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# Tragacanth, a Novel and Cheap Gelling Agent in Carnation and Miniature Rose Tissue Culture Media

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Abstract

The natural tragacanth gum, the sap of *Astragalus gummifer*, has been successfully used as a gelling agent in tissue culture media for in vitro shoot development and proliferation of miniature rose and carnation. Shoot development of nodal segments excised from greenhouse grown plants, was investigated on MS medium solidified with either 2.5, 3.0, 3.5 and 4.0% tragacanth or 0.8% agar as control. The media solidified with tragacanth were more transparent than the media solidified with agar. Viscosity of the tragacanth-gelled media was less than the agar-gelled media, and therefore, putting the explants on the media sometimes resulted in submersion of them into the media and some cases of development of hyperhydric shoots were observed. To increase the firmness of the media, the combinations of agar (0.2 and 0.3%) and tragacanth (2 and 3%) were used. The viscosity of the combinatory media was as good as the medium containing only 0.8% agar (control). Moreover, the explants productivity and growth obtained in these combinations were better than those recorded on the control medium. Being a plant product, biodegradable and environmental friendly, and 10 times cheaper than agar, tragacanth gum would definitely be useful for reducing costs, particularly in the plant tissue culture industry.

**Keywords:** Agar substitute, *Dianthus*, Natural gum, Nodal segment, Rosa.

## INTRODUCTION

Plant tissue culture is a technology which has been developed for more than a half century. It brought about enormous changes in plant technology. Nevertheless, the technology still has some disadvantages such as the expensive cost of equipment and chemicals which restrict its development for worldwide uses (Gebre and Sathyanarayana, 2001; Gonçalves and Romano, 2005). Agar contributes to 70% of tissue culture medium costs (Gebre and Sathyanarayana, 2001). More than 100 years ago, Robert Koch, the famous microbiologist, introduced agar as a gelling agent for media used in raising pure cultures of microbes. Agar was used for the first time in 1930's in plant tissue culture by White. Since the time, agar has remained the most frequently used gelling agent for plant tissue culture media (Gonçalves and Romano, 2005). The properties of agar gel, which make it a choice for plant tissue culture media, are its stability, high clarity and resistance to metabolism during culture (Henderson and Kinnersley, 1988). However, some doubts have been raised about its biological inertness and nontoxic nature (Debergh, 1983; Arnold and Ericksson, 1984; Singha, 1984). In addition, the risks of overexploitation of its natural sources and above all the high cost of agar have necessitated intensive efforts to look for alternative gelling agents (Babbar and Jain, 1998; Jain and Babbar, 2002).

During the last three decades, there have been increased efforts to explore suitable substitutes for agar namely, carrageenan (Lines, 1977), alginates (Scheurich *et al.*, 1980), ficol (Kao, 1981), gelrite (Pasqualetto et al., 1988), starch (Henderson and Kinnersley, 1988; Nene *et al.,* 1996), isubgol (Babbar and Jain, 1998), and katira gum (Jain and Babbar, 2002). Consequently, a number of substances have been used with reasonable success as a substitute for agar. These agents are not expected to reach universal acceptance due to various reasons. Starch, the cheapest of the gelling agents used, is not expected to find universal acceptance because of its inferior gelling ability and poor clarity than agar; and it metabolizes too readily (Kuria *et al.*, 2008). Carrageenan and alginates gel only in the presence of specific ions, and agarose and ficoll are cost prohibitive (Jain and Babbar, 2002). Gelrite, another gelling agent, though not a perfect replacement for agar, has found wide acceptance for plant tissue culture media (Pasqualetto *et al*., 1988). Isubgol, a highly cost-effective gelling agent, has all the desirable properties, however, its higher melting point (~70 °C) necessitates adjustments of pH and quick dispensing (Babbar and Jain, 1998; Jain and Babbar, 2002; Jain and Babbar, 2005). Guar gum is not expected to be used for routine purposes for the same reasons (Jain and Babbar, 2005).

Tragacanth gum, a Persian gum, is the sap of several species of Middle Eastern legumes of the genus *Astragalus*, including *A. adscendens, A. gummifer*, and *A. tragacanthus*. More than 20 taxa of *Astragalus* have been reported to yield gum (Gentry *et al.,* 1990) and probably all belong to the Tragacantha group. After incision of stem, the gum seeps from the plant in twisted ribbons or flakes which can be powdered. When added to water, tragacanth absorbs water and becomes a gel. The gel is viscous, odorless and tasteless which consists of water-soluble mixture of polysaccharides, mainly poly-D-galacturonicacid and bassorin (Mohamadnia *et al.*, 2008). One part of the gum is miscible and the other part forms a gel of exceptional quality. It is the most viscous water soluble natural gums and it is an excellent emulsifying agent with good stability to heat, acidity, and age (Gentry *et al.*, 1990).

The objective of this study was to estimate the physical and biological effects of using tragacanth as a gelling agent in plant tissue culture media. We tested the gum with MS medium for shoot proliferation of miniature rose, as a woody model plant, and carnation, as a herbaceous model plant.

## MATERIALS AND METHODS

Single node explants, excised from greenhouse grown plants of miniature rose (*Rosa chinensis* var. minima 'Little Buckaroo') and carnation (*Dianthus caryophyllus* 'Panorama') were surface-sterilized using 70% ethanol for 4 min, and 10% (v/v) clorox (containing 5.25% of sodium

hypochlorite) for 15 min, and then washed thoroughly with sterilized distilled water (Salehi and Khosh-Khui, 1997; Salehi, 2006). Basal MS medium supplemented with 3% sucrose was used for culture of the explants. The medium was supplemented with 1 mg $l^{-1}$ benzyl adenine (BA) and 0.1 mg $l^{-1}$ naphtaleneacetic acid (NAA), and 2 mg $l^{-1}$ BA and 0.1 mg $l^{-1}$ NAA for carnation and miniature rose, respectively. The pH of media was adjusted to 5.7 before addition of the gelling agents. The media were solidified with agar (control), various concentration of tragacanth (T1-4: 2.5, 3.0, 3.5, and 4%), and various mixtures of them (A1T1-A2T2: 0.2 + 2.0, 0.2 + 3.0, 0.3 + 2.0, and 0.3% Agar + 3.0% tragacanth). Fifteen ml aliquots of the media were dispensed in individual vessels and autoclaved at 103.4 kPa, 121°C for 15 min. The cultures were incubated at 25 ± 2°C under 16-h photoperiod of 17.76 µmol $m^{-2}$ $s^{-1}$ provided by cool white fluorescent tubes (40 W, PARS, Iran).

Individual cultures were scored for the number of shoots and leafs, fresh weight, shoot length, and the presence of hyperhydricity after 30 days in culture. The experimental design was a Randomized Complete Design (RCD), repeated twice, each time with 10 replications per treatment, and 2 explants per vessel. Data were analyzed by SPSS (v. 16.0) and means were separated with Duncan's multiple range test (DMRT) at $P< 0.05$.

## RESULTS

Shoot initiation took place less than 2 weeks after incubation. Growth of the explants on media containing tragacanth was started 4 to 6 days earlier than on agar media. Although performance of carnation on the tragacanth-gelled media was better than the agar-gelled media, the percentage of developed miniature rose explants on the tragacanth-gelled media was lower than the control (Fig. 1). The lowest frequency of developed carnation explants (25%) was found on T1 treatment which was similar to the control. On the other hand, frequency of developed miniature rose explants on T1-T4 (10-30%) was significantly lower than agar treatment (50%). The highest percentage of developed explants of the both species (95%) was found on the combinatory tragacanth-agar gelled media (A1T1-A2T2 treatments).

Table 1 represents the growth parameters of carnation at the end of the experiment. The nodal segments cultured on media gelled with either agar, gum tragacanth, and on their mixtures, developed multiple shoots. The highest shoot number of carnation was observed in A1T1 treatment (4.7 shoots) and the lowest was found in T1 treatment (1.3 shoots). Shoot length of carnation obtained on tragacanth-agar combinatory media was higher than on agar or tragacanth-solidified media. On the combinatory media (A1T1-A2T2 treatments), number of leaves was significantly higher than the other treatments. Leaf number obtained on tragacanth media (T1-T4) ranged between 3.1 to 5.9 leaf, which was statistically similar to the one obtained on the control medium (3.9 leaf). Fresh weight of carnation plantlets was significantly higher on the combinatory media

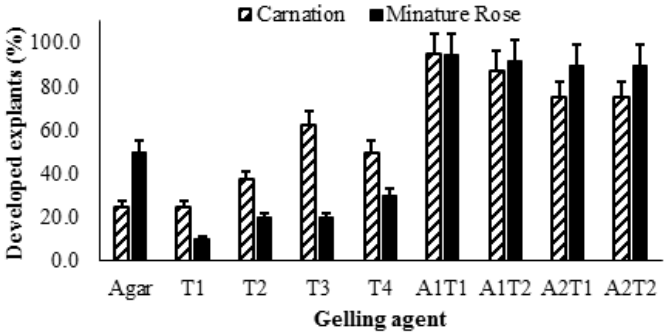


Fig. 1. Percentage of developed explants of carnation and miniature rose on media solidified by tragacanth (T1-T4: 2.5, 3.0, 3.5, and 4%), agar (0.8%) and their combination (A1T1-A2T2: 0.2 + 2.0, 0.2 + 3.0, 0.3 + 2.0, and 0.3% Agar + 3.0% tragacanth).

Table 1. Growth and frequency of hyperhydricity syndrome of carnation explants on media solidified by tragacanth, agar and their combination.

| Gelling agent | Shoot No. | Shoot length (mm) | Leaf No. | FW (mg) | Hyperhydricity (%) |
|---|---|---|---|---|---|
| Agar | 1.6$^{bc\dagger}$ | 6.1$^{b}$ | 3.9$^{b}$ | 110.0$^{c}$ | 0.0$^{b}$ |
| T1 | 1.3$^{c}$ | 4.9$^{b}$ | 3.1$^{b}$ | 81.4$^{c}$ | 6.2$^{a}$ |
| T2 | 1.7$^{bc}$ | 5.2$^{b}$ | 5.9$^{b}$ | 100.0$^{c}$ | 7.1$^{a}$ |
| T3 | 1.4$^{bc}$ | 6.7$^{b}$ | 4.3$^{b}$ | 82.3$^{c}$ | 5.7$^{a}$ |
| T4 | 1.4$^{bc}$ | 6.1$^{b}$ | 3.5$^{b}$ | 77.1$^{c}$ | 5.0$^{a}$ |
| A1T1 | 4.7$^{a}$ | 19.0$^{a}$ | 21.9$^{a}$ | 274.3$^{ab}$ | 0.0$^{b}$ |
| A1T2 | 3.0$^{ab}$ | 27.0$^{a}$ | 27.7$^{a}$ | 324.3$^{a}$ | 0.0$^{b}$ |
| A2T1 | 2.4$^{abc}$ | 19.4$^{a}$ | 16.6$^{ab}$ | 321.3$^{a}$ | 0.0$^{b}$ |
| A2T2 | 3.1$^{ab}$ | 21.1$^{a}$ | 20.8$^{a}$ | 251.4$^{b}$ | 0.0$^{b}$ |
| EMS | 1.54$^{*}$ | 2.19$^{**}$ | 1.88$^{**}$ | 1.05$^{*}$ | 1.75$^{*}$ |
| Error | 0.85 | 0.67 | 0.76 | 0.83 | 0.91 |

† Means followed by the same letter are not significantly different according to DMRT (P<0.05).
*, **: Significant at P<0.05 and P<0.01, respectively.

and the highest fresh weight was found in A1T2 and A2T1 media (324.3 and 321.3 mg, respectively). No significant difference was found between fresh weight of plantlets in tragacanth-gelled media and the control treatment. Hyperhydric carnation explants were only observed on T1-T4 media (5.0-7.1%) and no significant difference was found between these treatments (Table 1).

The effects of using different gelling agents on growth parameters and frequency of hyperhydric explants of miniature rose are shown in Table 2. Number of developed shoots of miniature rose was significantly higher in A1T1 and A1T2 treatments (3.2 and 3.6 shoot, respectively) and the lowest values were found in T1-T4 and agar treatments (1.4 shoot). The lowest shoot length was obtained on T1-T2 media (6.1 and 8.5 mm, respectively). Shoot length of miniature rose obtained on the combinatory media (A1T1-A2T2) was significantly higher than the other treatments. The highest numbers of developed leaves were observed on A1T2 medium (14.2 leaf). Leaf numbers of the explants on T1-T4 media (1.4-3.1 leaf) were similar to the one obtained on agar medium (3.4 leaf). Although fresh weight of the explants of T1-T4 treatments was similar to the agar treatment (65.2 mg), it was significantly increased on the combinatory media and the highest fresh weights were observed on A1T2 (187.5 mg) and A2T1 (170.7 mg) media. The highest frequency of hyperhydricity (12.7%) was observed on T1 media and no hyperhydric explant was observed on agar or on the combinatory media.

Table 2. Growth and frequency of hyperhydricity syndrome of miniature rose explants on media solidified by tragacanth, agar and their combination.

| Gelling agent | Shoot No. | Shoot length (mm) | Leaf No. | FW (mg) | Hyperhydricity (%) |
|---|---|---|---|---|---|
| Agar | 1.4$^{b\dagger}$ | 15.0$^{bc}$ | 3.4$^{c}$ | 65.2$^{c}$ | 0.0$^{c}$ |
| T1 | 1.2$^{b}$ | 6.1$^{c}$ | 3.1$^{c}$ | 57.1$^{c}$ | 12.7$^{a}$ |
| T2 | 1.4$^{b}$ | 8.5$^{c}$ | 1.6$^{c}$ | 35.9$^{c}$ | 9.8$^{ab}$ |
| T3 | 1.3$^{b}$ | 16.0$^{bc}$ | 3.1$^{c}$ | 45.1$^{c}$ | 6.0$^{b}$ |
| T4 | 1.2$^{b}$ | 28.6$^{b}$ | 1.4$^{c}$ | 47.4$^{c}$ | 6.4$^{b}$ |
| A1T1 | 3.2$^{a}$ | 93.4$^{a}$ | 10.5$^{ab}$ | 152.1$^{b}$ | 0.0$^{c}$ |
| A1T2 | 3.6$^{a}$ | 98.7$^{a}$ | 14.2$^{a}$ | 187.5$^{a}$ | 0.0$^{c}$ |
| A2T1 | 2.8$^{ab}$ | 81.4$^{a}$ | 6.8$^{bc}$ | 170.7$^{a}$ | 0.0$^{c}$ |
| A2T2 | 2.4$^{ab}$ | 79.4$^{a}$ | 9.5$^{ab}$ | 155.5$^{ab}$ | 0.0$^{c}$ |
| EMS | 1.60$^{*}$ | 3.22$^{**}$ | 1.75$^{*}$ | 4.06$^{**}$ | 3.95$^{**}$ |
| Error | 0.89 | 0.59 | 0.86 | 0.43 | 0.45 |

† Means followed by the same letter are not significantly different according to DMRT (P<0.05).
*, **: Significant at P<0.05 and P<0.01, respectively.

## DISCUSSION

There are many publications reporting on agar substitutes and supporting materials in plant tissue culture medium (Jain and Babbar, 2005; Kuria *et al*., 2008). In this study, tragacanth gum was evaluated as an agar substitute for in vitro plant tissue culture. Media gelled with either tragacanth gum or mixture of the gum and agar, were somewhat more transparent than agar gelled media (Fig. 2). Clarity of medium is essential for detection of contaminations or monitoring growth responses of explant. Lack of clarity may result in non-acceptance or non-use of a gelling agent for application in plant tissue culture media (Kuria *et al.*, 2008).

The media that gelled with only tragacanth, were not as firm as agar and therefore, putting the explants in such media often resulted in submersion of them. The higher mortality of the plantlets in tragacanth media was attributable to submergence of the explants in the media. Moreover, submergence in most cases resulted in development of shoots with hyperhydricity syndrome (Fig. 2B). However, the explants that were not submerged in the media containing tragacanth alone responded better than agar (Fig. 2C). As reported by Bakir *et al.* (2016), the hyperhydric plantlets appeared glassy and tend to exhibit a series of profound morphological alterations including the formation of a bushy habit and thickened and malformed stems and leaves. Morphological and physiological anomalies due to hyperhydricity are not acceptable and significantly reduce *in vitro* propagation efficiency. Our results were in agreement with Kevers, *et al.* (2004); Van de Dries *et al*. (2013) which stated hyperhydricity is a result of waterlogging stress. The frequency of hyperhydric plantlets can be reduced by solidifying media with high concentration of gelling agent or a gelling agent with a higher gel strength (Kuria *et al.*, 2008), increasing the calcium and use of fructose or galactose as a carbon source (Debergh *et al.*, 1992). Therefore, significant reduction in hyperhydricity by elevating tragacanth concentration in medium or by using tragacanth in combination with agar was due to enhancement of the media firmness and limiting excess water in the media.

Carnation explants were less sensitive to submergence than miniature rose explants, as

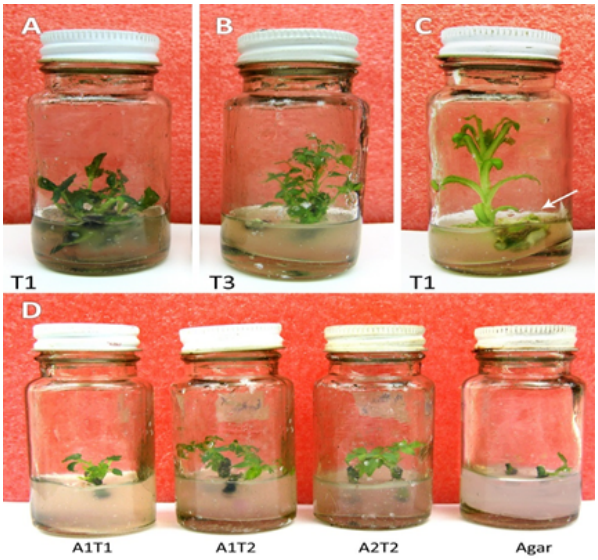


Fig. 2. The media gelled with tragacanth or its combination with agar were generally clearer than agar medium. A) Development of hyperhydric shoot after explant submergence in media, B) Vigorous growth of miniature rose on T3 medium solidified by 3.5% tragacanth, C) Development of a normal shoot by a submersed carnation explant into tragacanth-gelled medium, D) Growth of the explants on the combinatory media was started faster than agar-gelled medium (2nd week after incubation).


Fig. 3. Vigorous growth of miniature rose and carnation explants obtained on media that solidified by combinations of agar and tragacanth gum.

many of the submerged explants successfully developed new shoots and less hyperhydricity frequency was observed in comparison with miniature rose (Fig. 2D). The best growth and proliferation were obtained from combinatory media, which were firm and provided a suitable support for holding the explants. On these media, explants growth of the explants were started earlier and no sign of hyperhydricity was observed (Fig. 2D).

Vigorous growth and enhanced proliferation frequency were observed on the combinatory media (Fig. 3). The effects of tragacanth on enhancing growth and development of the explants may be due to its nutritional, growth stimulator substances, higher water availability and high carbohydrate contents. On the other hand, some studies have showed that agar contains some growth inhibitors which may adversely affect growth of plantlets (Debergh, 1983; Arnold and Ericksson, 1984; Singha, 1984). Moreover, enhanced growth and multiplication of the explants on tragacanth and the combinatory media could be a consequence of higher availability of water and inorganic nutrients due to a lower resistance to diffusion in the medium (Pierik, 1997). Since nutrient uptake is closely associated with the rate of water influx into tissues (George, 1993), reducing matrix potential of medium by reducing agar concentration in the medium may enhance diffusion of nutrients to explant and preventing nutrient stress and growth inhibition (Debergh and Zimmerman, 1991). Hence, lower viscosity of tragacanth and the less concentration agar in the combinatory media may explain why plantlets performed better in these media as compared to plantlets on agar gelled medium. These observations are in accordance with Mbanaso *et a*l. (2001) and Kuria *et al*. (2008).

The data presented here suggest that using tragacanth gum in tissue culture media can improve the performance of explants in comparison with agar. However, as a gelling agent, tragacanth alone may not provide sufficient support for holding the explants. Moreover, as tragacanth hydrates rapidly at room temperature and becomes a viscous gel, dispensing media gelled with tragacanth in vessels is troublesome. Using 3.0% tragacanth in combination with 0.2% agar may be suggested to solve these problems. Being a plant product, biodegradable, environmental friendly and 10 times cheaper than agar, tragacanth gum would definitely be useful for reducing costs, particularly in the commercial plant tissue culture.

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