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Feasibility of Somatic Embryo Development in *Musa, cv. Bluggoe*

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ABSTRACT. The uppermost part of proliferating buds (in vitro) of Musa, ABB, cv. Bluggoe, produced meristematic globules in basic Murashige and Skoog liquid medium (Murashige and Skoog, 1962), supplemented with 2,4 D (5.10^{-6} M) and BAP (10^{-6} M). Individual meristematic globules of size <= 2 mm diameter were confirmed as somatic pro-embryos.

Somatic embryos were obtained upon successive transfer of pro-embryos initially into a medium with ABA (10^{-5} M), and then to a medium without any growth regulators and finally to BAP (10^{-6} M) and IAA (10^{-6} M).

The embryo proper (shoot and root apex) and continuous pro-cambial strands between the shoot and root apices were observed in these structures.

INTRODUCTION

Banana and plantain are the staple food in most tropical and sub-tropical parts of Africa. They are also an important fruit to the whole world and a largely consumed fruit in Asia.

Banana and plantain are classified under the genus *Musa*. Simmonds and Shepherd (1955) reported that majority of the edible *Musa* are inter-specific hybrids of the two wild species, *Musa balbisiana* (BB) and *Musa acuminata* (AA). Naturally occurring AA, AB, BB, AAA, AAB, ABB and ABBB groups have been identified (Stover and Simmonds,

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1987). These include between 300 – 500 cultivars distributed throughout the world (De Langhe, 1987).

The banana and plantain cultivation throughout the world is threatened by serious pests and diseases. Many efforts are being made to combat these diseases mainly by chemicals. However, this is expensive, and De Langhe (1987) reported that there is a growing belief that genetic improvement is probably the answer to the serious threat of diseases and pests.

Genetic improvement of plants depends on the availability of a fast and safe way to regenerate new plants from genetically modified cells. The cells should be regenerated into a complete plant to express the desired characters. Somaclonal variation should be minimized during this process. This is where somatic embryogenesis become important, as development of a somatic embryo from a single cell or at least a cluster of cells has a background of low somaclonal variations. Somatic embryogenesis, in general, is the development of embryos from somatic haploid or diploid cells, without fusion of gametes.

Some efforts have been made in recent years to obtain somatic embryos from vegetative (pure somatic) tissues of *Musa* (Cronauer and Krikorian, 1983; Jarret *et. al.*, 1985; Bakry and Rossignol, 1985; Benerjee and De Langhe, 1987; Krikorian, 1987). Also research has been carried out with zygotic embryos to obtain somatic embryos (Escalant and Teisson, 1988; Cronauer and Krikorian, 1988). The present study was done with the objective of obtaining somatic pro – embryos (*in vitro*) in large numbers from proliferating buds of *Musa*. It was also attempted to study the development process of the pro – embryos into embryos.

MATERIAL AND METHODS

A piece of tissue cut from the uppermost part of proliferating buds was the starting – material (explant). Each piece was 2 – 3 mm thick and had a surface area of 5 – 6 mm^2. Each piece of tissue contained 4 – 5 buds (Figure 1), each bud had 5 – 6 layers of meristematic cells just below the epidermis (Figure 2).

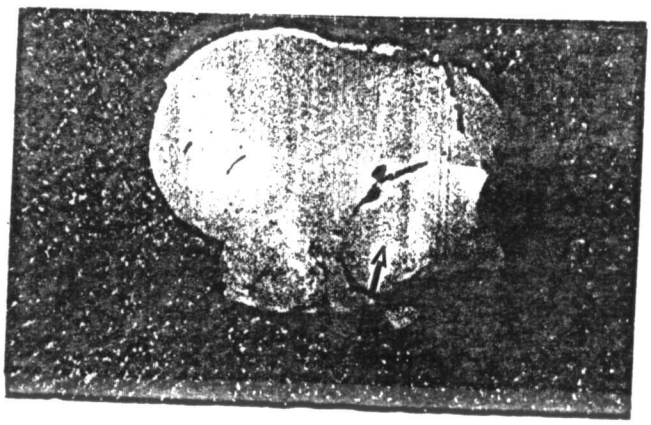


Figure 1. Explant: The uppermost part of proliferating buds (external appearance). Arrow indicates a bud.

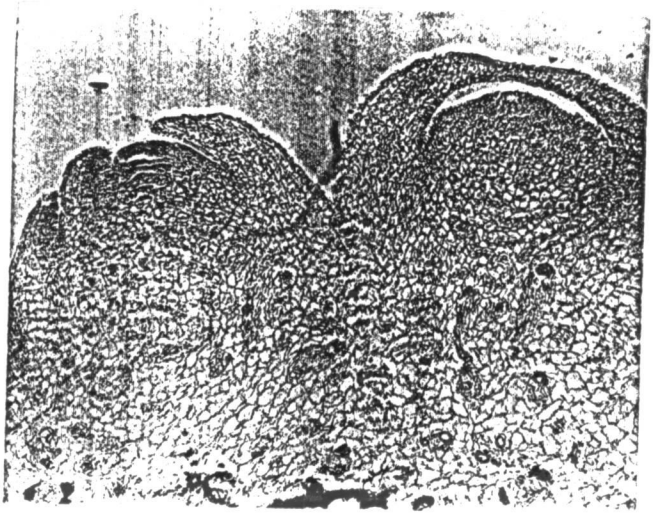


Figure 2. Longitudinal section of proliferating buds (LS of Fig 1) m = meristematic cells.

Culture medium

The basic culture medium was modified Murashige and Skoog (1962) liquid medium supplemented with different types of growth regulators at varying concentrations. The culture media were sterilized by autoclaving at 103 – 104 kPa and 121 C for 30 min.

Experimental conditions

Whenever light was given, the irradiance was 2.52 $\pm$ 40% Wm^{-2} (an illuminance of 900 $\pm$ 40% lx). This was provided by 36 W Osram cool white fluorescent tubes. Temperature was 25 $\pm$ 2 C. Cultures were incubated under continuous shaking at 60 rpm on an orbital shaker (MKV Model). Thirty culture flasks each containing 5 explants were used. The culture conditions and growth regulators used are given under the results.

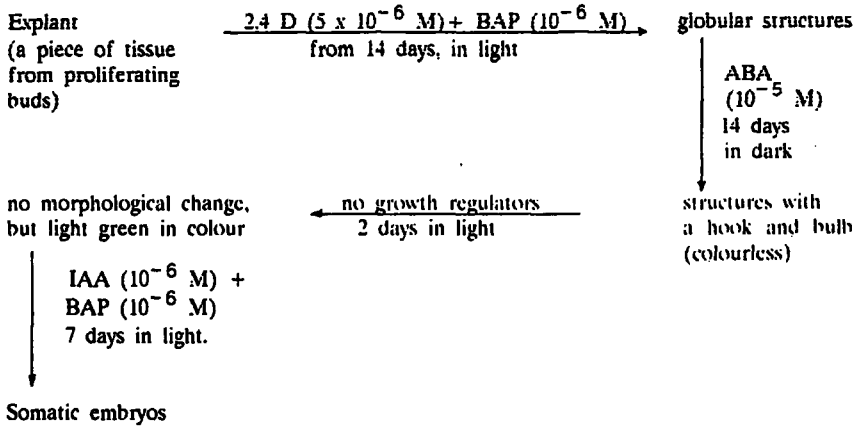
Morphological and histological observations were made every two days for 20 weeks. Change in the bud structure of the explant, appearance of any other structures and their morphological features were observed.

Histological studies

For histological studies, samples of explant were taken every 2 days up to 3 weeks, and then at 4, 6, 8, 10, 15 and 20 weeks after inoculation. Each time, two samples were taken randomly and were fixed in FAA to prepare microtome sections on paraffin (Berlyn and Miksche, 1976).

RESULTS AND DISCUSSION

Somatic pro – embryos and somatic embryos *in vitro* were developed under the specific conditions given in Scheme 1.



Scheme 1. Developmental procedure of somatic embryos.

From about the 2nd week, some changes of the explant were seen; the multiple buds became indistinct by producing yellowish – white nodular tissues on the upper surface. By about the 6th week, bud structures were not seen on the explant. Also, isolated globular structures were seen at the bottom of the flask from about the 2nd week. The number of globules increased with the time and exceeded 100 around the 8th week.

The size of globules ranged from 0.5 – 3.5 mm diameter. The globules were smooth and compact, yellowish – white in colour. The globules smaller than 2 mm in diameter had mainly meristematic cells (Figure 3). They were termed as "meristematic globules". These globules contained cells with embryogenic characters: dense cytoplasm, a large nucleus and a prominent nucleolus, hardly any vacuoles, and presence of starch and protein granules in the cytoplasm.

Morphologically, meristematic globules resemble "globular callus masses" described in *Musa* by Jarret *et. al.*, (1985), and those reported by Novak *et. al.*, (1989). They also resemble meristematic nodules of Date Palm (Tisserat and De Mason, 1980) and Oil Palm (Schwendiman *et. al.*, 1988).

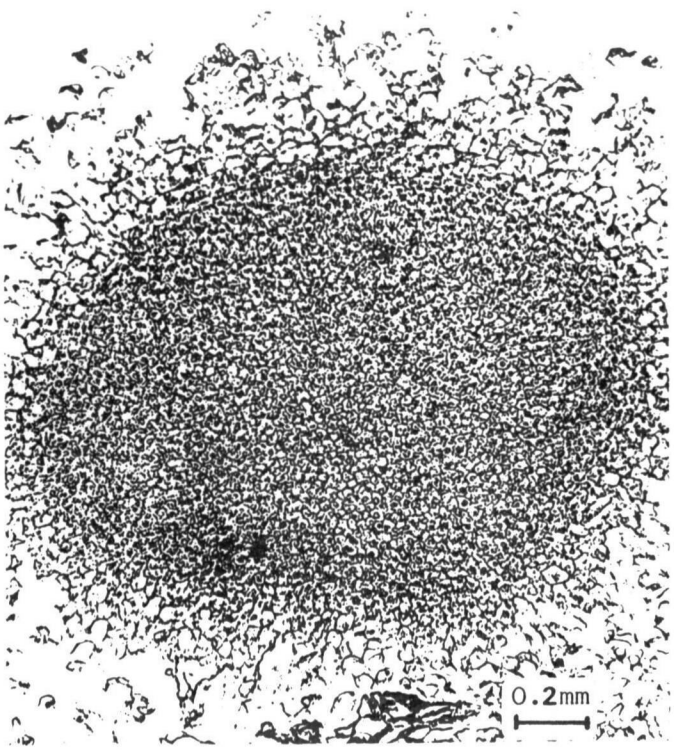


Figure 3. A cross section of a globular structure of 2 mm diameter.

Formation of somatic embryos

Meristematic globules upon transfer to three successive media in Scheme 1, developed into bipolar structures.

The structures were termed as somatic embryos as they had the following criteria defined for a somatic embryo.

a) Presence of shoot and root meristems (embryo proper). The embryo proper was identified as an essential part of the *in vitro* somatic embryo (Sannasgala, 1989).

b) Continuous pro-cambial strands between the two meristems.

The meristematic globules also had the following features.

a) The very early stage in the development pattern involved demarcations with the isolation of group of cells by a thick wall: a reported feature during the development of somatic embryo (Thomas *et. al.*, 1972; Mc Williams *et. al.*, 1974; Williams and Maheswaran, 1986).

b) Non differentiated mass of cells. Also a reported feature of pro-embryogenic stages (Natesh and Rao, 1984).

c) The fact that meristematic globules can be developed into somatic embryos strongly suggest that meristematic globules are somatic pro-embryos.

The possibility to obtain many somatic embryos *in vitro* is an important aspect in micro-propagation work. Since the embryos are isolated and individual structures, they have an advantage over clusters of somatic embryos. For example, studies on plant development could be more precisely done. Techniques such as irradiation of pro-embryos causing variations can be more effective. However, further studies are necessary where somatic embryos develop into complete plants. Also, additional features such as lipo-proteins and studies on RNA of pro-embryos would contribute for their further confirmation as pro-embryos.

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