# Somatic Embryogenesis Formation from Immature Male Flower of Banana cv. Nangka

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**Abstract**

*The plantain banana (cultivar nangka) is among the most valued crop plants in the tropical world. However, the commercially attainable yields are very low compared to other food crops. Somatic embryogenesis has the great potential for rapid and efficient regeneration of plantlets and offers opportunities for large-scale production of plant material. Genetic modification on embryogenic structure offers the opportunity to add desirable gene characteristic. The objective of this study was to evaluate the different medium combination for the induction and optimization of embryogenic formation from immature male flowers. The explants of inflorescence was sampled one month after bunch emergence and approximately 15-20 cm below the last female flower hand. Response of explants and percentage of inflorescence clusters forming nodular callus were recorded and noted. Result of the experiment showed that embryogenic callus could be induced from immature male flowers. The initiation of callus indicated of flower cluster responded to form globular embryogenic callus (49.7% ± 5.2), whereas the percentage of flower cluster responding to form nodular callus was 39.2 % ± 5.3. The highest response of explants forming nodular callus was from flower of rows 12 and 13 (60%).*

***Key words**: somatic embryogenesis, banana cv. Nangka, immature male flower, callus*

## Introduction

Plantains and bananas belong to the family Musaceae (Haarer, 1964; Stover and Simmonds, 1987). They are large herbs with pseudostems built up of leaf sheaths and the leaves stand in a spiral with new leaves arising from an underground true stems or rhizomes (Stover and Simmonds, 1987). According to Cronauer *et al.* (1984) and Tezenas du Montcel (1987) the plantain is normally propagated vegetatively using suckers and they are cultivated in the moist tropics and seldom grow alone but in combination with certain industrial crops and other food crops such as cassava, maize and groundnuts.

Banana provides a good source of carbohydrates, minerals such as potassium, magnesium, phosphorous, calcium, iron and also vitamins A and C (Horry and Jay, 1990; Kodym and Zapata-Arias, 2001). Latham (1979) reported that banana starch is easily digested and is thus suitable for the preparation of food for infants. On the other hand, the nature of the carbohydrates varies widely between cultivars and within a particular cultivar during different stages of ripening (Vuylsteke and Swennen, 1990). The plantains generally have starchy flesh and at maturity, they are usually unpalatable unless boiled (Robinson, 1996) and also easily differentiated from the dessert bananas by the speed of starch conversion into sugars. With plantain bananas, the starch is converted slowly into sugars, while with dessert bananas the development is rapid (Tezenas du Montcel, 1987).

Most edible bananas are triploids (2n = 33) and including the plantains, AAB and ABB (Tezenas du Montcel, 1987 and Swennen, 1990). These triploid genotypes are virtually or completely sterile and develop their fruit by vegetative parthenocarpy (Crouch *et al*., 1998). According to Robinson (1996) the plantain group (AAB) is well known for its important floral morphological heterogeneity. The French type has a complete female and male floral while the Horn type produces only female flowers or even none. An important intermediary type is the False Horn, which has a degenerating male bud.

Banana production is hampered by several diseases such as black Sigatoka, *Fusarium* wilt and banana bunchy top virus (BBTV) disease, which resulted in an

increased effort to genetically improve the crop (Vuylsteke *et al*., 1993; Lee *et al*., 1997). Conventional breeding of *Musa* spp. poses problems such as high sterility and polyploidy nature of most of the edible cultivars (Vuylsteke *et al*., 1993; May *et al*., 1995; Schoofs, *1997*).

Therefore, biotechnological approaches such as through mutation breeding, somaclonal variation or genetic engineering may have great potential in the genetic enhancement of bananas. Crouch *et al*. (1998) stated that genetic modification of *Musa* spp. offers the opportunity to add desirable gene characteristics into target tissue by genetic transformation technique. One of the target materials used in the transformation of *Musa* spp. is somatic embryo. Somatic embryos have proven to be the ideal materials as plantlets produced are non-chimerical and the multiplication is rapid (Panis and Swennen, 1993). Moreover, somatic embryogenesis is useful in agriculture because it fixes the genotype to that of the female parent (Grapin *et al*., 2000). Somatic embryogenesis leading to embryo production and plant regeneration offers advantages for mass propagation of elite cultivars and also provides a useful system for genetic manipulation (Ganapathi *et al.,* 1999). Somatic embryogenesis is divided into 3 stages, induction of somatic embryos with auxin, secondary somatic embryogenesis or multiplication of somatic embryos, and the germination and development of somatic embryos to whole plantlets. During the first stage, it was demonstrated that the presence of an auxin was critical for embryo initiation. Meanwhile the lowering of the auxin concentration or its complete absence fostered maturation of the embryos (Ammirato and Yamada, 1983). A very low level of auxin (0.01–0.1 mg/l) usually induced the maturation of embryos (Nikam, 1997). However, in banana and plantain, regeneration following an embryogenic pathway via somatic embryogenesis has been limited to specific genotype Today, there are few reports on somatic embryogenesis and plant regeneration from local cultivars of plantain banana.

## Materials and Method

Immature male inflorescence was sampled one month after bunch emergence and approximately 15-20 cm below the last female flower hand. The bracts were progressively removed until the inflorescence reached 4-5 cm in length (Figure 1).

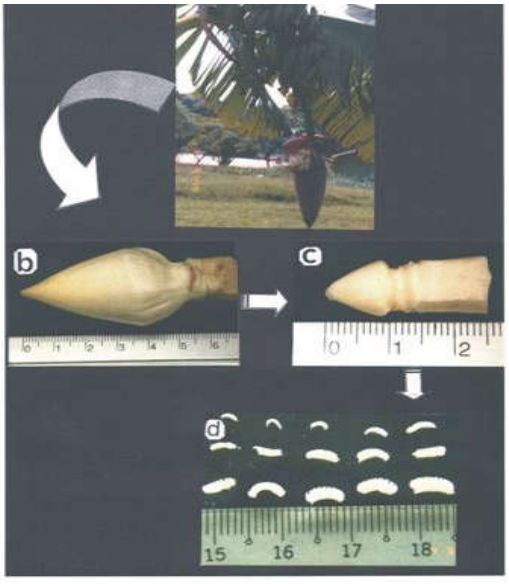



Figure 1: Preparation of immature male flowers for callus initiation (a) in cultivar Nangka, with the bracts removed until the inflorescence size reached ± 5 cm (b), ± 1 cm (c) and (d) flower clusters ranging from (1-15).

The reduced inflorescence was sterilized with 70% (v/v) ethanol for ten minutes and washed 3 times with sterile distilled water in the laminar flow hood. The bracts were further removed until the flower bud reached 1.0–1.5 cm in length (Plate 1) and with a stem to grip. The flower hands were removed under a dissecting microscope, till ± 1 mm long meristem remained and counting backwards through progressively larger flowers to # 15. These clusters were cultured on immature male flower medium.

MS basal medium (MI) containing 5.7 µM IAA, 18.0 µM 2,4-D, 5.4 µM NAA, 4.1 µM biotin and 3% (w/v) sucrose (Navarro *et al*., 1997) was prepared. The pH of the media was adjusted to 5.3 with 0.1 N NaOH prior to autoclaving. The cultures were maintained for three months without subculture. Subsequently they were transferred to proliferation medium (M 2) containing MS salts supplemented with 5.7 µM IAA, 4.5 µM 2,4-D, 5.4 µM NAA and 4.0 µM biotin (Escalant *et al*., 1994 and Navarro *et al*., 1997). The pH of media was adjusted to 5.7 with 0.1 N NaOH. After two months of culture the proliferated callus was transferred to SH macro and MS (Murashige and Skoog, 1962) micro medium (M 3) containing 1.0 µM NAA, 0.5 µM BAP, 0.1 g/L (w/v) glutamine, 0.1 g/L (w/v) malt extract and supplemented with 45 g/L (w/v) sucrose (Navarro *et al*., 1997) for stimulating the somatic embryo formation. The cultures were incubated under continuous darkness at 27 ± 1° C. The experiment was conducted in five replicates.

## Results and Discussion

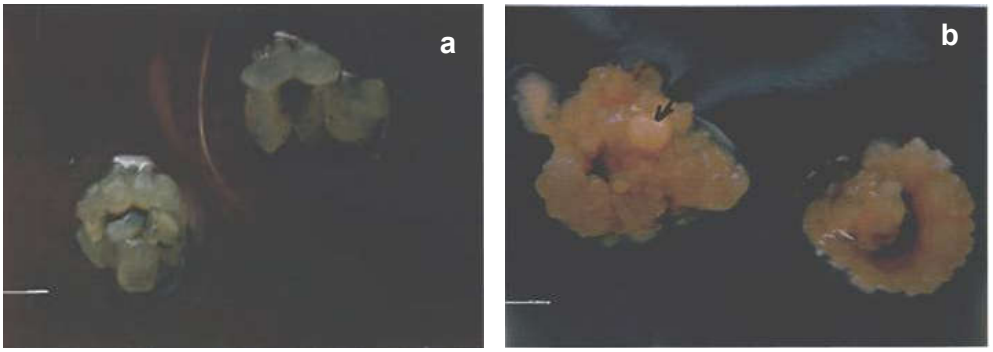
The immature male flowers of cultivar Nangka were cultured on different media composition to obtain and initiate embryogenic cultures. The response of immature male flowers throughout the culture period is indicated in table 1.

Table 1. Response of immature male flower explants of cultivar Nangka during the culture period.

| Media Composition | Response of explants | Duration (Weeks) |
|---|---|---|
| M1 | % of flower cluster responding to form nodular callus: 39.2 ± 5.3 | 12 |
| M2 | % of flower cluster responding to form embryogenic callus: 49.7 ± 5.2<br>- average number of globular embryogenic callus: 2.1 ± 0.9 | 20 |
| M3 | - average number of somatic embryos formed:0 | 24 |

Note: The experiment was conducted in three replicates with five explants per replicate

The explants became brown within a week of culture, and necrosis occurred in the smallest hands from 1 to 4 (table 2). Three weeks following culture, explants began to swell and increased in size due to active cell division at the meristematic region (Figure 2a). Three months later, the nodular and yellowish callus emerged from the flower clusters and became prominent during the fourth month of culture (Figure 2b). After the fourth months, 49.7% of the explants produced embryogenic callus on M 2 medium, which could have possibly been stimulated by the varied auxin combination in the medium. Flower clusters between rows 5 to 15 responded to form nodular callus and the highest percentage of nodular callus formation was attained from rows 12 and 13 (60 %) (Table 2). Escalant *et al*. (1994) reported that flower clusters between rows 7 to 13 responded to form embryogenic callus for Grande Naine (AAA), Gros Michel (AAA), Yangambi (AAA), Dominico (AAB), French Sombre (AAB), Mysore (AAB) and Silk (AAB). Auxin is required for callus formation and triggers a specific step in the cell cycle. According to Grapin *et al*. (2000) percentages of male buds forming embryogenic callus depended on the genotype.




Note: Arrow indicates the globular embryogenic callus (Bar = 2.5 mm)

Figure 2 : Induction of embryogenic callus from immature male flower of cultivar Nangka after 3 week of cultures (a) and globular embryogenic callus from cluster # 12, after 20 weeks of cultures.

Table 2. Percentage of immature male flower clusters forming nodular callus according to their position

| Position of the cluster from the meristem tip | % explants forming nodular callus |
|---|---|
| 1 | browning |
| 2 | browning |
| 3 | browning |
| 4 | browning |
| 5 | 6.7 ± 9.4 |
| 6 | 13.3 ± 9.4 |
| 7 | 20.0 ± 16.3 |
| 8 | 46.7 ± 9.4 |
| 9 | 40.0 ± 16.3 |
| 10 | 33.3 ± 9.4 |
| 11 | 40.0 ± 16.3 |
| 12 | 60.0 ± 16.3 |
| 13 | 60.0 ± 0.00 |
| 14 | 26.7 ± 9.4 |
| 15 | 0.0 |

* The position of the cluster after removed under a dissecting microscope, (± 1 mm long meristem remained) and counting backwards through progressively larger flowers to # 15.

However, from their work with banana (*Musa* spp.) cv. Dominico AAB French type, the first callus with proembryos appeared after the third months in culture. Escalant *et al*. (1994) reported that the first callus appeared after 1 to 2 months in culture and 40% of the floral primordia explants gave an embryogenic response in banana cvs. Grande Naine (AAA), Gros Michel (AAA), Yangambi (AAA), Dominico (AAB), French Sombre (AAB), Mysore (AAB) and Silk (AAB). Proliferation of globular callus became more vigorous by the fifth months counting at 2.1 globular embryogenic callus per flower cluster. After 6 months of culture, there was no occurrence of somatic embryogenesis and complete plantlets formation. This may be due to genotypic differences. The chemical and physical environments have profound effects on somatic embryogenesis. The most important chemical factors involved in the induction of somatic embryogenesis are the exogenous auxin content of the medium, and the composition of nitrogen compounds (Banerjee and De Langhe, 1985). Furthermore, the use of immature male flowers to initiate embryogenic cultures can be applied to a wide range of banana genotypes (Becker *et al*., 2000).

## Conclusion

In this study, the effect of several factors such as explants type; basic media formulation and different hormone combination and concentration on induction of somatic

embryogenesis and plant regeneration have been observed. The plant materials used in this study were immature male flowers of cultivar Nangka.

In the induction of embryogenic callus from immature male flowers, 49.7% flower cluster responded to form globular embryogenic callus after fourth months of culture, and the clusters that formed the globular callus were from rows 12 and 13 (60.0%). This maybe due to genotypic differences or unsuitable of the hormones combination in the media used. The suitable hormones combination required for the callus and somatic embryogenesis induction to support their growth.

However, there is no shoot regeneration was achieved from this study, but experiment need to be optimized and improved to enable regeneration of plantlet.

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## References

Ammirato, P.V and Y. Yamada. 1983. Handbook of Plant Cell Culture. Vol.I Techniques for Propagation & Breeding. Mac Millan. New York: 82-120.

Banerjee, N and E. De Langhe. 1985. A tissue culture technique for rapid clonal propagation and storage under minimal growth conditions of *Musa* (Banana and Plantain). Plant Cell Rep 14: 351-354.

Becker, D.K, Dugdale B, Smith M.K, Harding R.M and J.L. Dale. 2000. Genetic transformation of cavendish banana (*Musa* spp. AAA group) cv.'Grand Naine' via microprojectile bombardment. Plant Cell Rep 19: 229-234.

Crouch, J.H, Vuylsteke D, and R.Ortiz. 1998. Perspectives on the application of biotechnology to assist the genetic enhancement of plantain and banana (*Musa* spp.). Plant Biotech 1 (1) : 1-18.

Cronauer, S and A.D. Krikorian. 1984. Rapid multiplication of bananas and plantains by *in vitro* shoot tip culture. Hort Sci 19 : 234 - 235.

Escalant, J.V, Teisson C and F. Cote. 1994 Amplified somatic embryogenesis from male flowers of triploid banana and plantain cultivars (*Musa* spp). *In vitro* Cell Div Biol 30P: 181-186.

Ganapathi, T.R, Suprasanna P, BapatV.A, Kulkarni V.M, and P.S Rao. 1999. Somatic embryogenesis and plant regeneration from male flower buds in banana. Plants Cell Rep 19 .

Grapin, A, Ortiz J.L, Lescot T, Ferriere and F.X. Cote. 2000. Recovery and regeneration of embryogenic cultures from female flowers of false horn plantain. Plant Cell Tiss Org Cult 61: 237- 244.

Haarer, A.E. 1964. Modern Banana Production. Leonard Hill , London. pp. 97.

Horry, J.P and M. Jay. 1990. A evolutionary back ground of bananas as deduced from flavonoids diversification. In : Identification of genetic diversity in the genus Musa. INIBAP. Montpellier. Jarret R.L (ed.): pp. 41 – 55.

Kodym, A and F.J. Zapata-Arias. 2001. Low-cost alternatives for the micropropagation of banana. Plant Cell Tiss Org Cult 66 : 67-71.

Latham, M.C. 1979. Human Nutrition in Tropical Africa. Rome, FAO : pp 92.

Lee, K.S, Zapata-Arias F.J, Brunner H and R. Afza. 1997. Histology of somatic embryo initiation and organogenesis from rhizome explants of *Musa* spp. Plant Cell Tiss Org Cult 51: 1 – 8.

May, G.D, Afza R, Mason H.S, Wlecko A, Novak F.J and C.J. Arntzen. 1995. Generation of transgenic banana (*Musa acuminata*) plants via. *Agrobacterium*-mediated transformation. Biotechnology (13): 486-492.

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

Navarro, C, Escobedo R.M and A. Mayo. 1997. *In vitro* plant regeneration from embryogenic cultures of a diploid and a triploid, cavendish banana. Plant Cell Tiss Org Cult 51: 17-25.

Nikam, T.D. 1997. High frequency shoot regeneration in *Agave sisalana*. Plant Cell Tiss Org Cult 51: 225-228. .

Panis, B and R. Swennen. 1993. Embryogenic *Musa* plant cell cultures: Current and Future Applications. In. INFOMUSA 2 (1): 3-6.

Robinson, J.C. 1996. Bananas and Plantains. Crop Production Science in Horticulture. CAB International. Cambridge: pp 231.

Schoofs, H 1997. The origin of embryogenic cells in *Musa*. Phd Thesis KU. Leuven, Belgium. Dissertationes de Agricultura N: 258-330.

Stover, R.H and Simmonds N.W. 1987. Bananas, 3rd ed. Longman Scientific & Technical, New York: pp 468.

Swennen, R. 1990. Plantain Cultivation under West African Conditions. A Reference Manual - International Institute of Tropical Agriculture. Ibadan. Nigeria : pp. 97.

Tezenas du Montcel. 1987. The core programs of INIBAP. In. Proceeding of a Regional Technical Meeting on Diseases Affecting Banana and Plantain in Asia and the Pacific. Brisbane, Australia: 10-20

Vuylsteke, D and R. Swennen. 1990. Somaclonal variation in African Plantains. IITA Research 1: 4-10.

Vuylsteke, D, Ortiz R and R. Swennen. 1993. Genetic Improvement of Plantains and Bananas at IITA. In: INFOMUSA 2: (1) : 10-12.