Available online at www.scholarsresearchlibrary.com

Scholars Research Library

***J. Nat. Prod. Plant Resour.*, 2014, 4 (5): 8-13**
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Effect of low cost macronutrients in the micropropagation of Banana (*Musa paradisiaca* L.)

S. Dhanalakshmi and R. Stephan*

Plant Biotechnology laboratory, PG & Research Department of Botany, Govt. Arts. College, Ariyalur, TN, India

---

ABSTRACT

Banana plantains are grown as staple food, significant cash crops and major export crops in many tropical and subtropical countries. The objective of this study was to assess in vitro regeneration response of Poovan and Monthan varieties of banana using low cost macronutrients source to reduce the cost of micro-propagule production. The five MS conventional macronutrients;viz, ammonium nitrate, potassium nitrate, calcium chloride, magnesium sulphate, and potassium hydrogen phosphate were substituted to the locally available urea fertilizer, potassium fertilizer, cal rich, magnesium sulphate fertiliser and trio powder respectively. The result show that the sword suckers banana shoots and roots compared to conventional medium significant differences (p>00.5). The use of locally available macronutrients significantly (p<0.05) reduced the cost of micropropagation of banana. The Poovan performed better the highly shoots and roots produced and compared to Monthan. There was 72.4 % savings in the cost of the macronutrients used in media preparation.

Keywords: low cost macronutrients; variety; banana; *in vitro* regeneration; acclimatization.

INTRODUCTION

Banana is the fourth most important food crop in the world as well as in India [4]. It is a staple food and export commodity. It contributes to the food security of millions of people in the developing world and, when traded in local markets, provides income and employment to rural populations. India is a leading country in the world from the perspective of banana production. India is a largest producer of banana and plantain with an annual production of 16.91 tonnes from 490 700 hectares, and accounts to 19% of total world production. Micropropagation or *in vitro* techniques were established for fast multiplication of bananas [19]. Commercial production of micropropagated bananas is now common in many countries and it is estimated that 25 million plants are produced worldwide each year.

In many developing countries, the establishment cost of facilities and unit production cost of micropropagated plants is high and often the return on investment is not proportional to the economic potential advantage of the technology [16]. Micropropagation of banana is highly efficient, allowing a large turnover of plants in a very short period of time within very little space [2, 3].

Although conventional plant tissue culture has been applied for decades, the high cost of tissue production is a drawback for laboratories with limited resources, especially in the developing countries. One of the most important factors governing the *in vitro* shoots regeneration is largely determined by the composition of the culture medium [14].The basic MS nutrients [11] are the most widely used media. However, the cost of tissue-culture based plant propagation is high in the developing countries, therefore there is a need to develop low cost micropropagation protocol to lower the cost of micropropagule to enable accessibility of tissue cultured planting material to farmers [15].

The shoot multiplication rate obtained in the low cost options was not less when compared to the other *in vitro* multiplication trails. [5] Used tap water, commercial grade sugar and reduced the salt components in medium for banana plantlet production and achieved a maximum cost reduction of 31.2 %. Use of the media, culture vessel and low cost substitutes for mass propagation was successful in several other species [17, 18 , 9 ,8, 13,7].

In the present study was to evaluate *in vitro* regeneration of banana varieties using low cost macronutrients to reduce the cost of micropropagation.

MATERIALS AND METHODS

Collection of Plant Materials

Two banana varieties, Poovan and Monthan sword suckers were used as a source of plants material.

Culture Media Preparation

The preparation of the media for banana tissue culture by Modified Murashige and Skoog (MMS) media was used in this study **(Table 1)**. In this conventional MS macronutrients ammonium nitrate, potassium nitrate, calcium chloride, magnesium sulphate, and potassium dihydrogen phosphate were substituted by low cost macronutrients as shown in **(Table 1).** While the other nutrients are retained the same. The conventional MS macronutrients were used as control. The pH of all the media was adjusted to 5.8 before adding the gelling agent agar. The media were sterilized at the temperature of 121ºC for 15 minutes.

Table 1: Conventional macronutrient and corresponding low cost macronutrient substituted

Conventional macronutrient	CM medium mg/l	Low cost Macronutrient	LCM medium mg/l
NH4NO3	1650	Urea fertilizer	20
KNO3	1900	Potassium fertilizer	2.0
CaCl2	440	Cal rich	6.6
MgSO4	370	Magnesium sulphate fertiliser	10.6
KH2PO4	170	Trio powder	1.0
Total	**4530**	**Total**	**40.2**

Preparation and Sterilization of Banana sword suckers

The sword suckers with medium size were carefully removed from field grown banana plant. The older parts were excised with stainless steel knife. The shoot tips about 3-4 cm length were excised. They were washed thoroughly with a solution of Tween - 20. (2-3 drops in 500 ml water). All traces were removed by repeated washings under running tap water for 4-5 times and finally with distilled water. These shoot tips were treated with 0.1 percent HgCl2 Solution for 10 minutes. The shoot tips were rinsed with sterile distilled water under aseptic conditions.

Plant regeneration

The shoot tips of two varieties were cultured into a solidified medium supplemented with 3 % table sugar and 2.0 mg/l BAP for shoot induction. The cultures were incubated in a growth chamber at a temperature of 27±1ºC, with a light intensity of 1000 lux provided by cool white fluorescent lamp with a photoperiod of 16/8h (day/night). The number of leaves and shoots formed were recorded after five weeks of culture. The initiation of roots was carried out in a Modified MS media containing half strength nutrients.

Acclimatization

The *in vitro* regenerated plantlets with well-developed shoot root and leaf systems were removed from the culture bottles and washed in running tap water to remove the nutrient media to avoid root fungal attack. The plantlets were transplanted onto pots containing a mixture of rice husks and red soil. The pots were covered with transparent polythene sheets and the plants were watered for two weeks for acclimatization. The plants were the transplanted into polythene bags containing a mixture of red soil and manure in a ratio 1:2:1. The survival percentage of the plants during hardening was recorded to assess the success of using alternative resources.

RESULTS AND DISCUSSION

Cost Efficiency

Cost savings of 79.1 % was recorded when urea fertilizer was used as a source of macronutrient. Similarly, cost saving of 67.2 % potassium fertilizer, 65.2% cal rich, 73.8% magnesium sulphate fertiliser and 55.5% trio powder was recorded when were used as alternative source of micronutrient respectively. Overall substitution of conventional macronutrients with low cost locally available macronutrients reduced the overall cost of micropropagation of banana by 72.4 % **(Table 2)**.

Table 2: Cost efficiency of conventional macronutrients compared with low cost Macronutrients

Conventional macronutrient (CM)	Substitute to the Low cost macronutrient (LM)	Cost in (Rs.)		
		Conventional MS medium mg/l cost in Rupees (CM)	Low cost Medium mg/l cost in Rupees (LM)	% of Cost saving
Ammonium Nitrate (NH4NO3)	Urea fertilizer	7.2	1.50	79.1
Potassium Nitrate (KNO3)	Potassium fertilizer	1.1	0.36	67.2
Calcium Chloride (CaCl2)	Cal rich	1.9	0.66	65.2
Magnesium Sulphate (MgSO4)	Magnesium sulphate fertiliser	1.3	0.34	73.8
Potassium Hydrogen Phosphate (KH2PO4)	Trio powder	1.8	0.80	55.5
Total cost		**13.3**	**3.66**	**72.4**

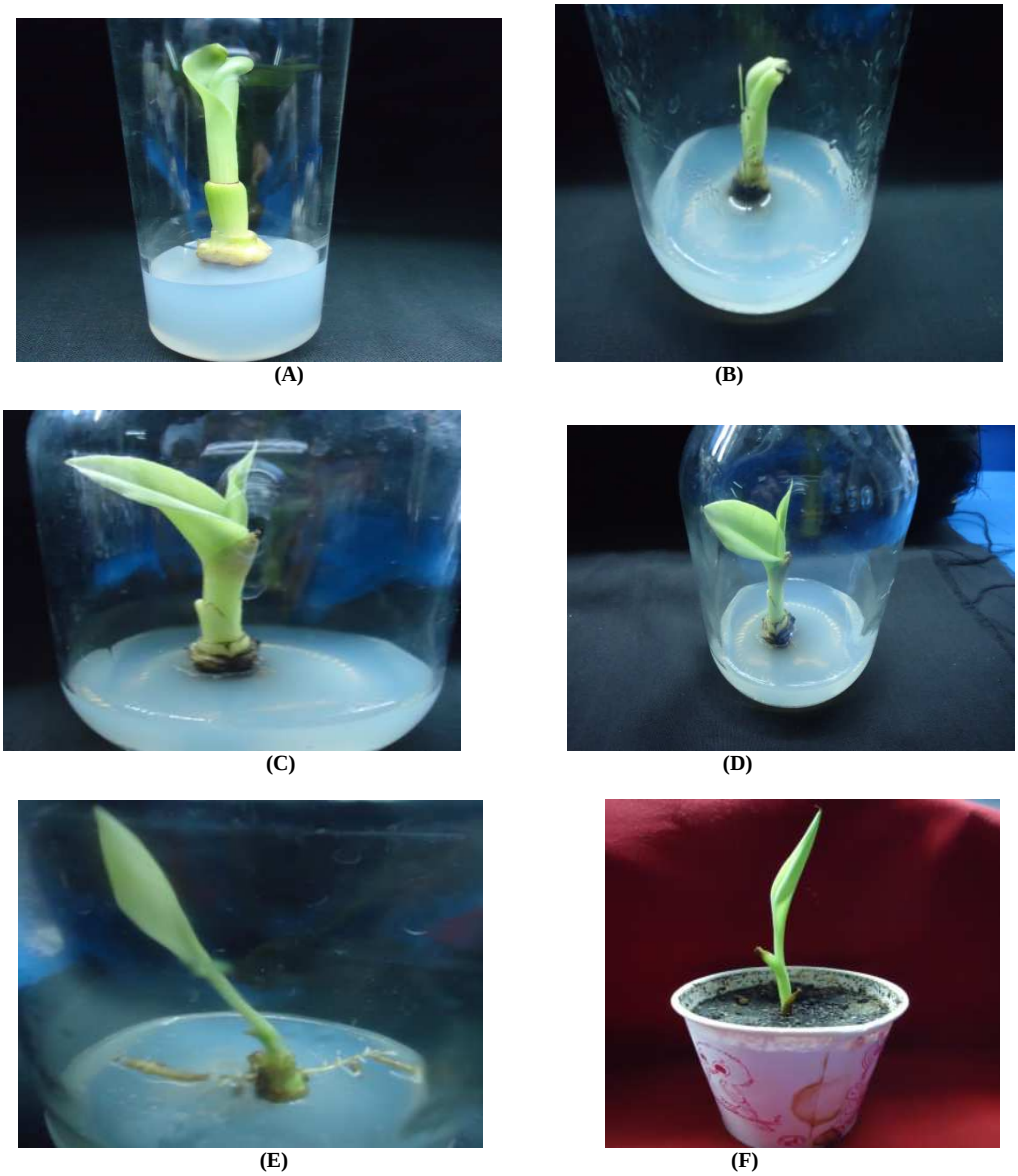

Figure 1: Regeneration response of banana verities on different media. A. Poovan plants on the medium containing conventional macronutrient (urea fertilizer); B. Poovan plants on the medium containing low cost conventional macronutrient; C. Monthan plants on the medium containing conventional macronutrient; D. Monthan plants on the medium containing low cost conventional macronutrient (urea fertilizer); .E, Root induction in modified MS media. F, *In vitro* regenerated banana plantlets in the greenhouse

Regeneration Response of Banana Varieties

Poovan and Monthan varieties regenerated from sword suckers cuttings in both conventional and low cost macronutrients. The two varieties was recorded in five-experimental set in term of shoots and roots formed between the low cost and conventional macronutrient sources (Fig. 1A-D).

There were significantly higher number of shoots and roots formed in both poovan and monthan in media containing low cost urea fertilizer compared to media with conventional NH4NO3 **(Table 3).** Significant differences were not detected ($p > 0.05$) on the number of shoots and roots formed in both poovan and monthan in media containing low cost potassium fertilizer compared with media containing conventional KNO3 **(Table 4)**. Significant differences were not detected ($p > 0.05$) on the number of shoots and roots formed in both poovan and monthan in media containing low cost calrich compared with media containing conventional CaCl2 **(Table 5)**. Significantly number of shoots and roots formed in both poovan and monthan in media containing low cost magnesium sulphate fertiliser compared to media with conventional MgSO4 **(Table 6)**. Significantly number of shoots and roots formed in both poovan and monthan in media containing low cost trio powder compared to media with conventional KH2PO4 **(Table 7)**.

The Mean number of shoots and roots formed on MMS media containing all the low cost macronutrients and conventional MS macronutrients culture of banana sword suckers. Poovan had significantly ($p < 0.05$) high number of shoots and roots overall compared with Monthan **(Table 8).**

Roots were formed in the same media used for regeneration when the cultures were left for one month and had no sub-roots in all the banana varieties tested. However root induction in Modified MS media containing MS nutrients formed sub-roots which grew vigorously (Fig. 1E). *In vitro* regenerated banana plantlets were successfully transferred into the soil in the greenhouse (Fig. 1F).

Table 3: Mean number of shoots and roots formed on MMS media containing Urea fertilizer and conventional NH4NO3 on 5th week culture of banana sword suckers

No. of shoots & roots				
Parameter	Variety	Conventional	Urea fertilizer NH4NO3 salt	Mean
Number of shoots	Poovan	6.40 ±0.26	4.95±0.38	5.68±0.53
	Monthan	5.50 ±0.20	3.95±0.30	4.73±0.32
Number of roots	Poovan	5.30±0.43	5.35±0.49	5.33±0.62
	Monthan	4.15±0.37	5.55±0.63	4.35±0.42

Table 4: Mean number of shoots and roots formed on MMS media containing Potassium fertilizer and conventional KNO3 on 5th week culture of banana sword suckers

No. of shoots & roots				
Parameter	Variety	Conventional	Potassium fertilizer KNO3salt	Mean
Number of shoots	Poovan	4.30 ±0.21	5.05±0.30	4.73±0.52
	Monthan	3.15 ±0.18	4.20±0.22	3.68±0.62
Number of roots	Poovan	4.15±0.12	4.55±0.18	4.35±0.28
	Monthan	2.90±1.30	2.50±0.26	2.70±0.22

Table 5: Mean number of shoots and roots formed on MMS media containing Cal rich and conventional CaCl2 on 5th week culture of banana sword suckers

No. of shoots & roots				
Parameter	Variety	Conventional	Cal rich CaCl2 salt	Mean
Number of shoots	Poovan	4.60 ±0.29	4.90±0.33	4.75±0.32
	Monthan	3.63 ±0.23	3.80±0.42	3.72±0.54
Number of roots	Poovan	4.25±0.47	4.89±0.23	4.57±0.26
	Monthan	3.82±0.82	2.08±0.20	2.95±0.92

Table 6: Mean number of shoots and roots formed on MMS media containing Magnesium sulphate fertiliser and conventional MgSO4 on 5th week culture of banana sword suckers

		No. of shoots & roots		
Parameter	Variety	Conventional	Magnesium sulphate MgSO4 fertiliser	Mean
Number of shoots	Poovan	6.20±0.93	4.98±0.68	5.59±0.54
	Monthan	5.30±0.42	4.25±0.49	4.77±0.33
Number of roots	Poovan	6.10±1.04	4.75±0.22	5.42±0.96
	Monthan	4.22±0.68	3.25±0.88	3.75±0.42

Table 7: Mean number of shoots and roots formed on MMS media containing Trio powder and conventional KH2PO4 on 5th week culture of banana sword suckers.

No. of shoots & roots				
Parameter	Variety	Conventional	Trio powder KH2PO4	Mean
Number of shoots	Poovan	4.70 ±0.44	4.30±0.51	4.50±0.62
	Monthan	3.63 ±0.26	3.80±0.49	3.72±0.52
Number of roots	Poovan	4.60±0.22	4.22±0.12	4.41±0.64
	Monthan	3.78±0.28	3.98±0.18	3.88±0.22

Table 8 : Mean number of shoots and roots formed on MMS media containing all the low cost macronutrients (urea fertilizer, potassium fertilizer, cal rich, magnesium sulphate fertiliser and trio powder) and conventional MS macronutrients on 5th week culture of banana sword suckers.

Bananan Var.	Number of Shoots (Mean±SD)			Number of Roots (Mean±SD)		
	CM	LCM	Mean	CM	LCM	Mean
Poovan	6.20±0.24	5.60±0.22	5.90±0.22	5.82±0.24	5.22±0.92	5.52±0.58
Monthan	5.20±0.68	4.80±0.25	5.01±0.46	4.80±0.62	5.10±0.24	4.95±0.43

In this study the cost of medium reduced when low cost macronutrients source were used **(Table 2).** This is in agreement with the findings by [6]. in which the cost was reduced by. 96.2 %, 93.1 % and 95.0 % respectively when low cost macronutrients (ammonium fertilizer, Epsom salt and potassium fertilizer) were used in the in the initiation and multiplication of banana plants. According to [12]. The use of ammonium quarry salt, Epsom salt and potassium fertilizer in the regeneration of cassava reduced the cost of macronutrients by 96.2 %, 93.0 % and 94.9 % respectively.

The banana sword suckers Poovan had the best regeneration response due to the higher number of shoots and roots formed compared to Monthan **(Table 8).** [6], developed a low cost protocol for micropropagation of local banana (*Musa spp*.) in Kenya. They used locally available fertilizers as a substitute of micronutrients [10] developed a low cost medium by replacing conventional sources of Murashige and Skoog (MS) salts with Easygro vegetative fertilizer containing both macro and micronutrients. Two grams of the fertilizer were supplemented with 30g/l of table sugar and 9g/l of agar for *in vitro* banana regeneration.

A low cost medium was developed for *in vitro* microprogation of (*cassava*) by [15]. Hydro Agri's fertilizer was used as a substitute for Murashige and Skoog macro and micronutrients. [1]. investigated on cost effective and economically cheaper alternatives to MS salts, agar and sucrose. In low cost media, tapioca was used as a substitute for agar and cane sugar in place of sucrose due to easy availability and low cost. Calcium ammonium nitrate (6.6gm/l) and sugar cane (30g/l) were used in place of MS salts.

Commercially available macronutrients occurring in the form of some locally fertiliser has also been used previously successfully as an alternative resource for *in vitro* micropropagation banana.

CONCLUSION

This research has shown that it is possible to reduce the cost of plantlet production during tissue culture. This can be achieved through the use of alternative sources of MS nutrients that are available locally. The low cost medium evaluated here can be adopted easily in the production of banana planting material. This will consequently lower the cost of the micro-propagation. However, further research is needed to verify different fertilizer effects on *in vitro* banana propagation, subsequent acclimatization and yield and its quality.

Acknowledgement
The authors are grateful to would like to thank University Grants Commission, New Delhi for providing financial Assistance (File No. 41/461/2012(SR)) to carry out this study.

REFERENCES

[1]Anoop Badoni, J.S, Chauhan. **(2011)** *Agricultural Research Center Journal international*, **2 (4),**161-167.
[2]Arias, O. **(1992)** *Inibap, San Jose, Costa Rica. pp*. 139-142.
[3]Arvanitoyannis, I.S, A.G, Mavromatis, G, Grammatikaki- Avgeli, and M, Sakellariou. **(2007)** *International Journal of Food Science and Technology* **43**:1871-1879.
[4]Ganapathi, TR, PS, Suprasanna, VA, Bapat, VM, Kulkarni, PS, Rao. **(1999)** *Current Science* **76**,1228-1231.
[5]Ganapathi, T.R, J.S.S, Mohan, V.A, Supasanna, V.A, Bapat and P.S, Rao, **(1995)** *Current Science*, **68**:646-649.

[6]Gitonga, N.M, O, Ombori, K.S.D, Murithi, and M, Ngugi. **(2010)** *African Crop Science Journal*,**18(4),** 243-251.
[7] Hung, D.C, K, Johnson, and F, Torpy. **(2006)** *In vitro Cell. Dev. Biol. Plant*, **42**:548-552.
[8] Kadota, M, and y, Niimi. **(2004)** *Science of . Horticulture.*, **102**: 461-466.
[9] Kodym, A, F.J.Z, Arias. **(2001)** *Plant Cell Tissue and Organ. Culture*, **66**:67-71.
[10]Kwame O, N.M, Gitonga, M, Maina, M.N, Michael. **(2012)** *Research journal of Biology*, **2(2)**.51-58.
[11]Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol.plant.* 15:473-497.
[12] Ogero, K.O, G.N, Mburugu, M, Mwangi, M.M, Ngugi, and O, Ombori. **(2012)** *Research Journal of Biology*, **2(2),** 51-58
[13] Piatezak, E, M, Wielanek, and H, Wysokinska. **(2005)** *Plant Science*, **168**:431-437.
[14] Rashid, H, K, Toriyama, A, Qureshi K, Hinata, AK, Malik. **(2000)** *Pakistan Journal of Biological .Science*, **3**:2229-2231.
[15]Santana, M.A, G, Romay, J, Matehus, J, Vicente Villardón and J.R, Demey. **(2009)** African Journal of *Biotechnology*, **8(16):** 3789-3897.
[16] Savangikar, V.A. **(2004)** *International Atomic Energy*, pp. **11**-16.
[17] Sujatha, M, K, Chandran. **(1997)** *Indian Journal of Experimental Biology*, **35:** 787-791.
[18]Varshney, A, V, Dhawan and P.S, Shrivastava. **(2000)** *In vitro* Cell Developmental *Biology Plant*, **36** : 383-391.
[19] Vuylsteke D, **(1989)** *International Board for plant Genetic Resources*, Rome.**56**.