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Micropropagation of lisianthus (*Eustoma grandiflorum*), an ornamental plant

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Abstract

Lisianthus (*Eustoma grandiflorum)* is an ornamental plant with beautiful flowers. Micropropagation is a powerful tool for large-scale propagation of ornamental plants. The shoot tips explants from Lisianthus were cultured on MS medium supplemented with concentrations of 0, 0.5, 1 and 2 mg/L of NAA and KIN. Here, we present a simple and reliable strategy for micropropagation of *Eustoma grandiflorum* in presence of the single growth regulator, KIN, which enables the production of stock plants. Multiple shoots containing roots can be obtained simultaneously on MS basal medium only supplemented with 0.5-1 mg/L KIN. Shoot tips media supplemented with 1 mg/L KIN without NAA resulted in the best shoot length per explant (2.058 cm) and shoot number per explant (2.62). Also, the most number of nodes per explant (8.86) was obtained in medium containing 0.5 mg/L KIN without NAA. The highest root number per shoot (2.40) was seen in medium supplemented with 2 mg/L KIN + 0.5 mg/L NAA. Shoot tips grown in medium containing 2 mg/L NAA without KIN showed the most callus formation. The results of this study revealed that the best shoot proliferation was achieved in MS medium supplemented with 0.5 or 1 mg/L KIN without NAA. Regenerated plants were transferred to peat and perlite (1:1) after hardening and they showed 100% survival.

Keywords: Lisianthus, *in vitro* propagation, kinetin, naphthalene acetic acid.

Abbreviations: KIN_kinetin; MS_Murashige and Skoog; NAA_naphthalene acetic acid.

Introduction

Lisianthus (*Eustoma grandiflorum*) (Gentianaceae), a relatively new floral crop to the international market, quickly ranked in the top ten cut flowers worldwide due to its rose-like flowers, excellent post-harvest life, and being available in various colors (Kunitake et al., 1995). The cut inflorescences typically have a vase life of 3 to 6 weeks (Dennis et al., 1989). Lisianthus is a moderately cold-tolerant annual or biennial plant native to the southern part of the United States and Mexico (Roh and Lawson, 1988). This plant grows to 50-75 cm in height with 20-40 flowers. By nature, Lisianthus initially forms a rosette and grows very slowly during the winter, stems elongate in the spring, and it flowers in summer (Roh et al., 1989). In recent decades, breeders have developed a variety of cultivars with respect to many traits such as uniform flowering throughout the year, lack of rosetting, heat tolerance, flower color, and flower size and form, including double flowers, etc. (Harbaugh, 2006). *Eustoma grandiflorum* is commonly propagated by seed or cutting. A large number of seedlings can be produced by seed propagation but the quality is not uniform due to variations in flowering time, plant height and the number of flowers. In some cultivars, such as those with marginal variegation, these seedlings show a wide range of variation because of their heterozygous character (Furukawa et al., 1990). Lisianthus has the qualities of an "ideal cut flower" (attractive flowers and long vase life) and should continue to increase in popularity throughout the next century. Micropropagation has been extensively used for the rapid production of many plant species and cultivars. The methods for micropropagation of *Eustoma grandiflorum* have been developed and many plants were regenerated from stem, leaf and meristem explants (Furukawa et al., 1990). Multiplication of selected clones through asexual aseptic culture should be valuable in Lisianthus as it enables the large scale multiplication of selected individual plants having desirable characteristics such as high productivity, dwarf habit, disease resistance or faster growth rate (Semeniuk and Griesbach, 1987).

The success of the micropropagation method depends on several factors like genotype, media, plant growth regulators and type of explants, which should be observed during the process (Pati et al., 2005; Nhut et al., 2010). The most frequently used growth regulators for micropropagation of ornamental plants by organogenesis, embryogenesis and axillary proliferation are naphthalenacetic acid (NAA), and benzyl adenine (BA) (Jain and Ochatt, 2010). Kinetin (KIN) has been applied for micropropagation of many plants (Jain and Ochatt, 2010). The objective of the present study was to evaluate the effects of different concentrations of KIN and

NAA on regeneration of shoot and root in *Eustoma grandiflorum*.

Results

Shoot multiplication

We studied the effect of different concentrations of KIN and NAA on micropropagation of Lisianthus (*Eustoma grandiflorum*), an ornamental plant. Studied characteristics were shoot length, shoot number, node number, and root number. The results are summarized in Table 1. Our data revealed that there are differences in the effect of the different concentrations of KIN, NAA and interaction between these two growth regulators on these characters. Shoot tips were excised and transferred on MS medium containing KIN (0-2 mg/L) and NAA (0-2 mg/L). Subsequently within the next 3-4 weeks, differences were observed. The medium supplemented with 1 mg/L KIN without NAA resulted in the best shoot length (2.058 cm) and shoot number (2.62) (Table 1 and Figs. 2 and 3). Medium containing 0.5 mg/L KIN with 2.61 shoots was a proper medium. This result was comparatively better than shoot length (1.14 cm) and shoot number (1.10) of the control. The lowest shoot length (0.856 cm) was obtained from plantlets grown in medium containing 2 mg/L NAA without KIN (Table 1, Fig. 2). The greatest callus was observed in the base of shoots grown on this medium (Fig. 2). The least shoot number (0.00) was seen in medium supplemented with 1 and 2 mg/L NAA without KIN (Table 1).

Data analysis showed that the effect of different concentrations of KIN and NAA were significant on the shoot number ($p \leq 0.01$) (Table 2). Also, NAA had significant effect on shoot length ($p \leq 0.01$), but the effect of KIN on shoot length was no significant (Table 2). The effect of KIN × NAA on the shoot length and shoot number was significant ($p \leq 0.05$) (Table 2). When the shoot tips were inoculated in the medium containing 0.5 mg/L KIN without NAA, the best result was observed for node number (8.86) (Table 1 and Fig. 4). The least node number (1.32) was calculated in medium supplemented with 2 mg/L NAA + 1 mg/L KIN (Table 1, Fig. 4). Analysis of variance showed that the effect of KIN, NAA and KIN × NAA on the node number were significant ($p \leq 0.01$) (Table 2).

Rooting and plant acclimatization

The largest number of roots was found when we used 2 mg/L KIN + 0.5 mg/L NAA (2.40) and 0.5 mg/L KIN without NAA (1.60) (Table 1 and Fig. 5). Data analysis showed that the effect of KIN, NAA and KIN × NAA were no significant on the root number (Table 2). Rooted plantlets were successfully transferred to the soil. The results of acclimatization showed that the 100% of plantlets were survived to grow under greenhouse conditions and were morphologically similar to mother plants (Fig. 6). A mixture of light soil with good drainage is suitable for acclimatization of Lisianthus (*Eustoma grandiflorum*).

Discussion

A micropropagation approach for Lisianthus, an ornamental plant, using KIN and NAA was developed. Cytokinins are

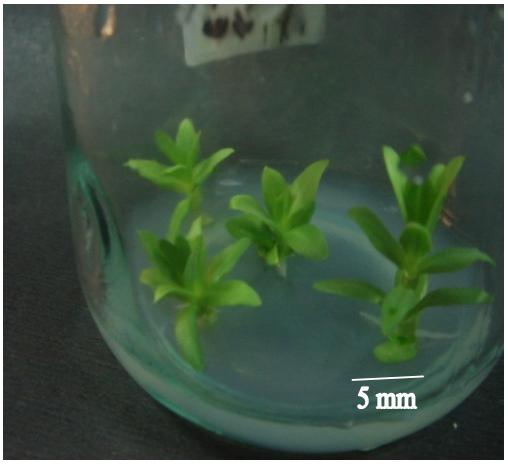

Fig 1. Seedlings of *Eustoma grandiflorum* in *in vitro* conditions, and shoot proliferation in solid MS medium. Shoot tips were cut from the mother plants as explants and cultured in MS medium containing plant growth regulators.

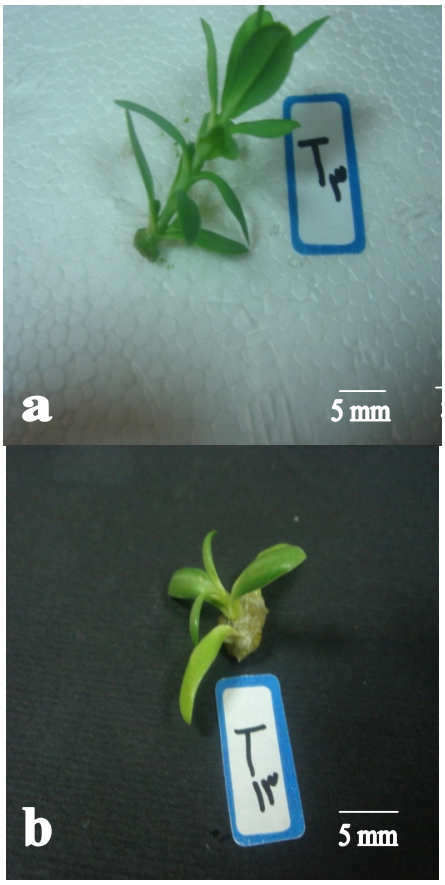

Fig 2. Effect of different concentrations of KIN and NAA on shoot length of *Eustoma grandiflorum*. a) highest shoot length (1 mg/L KIN without NAA), and b) lowest shoot length (2 mg/L NAA without KIN). Cytokinin KIN has important role in increasing shoot length of Lisianthus.

Table 1. Mean comparison of the effect of different concentrations of KIN and NAA on shoot length, shoot number, and shoot and root number of *Eustoma grandiflorum*.

Treatments (KIN + NAA) (mg/L)	Shoot length (cm)	Shoot No.	Node No.	Root No.
0 + 0	1.140^{bd}	1.10^{cd}	2.72^{d}	0.60^{c}
0.5 + 0	1.434^{bc}	2.61^{a}	8.86^{a}	1.60^{bc}
1 + 0	2.058^{a}	2.62^{a}	4.28^{bc}	0.00^{c}
2 + 0	1.554^{b}	2.20^{ab}	4.70^{b}	0.00^{c}
0 + 0.5	1.270^{bd}	0.40^{de}	3.04^{cd}	0.00^{c}
0.5 + 0.5	1.160^{bd}	1.40^{bd}	2.80^{cd}	0.00^{c}
1 + 0.5	1.178^{bd}	1.20^{bd}	2.88^{cd}	0.60^{c}
2 + 0.5	1.150^{bd}	2.00^{ac}	2.96^{cd}	2.40^{a}
0 + 1	0.974^{d}	0.00^{e}	2.19^{de}	0.00^{c}
0.5 + 1	1.080^{cd}	2.00^{ac}	3.08^{cd}	0.00^{c}
1 + 1	0.976^{d}	1.18^{bd}	2.72^{d}	2.20^{b}
2 + 1	0.860^{d}	2.19^{ab}	1.94^{de}	0.00^{c}
0 + 2	1.202^{bd}	0.40^{de}	2.50^{de}	0.00^{c}
0.5 + 2	1.016^{cd}	0.40^{de}	2.14^{de}	0.00^{c}
1 + 2	0.858^{d}	0.00^{e}	1.32^{e}	0.00^{c}
2 + 2	1.130^{cd}	1.40^{bd}	3.15^{cd}	0.00^{c}

In each column, means with the similar letters are not significantly different at 5% level of probability using Duncan's test.

Table 2. Analysis of variance (ANOVA) for the effect of different concentrations of KIN and NAA on the root length and number, callugenesis percent and fresh weight of *Matthiola incana*.

Source of variations	df	M.S.			
		Shoot length	Shoot No.	Node No.	Root No.
KIN	3	0.05^{ns}	1.28**	10.44**	0.06^{ns}
NAA	3	1.29**	1.34**	34.07**	0.21^{ns}
KIN × NAA	9	0.28*	0.21*	9.50**	0.37^{ns}
Error	64	0.11	0.09	0.68	0.23
Total	79	-	-	-	-
c.v.		27.99	24.11	25.89	56.56

**: Significant at α = 1%, *: Significant at α = 5%, ns=Not significant

usually used on the micropropagation media to stimulate axillary shoot proliferation (Nitsch et al., 1967; Jain and Ochatt, 2010). This study showed important role of KIN on micropropagation of Lisianthus. Studies of Xue-hua et al. (2009) on micropropagation of Lisianthus (*Eustoma grandiflorum*) showed that MS basal medium supplemented with 0.1-0.5 mg/L BA + 0.05 mg/L NAA was suitable for adventitious shoot differentiation; 0.1 mg/L BA + 0.02-0.05 mg/L NAA for sub-multiplication, and 0.5-1 mg/L IBA for adventitious root formation. Ordogh et al. (2006) revealed that the highest number of shoots in Echo cultivars of *Eustoma grandiflorum* was obtained on MS medium with 0.1 mg/L BA. Reduction of the shoot number occurred on the medium without BA. The highest percent of roots was found on medium with 1.0 mg/L NAA. Higher concentrations (2.0 and 3.0 mg/L) of NAA reduced the number of roots. Studies of Ming-xia et al. (2008) on *Eustoma grandiflorum* showed that the best proliferation medium was MS + 0.8 mg/L BA + 0.04 mg/L NAA. Evaluation of Paek and Hahn (2000) on *Eustoma grandiflorum* demonstrated that BA and KIN at high concentrations (13-22 and 14-23 µM) resulted in good shoot formation, but high percentages of hyperhydric shoots. Increased indole-3-acetic acid (IAA) and IBA concentrations favored root formation, while increased NAA concentrations adversely affected root formation and led to increased callus formation. Pierik (1987) indicated that NAA is a strong auxin and relatively low concentrations are needed for root formation. With high concentrations of NAA, root formation fails to occur and callus formation takes place. Used concentrations of NAA in our studies had no significant effect on root induction of Lisianthus and caused callus formation on the base of shoots. Lower concentrations of NAA must be examined. Studies of Fukai et al. (1996) showed that the medium containing 0.1 mg/L BA + 0.01 mg/L NAA produced the highest number of healthy shoots per explants in *Eustoma grandiflorum*. Explants of shoot tips of Lisianthus developed into multiple shoots on a medium supplemented with 3 mg/L BA + 0.2 mg/L NAA (Semeniuk and Griesbach, 1987). Rooting was induced in culture with multiple shoots by sub-culturing explants on a ½MS medium supplemented with 2 mg/L IAA. The addition of NAA to the medium containing BA did not increase the number of shoots produced. Studies on other ornamental plants showed the role of cytokinins especially KIN on proliferation (Jain and Ochatt, 2010). Gomes et al. (2010) found that KIN was more effective in promoting shoot growth of *Arbutus unedo* L. than other cytokinins. In current study the highest rates of shoot production were obtained when shoot tips were cultured on the medium supplemented with 0.5 and 1 mg/L KIN without NAA. In accordance with our finding, Gomes et al. (2010) showed that NAA was unable to improve the multiplication rate. Best results were achieved on media without NAA. Some species may require a low concentration of auxin in combination with high levels of cytokinins to increase shoot proliferation (Van Staden et al., 2008; Hashemabadi and Kaviani, 2010).

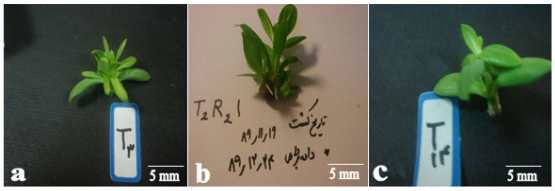

Fig 3. Effect of different concentrations of KIN and NAA on shoot number of *Eustoma grandiflorum*. a) largest shoot number (1 mg/L KIN without NAA), b) largest shoot number (0.5 mg/L KIN without NAA), and c) lowest shoot number (0.5 mg/L KIN with 2 mg/L NAA). Cytokinin KIN has important role in increasing shoot number of Lisianthus.

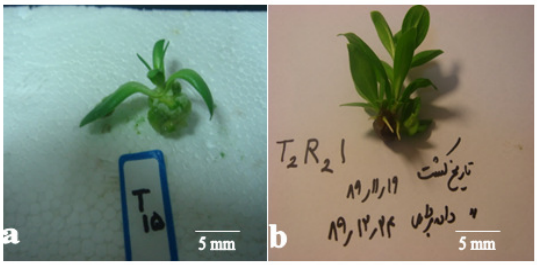

Fig 4. Effect of different concentrations of KIN and NAA on node number of *Eustoma grandiflorum*. a) Lowest node number (1 mg/L KIN with 2 mg/L NAA), and b) largest node number (0.5 mg/L KIN without NAA). Cytokinin KIN has important role in increasing node number of Lisianthus.

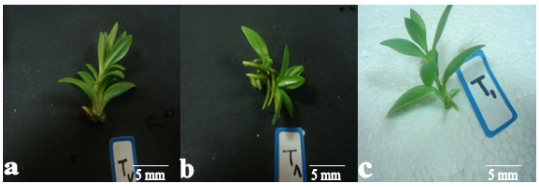

Fig 5. Effect of different concentrations of KIN and NAA on root number of *Eustoma grandiflorum*. a) Lowest root number (1 mg/L KIN with 0.5 mg/L NAA), b) largest root number (2 mg/L KIN with 0.5 mg/L NAA), and c) lowest root number (control). Suitable proportions of auxin NAA and cytokinin KIN have important role in increasing root number of Lisianthus.

Contrary to our results, studies of Fuller and Fuller (1995) on the micropropagation of *Brassica* spp. showed that the most shoot percentage (88.3%) obtained in medium containing 2 mg/L IBA + 4 mg/L KIN. Studies of Tatari Vernosefadrani et al. (2009) on micropropagation of *Gerbera jamesonii* using different growth regulators showed that the most proliferation and plantlets length obtained in medium containing 2 mg/L KIN. In contrast with our results, studies on *Bambusa arundinacea* showed that the highest multiplication was shown in medium without KIN (Nayak et al., 2010). Our findings demonstrated that the addition of NAA in culture media was no effective for increasing the root number and length. Some studies showed the positive effect of NAA on rooting (Gautam et al., 1983; Xilin, 1992; Hammaudeh et al.,

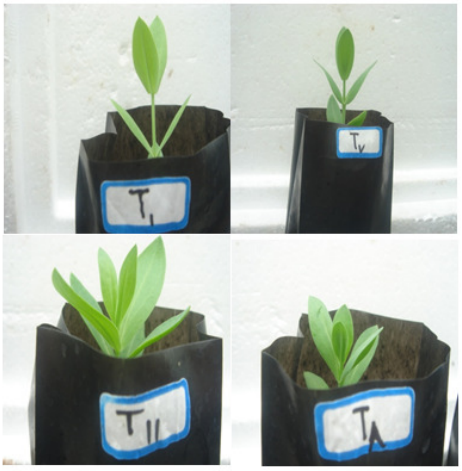

Fig 6. Hardening process of *Eustoma grandiflorum* plantlets. Plantlets were transferred to plastic pots containing a mixture of peat and perlite (1:1). Pots were kept in a greenhouse. The 100% of plantlets were survived to grow under greenhouse conditions.

1998; Lee-Epinosa et al., 2008; Jain and Ochatt, 2010). Rooting is a crucial step to the success of micropropagation. Some studies showed the positive effect of cytokinins on rooting (Gomes et al., 2010). Contrary to our findings, root formation was inhibited in the medium culture of *Lilium longiflorum* Georgia containing BA (Han et al., 2004). Also, Fuller and Fuller (1995) demonstrated that the most percentage of explants regeneration with root percent (65.0%) in *Brassica* spp. obtained in culture medium supplemented with 2 mg/L IBA without KIN. Studies of Gomes et al. (2010) on *Arbutus unedo* L. showed that shoots produced on higher cytokinin-containing medium are more amenable to root induction than shoots obtained with the lowest concentrations of BA. In a study on *in vitro* micropropagation of orchid, NAA stimulated root growth (Kalimuthu et al., 2007). Hartmann et al. (1997) have recommended brief exposure to auxins for root induction and not for prolonged growth.

Materials and methods

Plant materials and surface sterilization

Lisianthus (*Eustoma grandiflorum*) mother plants were prepared from a commercial greenhouse, Iran. Shoot tips were cut from the mother plants as explants and washed thoroughly under running tap water and a few drops of hand washing for 20 min. After three times rinses with distilled water, explants were sterilized for 30 sec in 70% ethanol followed by three times rinses with sterile distilled water (15 min each). Then, explants were disinfected with a 2% NaOCl aqueous solution and Tween-20 for 15 min then rinsed three times in sterile distilled water (10 min each).

Culture media and culture conditions

Five explants were cultivated in culture flasks on half strength macro- and micro salts of MS (Murashige and

Skoog, 1962) basal medium supplemented with 0, 0.5, 1 and 2 mg/L of KIN and 0, 0.5, 1 and 2 mg/L of NAA (16 treatments) (Fig. 1). The media were adjusted to pH 5.6-5.8 and solidified with 7 g/L Agar-agar. The media were pH adjusted before autoclaving at 121°C, 1 atm. for 30 min. The cultures were incubated in growth chamber whose environmental conditions were adjusted to 26±2°C and 75-80% relative humidity, under a photosynthetic photon density flux 50 µmol/m^2/s with a photoperiod of 16 h per day.

Measurements and acclimatization

Characters including shoot length, shoot number, node number, and root number were calculated after 45 days. Matured plantlets were washed with sterile distilled water and transferred into the plastic bags (10-cm in diameter) containing a mixture of peat and perlite (1:1). Plantlets were kept in a greenhouse at 24 ± 2°C and 70% RH with periodic irrigation.

Statistical analysis

The experimental design was R.C.B.D. Each experiment was carried out in five replicates and each replicate includes five specimens (totally; 25 specimens for each treatment). Data processing of the results was carried out by an EXCEL. Analysis of variance (ANOVA) was done using MSTATC statistical software and means were compared using Duncan's test.

Conclusion

The results of our experiment indicate that KIN is a suitable plant growth regulator on micropropagation of Lisianthus (*Eustoma grandiflorum*) as a single hormone. Also, proper types and concentrations of growth regulators (KIN and NAA) lead to increased shoot and root formation of Lisianthus cultured *in vitro*.

References

Dennis DJ, Ohteki T, Doreen J (1989) Responses of three cut flower selections of lisianthus (*Eustoma grandiflorum*) to spacing, pruning and nitrogen application rate under plastic tunnel protection. Acta Hortic 246: 237-246.

Fukai S, Miyata H, Goi M (1996) Factors affecting adventitious shoot regeneration from leaf explants of prairie gentian (*Eustoma grandiflorum* (Raf.) Shinners). Technical Bulletin of Faculty of Agriculture-Kagawa University 48 (2): 103-109.

Fuller MP, Fuller FM (1995) Plant tissue culture using Brassica seedlings. J Biol Edu 20 (1): 53-59.

Furukawa H, Matsubara C, Shigematsu N (1990) Shoot regeneration from the roots of Prairie gentian (*Eustoma grandiflorum* (Griseb.) Schinners). Plant Tiss Cult Letters 7 (1): 11-13.

Gautam VK, Mittal A, Nanda K, Gupta SC (1983) *In vitro* regeneration of plantlets from somatic explants of *Matthiola incana*. Plant Sci Letters 29: 25-32.

Gomes F, Simões M, Lopes ML, Canhoto M (2010) Effect of plant growth regulators and genotype on the micropropagation of adult trees of *Arbutus unedo* L. (strawberry tree). New Biotech 45 (1): 72-82.

Hammaudeh HY, Suwwan MA, Abu Quoud HA, Shibli RA (1998) Micropropagation and regeneration of Honeoye strawberry. Dirasat Agric Sci 25: 170-178.

Han BH, Yu HJ, Yae BW, Peak KY (2004) *In vitro* micropropagation of *Lilium longiflorum* 'Georgia' by shoot formation as influenced by addition of liquid medium. Scientia Hortic 103: 39-49.

Harbaugh BK (2006) Lisianthus, *Eustoma grandiflorum*. In: Anderson NO (Ed), Flower Breeding and Genetics, Springer, Netherlands, pp 645–663.

Hartmann HJ, Kester DE, Davies FT, Geneve RT (1997) Plant Propagation: Principle and Practices. 6th ed, Prentica-Hill, Englewood Cliffs, NJ.

Hashemabadi D, Kaviani B (2010) *In vitro* proliferation of an important medicinal plant Aloe- A method for rapid production. Aus J Crop Sci 4 (4): 216-222.

Jain SM, Ochatt SJ (2010) Protocols for *In Vitro* Propagation of Ornamental Plants. Springer Protocols. Humana Press.

Kalimuthu K, Senthilkumar R, Vijayakumar S (2007) *In vitro* micropropagation of orchid, *Oncidium* sp. (Dancing Dolls). Afr J Biotech 6 (10): 1171-1174.

Kunitake H, Nakashima T, Mori K, Tanaka M, Mii M (1995) Plant regeneration from mesophyll protoplasts of lisianthus (*Eustoma grandiflorum*) by adding activated charcoal into protoplast culture medium. Plant Cell Tiss Org Cult 43: 59-65.

Lee-Epinosa HE, Murguia-Gonzalez J, Garcia-Rosas B, Cordova-Contreras AL, Laguna C (2008) *In vitro* clonal propagation of vanilla (*Vanilla planifolia* Andrews). HortSci 43: 454-458.

Ming-xia G, et al. (2008) Study on rapid propagation technology of *Eustoma grandiflorum in vitro*. J Anhuo Agric Sci, Abstract, p 9.

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

Nayak S, Hatwar B, Jain A (2010) Effect of cytokinin and auxins on meristem culture of *Bambusa arundinacea*. Der Pharmacia Letter 2 (1): 408-414.

Nhut DT, Hai NT, Phan MX (2010) A highly efficient protocol for micropropagation of *Begonia tuberous*. In: Jain SM, Ochatt SJ (eds) Protocols for *In Vitro* Propagation of Ornamental Plants, Springer protocols. Humana Press pp 15-20.

Nitsch JP, Nitsch C, Rossini LME, Ha DBD (1967) The role of adenine on bud differentiation. Photomorph 17: 446-453.

Ordogh M, Jambor-Benczur E, Tilly-Mandy A (2006) Micropropagation of Echo cultivars of *Eustoma grandiflorum* (ISHS) Acta horticulture: V International Symposium on *In Vitro* Culture and Horticultural Breeding 725:457-460.

Paek KY, Hahn EJ (2000) Cytokinins, auxins and activated charcoal affect organogenesis and anatomical characteristics of shoot-tip cultures of lisianthus [*Eustoma grandiflorum* (Raf.) Shinn]. In Vitro Cell Dev Biol-Plant 36: 128-132.

Pati PK, Rath SP, Sharma M, Sood A, Ahuja PS (2005) *In vitro* propagation of rose-a review. Biotech Advances 94-114.

Pierik RLM (1987) *In vitro* culture of higher plants. Dordrecht, The Netherlands, Martinus Nijhoff 45–82.

Roh MS, Halevy AH, Wilkins HF (1989) *Eustoma grandiflorum*. In: Halevy AH (Ed), Handbook of Flowering, CRC Press, Boca Raton, FL, pp 322–327.

Roh SM, Lawson RH (1988) Tissue culture in the improvement of *Eustoma*. HortSci 23: 658.

Semeniuk P, Griesbach RJ (1987). *In vitro* propagation of prairie gentian. Plant Cell Tiss Org Cult 8: 249-253.

Tatari Vernosefadrani M, Askari Raberi N, Nosrati SZ (2009) Optimization of *in vitro* culture for *Gerbera* cv. Tropic Blend. J Sapling Seed 2 (25): 389-401 (In Persian).

Van Staden D, Zazimalora E, George EF (2008) Plant growth regulators, II: cytokinins, their analogues and inhibitors. In: Plant Propagation by Tissue Culture (edn 3) (George EF, et al eds), pp 205-226, Springer.

Xilin H (1992) Effect of different cultivars and hormonal conditions on strawberry anther culture *in vitro*. J Nanjing Agric Univ 15: 21-28.

Xue-hua J, Wei Y, You-lin L, Xiang-ying K, Xiu-chun P (2009) Aseptic seeding and establishment of plantlet regeneration system in *Eustoma grandiflorum*. J Anhui Agric Sci, Abstract, p 5.