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BRIEF COMMUNICATION

Esterase as a marker to study the genetic fidelity of micropropagated banana

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Abstract

Isozymic profiles of different micropropagated banana (*Musa* spp.) cultivars (Giant Governor, Dwarf Cavendish, Robusta, Champa, Kachakel and Chatim) of West Bengal, India were assessed at different subcultural passages. Variation with respect to the banding pattern was noticed only in esterase but not in peroxidase and acid phosphatase. Of the six cultivars, four showed variation both at isozymic and yield level. Two cultivars (Kachakel and Chatim) maintained their esterase profile and genetic stability even after twenty subcultural passages.

Additional key words: acid phosphatase, isozyme, *Musa* spp, peroxidase, subcultural passage.

Application of micropropagation for large scale production of elite clones is an effective and superior alternative to seed and conventional cuttings. *Musa* spp. are often micropropagated (Cox *et al.* 1960, Croaneur and Krikorian 1984, May *et al.* 1995, Mendes *et al.* 1999). Six banana cultivars (Giant Governor, Dwarf Cavendish, Robusta, Champa, Kachakel and Chatim) were established *in vitro*. Plantlets from these cultures were supplied to the farmers. The field performance was very encouraging in the first year but discouraging for some cultivars in the second year although the agronomic practices were the same. It was, therefore, essential to check the tissue cultured clones exhibiting reduced yield. Fresh cultures of the same cultivars were initiated and comparative isozymic profiles of cultured clones were studied at different stages with respect to their mother populations to assess the genetic fidelity of the cultured plants.

Cultivars of *Musa* spp. used were: Giant Governor, Dwarf Cavendish and Robusta (all AAA genomes); Champa (AAB), Kachakel (ABB) and Chatim (AB). Elite mother plants of first four cultivars were procured from Banana Research Station, Chinsura, West Bengal, India, while the last two were locally available hybrids between *M. acuminata* (AAA) × *M. balbisiana* (BBB).

The selected cultivars were established *in vitro* according to Balakrishnamurthy and Sri Rangaswamy (1992). Cultures were initiated in MS basal medium (Murashige and Skoog 1962) supplemented with 0.55 mM myoinositol, 80 mM sucrose, 11.42 μM indole-3-acetic acid (IAA) 22.19 μM N^6-benzyladenine (BAP). The same culture medium was used for multiplication but for rooting MS basal medium was supplemented with 9.80 μM indole-3-butyric acid (IBA). Cultures were kept under 16-h photoperiod (irradiance of 40 - 80 μmol $m^{-2} s^{-1}$), temperature of 25 ± 1 °C and relative humidity of 78 %. Before transplanting agar was removed from the roots in running water, plantlets were dipped in 0.5 % (m/v) *Bavistin* and transferred to polythene bags containing sand:farm yard manure:loamy soil in the ratio of 1:1:1. For hardening, the plantlets were grown in a humidity tent for a month. Then the plantlets were transferred to

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Abbreviations: ACP - acid phosphatase; BAP - N^6-benzyladenine; EST - esterase; IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; MS - Murashige and Skoog's basal medium; PRX - peroxidase.
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nursery for two months before subsequent distribution to the farmers. The yield data were collected both from the farmers' and experimental plots. Student's *t*-test was done to check the significance of the differences between plants of mother and tissue cultured population of each cultivar.

Prior to isozyme analysis fresh cultures were raised and samples were collected from *in vitro* plantlets (in culture vessel) after ten and twenty subcultural passages. Samples were also collected from the plants of mother population and suckers (*in vivo*) and from the tissue culture-raised plants transferred to field after twenty subcultural passages. All the samples, excepting the *in vitro* grown ones, were collected in pre-flowering stage. Isozymic analyses of three enzymes - peroxidase (PRX, E.C.1.11.1.7), acid phosphatase (ACP, E.C.3.1.3.2) and esterase (EST, E.C.3.1.1.1) were done after extraction of 2 g leaf tissue in each case (Wetter and Dyck 1983, Das and Mukherjee 1997). Leaf samples were homogenized at 4 °C with 1.5 cm^3 of 0.2 M Tris-HCl buffer (pH 8.5) containing 1 M sucrose and 0.056 M 2-mercaptoehanol. The macerate was centrifuged at 16 000 *g* for 20 min at 4 °C; the supernatant was used as source of enzyme. The protein content was estimated by the Folin-phenol method (Lowry *et al.* 1951). Equimolar amounts of protein (50 μg) of mother plant (*in vivo*), sucker of same mother plant (*in vivo*), tissue culture derived plant both in *in vivo* and *in vitro* condition were loaded in gels. Electrophoretic runs were made for 3 - 4 h at 2 mA per lane at 4 °C. The gels were developed following Das and Mukherjee (1997) method. Densitometric scan of the gels was done using *Biorad Gel Documentation System* (*Gel Doc 1000*, version *1.5*).

Two yield attributes, number of fruits and bunch mass were recorded. No significant difference in the yield attributes was noticed between mother population and clones, transplanted to the soil within ten passages of subculture (data not shown). However, both number of fruits and bunch mass of the tissue cultured plants declined ($P < 0.01$) in comparison to the plants of mother population in Giant Governor, Dwarf Cavendish and Champa (Table 1). In case of Robusta, bunch mass of tissue cultured plants was identical (difference statistically insignificant) to that of mother population but a significant decline ($P < 0.001$) of number of fruits per plant was noticed. Number of fruits of the tissue cultured plants increased significantly in comparison to those of mother population but bunch mass per plant remained identical in Kachakel and Chatim.

The results of the isozyme analysis of the four cultivars manifesting lower yield were comparable. For convenience only the results of Giant Governor is provided. Furthermore, as there was no difference in banding profile of mother and tissue culture-raised plants after ten subcultural passages, the results presented here are only those of *in vitro* plants after twenty subcultural passages. Peroxidase and acid phosphatase profiles of mother, its sucker, *in vitro* plant and *in vitro* plant established in soil were almost identical but differences were noted in relative mobility and intensity of bands. Bands of mother plant (*in vivo*) showed the highest enzyme activity while those of micropropagated plant (in culture vessel) showed lowest activity (Fig. 1*A,B*). Esterase profile of mother, its sucker though was almost identical but distinct difference in banding profile were noted in the micropropagated plants (in culture vessel) and the transplanted plants as two fast migrating new esterase bands appeared in the latter cases (Fig. 1*C*).

Table 1. Comparison of yield of mother plant and tissue culture (TC) derived plants (transplanted after 20 passages of subculture) (means ± SE, $n = 10$; * - $P < 0.05$, ** - $P < 0.001$, [a] - yield increased in the tissue cultured plants).

Cultivar	Source	Fruit number [plant^{-1}]	Bunch mass [kg plant^{-1}]
Giant Governor	mother	125 ± 1.22	15 ± 0.77
	TC	115 ± 2.61*	11 ± 0.67*
Dwarf Cavendish	mother	120 ± 1.74	15 ± 0.73
	TC	100 ± 3.66*	12 ± 0.84*
Robusta	mother	155 ± 3.10	18 ± 1.03
	TC	139 ± 2.59**	17 ± 0.67
Champa	mother	224 ± 3.70	16 ± 0.68
	TC	172 ± 2.19**	12 ± 1.00*
Kachkel	mother	100 ± 4.79	15 ± 0.84
	TC	126 ± 1.27**[a]	16 ± 0.92
Chatim	mother	102 ± 4.13	10 ± 0.83
	TC	114 ± 2.72*[a]	10 ± 1.11

Genetic fidelity is the maintenance of the genetic constitution of a particular clone through out its growth span (Chaterjee and Prakash 1996). Maintaining genetic fidelity is the quintessential factor in meeting the ever-rising demand of plant materials required for micro-propagated crops. While assessing the genetic fidelity from the agronomical point of view it was observed that a distinct decline of yield of tissue cultured clones had taken place in four out of the six banana cultivars under present programme (Table 1). It has been reported that genetic stability of tissue cultured clones mainly depends on genotype, the chimeric nature of the explant used, cultural conditions, maintenance of cultures (Haisel *et al.* 2001) and number of subcultural passages (Chaterjee and Prakash 1996). In the present case genetic fidelity was not maintained beyond certain time limit, the factor of sub cultural duration so was considered seriously and to ascertain the exact limit of that critical period, identification of a proper marker system seemed to be of utmost importance.

The genetic fidelity of the tissue cultured clones is often examined through isozymic patterns (Gupta and

Varshney 1999, Rout *et al.* 2000). An isozymic profile which is very stable during the growth of cultured tissue and not much affected by the environmental factors, should be considered as a marker (Chaterjee and Prakash 1996). Of the three isozymes studied, the profiles of peroxidase and acid phosphatase did not reveal any marked change in banding patterns, the only thing noteworthy was the differential staining intensity of the major band(s). The activity of both the enzymes was most pronounced in case of the samples derived from the *in vivo* plant of mother populations. This probably reflected the inherent defense mechanism of that plant against external environmental stimuli as peroxidase, in particular, had been reported to act as a barrier against both abiotic and biotic stresses (Edreva *et al.* 1989, Gangopadhyay *et al.* 1996). The diminishing intensity of the isozymic band(s) of the sample derived from the *in vitro* plant growing in the culture vessel was naturally predictable.

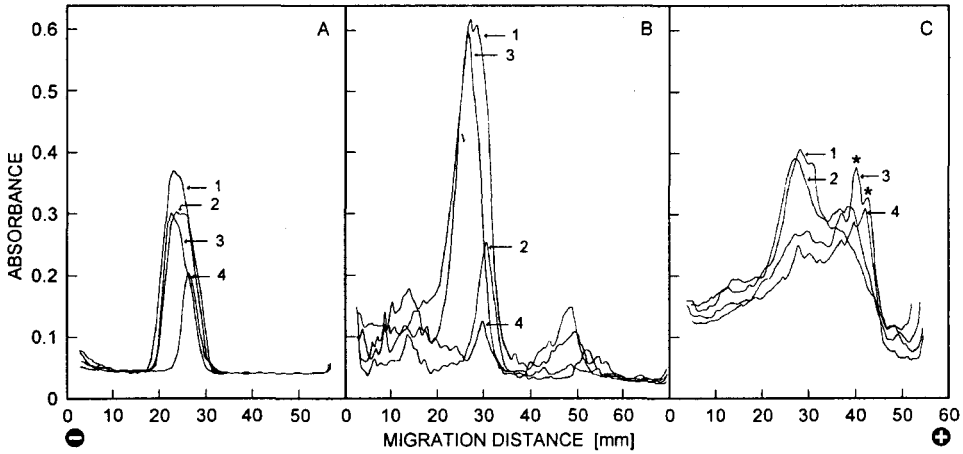

Fig. 1. Densitometric profile of peroxidase (*A*), acid phosphatase (*B*) and esterase (*C*) of banana (cv. Giant Governor): 1 - mother, 2 - sucker, 3 - *in vitro* plant, 4 - *in vitro* plant after transplantation, * - appearance of new bands.

Esterase profile, on the other hand, showed distinct alteration in banding pattern as two new bands appeared in the profiles of *in vitro* plants maintained in culture beyond twenty passages of subculture. This result provides the circumstantial evidence that recurrent subculture for indefinite period hinders maintenance of genetic fidelity in the four tissue cultured clones. Continuation of the cultures beyond a critical subcultural passage followed by transplantation and distribution of those plants to the farmers is probably the cause of declining yield. These results also indicate that for assessing genetic fidelity of tissue cultured clones of banana, esterase isozymic profile is plausibly the appropriate marker to identify the critical subcultural passage beyond which variation can occur. The present study also reveals that tissue culture derived plants of two out of the six cultivars Kachakel and Chatim manifest higher yield potential compared to their respective mother plants even after a prolonged tenure in culture (beyond twenty subcultural passages). These two cultivars are local. Hybrid nature of these genotypes, prolonged adaptation to the present agro climatic region along with a positive feedback support from the cultural milieu perhaps are the contributing factors for these varieties to show better vigor and maintain genetic fidelity.

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