IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
Volume 9 | Issue 1 | January, 2014 | 46-51

RESEARCH ARTICLE

Low-cost gelling agents for micro-propagation of banana (*Musa acuminate)* cv. 'GRANDE NAINE'

■ G. PRABHULING, A.B. MASTIHOLI AND M.G. KERUTAGI

SUMMARY

Bananas (*Musa* spp.) are important staple crop in tropical and sub-tropical countries providing a good source of carbohydrates, minerals and vitamins. Their trade also creates a considerable income as a cash crop. Micropropagated plants are increasingly becoming the planting material of choice, but the higher costs of plantlets have prevented the growers from benefiting from tissue culture technology. Agar is the most commonly used gelling agent for preparation of media, which adds significantly to the cost of media. The use of cheaper alternative to agar eliminates the need of agar. Therefore, the efficacies of sago, isubgol, semolina, starches of tapioca, corn, wheat, rice and ragi as a gelling agents have been tested to reduce the cost of plantlets. The performance of low cost gelling agent's sago and tapioca starch were found to be best and could compare well with that of agar. The results showed the potential of the cheaper substitutes for economic commercial tissue culture production of banana cv. 'Grande Naine' replacing the costliest gelling agent agar.

Key Words : Banana, Low-cost, Sago, Tapioca starch

How to cite this article : Prabhuling, G., Mastiholi, A.B. and Kerutagi, M.G. (2014). Low-cost gelling agents for micro-propagation of banana (*Musa acuminate)* cv. 'GRANDE NAINE'. *Internat. J. Plant Sci.*, **9** (1): 46-51.

Article chronicle : Received : 12.08.2013; **Revised :** 23.09.2013; **Accepted :** 09.10.2013

Banana and plantains together rank fourth as the most important food commodity in the world only after rice, wheat, milk or milk products. The world's annual production is 97.50 million tones with an area of 10 million hectares. Currently, banana is the largest fruit crop accounting for almost 32% of total fruit production. India is the largest producer of banana contributing about 26% of total world production with the production of about 26.91 million tones covering 0.49 million hectares.

Banana is propagated conventionally through suckers because being triploid plant, seed setting and propagation by seed is not possible. The major problem in propagation through conventional method is the transmission of soil borne disease through rhizome and viral infection causing bunchy top. Besides, this method is slow and season bound. *In vitro* propagated plants are increasingly becoming the planting material of choice because of disease control, uniformity and the possibility of rapid multiplication. However, growers have to face higher costs and pay up to five times more than for suckers. Sucker derived banana is still in demand owing to low cost and easy availability. The cost of fully hardened banana is Rs. 10-12/plant while the cost of sucker derived banana is Rs. 2-3/plant. Today only big farmers can afford the micropropagated plants. The high cost of plant is due to high price of purified agar (Nene *et al*., 1996), tissue culture grade sucrose, gelrite and artificial light (Kodym and Zapata-Arias, 2001) and high energy tariff (Bhat and Bhat, 2003). The present study was, therefore, undertaken with a leading commercial variety 'Grande Naine' with the objective to identify low cost alternative gelling agents.

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
G. PRABHULING, K.R.C. College of Horticulture, Arabhavi, BELGAUM (KARNATAKA) INDIA
Email: gprabhuling@gmail.com

Address of the Co-authors:
A.B. MASTIHOLI AND M.G. KERUTAGI, K.R.C. College of Horticulture, Arabhavi, BELGAUM (KARNATAKA) INDIA

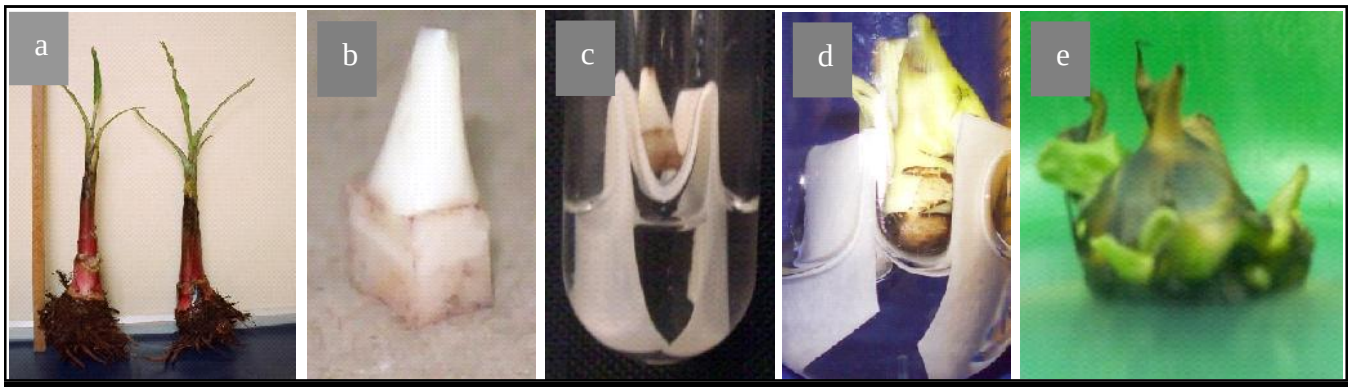

Fig. 1 : Initiation of aseptic culture. a) sword suckers, b) shoot-tip, c) - d) shoot-tip culture, e) aseptic shoot-tip culture

MATERIAL AND METHODS

The present study was conducted at the plant culture laboratory, division of horticulture, University of Agricultural Sciences, GKVK, Bangalore. Healthy and vigorously growing sword suckers of cv. 'Grande Naine' (3-4 month age), free from viruses and other diseases, were selected as a source of explant (Fig. 1a).

Preparation of explant:

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 of 1% bavistin for half an hour, to further clean the planting 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 0.5% bavistin + 0.05% streptocycline for eight hours. 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 0.05% cetrimide for 30 minutes. After removing one more layer, the shoot tips were surface sterilized with 0.1% mercuric chloride in a closed container for 15-20 minutes. 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.

Initiation of aseptic culture:

Shoot tip explants were incubated in MS liquid culture media containing 2 mg/l BAP and 75 mg/l adenine sulphate for two weeks maintaining standard culture conditions of 25 ± 2^0 C temperature, 70 % RH and photoperiodic cycle of 16 hours light and 8 hours dark period (Fig. 1b-c).

After two weeks of incubation, all the explants (Fig. 1d) 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 semisolid media supplemented with BAP (2 mg/l) and adenine sulphate (75 mg/l) and 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 in terms of per cent establishment. The successfully established explants (Fig. 1e) were excised by trimming the discoloured tissues, then 2-4 vertical cuts were made at the tip of each explant and the used to carry out further experiments.

Low cost gelling agents used in the present study were sago, isubgol, tapioca starch, corn starch, semolina, rice powder, wheat starch and ragi flour along with agar (control). At the end of experiments, morphological characteristics (number of shoots/explant, shoot length, number of leaves, roots/shoots, fresh weight etc.) were measured. An analysis of variance (ANOVA) was conducted on data concerning shoot and root morphological parameters using the statistical program wax vms fortran.

RESULTS AND DISCUSSION

The experimental findings obtained from the present study have been discussed in following heads:

Role of different gelling agents in shoot proliferation and in vitro rooting:

The extent to which micro-propagation can be practiced commercially is often being limited by production costs. The cost of micropropagation is influenced by a number of factors. Though there is a difference in opinion whether media costs really contribute a significant portion of the total cost but refined agar powder generally used in plant tissue culture media, no doubt, is a costly commodity (Gangopadhyay *et*

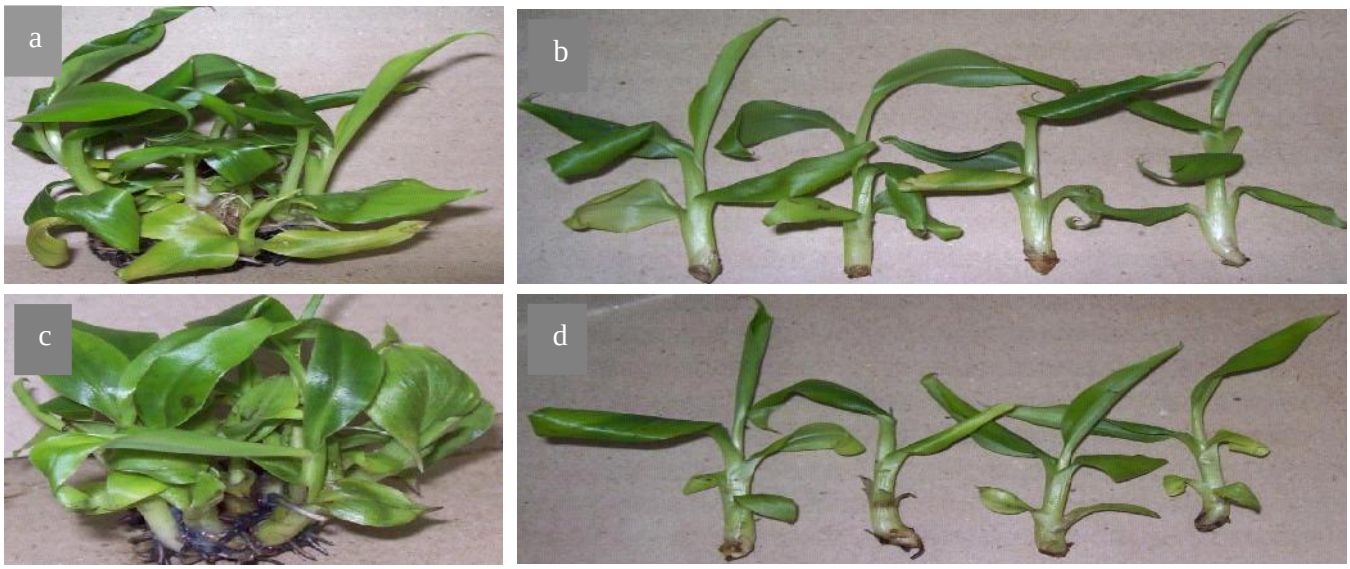

Fig. 2 : Multiple bud clump and microshoots obtained from medium gelled with a)-b) agar 6 g/l, c)-d) sago 80 g/l

al., 2009). Agar is widely used as a gelling agent for tissue culture media (George, 1993 a and b). Several suitable substitutes are now available for gelling plant media but the results obtained differ considerably. Often, the efficiency and economy of a product determines its usefulness as an agar substitutes for tissue culture production (George, 1993a).

In the present study gelling agents had a significant influence on shoot proliferation and *in vitro* rooting (Table 1 and 2, Fig. 2-3). Explants cultured on the medium gelled with commercial grade agar at 6 g/l produced maximum number of shoots per explant and highest propagation rate which did not vary with sago at 80, 100, and 120 g/l. However, quality wise (shoot length, number of leaves per shoot, shoot diameter, number of primary roots, plantlet diameter and fresh weight of plantlets) medium solidified with sago was found superior to commercial grade agar. This is because of the reason that rich nutrients present in sago might have supplemented the growth and development (Prabhakara and Reddy, 2004 and Shailaja and Patil, 2004). Sago at 7 % gave proper solidification and normal culture growth in ginger and turmeric (Prakash, 1993). Besides starch, sago contains small amount of sugars, fibre, protein, calcium, and other minerals. On heating, this starch gets converted into a complex polysaccharide, dextrin. Along with the nutrient media, the polysaccharide dextrin supports the growth of cells (Prabhakara, 1999). Nene and Sheila (1997) explained that sago unlike agar can be used as a gelling agent and a carbohydrate component in the nutrient media. The better response on sago starch gelled medium could be also due to the absence of inhibitors which have been reported to be

Fig. 3 : *In vitro* rooted plantlets obtained with medium gelled with a) agar 6 g/l, b) sago 80 g/l

present in agar (Debergh, 1983). And also, the promontory effect of sago starch may be probably because, starch can act as an additional carbon source and beneficial compounds present which act as a ionic supplements.

Root growth was better with the medium solidified with tapioca, corn starch and sago as compared to agar gelled medium. This may be due to richness of starch in nutrients like carbohydrates, amino acids, minerals etc. Better root growth might be due to the presence of amino acid (thiamine) in sago (Prabhakara, 1999). Microplants of potato cultured on sago medium did not show any growth abnormalities and there was no adverse effect on rooting (Naik and Sarkar, 2001). Sago gelled medium is easily removed from the roots as reported by Bhattacharya *et al*. (1994).

With increase in the concentration of gelling agents, poor response in terms of shoot and root growth was observed. This may be attributed to increase in surface hardness of the medium, which resulted in poor contact between the medium surface and explants and poor absorption of nutrients. Debergh (1983) opined that gel must be firm enough to support explant or shoot cluster. But, if it is too hard, it may prevent adequate contact between the medium and tissue. Due to solidity, water stress is created, which affect severely the growth and development of tissue culture.

In the present study, the media gelled with starches such as isubgol, tapioca, corn and semolina gave poor response in terms of shoot growth as compared to agar and sago gelled media. This might be due to the presence of lesser supporting nutrients. Starches, upon autoclaving, yields sugars that may further increase the osmotic potential of the storage medium resulting in reduced availability of water to the growing cultures (Naik and Sarkar, 2001). Isubgol gave poor results at all the concentrations used (Prabhkara and Reddy, 2004, Shailaja and Patil, 2004). This was due to poor absorption of nutrients as a result of hardness in its surface (Shailaja and Patil, 2004). Practical handling during pouring is a problem, removal of plantlets is laborious and results in damages to plantlets as observed by Prabhkara and Reddy (2004).

Further, media solidified with rice flour, wheat starch and ragi flour did not produce a satisfactory gel. Moreover, culture growth was very poor and the media turned black during the course of culture period. These results are in agreement with the report of Kodym and Zapata-Arias (2001). Prakash (1993) achieved proper media solidification with wheat flour and laundary starch but the culture growth was below average in ginger and turmeric. Starches at concentrations that supported plant growth was difficult to dissolve, pour and clean as observed by Kodym and Zapata-

Table 1 : Effect of different gelling agents on shoot proliferation of banana cv. 'GRANDE NAINE'

Sr. No.	Treatments	No. of shoots/ explant	Shoot length (cm)	No. of adv. buds/ explant	No. of leaves/ shoot	Shoot dia. (mm)
1.	Agar 6 g/l	12.75	3.68	4.37	3.36	3.76
2.	Sago 80 g/l	10.35	5.01	1.55	3.40	4.31
3.	Sago 100 g/l	11.55	4.78	1.10	3.23	3.73
4.	Sago 120 g/l	9.70	4.16	2.60	2.41	2.91
5.	Sago 140 g/l	7.40	4.02	1.30	2.66	2.64
6.	Isubgol 20 g/l	7.85	5.63	0.45	4.29	4.26
7.	Isubgol 30 g/l	6.80	4.52	1.90	2.99	3.76
8.	Isubgol 40 g/l	6.17	3.91	1.95	3.39	2.85
9.	Isubgol 50 g/l	4.60	3.12	1.60	2.80	2.66
10.	Tapioca starch 80 g/l	6.10	3.39	1.30	3.04	3.81
11.	Tapioca starch 100 g/l	7.65	4.22	1.15	3.37	3.66
12.	Tapioca starch 120 g/l	5.25	2.63	2.30	2.24	3.37
13.	Tapioca starch 140 g/l	3.35	2.99	1.73	2.36	3.05
14.	Corn starch 80 g/l	6.30	3.49	1.75	2.91	3.66
15.	Corn starch 100 g/l	5.50	2.65	3.30	2.02	3.46
16.	Corn starch 120 g/l	3.65	2.53	1.85	2.25	1.73
17.	Corn starch 140 g/l	3.35	2.59	1.21	2.37	1.63
18.	Semolina 50 g/l	4.15	3.27	0.75	2.47	3.87
19.	Semolina 60 g/l	3.30	3.18	0.76	2.81	3.74
20.	Semolina 70 g/l	3.25	3.15	2.55	2.49	2.02
21.	Semolina 80 g/l	3.00	2.72	0.95	3.35	1.93
	S.E. ±	1.22	0.21	0.53	0.23	0.16
	C.D. (P=0.01)	4.60	0.81	2.01	0.88	0.62

Table 2: Effect of different gelling agents on *in vitro* rooting of banana cv. 'GRANDE NAINE'

Sr. No.	Treatments	Per cent rooting (%)	No. of primary roots/shoot	Root length (cm)	No. of secondary roots/shoot	Plantlets dia. (mm)	Fresh weight of plantlets (mg)
1.	Agar 6 g/l	100 (89.63)	5.12	4.70	13.21	3.93	2310.00
2.	Sago 80 g/l	100 (89.63)	5.95	3.80	7.85	4.70	3013.50
3.	Sago 100 g/l	100 (89.63)	4.90	2.98	7.30	5.35	2616.50
4.	Sago 120 g/l	100 (89.63)	2.95	1.97	1.60	4.65	2109.50
5.	Sago 140 g/l	100 (89.63)	2.75	1.80	0.70	4.50	1963.00
6.	Isubgol 20 g/l	100 (89.63)	5.75	3.17	3.60	4.34	2578.80
7.	Isubgol 30 g/l	100 (89.63)	4.35	2.95	2.90	3.69	1880.00
8.	Isubgol 40 g/l	100 (89.63)	4.00	3.05	1.45	2.96	1807.00
9.	Isubgol 50 g/l	100 (89.63)	3.15	1.19	1.20	2.84	889.05
10.	Tapioca starch 80 g/l	100 (89.63)	8.50	3.46	7.10	3.93	2269.35
11.	Tapioca starch 100 g/l	100 (89.63)	8.50	3.43	10.10	4.15	1994.25
12.	Tapioca starch 120 g/l	100 (89.63)	7.90	3.32	9.10	4.16	2315.45
13.	Tapioca starch 140 g/l	100 (89.63)	5.95	2.62	3.60	3.07	1482.00
14.	Corn starch 80 g/l	100 (89.63)	6.50	3.38	2.80	3.59	2130.80
15.	Corn starch 100 g/l	100 (89.63)	8.70	2.78	6.80	3.24	1199.00
16.	Corn starch 120 g/l	100 (89.63)	5.00	2.36	2.40	2.92	1064.05
17.	Corn starch 140 g/l	100 (89.63)	3.70	2.23	2.00	2.70	882.90
18.	Semolina 50 g/l	100 (89.63)	6.10	2.03	2.45	3.87	2186.20
19.	Semolina 60 g/l	100 (89.63)	6.40	2.20	12.40	3.83	1869.05
20.	Semolina 70 g/l	100 (89.63)	5.53	2.05	7.86	3.42	1501.66
21.	Semolina 80 g/l	100 (89.63)	7.73	1.61	5.46	3.40	2161.96
	S.E. ±	NS	0.49	0.15	1.17	0.23	170.28
	C.D. (P=0.01)	---	1.86	0.56	4.43	0.90	639.63

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

Table 3 : Differential cost for a litre of media with different gelling agents

Gelling agents	Quantity / l g)	Price/kg Rs.	Price / l (Rs.	Cost reduction over control (%)
Agar (control)	6	2200	13.20	0
Sago	80	50	4.00	69.69
Sago	100	50	5.00	62.12
Sago	120	50	6.00	54.54
Sago	140	50	7.00	46.96
Isubgol	20	200	4.00	69.69
Isubgol	30	200	6.00	54.54
Isubgol	40	200	8.00	39.39
Isubgol	50	200	10.00	24.24
Tapioca starch	80	45	3.60	72.72
Tapioca starch	100	45	4.50	65.90
Tapioca starch	120	45	5.40	59.09
Tapioca starch	140	45	6.30	52.27
Corn starch	80	35	2.80	78.78
Corn starch	100	35	3.50	73.48
Corn starch	120	35	4.20	68.18
Corn starch	140	35	4.90	62.87
Semolina	50	30	1.50	88.63
Semolina	60	30	1.80	86.36
Semolina	70	30	2.10	84.09
Semolina	80	30	2.40	81.81

Arias (2001).

Economics:

Considering all the aspects (number of shoots, shoot length, number of leaves, length of primary roots, plantlet diameter and fresh weight of plantlets), sago at 80 g/l was found to be a suitable replacement for agar. With respect to number of shoots per explant agar and sago were found statistically at par with each other. However, quality wise (shoot length, number of leaves, shoot diameter, number of primary roots, plantlet diameter and fresh weight of plantlets) sago was found superior to agar. With regard to cost (Table 3), it reduced the cost by 69.69 per cent when compared with commercial grade agar.

From these results, it can be concluded that agar can be successfully replaced by sago as it minimizes the cost of plantlets to a large extent besides improving the quality of plantlets in commercial propagation of banana.

REFERENCES

Bhattacharya, P., Dey, S. and Bhattacharyya, B.B. (1994). Use of low-cost gelling agents and support matrices for industrial scale plant tissue culture. *Plant Cell Tiss. Org. Cult.,* **37** (1) : 15-23.

Bhatt, P. and Bhatt, D. (2003). Banana micropropagation – a success story. In: *Comprehensive micropropagation of horticultural crops*, International Book Distributing Co., Lucknow. pp 212-220.

Debergh, P.C. (1983). Effects of agar brand and concentration on the culture medium. *Plant Physiol.,* **59** (2) : 270-276.

Gangopadhyay, G., Roy, S.K. and Mukherjee, K. (2009). Plant response to alternative matrices for *in vitro* root induction. *Afr. J. Biotech.,* **8**(13): 2923-2928.

George, E.F. (1993a). *Plant propagation by tissue culture. Part 1-The technology,* London: Exogetics Limitted.pp.377-356.

George, E.F. (1993b). *Plant propagation by tissue culture. Part 2-In practice,* London: Exogetics Limitted.

Kodym, A. and Zapata-Arias, F.J. (2001). Low- cost alternative for the micropropagation of banana. *Plant Cell Tiss. Org. Cult.,* **66**: 67-71.

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.

Nene, L.Y. and Sheila, K.V. (1997). A manual on Tapioca- a potential substitute for agar in microbiology and tissue culture media. ICRISAT, Andhra Pradesh, India.

Naik, P.S. and Sarkar, D. (2001). Sago: an alternative cheap gelling agent for potato *in vitro* culture. *Biol. Plant.,* **44** (2) : 293-296.

Prabhakara, H.L. (1999). Studies on *in vitro* propagation of *Anthurium andreanum* Lind. M.Sc. (Ag.) Thesis, University of Agricultural Sciences, Dharwad, KARNATAKA (INDIA).

Prabhakara, H.L. and Reddy, B.S. (2004). A low cost strategy for tissue culture of anthurium I. Gelling agents. *J. Orn. Hort.,* **7**(3-4): 251-261.

Prakash, S. (1993). Production of ginger and turmeric through tissue culture methods and investigations into making tissue culture propagation less expensive. Ph. D. Thesis, Bangalore University, Bangalore, KARNATAKA (INDIA).

Shailaja, P.V. and Patil, S.S. (2004). Potential substitutes for agar in tissue culture media. *J. Orn. Hort.,* **7**(3-4): 247-250.