Short communication

Micropropagation in banana var. Korangi for *in vitro* conservation in Bay Islands

Asit B. Mandal*, Sonali Das and R. Elanchezhain

Biotechnology Section, Central Agricultural Research Institute, Port Blair 744 105

Biodiversity needs to be conserved for ecological sustainability, economic security and self-reliance of a country (Bhagyalakshmi and Singh, 3). The union territory of Andaman and Nicobar Islands represents one of the richest biodiversity centres in south and south-east Asia harbouring about 2,500 angiosperm species belonging to 84 diverse families. A large number of banana cultivars including indigenous wild stocks are available these islands in (Balakrishnamurthy and Sree Rangaswamy, 1). One such germplasm var. Korangi is dwindling because of ruthless deforestation. Their number has depleted alarmingly. The cultivar seems to have wide adaptability to thrive under humid tropics endowed with ~ 3100 mm rainfall. It also possesses tolerance to prevailing insect and disease pests, which are rampant in these warm humid tropics. To conserve this precious germplasm, in vitro micropropagation technique was adopted for mass multiplication, which has been proved to be effective in short time at low cost in many species.

The present study was aimed at evolving a suitable micropropagation protocol in banana var. Korangi. Various mass multiplication systems were utilized in banana, *e.g.* culture of shoot tips (Swaminathan, 9) and floral apices (Banerjee, 2) by employing diverse media and hormonal supplements (Doreswamy and Sahijram, 4; Ganapathi *et al.*, 5). In the present endeavour efforts were made to optimize mass multiplication of a few available plants of Korangi and their replanting *in situ*, so that propagules could be produced in plenty for establishing a fair population of this precious germplasm in Bay Islands.

Explants were obtained from lateral buds and suckers of field grown banana plants (var. Korangi) from south Andaman (6°45' and 13°41' N latitude and 92°12' and 93°57' E longitude). Individual shoot tips were isolated after removing sheath leaf bases. The white shoot tips (ca 3-3.5 × 2-2.5 cm) were treated with the fungicide bavistin (carbendazim 50%); 1% aqueous solution) for 20 min. After repeated rinsing in distilled water the shoot tips were kept in sterile

antioxidant solution containing 150 mg/l citric acid in 100 mg/l ascorbic acid as practised by (Doreswamy and Sahijiram, 6). They were trimmed to about 4-4.5 mm in length including the growing tip and a little corm tissue underneath. Trimmed explants were dipped in 70% ethanol for 30 and transferred directly into 0.1% HgCl_a for 10 min. Those were thoroughly washed with sterile distilled water for four times with vigorous shaking manually. Finally, sterile explants were placed onto (Murashige and Skoog, 7) basal medium with 500 mg/l casein hydrolysate, 200 mg/l tyrosine, 50 mg/l ascorbic acid and 30 mg/l sucrose. The pH of the medium was adjusted to 5.8 prior to addition of 0.8% agar (Sigma Chemcial Company, USA). All cultures were maintained at 25 ± 2°C with 16/18 h light (2000 lux)/dark cycle. The medium is supplemented with 1 to 4 mg/l kinetin or BAP singly or in combination. In all treatments, 0.5 mg/l of NAA was used. To break apical dormancy a vertical incision was given to each bud before final placement on culture medium.

Shoot elongation and rooting of in vitro developed adventitious buds were achieved by placing the developing shoot buds (2-3 cm height) on MS basal medium with various concentrations of IAA and IBA (0.1, 0.5, 1.0 and 2.0 mg/l each) singly and in combination. The in vitro response was scored after four weeks of culture. Plantlets were taken out from the culture tubes carefully and the agar adhered to roots was removed by thorough washing with water. Clusters of plantlets were separated into individuals manually. Before transplanting in sterile soil, bavistin (25% solution) treatment to individual plant was given for 30s. The plants were watered as and when required and for the first few days, water was sprayed 3-4 times per day. The established plants were finally transferred to field for evaluation.

A suitable micropropagation system for var. Korangi was finally developed. Cultured buds swelled up with visible proliferations within 20-25 days. Clusters of buds were developed on the explants. Those smaller buds were separated manually and individual buds were cultured onto freshly prepared multiplication medium of same composition. It led to the development of clusters of bud again and the cycle was found to be

^{*}Corresponding author's present address: Project Director, Directorate of Seed Research, Mau 275 101; E-mail: pd_dsr2005@yahoo.co.in

Hormone (mg/l) combination*	No. of buds per culture per cycle**	<i>Ex-vitr</i> o survival (%)
3.0 kinetin	5.2	44.5
4.0 BAP	7.6	30.7
2.0 kinetin + 1.0 BAP	6.3	42.6
2.0 kinetin + 2.0 BAP	7.8	34.8
3.0 BAP + 1.0 kinetin	9.2	35.9
2.0 BAP + 3.0 kinetin	6.5	27.9

Table 1. Effective hormonal supplements for micropropagation of indigenous banana var. Korangi.

*In all treatments, 0.52 mg/I NAA was a common auxin additive to MS basal medium.

**Cycle of 40 days duration

repeated. Those buds were sub-cultured repeatedly by dividing them into groups of 1 to 2 buds. Each individual bud produced 4 to 6 buds within a month. Maximum buds obtained were 9.2 in this experiment



Fig. 1. Micropropagation of banana in a var. Korangi. a. Proliferating cultural shoot apex; b. Initiation of lateral buds; c. Buds on multiplication medium; d. *In vitro* developed plantlets on rooting medium; e. Tender plants with roots; f. A plant established on soil in a plastic pot; g. A complete plant in the field; h. a fully grown bunch from the micropropagated plant.

on MS basal medium with 3.0 mg/l BAP and 0.5 mg/l NAA (Table 1). This was corroborative to the earlier reports of inflated multiplication by using higher concentration of BA in the medium (Doreswamy and Sahijram, 4). BAP and NAA were used successfully in multiplication of banana cultivars in many studies (Doreswamy and Sahijiram, 4; Swaminathan, 9; Ganapathi *et al.*, 6). However, the effective concentration of these growth regulators in the media varied with different banana cultivars. A combination of 0.2 mg/l IAA and 2 mg/l BAP (4) and 1 mg/l NAA and 5 mg/l BA (Singh *et al.*, 8) in MS were found to be effective in the concentration and development of banana plantlets.

In root development IAA and IBA in combination was found to be superior than using alone. Maximum roots of an average of 8 per plantlets in ~78% cultures were obtained on MS supplemented with 1.0 mg/l IBA with 0.5 mg/l IAA. In the present study, it was found that rooting in vitro was not essential for ex vitro survival. This was in conformity to the finding of Bhagyalakhsmi and Singh (5). In vitro grown plantlets were transferred to the experimental net house. The maximum ex vitro survival percentage of plantlets was 44.4% obtained from the multiplication medium containing 3 mg/l kinetin and 0.5 mg/l NAA treatment (Table 1). Bhagyalakhsmi and Singh (5) also reported 3 50% survival rate in dwarf Cavendish, Bluggoe and Silk varieties with 1 mg/l BA. However, the number of buds was found to increase and the ex vitro survival decreased with increased concentration of BAP in the medium (Table 1). This prospects in vitro micropropagation as an appropriate proposition for in vitro conservation of this indigenous germplasm of Bay Islands.

ACKNOWLEDGEMENT

The authors are grateful to the Director, Central Agricultural Research Institute, Port Blair for the facilities provided.

REFERENCES

- 1. Balakrishnamurthy, G. and Sree Rangaswamy, S.R. 1988. Regeneration of banana plantlets from *in vitro* culture of floral apices. *Curr. Sci.* 57: 271-72.
- Banerjee, N. 1989. *In vitro* technology for genetic conservation for banana- A case study. *Plant Genet. Resources* 2: 95-102.
- 3. Bhagyalakshmi, N. and Singh, N.S. 1995. Role of liquid versus agar gelled media in mass propagation and *ex vitro* survival in bananas. *Plant Cell, Tissue Organ Cult.* **41**: 71-73.
- Doreswamy, R. and Sahijram, L. 1991. Tissue culture strategies for banana. In: *Horticulture-New Technologies and Applications* (eds. Prakash, J. and Pierik, R.L.M.), Kluwer Academic Publishers, Netherlands, pp. 219-23.
- Ganapathi, T.R., Suprasanna, P., Bapat, V.A. and Rao, P.S. 1992. Propagation of banana through encapsulated shoot tips. *Plant Cell Rep.* 11: 571-75.

- Ganapathi, T.R., Suprasanna, P., Bapat, V.A. and Rao, P.S. 1994. Stimulatory effect of cyanobacteria extract on banana shoot tip cultures. *Trop. Agric.* (*Trinidad*) 71: 299-302.
- 7. Murashige, T. and Skoog, F. 1962. A revised medium and rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15**: 473-97.
- Singh, D. B., Sharma, T.V.R.S. and Suryanarayan, M.A. 1996. Evaluation of banana cultivar under rainfed condition in Andamans. *South Indian Hort.* 44: 90-92.
- Swaminathan, M.S. 1998. Biotechnology and agricultural betterment in the developing countries. In: *Biotechnolgy in Agricluture*. Natesh, S., Chopra, V.L. and Ramachandra, S. (eds), Oxford and IBH Pub. Co. Pvt. Ltd. New Delhi. pp. 1-11.

Received: July, 2005; Revised: November, 2009; Accepted : December, 2009