Middle East Journal of Agriculture Research
ISSN 2077-4605
Volume : 05 | Issue : 03 | July-Sept. | 2016
Pages: 313-323

CRW CURRENT RESEARCH WEB

In vitro Growth Regulators, Gelling Agents and Sucrose Levels Affect Micropropagation of *Gypsophila paniculata* L.

Nabil Toaima, Hussein Bosila and Ahmed Abd El-Fattah El-Ateeq

Department of Horticulture, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt.

Received: 25 June 2016 / Accepted: 27 July 2016 / Publication date: 10 August 2016

ABSTRACT

The effect of plant growth regulators, gelling agents and sucrose on micropropagation and hyperhydricity of *Gypsophila paniculata* L. was examined. Shoot tips were tested at different levels and combinations of 6-benzyl adenine (BA) and α-naphthaleneacetic acid (NAA) for shoot induction. Shoot proliferation and hyperhydricity were evaluated under different levels of sucrose, agar and gelrite. Highest efficiency of shoot formation was obtained with Murashige and Skoog's (MS) medium containing 0.5 mg/l each of BA and NAA. Here, 98.7 % explants produced 7.5 shoots per shoot tip with 4 cm. length per shoot. Gelrite at recommended used concentrations, 0.2-0.25 %, were not adequate for micropropagation of *G. paniculata*, or this type of gelling agents was not suitable for this species. The lowest degree of hyperhydricity (13.4 and 8%) was recorded when 8 and 10 g/l agar, respectively, were used. Higher concentrations of sucrose (40-60 g/l) recorded inhibitory effect on shoot formation, shoot number, shoot length and reduced hyperhydricity rate. For *in vitro* rooting, half strength MS media supplemented with different combinations of NAA and 3-indolebutyric acid (IBA) were tested, out of which 1 NAA + 3 IBA mg/l and 3 mg/l IBA were found to be favorable with 95 % rooting.

Key words: *Gypsophila paniculata*, hyperhydricity, sucrose, gelling agents, micropropagation

Introduction

Genus *Gypsophila* L. (Caryophyllaceae) comprises about 125 species which are native to temperate region of Eurasia and they include annuals, biennials and perennials out of which *Gypsophila paniculata* L., also known as baby`s breath, is perennial in nature often grown commercially as an annual crop. It is grown both as garden plants and also valuable as an important cut flower in commercial floriculture production to add as a filler to flower bouquets (Thakur *et al*., 2013; Wang *et al*., 2013). Phytochemical examination of genus *Gypsophila* revealed the presence of various groups of biological active compounds such as triterpene saponins and flavonoids (Yotova *et al*., 2012; Zdraveva *et al*., 2015). Saponin (E. Merck No. 7695), also known as Saponin album, is a crude saponin fraction obtained from roots and rhizomes of *G. paniculata* L. and *G. arrostii* Guss. which used to be commercially available (Yotova *et al*., 2012). *G. paniculata* L. and *G. arrostii* Guss. have long been used as an expectorant in Arabic countries (Kocaoğlu Kavas and Yorgancılar, 2016). Pensec *et al*. (2013) showed that *G. paniculata* saponins are an efficient and environmentally friendly treatment against some nematodes.

Propagation of *G. paniculata* through vegetative means is not so easy and plant has always forced difficulties in rooting its terminal cutting (Thakur *et al*., 2013). Also, the flowers of commercial *G. paniculata* are sterile and do not produce seeds, therefore, breeding programs are severely restricted (Shillo, 1985). Thus, there is a need to explore other more effective methods of propagation. Hence, the use of tissue culture techniques for propagate *G. paniculata* becomes important and may overcome these problems, and can be the alternative for the continuous provision of plantlet stocks for large scale field cultivation.

Hyperhydricity (or vitrification), a physiological disorder characterized by excessive water retention, is a common abnormality observed during tissue culture. This phenomenon limits the application of *in vitro* propagation methods and affects the commercial micropropagation industry (Tian *et al*., 2016). This phenomenon is well known in micropropagation of *G. paniculata* and hampers the success of tissue culture technique due to the poor rate of survived plantlets obtained (Rady, 2006). Han *et al*. (1991b) and Rady (2006) studied hyperhydricity in *G. paniculata* tissue culture and found its relation with agar concentration in the culture medium.

In plant cultured *in vitro*, a continuous supply of carbohydrates from the medium is necessary, since the photosynthetic activity is reduced under low light intensity, limited gas exchange and high relative humidity (Kozai, 1991). Sucrose is the main source of energy during micropropagation. Moreover, as an osmoticum, it can also affect shoot proliferation. The optimum concentration of sucrose should be sufficient for all basic energy requirements for cell division and plantlet regeneration (Bhojwani and Razdan, 1996)

Corresponding Author: Ahmed Abd El-Fattah El-Ateeq, Department of Horticulture, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt.
E-mail: ahmed_elateeq@yahoo.com

This experiment sought to establish the optimum concentration of BA and NAA combination required for shoot production from shoot tips explants of *G. paniculata* L. cv. Perfecta and developed a rooting medium for regenerated shoots, as well as investigate the influence of various types and levels of gelling agents (solidifying agents) and sucrose levels on shoot proliferation and hyperhydricity degree in regenerated shoots of *G. paniculata*.

Materials and Methods

This experiment was carried out in the Laboratory of Biotechnology, Horticulture Department, Faculty of Agriculture, Al-Azhar University.

Plant material:

Gypsophila paniculata L. cv. Perfecta plants were kindly gifted from the Floramix Egypt Company for cut flowers, Giza, Egypt.

Explant preparation:

Shoot tips were excised (Fig. 1A) and washed several times with commercial detergent and tap water, and surface sterilized with 70% ethanol for 1 min., followed by 20% commercial Clorox (contained 5.25% sodium hypochlorite) with a few drops of Tween 20 for 20 min., then rinsed three times in sterile distilled water to remove the residual sodium hypochlorite.

Effect of BA and NAA on shoot induction:

The sterile shoot tips were placed in Murashige and Skoog (1962) basal medium (MS) with 30 g/l sucrose and supplemented with different combinations of BA (0.5 and 1 mg/l) and NAA (0, 0.5 and 1 mg/l), and solidified with 8 g/l agar for shoot induction.

Effect of gelling agents and sucrose levels on shoot proliferation and hyperhydricity:

Nodal explants ((single nodes containing two leaves)) taken from *in vitro* healthy shoot cultures were transferred to MS medium supplemented with 0.5 mg/l each of BA and NAA, as the best result recorded. For gelling (solidifying) agents experiment, the media were gelled with agar (6, 8 and 10 g/l) or gelrite (1.5, 2 and 2.5 g/l) or in combination (4 agar + 1 gelrite g/l), and 30 g/l sucrose was added. To test the effect of sucrose, the media were gelled with 8 g/l agar and sucrose was added at different levels (0 – 60 g/l).

Effect of NAA and IBA on in vitro rooting:

Healthy shoots (2-3 cm.) excised from *in vitro* shoot cultures were cultured on half strength MS medium (½ MS), gelled with 8 g/l agar and supplemented with different concentrations and combinations of NAA (0, 1, and 3 mg/l) and IBA (0, 1, and 3 mg/l).

Well-developed plantlets were subsequently grown in pots containing a mixture of peat-moss and sand in the greenhouse for acclimatization.

Culture conditions:

The pH value of all tested media was adjusted to 5.8 with NaOH or HCL (1 N) prior to the addition of gelling agents. Forty millilitre of medium was poured into 350 ml tissue culture jars capped with polypropylene lids and autoclaved at 121ºC at 1.2 kg. F. /cm^2 for 20 min. Three explants were cultured per jar. Each treatment comprised 18 explants and each experiment was repeated twice The cultures were incubated for 4 weeks in growth chamber under 25±2ºC and 16-h light/8-h dark cycle with illumination from cool white fluorescence lights 40 $\mu mol^{-2}\ s^{-1}$.

Data collection and statistical analysis:

Different measurements were recorded after 4 weeks incubation period, shoot formation (%), shoot number per explant, shoot length (cm.), hyperhydricity (%) (by visual observations), water content (%), callus formation (%), rooting (%), root number per explant, root length (cm.) were recorded. Chlorophyll content (Chl a+b µg/g fresh weight (FW)) was determined spectrophotometry in 80% acetone extract according to

Lichtenthaler (1987). The experiment was conducted in a complete randomized design (CRD). The data were subjected to Analysis of Variance (ANOVA) using COSTAT computer package ver. 6.4 (CoHort software Monterey, California, USA). The significance of differences among means was carried out using Duncan's multiple range test (DMRT) (Duncan, 1995) at $p<0.05$.

Results and Discussion

Effect of BA and NAA on shoot induction:

Shoot tips (Fig. 1A) were used to study the effect of BA and NAA on adventitious shoot induction of *G. paniculata* in a preliminary experiment (Table 1, Fig. 1B). Significant differences were recorded between treatments for all measurements. In the absence of growth regulators (BA and NAA), control, recorded a significant reduction in shoot formation, shoot number per explant and hyperhydricity rate. The highest significant values for shoot formation (98.7 %), shoot number per explant (7.5) and shoot length (4 cm.) were recorded at 0.5 mg/l each of BA and NAA. At this treatment, hyperhydricity rate recorded 23 % compared to higher concentration of BA. Cytokinins are essential factors for breaking the apex dormancy and inducing auxiliary shoot proliferation that influence the success of *in vitro* multiplication (Silva *et al*., 2003). BA cytokinin induces cell division, shoot multiplication and lateral bud formation while inhibiting root development (Sutter, 1996). Callus formation was observed in all treatments (Fig. 1C) and recorded 100 % at higher level of NAA (1 mg/l) within both BA concentrations.

High percentages of hyperhidric shoots (40 and 30.3) were observed at high level of BA (1 mg/l) alone or combined with 0.5 mg/l NAA, respectively, indicating an upper limit on BA concentration for the multiplication of this species. High concentrations of cytokinins have been reported to be one of the most important factors involved in the induction and/or development of hyperhydricity during *in vitro* culture of *G. paniculata* (Rady, 2006; Ayeh *et al*., 2009) and also in some other species (Badr-Elden *et al*., 2012; Rathore *et al*., 2015). In this respect, the synergistic effect between cytokinins and the stressing hyperosmotic shock in ethylene production might be suspected to occur and be connected with hyperhydricity (Gaspar, 1991).

A synergistic effect of NAA on increasing shoot formation and decreasing hyperhydricity percentages was observed when employed with BA compared to BA alone. Similar observation was found in *Spathiphyllum cannifolium* by Dewir *et al*. (2006) and on *Ipomoea sepiaria* by Cheruvathur *et al*. (2015). The beneficial effects of BA and NAA combination at 0.5 mg/l on shoot formation and proliferation of *G. paniculata* was also confirmed by Rady (2006) and Barakat and El-Sammak (2011). Therefore, the combination of 0.5 mg/l BA and 0.5 mg/l NAA was applied to further experiments.

Table 1: Effect of different combinations of BA and NAA on shoot induction from shoot tips of *G. paniculata* after 4 weeks incubation periods.

Growth regulators mg/l		Shoot formation	Shoot number	Shoot length	Hyperhydricity (%)	Callus
BA	NAA	(%)	per explant	(cm.)		formation (%)
0	0	75 c	4 c	3 b	20 c	25 e
0.5	0	90 b	5 bc	2.5 b	25 bc	50 d
0.5	0.5	98.7 a	7.5 a	4 a	23 c	90 ab
0.5	1	90.5 b	6.5 ab	3 b	20.3 c	100 a
1	0	88 b	7.5 a	3 b	40 a	80 b
1	0.5	93.3 ab	7 ab	3.5 ab	30.3 b	66.7 c
1	1	88.6 b	6.5 ab	3 b	25 bc	100 a

Mean values followed by different letters in the column are significantly different according to DMRT at $p<0.05$.

Rady and Bekheet (2008) mentioned that shoot cultures of *G. paniculata* cv. Bristol fairy were established from shoot tips on MS medium supplemented with 0.5 mg/l each of BA and NAA, also, Wang *et al*. (2013) used 0.5 mg/l BA + 0.1 mg/l NAA for micropropagation of *G. paniculata*. Han *et al*. (1991a) reported that BA (0.5 - 2 mg/l) found to be the most effective cytokinin for shoot proliferation of *G. paniculata* shoot tip. They found that a combination of BA and Indole-3-acetic acid (IAA) each at 0.1 - 0.3 mg/l gave better shoot proliferation and growth than BA alone.

Effect of gelling agents on shoot proliferation and hyperhydricity:

Agar and gelrite are natural polysaccharides with high capability of gelation. Their gels combine with water and absorb other compounds (Ebrahim and Ibrahim, 2000). Agar is the most commonly used gelling agent in media for plant tissue culture. Since its introduction more than 100 years ago, it has remained the most frequently used gelling agent in culture media employed for microbes as well as plants. The properties of agar that make it the gelling agent of choice are stability, high clarity and resistance to metabolism during culture (Jain and Babbar, 2002). Gelrite appears to be an economical gelling substitute. It is characterised by a

consistent quality and high purity, and substantially smaller quantities produce gels of hardness comparable to agar (Ivanova and Van Staden, 2011).

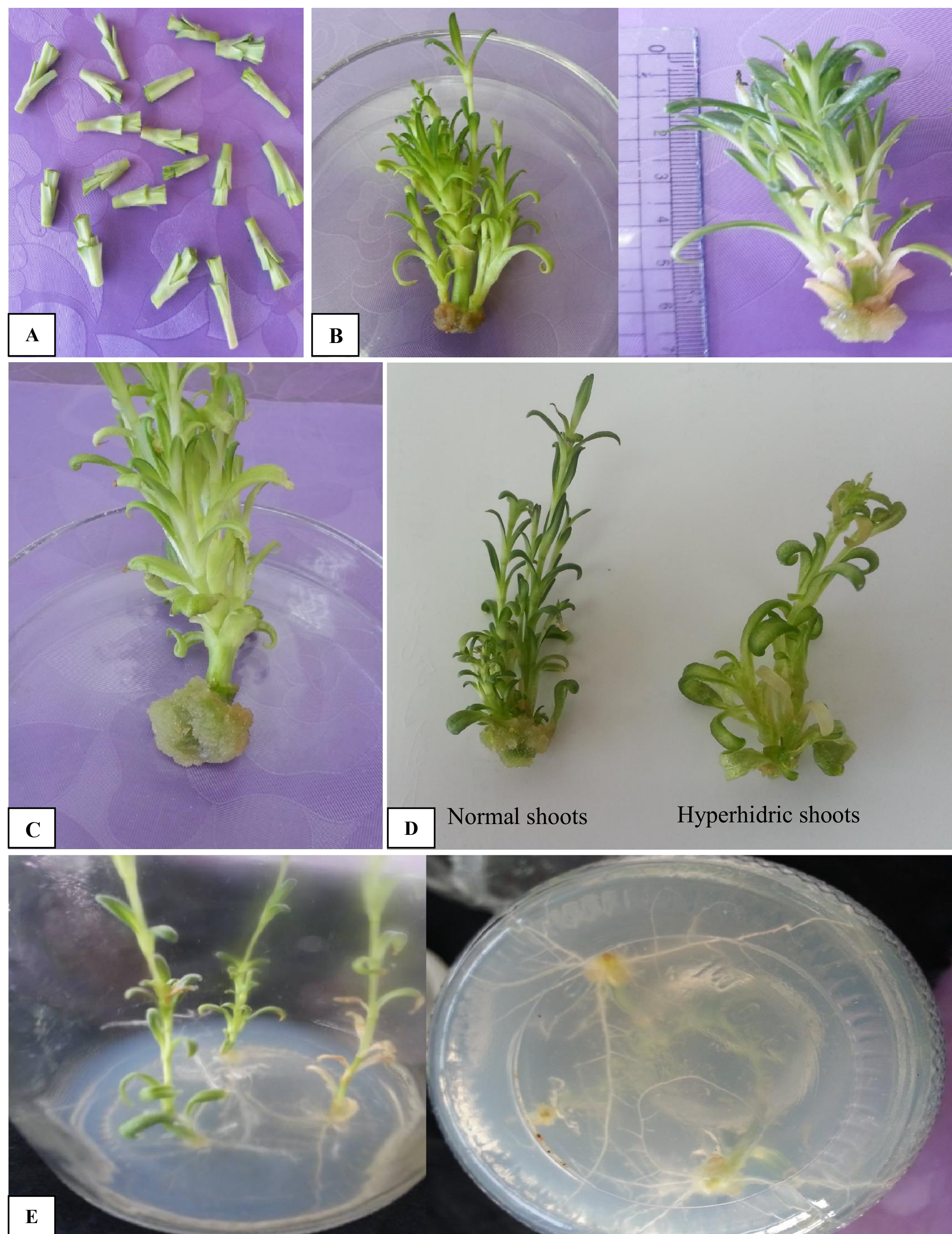

Fig. 1: Micropropagation of *G. paniculata*, **A.** shoot tips explants, **B.** shoots induction from shoot tips, **C.** callus developed on cut end of shoot tips, **D.** normal and hyperhidric shoots, **E.** *in vitro* rooting of *G. paniculata*.

Stem nodal segments were excised from *in vitro* shoot multiplication of *G. paniculata* and used in this experiment. Data presented in Table 2 and Fig. 2, 3A demonstrated that proliferation, growth and hyperhydricity rates were significantly influenced by both type and the concentration of solidified agents. Comparing agar and

gelrite with MS medium containing 0.5 mg/l each of BA and NAA, agar appeared superior in shoot formation, shoot number per explant, chlorophyll content and callus formation. Agar at 8 and 10 g/l resulted in the highest shoot formation percentage (94.4), while 6 g/l agar recorded the highest shoot number (8.4) per nodal explant. Increasing agar level to 8 and 10 g/l caused a reduction in shoot proliferation (7.1 and 6.9 per explant, respectively) which also observed in watermelon cultured *in vitro* (Badr-Elden *et al*., 2012). Gelrite at its different levels and its employ with agar reduced the percentage and number of proliferated shoots.

Table 2: Effect of gelling agents on shoot proliferation from nodal explants and hyperhydricity of *G. paniculata* after 4 weeks incubation periods.

Gelling agents g/l		Shoot formation (%)	Shoot number per explant	Shoot length (cm.)	Hyperhydricity (%)	Water content (%)	Chlorophyll content µg/g FW	Callus formation (%)
Agar	Gelrite							
6	0	88.9 b	8.4 a	2.5 bc	18.2 c	92.9 bc	452.3 d	83.3 b
8	0	94.4 a	7.1 b	3.5 ab	13.4 d	91.9 bc	489.1 b	88.9 b
10	0	94.4 a	6.9 bc	4 a	8 e	90.9 c	514.4 a	100 a
0	1.5	77.8 d	4.2 e	2.4 c	100 a	95.5 a	325.3 f	27.8 d
0	2	83.3 c	5.6 d	3.2 abc	75.2 b	94.2 ab	348.5 e	16.7 e
0	2.5	83.3 c	4.9 de	4 a	75.3 b	94.4 ab	451.7 d	33.3 d
4	1	77.8 d	5.7 cd	2.5 bc	19.6 c	92.8 bc	470.5 c	72.2 c

Mean values followed by different letters in the column are significantly different according to DMRT at p<0.05.

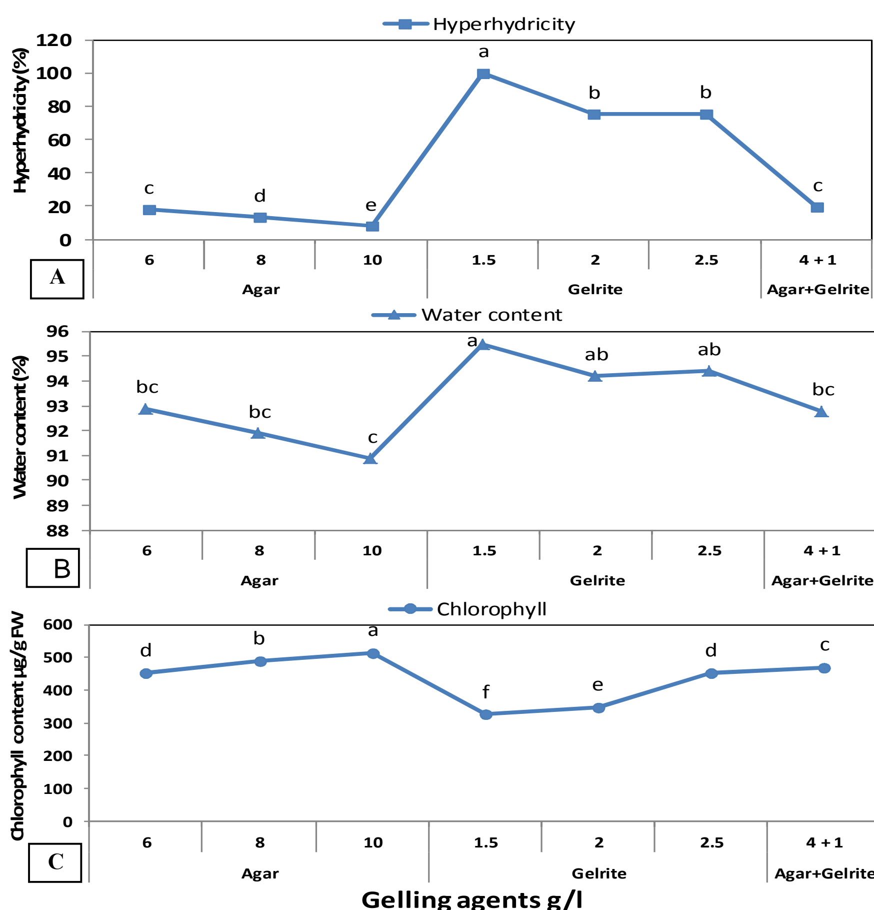

Fig. 2: Effect of gelling agents (agar 6, 8, 10, gelrite 1.5, 2, 2.5 or 4 agar + 1 gelrite g/l) on **A.** hyperhydricity (%), **B.** water content (%) and **C.** chlorophyll content (µg/g FW) of shoots developed from nodal explants of *G. paniculata* after 4 weeks incubation periods. Significant differences (p<0.05) between treatments are indicated by different letters according to DMRT.

Shoot length increased with increasing levels of both gelling agents reached 4 cm. for 10 agar and 2.5 gelrite g/l. Respecting the effect of both gelling agents on hyperhydricity and quality of proliferated shoots; visual observation (Fig. 1D) demonstrated that agar solidified media reduced the hyperhidric shoots to the minimal

rates (18.2, 13.4 and 8 % for 6, 8 and 10 g/l agar, respectively), while gelrite aggravated the occurrence of hyperhydricity in *G. paniculata* (Table 2) as 100, 75.2 and 75.3 % of the new shoots were affected when 1.5, 2 and 2.5 g/l gelrite used, respectively. This result was in agreement with other authors who reported that in *G. paniculata* cultures that raising agar content could reduce hyperhydricity of produced shoots (Han *et al*., 1991a, b; Rady, 2006) and also agree with Badr-Elden *et al*. (2012) on watermelon.

Water content takes the same trend recorded for hyperhydricity with all treatments (Fig. 2) as hyperhidric shoots characterize with excessive water retention in their tissues (Tian *et al*., 2016). This observation is consistent with findings on the micropropagated plantlets of *Dianthus caryophyllus* (Casanova *et al*., 2008). The largest water contents were measured when MS medium was solidified with gelrite alone. In contrast to water content, chlorophyll level recorded the highest values in agar solidified media, and also increased by raising the concentrations of both gelling agents as hyperhydricity rate decreased (Fig. 2). Also hyperhidric shoots (Fig. 1D) characterize with low chlorophyll content (Badr-Elden *et al*., 2012). Callus formation response was also enhanced with medium solidified with agar even when combined with gelrite. These observations could be attributed to the difference between both gelling agents in most characteristics mentioned above.

In this concern, hyperhydricity was stimulated in 'Jonagold' apple shoots grown *in vitro* for one multiplication cycle by replacing the gelling agent agar with gelrite (Tabart *et al*., 2015). Gelrite has been found to increase hyperhydricity in some species (Franck *et al*., 2004; Ivanova and Van Staden, 2011). The physical structure of gelrite allows better absorption of substances such cytokinins, ammonium ions, and water, which are thought to play a key role in the development of hyperhydricity (Franck *et al*., 2004; Ivanova and Van Staden, 2011). The low frequency of hyperhydricity on medium with agar has been suggested to be due to a sulphated galactan in agar (Nairn *et al*., 1995), which is able to control hyperhydricity (Ivanova and Van Staden, 2011). It was indicated that increasing agar concentration decreased hyperhydricity and insured obtaining healthy vigorous shoots. The beneficial effect of raising agar concentration should be explained by decreasing the availability of cytokinins (Gaspar, 1991). Some plants, however, vitrify in the absence of cytokinins, which leads to the suggestion that cytokinins are not inducers of the process but may act synergistically with other factors (Gaspar *et al*., 1987).

It is clear from our data that gelrite at recommended used concentrations, 0.2-0.25% (Pierik, 1987), were not adequate for micropropagation of *G. paniculata*, or this type of gelling agents was not suitable for this species. For our knowledge, all published papers on micropropagation of *G. paniculata* used agar as gelling agents in their studies.

Effect of sucrose levels on shoot proliferation and hyperhydricity:

Carbohydrates serve as an energy source in the tissue culture medium and act to supplement osmotic pressure in the culture of plant cells (Huh *et al*., 2016). In particular, sucrose is considered as the important carbon and energy source, because it is the most common carbohydrate in phloem sap and involved in controlling developmental processes (Gibson, 2000). It could provide a balanced carbon source for cell growth with the released hexoses directly participating in glycolytic and pentose phosphate pathways (Zha *et al*., 2007). For most plant tissue culture protocols, sucrose is the preferred carbon source as it is an easily assimilated macronutrient that rapidly provides energy (Saraswathi *et al*., 2016).

The effect of sucrose levels on shoot proliferation and hyperhydricity of *G. paniculata* was evaluated, and the results obtained are present in Table 3, Fig. 3B and Fig. 4. MS medium with 0.5 mg/l BA and NAA was cultured with nodal segments obtained from *in vitro* shoot cultures. Sucrose was added at various levels ranged 0 – 60 g/l. Results indicated that proliferation of *G. paniculata* cultures in this study was strongly affected significantly by sucrose concentrations (Table 3).

Table 3: Effect of sucrose levels on shoot proliferation from nodal explants and hyperhydricity of *G. paniculata* after 4 weeks incubation periods.

Sucrose g/l	Shoot formation (%)	Shoot number per explant	Shoot length (cm.)	Hyperhydricity (%)	Water content (%)	Chlorophyll content µg/g FW	Callus formation (%)
0	-*	-	-	-	-	-	-
10	94.4 a	4.4 c	4 a	55 a	93.5 a	340.0 e	72.2 b
20	94.4 a	5.7 b	3.6 a	15.5 b	92.4 b	441.9 c	66.7 c
30	94.4 a	7.1 a	3.5 a	13.4 b	91.9 b	489.1 b	88.9 a
40	77.8 b	5.8 b	2.4 b	3.8 c	90.6 c	557.9 a	44.4 d
50	77.8 b	5 bc	2 b	1 c	90.1 c	486.4 b	43.5 d
60	50 c	2.3 d	1.2 c	0.5 c	88.6 d	409.8 d	38.9 e

Mean values followed by different letters in the column are significantly different according to DMRT at p<0.05.
** Indicated not determine due to no response of nodal segments.*

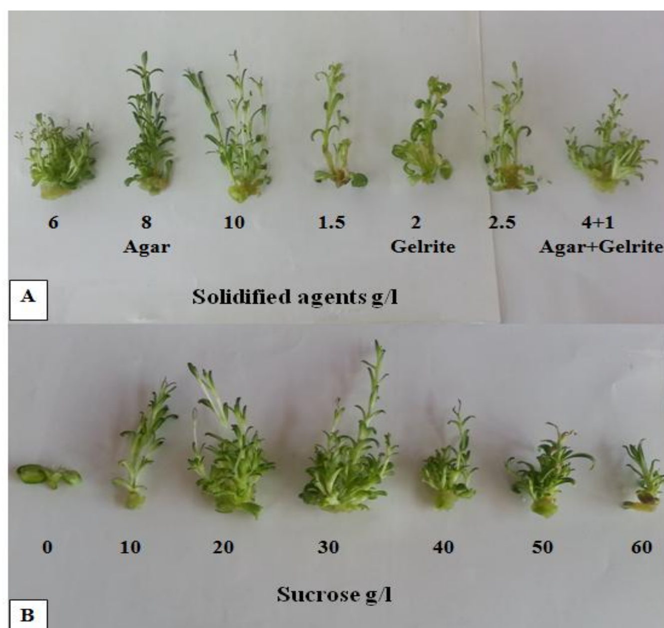

Fig. 3: Effect of different levels of A. solidified agents (agar 6, 8, 10 and gelrite 1.5, 2, 2.5 g/l) and B. sucrose (0-60 g/l) on shoot proliferation from nodal explants and hyperhydricity of *G. paniculata* after 4 weeks incubation periods.

The explants (single nodes containing two leaves) cultured on medium free of sucrose but containing growth regulators (0.5 mg/l BA and NAA) didn't produce any shoots or callus and the leaves turned to light green. The highest formation of shoots (94.4 %) was recorded with low and moderate levels of sucrose (10, 20 and 30 g/l). Shoot proliferation was increased by increased sucrose levels from 10 to 30 g/l and then decreased gradually by continuous increasing. Medium supplemented with 30 g/l sucrose greatly enhanced shoot proliferation (7.1 shoots per explant). Shoot elongation is a critical step in the micropropagation system, closely related to the nutritional composition of the media (Chen *et al*., 2003). In this connect, 10, 20 and 30 g/l sucrose recorded the highest shoot lengths without significant differences ($p<0.05$) (4, 3.6 and 3.5 cm, respectively).

Hyperhydricity, a great problem in *G. paniculata* tissue culture (Fig. 1D), was significantly affected by sucrose levels. Raising the concentration of sucrose correlated inversely to the frequency of hyperhydricity, which decreased gradually (Fig. 4A), but a reduction in shoot formation, shoot number, shoot length were also recorded. Water content takes the same trend recorded for hyperhydricity as it decreased gradually (Fig. 4A, B). Chlorophyll content recorded an increase in its level when sucrose raised from 10 to 20, 30 and 40 g/l and decreased again at 50 and 60 g/l as shown in Fig. 4C. Here, the highest chlorophyll content (557.9 µg/g FW) was measured in shoots developed on medium containing 40 g/l.

Our study indicated that a reduction in shoot formation, shoot number, shoot length, hyperhydricity rate, water content and callus formation were observed at the highest (40-60 g/l sucrose) concentrations used. This observation confirms data of Gabryszewska (2011) and Ilczuk *et al*. (2013) who reported an inhibitory effect of high concentration of sucrose in growth of axillary shoots of *Syringa vulgaris* and *Physocarpus opulifolius*, respectively. In this study we can found that high concentration of sucrose also inhibited the growth of produced shoots of *G. paniculata*. Higher amount of sucrose could retard the development of cultured cells by causing a cessation of the cell cycle when nutrients were limited (Wu *et al*., 2006). Other researchers also claimed that the addition with high concentration of sucrose in the culture medium might have inhibitory effect on nutrient uptake by lowering water potential of the medium (Shim *et al*., 2003), and induce the osmotic stress (Shohael *et al*., 2006). Steinitz (1999) and Teixeira da Silva (2004) suggested that carbohydrates are perceived by cells as chemical signals, with very high concentrations *in vitro* acting as stress agents. Negative effect of low sucrose amount (10 mg/l) on hyperhydricity (55 %) could be attributed to the medium high water potential which makes water more available for explant (Sadeghi *et al*., 2015) and subsequently improves hyperhydricity.

It can be asserted that sucrose at 30 g/l (recommended concentration for plant tissue culture) found to be optimum for *G. paniculata* micropropagation.

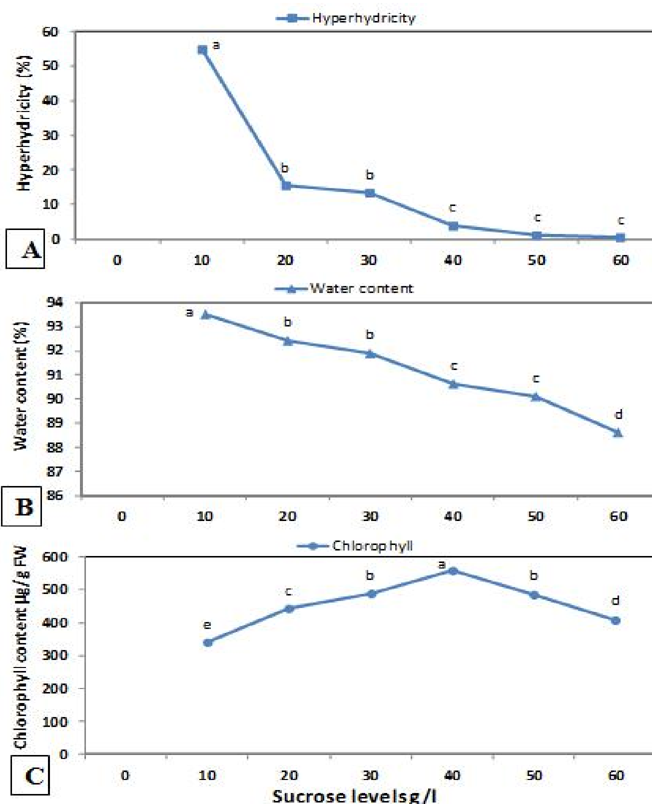

Fig. 4: Effect of different levels of sucrose (0-60 g/l) on **A.** hyperhydricity (%), **B.** water content (%) and **C.** chlorophyll content (µg/g FW) of shoots developed from nodal explants of *G. paniculata* after 4 weeks incubation periods. Significant differences ($p<0.05$) between treatments are indicated by different letters according to DMRT.

Effect of NAA and IBA on in vitro rooting:

The induction of roots on explants, in the rooting stage, from *in vitro* culture is crucial part in any micropropagation process (Molassiotis *et al*., 2003). Table 4 displayed the response of *G. paniculata* shoots to *in vitro* rooting using half strength MS media supplemented with various combinations of auxins (NAA and IBA). Based on the results obtained, addition of IBA and combined it with NAA enhanced the formation of adventitious roots (Fig. 1E). IBA applied at 3 mg/l alone or in combination with 1 mg/l NAA recorded the highest significant percentage of root formation (95 %). Rooting percentage recorded 70 % in control treatments (without auxins).

There was significant level of reduction in rooting formation from 66.7, 76.5 and 95 to 50, 75.3 and 76.2 % as the concentration of NAA increased from 1 to 3 mg/l within IBA concentrations (0, 1 and 3 mg/l, respectively). This might be due to inhibition of rooting at higher concentration of NAA. Higher concentrations of NAA reduce root induction as it promotes the biosynthesis of ethylene which has inhibitory effect in rooting (Tesfa *et al*., 2016). The highest root number (3.7 roots per explant) was recorded for 3 mg/l IBA and 3 NAA + 1 IBA mg/l. Using IBA at 1 and 3 mg/l and 1 NAA + 3 IBA resulted in the highest root length (2, 2.1 and 2 cm., respectively).

Auxins, such as IBA and NAA, are used to promote effective rooting more often than IAA because of their greater stability within tissue and during storage (Hartmann *et al*., 1996) and less sensitive to auxin degrading enzymes (Riov, 1993). It has been stated that IAA is metabolized by peroxidase enzymes rapidly (Nag *et al*., 2001). Rady (2006) found that 3 mg/l IBA recorded the great values for *in vitro* rooting of *G. paniculata*, also, Barakat and El-Sammak (2011) and Wang *et al*. (2013) used MS medium containing 3 mg/l IBA for *in vitro* rooting of *G. paniculata* shoots. Thakur *et al*. (2013) found that 0.93 mg/l NAA + 1 mg/l IBA recorded the best rooting of shoot (100%) for *G. paniculata* L. cv. Bristol Fairy.

In vitro plantlets with well-developed roots were acclimated in the greenhouse with a survival percentage of over 70%.

Table 4: Effect of different combinations of NAA and IBA on *in vitro* rooting of *G. paniculata* shoots after 4 weeks incubation periods.

Growth regulators mg/l		Rooting (%)	Root number per explant	Root length (cm.)
NAA	IBA			
0	0	70 cd	1.8 c	1.6 bcd
0	1	80.2 b	3 ab	2 a
0	3	95 a	3.7 a	2.1 a
1	0	66.7 d	3 ab	1.8 abc
1	1	76.5 b	3.3 ab	1.9 ab
1	3	95 a	3.5 ab	2 a
3	0	50 e	2.5 bc	1 e
3	1	75.3 bc	3.7 a	1.5 cd
3	3	76.2 b	3.3 ab	1.3 de

Mean values followed by different letters in the column are significantly different according to DMRT at $p<0.05$.

In conclusion, the present investigation reveals the varied response of *G. paniculata* L. cv. Perfecta shoot tips to BA and NAA combinations. Hyperhydricity was controlled by the high level of agar in the culture medium, as the use of gelrite resulted in high hyperhydricity. The recommended sucrose concentration used for plant tissue culture (30 g/l) found to be adequate for *G. paniculata* micropropagation. For *in vitro* rooting, ½ MS medium supplemented with 1 NAA + 3 IBA mg/l or 3 mg/l IBA were found to be favorable with 95 % rooting.

References

Ayeh, K.O., R. Selliah and A.K. Hvoslef-Eide, 2009. Effect of media on some *in vitro* and *ex-vitro* parameters in micropropagation of *Gypsophila paniculata* L. Academic J. Plant Sci., 2(4): 260-266.

Badr-Elden, A.M., A.A. Nower, I.A. Ibrahim, M.K.H. Ebrahim and T.M. Abd Elaziem, 2012. Minimizing the hyperhydricity associated with *in vitro* growth and development of watermelon by modifying the culture conditions. Afr. J. Biotechnol., 11(35): 8705-8717.

Barakat, M.N. and H. El-Sammak, 2011. *In vitro* culture and plant regeneration from shoot tip and lateral bud explants of *Gypsophila paniculata* L. J. Med. Plant. Res., 5(15): 3351-3358.

Bhojwani, S.S. and M.K. Razdan, 1996. Plant tissue culture: theory and practice. 2nd ed. Elsevier Publishers, Amsterdam,The Netherlands, 767.

Casanova, E., L. Moysset and M.I. Trillas, 2008. Effects of agar concentration and vessel closure on the organogenesis and hyperhydricity of adventitious carnation shoots. Biol. Plant., 52(1): 1-8.

Chen, Y., S. Lin, S. Dujuid, P. Dribnenki and E. Kenasehuk, 2003. Effect of sucrose concentration on elongation of shoots from Flax anther cultures. Plant Cell Tiss. Org. Cult., 72: 181-183.

Cheruvathur, M.K., J. Abraham and T.D. Thomas, 2015. *In vitro* micropropagation and flowering in *Ipomoea sepiaria* Roxb. An important ethanomedicinal plant. Asian Pacific J. Reprod., 4(1): 49-53.

Dewir, Y.H., D. Chakrabarty, E.J. Hahn and K.Y. Paek, 2006. A simple method for mass propagation of *Spathiphyllum cannifolium* using an airlift bioreactor. In Vitro Cell. Dev. Biol.—Plant, 42: 291-297.

Duncan, D.B., 1995. Multiple range and multiple F tests. Biometrics, 11: 1-42.

Ebrahim, M.K.H. and I.A. Ibrahim, 2000. Influence of medium solidification and pH value on *in vitro* propagation of *Maranta leuconeura* cv. Kerchoviana. Sci. Hort., 86: 211-221.

Franck, T., C. Kevers, T. Gaspar, J. Dommes, C. Deby, R. Greimers, D. Serteyn and G. Deby-Dupont, 2004. Hyperhydricity of *Prunus avium* shoots cultured on gelrite: a controlled stress response. Plant Physiol. Biochem., 42: 519-527.

Gabryszewska, E., 2011. Effect of various levels of sucrose, nitrogen salts and temperature on the growth and development of *Syringa vulgaris* L. shoots *in vitro*. J. Fruit Ornam. Plant Res., 19(2): 133-148.

Gaspar, T., 1991. Vitrification in micropropagation. In: Bajaj Y.P.S., (ed.), Biotechnology in agriculture and forestry. Vol. 17. High-tech and micropropagation I. Springer-Verlag Berlin Heidelberg, 116-126.

Gaspar, T.H., C. Kevers, P. Debergh, L. Maene, M. Pâques and P.H. Boxus, 1987. Vitrification: morphological, physiological, and ecological aspects. In: Bonga J.M. and D.J. Durzan, (eds.), Cell and tissue culture in forestry. Vol. 1. General principles and biotechnology. Martinus Nijhoff, Dordrecht., 152-166.

Gibson, S.I., 2000. Plant sugar-response pathways. Part of a complex regulatory web. Plant Physiol., 124: 1532-1539.

Han, B.H., K.Y. Paek and J.K. Choi, 1991a. Micropropagation of *Gypsophila paniculata* using shoot tip culture *in vitro*. J. Korean Soc. Hort. Sci., 32: 394-400.

Han, B.H., K.Y. Paek and J.K. Choi, 1991b. Prevention of vitrification of *Gypsophila paniculata* regenerated *in vitro*. J. Korean Soc. Hort. Sci., 32: 518-524.

Hartmann, H.T., D.E. Kester, F.T. Davies and R.L. Geneve, 1996. Plant propagation: principles and practices. Prentice-Hall, Englewood Cliffs, NJ, USA.

Huh, Y.S., J.K. Lee, S.Y. Nam, E.Y. Hong, K.Y. Paek and S.W. Son, 2016. Effects of altering medium strength and sucrose concentration on *in vitro* germination and seedling growth of *Cypripedium macranthos* Sw. J. Plant Biotechnol., 43: 132-137.

Ilczuk, A., K. Jagiełło-Kubiec and E. Jacygrad, 2013. The effect of carbon source in culture medium on micropropagation of common ninebark (*Physocarpus opulifolius* (L.) maxim.) 'Diable D'or'. Acta Sci. Pol., Hortorum Cultus, 12(3): 23-33.

Ivanova, M. and J. Van Staden, 2011. Influence of gelling agent and cytokinins on the control of hyperhydricity in *Aloe polyphylla*. Plant Cell Tiss. Org. Cult., 104: 13-21.

Jain, N. and S.H. Babbar, 2002. Gum katira - a cheap gelling agent for plant tissue culture media. Plant Cell Tiss. Org. Cult., 71: 223-229.

Kocaoğlu Kavas, M. and M. Yorgancılar, 2016. Determination of the response of *Gypsophila arrostii* Guss. to boron under *in vitro* conditions. J. Elem., 21(1): 67-76.

Kozai, T., 1991. Micropropagation under photoautotrophic conditions. In: Debergh P.C. and R.H. Zimmerman, (eds.), Micropropagation: technology and application. Kluwer Academic Publishers, Dordrecht, 447-469.

Lichtenthaler, H.K., 1987. Cholorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol., 148: 350-382.

Molassiotis, A.N., K. Dimassi, I. Therios and G. Diamantidis, 2003. Fe-EDDHA promotes rooting of rootstock GF-677 (*Prunus amygdalus* × *P. persica*) explants *in vitro*. Biol. Plant., 47(1): 141-144.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.

Nag, S., K. Saha and M.A. Choudhuri, 2001. Role of auxin and polyamines in adventitious root formation in relation to changes in compounds involved in rooting. J. Plant Growth Regul., 20(2): 182-194.

Nairn, B.J., R.H. Furneaux and T.T. Stevenson, 1995. Identification of an agar constituent responsible for hydric control in micropropagation of radiata pine. Plant Cell Tiss. Org. Cult., 43: 1-11.

Pensec, F., A. Marmonier, A. Marchal, S. Gersch, N. Nassr, J. Chong, M. Henry, G. Demangeat and C. Bertsch, 2013. *Gypsophila paniculata* root saponins as an environmentally safe treatment against two nematodes, natural vectors of grapevine fanleaf degeneration. Aust. J. Grape Wine Res., 19: 439-445.

Pierik, R.L.M., 1987. *In vitro* propagation of higher plants. Martinus Nizhoof Publisher, Boston.

Rady, M.R., 2006. *In vitro* culture of *Gypsophila paniculata* L. and random amplified polymorphic DNA analysis of the propagated plants. Biol. Plant., 50(4): 507-513.

Rady, M. and S. Bekheet, 2008. Effect of temperature, sugar and vessel closure on *in vitro* growth of *Gypsophila paniculata*. Floricul. Ornam. Biotechnol., 2(2): 73-76.

Rathore, M.S., S. Yadav, P. Yadav, J. Kheni and B. Jha, 2015. Micropropagation of elite genotype of *Jatropha curcas* L. through enhanced axillary bud proliferation and *ex vitro* rooting. Biomass Bioenergy, 83: 501-510.

Riov, J., 1993. Endogenous and exogenous auxin conjugates in rooting of cuttings. Acta Hortic., 329: 284-288.

Sadeghi, F., A. Yadollahi, M.J. Kermani and M. Eftekhari, 2015. Optimizing culture media for *in vitro* proliferation and rooting of Tetra (*Prunus empyrean* 3) rootstock. J.Genetic Engineering Biotechnol., 13: 19-23.

Saraswathi, M.S., S. Uma, G. Kannan, M. Selvasumathi, M.M. Mustaffa and S. Backiyarani, 2016. Cost-effective tissue culture media for large-scale propagation of three commercial banana (*Musa* spp.) varieties. J. Hort. Sci. Biotechnol., 91(1): 23-29.

Shillo, R., 1985. *Gypsophila paniculata*. In: Halevy A.H. (ed.), Hand–book of flowering. CRC press, Inc, Boca Raton, Florida, 3: 83-87.

Shim, S.W., E.J. Hahn and K.Y. Paek, 2003. *In vitro* and *ex vitro* growth of grapevine rootstock '5BB' as influenced by number of air exchanges and the presence or absence of sucrose in culture media. Plant Cell Tiss. Org. Cult., 75: 57-62.

Shohael, A.M., D. Chakrabarty, M.B. Ali, K.W. Yu, E.J. Hahn, H.L. Lee and K.Y. Paek, 2006. Enhancement of eleutherosides production in embryogenic cultures of *Eleutherococcus sessiliflorus* in response to sucrose-induced osmotic stress. Process Biochem., 41: 512-518.

Silva, A.L. da, M. Rogalski and M.P. Guerra, 2003. Effects of different cytokinins on *in vitro* multiplication of *Prunus* 'Capdeboscq' rootstock. Crop Breed. Appl. Biotechnol., 3(2): 149-156.

Steinitz, B., 1999. Sugar alcohols display nonosmotic roles in regulating morphogenesis and metabolism in plants that do not produce polyols as primary photosynthetic products. J. Plant Physiol., 155: 1-8.

Sutter, E.G., 1996. General laboratory requirements, media and sterilization methods. In: Trigiano R.N. and D.J. Gray (eds.), Plant tissue culture concepts and laboratory exercises. CRC Press, New York, 11-25.

Tabart, J., T. Franck, C. Kevers and J. Dommes, 2015. Effect of polyamines and polyamine precursors on hyperhydricity in micropropagated apple shoots. Plant Cell Tiss. Organ. Cult., 120: 11-18.

Teixeira da Silva, J.A., 2004. The effect of carbon source on *in vitro* organogenensis of Chrysanthemum thin cell layers. Bragantia Campinas., 63(2): 165-177.

Tesfa, M., B. Admassu and K. Bantte, 2016. *In vitro* rooting and acclimatization of micropropagated elite sugarcane (*Saccharum officinarum* L.) genotypes - N52 and N53. J. Tiss. Sci. Eng., 7: 164. doi:10.4172/2157-7552.1000164

Thakur, S., S.R. Dhiman, Y.D. Sharma and A.K. Singh, 2013. *In vitro* regeneration using nodal sections and shoot tips of gypsophila cv. `Bristol Fairy`. J. Hill Agric., 4(2): 74-77.

Tian, J., Y. Cheng, X. Kong, M. Liu, F. Jiang and Z. Wu, 2016. Induction of reactive oxygen species and the potential role of NADPH oxidase in hyperhydricity of garlic plantlets in vitro. Protoplasma, DOI 10.1007/s00709-016-0957-z

Wang, S.M., X.C. Piao, S.Y. Park and M.L. Lian, 2013. Improved micropropagation of *Gypsophila paniculata* with bioreactor and factors affecting *ex vitro* rooting in microponic system. In Vitro Cell.Dev.Biol.-Plant, 49: 70-78.

Wu, C.H., Y.S. Dewir, E.J. Hahn and K.Y. Paek, 2006. Optimization of culturing conditions for the production of biomass and phenolics from adventitious roots of *Echinacea angustifolia*. Plant Biol., 49: 193-199.

Yotova, M., I. Krasteva and S. Nikolov, 2012. Triterpenoid saponins from genus *Gypsophila* L. (*Caryophyllaceae*). In: Koh R. and I. Tay (ed.), Saponins: properties, applications and health benefits. Nova Science Publishers, United States., 99-122.

Zdraveva, P., I. Pencheva, P. Popova, I. Ionkova and I. Krasteva, 2015. Production of saponarin in *in vitro* cultures of *Gypsophila* species. J. Chem. Pharm. Res., 7(1): 829-832.

Zha, X.Q., J.P. Luo, S.T. Jiang and J.H. Wang, 2007. Enhancement of polysaccharide production in suspension cultures of protocormlike bodies from *Dendrobium huoshanense* by optimization of medium compositions and feeding of sucrose. Process Biochem., 42: 344-351.