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The usefulness of apricot gum as an organic additive in grapevine tissue culture media

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Key words: apricot gum, callus culture, carrot, grapevine, rooting, stevia, tissue culture.

Abstract: The growth and morphogenesis of cultured plant tissues can be improved by small amounts of some organic elements. In addition to being a natural source of carbon, organic additives may contain natural vitamins, phenols, fiber, hormones and also proteins. Hence, the physiological effects of apricot gum on the regeneration capacity and growth rate of three different plant species i.e. carrot (as a model plant), stevia (as an herbaceous plant), and grapevine (as a woody plant) were examined. The proliferated callus cultures of carrot and *in vitro*-derived microcuttings of stevia and grapevine were inoculated on their respective standardized proliferation media supplemented with 2.0-6.0 g/l apricot gum. The growth parameters of treated samples were measured and compared to gum-free medium. Earlier callus initiations with greater fresh weight, volume, as well as improved pigmentation were recorded in media fortified with apricot gum. The usefulness of gum application was also obvious in both stevia and grapevine with respect to better shoot multiplication and rooting parameters. Due to positive effects of apricot gum, longer vines with a higher number of lateral shoots, internodes and leaf area were achieved. Overall, the gum at the rate of 4.0 g/L was found to be a logical concentration with respect to encouraging response in all three species. Owing to promising results evolved in the present research, the application of gum in commercial tissue culture protocols is highly recommended. However, further studies are needed to exploit plant derived gums as an alternative carbon source in plant tissue culture media.

1. Introduction

The degree of success in any technology employing plant cell, tissue or organ culture is related to quite a few major factors. A significant factor is the choice of nutritional components and growth regulators (Gamborg, 1991). A plant tissue culture medium is composed of necessary and optional components required for plant growth, which vary according to the plant species, cultivar, or explant type that is used and must be experimentally defined for each particular case. Moreover, all the nutrients in a medium should be present in optimum concentrations to ensure the best possible growth of explants (George, 1993). Under *in vitro* conditions, an intact plant requires macronutrients, micronutrients, plant growth regulators, vitamins, amino acids and other nitrogen supplements and sugars (Gamborg, 1991). Another important component in plant tissue culture media is the carbon source because it supplies energy to the plants, especially when they are not ready to photosynthesize their own food during the early stage of tissue culture (Al-Khateeb, 2008 b). Carbon source can be in the form of simple or complex sugars (Akter *et al.,* 2007). Normally, sucrose is used as the carbon source in plant tissue culture. A range of other organic additives have been used in plant tissue culture to promote the growth of the plants, including coconut milk, banana pulp, potato homogenate and juice, honey, date palm syrup, corn extract, papaya extract, guar gum, and isubgol (Islam *et al.*, 2003; Jain and Babbar, 2005; George *et al.*, 2008; Murdad *et al.,* 2010; Nambiar *et al.,* 2012). Such additives are commonly known as organics with undefined compositions (Torres, 1989). The advantages of adding such organic materials to medium have already been reported by some researchers, for example, the organic additives help to produce more PLBs, shoots and leaves in orchid (Akter *et al.,* 2007), increase the size of date palm somatic embryos (Al-Khateeb, 2008 a), and also promote growth and development of asymbiotic seeds and regeneration

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of *Cymbidium* plantlets (Tawaro *et al.,* 2008). The reasons for application of organic additives into culture medium, in addition to being a natural source of carbon, are because they contain natural vitamins, phenols, fiber, hormones, and also proteins (Gnasekaran *et al.,* 2010). It was mentioned in a report by Al-Khateeb (2008 a) that organic additives contained not only sugar but also other nutrients such as proteins, lipids and minerals.

In recent years, plant-derived polymers have evoked remarkable attention in various industries due to their diverse applications as food emulsifiers, stabilizers and thickeners, pharmaceuticals, cosmetics, textiles, and in art. They are also used as gelling agents in gels and bases in suppository (Nussinovitch, 1996). These polymers are biocompatible, biodegradable and are preferred to semi synthetic and synthetic excipients because of their lack of toxicity, low cost, soothing action, and non irritant nature (Deogade *et al.*, 2012). Environmental-friendly processing and local availability, especially in developing countries, are considered additional advantages for application of these natural products (Jain and Babbar, 2005). The Rosaceae family, *Prunus* genus, consists of peach, plum, apricot, cherry, and almond trees, all of which can produce exudate gums. Herbal exudate gums normally secrete from bark, branch and fruit of trees due to their protection impact against mechanical damage or microbial attacks. Gum secretion may also occur due to adaptation to climate of some trees, called physiological gummosis (Simas *et al.*, 2008; Simas-Tosin *et al.*, 2009). A large number of complex natural additives can be very effective in providing an undefined mixture of organic nutrients and growth factors. In the context of carbohydrates, gums are usually considered to be non-starch, water-soluble polysaccharides with commercial importance. Gums are typically more or less sticky in nature and are translucent and amorphous substances which are degradation products of the cell wall of woody species which exude from trees. Natural gums (gums obtained from plants) are hydrophilic carbohydrate polymers having high molecular weights, generally composed of monosaccharide units joined by glucosidic bonds (Khorsha, 2014). While thorough reports on gum structure (Saniewski *et al.*, 2001; Lluveras-Tenorio *et al.*, 2012), its rheological properties (Wang *et al.*, 2008), and its applications in different industries (Verbeken *et al.*, 2003) were previously reported, the influence of such natural products as organic addenda in plant tissue culture media has yet to be studied. Therefore, the present investigation was conducted to evaluate the effectiveness of apricot gum as an organic additive on growth and *in vitro* regeneration of three different plant species: carrot (as a model plant), stevia (as herbaceous species) and grapevine (as woody plant). The results of the current study should be applicable to other plant species following minor modifications.

2. Materials and Methods

The present research work was conducted in the plant tissue culture laboratory of the Horticulture department, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. The physiological effects of apricot gum on regeneration capacity and growth rate of three different plant species i.e. carrot (as model plant), stevia (as herbaceous plant) and grapevine (as woody plant) were examined. The apricot gum was obtained dried on bark and shoots of apricot trees immediately after secretion, collected from a commercial apricot orchard (Gonabad, Khorasan, Eastern Iran) through scraping it from bark surfaces. The bark residues were removed from the collected mass and the clean, pure gum was powdered prior to addition to the culture medium.

Plant materials and in vitro *culture establishement*

Carrot. Healthy, undamaged roots of carrot (*Daucus carrota* cv. Nantes), 3-4 cm diameters long, were selected and washed thoroughly with normal tap water (30 min) and surface sterilized with ethanol (70% v/v for 40 s) followed by sodium hypochlorite solution (35% v/v plus two drops of tween-20 for 15 min). The clean, sterilized roots were cut transversely, employing a scalpel, to prepare root explants as slices (10×10 mm and 1mm thickness). Each explant was prepared in such a way to consist of xylem, secondary phloem and a small part of cambium tissues (Hall, 1991).

Stevia. Stevia (*Stevia rebaudiana*) mother plants were procured from Golsaran-e-Shomal Corporation, as tissue cultured pot plants and were transferred to our laboratory. Single node explants were disinfected with $HgCl_2$ (0.1% for 6 min) and washed three times with sterilized distilled water inside a laminar hood cabinet. The single node explants were cultured and *in vitro* culture was established following an already standardized protocol (Taherian, 2012).

Grapevine. Single node explants of grapevine (*Vitis vinifera* cv. Laal) were utilized following the pre-

viously standardized micropropagation protocol developed by Alizadeh *et al.* (2010). Laal is an Iranian, commercially grown variety of grapevine. Single node explants (2-4 cm length) were pre-washed in 0.1-0.2% commercial detergent (JAM dish-washing liquid, Iran) followed by stirring in Mancozeb (2 g/L) solution for 45 min. The explants were surface disinfected using 60% (v/v) NaOC1 solution (5% available chlorine) for 30 min. After four to five rinses in sterile distilled water, single node cuttings (2-4 cm) were inoculated for *in vitro* culture establishement.

Culture media and incubation conditions

Basal MS (Murashige and Skoog, 1962) medium was used during the whole experiment for all three plant species. The various media along with their growth regulators for each plant species are reported in Table 1. The pH was adjusted to 5.8 prior to the addition of 0.8% agar, and the media were autoclaved at 121°C and 15 PSI for 15 min. Carrot root cultures were kept in growth chamber in darkness and low light intensity for 7-14 days and the explants were investigated for callus formation. The callus mass was sub-cultured on fresh medium supplemented with apricot gum. The stevia and grapevine single node cultures were incubated at 25±2°C under continuous light (50 µmol m^{-2} s^{-1}). The established and sprouted cultures were sub-cultured as double-node explants on proliferation media (Table 1) supplemented with different concentrations of apricot gum.

Table 1 - Different culture media and growth regulators for each plant species

Plant species	*In vitro* establishment	Shoot proliferation	Rooting
Carrot	2,4-D (1.0 mg/l)	-	-
Stevia	Hormone-free medium	IBA (2.0 mg/l)	IBA (2.0 mg/l)
Grapevine	BA (2.0 mg/l) + NAA (0.2 mg/l)	IBA (2.0 mg/l)	IBA (2.0 mg/l)

Measured parameters

Due to the utilization of explants with different origins, dissimilar parameters were recorded in each species. In the case of carrot callus cultures, days to callus initiation, callus color, firmness, volume and fresh/dry weight were recorded 30 days after caulogenesis. In the case of stevia and grapevine, initially the *in vitro* cultures were established as explained in above. Then, the double-node explants procured from *in vitro* proliferated cultures were inoculated on their respective proliferation media supplemented with different concentrations of apricot gum. The parameters such as days taken to bud sprouting, number of leaves, leaf area, total chlorophylls and cartenoides, intermodal length, shoot length, days to root initiation, number of roots, root length, rooting percentage and root/shoot fresh and dry weights were measured in both stevia and grapevine regenerated cultures.

Experimental design and data analysis

The present experiment was conducted as a complete randomized design with four replications. The percentage data were transformed using root square method (√ % + 0.5) prior to analysis. The results were analyzed using SAS software (SAS Institute Inc., 2003) and the mean values were compared by least significant difference (LSD) test in $p<0.01$ probability.

3. Results

The present research was undertaken to ascertain the likely positive effects of apricot gum as an organic additive to plant tissue culture media. The gum was primarily supplemented to carrot callus cultures and, owing to its positive response, was tested in two other plant species, stevia and grapevine, for which *in vitro* culture propagation protocols were already standardized in our laboratory (Alizadeh *et al.*, 2010; Taherian, 2012).

Iran is the world's second producer of apricot (FAOSTAT, 2012) and apricot gum is readily available throughout the country at a very low price. In order to optimize the concentrations of the apricot gum, some preliminary tests were performed and the rate of 2.0-6.0 g/L was found to be a logical range because the higher concentration formed a dense solution that was difficult to dispense in culture vessels. Furthermore, in high concentration, it negatively interacted with agar solidification (Khorsha, 2014).

Carrot callus growth

The application of gum surprisingly enhanced the performance of *in vitro* callus initiation and further growth of carrot explants. Furthermore, it significantly reduced the time taken to callus initiation and among the treatments caulogenesis occurred even less than two weeks following inoculation of the explants on medium supplemented with 6.0 g/L apricot gum. The callus initiation in control explants was found to start normally after 5 weeks, therefore, precocious callus formation (nearly 20 days earlier than control) is considered a promising result for *in vitro* application of apricot gum. In addition, callus morphological traits such as callus volume, color, and tissue firmness were also affected by gum treatments

(Table 2). The callus mass produced on control medium (30 days after inoculation) were light brown in color and had intermediate firmness. However, with gum application the color was greenish and the tissue mass volume was increased corresponding to gum concentration (Fig. 1). The callus fresh weight was also improved in all the treatments, however the dry weight was the same and was determined to be lower than control media (Table 3). It is worth noting that the treatment with 6.0 g/L apricot gum may be toxic for carrot callus; however callus fresh weight was not statistically significant compared to 2 g/L level (Table 3).

Due to the positive and promising results with carrot callus cultures, the effect of apricot gum was evaluated on *in vitro* proliferation of an herbaceous medicinal plant, stevia, for which its proliferated cultures were already established in our laboratory.

Table 2 - The qualitative traits of carrot callus as affected by gum application 30 days after inoculation

Qualitative traits	Apricot gum concentration (g/l)			
	Control	2.00	4.00	6.00
Callus volume	<30%	30-50%	50-100%	≥100%
Callus color	Light brown	Beige to light green	Beige to greenish	Beige to pistachio green
Tissue firmness	+++	+++	++++	++++

+++ indicates medium hadness, ++++ indicates medium hardness and fragile callus tissue.

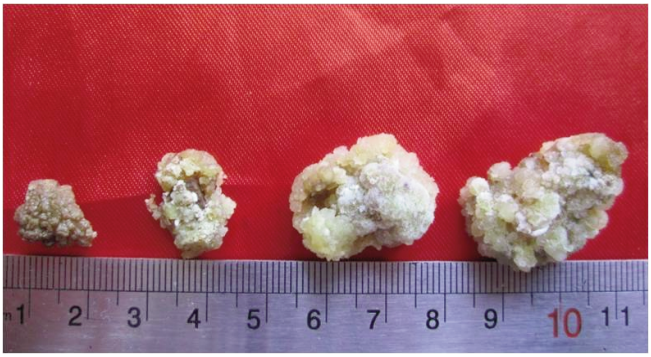

Fig. 1 - The effect of apricot gum on *in vitro* growth and proliferation of carrot callus cultures 30 days after inoculation (left to right: Control, 2.0, 4.0, 6.0 g/l apricot gum).

Table 3 - The growth parameters of carrot callus as affected by gum application 30 days after inoculation

Qualitative traits	Apricot gum concentration (g/l)			
	Control	2.00	4.00	6.00
Days to callus initiation	35.75 a	29.80 b	19.60 c	13.70 d
Callus fresh weight (g)	0.121 c	0.269 cb	1.01 a	0.364 b
Callus dry weight (g)	21.54 a	10.08 b	11.32 b	13.66 b

Means in the same row followed by different letters are significantly different at $P<0.01$ using LSD-Test.

Stevia shoot multiplication and rooting

Stevia is an herbaceous plant with rapid *in vitro* proliferation. In our laboratory, stevia cultures were normally sub-cultured at three- to four-week intervals. The growth parameters of stevia shoot proliferation and rooting four weeks after inoculation are shown in Table 4. It is clear that apricot gum added to stevia medium was particularly beneficial. The gum significantly reduced the time taken to root initiation and the micro-cuttings inoculated on medium containing gum (6.0 g/L) achieved root initiation in less than a week (6.5 days only), while the control plantlets came into rooting after at least 10 days. Root length also increased in the presence of gum, however, the number of roots were not considerably different among treatments. *In vitro* plantlet length and leaves were reduced on gum-supplemented media, but instead the leaf area and lateral shoots were enhanced (Table 4). It seems the lower concentrations of apricot gum (2.0 and 4.0 g/L) led to better vegetative response in stevia cultures. A similar trend was also recorded with respect to stevia leaf pigments. Thus, at the rate of 2.0 g/L, gum was more effective in pigmentation and production of dark green leaves. Overall, it may be stated that apricot gum had a positive influence on most of the *in vitro* traits measured in stevia tissue-cultured plants.

Table 4 - The growth parameters of stevia shoot proliferation and rooting 28 days after inoculation

Growth trait	Apricot gum concentration (g/l)			
	Control	2.00	4.00	6.00
Plantlet length (cm)	20.00 ab	10.95 bc	22.72 a	8.60 c
Number of shoots	2.25 b	3.75 ba	3.25 ba	5.25 a
Number of leaves	68.25 a	27.00 b	36.75 b	47.5 ab
Average leaf area (cm^3)	0.06 c	1.75 a	1.16 ab	0.68 bc
Days to root initiation	10.75 a	10.25 a	7.25 a	6.50 a
Number of roots	35.00 a	29.50 a	35.00 a	17.50 a
Root length (cm)	0.56 b	2.21 a	1.86 a	0.85 b
Chlorophyll a (mg.g F.W.)	7.50 c	14.45 a	10.96 b	10.68 b
Chlorophyll b (mg.g F.W.)	4.33 ab	5.43 a	2.77 b	3.87 ba
Total chlorophyll (mg.g F.W.)	11.91 b	19.97 a	13.76 b	14.72 b
Carotenoid (mg.g F.W.)	3.15 b	5.95 a	4.25 b	4.53 ba

Means in the same row followed by different letters are significantly different at $P<0.01$ using LSD-Test.

Grapevines shoot multiplication and rooting

It has previously been reported that shoot proliferation and rooting occur simultaneously in grapevine (Alizadeh *et al.*, 2010), hence, in the present experiment, it was possible to measure related

parameters collectively in a single medium (shoot proliferation *cum* rooting medium supplemented with apricot gum). Furthermore, the results of our grapevine experiment were more obvious and noticeable than for the other species. These distinct effects on grapevine shoot proliferation and rooting are reported in figure 2 and Table 5. In the case of rooting parameters, the gum considerably reduced the time taken to root initiation (6 days against 14 days in control explants) and the rooting percentage was also improved, reaching nearly 100 % in the case of 6.0 g/L gum-supplemented media. Root length also increased: the greatest length was found in medium containing 4.0 g/L gum. However, all gum treatments showed longer roots compared to control explants. A similar trend was also observed in stevia samples (Table 4). Shoot proliferation was vigorously enhanced following application of gum to the media (Fig. 2 and 3). Thanks to the positive effects of apricot gum, longer vines with more lateral shoots, internodes and greater leaf area were achieved (Fig. 3). Although all three concentrations of gum were found to be effective with regard to the aforementioned vegetative traits as compared to gum-free medium, the efficiency of 4.0 g/L was evident.

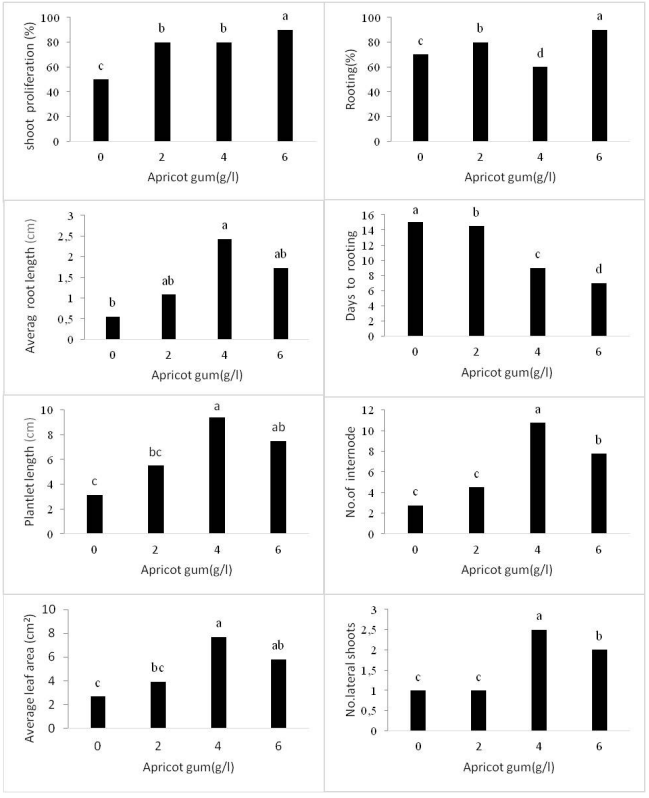

Fig. 2 - The effect of apricot gum on *in vitro* growth performance of grapevine explants.

Table 5 - The effect of apricot gum on certain growth parameters of grapevine 30 days after inoculation

Growth trait	Apricot gum concentration (g/l)			
	Control	2.00	4.00	6.00
Appearance of the first leaf	17.25 a	7.00 b	5.00 c	4.75 c
Number of shoots	1.00 c	1.00 c	2.5 a	2.00 b
Number of leaves	3.00 b	4.75 b	9.5 a	9.50 a
Number of roots	20.25 b	32.5 b	77.25 a	65.75 a
Internode length (cm)	0.94 c	1.02 bc	1.41 a	1.29 ab

Means in the same row followed by different letters are significantly different at $P<0.01$ using LSD-Test.

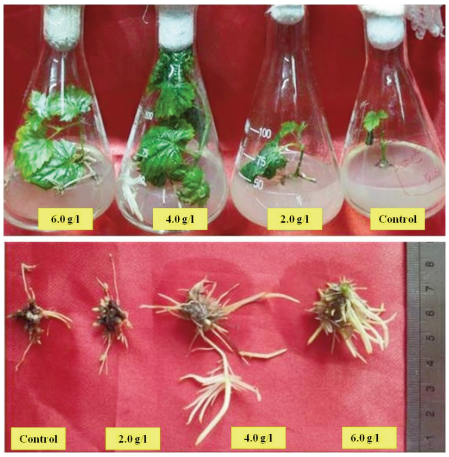

Fig. 3 - The effect of apricot gum on *in vitro* shoot proliferation (above) and rooting (below) of grapevine.

4. Discussion and Conclusions

Cultured plant tissues need a continuous supply of carbohydrates from the medium to encourage growth and survival *in vitro* (Kozai, 1991). Therefore, sugars such as sucrose, glucose, and sorbitol are generally added as a carbon source (Kadota and Niimi, 2004). Furthermore, reliable callus proliferation and subsequent plant regeneration are important for efficient micropropagation and genetic manipulation of plant tissues (Khorsha, 2014). Carbohydrates play an important role in *in vitro* cultures as an energy and carbon source, as well as an osmotic agent (George *et al.*, 2008). There are numerous reports on the effects of various carbon sources on *in vitro* callus growth and regeneration. For example, Huang and Huang (1999) demonstrated that sorbitol acts as a carbon source, providing energy for callus growth and plantlet regeneration. Other studies suggest that it acts only as an osmotic regulator to adjust osmotic pressure in calli (Al-Khayri and Al-Bahrany, 2002; George *et al*., 2008). The capacity of various carbohy-

drates to support growth of Japanese morning glory callus was examined and it was found that sucrose was the most effective compound but glucose, fructose, trehalose, maltose, cellobiose, raffinose and soluble starch were also found to be significant (Hisajima and Thorpe, 1985).

Apricot gum and callus growth and proliferation

Gums are typically more or less sticky in nature and translucent and amorphous. Chemical analysis of apricot gum has already been performed by Lluveras-Tenorio *et al.* (2012). They found total sugars (60%), galactose (43%), mannose (4%), arabinose (44%), xylose (7%) and ramnose (1%) in apricot gum. It is clear that such composition may be different in each lot, from one tree to another, and the results may not be reproducible in different tissue culture laboratories. However, the present study was performed in five replications and positive responses were found with carrot callus growth. The callus volume produced on media fortified with 4.0-6.0 g/L apricot gum was at least three to four times that of gum-free medium (Fig. 1). The enhanced callus growth induced by apricot gum may be considered a significant logical reason for application of gum in plant tissue culture media, especially in species for which indirect regeneration (plant regeneration from callus) is to follow.

Apricot gum and shoot/root proliferation

Different types of basal media used in plant tissue culture vary with regard to the concentration of macro and microelements, greatly affecting the *in vitro* growth and multiplication of shoots. Apart from this, type and concentration of carbon sources in the medium affect the physiology and differentiation of tissues (Lipavska and Konradova, 2004) while serving as osmotic and energy source (George *et al.*, 2008). In our experiment, the utilization of apricot gum caused reasonable shoot proliferation both in stevia and grapevine (Table 4, Fig. 3), not to mention its very low cost and economic advantages, making it useful in commercial tissue culture laboratories seeking economical and feasible protocols for *in vitro* propagation of horticultural crops. There are some reports on the utilization of such low-priced and organic materials as a potential plant source with a high amount of sucrose and other sugars which could possibly be used as an alternative carbon source, for example molasses (Dhamankar, 1992), sugar cane juice (Buah *et al*., 2011), and date palm syrup (Al-khateeb, 2008 a). Furthermore, in a review presented by Yaseen *et al.* (2012), factors determining the efficacy of a carbon source including its type, concentration, and their mutual interaction are reported. In our study, apricot gum was exploited, although the application of almond gum or sweet cherry gum would also be possible, since these types of gum are also readily available throughout the country at similar price. However, there may be some differences in *in vitro* responses of gums from different origins. With regard to concentration, for tissue culture media, 4.0-6.0 g/L may be considered as a feasible range, because beyond this dose the solubility of gum would be difficult even with heat treatments. Moreover, in high concentrations the culture media cannot be solidified properly (Khorsha, 2014). The mutual interaction between gum and explants is also an important matter. Although, in the present experiment all three plant species showed positive responses for most of the measured parameters, however grapevine *in vitro* shoot proliferation was greatly enhanced in gum-supplemented media (Fig. 3).

Rooting is considered a difficult step in micropropagation of many woody plants (George, 1993) and it is regulated by a number of physiological, biochemical, and genetic factors (Pawlicki and Welander (1995). Generally, rooting occurs in an auxin-enriched medium. On the other hand, Hassan *et al.* (2009) mentioned that sucrose at the highest level considerably increased rooting of palm tissue cultures. The results of the present research on rooting parameters of stevia and grapevine correspond with those of Al-Khateeb (2008 b) on date palm. He observed an enhanced root formation as the sugar concentration increased (60 g/L and above).

In conclusion, the present study considered addition of apricot gum as a complex organic addendum in tissue culture of carrot callus, stevia and grapevine. It was found that addition of gum not only was without negative effects, but its usefulness was definitely confirmed. Apricot gum significantly increased *in vitro* growth and proliferation of carrot callus tissues. Furthermore, shoot/root vegetative parameters of gum-fortified media were actually promising in stevia and grapevine. Overall, gum at the rate of 4.0 g/L was found to be a logical concentration with respect to the positive response in all three species. Owing to the promising results found with the present research, the application of gum in commercial tissue culture protocols is highly recommended. However, further studies would be required to fully exploit plant-derived gums as an alternative carbon source.

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