Kampala, Uganda

S. M. Magambo
Crop Science, Makerere University, P.O. Box 7062, Kampala,
Uganda

minerals such as iron. Unfortunately, genetic improvement of bananas through conventional breeding is hampered by limited genetic diversity, inherent biological and genetic characteristics of the plant, and long cropping cycles. However, applying biotechnology techniques in particular, genetic engineering, offers an alternative strategy to improve the infertile and poor seeding cultivars for traits such as resistance to biotic and abiotic stresses, producing higher yielding varieties with increased nutritional content, shortened maturity period, and improved post-harvest qualities.

Successful application of genetic transformation technology in plants requires an efficient *in vitro* regeneration system for producing true-to-type plants from regenerable target tissue. In the case of banana, somatic embryogenesis and cell suspension cultures have made it possible to obtain plants *in vitro* (Escalant and Teisson 1989; Novak *et al.* 1989). Somatic embryogenesis has two objectives: the development of micropropagation techniques and cell regeneration systems. The main characteristic of such a system is that normal true-to-type plants can be regenerated from the explant tissue at high frequency (Sagi *et al.* 1995), which has been achieved using cell or protoplast cultures derived from meristems (Novak *et al.* 1989; Panis *et al.* 1993; Schoofs 1997), zygotic embryos (Escalant and Teisson 1989), young male flower tissue (Escalant *et al.* 1994; Côte *et al.* 1996; Grapin *et al.* 1998; Becker *et al.* 2000; Becker and Dale 2004; Meenakshi *et al.* 2011), and AAB false horn plantains (Grapin *et al.* 2000). Although different explants have been used to regenerate banana plants *in vitro*, these procedures remain genotype specific (Vuylsteke *et al.* 1998). In addition, most banana transformation systems use embryogenic cell suspensions due to high regeneration efficiency and chances of generating true-to-type plants through somatic embryogenesis (Ganapathi *et al.* 2001; Khanna *et al.* 2004; Ghosh *et al.* 2009).

While development of cell suspensions and subsequent regeneration has been demonstrated for a few *Musa* spp. including AAA triploids (Cavendish and plantains), previous attempts to induce somatic embryogenesis from proliferating meristems of EAHB cultivars were not successful (Nahamya 2001). This paper presents the first reports for the induction of somatic embryogenesis and regeneration of true-to-type banana plants from embryogenic cell suspensions established from immature male flowers of EAHB cv. 'Nakyetengu'.

Material and Methods

Induction of embryogenic callus. Four cultivars representing the four clone sets of *Musa* spp. AAA-EA cooking banana group were selected for screening to identify which of these infertile varieties were able to produce embryogenic cells. Cultivars tested were 'Nakyetengu' belonging to the Nakabululu clone set, 'Namwezi' of Nfuuka clone set,

'Nakitembe' of Nakitembe clone set, and 'Kisansa' of Musakala clone set (Karamura 1998). Immature male flowers were used as explants. Ten male buds of each cultivar were harvested from growing plants in the field after bunch formation. Bracts were removed up to a final size of about 1.5 cm width and 2 cm length. These were disinfected in 70% (v/v) ethanol for 2–3 min and then rinsed in sterile-distilled water. Immature flowers (hands) were aseptically isolated from positions 8 to 15 under a binocular microscope in laminar flow hood. The flowers (eight flowers per bud) were inoculated on Murashige and Skoog (MS)-based callus induction medium optimized for *Musa* AAA-EA (Namanya 2003; Namanya *et al.* 2004) in 90×15-mm Petri dishes. The medium consisted of standard MS salts and vitamins (Murashige and Skoog 1962), supplemented with 1 mg/L biotin, 1 mg/L indole acetic acid (IAA), 1 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), 1 mg/L naphthalene acetic acid (NAA), 20 mg/L ascorbic acid, 3% (w/v) sucrose, and 7.2 mg/L agarose. The pH was adjusted to 5.8 with 1 N NaOH and 1 N HCl before autoclaving. Culture dishes were sealed with Parafilm™ to avoid contamination, placed in the dark at 28±1°C and 67% relative humidity, and monitored for 4–6 mo for embryogenic callus formation. All male buds were obtained from a banana plantation at National Agricultural Research Laboratories—Kawanda, Wakiso District, Central Uganda, and *in vitro* experiments were conducted in the tissue culture laboratory at the same institute.

Initiation of cell suspension cultures. The embryogenic callus was cultured in liquid medium MA2 (Côte *et al.* 1996). This consisted of MS salts and vitamins supplemented with 4.5 µM 2,4-dichlorophenoxyacetic, 100 mg/L glutamine, 100 mg/L malt extract, and 45 g/L sucrose. The pH was adjusted to 5.3 with 1 N NaOH and 1 N HCl before autoclaving. Three cell suspension lines 11AA, 12AA, and 12BB were initiated in 10 mL of MA2 in 25-mL Erlenmeyer flasks. These were placed on a rotary shaker at 80 rpm in the dark at 28±1°C. Flasks were sealed with sterile aluminum foil and Parafilm™ to prevent contamination. Culture medium was refreshed twice each week in the first month and once every 10 d thereafter. Observations for cell growth and proliferation were made using an inverted microscope (Zeiss Axiovert 25 Inverted microscope, Carl Zeiss, Regensburg, Germany). In order to maintain a clean culture, blackened or discolored cell clusters were removed using a sterile pipette. All images were taken using a zoom digital camera (Mavica, MVC-FD 75, Sony, Tokyo, Japan).

Embryo growth and development. To determine the embryogenic potential of the cell suspension cultures, samples from the suspension cultures were plated periodically. Cells were placed on regeneration medium MA3 (Côte *et al.* 1996) comprised of half-strength MS salts, MS vitamins, 20 mg/L

ascorbic acid, 30 g/L sucrose, and 100 mg/L *myo*-inositol and solidified with 2.3 g/L Phytagel™. The pH was adjusted to 5.3 with 1 N NaOH and 1 N HCL before autoclaving. Sterile Whatman™ filter paper was placed on the semisolid media in Petri dishes and 1 mL of cell suspension containing 0.1 mL sedimented cell volume (SCV) spread over the top. These were incubated in the dark at 28±1°C and monitored for embryo development. The time taken (number of d) to embryo formation and maturation was recorded. Observations were made using a stereomicroscope (Axiostar Leica, Jena, Germany). The quality of embryo formation was scored according to the characteristics codes in Table 1.

Embryo germination and plant regeneration. Somatic embryos regenerated from cell suspension cultures were transferred to germination medium MA4 (Escalant *et al.* 1994) which consisted of standard MS salts supplemented with Morel vitamins (1 mg/L calcium panthotenate, 100 mg/L *myo*-inositol, 1 mg/L nicotinic acid, 1 mg/L thiamine, 1 mg/L pyridoxine, and 0.01 mg/L biotin) (Morel and Wetmore 1951), 0.22 µM benzylaminopurine (BAP), 1.14 µM IAA, and 87 µM sucrose and solidified with 7.2 g/L agarose. Embryo germination was determined as the number of embryos with either shoot or root and shoot development. Percent embryo germination was recorded for the different cell lines. Plant development (root and shoot elongation) was achieved by transferring germinated plantlets onto MS medium free of plant growth regulators and incubating at 28 28±1°C with 14/10-h light/dark photoperiod. The time taken (number of d) to achieve root development and shoot elongation was recorded. Plants with sufficient roots and at least four open leaves were acclimatized in the nursery for preliminary evaluation and observation for somaclonal variation before planting in the field.

Field evaluation of plants derived from somatic embryos involved counting the number of suckers, measuring leaf length and width, plant height, stem girth, total number of leaves at flowering, flowering time, fruit bunch weight, and number of hands per bunch. The field was established at the National Agricultural Research Laboratories, Kawanda,

Uganda in a completely randomized block design, randomized individually at each plot; each treatment had eight lines which were replicated four times.

Data collection. Embryogenic callus formation was recorded as percent response. This was taken as the number of hands (with embryogenic callus) out of the total number of flowers used. Somatic embryo germination was recorded as the number of embryos with root and/or shoot formation from the total number of embryos cultured (Strosse *et al.* 2003). Agronomic data from the field trial was analyzed for variance using SAS 9.0, 2002 (SAS institute Inc., Cary, NC). In addition, means were separated by Duncan's multiple range tests using the SAS.

Results

Embryogenic callus production and subsequent initiation of embryogenic cell suspensions (ECS). The results describe callus induction, initiation, and establishment of cell suspensions and plant regeneration of *Musa* spp. EAHB cultivar 'Nakyetengu'. No embryogenic response was obtained from the other three cultivars 'Namwezi', 'Nakitembe', and 'Kisansa' tested in this study. Embryogenic callus was obtained from immature male flowers of cultivar 'Nakyetengu' after 4 mo of culture on callus induction medium optimized for this variety. The flowers differentiated mainly into yellow callus and white compact tissue. By 4 mo, well-developed embryos were observed attached to colorless friable tissue (Fig. 1A). Embryogenic callus was observed on 2/80 flowers derived from ten buds representing a 2.5% embryogenic response. Out of the two calli observed, one had friable tissue. Upon transfer into liquid medium MA2 for cell suspension initiation, the friable tissue dispersed into cell clusters with dense cytoplasm and proliferating edges (Fig. 1B; Georgett *et al.* 2000; Pere *et al.* 2001). In the first month after initiating the liquid culture, some embryogenic cells were still attached to groups of young embryos. However, with continued agitation on a rotary shaker, the cells detached from the embryos. The embryos gradually turned into spherically shaped type IV cells opaque to light with loose vacuolated cells at the periphery (Fig. 1C) as described by Georgett *et al.* (2000). In order to keep a healthy cell suspension culture, type IV cells (which usually release empty, non-embryogenic cells into the culture) were regularly removed. Over a period of 4.5 mo, an average cell growth rate of 1.5–2.0 SCV per month was achieved. The cell volume increased sixfold during this period (Fig. 2).

Embryo formation and germination. The initial signs of embryo formation from the ECS (characterized by translucent structures) were observed 3 d after transferring the cells to embryo formation media. Clear embryos were visible after 7 d

Table 1. Quality codes for embryos regenerated from embryogenic cell suspensions

Quality code	Characteristics
0	All the plate is black and nothing is growing
1	Only very limited number of embryos, a lot of black spots
2	A few well-developed embryos and embryo clusters, some embryos may have aborted, blackening less than in "1" (above)
3	Almost all embryos well developed irrespective of their size

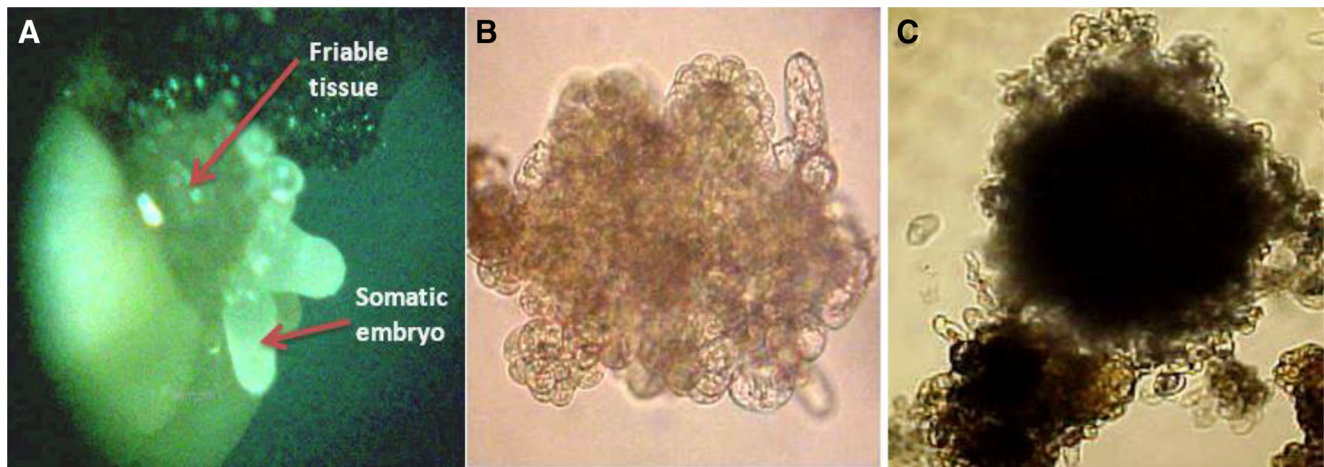


Figure 1. Embryogenic tissues of cultivar 'Nakyetengu' showing (A) embryogenic callus; (B) a proliferating embryogenic cell cluster; and (C) cell type IV in culture.

of culture. At the end of 30 d, the embryos were fully developed in shape, size, and color (Fig. 3). The quality of the plated embryos, an indication of the embryogenic potential of the cell suspension, was rated according to the description given in Table 1. The number of embryos obtained from 1 mL SCV after 1.5–2.5 mo on regeneration medium was approximately 2.10×10^3 for cell line 12AA and 2.18×10^3 for cell line 12BB. The quality of the embryos initiated from proliferating cell suspension cultures ranged from 1 to 3 (Table 1 and Fig. 3). When somatic embryos were cultured on embryo germination medium, they increased in size and developed small root hairs within 7 to 10 d of culture. Germination with a clear root and shoot structure was complete after 14 d (Fig. 4A).

Two types of germination were observed. In the first type, roots and shoots developed as part of a single germination event (Fig. 4A). In the second type, only a shoot or a single leaf was observed (Fig. 4B, C). However, when transferred to MS media free of plant growth regulators, normal plantlet development, *i.e.*, shoot elongation and root development, was achieved from both forms of germinating embryos at

varying percentages. After 2–3 mo, the plantlets had at least four open leaves and were ready for acclimatization (Fig. 4D). Some embryos, however, formed non-embryogenic callus, and others formed only roots without shoots. From a total of 420 mature embryos, the percentage of somatic embryo germination was $2.8 \pm 1.2\%$ and $6.2 \pm 2.3\%$ for embryogenic cell suspension lines 11AA and 12AA, respectively. However, some individual plates had up to 16.7% and 21% embryo germination for 11AA and 12AA, respectively. Recovery of normal plants from germinating embryos was 60% and 100% for cell lines 11AA and 12AA, respectively.

Subsequent plantlets derived from these cell suspensions were all normal at nursery stage through to the field with no somaclonal variations being observed. In the field, during the vegetative and reproductive stages, plants derived from somatic embryos showed morphological growth patterns similar to those of the control plants derived from suckers (Figs. 5 and 6). All plants were morphologically normal and completed the development stages of the cropping cycle of flowering and bunch maturation. No significant differences were observed ($P \leq 0.05$) between control plants derived from suckers and somatic embryo-derived plants for number of suckers, leaf length/width, total number of leaves at flowering, bunch weight, or number of hands per bunch (Table 2 and Figs. 5 and 6). Statistical differences were, however, obtained between controls and plants derived from 12AA, but not 11AA, for flowering time and stem girth. Similarly, there were statistical differences between controls and plants derived from 11AA, but not 12AA, for plant height.

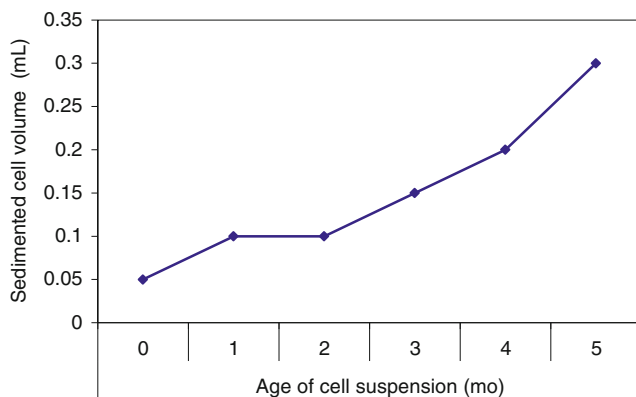


Figure 2. Cell growth rate of cell suspension line 12AA of cv. 'Nakyetengu'.

Discussion

The results presented confirm the initiation and regeneration of embryogenic cell suspensions from ideal callus induced

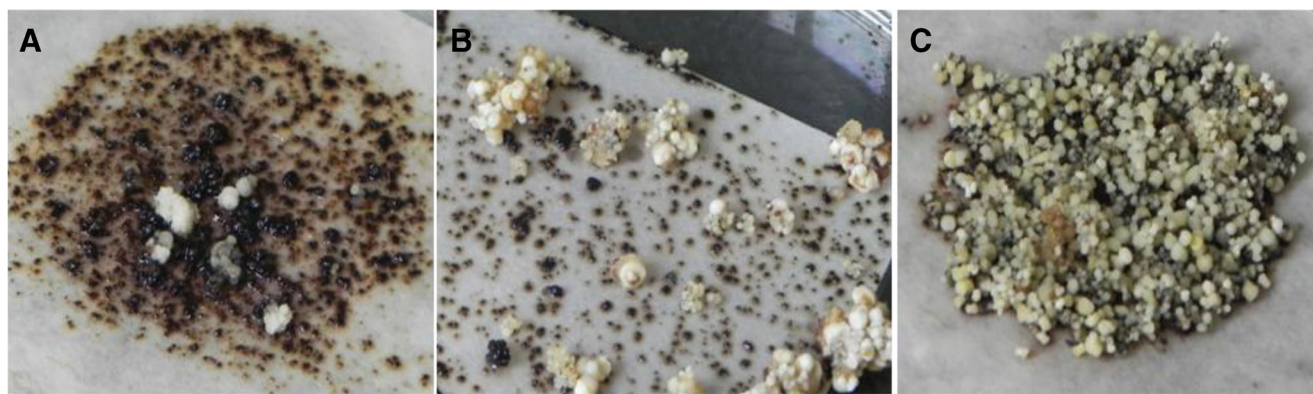


Figure 3. Embryo development showing different qualities of somatic embryos generated from 0.1 mL SCV per plate. (A) Quality 1; (B) Quality 2; (C) Quality 3 (refer to Table 1 for quality descriptors).

from immature male flowers of *Musa* AAA-EA highland cultivar 'Nakyatengu'. Embryogenic callus with friable tissue and somatic embryos was ideal for initiation of cell suspensions. When transferred to liquid media, the cells dispersed into multiple cell clusters with proliferating edges characteristic of multiplying cell suspensions. The cell suspensions initiated had a cell growth rate of 1.5–2.0 SCV per mo. This was comparable to two- to fivefold packed cell volume monthly growth rate reported for Cavendish (Côte *et al.* 1996) and also commonly observed in embryogenic cell suspensions of other monocotyledons. The starting volume to initiate the ECS of 'Nakyatengu' was 0.05 mL. At 4.5 months, a cell growth of up to sixfold was obtained. Larger cell aggregates tended to differentiate into embryos. According to Schoofs *et al.* (1999),

such cell clusters tend to accumulate starch in the outer cells thereby producing high amounts of polyphenols and reducing the quality of the cell suspension culture overall. This was also shown by the low quality of embryos (Fig. 3A, B) obtained from such cells when tested for embryo formation and could be attributed to oxidation/blackening, which was encountered in the cell suspension lines used. However, low cell suspension quality may also result from prolonged cell culture periods.

Musa spp. AAA-EA bananas exhibit high levels of phenolic compounds from leaf and corm tissue often observed during shoot tip *in vitro* micropropagation or scalp cultures (Nahamya 2001). Studies on cytotoxicity and antiproliferative activities of phenolic compounds conducted on plant cells suggest a modest correlation between their oxidative nature and their antiproliferative effects (Yáñez *et al.* 2004). Based on these findings, it is probable that the phenolic compounds in banana tissues contribute to retarding tissue dedifferentiation into callus and also slowing down the proliferation rate of cell suspensions. This could possibly explain the slow growth and subsequent cell oxidation observed with the banana cell lines generated in this study. Therefore, there is a need to identify the nature of these compounds and their effect on cell viability and understand the role they play in hampering generation of ECS from different cultivars.

The ECS were regenerable into somatic embryos with approximately 2.2×10^3 embryos obtained from 1 mL SCV. Reports by Grapin *et al.* (2000) and Côte *et al.* (1996) on *Musa* spp. obtained approximately 1×10^5 to 5×10^5 and 3.7×10^5 embryos using 1 mL SCV from both female flowers of false horn plantain and male flowers of Cavendish banana. The differences in reported regeneration capacity appear to reflect the effect of cultivar dependency and have been reported to arise from differences in the physiological state that is characteristic of cell suspensions. In this study, callus was obtained from only one of four cultivars tested. In related somatic embryogenesis studies on banana (Gamborg *et al.* 1995; Grapin *et al.* 2000; Côte *et al.* 1996), varied responses

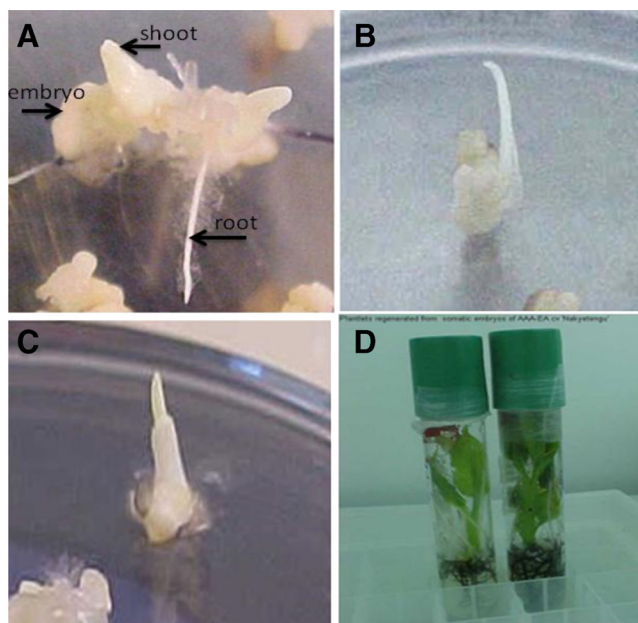


Figure 4. Germination of somatic embryos derived from embryogenic cell suspensions of banana cultivar 'Nakyatengu'. Embryos with (A) shoot and root; (B) leaf only; and (C) shoot only. (D) Plantlets ready for weaning 3 mo after germination.

Figure 5. Morphological appearance of suckering and vegetative stage of somatic-derived plants of ‘Nakyetengu’ showing mats of (A) somatic embryo-derived plant and (B) sucker-derived plant (control).



to specific induction media were attributed to cultivar dependency. Furthermore, although totipotency is an important characteristic of cells cultured *in vitro*, under specific conditions, only a limited fraction of the cells within populations or tissue type are totipotent (Quiroz-Figueroa *et al.* 2006). Somatic cells undergo restructuring through an embryogenic pathway involving dedifferentiation of cells, activation of cell division, and reprogramming of their physiology, metabolism, and gene expression patterns in order to generate embryogenic cells (Yang and Zhang 2010). In *Arabidopsis* for example, *in vitro* organogenesis is likely to be regulated by ecotypes

through mediating endogenous hormonal biosynthesis (Zhao *et al.* 2013). Therefore, we can conclude that although each of the banana cultivars tested in this study belong to the same genomic group AAA-EA (*Musa* spp.), the various factors influencing the embryogenic pathway likely explain the differences observed in their potential to form embryogenic cells. It has also been established that coordination of cell division and morphogenesis is linked to a wide variety of extracellular complex compounds that cells release into liquid medium including polysaccharides, amino acids, and low and high molecular weight compounds (Quiroz-Figueroa *et al.* 2006).

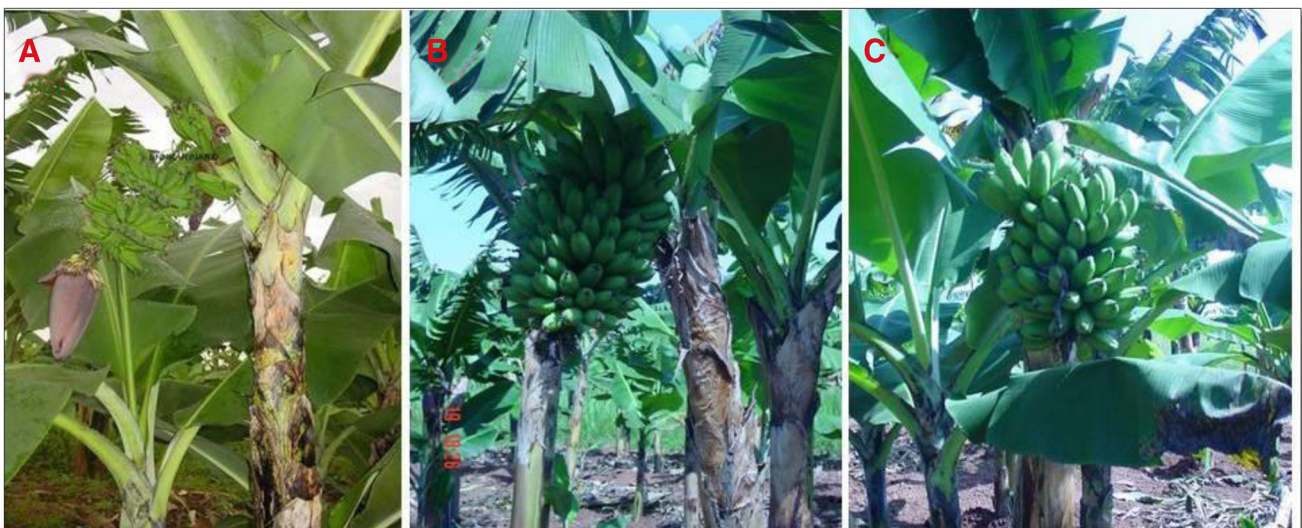


Figure 6. Reproductive stage of somatic embryo-derived banana plants of East African Highland banana cv. ‘Nakyetengu’ showing (A) first wk of flowering with normal fruit emergence; (B) plant derived from somatic embryos; and (C) plant derived from suckers (control plant).

Table 2. Vegetative and reproductive characteristics of somatic embryo derived plants for banana cv. 'Nakyetengu'

Genotype	Flowering time (mo)	No. of suckers	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	No. of leaves	Bunch weight (kg)	No. of hands	Stem girth (cm)
Control	16.5a	3.0a	201.8a	57.4a	164.0b	6.3a	6.5a	5.8a	49.5a
11AA	16.9ab	2.8a	171.5b	54.3a	185.9a	6.1a	7.3a	5.6a	46.2ab
12AA	18.3b	2.5a	168.6b	54.2a	169.9ab	6.3a	6.4a	5.6a	44.7b

Means with the same *letter* in the same *column* are not significantly different at $P \leq 0.05$

Such compounds will need to be investigated in the future to understand cell suspensions of East African Highland banana varieties.

In order to achieve embryo formation, auxin was removed from the medium during the plating stage. This was previously reported to be useful for completion of embryo development (Zimmermann 1993). It is believed that when embryos are exposed to too much auxin during development, they fail to accumulate storage proteins and this leads to low germination frequency (Zimmermann 1993). Furthermore, the quality ranking at 1 or 2 for the somatic embryos formed depicts a state of low cell suspension potential which is often a characteristic of “non-ideal” callus. In addition, a low quality embryo ranking can be indicative of a degenerating cell suspension culture. Degeneration of a cell suspension culture could be attributed to the production of phenolic compounds leading to cell oxidation as was encountered for the cell suspensions used in this study. However, other factors such as prolonged *in vitro* culture periods may lead to degeneration of cell cultures. Cell suspensions from different cultivars may exhibit longer healthy culture periods than others. Therefore, optimal culture periods, beyond which a cell culture loses embryogenicity, may need to be established for individual cell lines.

The rate of germination for embryos obtained was 2.8% and 6.2%. These were comparable to previous reports on *Musa* spp. where germination percentages of 5% for male flower-derived cultures of ‘Grand naine’ (Côte *et al.* 1996), 10–23% for a culture of ‘Bluggoe’ cultivar derived from proliferating meristems (Dhedda *et al.* 1991), and 1.5–12% for dessert and cooking banana cultures derived from rhizomes (Novak *et al.* 1989) were attained. Cell line 12AA had a higher regeneration capacity (6.18%) that is reflected by a higher percentage plant recovery (up to 100%). Some embryos, however, formed non-embryogenic callus, and others formed only roots without shoots. These were not considered successful germination since fully developed plants were not derived from them. It is believed that separate embryogenic development events are distinct, and subsequent cell suspensions or somatic embryos derived can behave differently (Côte *et al.* 1996). In this study, the different cell lines, though from the same cultivar, clearly had different regeneration potentials. In studies to understand factors affecting somatic embryogenesis, cultivar dependence on

production of embryogenic callus has been emphasized (Gamborg *et al.* 1995). Other factors also include genotype and the ontogenetic developmental stage of the tissue (Dixon and Gonzales 1994). Results from this study suggest that East African Highland banana varieties require lower levels of the auxin 2,4-D for embryogenic callus formation compared to *Musa* spp. AAA Cavendish group or plantains. Although auxins and cytokinins are known to facilitate tissue differentiation, the precise mechanisms involved in plant morphogenesis or somatic embryogenesis at the cellular level remain to be elucidated. Early principles of differentiation point to genetic factors, environmental factors, cell position effects, field effects, factors related to cell division and physiology, and the interaction among these factors (Salisbury and Ross 1992). In the recent years, progress has been made in understanding the molecular and cellular basis of differentiation and morphogenesis (Yang and Zhang 2010). Therefore, in future studies, such factors need to be considered when experimenting with somatic embryogenesis from different banana cultivars.

Plants derived from cell suspensions were planted in the field together with plants raised from suckers of the same cultivar serving as controls to ascertain trueness to type. From the field observations, their vegetative growth, vigor, and stature were comparable to the control plants propagated from suckers. All plants were morphologically normal, completed the development stages of the cropping cycle, *i.e.*, suckering, and flowering, and no significant statistical differences were observed ($P \leq 0.05$) for the number of suckers, leaf length/width, total number of leaves at flowering, bunch weight, or number of hands per bunch. The differences between controls and somatic embryo-derived plants for stem girth, plant height, and flowering time (Table 2) were overlapping whereby controls were different from plants derived from either 12AA but not 11AA or 11AA but not 12AA. Due to the overlapping differences, the test plants were considered true to type. In related studies, plants of *Musa* spp. AAA cv. Grand naine derived from cell suspensions showed agronomic characteristics similar to those observed in plants produced conventionally by *in vitro* shoot tip culture method (Cote *et al.* 2000). However, as this study being the first of its kind to be conducted on *Musa* spp. AAA-EA cultivars, it necessitates additional investigations on protocol optimization, factors affecting regeneration potentials, and deciphering the

mechanisms involved in embryogenesis of banana. The findings are critical towards application of genetic transformation techniques to improve infertile *Musa* spp. AAA-EA bananas. Therefore, the introduction of genes of desired traits such as pest and disease resistance will be done using the *in vitro* cell regeneration system developed during this study. Cultivated bananas have been termed recalcitrant for a long time because they are infertile and are not readily amenable to tissue or cell culture. However, with this success, the future efforts to improve infertile 'Matooke' varieties against numerous constraints are promising for the banana farmer and consumer. The initiation and regeneration of cell suspensions from cv. 'Nakyetengu' confirms the embryogenic potential of EAHB (Matooke) cultivars where development of regenerable cell suspensions has previously been difficult. Furthermore, results from field evaluation confirmed that true-to-type plants of East African Highland banana cultivars can be generated through somatic embryogenesis. Initial *in vitro* procedures developed from this study have already been successfully applied to selected popular East African Highland banana varieties to generate embryogenic cell suspensions which are currently being utilized at the National Agricultural Research Laboratories (NARL-NARO, Uganda) in ongoing genetic engineering activities targeting pest, disease, and nutrient content improvement.

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