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EFFECT OF VARIOUS MEDIA ALTERNATIVES ON *IN VITRO* PROPAGATION OF BANANA (G-9) THROUGH TISSUE CULTURE

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Abstract: *Cost of media for explant propagation is one of the most significant factors affecting economy of tissue culture units. In this study, we evaluated effect of selective media alternatives on micropropagation of Musa cavendish cultivar G-9. Effect of using blotting paper as support matrix to replace agar (LCM II) and effect of using vermicompost and coconut water to replace MS nutrients(LCM I) was evaluated .Highest number of shoots were produced in control media during shoot induction and shoot mulitiplication stages. Among low cost media LCM II produced more number of shoots than LCM I. There was no significant difference in the number of roots produced on control, LCMI and LCMII. 52.20 % cost reduction was possible using LCM II and 38.68 % cost reduction was found possible using LCM I in place of control media.*

Keywords: *Banana, low cost tissue culture, micropropagation, vermicompost.*

Introduction

Banana (*Musa* spp.) is the most important and most widely grown fruit crops in India. Banana is a good source of carbohydrates, proteins and other vitamins. Because of its high degree of sterility and polyploidy of the edible varieties genetic improvement through breeding is very difficult (Stover and Simmonds, 1987) In order to augment conventional breeding and to avoid constraints imposed by some pests and pathogens, transgenic and in vitro approaches are being considered (Tripathi, 2003). Micropropagation has proved to be an alternative tool for rapid mass multiplication, disease free production and year round availability of banana planting material. With the increasing demand and vast export potential coupled with the farmer's desire to grow in-vitro propagated banana on a large area, it is becoming increasingly important for rapid multiplication of quality planting material (Ray *et al.*, 2006). The experimental plant material of cv. Grand Naine was raised through tissue culture on MS medium using suckers as explants. The results showed that out of different potting mixtures used for hardening soil: sand and FYM (2:1:1 v/v/v) showed cent percent survival. (Shahnavaz *et al.*, 2014)

In Vietnam, a simple low - cost rapid multiplication system has been developed for farmers using *in vitro* plantlets. These are multiplied in vitro, producing single node cuttings that are transferred and rooted in sand beds at high density. Apical and axillary cuttings are taken and rooted in beds with subsoil-manure mixture. Then cuttings are taken for rooting in the small banana leaf pots. Three in vitro plantlets can provide sufficient material to plant 1 ha in 7 months. (Uyen and Van der Zaag, 1983) Tapioca and table sugar are the best alternative of agar and sucrose respectively, to reduce the cost of media. In the place of MS nutrients, LC nutrients may be used and the cost of whole media may be reduced 100%, without any adverse effect. through reduction of the cost on the techniques, the cost of the product automatically also be reduced and farmers get benefited using low cost, disease free and clonal planting material with high production and saving land resources. (Badoni and Chauhan, 2011)

In India, there are about 100 commercial plant tissue culture units with a minimum production capacity of about 1 million plants per year from each of the units. Among these, at least 20 of the units have larger production capacities, with 5 to 10 million plants/year. In addition, there are more than

a dozen smaller units with 0.2 to 0.5 million plant production capacities where single crops are being produced. The Government of India has identified micro propagation industry as a priority area for further research, development and commercialization. In 1991, there was a decline and only 20% of the target was achieved. In 1996, there was an increase in the number of plant tissue culture units and as a result, most of the units had to suffer under-utilization of their facilities. This trend resulted in better capacity utilization of the existing facilities by 2002 and additional facilities are now being set up to increase the total installed capacity in the country to 300 million plants per annum. (Annual report 2013-2014)

The cost of the culture medium is very high because it requires expensive chemicals like agar, vitamins growth regulators etc. In order to increase application of tissue culture technology in producing commercial horticultural crops, it is essential to lower the cost of micropropagule production. The investigations were carried out to study the effects of low cost alternatives on *in vitro* development of banana (G-9).

Material and Method

For banana propagation the method followed by Dhanalakshmi and Stephan (2014) was used with slight modifications. At least 20 explants were used for each treatment and all the experiments were conducted twice. Results were recorded and statistically analysed.

Plant material: In the present study, *Musa cavendish* L.var.G9 was used as mother plant. The source of the explant used for micropropagation was sword suckers of the mother plant.

Media preparation: Murashige and Skoog (1962) basal medium with slight modifications for banana micropropagation was used as control. In alternative media either support matrix or nutrient source of control media was replaced with alternative components. In all media compositions, growth regulators namely BAP (2.5 mg/lit) and IAA (1.0 mg/lit) were added for shoot induction and shoot multiplication study. To study root induction the media was supplemented with IAA (0.5mg/l) and NAA (0.5mg/lit). For carbon source in the control media, sucrose (30 g/lit) was used and in alternative media, table sugar (30 g/lit) was used.

The glassware and other utensils were washed, cleaned, dried and autoclaved or heat sterilised as per experimental requirement.

Sterilization and initiation of the cultures: The sword suckers with medium size were carefully removed. The older leaves were excised with stainless steel knife. The shoot tips were finally brought to the size of 5-8 mm with the base and shoot apex. The shoot tips of 3-4 cm length were excised and washed thoroughly with Dettol solution (2-3 drops in 500 ml water), 1% $HgCl_2$ solution for 10 min, 0.1% citric acid solution for 30 min and washed under running tap water for 4 to 5 times. Finally the sword sucker tips were treated with 0.1% hydrogen peroxide solution (H_2O_2) for 5 min. Then, they were washed with sterile distilled water and inoculated on sterile shoot induction media under aseptic condition. The culture was incubated at a temperature of 25 ± 2^0C and a photoperiod of 16 h light and 8 h darkness at a light intensity of 2000 lux.The number of shoots and roots was determined and recorded after 6 weeks. The experiment was repeated twice to test the reproducibility of the results.

Multiplication: Multiplication was carried out twice to increase the number of plantlets. The plantlets that had 4-5 shoots were selected and spliced into sucker cuttings. The sucker cuttings were put in the fresh medium of the same composition as the initiation medium. Morphological changes were observed and the number of shoots and roots were recorded after the 6^{th} week of culture.

Result and Discussion

The cost of production of planting material through tissue culture depends on many factors of which media cost is also a significant factor. The support matrices like agar are significant contributors in enhancing the cost of media and hence effective low cost substitutes to these costly materials are required.

In this work use of blotting paper as alternative to agar(LCM II) as support matrix and vermicompost and coconut water as nutrient alternative to M.S. salts and vitamins (LCM I) was studied. The control media contained M.S. salts, vitamins, amino acids and agar.

I) Effect of media on the initiation and multiplication of shoots

During shoot induction stage, the number of shoots produced per plantlet was significantly higher in control as compared to all low cost alternative media. The same trend was also observed in the shoot multiplication stage. Among the low cost alternatives, LCM II produced more number of shoots than LCM I in both stages.(Table 1)

Table 1: Effect of media alternatives on shoot induction and multiplication

Medium	Control	LCM I	LCM II
Shoot induction	4.90±0.29 ^{xa}	3.60±0.25 ^{yc}	4.2±0.34 ^{yc}
Shoot multiplication	5.30 ±0.22 ^{xb}	3.80±0.29 ^{yc}	4.7±0.28 ^{xa}

*(Values are expressed as mean ± standard errors of the mean. Same letters represent values without significant differences. x and y represent comparison between shoot induction and shoot multiplication stages(within rows) while a,b represent comparison between media (within columns).

II) Effect of media alternatives on the formation of roots: The two low cost media alternatives (LCM I and LCM II) and control had no significant difference ($p>0.05$) in the number of roots produced.(Table 2)

Table 2: Effect of media on the formation of roots

Medium	Control	LCM I	LCM II
Average number of roots per plantlet	4.90±0.29 ^{a}	4.60±0.25 ^{a}	4.8±0.34 ^{a}

*Values are expressed as mean ± standard errors of the mean. Same letters represent values without significant differences.

III) Cost analysis: As compared to control the use of vermicompost and coconut water as the alternative source of conventional MS nutrients (LCM I)reduced the cost of medium by 38.67%, while the use of blotting paper to replace agar(LCM II) reduced the cost by 52.20%.(Table 3).

Table 3: Cost comparison between control and low cost media alternatives.

Sr. No.	Attribute	Control	L.C.M.I	L.C.M II
1	**Cost per litre media(Rs./lit)**	161.24	98.88	77.07
2	**Total Cost reduction (%)**	-	38.67	52.20

Several low cost alternatives have been reported for significant reduction in the cost of media. A low cost protocol for multiplication of healthy banana seedlings has been reported by Gitonga *et al.* (2010). In the plant propagation medium sucrose substituted with table sugar reduced the cost of medium by 96.8%. (Dhanalakshmi and Stephan, 2014)

Several low cost substitutes have been reported for replacing agar as gelling agent. These include glass beads (Goel *et al.*, 2007) Corn starch, (Zimmerman *et al.*, 1995) Potato starch, (Mohamed *et al.,* 2010) Polyurethane foam, coconut coir, (Sharifi *et al.*, 2010)

Vermicompost is reported to improve soil fertility, supply nutrients for plant growth and capable of reducing chemical fertiliser requirements. (Lazcanoa and Domínguezb, 2011). Hence vermicompost as low cost nutrient alternative was evaluated in the present work as a substitute to MS salts.

Coconut water is also a rich source of essential vitamins, amino acids and micronutrients for plant development. Use of coconut water for tissue culture has been reported by Khawaj *et al.*,(2015).

A combination of vermicompost and coconut water has not been used as low cost alternative for banana propagation media. Our work suggest successfull use of these low cost alternatives in banana propagation media.

Use of blotting paper as a low cost alternative is also a significant cost reducing substitution which has not been reported earlier.

The acclimatisation and hardening was successfully completed without any significant difference in survival percentage between low cost media and control. This suggest that the combination of vermicompost and coconut water and use of blotting paper are very effective low cost substitutes which can be used in commercial banana tissue culture.

Conclusion

Two low cost media alternatives have been developed. One replacing conventional nutrients and the other for gelling agent. Both reduced the cost of media significantly and hence can be used by commercial producers of tissue cultured plantlets as well as farmers interested in producing tissue cultured plantlets on small scale.

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