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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 4 (5): 562-563, 2001

An Efficient and Economical Method of Mass Multiplication of Virus and Disease Free Banana Using Plant Tissue Culture Techniques

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Abstract: Of the five supportive materials tried, MS with cotton fiber was found to be the best for rapid growth and plants were ready for further sub-culturing after two weeks while the control showed the same results after six weeks. Addition of table sugar was tested against sigma sucrose. Both gave the same growth of banana plants.

Key words: Micropropagation, banana, *in-vitro* MS, cytokinine

Introduction

Traditionally Banana is propagated vegetatively by means of suckers. This results in the wide spread of diseases due to the infected plant material. So it is necessary to replace the infected plant materials by healthy ones. Micropropagation offers an efficient method for rapid and disease free clonal propagation of defined banana varieties. Apical meristem culture offers an efficient method for rapid clonal propagation, production of pathogen-free material and germplasm preservation in plants (Wang 1986). But *In vitro* propagation of different cultivars through excised meristem required different culture media for shoot multiplication and root differentiation (Khanum *et al.*, 1996).

In this study, an exotic cultivar of banana imported from Dhaka named Amrit Sagar has been used. The introduction of this type of variety will not only increase the production, but will also give exportable quality. To compete with international market, an exotic commercial variety was selected to standardize the protocol for commercial-scale multiplication on cost-effective system.

Agar contributes to the matrix potential, the humidity, and it also affects the availability of water and dissolved substances in the sealed container (Debergh, 1983). Explants are either supported on top of the medium or are submerged in it. For the culture of cells, tissues and organs, it is necessary to incorporate a carbon source into the medium. Sucrose is almost universally used for micropropagation purposes, as it is generally utilizable by tissue cultures. Sucrose was found to be the best source of carbon followed by glucose, maltose and raffinose.

In this paper, we tried to workout some alternative sources of agar and sucrose for establishing an economical system of micropropagation of Banana on commercial-scale.

Materials and Methods

Micropropagated cultures of Banana cv. Amrit Sagar (Genome AAA) having good growth were selected for subculturing. Cotton fiber, wood chips, paper pieces, sawdust and coarse sand were selected as alternative for agar to support explants. Commercial sugar (Rs. 22/kg household) was selected as a "Carbon Source" instead of sigma sucrose". Cost of Sigma products during the experiment (1998) were as in Table 1.

Table 1: Cost of sigma products during the experiment (1998).

Catalog #	Name	Quantity	US $	RS.
A.9915	Agar Powder	1 kg	132.50	6095.00
S.1888	Sucrose >99.5%	1 kg	39.80	1830.80

The explants were subcultured on MS liquid medium with BAP 3.75 mg/L and Kinetin 2 mg/L, commercial sugar and Sigma sucrose were used at 30 g/L. The pH of medium was adjusted to 5.7 by 1M NaOH and 1M HCl. The supporting materials were added in 250 ml jars to the height of 2 cm and liquid media was added about 30 ml to cover the supporting material. The medium was sterilized by autoclaving. Each treatment contained ten replicates. Solid media was also prepared by adding agar 6 g/L as control. All cultures were incubated at 25 ± 1°C with a 16 h-photoperiod (2000-lux) provided by cool white fluorescent tubes. Regular observation and data recording provide interesting results, The differentiated explants were cultured in half strength MS supplemented with IBA, 1 mg/L for rooting.

Results and Discussion

Healthy shoot regeneration potential of subcultured banana plants on different supporting, materials was investigated. The presence of cotton fiber as a supporting material significantly effects the growth of subcultured banana explants within two weeks. Same growth of banana plants was seen after six weeks in solid media (control treatment). Within two weeks subcultured plants with different supporting materials that are T_0-Control, T_1-Wood chips, T_2-Paper pieces, T_3-Cotton fiber, T_5-Coarse Sand started to regenerate shoots except T_4-Sawdust. (Fig. 1) By the comparison of all treatments it is obvious that cotton fiber showed significantly high degree of shoot induction and showed best response towards multiple shoot regeneration, subcultured with cotton fiber support. The highest number of shoots (4) were produced with cotton fiber support as wel as the highest elongation was also observed in explants supported with cotton fiber (Fig. 1).

Use of cotton fiber enhanced the availability of nutrients and inoculated plants showed vigorous growth and maximum number of buds. As compared to agar, cotton also showed good absorptive properties for phenolic compounds secreted by inoculated plants. In cotton fiber system phenolics and other toxic compounds released by plants immidiately diluted in the media and become less toxic. This research successfully found a cost-effective system and cotton fiber proved to be an economicaly supportive medium. Above research was also conducted with sigma sucrose and table sugar, both carbon sources showed same results. Table sugar provides an economical alternative to Sigma sucrose which ultimately

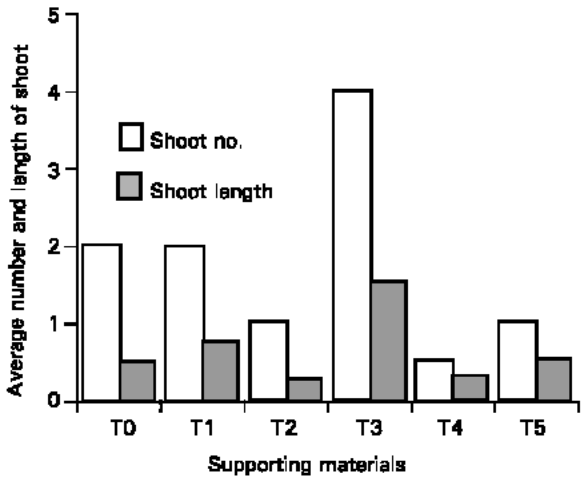

Fig. 1: Data was recorded after two weeks. T_0-Agar, T_1-Wood chips, T_2-Paper pieces, T_3-Cotton fiber, T_4-Sawdust, T_5-Coarse sand. (A) Average number. of shoots per culture. Each bar is the mean of 30 explants. (B) Average shoot length per culture in cm.

Fig. 2: Rooting observed in banana 4 weeks after culturing on MS media supplemented with IBA

affects the final production cost of tissue cultured plants.

For rooting, the shoots of banana were cultured onto MS media supplemented with IBA (Fig. 2). According to Ghose (1993) plants cultured on media supplemented with IBA showed good rooting within a period of four weeks. After weaning, rooted plants were transferred to small polythene bags and kept in net house. All plantlets survived and showed cent per cent result with good growth. Now these are ready to be sold.

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