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In vitro response as a reflection of genomic diversity in long-term cultures of *Musa*

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Summary. Ten commercial cultivars of *Musa* representing five different types of genomic constitutions were studied for in vitro multiplication through meristem culture. In addition, the effects of various genomic constitutions at different ploidy levels on growth and meristem proliferation in long-term cultures were analysed statistically. Plantlets were readily obtained by culturing the excised meristems on MS semisolid medium supplemented with IAA, IBA and BAP at various concentrations. The regenerative potential of all cultivars of *Musa*, irrespective of their genomes, remained unaffected in long-term culture, even after 28–30 months. The genomic influence on both the nature and rate of proliferative growth was evident. Statistical analysis revealed that the rates of meristem proliferation between different cultivars of the same passage and between different passages of the same cultivar were significantly different. Those cultivars having only an A genome showed a low rate of meristem proliferation, while under the same culture conditions, cultivars having one or two B genomes in addition to the A exhibited a very high rate.

Key words: *Musa* – Meristem proliferation – Genomic constitution – Long-term culture – Plantlet regeneration

Introduction

Most of the commercial cultivars of *Musa* are triploids and seed sterile. These triploid cultivars have been subdivided into several groups on the basis of their genomic constitution (Simmonds 1966). The most common edible cultivars are included in the AAA, AAB and ABB groups where A and B represent the haploid genomes of *Musa acuminata* and *Musa balbisiana*, respectively. Some diploid and tetraploid forms (AA and AAAA) of *Musa acuminata* are also grown for commercial purpose. The clonal propagation of *Musa* cultivars using conventional techniques has serious limitations because of the low rate of multiplication of these cultivars (5–10 years) (Barker 1959; Hamilton 1965; Ascenso 1967).

The prevalence of disease problems and the need for generating disease-free plant material have recently stimulated an upsurge of interest in the regeneration of clonal plantlets using various in vitro techniques (Cronauer and Krikorian 1984; Banerjee and De Langhe 1985; Jarret et al. 1985; Banerjee et al. 1986, Vuylesteke and De Langhe 1986; Wong 1986; Banerjee et al. 1987). Despite a number of published works, reports on the effects of genomic diversity and hormonal composition on the growth and rate of multiplication in long-term cultures of *Musa* are scarce.

This paper, therefore, describes a systematic study of in vitro rapid multiplication and the effects of various genomes at different ploidy levels on the growth and proliferation of meristem tips in long-term cultures of some selected cultivars of *Musa*.

Materials and methods

Materials

Ten commercial cultivars of *Musa* having a genomic combinations of AA, AAA, AAB, ABB or AAAA were obtained through the courtesy of Laboratory of Tropical Crop Physiology, Catholic University of Leuven, Belgium, and grown in the experimental garden of the Department of Botany, University of Calcutta.

Cultural techniques

For isolation of shoot apical meristems, explants from meristems of suckers, peepers, dormant eyes and parental pseu-

dostem were used. The method of shoot meristem isolation has been described earlier (Banerjee et al. 1986).

Isolated shoot apices of 2 cm^3 were surface disinfected by immersion in a 0.1% mercuric chloride solution for 15–20 min, followed by repeated washings in sterile water. Pieces of shoot apices of 2–4 mm, were then microscopically dissected out. Explants were cultured on MS basal medium (Murashige and Skoog 1962) supplemented with various growth regulators at different concentrations. Ascorbic acid (10 mg/l) was added to the medium as an antioxidant to reduce blackening possibly due to oxidized polyphenols. Cultures were maintained at 22° ± 2 °C under a 16 h daily illumination of 3,000 lux. Subculturing was done every 8 weeks by subdividing the multiple shoot clusters or meristem tips. Counts were kept of the number of shoot meristems in every passage just before subculturing. For each passage, the mean value of the number of meristem tips was calculated from ten readings.

Statistical analysis

For the statistical analysis, the mean numbers of meristem tips per explant in long-term culture were used for the two-way Anova test (Sokal and Rohlf 1973) followed by Duncan's multiple range test (Harter 1960) to find out the significant differences, if any, between different passages of each cultivar and between cultivars in each passage.

Results and Discussion

In vitro growth response and plantlet regeneration

The small explants comprising 2–3 young leaf primordia and the shoot apex turned green within 15–20 days of inoculation. Both the explant as well as the surrounding medium slowly turned black, a possible consequence of the oxidation of polyphenols (Simmonds 1966); this occurred in all cultivars regardless of their genotypic status. The blackening could be controlled by adding ascorbic acid (10 mg/l) to the medium and also by transferring the explant frequently onto fresh medium. Two distinct types of growth response were noticed. In MS medium supplemented with IAA (0.2 mg/l) and BAP (0.2 mg/l), a single shoot regenerated from an excised shoot tip; upon increasing the BAP level to 2 mg/l while keeping the IAA level constant, multiple shoot buds originated. These multiple meristem tips developed as a uniform meristematic layer on the surface. This layer originated through the rapid extension and subsequent flattening of the initial meristematic dome (Banerjee et al. 1986). In the shoot regeneration medium, the outermost leaf primordium slowly opened at the tip and unfurled the lamina within 4 weeks. There was a gradual emergence of the younger leaf primordia (Fig. 3). In this medium the response of all genotypes was more or less similar. Shoot outgrowth was extremely slow when kinetin was used instead of BAP. After 2 weeks of growth in MS semisolid medium containing a half concentration of macrosalts and IBA (0.2 mg/l), rooting from the base of the established shoots could be achieved (Fig. 4). As soon as the roots attained a length of 2–3 cm, the plantlets were transferred to 250 ml Earlenmeyer flasks containing 50 ml of MS semisolid medium supplemented with a half concentration of macrosalts, but no growth regulators. Growth was very fast, with the plantlets attaining a height of 8–10 cm. These plantlets were finally transferred to soil.

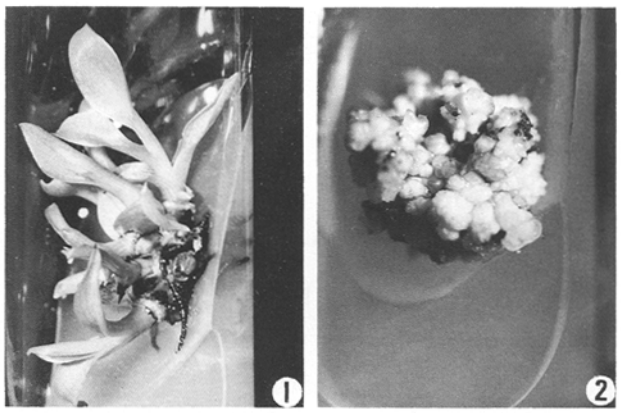

Fig. 1. Proliferation of shoot buds of *Musa* cv 'Valeri' AAA. Note the emergence of a bunch of leafy shoots

Fig. 2. Proliferation of meristem tips of *Musa* cv 'Espermo' ABB. Note the formation of a large number of white bulbous structures bearing tiny meristems on their surface

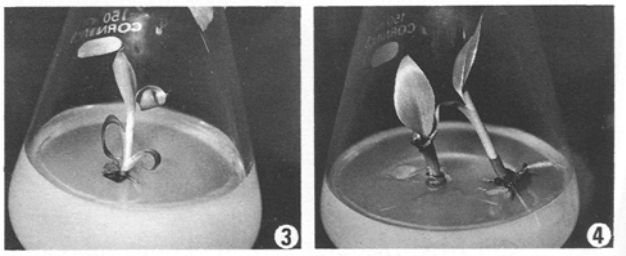

Fig. 3. Regeneration of leafy shoot from an excised meristem tip of *Musa* cv 'Valeri' AAA after 4 weeks

Fig. 4. Root induction and subsequent development of the plantlet

Regeneration potential in long-term cultures

The regeneration potential of all the cultivers in long-term culture was studied in different passages. Plantlets of each cultivar could be frequently regenerated when individual meristems excised from multiple shoot apices were placed on MS medium containing a reduced level of BAP (0.2 mg/l). The meristems of cultivars having only the A genome showed a quicker response in shoot outgrowth as well as a further elongation of the plantlet than that shown by cultivars having the AAB and ABB genomic constitution. With increasing age of the cultures, there was no decrease in regeneration potential. In a few

Table 1. Mean number of meristem tips of different cultivars of *Musa* in long-term culture

Cultivar and genotype	Mean number of meristem tips/explant							
	2 months	6 months	10 months	14 months	18 months	22 months	26 months	30 months
Pisang tongat (AA)	4.0	6.0	6.4	12.4	12.5	10.0	12.2	9.5
Valeri (AAA)	2.5	6.8	8.0	18.5	18.3	18.6	17.8	15.6
Musa acuminata (AAA)	4.8	10.2	10.6	15.4	9.5	13.3	12.4	10.5
IC-2 (AAAA)	3.5	10.2	8.5	12.6	10.6	6.0	11.4	9.5
Monthan (AAB)	10.5	14.8	21.0	30.2	27.5	31.7	27.4	28.7
Mulolou (AAB)	4.2	12.2	20.4	23.2	17.9	20.4	26.4	31.2
Silk (AAB)	3.5	10.3	10.8	13.9	24.3	21.4	24.8	28.4
Bluggoe (ABB)	7.4	45.8	26.4	38.0	34.7	39.4	34.2	31.6
Espermo (ABB)	4.8	10.2	21.6	25.6	34.5	26.0	36.5	30.0
Pisang Abu Perak (ABB)	2.4	3.8	12.0	20.8	27.6	34.2	24.5	22.4

Table 2. Two-way anova test showing significant differences in the rates of meristem proliferation within and between cultivars of *Musa*

Source	Degrees of freedom	Sum of squares	Mean sum of squares	'F' ratio
Within cultivars, between subcultures	9	3265.75	362.86	17.56**
Within subcultures, between cultivars	7	3239.57	462.79	20.40**
Error	63	1384.00	20.66	

** Significant both 5% and 1% levels

cultivars, we registered a slight fall in rate of shoot meristem multiplication towards the end (after 22 months). In general, the regenerative potential of all the cultivars irrespective of their genomes, remained unaffected even after 28–30 months of culture.

Shoot meristem proliferation and genotypic diversity

Shoot meristems proliferation was recorded on MS medium supplemented with IAA (0.2 mg/l) and BAP (2 mg/l). In the diploid, triploid and tetraploid cultivars possessing only the A genome, there was adventitious growth of a number of leafy shoots (Fig. 1). In cultivars having the AAB and ABB genomes, on the other hand, numerous fleshy bulbous structures developed from the explant, each in turn bearing tiny meristem tips on their surface (Fig. 2). Thus, genomic influence on the nature of proliferative growth was evident.

The rate of shoot meristem proliferation was recorded for every passage between 2 and 30 months. Statistical analysis revealed, in general, a high level of significant difference (Tables 1 and 2) in the rate of proliferation between different varieties of the same passage and also between different passages of the same variety. At the initial stage, 2 months after inoculation, there were no marked differences in the rate of proliferation between different genotypes: mostly it ranged from 2.4 to 7.5,

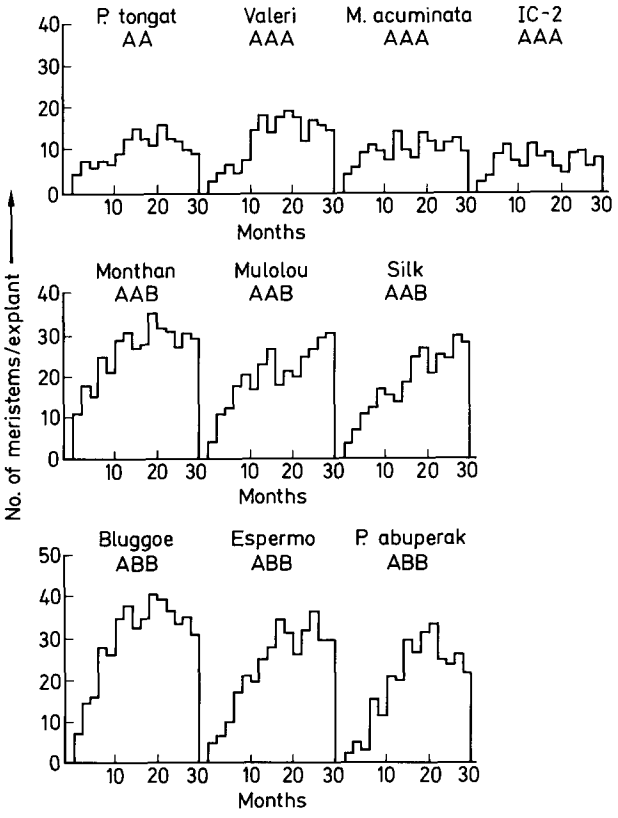

Fig. 5. Histograms representing the rates of meristem tip proliferation in different subcultures of all the cultivars tested belonging to the five genomic classes

with the exception of 10.5 recorded in 'Monthan', an AAB cultivar. There were marked differences, however, in later subcultures. Those cultivars having only the A genome ('Pisang tongat', 'Valeri', 'IC-2', etc.) showed a slow increase in the rate of proliferation achieving a maximum of 15–20 tips/explant during 20–22 months of culture, followed by a slight decline in the later period. Under the same cultural set-up, cultivars having one or two B's in addition to A exhibited a sudden spurt in the rate of proliferation after one or two initial subcultures.

Table 3. Non-significant blocks of cultivars in 2-, 18-, and 30-month-old cultures

Age in months										
	Pisang Abu Perak (ABB)	Valeri (AAA)	Silk (AAB)	IC-2 (AAAA)	Pisang tongat (AA)	Mololou (AAB)	Espermo (ABB)	Musa acuminata (AAA)	Bluggoe (ABB)	Monthan (AAB)
2	2.4	2.5	3.5	3.5	4.0	4.2	4.8	4.8	7.4	10.5
	Musa acuminata (AAA)	IC-2 (AAAA)	Pisang tongat (AA)	Mulolou (AAB)	Valeri (AAA)	Silk (AAB)	Monthan (AAB)	Pisang Abu Perak (ABB)	Espermo (ABB)	Blaggoe (ABB)
18	9.5	10.6	12.5	17.9	18.3	24.3	27.5	27.6	34.5	34.7
	IC-2 (AAAA)	Pisang tongat (AA)	Musa acuminata (AAA)	Valeri (AAA)	Pisang Abu Perak (ABB)	Silk (AAB)	Monthan (AAB)	Espermo (ABB)	Bluggoe (ABB)	Mulolou (AAB)
30	9.5	9.5	10.5	15.6	22.4	28.4	28.7	30.0	31.2	31.6

A maximum of 35–40 tips/explant during 20–24 months of culture was recorded (Fig. 5). The influence of the B genome on the acceleration of the proliferative response is clearly indicated: an increase in the dosage of B, as in 'Bluggoe' and 'Espermo' (ABB), leads to a higher acceleration than that found in cv 'Silk' (AAB). In cultivars with only the A genome, the addition of A neither retards nor accelerates growth further (Fig. 5).

The analysis of the variance test revealed that, in general, there was a highly significant difference in the rate of meristem proliferation between cultivars in different passages (Tables 1 and 2). However, a number of non-significant blocks of cultivars were recorded from Duncan's multiple range test (Harter 1960). Thus, in 2-month-old cultures, cultivars belonging to all five different genotypes form non-significant blocks (Table 3). In 18-month-old cultures, four non-significant blocks were found: one consisted of three cultivars possessing only the A genome; one, only the ABB; the remaining two blocks contained cultivars with the AAB, ABB and AAA genomic constitution. Finally, after 30 months, two fairly large non-significant blocks were recorded where one consisted of cultivars with only the A genome while the other block consisted of cultivers with either one or two B genomes in addition to A. Such non-significant blocks at different stages of growth may be an index of genomic affinities. The formation of two distinct blocks with and without the B genome, respectively after 30 months reflects the significant influence of B in the proliferative response.

The present study shows the potential of proliferative capacity in long-term cultures of different genotypes of *Musa*. The influence of genomic interaction on the nature and rate of in vitro proliferation is clearly indicated.

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