International Journal of Innovative Research in Science and Engineering
Vol. No.3, Issue 01, January 2017
www.ijirse.com
ISSN (O) 2454-9665
ISSN (P) 2454-0663

GELLING AGENTS FOR PLANT TISSUE CULTURE MEDIA: A COMPARATIVE STUDY

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ABSTRACT

Different alternative gelling agentssuch as gelrite, gum katira, isubgol, and sago starch were used for a comparative evaluation with newly introduced gelling agents such as guar gum and xanthan gum for different morphogenic responses, namely in vitro seed germination,caulogenesis, and rhizogenesis of Albizzia lebbeck, *and somatic embryogenesis of* Calliandra tweedii. *Media gelled with guar gum and xanthan gum supported all these morphogenic responses. The caulogenic response in terms of number of shoots per responding explant was best on xanthan gum followed by agar- and guar-gum-gelled media. Rhizogenic response was found to be the best on guar-gum-gelled media.However, the average number of embryos per responding culture varied significantly among different treatments with the best being on guar gum followed by on xanthan gum.*

Keywords: Embryogenesis, Gelling Agents, Morphogenesis, Rhizogenesis

I INTRODUCTION

Biotechnology industry in India, at present, is seeing tremendous growth. The rapid developments in this sector are expected to touch almost every aspect of Indian market. The most popular and widely commercialized application of plant biotechnology, the world over, perhaps is plant tissue culture. The success of plant tissue culture largely depends on the composition of culture media. Gelling agents, an important component, is added to make medium viscous enough to afford the floatation of tissues [1]. Agar has remained the most commonly used gelling agent for microbial and plant tissue culture media [2, 3]. However, some doubts have been raised about its suitability [4–9]. Above all, overexploitation of its limited resources and prohibitive prices are other reasons because of which attempts have been made to identify its suitable substitutes.

The suitability of guar gum and xanthan gum as gelling agents for microbial and plant tissue culture media [10–13] has already been established. The aim of this study was to compare and evaluatethe above-mentioned, newly introduced gelling agents with other alternative gelling agents, namely gelrite, gum katira, isubgol, and sago starch for *in vitro* seed germination, caulogenesis, rhizogenesis, and somatic embryogenesisthe morphogenic phenomenon for which semisolid media are commonly used.

Guar gum is derived from the endosperm of *Cyamopsis tetragonoloba*, a leguminous annual herb grown widely in India.Guar gum is a creamy white-colored powderand possesses very high viscosity with pH ranging between 5.5 and 6. Xanthan gum is a microbial dessication-resistant polysaccharide prepared commercially by aerobic

ISSN (O) 2454-9665
ISSN (P) 2454-0663

submerged fermentation from *Xanthomonas campestris*[14].Agar–agar is a colloidal polysaccharide extracted from red algae, *Gelidium* spp., *Gracilaria* spp., *Pterocladia* sp., *Acanthopeltis japonica*etc., collectively termed as agarophytes [2, 15, 16]. Gelrite is a bacterial polysaccharide discovered through the screening of thousands of bacteria. It is prepared commercially by aerobic submerged fermentation of strain S-60 of *Sphingomonas elodea* [17]. Gum katira is a gum exuded from the deeply furrowed fibrous bark of *Cochspermum religiosum*. Isubgol is a colloidal polysaccharide derived from the seeds of *Plantago ovata*, whereassago starch is a complex polysaccharide serves as a storage product in variety of plants.

II MATERIALS AND METHODS

Media used for the present investigations were gelled with 0.9% (w/v) agar (bacteriological grade; Qualigens Fine Chemicals, Mumbai), 3% (w/v) guar gum (HiMedia Laboratories, Pvt. Ltd., Mumbai), 0.3% gelrite (Sigma–Aldrich Corp., St. Louis, Missouri), xanthan gum (Shree Krishna Pharmaceuticals, Delhi), 3% (w/v) isubgol (Telephone brand, Sidhpur), 3% (w/v) gumkatira,and 15% sago (from a local grocery shop).

2.1 Preparation of Culture Media

For preparing agar- or gelrite-gelled media, gelling agent along with sucrose was added in an amount of water less than the total volume of the medium to be made. Further procedure was followed as described [10]. For preparing guar-gum- or sago-gelled media, sucrose was mixed with other constituents and volume raised to the required level. pH of this solution was adjusted to 5.8. Aliquots of a liquid medium were added to individual culture tubes in which required amount of a gelling agent was already present and autoclavedat 1.06 kg cm^{-2} and 121°C for 15 min. For preparing media containing isubgol or xanthan gum, gelling agent along with sucrose was directly added to other constituents and volume raised to the required level. pH was adjusted to 5.8 before dispensing in culture tubes and autoclaved. For preparing gum katira medium, gum was soaked in the liquid medium overnight. Further procedure was followed as mentioned above. In case of liquid media, explants were cultured on Whatman No. 1 paper bridge with their ends dipping in liquid mediapoured in culture tubes.

2.2 Seed Germination

Seeds of *Albizzia lebbeck* (Pratap Nursery and Seed stores, Dehradun) scarified at the chalazal end and surface sterilized by treating with Teepol for 5 min and 0.1% $HgCl_2$for 7–8 min and then washed thoroughly with autoclaved distilled water. Seeds are implanted on B_5 medium [18] gelled with different gelling agents containing 2% sucrose.

2.3 Caulogenesis and Axillary Shoot Proliferation

For *in vitro* caulogenesis,hypocotyl segments of *A.lebbeck*(1-cmlong), excised from *in vitro* raised 11-day-old seedlings, were cultured on the same medium as used for seed germination.

2.4 Rhizogenesis

For *in vitro* rhizogenesis, microshoots of *A. lebbeck* (2.5–3.0-cm long), developed from hypocotyl explants, were excised and implanted on the B_5 basal medium gelled with different gelling agents.

ISSN (O) 2454-9665
ISSN (P) 2454-0663

2.4 Somatic Embryogenesis

Calli from intermodal segments of *C. tweedii*, raised and maintained on MS medium [19]and supplemented with 0.1 μM 6-benzylaminopurine (BAP) and 2% sucrose, were transferred to the same medium containing 0.4 μM BAP and gelled with gelling agents.

For all experiments, cultured tubes were closed with cotton plugs (nonabsorbent cotton wrapped in a layer of cheese cloth). All cultures were maintained in continuous light of 17.76 mol m^{-2} $s^{-1}$for 16 h by cool day tubes (40 W) and incubated at 25 ± 2°C with 50–60% humidity. All experiments described here were repeated at least twice. To test whether observed differences were significant, the data were subjected to one-way analysis of variance (ANOVA, $p = 0.01$) and comparisons between mean values of treatments were made by Duncan's multiple range test.

III RESULTS AND DISCUSSION

Six gelling agents (gelrite, guar gum, gum katira, isubgol, sago starch, and xanthan gum) were compared for their effect on *in vitro* morphogenesis. Agar-gelled and liquid media with filter paper bridges served as controls.

Seeds of *A. lebbeck* started germinating after 1day of culture in all the above-mentioned treatments. The percentage of seed germination on all gelling agents except guar gum or xanthan gum was not significantly different (Table 1). The lower number of seed germination on guar-gum- or xanthan-gum-gelled media was because of low viscosity of media. On gum katira, all seeds sank and therefore could not germinate. It could be because of poor quality of gum katira. It would be worthwhile to mention here that throughout the course of this study, it was never possible to procure gum katira of consistent quality, as judged by its appearance. However, seeds that germinated irrespective of the gelling agent produced normal plants.

Irrespective of the treatment, the hypocotyl segments developed shoots after 30 days of culture. Numerically, the percentage of responding explants was the best on agar followed by guar-gum- and xanthan-gum-gelled medium (Table 2, Fig. 1). However, observed differences are not statistically significant. The caulogenic response in terms of number of shoots per responding explant was best on xanthan gum followed by agar- and guar-gum-gelled media. Observed differences in morphogenic responses on media gelled with different gelling agents could be due to physical effects, which are also known to influence the morphogenic response in agar gels [20]. The same is evident by chain of experiments conducted by the author in the Department of Botany, University of Delhi.

After transferring shoots on the B_5basal medium, roots were initiated from the lower cut end of microshoots after 1 month of transfer in all treatments. The percentage of shoots developing roots on different media wasstatistically identical, though numerically it was the best on guar gum and gelrite (Table 3). On gum katira medium, the percentage of rhizogenic shoots decreased significantly.

Ten plants from each treatment were transferred to paper cups and finally to pots in natural conditions. The number of plants surviving from the hardening process varied from four to seven in different treatments.

Calli of *C. tweedii* transferred to different gelling agents differentiated embryos with the percentage of embryogenic cultures not varying significantly among different treatments. However, the average number of

ISSN (O) 2454-9665
ISSN (P) 2454-0663

embryos per responding culture varied significantly among different treatments, with the best being on guar gum followed by xanthan gum (Table 4, Fig. 2). In other treatments, the average number of embryos per culture was half of that recorded for guar gum with the least on the liquid medium.

During the past 25 years, a number of polysaccharides of plant or microbial origin have been tested as alternatives to agar for microbial or plant tissue culture media [11, 21].Because of the one or other inherent problems with these alternative gelling agents, none of these is being used as routinely as agar [13]. The present study shows the comparison of newly identified gelling agents, guar gum and xanthan gum, with other earlier known gelling agents for plant tissue cultures techniques.

From the above, it becomes evident that the overall performance of guar gum and xanthan gum was not inferior to other known alternative gelling agents. Rather, for some of the morphogenic responses, guar gum and xanthan gum proved to be better than the other gelling agents. The foremost advantage of guar gum and xanthan gum is their cost effectiveness. Guar gum is ~10 and 80 times cheaper than agar (HiMedia, used in the present study) and Difco-Bacto agar, respectively,whereas xanthan gum, though not as cheap as guar gum, is five and 40 times cheaper than two brands of agar.

IV CONCLUSIONS

After establishing the suitability of guar gum and xanthan gum as gelling agents for microbial and plant tissue culture media, their effects on growth and differentiation were compared with few other known gelling agents. For different morphogenic responses, guar gum and xanthan gum proved to be better than other gelling agents.Apart from costeffective, both the gelling agents are biocompatible and biodegradable; therefore, pose no threat to the environment on being disposed after use.

Table 1: Seed Germination of *A. lebbeck* on B_5Basal Medium Gelled with Different Gelling Agents

Gelling agents	No. of seeds	Germination (%)	Shoot length (cm)
LM	48	79.1^{a}	$5.74^{c} \pm 2.43$
0.9% Ag	48	85.4^{a}	$8.07^{a} \pm 2.02$
0.3% GE	48	75.0^{a}	$7.50^{a} \pm 1.82$
3% GG	48	47.9^{b}	$5.35^{c} \pm 1.96$
3% Is	48	87.5^{a}	$5.93^{bc} \pm 1.65$
15% S	48	79.1^{a}	$7.68^{ab} \pm 2.81$
1% Xan	48	35.4^{b}	$4.77^{c} \pm 1.82$

Ag, agar; GE, gelrite; GG, guar gum; Is, isubgol;LM, liquid medium; S, sago; Xan, xanthan gum.

International Journal of Innovative Research in Science and Engineering
Vol. No.3, Issue 01, January 2017
www.ijirse.com
ISSN (O) 2454-9665
ISSN (P) 2454-0663

Table 2: Caulogenic Response of *A. lebbeck* on B_5Basal Medium Gelled with Different Gelling Agents

Gelling agents	No. of explants	Response (%)	No. of shoots per responding explants	Averageshoot length (cm)
LM	48	62.5[a]	3.24[bc]	0.94[a] ± 0.78
0.9% Ag	48	83.3[a]	4.07[a]	1.16[a] ± 0.87
0.3% GE	48	75.0[a]	3.07[b]	1.19[a] ± 0.71
3% GG	48	81.2[a]	3.99[a]	1.18[a] ± 0.88
3% GK	48	60.9[a]	2.11[d]	0.98[a] ± 0.51
3% Is	48	68.7[a]	3.46[b]	1.01[a] ± 0.77
15% S	48	60.5[a]	2.73[cd]	0.92[a] ± 0.70
1% Xan	48	79.1[a]	4.13[a]	1.07[a] ± 1.10

Ag, agar; GE, gelrite; GG, guar gum; GK, gum katira; Is, isubgol; LM, liquid medium;S, sago; Xan, xanthan gum.

Table 3: Rhizogenic Response of *A. lebbeck*Microshoots on B_5Medium Gelled with Different Gelling Agents

Gelling agents	No. of shoots	Response (%)	No. of roots per explants	Averageshoot length(cm)
LM	47	42.5[a]	2.05[ab]	2.45[a]±1.47
0.9% Ag	50	50.0[a]	2.72[a]	2.64[a]±2.02
0.3% GE	52	57.0[a]	2.20[a]	2.61[a]±1.94
3% GG	49	57.1[a]	2.50[a]	2.67[a]±1.61
3% GK	48	20.8[a]	1.66[b]	1.41[b]±0.98
3% Is	48	45.8[a]	2.54[a]	2.05[ab]±1.49
15% S	48	37.5[a]	2.25[a]	2.17[a]±1.07
1% Xan	50	52.0[a]	2.65[a]	2.70[a]±1.89

Ag, agar; GE, gelrite; GG, guar gum; GK, gum katira; Is, isubgol; LM, liquid medium; S, sago; Xan, xanthan gum.

ISSN (O) 2454-9665
ISSN (P) 2454-0663

Table 4: Somatic Embryogenesis in Callus Cultures of *C. tweedii*Maintained on Media Gelled with Different Gelling Agents

Gelling agents	No. of explants	Response (%)	No. of embryos per responding culture
LM	70	87.1[a]	5.39[d]
0.9% Ag	72	91.6[a]	7.83[c]
0.3% GE	71	88.7[a]	8.76[c]
3% GG	72	98.6[a]	19.80[a]
3% Is	70	88.5[a]	7.22[cd]
15% S	72	81.9[a]	6.79[cd]
1% Xan	72	95.8[a]	14.00[b]

Ag, agar; GE, gelrite; GG, guar gum; Is, isubgol; LM, liquid medium; S, sago; Xan, xanthan gum.

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Figure 1 A--H: Initiation of shoots from hypocotyl explants of *A. lebbeck* after 30days of culture on B_5 basal medium (A) or the same gelled with agar (B), gelrite (C), guar gum (D), gum katira (E), isubgol (F), sago starch (G), or xanthan gum (H)

International Journal of Innovative Research in Science and Engineering
Vol. No.3, Issue 01, January 2017
www.ijirse.com
ISSN (O) 2454-9665
ISSN (P) 2454-0663

Figure 2 A--F: Somatic embryogenesis of *Calliandra tweedii* in calli cultured for 60 days on liquid medium (A) or the same gelled with agar (B), gelrite (C), guar gum (D), isubgol (E), or sago starch (F).