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Evaluation of Blends of Alternative Gelling Agents with Agar and Development of Xanthagar, A Gelling Mix, Suitable for Plant Tissue Culture Media

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ABSTRACT

The present study was undertaken to evaluate various blends of three alternative gelling agents, viz., guar gum, isubgol or xanthan gum, with agar for their rheological properties and suitability for plant tissue culture as substitute of agar, an expensive component of culture media. In past, many attempts have been made to replace this with cheaper alternatives. Guar gum, isubgol and xanthan gum, three of the suggested alternatives, do not provide culture media capability of forming stable slants. Moreover, such media are difficult to dispense. To overcome these problems, 55 blends of agar and one of the alternative gelling agents, were compared for their capability of forming stable slants and ease of dispensing. Eighteen of these provided culture medium capability of forming stable slants. Among these, only xanthan gum+agar blends possessed ease of dispensing comparable to agar. As guar gum+xanthan gum, isubgol+guar gum and isubgol+xanthan gum blends spurted during autoclaving, only the blends of guar gum, xanthan gum or isubgol with agar, were compared for their rheological properties (viscosity and texture) and their capability of supporting morphogenic response. Although, blending of guar gum, xanthan gum or isubgol with agar increased viscosity and firmness, none had rheological properties equivalent to agar medium. However, *in vitro* seed germination, shoot differentiation and rooting of *Albizzia lebbeck* on media gelled with any one of these gelling mixes, except one having isubgol (2.6%)+agar (0.4%), were comparable to or better than the controls. Thus, because of their suitability comparable to agar and a distinct cost advantage, xanthagar [xanthan gum+agar (6:4)] gelling mix is recommended as a possible alternative to agar.

Key words: Alternative gelling agents, xanthan gum, viscosity, texture analysis, *in vitro* morphogenesis, *Albizzia*

INTRODUCTION

Besides an essential and baseline technology for plant biotechnology, plant tissue culture is widely used the world over for commercial mass propagation of many plants. In recent past, several plant tissue culture laboratories and commercial facilities have been set up, which are generating a large number of tissue culture-raised plants of commercial crops and forest trees (http://dbtmicropropagation.nic.in/about.htm). The expansion of this activity is mainly hampered due to the high cost of plants regenerated through tissue culture. Culture media ingredients, especially agar that is used for making the media semi-solid, adds to the cost of regenerated plants (Ezekiel, 2010). In the past, many attempts have been made to identify a suitable cheaper

alternative of expensive agar as a gelling agent for microbial and plant tissue culture media. Investigations carried out in our laboratory during the last one decade demonstrated the suitability of a few colloidal polysaccharides of microbial or plant origin as potential alternative gelling agents. These are isubgol (Jain *et al*., 1997; Babbar and Jain, 1998), gum katira (Jain and Babbar, 2002), guar gum (Babbar *et al*., 2005; Jain and Babbar, 2005; Jain *et al*., 2005) and xanthan gum (Babbar and Jain, 2005; Jain and Babbar, 2006). Prior to our report, use of isubgol for tissue cultures of Chrysanthemum was reported (Bhattacharya *et al*., 1994). Recently, it has been successfully used for the culture of organisms as varied as prokaryotic and eukaryotic microalgae and commercially important plants, such as, turmeric, tobacco, blueberry, woad plant and banana (Atici *et al*., 2008; Tyagi *et al*., 2007; Ozel *et al*., 2008; Fira *et al*., 2008; Saglam and Ciftci, 2010; Agrawal *et al*., 2010). However, despite all these having a distinct cost advantage over agar, none is likely to be used as routinely as agar because of some inherent drawbacks. Isubgol and guar gum remain highly viscous even at high temperature and therefore, pose problem in adjustment of pH and dispensing of the medium to culture vessels (Jain and Babbar, 2006). Because of their flowing nature even at room temperature, neither of these forms stable slants in culture tubes (Personal observation). Gum katira gelled media remain viscous liquid (Jain and Babbar, 2005) and therefore, cannot be used for overlaying of heavy explants. Xanthan gum media, though more viscous than gum katira gelled media, too remain in liquid state and are also incapable of forming stable slants (Personal observation). It was envisaged to overcome these shortcomings by developing gelling mixes having one gelling agent from among the alternative gelling agents and agar.

Based on the comparisons of rheological properties of 55 blends and their component gelling agents and the study of comparative plant morphogenic responses on selected sixteen of these, the present communication reports successful development of a gelling mix, Christianized as xanthagar. Xanthagar, a mix of xanthan gum and agar, possesses all desirable properties comparable to agar and offers a substantial cost benefit over agar.

MATERIALS AND METHODS

The media used in the present study were gelled with agar, isubgol, guar gum and xanthan gum either individually or in different combinations as described below:

- Guar gum (2.6-2.9%) + Agar (0.1-0.4%)
- Xanthan gum (0.6-0.9%) + Agar (0.1-0.4%)
- Isubgol (2.6-2.9%) + Agar (0.1-0.4%)
- Guar gum (1.5-2.9%) + Xanthan gum (0.1-1.5%)
- Isubgol (0.2-2.8%) + Guar gum (0.2-2.8%)
- Isubgol (1.6-2.9%) + Xanthan gum (0.1-1.4%)

The total concentration of the gelling agents in xanthan gum-agar blends was 1%, while in rest it was 3%. The increments for each gelling agent in all these blends were of 0.1%, except for isubgol-guar gum mixes where it was 0.2%.

Basal media used were MS (Murashige and Skoog, 1962) and B_5 (Gamborg *et al*., 1968). Before autoclaving at 1.06 kg cm^{-2} and 121°C for 15 min, pH was adjusted to 5.8 with 0.1 N NaOH or 0.1 N HCl. The media gelled with agar, isubgol, guar gum or xanthan gum were prepared as has been described earlier (Jain and Babbar, 2005, 2006). Twenty ml aliquots of media were dispensed

in individual culture tubes (25×150 mm), which were plugged with cotton plugs (non-absorbent cotton wrapped in a layer of cheesecloth).

Rheological properties: In a preliminary experiment, flow characteristics of MS basal medium containing 3% sucrose and gelled with various gelling agents or their blends were compared for their ease of dispensing and capability of forming stable slants. For the latter, culture tubes containing media after autoclaving were kept in inclined position (45°) for 12 h followed by 24 h in erect position.

A rotational viscometer (Brookfield, USA) with spindle S-34, rpm -0.3 for 5 min at 25°C, was used for measurement of viscosities. Texture analyses of the MS medium gelled with each of the gelling agent or gelling mix was carried out by back extrusion method using a Texture Analyser (TA.XT Plus, Stable Micro Systems, USA) with probe having a diameter of 35 mm and load cell of 5 kg. The other variable parameters were: pre-test and test speeds of 2 mm sec^{-1}, post-test speed of 10 mm sec^{-1}, distance as 5 mm and trigger force of 5 g. The measurements were made at 28±1°C with data acquisition speed of 200 pps.

Tests were carried out by filling 75% of the standard sized container (50 mm diameter) with the gelled medium. The probe was positioned centrally over the sample container. When the probe was returning to its original position, the container was held to prevent it from lifting along with the probe. For each gelled medium, viscosity measurement and texture analysis were done in triplicate and mean of three readings are presented for each parameter.

Tissue culture: For comparing the ability of different gelling agents, individually or in blended forms, *in vitro* morphogenic responses, viz., seed germination, caulogenesis and rhizogenesis of *Albizzia lebbeck* were studied on media gelled with one of the gelling agents or mixes as per the protocols described earlier (Jain and Babbar, 2006).

All experiments were repeated at least twice. The data were subjected to one-way Analysis of Variance (ANOVA, $p = 0.05$) using SPSS version 10 to test the significance of observed differences and comparisons between the mean values of the treatments were made by Post Hoc- Tukey HSD (Honestly Significant Different) test at $p = 0.05$.

The studies presented here were conducted during 2004-2005 at the Department of Botany, University of Delhi. However, texture analysis of the media was carried out at Birla Institute of Scientific Research, Jaipur during March 2004 and September 2005.

RESULTS AND DISCUSSION

During the last twenty five years, a number of polysaccharides of plant or microbial origin have been tested as alternatives to agar for microbial or plant tissue culture media (Babbar and Jain, 2005; Jain and Babbar, 2006). However, because of the one or other inherent problem, none of these alternative gelling agents is being used as routinely as agar (Jain and Babbar, 2005). One way to improve gelling of these alternative gelling agents, especially guar gum, is by using cross-linkers like borax or cations (Pezron *et al.*, 2003). Borax provides gelling characteristics to guar gum only at alkaline pH (7.0-8.3) (Whittier, 2002), which is generally not conductive for growth of plant tissues. Likewise, cations could have adverse effect on the growth and differentiation of cultured tissues. These problems could possibly be overcome by using a combination of two or more gelling agents. Therefore, for the present investigations, second possibility was explored.

Table 1: The gelling mixes providing the culture medium capability of forming stable slants and/or ease of dispensing after autoclaving

Gelling mix	Stable slants	Ease of dispensing
Guar gum and agar		
2.9% GG + 0.1% Ag	√	×
2.8% GG + 0.2% Ag	√	×
2.7% GG + 0.3% Ag	√	×
2.6% GG + 0.4% Ag	√	×
Xanthan gum and agar		
0.9% Xan + 0.1% Ag	×	√
0.8% Xan + 0.2% Ag	×	√
0.7% Xan + 0.3% Ag	√	√
0.6% Xan + 0.4% Ag	√	√
Isubgol and agar		
2.9% Is + 0.1% Ag	√	×
2.8% Is + 0.2% Ag	√	×
2.7% Is + 0.3% Ag	√	×
2.6% Is + 0.4% Ag	√	×
Guar gum and xanthan gum		
2.4% GG + 0.6% Xan	√	×
2.3% GG + 0.7% Xan	√	×
2.2% GG + 0.8% Xan	√	×
2.1% GG + 0. 9% Xan	√	×
2.0% GG + 1.0% Xan	√	×
Isubgol and guar gum		
2.8% Is + 0.2% GG	√	×
2.6% Is + 0.4% GG	√	×
Isubgol and xanthan gum		
2.9% Is + 0.1% Xan	√	×

Ag: Agar; GG: Guar gum; Xan: Xanthan gum

Of the 55 tested combinations, only eighteen formed stable slants. Of these, only with two having agar and xanthan gum, dispensing was as easy as with 0.9% agar (Table 1). However, in other combinations, it was not a disabling feature, at least for experiments in which media had to be poured in the culture vessels before autoclaving. Of the eighteen combinations, those involving guar gum+xanthan gum, guar gum+isubgol and xanthan gum+isubgol spurted on during autoclaving. Sometimes, spurting was so acute that medium came out of the vessel. Therefore, for further experimentation these gelling mixes were not used.

A gelling agent is added to increase its viscosity/firmness to a level where it can support overlaying of explants. Among individual gelling agents, agar had the highest viscosity. The viscosity of xanthan gum at 1% was twenty times less than that of 0.9% agar. Among the alternative gelling agents, isubgol (3%) had the maximum viscosity, which was however, half of the agar (0.9%). Addition of agar to these gelling agents increased the viscosity with the increase being directly proportional to the concentration of agar in the gelling mix. Gelling mix having 2.6% isubgol and 0.4% agar had the highest viscosity, which was almost 90% of the agar at 0.9% (Table 2). However, it seems such a viscosity is rather not required for the gelling agent to be effective. Thus, viscosity of the medium gelled with 0.9% xanthan gum + 0.1% agar, though minimum among the gelling mixes tested, was sufficient to prevent sinking of the explants. Rather, these lower viscosities might have afforded better diffusion in the medium, which could have been the cause of better morphogenic response in some of the gelling mixes. Diffusibility within the

Table 2: Viscosities and different parameters of texture analysis of gelling agents alone or in various combinations

Gelling agents	Viscosity (Pa.S)	Rupture strength (g)	Brittleness (mm)	Firmness/toughness (g s)	Adhesiveness (g s)
0.9% Ag	219.98	413.7	2.52	376.650	5.235
3% GG	6.78	60.1	2.48	102.505	95.610
2.9% GG + 0.1% Ag	105.52	107.2	2.54	145.510	72.100
2.8% GG + 0.2% Ag	136.38	116.7	2.56	201.760	62.310
2.7% GG + 0.3% Ag	163.02	183.8	2.50	253.560	49.300
2.6% GG + 0.4% Ag	182.92	261.5	2.45	345.360	46.110
1% Xan	13.58	23.3	2.45	39.164	18.190
0.9% Xan + 0.1% Ag	43.24	32.7	2.45	66.370	40.153
0.8% Xan + 0.2% Ag	67.56	34.1	2.44	96.596	33.970
0.7% Xan + 0.3% Ag	119.32	42.1	2.56	120.220	32.510
0.6% Xan + 0.4% Ag	140.36	60.1	2.47	174.340	29.510
3% Is	115.56	84.0	2.45	114.220	42.380
2.9% Is + 0.1% Ag	140.76	92.3	2.50	143.950	27.960
2.8% Is + 0.2% Ag	179.58	143.1	2.47	170.510	23.260
2.7% Is + 0.3% Ag	182.48	168.5	2.50	232.516	22.200
2.6% Is + 0.4% Ag	193.50	176.5	2.49	323.750	20.560

Ag: Agar; GG: Guar; Is: Isubgol; Xan: Xanthan gum; Pa.S: Pascal second

medium is an important attribute to avoid accumulation of toxic metabolites in the cultured cells or around them (if leached out) and for uptake of ions. Besides providing firmness to the medium, presence of gelling agent in the medium also affects diffusion within it (Romberger and Tabor, 1971; Faye *et al*., 1986). Diffusion depends on viscosity/gel strength of the medium (Ackers and Steere, 1961, 1962; George, 1993) which in turn depends on physical and chemical characteristics, and concentration of the gelling agent. Lucyszyn *et al*. (2005) ascribed better diffusion in agar/galactomannan blend than in agar due to the lower gel strength of the former.

Texture analysis is infinitely more descriptive of gel texture than simple gel strength measurements (Sanderson *et al*., 1989). This analysis reveals rupture strength, brittleness, toughness and adhesiveness of the media (Sanderson *et al*., 1989). The maximum force required to rupture the gel is the 'rupture strength'. The total 'work' required for the penetration of the probe for pre-adjusted distance and a constant speed is measure of toughness of the gel. The distance that the probe penetrates in the gel before this rupture (break) occurs is indicative of the gels elasticity ('brittleness'). The work required to be performed during the withdrawal of the probe is adhesiveness (Manufacturer Catalogue, Stable Microsystems, USA). In comparison, gel strength only tells about the amount of force required to rupture the gel (Chapman and Chapman, 1980). Thus, texture analysis provides better means for comparison of different gels than only gel strength or viscosity. Textural measurements on gels were made using the technique known as texture profile analysis, originally developed by Szczesniak *et al*. (1963).

Texture analysis of the MS medium gelled with 16 gelling agents/mixes revealed that agar (0.9%) and xanthan gum (1%) possessed maximum and minimum rupture strength, respectively. Although, addition of agar to the alternative gelling agents (guar gum, isubgol or xanthan gum) increased the rupture strength it was nowhere near to that of agar. The elasticity/brittleness of the gel is indicated by the distance that the probe penetrates in the gel before its rupture. The elasticity due to all other gelling agents was similar to agar (0.9 %). The firmness of the medium gelled with isubgol (3%), guar gum (3%) or xanthan gum (1%) was much less than that of agar (0.9%) medium. However, addition of agar to these increased the firmness with increase directly

proportional to the concentration of agar. Thus, the firmness of the medium with 2.6% guar gum + 0.4% agar was almost comparable to that of agar (0.9%) medium. The adhesiveness of the agar (0.9%) medium was the minimum. Among the three alternative gelling agents, guar gum (3%) provided maximum adhesiveness to the medium with minimum being due to xanthan gum (1%). Addition of agar along with these gelling agents decreased the adhesiveness of guar gum and isubgol media, with the decrease being inversely proportional to the concentration of agar. However, addition of 0.1% agar along with 0.9% xanthan gum almost doubled the adhesiveness of the medium, when compared with 1% xanthan gum medium. Further increase in the agar concentration decreased adhesiveness of the medium. However, adhesiveness of the medium with 0.6% xanthan gum + 0.4% agar was still higher than that of the medium with 1% xanthan gum alone (Table 2). Though, not for developing gelling agents for culture media, in past, blends of different polysaccharides have been used for augmenting the desired rheological properties desirable for various applications, such as, ceramic processing (Sikora *et al*., 2004), oil recovery (Mothé *et al*., 2006), development of nanofibers (Safi *et al*., 2007) and drug delivery (Patel and Patel, 2007; Yi and Zang, 2006).

Among the four parameters defining texture, only firmness of the medium appears to have direct relevance to the plant tissue culture as it indicates the ability of the medium to retain explant on the surface. Adhesiveness would be of limited importance, especially when plants developed on a medium have to be transferred. In case of highly adhesive medium, additional efforts would be required to wash away the culture medium.

The suitability of selected gelling mixes was tested for *in vitro* seed germination, shoot differentiation and rhizogenesis of *A. lebbeck*. For each, the corresponding media gelled with only agar, guar gum, isubgol or xanthan gum served as respective controls.

Seeds of *A. lebbeck* started germinating after a day of culture in all the gelling mixes and their corresponding controls. The percentages of seed germinating in all treatments were not significantly different except in case of guar gum (3%) and xanthan gum (1%). On these media, due to the submergence of some seeds in the culture medium, many failed to germinate. The percentage germination was best on gelling mix of 0.6% xanthan + 0.4% agar. The growth of seedlings as assessed by shoot length was best on 2.8% isubgol + 0.2% agar followed by 2.6% guar gum + 0.4% agar, whereas, least response was on 3% guar gum. Average root length was best on 2.6% guar gum + 0.4 % agar (Table 3).

Hypocotyl segments developed shoots after 30 days of culture in all the treatments. Numerically, the percentage of responding explants was the best on 0.6% xanthan+0.4% agar. Figure 1 A-D depicts representative cultures showing differentiation of shoots from the hypocotyls explants cultured on the medium gelled with agar, xanthan gum or gelling mixes of xanthan gum and agar. However, observed differences were not statistically significant, except on medium gelled with isubgol and agar with the minimum on the medium gelled with 2.6% isubgol+0.4% agar. Caulogenic response in terms of number of shoots per explant varied among different treatments with the best being on 0.7% xanthan+0.3% agar. However, significant decrease was observed on the medium gelled with isubgol+agar combination, i.e., 2.6% isubgol+0.4% agar. The growth of shoots in all the treatments was statistically identical (Table 4).

Shoots transferred to media gelled with all gelling mixes with their respective controls, started rooting after a month of subculture. The percentages of shoots developing roots on all the gelling mixes, except the isubgol+agar combinations, were not significantly different. On media gelled with isubgol+agar, percentage of rooting declined progressively with the total inhibition being in 2.6% isubgol+0.4 agar (Table 5).

Table 3: Seed germination of *A. lebbeck* on media gelled with agar (Ag), guar gum (GG), xanthan gum (Xan) or isubgol (Is) alone or in various combinations

Gelling agents	No. of explants	Germination (%)	Av. shoot length (cm)	Av. root length (cm)
0.9% Ag	96	82.2^{a}	7.77±3.45^{bc}	5.40±2.90^{bc}
3% GG	92	47.9^{b}	5.35±2.72^{c}	4.33±2.25^{c}
2.9% GG + 0.1% Ag	96	81.2^{a}	8.57±2.98^{ab}	5.98±1.97^{bc}
2.8% GG + 0.2% Ag	96	73.9^{a}	9.21±3.04^{ab}	6.77±2.46^{ab}
2.7% GG + 0.3% Ag	96	80.2^{a}	8.66±2.63^{ab}	5.83±2.50^{bc}
2.6% GG + 0.4% Ag	96	85.4^{a}	10.70±1.30^{a}	8.03±2.13^{a}
1% Xan	96	52.0^{b}	6.10±1.92^{c}	4.05±1.76^{c}
0.9% Xan + 0.1% Ag	96	71.8^{a}	8.21±2.88^{b}	6.57±2.55^{ab}
0.8% Xan + 0.2% Ag	96	86.4^{a}	8.08±2.42^{b}	5.52±2.31^{bc}
0.7% Xan + 0.3% Ag	96	76.0^{a}	8.27±2.12^{b}	5.61±2.44^{bc}
0.6% Xan + 0.4% Ag	96	89.5^{a}	9.09±2.97^{ab}	7.24±2.52^{ab}
3% Is	96	83.3^{a}	10.00±2.97^{ab}	6.61±2.68^{ab}
2.9% Is + 0.1% Ag	96	81.2^{a}	9.79±2.34^{ab}	5.51±2.53^{bc}
2.8% Is + 0.2% Ag	96	86.4^{a}	10.79±1.96^{a}	5.62±1.82^{bc}
2.7% Is + 0.3% Ag	96	87.5^{a}	10.66±2.20^{a}	6.39±1.79^{b}
2.6% Is + 0.4% Ag	95	82.1^{a}	10.56±1.85^{a}	6.71±1.59^{ab}

Ag: Agar; GG: Guar gum; Is: Isubgol; Xan: Xanthan gum. The values followed by same superscript(s) in a column are not significantly different (p = 0.05)

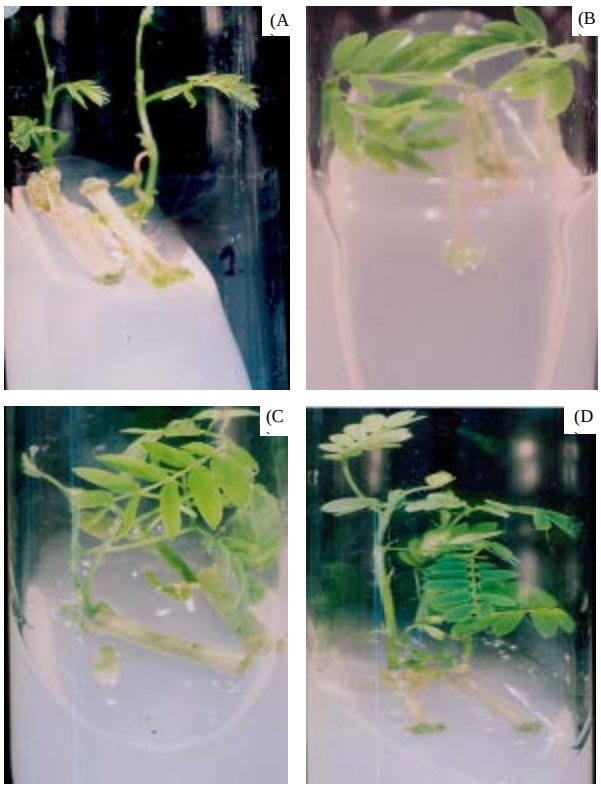

Fig. 1: (A-D) depicts representative cultures showing differentiation of shoots from the hypocotyls explants cultured on the medium gelled with agar, xanthan gum or gelling mixes of xanthan gum and agar

Table 4: Caulogenic response of *A. lebbeck* on media gelled with agar (Ag), guar gum (GG), xanthan gum (Xan) or isubgol (Is) alone or in combinations of these

Gelling agents	No. of explants	Response (%)	No. of shoots per responding explant	Av. shoot length (cm)
0.9% Ag	72	83.3^{ab}	4.00^{ab}	1.02±1.10^{a}
3% GG	68	77.9^{ab}	4.09^{ab}	1.05±0.78^{a}
2.9% GG + 0.1% Ag	71	90.1^{ab}	4.10^{a}	1.12±1.11^{a}
2.8% GG + 0.2% Ag	72	91.6^{a}	4.18^{a}	1.15±1.10^{a}
2.7% GG + 0.3% Ag	72	84.7^{ab}	4.01^{ab}	1.09±0.91^{a}
2.6% GG + 0.4% Ag	72	75.0^{ab}	2.60^{de}	0.98±0.77^{a}
1% Xan	72	80.5^{ab}	4.07^{ab}	1.06±1.00^{a}
0.9% Xan + 0.1% Ag	72	84.7^{ab}	3.95^{abc}	1.14±1.01^{a}
0.8% Xan+ 0.2% Ag	72	84.7^{ab}	4.17^{a}	1.21±1.09^{a}
0.7% Xan + 0.3% Ag	72	88.8^{ab}	4.29^{a}	1.21±1.23^{a}
0.6% Xan + 0.4% Ag	71	92.9^{a}	4.28^{a}	1.23±1.17^{a}
3% Is	72	70.8^{ab}	3.13^{bcd}	1.12±0.81^{a}
2.9% Is + 0.1% Ag	71	73.2^{ab}	3.09^{bcd}	1.13±0.68^{a}
2.8% Is + 0.2% Ag	70	68.5^{b}	3.04^{bcd}	0.92±0.73^{a}
2.7% Is + 0.3% Ag	72	65.2^{b}	2.29^{de}	1.00±0.58^{a}
2.6% Is + 0.4% Ag	70	44.2^{c}	1.93^{e}	0.94±0.69^{a}

Ag: Agar; GG: Guar gum; Is: Isubgol; Xan: Xanthan gum. The values followed by same superscript(s) in a column are not significantly different (p = 0.05)

Table 5: Rhizogenic response of *A. lebbeck* on the media gelled with agar (Ag), guar gum (GG), xanthan gum (Xan) or isubgol (Is) alone or in combinations of these

Gelling agents	No. of explants	Response (%)	No. of roots per responding explant	Av. length of root per explant (cm)
0.9% Ag	70	55.7^{a}	2.56^{a}	2.52±1.96^{ab}
3% GG	71	61.9^{a}	2.56^{a}	2.40±2.15^{abc}
2.9% GG + 0.1% Ag	69	59.4^{a}	2.20^{a}	2.42±2.14^{ab}
2.8% GG + 0.2% Ag	70	57.1^{a}	2.32^{a}	2.43±1.91^{ab}
2.7% GG + 0.3% Ag	71	53.5^{a}	1.97^{a}	2.09±1.63^{abc}
2.6% GG + 0.4% Ag	72	55.5^{a}	2.07^{a}	2.30±1.91^{abc}
1% Xan	72	56.9^{a}	2.68^{a}	2.52±1.85^{ab}
0.9% Xan + 0.1% Ag	70	65.7^{a}	2.72^{a}	3.10±1.78^{a}
0.8% Xan + 0.2% Ag	71	56.3^{a}	2.67^{a}	2.67±2.01^{ab}
0.7% Xan + 0.3% Ag	72	52.7^{a}	2.57^{a}	2.66±1.84^{ab}
0.6% Xan + 0.4% Ag	69	56.5^{a}	2.80^{a}	2.91±2.37^{a}
3% Is	72	47.2^{ab}	2.23^{a}	1.64±1.13^{bc}
2.9% Is + 0.1% Ag	68	47.0^{ab}	2.12^{a}	1.64±1.11^{bc}
2.8% Is + 0.2% Ag	71	29.5^{bc}	1.95^{a}	1.85±1.49^{bc}
2.7% Is + 0.3% Ag	72	13.8^{c}	2.60^{a}	1.26±0.87^{c}
2.6% Is + 0.4% Ag	70	0.00^{d}	0.00^{b}	0^{d}

Ag: Agar; GG: Guar gum; Is: Isubgol; Xan: Xanthan gum. The values followed by same superscript(s) in a column are not significantly different (p = 0.05)

From the above, it is obvious that the tested morphogenic response of plants was supported by all the gelling mixes except the rhizogenic response on the mix containing 2.6% isubgol and 0.4% agar. In some respects, the gelling mixes performed better than their individual alternative gelling agent components. The percentage of seed germination improved significantly when the agar was mixed with xanthan gum or guar gum, thus improving over the performance of xanthan gum or guar gum when used alone. The other common feature was the reduction in caulogenic response

with increase of agar to 0.4% in gelling mixes with isubgol and guar gum. This again could be only because of the physical attributes, such as reduced diffusion because of increased viscosity (Romberger and Tabor, 1971; Faye *et al*., 1986) or decreased availability of nutrients because of reduced water influx (George, 1993). Improvement of the performance of individual gelling agent by using blends has already been attempted (Lucyszyn *et al*., 2005, 2006, 2007; Tiwari and Rahimbaev, 1992; Zimmerman *et al*., 1995). Tiwari and Rahimbaev (1992), investigated the effect of barley starch, agar and agarose used individually or in combination in anther cultures of barley. They observed that a combination of agarose and barley starch provided a better gelling effect as it provided a firm surface throughout and prevented the sinking of explants even after enzymatic degradation of starch. Zimmerman *et al*. (1995) used a blend of starch and gelrite to propagate five apple and two pear cultivars. Lima-Nishimura *et al*. (2003) demonstrated the use of xyloglucan as a partial substitute for agar in tissue culture media for micropropagation of the Marubakaido apple rootstock (*Malus prunifolia*) and the apple cultivar Jonagored (*Malus domestica*). Lucyszyn *et al*. (2005, 2006, 2007) tried blends of agar and guar gum for Marubakaido apple rootstock proliferation. Gonçalves and Romano (2005) described successful use of locust bean gum in combination with agar for shoot multiplication and rooting of carob tree and Italian rose shoots. The blend of xanthan gum and agar described presently has been successfully used for microbial culture media (Babbar and Jain, 2005) and demonstration of its suitability for plant tissue cultures extends its application.

From the above it can be concluded that in physical attributes, viscosity, rupture strength and firmness, all gelling mixes tested were not comparable to agar. However, in their capability of supporting growth and differentiation of plants, they were either equal to or better than the component alternative gelling agents, viz., guar gum, isubgol and xanthan gum. The inference which could be drawn from these observations is that physical attributes such as viscosity, firmness, etc., comparable to agar (0.9%) may not be necessary for supporting the growth and differentiation of plant tissues. This is quite evident from the fact that gelling mixes having much lower viscosity and firmness provided matrices which supported morphogenic responses comparable to or better than agar medium. Among twelve gelling mixes, xanthagar having xanthan gum and agar (6:4) appeared to be the best. It had all the desirable characteristics such as, requisite firmness affording overlaying of explants, capability of forming stable slants and liquid state at high temperatures, facilitating adjustment of pH and easy dispensing. The clarity of xanthagar media, an important attribute for discerning contamination, was comparable to that of agar media. Even in its capability of supporting growth and differentiation of plant tissues, its overall performance was better than their respective controls. Moreover, xanthagar, despite having a considerable proportion of agar retains a distinct cost advantage over agar when used alone. The prices of Qualigen agar (used in the present study) and Difco-bacto agar are about 1.7 and 7.4 times more than that of xanthan gum (HiMedia). Xanthan gum, the major component of xanthagar, is produced commercially under controlled conditions using renewable sources. Thus, its increased demand would put relatively less pressure on agar sources. Because of lower degree of syneresis (Chapman and Chapman, 1980), the media gelled with xanthagar crack and dry slowly as compared to agar media. This attribute makes xanthagar a better choice as a gelling agent for media used for long term maintenance of cultures. Finally, because of the characteristics comparable to or better than agar, xanthagar appears to have potential to become an ideal replacement of agar and much better option than xanthan gum used alone. However, this needs to be tested for the culture of tissues of a range of plants, including crop plants. Such studies would decide whether this gelling mix can become an ideal replacement of agar.

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