IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
e ISSN-0976-593X Volume **12** | Issue 1 | January, 2017 | 85-89

DOI: 10.15740/HAS/IJPS/12.1/85-89
Visit us - *www.researchjournal.co.in*

RESEARCH ARTICLE

Liquid medium culture method for rapid multiplication of banana (*Musa acuminata*) cv. 'GRAND NAINE' through tissue culture

■ **G. PRABHULING AND B.N. SATHYANARAYANA**

SUMMARY

For an increasing number of plantlets, liquid culture methods have demonstrated a number of important advantages over conventional semi-solid micropropagation, including several fold increase in multiplication rate, reduction in medium cost and also space, energy and lobour requirements. These cost-saving advantages have been the driving force for increased attention to the use of liquid systems. Effects of liquid medium using different simple low cost culture containers on *in vitro* propagation of banana cv. 'GRAND NAINE' were investigated and compared with conventional solid medium. The treatments studied included: Growtech container with nylon wire mesh support; simple polypropylene container with cotton fibre support; simple polypropylene container with partial immersion, simple polypropylene container with full immersion and conventional baby jar bottle with agar gelled medium. Simple polypropylene container with cotton fibre support was found effective than that of agar-gelled medium. The plantlets produced were also sturdier and better quality.

Key Words : *In vitro*, Aseptic cultures, Support matrix, Microshoots, Vitrification

How to cite this article : Prabhuling, G. and Sathyanarayana, B.N. (2017). Liquid medium culture method for rapid multiplication of banana (*Musa acuminata*) cv. 'GRAND NAINE' through tissue culture. *Internat. J. Plant Sci.*, **12** (1): 85-89, **DOI: 10.15740/HAS/IJPS/12.1/85-89**.

Article chronicle : Received : 16.08.2016; **Revised :** 30.11.2016; **Accepted :** 27.12.2016

Banana (*Musa acuminata*) cv. 'GRAND NAINE' belongs to the important Cavendish subgroup and is valued for its good horticultural characteristics. India is the largest producer of banana contributing about 29 per cent of total world production with the production of about 23.20 million tones covering 0.649 million hectares. *In vitro* propagated plants are increasingly becoming the planting material of choice because of choice due disease control, uniformity and the possibility of rapid multiplication. However, growers have to face higher costs and pay upto five times more than for suckers (Robinson, 1996). The technique is costly due to high price of purified agar (Nene *et al*., 1996) and intensive hand manipulation of the various culture phases (Ziv, 2000).

The advantages of liquid media for enhancing shoot propagation (Harris and Mason, 1983), growth (Skidmore

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
G. PRABHULING, Center for Horticulture Biotechnology, Directorate of Research, University of Horticultural Sciences, BAGALKOT (KARNATAKA) INDIA
Email: gprabhuling@gmail.com

Address of the Co-authors:
B.N. SATHYANARAYANA, Division of Horticulture, University of Agricultural Sciences, G.K.V.K. BENGALURU (KARNATAKA) INDIA

et al., 1988) or somatic embryogenesis (Gawel and Robacker, 1990) have been reported. The absence of a gelling agent may increase availability of water and dissolved substances to the explants (Debergh, 1983). However, the use of liquid media involves the problem of asphyxia of explants as a result of immersion. The most commonly used preventive methods are based on the principle of partial immersion of explants to ensure aeration. Inert absorbent substances are used to maintain contact between the medium and the lower part of the explants (filter paper, cellulose, rockwool etc.) or a depth of medium is used to enable partial submergence of the explants tissue. The liquid medium culture methods have been tested on different species and not have been compared directly. The present study was, therefore, carried out with the objective to standardize protocol for liquid medium culture for rapid mass multiplication in banana (*Musa acuminata*) cv. 'GRAND NAINE' (AAA).

MATERIAL AND METHODS

Preparation of explants :

Healthy and vigorously growing sword suckers of cv. GRAND NAINE (3-4 month age and in active growth phase), free from viruses and disease, were selected as a source of explant (Fig. A i). The procedure described by Besagarhally (1996) was followed for initiation of aseptic culture with certain modifications. The plant material obtained from the field was thoroughly washed in running tap water followed by washing with a detergent solution to remove adhering soil particles. Later, rhizomes were kept immersed in a fungicide solution Bavistin (w/v) 1 per cent for 30 min to further clean the plant material. The outer leaves, leaf base and corm tissue were trimmed using a sterilized stainless steel knife until the length of explant was 4-6 cm and the diameter 3-4 cm. These trimmed suckers enclosing the shoot tip were washed with double distilled water. After trimming one more outer layer, they were soaked in a solution of Bavistin (w/v) 0.50 per cent and Streptocycline (w/v) 0.05 per cent for 8 hr. After thoroughly washing with double distilled water, they were trimmed again, so that trimmed suckers were of 2-3 cm in length and 2-2.5 cm in diameter. These shoot tips were soaked in cetrimide (w/v) 0.05 per cent for 30 minutes. After removing one more layer, the shoot tips were surface sterilized with mercuric chloride (w/v) 0.10 per cent in a closed container for 15-20 min. Further operations such as washing several times with sterile distilled water to remove all traces of chlorine, trimming of explant and inoculation in liquid culture media were carried out under a laminar air flow chamber (Kleanzone systems, Chennai).

Raising of aseptic cultures and regeneration of plantlets :

Shoot tip explants were incubated in MS liquid culture media containing Benzyl aminopurine (BAP) 2 mg lit^{-1} and adenine sulphate (AS) 75 mg lit^{-1} for two weeks maintaining standard culture conditions of 25 ± 2^0 C, 70 per cent RH and photoperiodic cycle of 16 hr light and 8 hr dark period (Fig. A ii-v). After two weeks of incubation, all the explants (Fig. A iv) were evaluated for their ability to establish in liquid media. Greening and swelling of the explants were utilized as important criteria for assessing the success in establishment. Shoot tips that had turned dark brown/black and which did not swell were considered as non-established. Healthy and contaminant free explants were excised by removing discoloured tissue and transferred to the semi-solid media supplemented with BAP mg lit^{-1} and AS 75 mg lit^{-1} and

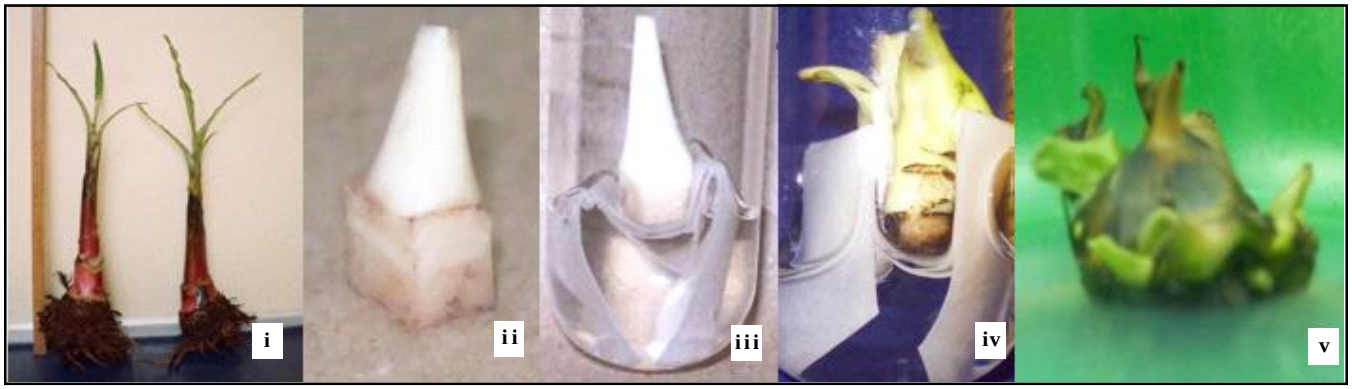

Fig. A: Initiation of aseptic culture by shoot-tip culture: (i) Sword suckers; (ii) Shoot-tip; (iii) and (iv) Shoot-tip culture: (v) Aseptic culture

incubated for four weeks maintaining standard culture conditions. The explants were observed for their bulging in the tips and morphogenetic activity. Such explants were counted and expressed and in terms of per cent establishment. The successfully established explants (Fig. A.v) were excised by trimming the discoloured tissues and then 2-4 vertical cuts were made at the tip of each explant. Multiple shoot clumps so obtained were cultured on liquid medium using simple culture containers and compared with conventional solid medium.The treatments studied included: Growtech container with nylon wire mesh support (treatment 1); simple polypropylene container with cotton fibre support (treatment 2); simple polypropylene container with partial immersion (treatment 3), simple polypropylene container with full immersion (treatment 4) and conventional baby jar bottle with agar gelled medium (treatment 5).

Liquid medium culture methods :

Following liquid medium culture methods were tried for shoot proliferation and *in vitro* rooting simultaneously.

Growtech container :

A simple container of 1 litre capacity (Titan Biotech Limited, Biwadi, Rajastan, India) consisted opening at the side for pouring the medium and fine wire mesh as a support at the base on which five explants were inoculated per container (Fig. B i). About 100 ml MS liquid medium was dispensed into each container and were sterilized by autoclaving.

Simple container with cotton fibre support :

A simple food grade autoclable plastic container of 1 litre capacity (K.R. Market, Bangalore, India) was used. Sterile cotton fibre of about 500 mg was compressed, then placed in each container (Fig. B iv) and sterilized by autoclaving. About 100 ml MS liquid medium was dispensed into each container and were sterilized by autoclaving. Five explants were inoculated per container.

Simple container with half immersion :

A simple food grade autoclable plastic container of 1 liter capacity (K.R. Market, Bangalore, India) was used and about 100 ml MS liquid medium was dispensed into each container (Fig. B vii) and were sterilized by autoclaving. Five explants were inoculated per container and were half immersed in the MS liquid medium.

Simple container with full immersion :

A simple food grade autoclable plastic container of 1 lit capacity (K.R. Market, Bangalore, India) was used and about 100 ml MS liquid medium was dispensed into each container (Fig. B x) and were sterilized by autoclaving. Five explants per container were inoculated per containers which were fully immersed in the MS liquid medium.

Conventional baby jar bottle with agar gelled medium :

Baby jar bottle (11 x 5.5cm) with polypropylene caps containing about 40 ml semisolid medium was used for comparison with other treatments (Fig. B xiii). Two explants per bottle were inoculated on the MS semisolid medium.

Multiplication cum rooting medium :

The significant aspect of the present study was that the shoot proliferation and *in vitro* rooting was induced simultaneously. The medium consisted of MS basal medium + 30 g lit^{-1} sucrose + 2 mg lit^{-1} BAP + 75 lit^{-1} AS + 2 mg/lit IBA + 1 mg/lit NAA.

Observations recorded and statistical analysis :

Shoot proliferation and *in vitro* rooting was induced simultaneously over a period of four weeks. The differences among treatments were analyzed in a Completely Randomized Block Design using analysis of variance (ANOVA) with wax vms fortran stat programme (Digital Equipment Corporation, USA).

Table 1: Effect of liquid medium culture methods on shoot multiplication of banana cv. 'GRAND NAINE'

Sr. No.	Treatments	Number of shoots/ explant	Shoot length (cm)	Number of leaves/shoot
1.	Growtech container	5.90	4.47	3.75
2.	Simple container with cotton fibre support	8.05	5.00	4.75
3.	Simple container with half immersion	4.45	4.02	3.75
4.	Simple container with full immersion	1.50	2.60	2.00
5.	Conventional baby jar bottle with agar gelled medium	5.12	3.30	3.00
	S.E.±	0.54	0.15	0.19
	C.D. (P=0.01)	2.28	0.64	0.80

Fig. B: ***In vitro*** **regenerated plantlets with; (i),(ii) and (iii) Growtech container; (iv),(v) and (vi) Simple container with cotton fibre support; (vii),(viii) and (ix) Simple container with half immersion; (x),(xi) and (xii) Simple container with full immersion; (xiii),(xiv) and (xv) Conventional baby jars with agar gelled medium**

Table 2 : Effect of liquid medium culture methods on *in vitro* rooting of banana cv. GRAND NAINE

Sr. No.	Treatments	Per cent rooting (%)	Number of primary roots/ shoots	Root length (cm)	Number of secondary roots/shoot
1.	Growtech container	100 (90)*	10.60	3.64	10.05
2.	Simple container with cotton fibre support	100 (90)	9.85	3.55	7.65
3.	Simple container with half immersion	100 (90)	7.55	3.28	6.65
4.	Simple container with full immersion	100 (90)	3.70	0.60	1.84
5.	Conventional baby jar bottle with agar gelled medium	100 (90)	8.05	3.16	3.00
	S.E. ±	NS	0.70	0.12	1.15
	C.D. (P=0.01)	---	2.95	0.49	4.79

*Figures in parenthesis indicate arcsin-transformed values NS= Non-significant

RESULTS AND DISCUSSION

After four weeks of cultures, the explants cultured in simple container with cotton fibre support matrix produced significantly maximum number of shoots, shoot length and number of leaves (8.05 shoots/explant; 5.00 cm and 4.75 leaves/shoot) as compared to conventional agar gelled medium (5.12 shoots/explant; 3.3 cm and 3.00 leaves/shoot) (Table 1 and Fig. B v).

No significant difference was found in per cent rooting of microshoots among the treatments (Table 2). A cent per cent rooting was recorded in all the treatments. In terms of root growth, microshoots cultured in growtech container and simple container with cotton fibre support matrix showed higher number of primary roots, root length and number of secondary roots (10.60; 3.64 cm; 10.05 and 9.85; 3.55cm; 7.65, respectively) than those grown in conventional baby jars with agar gelled medium (8.05; 3.16 cm and 3.00) (Table 2 and Fig.B iii, v and xv).

The plantlets produced in growtech container and simple container with cotton fibre support matrix were also sturdier and of better quality when compared with conventional agar gelled medium (Fig. B iii and vi).

It is evident that the type of liquid medium application greatly influences the development of banana explants in micropropagation. Upto five-fold differences were observed with the same medium composition. Probably, the wire mesh and cotton fibre matrices may have afforded better aeration to explants. Besides, the absence of gelling agent might have led to increased availability of water and dissolved substances to the explants. These observations are in conformity with the findings of Debergh (1983); Harris and Mason (1983) and Skidmore *et al*. (1988).

Differences in culture growth between the explants fully immersed in a liquid medium and those cultured in liquid medium with support matrices suggest that the lack of oxygen to explants in liquid media was a major limiting factor for explants growth. This may be attributed to problem of vitrification resulting in poor culture growth (Fig. B x, xi and xii). These findings are in accordance with the reports of Alvard *et al*. (1993).

REFERENCES

Alvard, D., Cote, F. and Teisson, C. (1993). Comparison of liquid medium culture for banana micropropagation, effects of temporary immersion of explants. *Plant Cell Tiss. Org. Cult.,* **32** : 55-60.

Besagarhally, R. (1996). Micropropagation and nutritional studies in of tissue cultured banana var. GRAND NAINE. Thesis, Ph.D. University of Agricultural Sciences, Bangalore, KARNATAKA (INDIA).

Debergh, P.C. (1983). Effects of agar brand and concentration on the tissue culture medium. *Plant Physiol*., **59**: 270 - 276.

Gawel, N.J. and Robacker, C.D. (1990). Somatic embryogenesis in two *Gossypium hirsutum* genotypes on semi-solid versus liquid proliferation media. *Plant Cell Tiss. Org. Cult.,* **23**: 201-204.

Harris, R.E. and Mason, E.B.B. (1983). Two machines for *in vitro* propagation of plants in liquid media. *Can. J. Plant Sci,*. **63**: 311-316.

Nene, Y.L., Sheila, V.K. and Moss, J.P. (1996). Tapioca a potential substitute for agar in tissue culture media. *Curr. Sci*., **70** : 493-494.

Robinson, J.C. (1996). *Banana and Plantains*. 104-127 p. CAB International University Press, Cambridge, U.K.

Skidmore, D.I., Simon, A.J. and Bedis (1988). *In vitro* culture of shoots of *Pinus caribaea* on a liquid medium. *Plant Cell Tiss. Org. Cult*., **14** : 129-136.

Ziv, M. (2000). Bioreactor technology for plant micropropagation. *Hort. Rev.,* **24**: 1-30.