# INDUCTION OF SOMATIC EMBRYOGENESIS FOR *IN VITRO GENETIC* MANIPULATION OF LOCAL BANANA CULTIVARS

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## Introduction

The production and export potential of our local banana cultivars are greatly hampered by diseases and undesirable post harvest qualities. Genetic improvement of the crop through classical breeding methods remains difficult due to high sterility and polyploidy nature of the crop. Interest is now diverted towards the genetic improvement of banana through gene transfer techniques. Prerequisite to the successful exploitation of the gene transfer technology is the establishment of a reliable *in vitro* culture system that allows regeneration of mature plants. Regeneration through somatic embryrogenesis have been reported in various cultivated triploids - Grand Naine (AAA Cavendish') (Novak et al. 1989), Bluggoe (ABB Bluggoe') (Dhed`a et al. 1991) and diploid *Musa* (AA) ssp. *malaccensis* (Navarro et al. 1997). An attempt at inducing somatic embryogenesis in our local banana cultivars, particularly in cv. Mas, is described.

## Materials and Methods

Shoot tips, immature male flowers and thin corm slices were used as explants. Shoot tips removed from suckers were sterilised in 80% ethanol (1 min) and in 100% clorox (30 min), and rinsed thrice in sterile distilled water. The shoot tips were placed on modified Murashige and Skoog (1962) (MS) medium supplemented with 10μM and 100μM benzylaminopurine (BAP) in combination with 1.0μM indole acetic acid (IAA). Subculture was performed at 4-week intervals. Scalps obtained were transferred to half strength liquid MS medium with 5.0μM 2,4-Dichlororphenoxyacetic acid (2,4-D) and 1.0μM zeatin and agitated at 70 rpm. The culture medium was refreshed at 3 weeks interval. Young male inflorescence, reduced to approximately 5.0 cm in size by removal of the external bracts, were sterilised in 70% ethanol (1 min) and the first 15 rows of flowers below the apical meristem were dissected and cultured on modified MS medium with 20μM 2,4-D, 5.0μM IAA and 5.0μM napthaleneacetic acid (NAA). Thin corm slices were obtained from below the shoot tip of *in vitro* plantlets and cultured on MS medium supplemented with 0.5, 10, 20, 40 and 80μM 2,4-D. Callus produced were subcultured onto MS medium with 4.7μM abscisic acid (ABA). Embryogenic callus that proliferated were transferred to various regeneration media: MS medium with 11μM IAA and 2.0μM BAP, MS medium with 5.0, 10 and 20μM BAP, and MS medium without growth regulators.

## Results and Discussion

From the seven cultivars of banana and plantain tested: Mas (AA), Berangan (AA), Intan (AA), Tandok (AAB), Raja (AAB), Gros Michel (AAA) and Grand Naine (AAA), cv. Mas responded the earliest to form scalps (after 5 subcultures). Scalps were obtained only after 6 to 8 subcultures inthe rest of the cultivars, and the scalps were inferior in quality compared to those of cv. Mas. MS medium with 100μM BAP and 1.0μM IAA promoted scalp formation, whilst MS medium with 10μM BAP and 1.0μM IAA produced only stunted shoots. Dhed`a et al. (1991) however, reported obtaining scalps on MS medium with 10μM BAP and 1.0μM IAA in cv. Bluggoe, thus indicating a cultivar specific response to scalp formation in banana. Scalps of cv. Mas placed in liquid medium responded to form meristematic globules on their surface and releasing them into the liquid medium after 5 to 8 weeks in culture. The number of meristematic globules released increased rapidly up to the third subculture and gradually stabilised in later subculture. With subculture, cells were simultaneously released from the meristematic globules. Cells released from meristematic globules of 5 to 6 month-old culture were embryogenic, whilst cells released earlier were more heterogeneous, elongated and highly vacuolated. In cv. Bluggoe, embryogenic cell suspension was attained from scalps after 4 to 7 months in liquid culture medium (Schoofs, 1997). Young male flowers from rows 5 to 10 responded to form callus after 1 month in culture. Three months later, whitish, globular and embryogenic callus appeared especially around each flower. Subculture on MS medium with 4.7μM ABA proliferated more of such callus. Thin corm slices developed yellowish callus after a month in culture on 0.5μM 2,4-D. Higher concentrations of 2,4-D caused blackening of the callus. More calluses proliferated and became globular and embryogenic after 3 months in culture. Embryogenic calluses were placed on regeneration media to form somatic embryos and germinate into whole plants.

## Conclusions

Various explants of banana are potential sources for embryogenic callus formation. Exogenous supplies of growth regulators, particularly auxins, are vital in inducing embryogenic structures. Somatic embryogenesis attained will be an ideal system for improving the local *Musa* spp. through non-conventional strategies such as genetic transformation, protoplast fusion, mutagenesis and somaclonal variation, and for mass propagation and cryopreservation.

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