Journal of Academia and Industrial Research (JAIR)
Volume 2, Issue 9 February 2014

ISSN: 2278-5213

RESEARCH ARTICLE

Low Cost Media Options for the Production of Banana (*Musa paradisiaca* L.) through Plant Tissue Culture

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Abstract

Banana serves as an ideal and low cost food source for developing countries where most of the population relies mostly on bananas for food. The objective of this study is to reduce the cost of banana tissue culture nutrients by using alternative nutrients sources. The conventional sources of Murashige and Skoog (MS) media were replaced by mixed nutrients containing both macro and micronutrients. The mixed nutrients were supplemented with 30 g/L of table sugar and 8 g/L agar. The conventional MS medium supplemented with 30 g/L sucrose and 8 g/L agar was used as control. Two banana varieties of Poovan and Monthan were regenerated on the two media. The mean number of shoots and roots were determined and comparisons were made between the two media. There was 61.4% reduction in the cost of the nutrients used in the media preparation. Significant differences were detected on the number of shoots produced by Poovan on the two media with shoots cultured on the low cost medium producing average of four shoots per plantlets.

Keywords: Banana, tissue culture nutrients, MS medium, table sugar, sucrose, Poovan, Monthan.

Introduction

Banana is one of the world's most important horticultural crops cultivated on five continents in about 120 countries. Current world production of banana is estimated at 97.5 million tones per year covering 10 million hectare (Kalloo, 2002; Singh, 2002). Banana is an important food crop in the world as well as in India (Ganapathi *et al.,* 1999). Micropropagation of banana is highly efficient allowing a large turnover of plants in a very short period of time with in a very little space (Arias, 1992; Arvanitoyannis *et al.,* 2007). Micropropagation techniques were established for fast multiplication of banana (Vuylsteke, 1989). Commercial production of micropropagated banana is now common in many countries and it is estimated that 25 million plants are produced worldwide each year. One of the most important factors governing the *in vitro* shoot multiplication is largely determined by the composition of the culture medium (Rashid *et al.,* 2000). Murashige and Skoog (1962) medium is widely used for banana propagation and a critical factor involved is the cost of the culture medium which requires chemicals that are often very expensive. In order to increase the tissue culture technology in banana farming, innovative approaches are needed to lower the cost of micropropagule production. Banana plant production via low cost technology in which cost reduction is achieved by improving process efficiency and better utilization of resources is reported by Savangikar (2002). Low cost options should lower the cost of production without compromising the quality of the micropropagules and plants (Prakash *et al.,* 2002).

It is necessary to develop low cost technologies by improving the process efficiency and better utilization of resources. Keeping the above facts in view, this study was aimed to reduce the cost of banana tissue culture nutrients by using alternative nutrients sources.

Materials and methods

Cost analysis: The cost of each compound used was calculated based on the quantities used per liter of the medium: Amount used in culture medium (g) x Price of amount bought (Rs.)/Amount bought (g). Differences in cost between the conventional and alternative nutrient sources were then determined and their percentages evaluated.

Plant material: In the present study, banana var. Poovan and Monthan maintained in the college garden were used as a source of mother plant and in this, the sword suckers were used as a source material.

Media preparation: Two types of media was prepared, one is conventional Murshige and Skoog (1962) media which is used as a control and the other media was low cost tissue culture medium where locally available nutrients was used as the alternative source. In these two media, growth regulators namely BAP (0.5 mg/L) and IAA (1.0 mg/L) with agar-agar (8 g/L) were added. For carbon source in the control media, sucrose (30 g/L) was added and in low cost media, table sugar (30 g/L) was used.

Table 1. Cost comparison between low cost medium and conventional medium.

Conventional Murashige and Skoog (1962) media	Low cost nutrients media	Cost in 1 L of the medium (in Rs.)		Cost reduction (%)
	Macro and micro mixed nutrients*	Conventional	Low cost	
Macronutrients				
$CaCl_2$		1.9		
KH_2PO_4		1.8		
KNO_3		1.1		
$MgSO_4$		1.3		
NH_4NO_3		7.2		
Sub total		13.3		
Micronutrients				
$CoCl_2$		0.2	8.8	
$CuSO_4.5H_2O$		0.04		
Na_2. EDTA		5.8		
$FeSO_4.7H_2O$		1.1		
H_3BO_3		3.7		
KI		11.2		
$MnSO_4.4H_2O$		1.4		
$Na_2MoO_4.2H_2O$		1.1		
$ZnSO_4.7H_2O$		4.2		
Sub total		28.74	8.8	
Total		42.04	8.8	79.0
Carbon source				
Sucrose	Table sugar	14.46	2.20	84.7
Agar-agar		62.78	35.0	
Total		119.28	46.0	61.4

* The products of Trio, Power-B, Chelated Baban, Calrich from Zuari Agro Chemicals Ltd. India.

Sterilization and initiation of the cultures: The sword suckers with medium size were carefully removed from the college garden. 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 in 10 min, 0.1% citric acid solution in 30 min and washed under running tap water for 4 to 5 times. Finally the sword sucker tips were treated with 0.1% sodium peroxide solution (H_2O_2) for 5 min. Then, shoot tips were washed with sterile distilled water under aseptic conditions. The culture was incubated at a temperature of 25±2°C and a photoperiod of 16 h light and 8 h darkness at a light intensity of 2000 lux. The number of shoots and roots were 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 suckers cuttings. The sucker cuttings were put in low cost fresh medium of the same composition as the initiation medium. Morphological changes were observed and the number of shoots and roots recorded after the 6th week of culture.

Results and discussion

Cost analysis per liter of medium: The use of mixed macro and micro nutrients as the alternative source of conventional MS nutrients reduced the cost of the nutrient medium by 79%, while the use of table sugar led to 84.7% respectively. A total cost reduction of 61.4% was obtained (Table 1).

Effect of media on the initiation of shoots: The banana variety produced significantly ($p<0.05$) higher number of shoots on the conventional medium compared to low cost medium. The cultivar of Monthan produced significantly ($p<0.05$) higher number of shoots on the low cost medium compared to Poovan. Monthan had an average of 4.6 shoots per plantlet at the end of the culture period while Poovan had an average of 4.5 shoots per plantlet. On the conventional medium, Poovan produced significantly ($p<0.05$) more shoots with an average of 5.1 shoots per plantlet compared to Monthan which had an average of 4.9 shoots per plantlet. Both varieties produced significantly higher number of shoots in conventional medium compared to the low cost medium.

Effect of media on the formation of roots: The two banana varieties had no significant difference ($p>0.05$) in the number of roots produced on the low cost medium.

Table 2. Mean number of shoots produced by two banana varieties cultured on low cost and conventional tissue culture medium.

Medium	Mean number of shoots*					
	Poovan			Monthan		
	1st subculture	2nd subculture	Mean	1st subculture	2nd subculture	Mean
Low cost medium	4.50 ± 0.33^{ax}	5.00 ± 0.31^{ax}	4.75 ± 0.25^{ax}	4.5 ± 0.38^{ax}	4.8 ± 0.42^{ay}	4.60 ± 0.10^{ax}
Conventional medium	5.40 ± 0.32^{bx}	4.90 ± 0.34^{cx}	5.10 ± 0.25^{by}	4.8 ± 0.37^{by}	5.0 ± 0.30^{ax}	4.9 ± 0.05^{ay}

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

Table 3. Mean number of roots produced by two banana varieties cultured on low cost and conventional tissue culture medium.

Medium	Mean number of shoots*					
	Poovan			Monthan		
	1st subculture	2nd subculture	Mean	1st subculture	2nd subculture	Mean
Low cost medium	3.40 ± 0.26^{ax}	3.80 ± 0.29^{ax}	3.6 ± 0.15^{ax}	4.0 ± 0.27^{ay}	3.50 ± 0.33^{by}	4.2 ± 0.15^{aby}
Conventional medium	4.60 ± 0.26^{bx}	4.10 ± 0.26^{bx}	4.35 ± 0.26^{bx}	3.0 ± 0.27^{ay}	3.20 ± 0.33^{by}	3.1 ± 0.10^{by}

But on the conventional medium, Poovan produced significantly ($p<0.05$) higher number of roots compared to Monthan. The Poovan variety produced an average of 3.6 roots per plantlet on the low cost medium, while Monthan had an average of 3.8 roots per plantlet after 6 weeks of culture. Poovan had an average of 4.3 roots per plantlet on the conventional medium, while Monthan had an average of 3.1 roots per plantlet.

Effect of media on the multiplication of shoots and roots: Significantly ($p<0.05$) higher number of shoots were produced in the conventional medium compared to the low cost medium during the first subculture, while no significant differences ($p>0.05$) were detected during the second subculture in the two varieties of banana as shown in Table 2. In overall, the two banana varieties did not show any significant difference ($p>0.05$) in the number of shoots produced on the low cost medium but Poovan had significantly more shoots on the conventional medium compared to Monthan. Poovan variety produced significantly ($p<0.05$) higher number of roots on the conventional medium compared to the low cost medium during both subcultures as shown in Table 3. Monthan did not show any significant differences ($p<0.05$) in the number of roots formed in the two media during the first subculture but in the second subculture more roots were produced on the low cost medium compared to the conventional medium. The Monthan variety produced significantly ($p<0.05$) higher number of roots compared to Poovan on the low cost medium during both subcultures. Poovan had better root production compared to monthan on the conventional medium (Table 3).

In vitro regeneration of banana tissue culture can significantly boost the production of the crop by availing healthy plant materials. However, this is usually constrained by high cost of shoot production. The successful regeneration of the two Banana cultivars indicates that locally available salts like mixed nutrients can be used as an alternative source which can greatly reduce the cost of media and hence, the cost of shoot production which in turn will lower the production of shoots.

Strategies to reduce the cost of tissue culture nutrient media have been reported on other plants. A low cost protocol for multiplication of healthy banana seedlings has also been reported by Gitonga *et al.* (2010). The use of mixed nutrients as the alternative source of MS media was on the basis that this foliar feed contains both the macro and microelements required for plant growth. The medium is supported to the plant growth hence, can easily be adopted for regeneration of banana. The carbon source such as grade sucrose that is often used in the micropropagation of plants at laboratory contributes about 34% of the production cost (Demo *et al.,* 2008). Sucrose has been reported as a source of both carbon and energy (Bridgen, 1994). There are reported success in reducing 90% cost of tissue culture banana plants by replacing sucrose. In the plant propagation medium Kaur *et al.* (2005) substituted sucrose with table sugar which reduced the cost of medium considerably by 96.8% similar to the present study. The findings of our study is in agreement with that of Prakash *et al.* (2002) who reported the reduction in the cost medium by 78 to 87% using common sugar. A good root system is essential for successful acclimatization of the shoots and subsequent growth in the fields since roots facilitate the absorption of nutrients from the soil (Xiansong, 2012). The two banana varieties produced roots without incorporating any auxin. Poovan produced more roots in the low cost medium was more appropriate for root formation in Poovan compared with the Monthan. This can be attributed to the differences in the genetic constitution between the two varieties. Plantlets were successfully acclimatized and then transplanted into the potted soil in the shade net. This shows that it is possible to develop a low cost tissue culture media for production of banana.

Conclusion

In this study, a low cost media options for banana propagation was developed. This can contribute to increase in banana production not only in Tamil Nadu but worldwide. Adoption of this protocol can empower farmers to set up low cost tissue culture laboratories in their localities to increase banana plant production.

Acknowledgements

Authors would like to thank University Grants Commission, New Delhi for providing financial Assistance (File No. 41-46/2012(SR)) to carry out this study.

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