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Somatic Embryogenesis for the Genetic Improvement of a Triploid Banana (Musa Spp. AAA Cv. Berangan) Using Three Different Media with Different Growth Regulators

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Abstract: **Plant regeneration by somatic embryogenesis was attempted with triploid banana Musa cv. (Berangan "AAA"). Cell suspension culture initiated from in vitro proliferating meristems. Embryogenic calli with globular structures developed from three different media. Induction media contained: 2,4 –Dichlorophenxy acetic acid (2,4-D); Naphthalene acetic acid (NAA) and Indole -3-acetic acid (IAA) for the triploid ;zeatin and kinetin were necessary for embryo maturity; 6-benzylamino purine (6-BA) and Inidol 3-acetic acid (IAA) were used for germination. Generally, auxin is not required for initiation of the embryo, but it is needed for the growth of callus prior to this stage if embryogenesis cannot be induced directly from the tissue of explants. Somatic embryos (embryoids) from entirely different origins have a striking similar sequence of embryo formation. However, the origin of a somatic embryo may be a single cell or even a protoplast, but it can also develop from multiple cells derived from the same origin. A yellow-green compact callus was initiated, which consisted of an actively dividing meristematic zone surrounded by several layers of starchy cells. A white and friable callus, characterized by the presence of proem bryonic cells, bicellular proem bryos and proem bryonal masses in its periphery gradually appeared which gave rise to somatic embryos from which plants were recovered.**

Keywords: **histology, Musa spp., somatic embryogenesis, regeneration.**

I. INTRODUCTION

Banana is the most important fruit crop in the world. It forms the primary food source for millions of people in many parts of the world and rank next only to rice and wheat (INIBAP, 1992). Bananas (Musa spp.) are giant perennial herbs belonging to the genus Musa and family Musaceae. They have a very narrow genetic base, being derived from a small number of clones that have been vegetatively propagated. Most cultivated bananas are triploids (2n = 3x), and triploidy leads to problems during meiosis due to uneven numbers of chromosomes. Generally improving banana by the application of traditional breeding methods has had limited success due to the high sterility and polyploidy of most cultivated bananas. Biotechnology offer important alternative methods in the genetic improvement of this crop. Some biotechnologies techniques applied to Musa include in vitro mutation induction, cell protoplast culture and somatic embryogenesis (Escalant, et al.1994; Assani, et al.2001; Strossea, et al.2006), molecular markers of genetic diversity and in vitro screening for disease resistance. Regeneration of Musa spp. via somatic embryogenesis has been established from immature zygotic embryos, immature male flowers, (Strossea, et al.2006; Aini, 2008; and Aiqing, et al. 2011) and somatic tissues such as in vitro leaf bases and corm slices(Aiqing, et al.2011; Wei, et al., 2007) and proliferating meristems (Yang, et al. 2003). Somatic embryogenesis is of great potential value to biology as well as agriculture since it provides an important tool for the study and analysis of molecular and biochemical events (Korzun, 2002). Moreover, this technique is fundamentally more efficient than separate root and shoots induction. In addition, the process is usually less elaborate, less diversity of media is required, and the plant material formed in direct embryogenesis is usually more uniform (Chung, et al.2006). In most species somatic embryos are initiated on a medium containing auxin e.g. 2,4-D, NAA, IAA and the lowering or absence of auxin was important for maturation. However, reduction of nitrogen is necessary for both initiation

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and maturation (Yang, et al. 2003; Chung, et al.2006).The objective of the present study was to assess plant regeneration via somatic embryogenesis of a triploid banana (Berangan) using mixture of 2,4-D, IAA and NAA.

II. MATERIALS AND METHODS

Plant material and explants:

"Berangan" (AAA), Male inflorescences (distal ends) were collected from commercial plantations, when all the female flowers of a bunch were completely exposed and had finished bending upward. The male inflorescences were reduced to 3 cm tips by removing the enveloping bracts and the male flower clusters subtended by them. The tips were washed in a 4% Dextran solution with 2 drops of Tween 80 (per 100 ml of solution) for 15 min, sterilized with 1.5% sodium hypochlorite (w/v) for 30 min and rinsed three times with sterile distilled water. Finally, the tips were reduced to 1 cm in length under sterile conditions. The very young enveloping bracts contained in the inflorescence tips were removed individually and the primordial of male flower buds were excised and inoculated in the culture medium.

Establishment of embryogenic culture and regeneration:

A sequence of different media was used to regenerate plants via somatic embryogenesis. The composition of media, the culture conditions and the duration of each step of induction and somatic embryo maturation is indicated in Table 1.

Histological techniques:

Samples were fixed in 20.5% glutaraldehyde solution (0.2 M phosphate buffer, pH 7.2), containing 1% caffeine which precipitated polyphenols in situ. They were dehydrated and embedded in plastic resin (JB-4 Glycol Methacrylate, POLYSCIENCES). Specimens were sectioned, stained with PAS (Periodic acid, Schiff base) -Regaud´s hematoxylin, and mounted in Permount (SP15-500, FISCHER SCIENTIFIC).

Table (1): Media composition and growth conditions used for plant regeneration through somatic embryogenesis in Musa (AAA) 'Berangan'. Lightintensity was 120 µmol m-2 s-1 and temperature + 27± 1°C

Culture compounds	Embryogenesis steps				
	Callus Induction (M)	Callus proliferation (MPE)	Somatic embryos Maturation (MM)	Germination (MG)	Plant growth (MC)
Macroelements	MS	MS	SHa	MS	MS
Microelements	MS	MS	MS	MS	MS
KH2PO4 (mg1-1)					
Sucrose	30	30	45	60	40
Vitamins	MS	MS	MS	MS	MS
Biotin (mg1-1)	1	1	1		
Malt extract (mg1-1)	100	100	100		
Glutamin (mg1-1)	100	100	100		
2,4-D (µM)	18	4.5			
IAA (µM)	5.7	5.7		11.4	
NAA (µM)	5.4	5.4	1.07		
Kinetin (µM)			0.46		
Zeatin (µM)			0.23		
6-BA (µM)				2.22	
Gellan gum (g1-1)	2	2	2	2	2
pH	5.3	5.8	5.8	5.8	5.8
Photoperiod (day/night)	Darkness	Darkness	Darkness	16 h / 8 h	12 h/12h
Time (wk)	12-16	4	4	8 – 12	4 - 6

A Schenk and Hildebrandt (1972)

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III. RESULTS

Somatic embryogenesis in Musa AAA 'Berangan':

Morphology:

The primordial of male flower buds were cultured on induction medium (M1, Table 1). Two or three weeks after culturing, they started to swell. Two months later, a small yellow-green, compact callus emerged through the stamen primordial (Fig.1-A). During the third to fourth month of culture, a white, hydric and translucent callus began to develop from the callus. Approximately 50% of the established explants formed yellow-green compact callus, but only 2 to 6% of the initial number of explants gave rise to embryogeneic white calli (Fig. 1B, Table 3). Normally, embryogenic callus grew faster than the yellow-green one, the size of the former increased an equivalent volume of the second in approximately half the time of culture. After two to three weeks on proliferation medium (MPE, Table1) somatic embryos appeared on the surface of the embryogenic callus. When subcultured on maturation medium (MM, Table1), the somatic embryos increased in size and the cotyledonary slit was evident. Approximately 13 to 25% of the somatic embryos regenerated plants on germination medium (MG, Tables 2 & 3) where the plumule emerged through the cotyledonary slit. The plants formed roots in vitro (MC medium, Table 1) and grew normally during acclimatization. The apical extremity of a male inflorescence bearing male floral primordial. The explants corresponded to the 5 to 20 youngest clusters of male flower primordial (Fig. 2). When excised, their meristems were enveloped by the stamen primordial; the flower buds were attached at their base where vascular region and a parenchymatous reserve tissue were present. From the meristem of each male flower bud an actively dividing meristematic region started to develop (Fig.2).

Table (2): Conversion frequency (Mean ± S.E.) from initial explant to embryo genic callus during the induction and proliferation steps for plant regeneration of Musa 'Berangan', Experiment 1, 2 and 3 were carried out at different dates.

Species (type of explant	Male inflorescences a		
Experiment	1	2	3
Number of plants	25	30	100
Number of replicates	1	3	5
% embryogenic calli	5.6	3.3 ± 0.4	± 0.6

A; each male inflorescence yields about 15 clusters of primordial of male flower buds.

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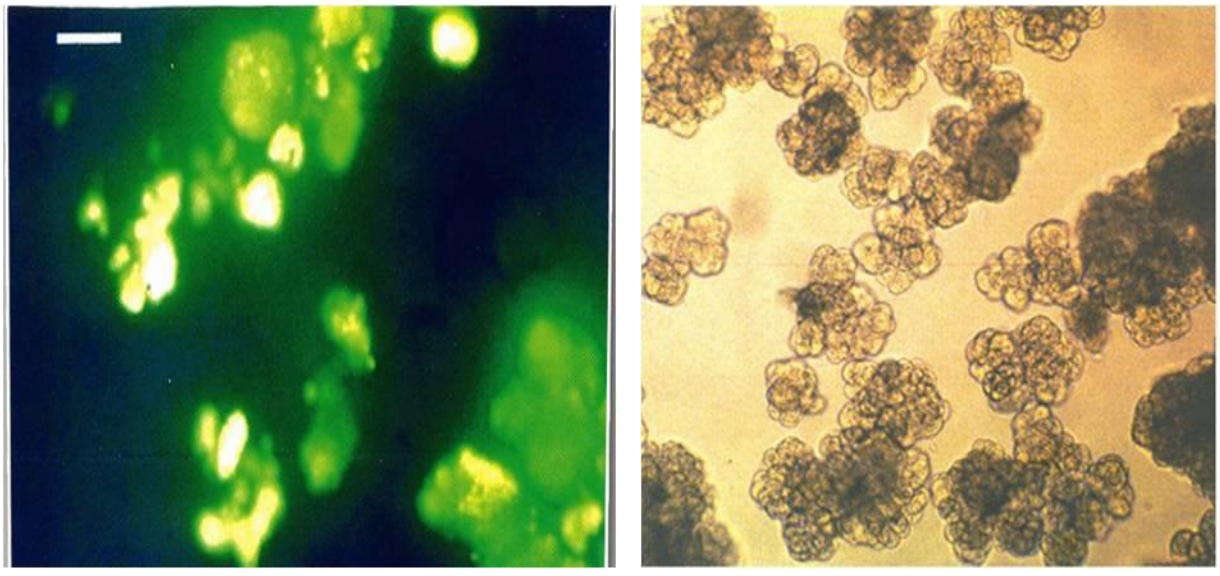

Figure (1): (1-A): Small yellow-green, compact (1-B): Somatic embryos Callus emerged through the stamen primordial.

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Histological analysis

Adjacent to it, another region formed by the proliferation of perivascular cells began to accumulate quantities of starch grains. These two regions of active cell division formed meristematic centers, surrounded by several layers of starchy cells as shown in (Fig. 2).

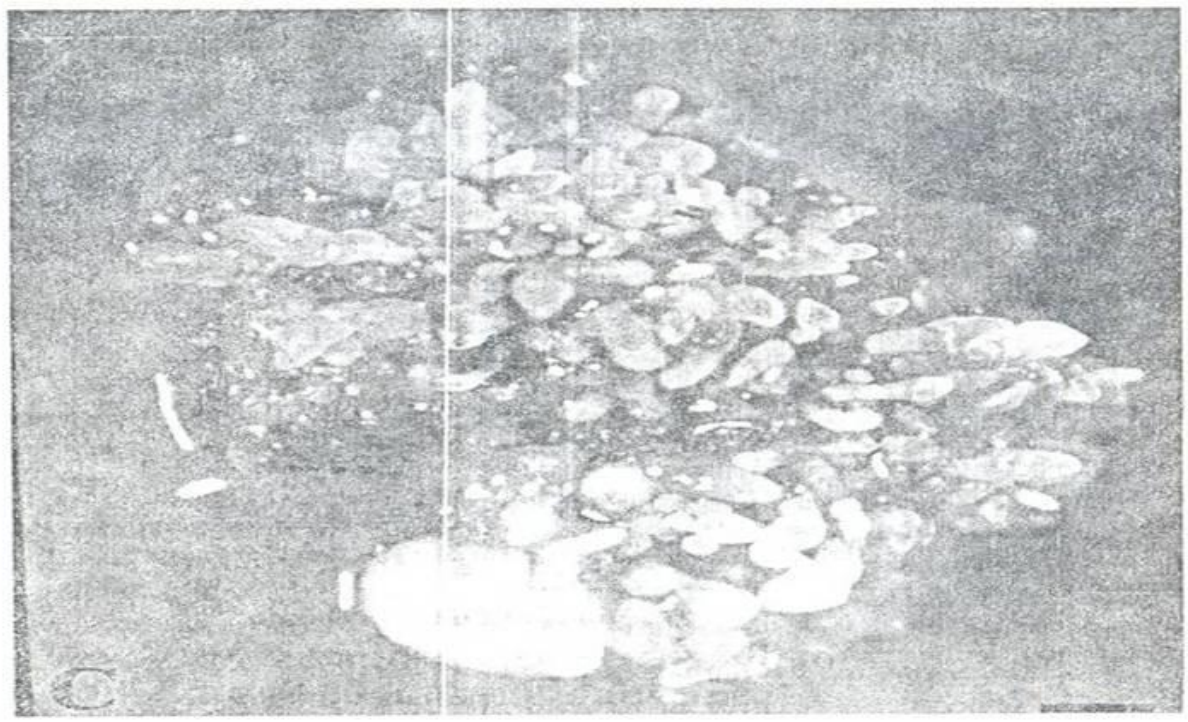

Figure (2): Histological analysis: Meristematic Centers surrounded by several layers of starchy cells

Table (3): Conversion frequency (Mean±S.E.) from somatic embryo to plant, during the maturation and germination steps for plant regeneration of Musa (AAA) 'Berangan'. Experiment 1 and 2 were carried out at different dates

Species (type of explant	Somatic embryos	
Experiment	1	2
Number of embryos	50	40
Number of replicates	3	3
% conversion		
(1 embryo: 1 plant)	13 ± 6.7	25 ± 7.7
(1 embryo : 2-3 plants)		2 ± 0.6

These structures corresponded morphologically to the globular yellow-green callus. The cells on the surface of a white callus are apparent, including embryogenic cells, bicellular proembryos and somatic embryos. They later formed clusters of globular somatic embryos which developed completely on maturation medium. The cotyledonary-like structure of the immature triploid somatic embryo had a conical shape enlarged at its base. During maturation of the somatic embryo, different forms of somatic embryos could be distinguished. In some cases, a denovo callus formation in the basal part of

the somatic embryos was observed and eventually secondary somatic embryogenesis occurred from it. It was difficult to distinguish the root initials at this stage.

IV. DISCUSSION

Somatic embryogenesis may arise directly from explants or through an intermediary callus, but some observations have revealed that organogenesis occurs in place of embryogenesis. There has been steady and significant progress on the regeneration of bananas and plantains from somatic embryos both on semi-solid medium from embryogenically competent units and from embryogenic or organogenic suspension in liquid. In this study three stages were recognized: induction, somatic embryos development and regeneration. However, the patterns of somatic embryo development: a) active division of meristematic cells (induction) (Tang, et al. 2000) accompanied by the proliferation of starchy cells (swelling of explants and/or appearance of a hyperhydric callus); b) formation of meristematic centers (yellow-green, globular compact callus or proembryogenic cell clumps); c) conversion of meristematic (or proembryogenic) cells into embryogenic cells. This process of indirect somatic embryogenesis requires exogenous auxin during both de- and re-differentiation phases (Tang, et al. 2000). Development of one to many-celled proembryos occurred on the periphery of embryogenic callus. Somatic emberyos maturation was completed in the presence of cytokinins. The number of somatic embryos recovered from a single callus was very low as compared to the number of embryogenic cells present on the surface of an embryogenic callus. These findings agree with that reported by (Grapin, et al.1996; Geng, et al., 2011).

Somatic embryogenesis may be used for mass clonal propagation, germplasm handling, and cryopreservation and for improving Musa spp. through non-conventional strategies. Similarly, (Wang, et al.2008; Wang et al. 2005 and Yan-na, et al. 2010) reports that in case with most monocots, embryogenesis appear to be the material of choice for non-conventional Musa breeding. Recently, the development of different methods for somatic embryogenesis has considerably assisted in vitro technologies for these species.

V. CONCLUSIONS

Biotechnology offers important alternative methods in the genetic improvement of Banana (*Musa spp*.). Regeneration of these cultivars, via somatic embryogenesis has been established from immature male flower. A sequence of different media was used to generate plantlets via somatic embryogenesis. There has been steady and significant progress on the regeneration of Banana from somatic embryos. The number of somatic embryos recovered from a single callus was very low as compared to the number of embryogenesis cells present on the face of an embryo genic callus. In addition, the embryo genic cells produced were more uniformed.

VI. RECOMMENDATIONS

Somatic embryogenesis appears to be the material of choice for non-conventional Musa breeding. Somatic embryogenesis arises directly from explants or through an intermediary callus. Three stages are recommended (induction, somatic embryos development and regeneration), by using three types of media supplemented with exogenous auxin during both de- and re-differentiation phases, to develop embryo genic cells. The development of different methods for somatic embryogenesis has considerably assessed in vitro technologies for Musa spp., moreover, most monocots.

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