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CELL SUSPENSION CULTURES OF *MUSA*. CV EMBUL (AAB), MONDAN (ABB) AND KOLIKUTTU (AAB)

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ABSTRACT

Three explants (shoot tip, anthers and fruit pulp) of three cultivars of Musa were tested for callus production under different culture conditions and for the development of cell cultures from the callus. It was observed that callus is produced from all three explants of the tested cultivars, both in liquid and semi-solid, under different culture conditions. There was a variation in the morphology of callus and the type of cells in culture. It could be revealed that factors, namely, the explant, growth regulators and light affect the cell types in suspension, irrespective of the cultivar. Shoot tip of all 3 cultivars produced an embryogenic callus and cell cultures while fruit pulp and anthers produced callus and cell cultures with predominantly parenchyma.

INTRODUCTION

A cell suspension culture ideally is single cells suspended in a liquid nutrient medium, which is continuously being agitated. But very rarely such a homogeneous suspension would be achieved. Mostly, a mixture of single cells, mini-clusters (2-8 cells) and cluster of cells are suspended in liquid medium. With the development of cell cultures in carrot (Steward et al. 1958), cell suspensions have been established in very many plant species (Shenuk and Hilderbrandt, 1972; Brackpool et al. 1986; Hall, 1991; Ho and Vasil, 1983). Such cell cultures have been applied for (1) basic cell physiology and biochemistry studies, (2) secondary metabolite production, (3) enzymological studies, (4) isolation of protoplasts, (5) embryogenesis and artificial seed synthesis. The application has improved to such an extent that bioreactors for large scale productions are now available for certain crops. One common feature in all these suspension cultures is the heterogeneity of the cell composition. Hall (1991) reported that the species/genotype and medium composition can greatly influence cell types in a culture. However, the effect of the explant (plant tissue) is not mentioned.

In vitro cell cultures are also reported in *Musa* spp. (Mohan Ram, 1964; Krikorian, 1987; Novak et al. 1989; Dhed'a et al. 1991, Dhed'a et al. 1992). In these studies, fruit pulp, the uppermost part of proliferating shoots *in vitro* and seeds (embryos) have been used as explants and different types of cells in suspensions are reported. However, the cultivar effect is not mentioned. But the *in vitro* multiplication rate of *Musa* is reported to be cultivar specific (Hirimburegama and Gamage, 1996). The present study compares the cells in suspension cultures derived from different explants of three *Musa* cultivars. It was also attempted to identify some factors affecting callus development and the cells in suspension cultures, so that it would be possible to have a control over the type of cells. This is important in the application of cell cultures. For example, in plant regeneration, cells in culture should actively divide (meristematic condition) than of cultures for secondary metabolite production.

Recently renewed attention has been given for breeding of bananas especially the triploids. This is mainly due to wide spread of diseases *in Musa* cultivars which had reduced the production especially in large scale monocultures. Therefore, increased efforts are being given to breeding programmes on disease resistance. Approach of molecular genetic techniques for banana improvement is given high prominence. Such programmes require successful regeneration of plants from single cells/protoplasts, and thus it is necessary to have a control over cells in culture.

MATERIALS AND METHODS

Musa cultivar Embul (AAB), Kolikuttu (AAB), Mondan (ABB) (Stove and Simmonds, 1987) were used in the study. Three types of explants: the uppermost part of proliferating shoots *in vitro*, fruit pulp (mesocarp) and anthers were tested. For callus initiation and growth, the basic Murashige and Skoog medium (MS) (1962) was supplemented with auxins and cytokinins as given in Table 1. The media were gelled with agar (BDH) at 5.0 g/L for semi-solid condition. The pH was adjusted to 5.8 ± 0.1. Aliquots of 15 ml of the culture media were dispensed into *pyrex* tubes (25 mm diameter, 150 mm length). Vessels were plugged and were autoclaved at 120 kPa at 121°C for 20 minutes. Altogether there were 36 treatments (3 x 3 x 4) and each treatment had 25 replicates.

Surface sterilization was not necessary for *in vitro* shoots. Anthers were sterilized by immersing in 50% (v/v) ethanol for 3 minutes. But for fruit pulp, an initial ethanol (70% v/v) treatment for 5 minutes was followed by 10 minutes in 5% Clorox (NaOCl).

For semi-solid media a piece of tissue (size of 5 x 5mm^2) either fruit pulp or the shoot tip were inoculated while five such tissue pieces were put to liquid media. Four anthers were inoculated per vessel. The semi-solid cultures were placed in complete dark at 26±1°C temperature and 80% RH. The liquid cultures were incubated at 16 h photoperiod provided by white cool fluorescent tubes (4.2 W/m^2) and were continuously agitated on an orbital shaker at 60 rpm. The dry weight of callus was determined every week.

Callus produced on semi-solid media were cut under sterile conditions, into 4 pieces, each portion of size 10 mm^2. Five such pieces were inoculated into similar but liquid media. All the vessels were continuously agitated on an orbital shaker at 60 rpm.

Samples from each liquid culture were taken every other day and the following were observed. (i) Different types of cells (meristematic, parenchyma, vascular cells) (ii) their number (iii) morphological changes of the explant, (iv) any other morphological change. The average number of each type of cells every week were compared for significance, using Turkey's HSD test at 5% level of significance.

Table 1
The culture media compositions tested on all 3 explants of the 3 cultivars

Number	Medium composition (on Murashige and Skoog basic)		
	auxin	cytokinin	culture conditions
1	2,4-D (5.0 mg/L)	-	semi-solid, dark
2	2,4-D (5.0 mg/L)	BAP (1.5 mg/L)	liquid, light (16 h)
3	2,4,5-T (5.0 mg/L)	-	semi-solid, dark
4	2,4,5-T 5.0 (mg/L)	BAP (1.5 mg/L)	liquid, light (16 h)

2,4-D: 2,4 dichlorophenoxy acetic acid, 2,4,5-T: 2,4,5 Trichloro phenoxy acetic acid, BAP: benzyl amino purine

RESULTS

Callus development

In all three cultivars, a callus was obtained with all three explants tested (fruit pulp, anther and shoot tips). However, there was a variation in the rate of callus formation among explants (Figure 1), highest being the fruit pulp. There were also variations in their morphology and texture as shown in Table 2 (Figure 2). With respect to the colour, all calluses were whitish yellow irrespective of some being cultured in 16 h photoperiod. There was a considerable variation in the cell composition of calluses. Heterogeneity in cell composition was highest in shoot tip callus while it was less in fruit pulp and anther callus. The latter was more homogeneous containing mostly parenchyma (Figure 3a). Also, the number of cells in division state was less in these two calluses. However, the shoot tip callus contained several types of cells: groups of meristematic cells, embryogenic cells (Williams and Maheswaran, 1986), vascular cells and parenchyma (Figure 3b). It was also observed that callus from the three types of explant was the same in all the replicates.

Table 2
Some characters of the callus of the three types of explants

Explant	Character			
	Colour	Texture	Cell composition	Cell division
Fruit pulp	white	loosely arranged	Parenchyma, Vascular elements	none
Anther	white	loosely arranged	Parenchyma, Vascular elements	a few
Shoot tip	yellow	compact	Mostly parenchyma, Meristematic cells, Embryogenic cells, Vascular elements	many

The general observation of a character is given

Cell cultures

Upon transfer to liquid cultures, callus produced a suspension within one week. Liquid media with shoot tips produced a milky suspension at the same time the callus was produced. Thus, within the same culture, callus as well as cells in suspension were present. Cell composition of suspension cultures of all three explants was similar to their respective callus. However, there was a variation among the explants. The fruit pulp and anther suspensions had mostly parenchyma while that of shoot tip had the three types of cells as in the callus (Figure 4). With respect to callus or cell culture formation and composition, there were differences among 3 explant types but a variation was not observed in the three cultivars tested; similar explants of all 3 cultivars behaved in the same manner.

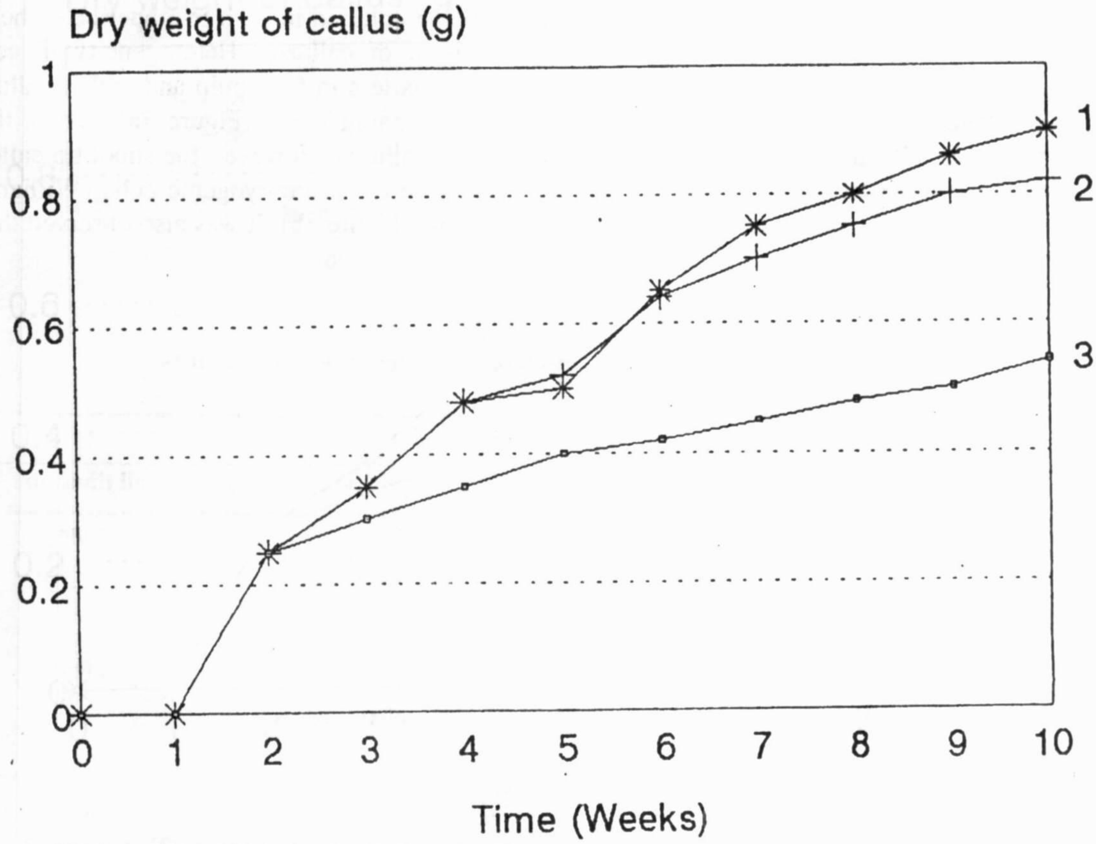

Figure 1. The rate of callus development in the three explants

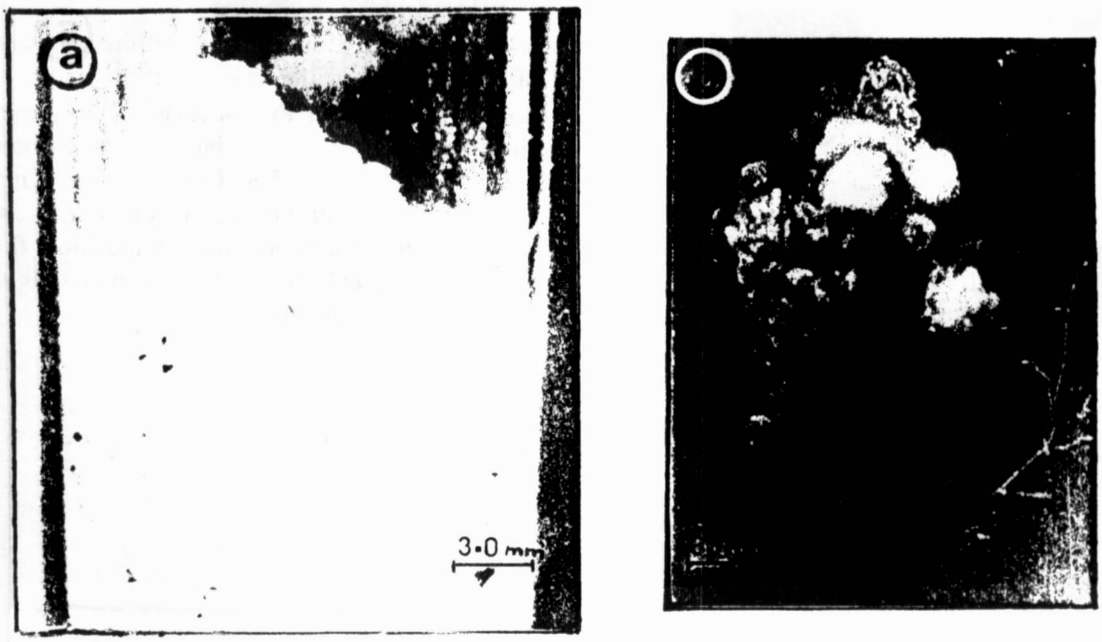

Figure 2. Morphology of callus of different explants
(a: fruit pulp; b: shoot tip)

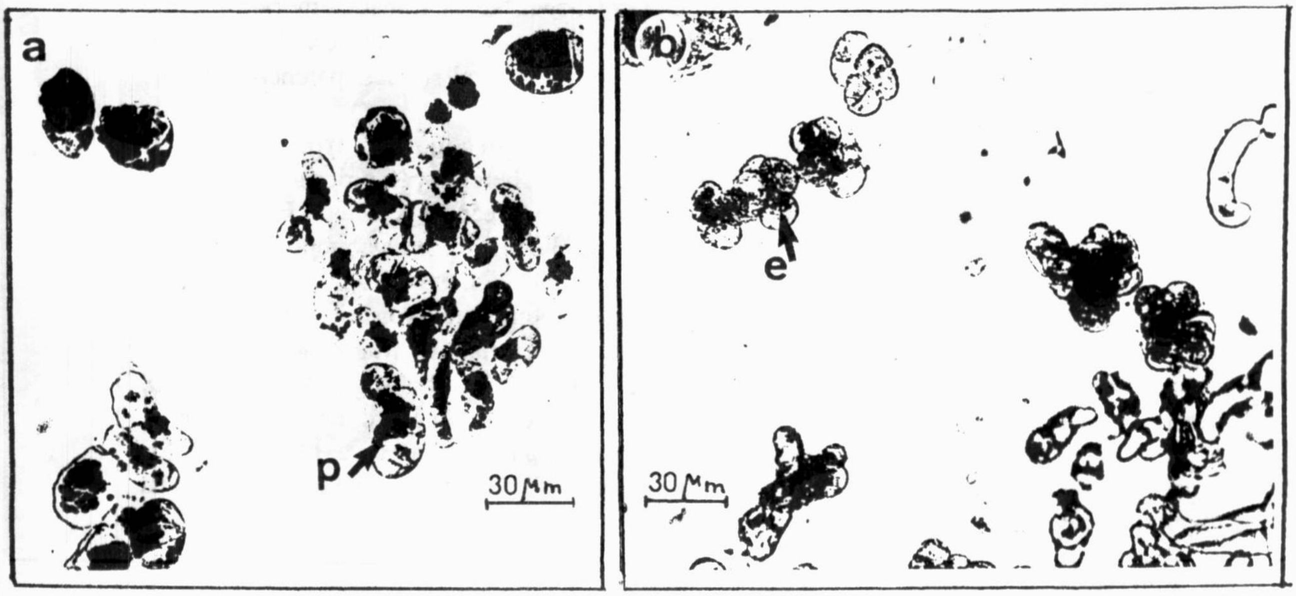

Figure 3. Cells in liquid cultures obtained from different explants (a: fruit pulp; b: shoot tip) (e: embryogenic; p: parenchyma)

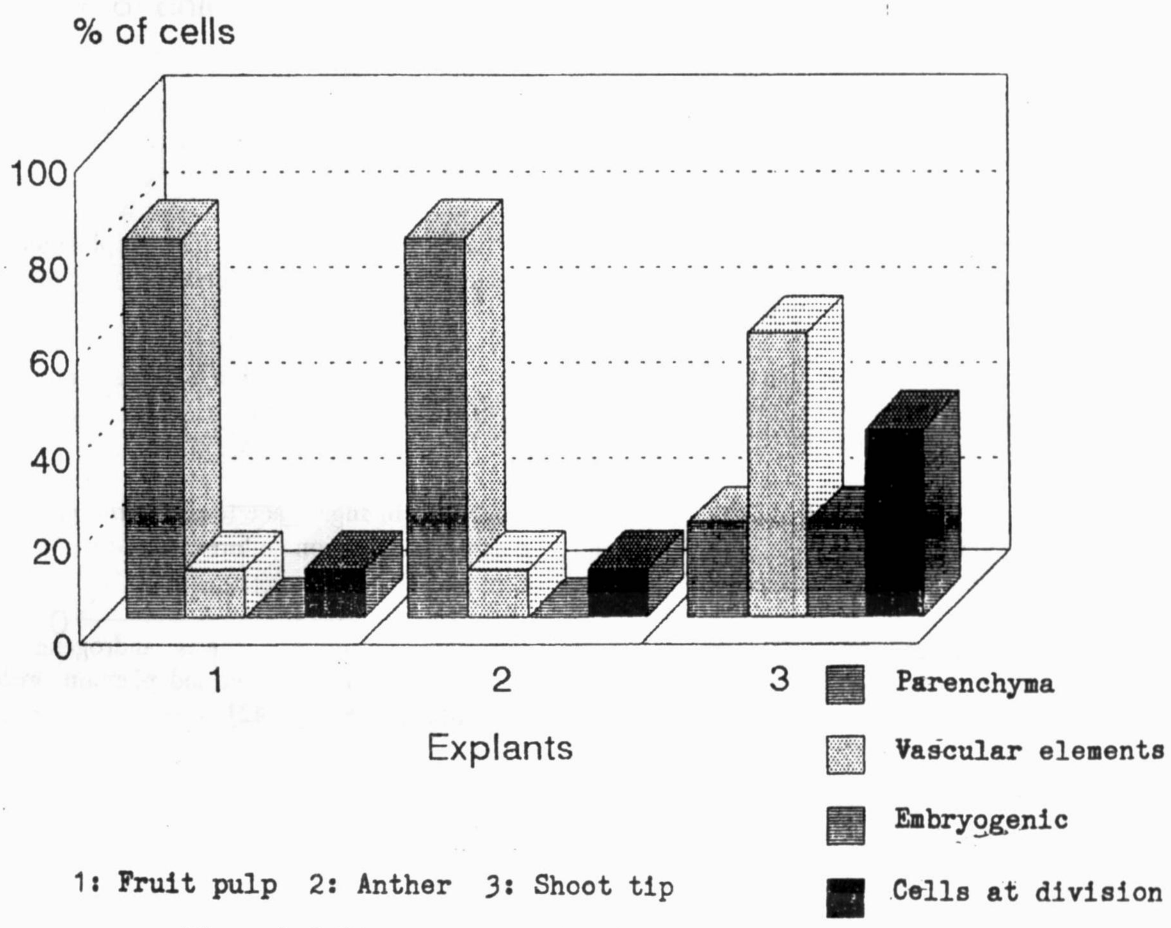

Figure 4. Cell types in suspension of the three explants

DISCUSION

The study shows the variation in the callus and cell cultures of different explants of the same cultivar. The study reveals that the explant tissue affect the texture of callus and the cell composition of the culture. It is also known that the type of cells in culture is important in its use ie. For plant regeneration (either through organogenesis or somatic embryogenesis), meristematic cells and/or embryogenic cells should be present in cell cultures. Whereas for secondary metabolite production and for some other physiological studies, parenchyma cells in cultures are important.

The study also reveals certain other conditions affecting the type of cells in suspension; light, growth regulators added to the medium. Therefore, in order to achieve the objectives of a study, a suitable explant and culture conditions can be selected. Embryogenic cells shown in callus and cell cultures of shoot tips of all 3 cultivars are also reported in *Musa* cv Bluggoe (ABB) (Sannasgala, 1989; Dhed'a et al. 1991; Panis, 1995). Therefore it is possible to conclude that the shoot tip tissues of *Musa* produce embryogenic suspensions on MS with 2,4-D and BAP. Also, the callus and cell suspensions of fruit pulp reported by Mohan Ram (1964) appears to be the same of all 3 cultivars. All this suggest that in *Musa*, irrespective of the cultivar, if the same environmental conditions are provided, similar explants would produce morphologically similar callus and similar type of cells. This suggests that in determining the characters of the callus and cell cultures, the explant plays an important role.

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