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Efficacy of Low Cost Gelling Agents and Carbon Source Alternatives during *in Vitro* Rooting of *Balanites aegyptiaca* and *Phyllanthus emblica* Microshoots

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ABSTRACT

Several attempts have been made to reduce the cost of tissue culture raised plants. The efforts have largely concentrated around using low cost alternatives to agar (gelling agent) and sucrose (carbon source). Isabgol-husk, guar gum and sago powder were used as low cost gelling alternatives and table sugar, raw sugar and jaggary were tested as low cost carbon sources in tissue culture media. These low cost alternatives were tested during *in vitro* rooting of microshoots of two plants of arid and semi-arid regions of India: *Balanites aegyptiaca* and *Phyllanthus emblica*. Sago powder, guar gum and isabgol husk solidified the media properly at 15.0, 5.0 and 5.0% (w/v), respectively. In the presence of isabgol, rooting response in microshoots of *B. aegyptiaca* was equivalent to that of agar and application of isabgol husk can reduce the cost of gelling agents per unit media by ~44%. Further, table sugar can be used for *in vitro* rooting of both the species at a commercial scale. As far as gelling agents are concerned, isabgol was found to be a suitable gelling agent for rooting with equally good rooting response comparable to agar.

Keywords: *Balanites aegyptiaca*, guar gum, isabgol, jaggary, *Phyllanthus emblica*, raw sugar, sago powder, table sugar
Abbreviations: INR, Indian National Rupee (India's currency)

INTRODUCTION

Plants produced through tissue culture are invariably costlier when compared to other methods of propagation. To make it economically more viable and attractive researchers as well as commercial tissue culture establishments around the world have been trying to reduce the cost of tissue culture raised plantlets. In plant production through micropropagation media chemicals cost a little less than 15% of the total cost (Prakash *et al*. 2004) but the cost of gelling agent per unit media is 73.53%. Out of media components, gelling agents such as agar contribute 70% to the cost of media. Therefore, agar is one of the major components to be swapped with low cost gelling alternatives, to be able to achieve cost reduction. Attempts have been made earlier to replace agar. A few low cost gelling agents had been tested in several laboratories viz., sago powder, isabgol husk, guar gum, cassava flour and xantham gum etc (Maliro and Lameck 2004; Jain and Babbar 2005, 2006).

In the present study the cost of MS media has been calculated to understand the cost contribution of different media components. The concentrations of alternative low cost gelling agents such as isabgol-husk, guar gum and sago powder were first standardized to solidify the nutrient media properly at pH 5.8. The effects of low cost alternatives of sucrose and agar on rooting efficiency of two medicinally important and reasonably salt and draught-tolerant plants of the Indian Thar Desert namely *Balanites aegyptiaca* (Balanitaceae) and *Phyllanthus emblica* (Euphorbiaceae) have been studied. *B. aegyptiaca* is known for its diosgenin content, which is used in production of oral contraceptive, steroids and sex hormone. *P. emblica* is well known for being a natural source of vitamin C. Micropropagation has already been developed for *B. aegyptiaca* and *P. emblica* in our laboratory (Gour *et al.* 2005; Gour and Kant 2007; Gour *et al*. 2007). Here we present the results of a study on the effectiveness of low cost alternatives of agar and sucrose under *in vitro* rooting of microshoots of the two tree species.

MATERIALS AND METHODS

Explants

Microshoots 2-3 cm long were harvested from actively growing cultures of both plants and used for rooting experiments.

Culture medium and growth conditions

B5 medium (Gamborg *et al*. 1968) and half-strength MS (Murashige and Skoog 1962) media were used in different experiments to evaluate efficacy of low cost alternatives of agar and sucrose. B5 medium was supplemented with plant growth regulators and a carbon source (2%, w/v) while half-strength MS medium was supplemented with 2.0 mg/l Indole-3-butyric acid (IBA) and a carbon source (1.5%, w/v). The pH of MS and B5 media was adjusted to 5.8 and 5.5, respectively using 1.0 N NaOH and/or 1.0 N HCl, and before adding gelling agents.

The cost of MS media was calculated using all chemicals from HiMedia Laboratories (India) to determine the contribution of agar and sucrose to the total cost of media. To determine the optimum concentration of low-cost gelling agents required to solidify the media, different concentration were tested (**Table 1**). To replace the extra-pure sucrose (HiMedia, India) low cost alternatives such as table sugar, raw sugar and jaggary (purchased from a local market at Jodhpur, India) were added to the media. 40 ml of medium was dispensed per glass jam jar of 400 ml capacity while 20 ml per borosilicate culture tube of 60 ml capacity. Cotton plugs made up of non-absorbent cotton were used throughout. The glassware, forceps, scalpels, media and distilled water were autoclaved for 20 min at 121°C and 15 psi pressure. The cultures were incubated for 16 hrs (1400 lux light intensity using 40W florescent

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Short Communication

Table 1 Costs of cheaper alternatives to gelling of tissue culture media.

Alternative	Cost per Kg		Percentage (w/v) for	Fails to solidify	Cost per liter media	
	INR	USD*	gelling D.W. (pH 5.8)	(% w/v)	INR	USD*
Agar Agar (HiMedia)	2816	60	0.8	Up to 0.6	22.53	0.48
Isabgol-husk (Deer Brand)	252	5.3	5	Up to 3	12.60	0.27
Sago powder	36	0.7	15	Up to 12	5.40	0.12
Guar Gum	40	0.8	5	Up to 3	2.00	0.04

*equivalent cost in USD where 1 USD = 47 INR

Table 2 Effect of gelling agents on rooting of micro shoots of *Balanites aegyptiaca.*

Gelling agents	Percentage rooting	No. of roots/ shoot*	Root length* (cm)
Control (liquid media)	0	0	0
15 % sago powder	66.67	4.2 ± 0.66	0.8 ± 0.2
5% guar gum	73.33	1.91 ± 0.25	0.84 ± 0.12
5% Isabgol	100	3.87 ± 1.04	1.92 ± 0.23
0.8% agar	100	4.73 ± 0.71	0.77 ± 0.10

*indicates values are presented as Mean ± SE of 15 replicates
Explant: micro shoots, source: MS + 1.0 BAP + 3.0% sucrose + 0.8% agar (pH 5.8)
Media: B5 + 0.02 NAA + 2.0% sucrose (pH 5.5), observation after 4 weeks

Table 3 Effect of gelling agents on rooting of micro shoots of *Phyllanthus emblica*.

Gelling agents	Percentage rooting	No. of roots/ shoot*	Root length* (cm)	Callusing %
Control (liq. media)	0	0	0	0
15 % sago powder	20	2.5 ± 0.5	0.95 ± 0.25	0
5% guar gum	0	0	0	20
5% Isabgol	0	0	0	21.42
0.8% agar	37.5	1.83 ± 0.48	0.87 ± 0.16	58.33

*indicates values are presented as Mean ± SE of 15 replicates
Media: MS ½ + 2.0 IBA + 1.5 % sucrose + pH 5.8, observation after 4 weeks

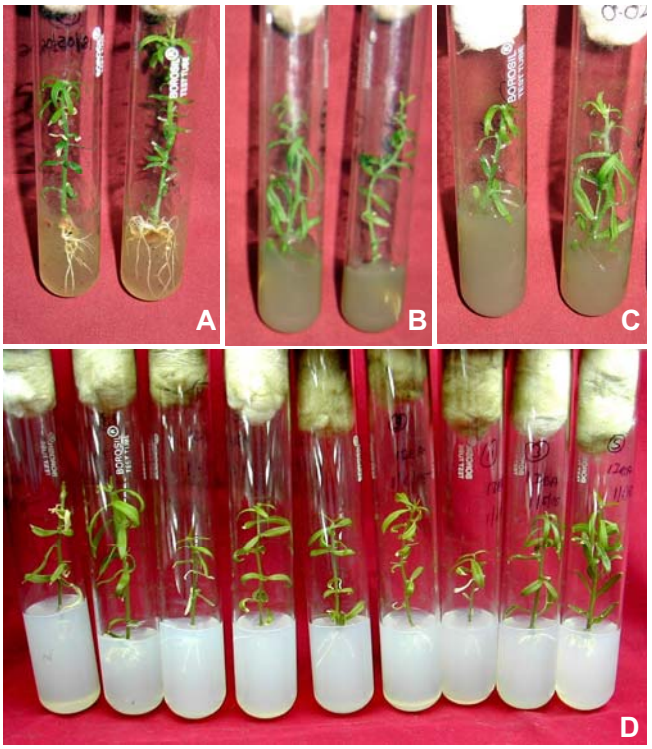

Fig. 1 Effect of low cost gelling agents on rooting. (**A**) Medium gelled with isabgoal; (**B**) medium gelled with sago; (**C**) medium gelled with guar gum; (**D**) medium gelled with agar (control).

lights (Surya HBT, India)) and an 8-hr photoperiod at 26 ± 2°C. Observations were recorded at one-week intervals. Plantlets were hardened as described by Gour *et al*. (2007). In each experiment 14-15 explants were used. For comparison of mean of number of roots/explant and root length in different media, the data were subjected to the analysis of variance, and the mean separation was performed using Duncan's multiple range tests at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

The cost of MS medium (including vitamins, 3% sucrose and 0.8% agar) as per the 2007-08 price-list issued by HiMedia Laboratories (India) was INR 30.64 (USD 0.65; currency conversion rate: 1 USD = 47 INR). Out of the various components of MS media, micronutrients (which are used at ≤100 mg/l), macro-nutrients (which are used at >100 mg/l), vitamins, sucrose and agar contributes 4.73, 0.59, 0.78, 20.36 and 73.53%, respectively. Thus, agar and sucrose are two major components of nutrient media that contribute the most towards the cost of media.

Sago powder, guar gum and isabgol husk solidified the media properly at 15.0, 5.0 and 5.0% (w/v), respectively (**Table 1**).

Out of the different gelling agents tested for *in vitro* rooting in *B. aegyptiaca* and *P. emblica*, agar was found to be the best media gelling agent (**Tables 2, 3**). No rooting was observed in liquid medium. A rooting percentage of 73 and 66% were obtained with guar gum and sago powder, respectively (**Table 2**). For isabgol, rooting percentage was comparable to that of agar. Media solidified with isabgol husk in place of agar can reduce the cost of gelling agents per unit media by ~44% (**Fig. 1**).

P. emblica also responded in a similar manner, resulting in 37.5% rooting on agar-supplemented media while no rooting was observed on guar gum- and isabgol-supplemented media (**Table 3**). Similar findings have been reported by Bhattacharya *et al.* (1994) who found that sago (from *Metroxylon sagu* Rottb.) and isubgol (from *Plantago ovata* Forsk.) were highly cost-effective gelling agents compared to agar. They studied the effect of these gelling agents on the micropropagation of chrysanthemum (*Dendranthema grandiflora* Tzvelev) plantlets. Crude agar was as good as AR grade agar in multiplication in *Wrightia tomentosa* (Joshi *et al*. 2009). Our results are also in agreement with those reported by Jain and Babbar (2005) who indicated that the cost of isabgol and guar gum/liter of medium is about 2.5–13 times less than different brands of agar; these are two highly cost-effective gelling agents which could be used for reducing the cost of *in vitro*-propagated orchid (*Dendrobium chrysiotoxum*) plants. Both, being of plant origin, are biodegradable and do not pose any threat to the environment once disposed after use. Guar gum was used as a gelling agent in an *in vitro* study of *Linum usitatissimum* and *Brassica juncea* (Babbar *et al*. 2005). It was found to be 8-80 times cheaper than agar and Difco bacto agar, respectively.

Experiments were carried out to determine a low-cost alternative of carbon source for *in vitro* rooting of microshoots of *B. aegyptiaca*. Sucrose is the second largest factor contributing to the cost of a tissue culture medium. Raw sugar, table sugar and jaggary were used as alternative carbon sources in place of sucrose, each at 2% (w/v). Replacement of table sugar, jaggary and raw sugar could reduce the cost of the carbon source up to 95.19, 90.38 and 85.58%, respectively (**Table 4**). Among these low-cost alternatives to sucrose in media for *in vitro* rooting of microshoots table

Table 4 Cost calculations of cheaper alternatives for C source for Tissue culture media.

Alternative	Cost per Kg		Cost of carbon source per liter media @ 2% w/v	
	INR	USD	INR	USD
Sucrose	208 (HiMedia)	4.42	4.16	0.89
Jaggery	20	0.43	0.40	0.008
Raw sugar	30	0.64	0.60	0.012
Table sugar	15	0.32	0.30	0.006

Table 5 Effect of carbon sources on rooting of micro shoots of *Balanites aegyptiaca*.

C source 2 % (w/v)	Percentage rooting	No. of roots/ shoot*	Root length* (cm)
Sucrose	100	5.57 ± 0.81	0.73 ± 0.11
Table sugar	75	6.78 ± 1.66	0.82 ± 0.14
Raw Sugar	20	1.33 ± 0.33	0.40 ± 0.20
Jaggary	28.57	1.50 ± 0.50	0.63 ± 0.19

*indicates values are presented as Mean ± SE of 14 replicates
Explant: micro shoots, source: MS + 1.0 BAP + 3.0 % sucrose +0.8% agar+ pH 5.8
Media: B5+ 0.02 NAA + 0.8% agar+ pH 5.5 + 2% C source, Observation after 4 weeks

Table 6 Effect of different carbon sources on rooting of micro-shoots of *Phyllanthus emblica*.

Carbon sources	Percentage rooting	No. of roots/ shoot*	Root length* (cm)
0	0	0	0
Jaggary	0	0	0
Raw sugar	20	1.00 ± 0.13	0.70 ± 0.06
Table sugar	41.67	1.60 ± 0.40	2.04 ± 0.60
Sucrose	37.5	1.83 ± 0.48	0.87 ± 0.16

*indicates values are presented as Mean ± SE of 15 replicates
Media: MS ½ + 2.0 IBA + 1.5 % carbon sources + pH 5.8, Observation after 4 weeks

sugar was found to be best with 75% at an average of 6 roots/explant (**Table 5**). However, 100% rooting response could be observed on media with sucrose. For *in vitro* rooting of microshoots at a large scale table sugar can be used as a good option to reduce cost.

In *P. emblica*, *in vitro* rooting percentage was 41.67% and mean root length was also higher when table sugar was used, higher than that when sucrose was used (**Table 6**). According to Prakash (2004) local sugar was as good as the high-grade laboratory sugar for the multiplication of banana (*Musa paradisica*). Maple syrup (from *Acer saccharum*) has been used for the multiplication (50 g/l) and rooting (34 g/l) of cherry (*Prunus avium*) rootstocks from nodal segments and shoot tips. The carbohydrate requirement for rooting of shoots depends upon the availability of auxins, nitrogen and light (Bonga and Aderkas 1992). It has a promoting effect during pre-meristemoid formation but imposes an inhibitory effect thereafter. The need of carbohydrate is species and stage specific. According to Joshi *et al*. (2009) the rate of multiplication of *Wrightia tomentosa* shoots was higher on sugar cubes- and agar-supplemented media in comparison to the control. The rate of multiplication was minimum on media supplemented with jaggary in *W. tomentosa*, in comparison to analytical reagent grade sucrose, sugar cubes, common sugar and jaggary. According to Prakash (1993), the use of common sugar in place of laboratory-grade sucrose reduces the cost of carbon source of the medium 78-87%. In the present study, the use of table sugar in place of sucrose could reduce the cost of carbon source for one unit MS media by ~93% (**Table 4**).

Therefore, table sugar can be used for *in vitro* rooting of the *B. aegyptiaca* and *P. emblica* instead of high purity grade sucrose in media. Substitution of agar requires further research work to obtain a product that is less sticky and easy to remove from plantlet roots.

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REFERENCES

Babbar SB, Jain R, Walia N (2005) Guar gum as a gelling agent for plant tissue culture media. *In Vitro Cellular and Developmental Biology – Plant* **41**, 258-261

Bhattacharya P, Dey S, Bhattacharya BC (1994) Use of low-cost gelling agents and support matrices for industrial scale plant tissue culture. *Plant Cell, Tissue and Organ Culture* **37**, 115-123

Bonga JM, Aderkas PV (1992) *In Vitro Culture of Trees*, Kluwer Academic Publishers, The Netherlands, pp 12-53, 110-113

Gamborg OL, Miller A, Ojima K (1968) Nutrient requirements of suspension cultures of soyabean root cells. *Experimental Cell Research* **50**, 151-158

Gour VS, Emmanuel CJSK, Kant T (2005) Direct *in vitro* shoot morphogenesis in desert date – *Balanites aegyptiaca* (L.) Del. from root segments. In: Tewari VP, Srivastava RL (Eds) *Multipurpose Trees in the Tropics: Assessment, Growth and Management*, Scientific Publishers, India, pp 701-704

Gour VS, Emmanuel CJSK, Sharma SK, Kant T (2007) A rapid *In vitro* morphogenesis and acclimatization protocol for *Balanites aegyptiaca* (L.) Del. – A medicinally important xerophytic tree. *Journal of Plant Biochemistry and Biotechnology* **16**, 151-153

Gour VS, Sharma SK, Emmanuel CJSK, Kant T (2007) Stomata and chlorophyll content as marker traits for hardening of *in vitro* raised *Balanites aegyptiaca* (L.) Del. plantlets. *The National Academy Science Letters* **30**, 45-47

Jain R, Babbar SB (2005) Guar gum and isubgol as cost-effective alternative gelling agents for *in vitro* multiplication of an orchid, *Dendrobium chrysotoxum. Current Science* **88**, 292-295

Jain R, Babbar SB (2006) Xanthan gum: an economical substitute of agar for *in vitro* multiplication of an orchid, *Dendrobium chrysotoxum* Lindl. *Current Science* **91**, 27-28

Joshi P, Trivedi R, Purohit SD (2009) Micropropagation of *Wrightia tomentosa*: Effect of gelling agents, carbon source and vessel type. *Indian Journal of Biotechnology* **8**, 115-120

Maliro FAM, Lameck G (2004) Potential of cassava flour as a gelling agent in media for plant tissue cultures. *African Journal of Biotechnology* **3**, 244-247

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473-497

Prakash S (1993) Production of ginger and turmeric through tissue culture methods and investigations into making tissue culture propagation less expensive. PhD thesis, Bangalore University, Bangalore

Prakash S, Hoque MI, Brinks T (2004) Culture media and containers. In: Low cost options for tissue culture technology in developing countries, FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, pp 29-40