

***In vitro* rooting in the tissue culture raised plantlets of Malbhog cultivar of Banana**

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Abstract

Banana plantlets propagated through shoot tip culture were used for *in vitro* rooting in ½ strength MS medium supplemented with different concentrations (0.2mg/l – 2.0mg/l) of three different auxins either alone or in combinations. ½ strength MS + 1.0 mg/l IBA + 0.5 mg/l IAA induced roots among 90% of the explants after 12th days of inoculation. The average number of roots in this condition was 6.0. Increase in the concentrations of the above two auxins had no promoting impact; rather the number of roots were reduced. Similarly IBA (1.5mg/l) alone induced rooting but the percentage response was reduced (66%) and time of initiation was increased (18th days). Lower concentration of IBA (0.5mg/l) + 0.5mg/l IAA had also no promoting impact. About 70% of the explants responded in this after 15th days of incubation. Higher concentration of IBA (1.5mg/l) + 0.5mg/l NAA, revealed reduced rate of response, similarly IBA at its different concentration revealed no better response with NAA at 0.2 or 0.5mg/l.

Keywords: Micropropagation, Plantlets, *in vitro*, Explants, Auxins, Inoculation.

Abbreviations: IAA – Indole-3-acetic acid; IBA – Indole-3-butyric acid; Kn – Kinetin; MS – Murashige & Skoog; NAA – Naphthaleacetic acid.

1. Introduction

Banana is one of the important horticultural crops in India. It is a good source of carbohydrates and proteins and other vitamins and minerals. Because of its high degree of sterility and polyploidy of the edible varieties (Stover and Simmonds, 1987), classical breeding is difficult. 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). It is getting more and more importance because the farmers are getting cash return within a short period in comparisons to other fruit crops. In Bihar “Malbhog cultivar” is the best cultivar and is being planted by the farmers on preference basis because of its market value. However, planting materials of this cultivar is not easily available; therefore, its plantation is not so popular.

In vitro culture is of great advantage for mass propagation of various vegetative propagated crops. Such approach for banana plantlets is an excellent alternative to provide large number of planting materials to the farmers. Plantlets produced through micro propagation method have been found to establish faster, healthier, stronger, shorter production cycle and higher yields than those produced through conventional methods (Ortiz and Vuylsteke, 1996). Now a day's micro propagation of an elite cultivar of banana is being done in different countries in the world like Israel (Israeli *et al.*, 1995), France (Cote *et al.*, 1996), Australia (Smith & Draw, 1990), Cuba and many African countries (Vuylsteke, 1998). Rooting in the micro propagated plantlets is an important aspect of banana micro propagation. However, there is still lack of information on *in vitro* rooting of banana. The present work is a part of micro propagation of “Malbhog cultivar” of banana. Experiments were carried out to study the efficiency of different concentrations of auxins and number of days for better rooting in the *in vitro* produced plantlets of “Malbhog cultivar” of banana.

2. Materials and Method

Suckers were collected from the field grown plants of “Malbhog cultivar” of banana from Muzaffapur, Bihar. They were brought to laboratory and properly surface sterilized. The shoot tip explants from the selected suckers were established on Murashige and Skoog's medium fortified with various concentrations of IBA, IAA and NAA either alone or in combination. Rapid multiplication was done through subcultures as reported by Cronver and Krikorian (1984). Well developed micro propagated plantlets were taken out from the culture jar and washed properly to remove the adherent agar on their base. These plantlets were used individually for rooting in ½ strength MS medium supplemented with various concentrations (0.2mg/l – 2.0mg/l) of three auxins viz IBA, IAA, NAA either alone or in combination. Observations were made on alternate days and rooting percentages, numbers of roots / explants were calculated in different culture media. Fifteen plantlets were inoculated in each concentration and data were obtained from the replica of three experiments. With the help of above results standard error was calculated.

Table 1: Initiation of root in tissue culture raised plantlets of “Malbhog cultivars” of banana in ½ strength MS medium fortified with different concentrations of three auxins either alone or in combination.

Growth regulators (µM)	Days taken for Root initiation	% of Responses	No. of Roots Initiated
IAA + IBA + NAA	-	-	-
0.2 - -	25	40	2.00 ± 0.24
0.5 - -	20	46	2.50 ± 0.22
1.0 - -	26	32	1.60 ± 0.18
1.5 - -	-	-	-
2.0 - -	-	-	-
- 0.2 -	20	48	3.20 ± 0.24
- 0.5 -	18	66	6.50 ± 0.16
- 1.0 -	19	60	3.60 ± 0.18
- 1.5 -	-	-	-
- 2.0 -	-	-	-
- - 0.2	24	32	1.64±0.14
- - 0.5	26	30	1.04 ± 0.12
- - 1.0	-	-	-
- - 1.5	20	44	1.80 ± 0.22
- - 2.0	22	44	3.64 ± 0.16
0.2 0.2 -	18	48	6.40 ± 0.40
0.2 0.5 -	20	42	3.46 ± 0.18
0.2 1.0 -	-	-	-
0.2 1.5 -	18	54	3.54 ± 0.16
0.2 2.0 -	15	70	4.64 ± 0.22
0.5 0.2 -	12	90	8.50 ± 0.18
0.5 0.5 -	16	68	5.20 ± 0.24
0.5 1.0 -	-	-	-
0.5 1.5 -	20	50	2.26 ± 0.16
0.5 2.0 -	18	58	4.62 ± 0.22
- 0.5 0.2	18	68	3.66 ± 0.12
- 1.0 0.2	20	52	2.30 ± 0.20
- 1.5 0.2	17	66	4.56 ± 0.18
- 0.5 0.5	18	62	3.30 ± 0.14
- 1.0 0.5	-	-	-
- 1.5 0.5	-	-	-

3. Results and Discussion

Well grown tissue culture raised plantlets were cultured in ½ strength MS medium supplemented with different concentrations of IAA, IBA and NAA either alone or in various combinations. The result is documented in Table 1. The treatment containing 1.0 mg/l IBA + 0.5 mg/l IAA showed the best rooting medium, with values around 8.5 roots each explant on 12th days of inoculation in 90% of the plantlets. Culture medium supplemented with 1.0 mg/l IBA alone induced rooting in 66% of explants and 6.5 roots/explants were noted on 18th days of incubation (Fig. 1-6). Medium fortified with 1.0mg/l IBA + 0.2mg/l NAA and 0.5 mg/l NAA were not more significant than the medium containing 1.0mg/l IBA alone. Medium containing 1.0 mg/l NAA and 1.5 mg/l NAA were less efficient with only 1.64 and 1.04 roots on an average basis. Table 1 also shows that an increase in IBA, IAA or NAA concentration decreased the number of roots/explant. Cronauer and Kaikorian, 1984; Jarret et al, 1985, reported that rooting in individual plantlet can be induced in tissue culture raised plantlets if cultured in ½ strength MS medium without auxin. However, auxins may induce further root initiation Vuylsteke (1989). The optimum IBA concentration was found to be 1µM/l by Vuylsteke and Laughe (1985). Hamid and Mustafa (2004) also reported that 1µM IBA/l 11.28 roots/plant. Micropropagation of different cultivars of banana has been carried out by different workers: Doraswamiet al (1983); Hawng et al (1984); Jarret et al (1985); Vuylsteke and Laughe (1985); Wong (1986) Vuylsteke (1998); Arias (1992) and Arinaitwe et al (2000). Findings in the present work may be corroborated with the above outcomes. Based on the above result in may be concluded that among the auxins, IBA is the best and if it is supplemented with 0.5 IAA then more promising results were achieved.

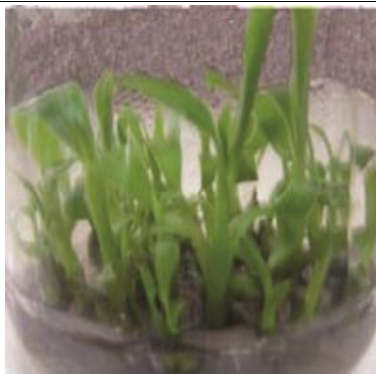


Fig. 1: Multiple shoots of banana



Fig.2: Multiple shoots separated and cultured for rooting



Fig.3: Shoots showing initiation of roots



Fig. 4: Shoots showing multiple roots



Fig. 5: 50 days old culture showing multiple shoots and roots



Fig. 6: 70 days old rooted plantlets

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References:

1. Arias O (1992) Commercial micro propagation of banana. In: *Biotechnology Applications for Banana and Plantain Improvement*. Inibap, San Jose, Costa Rica. pp. 139-142.
2. Arinaitwe G, Rubaihayo PR, and Magambo MJS. (2000) Proliferation rate effects of cytokinins on banana (*Musa* spp.) cultivars. *Scientia Horticulturae*. 86, 13-21.
3. Cote FX, Domergue R, Monmarson S, Schwendiman J, Teisson C, and Escalant JV. (1996) Embryogenic cell Suspension from the male flower of *Musa* AAA cv. Grand Nain. *Physiology Plant*. 97, 285–290.
4. Cronauer SS, Kerkorian AD. (1984) Multiplication of *Musa* from excised stem tips. *Ann Bot (London)* 53: 321-328.
5. Dore Swamy R, Srinivasa Rao NK, and Chacko EK. (1983) Tissue culture propagation of banana. *Scientia Horticulturae*.18, 247- 252.
6. Drew RA, and Smith MK. (1990) Field evaluation of tissue-cultured bananas in south-eastern Queensland. *Aust. Journal of Experimental Agriculture*.30, 569–574.
7. Hamide Gubbuk and Mustafa Pekmezci (2004) In Vitro Propagation of Some New Banana Types (*Musa* pp.).*Turkish Journal of Agriculture*. 28, 355-361.
8. Israeli Y, Bassat DB, and Reuveni O. (1996) Selection of stable clones which do not produce dwarf soma clonal variants during in vitro culture. *Science Horticulture*, 67, 197–205.
9. Jarret RL, Rodriguez W, and Fernandez R. (1985) Evaluation, tissue culture propagation and dissemination of oSabao and oPelipitao plantains in Costa Rica. *Scientia Horticulture*. 25, 137-147.
10. Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture, *Physiologia PL*.15, 473-497.



11. Ortiz R, and Vuylsteke D. (1996) Recent advances in Musa genetics, breeding and biotechnology. *Plant Breed. Abs.* 66, 1355-1363.
12. Smith MK, Drew RA. (1990) Growth and yield characteristics of dwarf off-types recovered from tissue cultured bananas. *Aust J Exp Agric* 30:575–578. doi:10.1071/EA9900575
13. Stover RH, and Simmonds NW. (1987) *Bananas*, Longman Scientific and Technical, Essex, UK.
14. Tripathi L. (2003) Genetic engineering for improvement of Musa production in Africa. *African Journal of Biotechnology*. 2(12), 503-508.
15. Vuylsteke D. (1998) Shoot-tip culture for the propagation, conservation and exchange of Musa germplasm. In: *International board for plant genetic resources*, Rome.
16. Vuylsteke D, and De Langhe E. (1985) Feasibility of in vitro propagation of bananas and plantains. *Tropical Agriculture*. (Trinidad). 62, 323-328.
17. Wong WC. (1986) In vitro propagation of banana (*Musa* spp.): Initiation, proliferation and development of shoot-tip cultures on defined media. *Plant Cell, Tissue and Organ Culture*.6,159-166.