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In vitro FLOWERING AND MICROPROPAGATION OF LISIANTHUS (*Eustoma grandiflorum*) IN RESPONSE TO PLANT GROWTH REGULATORS (NAA AND BA)

Behzad Kaviani[1], Forouzan Zamiraee[1], Sahar Bohlooli Zanjani[2], Alireza Tarang[2], Ali Mohammadi Torkashvand[1]

[1]Rasht Branch, Islamic Azad University, Rasht, Iran

[2]Agricultural Biotechnology Research Institute of Iran (North Region), Rasht, Guilan, Iran

Abstract. *In vitro* flowering and micropropagation are useful for plant breeding programs and commercial production of important ornamental plants. *In vitro* conditions including media components, kind, concentration and ratio of plant growth regulators and culture conditions significantly affect *in vitro* flowering and micropropagation. There is no any report dealing with the *in vitro* flowering of Lisianthus (*Eustoma grandiflorum*). Here, a protocol was developed for flowering and high frequency *in vitro* micropropagation of *E. grandiflorum*, an ornamental plant. Micropropagation is an effective tools for propagation of ornamental plants in large scale. The aim of the present study was to evaluate the effect of different concentrations of NAA and BA on micropropagation and flowering of Lisianthus, *in vitro*. Used culture medium was MS enriched with 0, 0.1, 0.2 and 2 mg L^{-1} of NAA and BA. In establishment process of explants, the most shoot length (2.07 cm per plant) was obtained on medium supplemented with 0.1 mg L^{-1} BA (without NAA). Maximum shoot number (5.80 per plant) was produced in medium containing 0.1 mg L^{-1} BA along with 0.2 mg L^{-1} NAA. Bud explants in culture media containing 0.2 mg L^{-1} NAA (without BA) and 0.1 mg L^{-1} NAA along with 2 mg L^{-1} BA produced maximum node number (3.20 per plant). The largest number of root (14.53 per plant) and root length (3.87 cm per plant) were produced on 0.2 mg L^{-1} NAA without BA, also 0.2 mg L^{-1} BA plus 0.2 mg L^{-1} NAA and 0.2 mg L^{-1} BA without NAA. Explants produced flower on medium containing 0.1 mg L^{-1} BA along with 0.1 mg L^{-1} NAA without transition of callus formation. Flower was produced from callus in medium containing 0.1 mg L^{-1} BA along with 2 mg L^{-1} NAA. Regenerated plants showed 98% survival in greenhouse during acclimatization. Acclimatized plants were morphologically similar to the mother plants.

Key words: axillary buds, callus, ornamental plants, plant tissue culture

Corresponding author: Behzad Kaviani, Department of Horticultural Science, Rasht Branch, Islamic Azad University, Rasht, Iran, e-mail: b.kaviani@yahoo.com

INTRODUCTION

Lisianthus (*Eustoma grandiflorum*) (Gentianaceae), quickly ranked in the top ten cut flowers worldwide due to its rose-like flowers and being available in various colours [Kunitake et al. 1995]. 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 colour, and flower size and form, including double flowers, etc. [Harbaugh 2006]. *E. 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. Lisianthus has the qualities of an "ideal cut flower" and should continue to increase in popularity throughout the next century.

The number of papers dealing with the *in vitro* propagation of *E. grandiflorum* is relatively low. Some studies on micropropagation of *E. grandiflorum* have been reported by Paek and Hahn [2000], Ordogh et al. [2006], Ming-xia et al. [2008], Xue-hua et al. [2009], Mousavi et al. [2012] and Ghaffari Esizad et al. [2012]. Most of these researchers have been used of shoot tips as explants and BA, KIN, NAA and IBA as plant growth regulators. 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].

Flowering stage is one of the most important process in plant development. Plant flowering is controlled by several factors, both genetic and environmental such as medium pH, carbohydrate, agar, light intensity and size of explants [Kulpa and Nowak 2011]. The most important media components for flowering are kind and concentration of plant growth regulators [Taylor and van Staden 2006]. Gibberellic acid (GA_3) and cytokinins are considered plant growth regulators responsible for induction of flower *in vitro* [Kulpa and Nowak 2011]. Some studies have been indicated flowering of ornamental plants *in vitro* such as in roses [Wang et al. 2002], orchids [Tee et al. 2008], *Spathiphyllum* [Dewir et al. 2007] and *Petunia* [Kulpa and Nowak 2011]. *In vitro* flowering is a useful tool for plant breeding programs and commercial production of important ornamental plants. The objective of the present study was to determine the influence of the concentrations of BA and NAA in the medium of *E. grandiflorum* propagated *in vitro* and also the choice of the optimal media for shoot and root production as well as the induction of flowering *in vitro*.

MATERIALS AND METHODS

Plant materials and surface sterilization. Lisianthus (*Eustoma grandiflorum*) mother plants were prepared from a commercial greenhouse, Mahallat city, Iran. Lateral buds were cut from the mother plants as explants and washed thoroughly under running tap water and a few drops of hand washing liquid for 20 min. After three times rinses with distilled water, explants were sterilized for 40 sec in 70% ethanol followed by three times rinses with sterile distilled water (15 min each). Then, explants were disin-

fected with 300 mg L^{-1} nano-silver 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, 0.1, 0.2 and 2 mg L^{-1} of both BA and NAA (16 treatments). The media were adjusted to pH 5.6–5.8 and solidified with 7 g L^{-1} 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, root number and flowering were calculated after 36 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 three replicates and each replicate includes five explants (totally; 15 explants 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.

RESULTS

Shoot multiplication. Studied characteristics on shoots were shoot length, shoot number and node number. The results are summarized in Tables 1 and 2. Our data revealed that there are differences in the effect of the different concentrations of BA, NAA and interaction between these two growth regulators on the characters except for the effect of BA on node number (fig. 1, tab. 2). When different BA concentrations were tested, the best results were obtained with 0.1 mg L^{-1}. When this concentration of BA was applied, the shoot length (2.07 cm per plantlet) and shoot number (5.80 per plantlet) were significantly higher than the other concentrations tested ($p \leq 0.01$) (tabs 1 and 2). The medium enriched with 0.2 mg L^{-1} BA along with 2 mg L^{-1} NAA and 2 mg L^{-1} NAA without BA resulted in the lowest shoot length (0.52 cm per plantlet) and shoot number (1.26 per plantlet), respectively (tab. 1). The results showed that BA was more effective than NAA on enhancing the shoot length and shoot number (tab. 1). The media containing 0.1 mg L^{-1} BA without NAA (with 4.68 shoots per plantlet), also 0.1 mg L^{-1} BA along with 0.1 mg L^{-1} NAA (with 4.13 shoots per plantlet) were proper treatments for increasing shoot number (fig. 1, tab. 1). When the explants were grown in the media containing 0.2 mg L^{-1} NAA without BA, also 2 mg L^{-1} BA along with 0.1 mg L^{-1} NAA, the best results were observed for node number (3.20) (Table 1, Fig. 1). The media containing 0.1 mg L^{-1} NAA without BA, also 0.1 mg L^{-1} BA along with 0.1 mg L^{-1} NAA (both with 3.13 node per plantlet) were suitable media for increasing node number. The least node number (1.60) was calculated in medium supplemented with 0.1 mg L^{-1} BA along with 2 mg L^{-1} NAA (tab. 1). Most callus was ob-

served in the base of shoots grown on the medium enriched with 0.1 mg L^{-1} BA along with 2 mg L^{-1} NAA. Analysis of variance (ANOVA) showed that the interaction effect of BA and NAA on the node number were significant ($p \leq 0.01$) (tab. 2).

Table 1. The mean comparison for the effect of different concentrations of BA and NAA on shoot length, shoot number, node number, root length and root number of *Eustoma grandiflorum*

Phytohormones (mg L^{-1})	Shoot length (cm)	Shoot number	Node number	Root length (cm)	Root number
BA 0 + NAA 0	1.96^{ab}	3.02^{c}	2.53^{bcd}	0.16^{h}	4.60^{cdef}
BA 0 + NAA 0.1	1.20^{cd}	1.73^{de}	2.80^{ab}	1.40^{def}	7.66^{bc}
BA 0 + NAA 0.2	1.18^{cd}	3.13^{c}	3.00^{a}	1.15^{de}	13.14^{a}
BA 0 + NAA 2	0.54^{e}	1.26^{e}	1.80^{fg}	1.44^{cd}	5.46^{bcd}
BA 0.1 + NAA 0	2.07^{a}	4.68^{b}	2.93^{abc}	1.52^{cd}	1.76^{efg}
BA 0.1 + NAA 0.1	1.83^{ab}	4.13^{b}	3.13^{ab}	1.86^{c}	0.33^{g}
BA 0.1 + NAA 0.2	1.49^{bc}	5.80^{a}	2.80^{bcd}	1.92^{c}	0.33^{g}
BA 0.1 + NAA 2	1.24^{cd}	1.66^{de}	1.60^{g}	2.96^{b}	1.13^{fg}
BA 0.2 + NAA 0	1.14^{cd}	2.63^{cd}	2.90^{abc}	3.87^{a}	3.53^{defg}
BA 0.2 + NAA 0.1	0.98^{de}	2.46^{cd}	2.86^{abcd}	0.16^{h}	4.80^{cde}
BA 0.2 + NAA 0.2	1.28^{cd}	2.40^{cd}	2.93^{abc}	0.17^{h}	14.53^{a}
BA 0.2 + NAA 2	0.52^{e}	1.80^{de}	2.00^{efg}	0.49^{gh}	13.40^{a}
BA 2 + NAA 0	1.61^{abc}	2.61^{cd}	2.35^{cdef}	0.57^{fgh}	7.66^{bc}
BA 2 + NAA 0.1	1.58^{bc}	2.06^{cd}	3.20^{a}	0.60^{fgh}	1.63^{efg}
BA 2 + NAA 0.2	0.90^{de}	2.13^{cd}	2.26^{def}	0.74^{efg}	5.40^{bcd}
BA 2 + NAA 2	0.66^{e}	1.33^{e}	2.06^{efg}	0.95^{efg}	8.60^{b}

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 BA and NAA on shoot length, shoot number, node number, root length and root number of *Eustoma grandiflorum*

Source of variations	df	MS				
		shoot length	shoot number	node number	root length	root number
BA	3	0.96^{**}	10.50^{**}	0.10^{ns}	8.05^{**}	157.52^{**}
NAA	3	1.92^{**}	8.57^{**}	3.26^{**}	4.06^{**}	65.49^{**}
BA × NAA	9	0.16^{*}	1.47^{**}	0.26^{*}	1.33^{**}	39.32^{**}
Error	32	0.06	0.313	0.10	0.07	3.73
CV (%)		20.48	20.88	12.59	22.09	22.56

** – significant at $\alpha = 1\%$, * – significant at $\alpha = 5\%$, ^{ns} – not significant

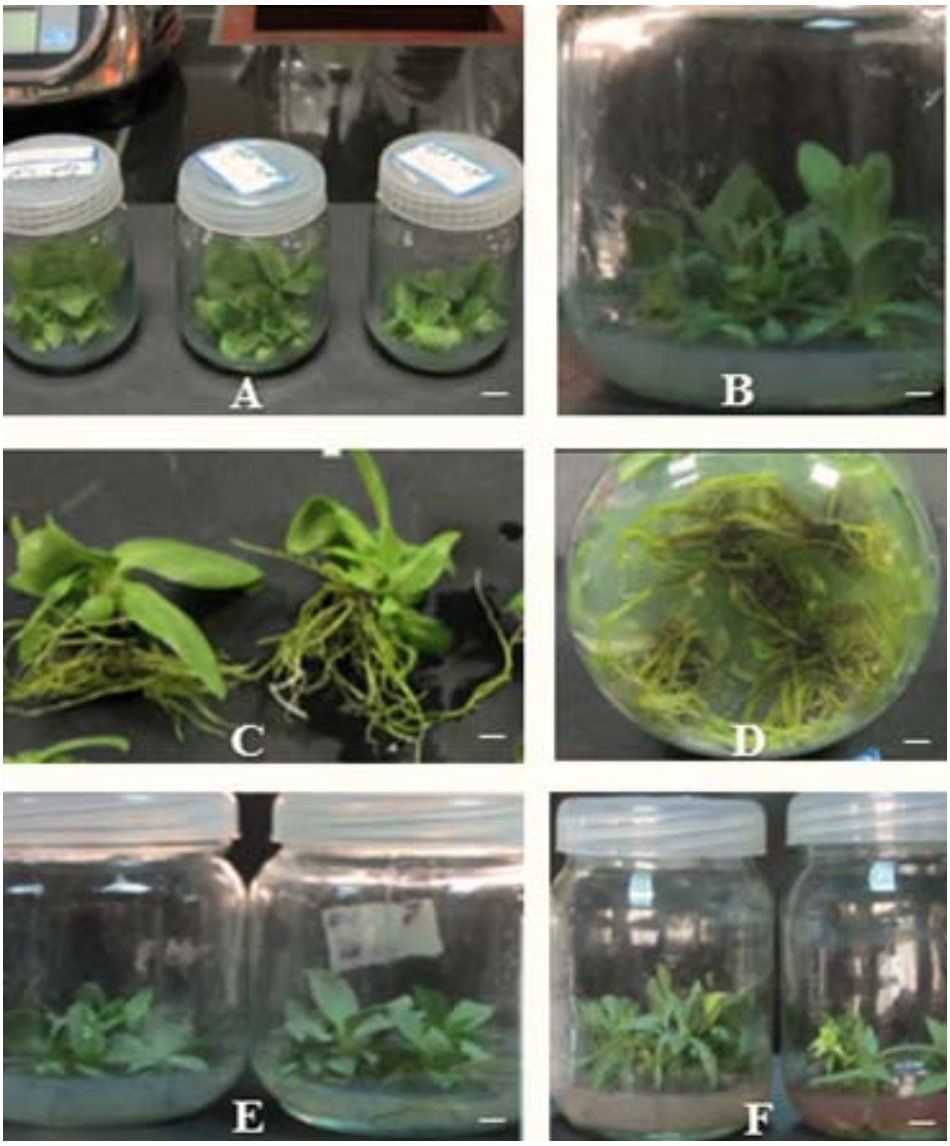

Fig. 1. Micropropagation process of *Eustoma grandiflorum* through lateral buds. A – lateral buds used as explants, Bar = 5 mm; B – lateral buds cultured on MS medium containing 0.2 mg L^{-1} NAA (largest number of node), Bar = 10 mm; C and D – lateral buds cultured on MS medium containing 0.2 mg L^{-1} BA along with 0.2 mg L^{-1} NAA (largest number of root), Bar = 5 mm; E – lateral buds cultured on MS medium containing 0.2 mg L^{-1} BA (maximum root length), Bar = 5 mm; F – lateral buds cultured on MS medium containing 0.1 mg L^{-1} BA (maximum shoot number and length and containing flower), Bar = 5 mm

Flower induction. The studies show that the addition of plant growth regulators, especially cytokinin to the media has a significant influence on *E. grandiflorum* flowering. Our study revealed the flower induction on the both of callus and shoots. Flower induction occurred on the shoots grown on the media containing 0.1 mg L^{-1} BA without and with 0.1 mg L^{-1} NAA (fig. 2). Flower also appeared on the callus produced on the medium supplemented with 0.1 mg L^{-1} BA along with 2 mg L^{-1} NAA (fig. 2). Normal floral structure was not observed in callus. Flower development in shoot tips grown on the mentioned above media was done completely (fig. 2).

Fig. 2. *In vitro* flowering. A and B – flower induction on lateral buds formed in medium supplemented with 0.1 mg L^{-1} BA without or with 0.1 mg L^{-1} NAA, Bar = 5 mm; C – flower induction on callus formed in medium supplemented with 0.1 mg L^{-1} BA along with 2 mg L^{-1} NAA, Bar = 5 mm

Fig. 3. The process of plantlets acclimatization. Acclimatization was done in a greenhouse using peat and perlite (1:1)

Rooting and plant acclimatization. When roots induced on the media containing different concentrations of BA and NAA, it was found that the highest frequencies of root formation (14.53 per plantlet) were obtained on a medium supplemented with 0.2 mg L^{-1} of both of BA and NAA (tab. 1, fig. 1). However, shoots cultured on the media enriched with 0.2 mg L^{-1} NAA without BA (with 14.13 roots per plantlet) and 0.2 mg L^{-1} BA along with 2 mg L^{-1} NAA gave the number of root higher than other treatments (tab. 1). Low number of root (0.33 per plantlet) was observed on media containing 0.1 mg L^{-1} BA along with both of 0.1 and 0.2 mg L^{-1} NAA (tab. 1). Shoots grown on the medium with 0.2 mg L^{-1} BA produced the longest root (3.87 cm per plantlets) (fig. 1). NAA was unable to improve the length of root. Comparing the root length

per explants showed that the medium without BA and NAA (control) and medium containing 0.2 mg L^{-1} BA along with 0.1 mg L^{-1} NAA were no effective for root length and induced minimum of that (0.16 cm per plantlet) (tab. 1). All media containing higher concentrations than 0.2 mg L^{-1} BA along with 0.1 mg L^{-1} NAA have negative effect on root length. The data clearly show that root number and root length are strongly affected by BA and NAA concentrations ($p \leq 0.01$) (tab. 2).

Rooted plantlets were successfully transferred to the soil (fig. 3). The results of acclimatization showed that the 98% of plantlets were survived to grow under greenhouse conditions and were morphologically similar to the mother plants. A mixture of light soil containing peat and perlite (1:1) with good drainage is suitable for acclimatization of Lisianthus (*E. grandiflorum*) (fig. 3).

DISCUSSION

The results indicate that the multiplication rate increased in the presence of optimal concentrations of BA and NAA (0.1 and 0.2 mg L^{-1}, respectively). Cytokinins are usually used on the micropropagation media to stimulate shoot multiplication [van Staden et al. 2008, Chawla 2009, Gomes et al. 2010]. The ideal concentrations differ from species to species and need to be established accurately to obtain the effective rates of multiplication [Gomes et al. 2010]. Our results showed that NAA at particular concentrations along with BA was able to increase the multiplication rate. Suitable concentrations of BA and NAA promoted callus formation. Current study showed important role of BA on micropropagation of Lisianthus. Study of Ghaffari Esizad et al. [2012] on micropropagation of Lisianthus (*E. grandiflorum*) showed the positive effect of cytokinin KIN on multiplication rate. Xue-hua et al. [2009] finding on micropropagation of Lisianthus (*E. grandiflorum*) showed that MS basal medium supplemented with 0.1–0.5 mg L^{-1} BA + 0.05 mg L^{-1} NAA was suitable for adventitious shoot differentiation. Our finding is in consistence with this result. Ordogh et al. [2006] revealed that the highest number of shoots in Echo cultivars of *E. grandiflorum* was obtained on MS medium with 0.1 mg L^{-1} BA. In the most cases, reduction of the shoot number occurred on the medium without BA. Study of Ming-xia et al. [2008] on *E. grandiflorum* showed that the best proliferation medium was MS + 0.8 mg L^{-1} BA + 0.04 mg L^{-1} NAA. Evaluation of Paek and Hahn [2000] on *E. 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. Study of Fukai et al. [1996] showed that the medium containing 0.1 mg L^{-1} BA + 0.01 mg L^{-1} NAA produced the highest number of healthy shoots per explants in *E. grandiflorum*. Shoot tip explants of Lisianthus developed into multiple shoots on a medium supplemented with 3 mg L^{-1} BA + 0.2 mg L^{-1} NAA [Semeniuk and Griesbach 1987]. Studies on the other ornamental plants showed the role of cytokinins on proliferation [Jain and Ochatt 2010, Kaviani et al. 2011, Ahmadi Hesar et al. 2011]. In current study the highest rate of shoot production was obtained when shoot tips were cultured on the medium supplemented with 0.1 mg L^{-1} BA. In contrary with our finding, Gomes et al. [2010] showed that NAA was unable to improve the multiplication rate. Some species may require a low concentration of auxin

in combination with cytokinins to increase shoot proliferation [van Staden et al. 2008, Hashemabadi and Kaviani 2010]. Fuller and Fuller [1995] showed that the most shoot percentage (88.3%) of *Brassica* spp. was obtained in medium containing 2 mg L^{-1} IBA + 4 mg L^{-1} KIN. Study of Mousavi et al. [2012] on *E. grandiflorum* revealed that cytokinins are essential to induce shooting on micro-cutting.

Our findings demonstrated that the addition of NAA in culture media was effective for increasing the root number but not for root length. Some studies showed the positive effect of NAA on rooting [Gautam et al. 1983, Xilin 1992, Hammaudeh et al. 1998, Lee-Epinosa et al. 2008, Jain and Ochatt 2010, Kaviani et al. 2011]. Rooting is a crucial step to the success of micropropagation. Some studies showed the positive effect of cytokinins on rooting [Gomes et al. 2010]. Our study demonstrated the positive effect of low concentration of BA (0.2 mg L^{-1}) with or without NAA on root number and root length. 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^{-1} 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. The highest root number per shoot (2.40) was seen in medium supplemented with 2 mg L^{-1} KIN + 0.5 mg L^{-1} NAA. Shoot tips grown in medium containing 2 mg L^{-1} NAA without KIN showed the most callus formation. Studies of Ghaffari Esizad et al. [2012] on micropropagation of Lisianthus (*E. grandiflorum*) showed that the largest number of root per shoot (2.40) was obtained in medium supplemented with 2 mg L^{-1} KIN + 0.5 mg L^{-1} NAA. Shoot tips grown in medium containing 2 mg L^{-1} NAA without KIN showed the most callus formation. Study of Mousavi et al. [2012] on *E. grandiflorum* revealed that the best development of root was obtained on medium containing 1.5 mg L^{-1} NAA. The results showed that auxin is necessary to induce rooting in *E. grandiflorum*.

In vitro flowering was observed in some ornamental plants [Wang et al. 2002, Zhang and Leung 2002, Dewir et al. 2007, Tee et al. 2008]. The study of Kulpa and Nowak [2011] on *in vitro* flowering of *Petunia* × *atkinsiana* D. Don using different concentrations of auxins, cytokinins and GA_3 showed that the nodes cultured on MS medium enriched with 0.5 mg L^{-1} KIN had the largest number of flower and the most flowering percentage. Some other observations confirm more impact of cytokinings on the flowering *in vitro* than the other growth regulators [Dielen et al. 2001, Lin et al. 2007]. Our study showed the positive effect of 0.1 mg L^{-1} BA on the flower induction of *E. grandiflorum*. Some researchers revealed that GA_3 and cytokinins are considered growth regulators responsible for inducing flowering *in vitro* [Dielen et al. 2001, Lin et al. 2007, Huang et al. 2009, Masmoudi-Allouche et al. 2010, Kulpa and Nowak 2011]. Flowering *in vitro* has been successful in many species using other conditions and products [Chaari-Rkhis et al. 2006].

CONCLUSION

1. The present study describes the protocol for miropropagation of Lisianthus (*E. grandiflorum*).

2. In the present study, BA successfully induced root length with a minimum concentration (0.1 an 0.2 mg L^{-1}).

3. The results have shown that BA successfully promoted shoot number with a minimum concentration (0.1 mg L^{-1}) individually as well as in combination with NAA (0.1 and 0.2 mg L^{-1}).

4. The most effective concentrations of NAA for root induction were 0.2 and 2 mg L^{-1} individually or in combination with 0.2 mg L^{-1} BA.

5. Flower induction on lateral buds was seen on medium enriched with 0.1 mg L^{-1} BA without or with 0.1 mg L^{-1} NAA.

REFERENCES

Ahmadi Hesar A., Kaviani B., Tarang A.R., Bohlooli S., 2011. Effect of different concentrations of kinetin on regeneration of ten weeks (*Matthiola incana*). Plant Omics J., 4(5), 236–238.

Chaari-Rkhis A., Maalej M., Ouled Messaoud S., Drira N., 2006. *In vitro* vegetative growth and flowering of olive tree in response to GA_3 treatment. Afr. J. Biotech., 5(22), 2097–2302.

Chawla H.S., 2009. Introduction to plant biotechnology. Springer.

Dewir Y., Chakrabart D., Ali M., Singh N., Hahn E., Paek K., 2007. Influence of GA_3, sucrose and solid medium/bioreactor culture on *in vitro* flowering of *Spathiphyllum* and association of glutathione metabolism. Plant Cell, Tiss. Organ Cult., 90, 225–235.

Dielen V., Lecouvet V., Dupont S., Kinet J., 2001. *In vitro* control of floral transition in tomato (*Lycopersicon esculentum* Mill.), the model for autonomously flowering plants, using the late flowering uniflora mutant. J. Exp. Bot., 52(357), 715–723.

Fukai S., Miyata H., Goi M., 1996. Factors affecting adventitious shoot regeneration from leaf explants of prairie gentian (*Eustoma grandiflorum* (Raf.) Shinners). Technic. Bull. Fac. Agric-Kagawa Univ., 48(2), 103–109.

Fuller M.P., Fuller F.M., 1995. Plant tissue culture using Brassica seedlings. J. Biol. Edu., 20(1), 53–59.

Gautam V.K., Mittal A., Nanda K., Gupta S.C., 1983. *In vitro* regeneration of plantlets from somatic explants of *Matthiola incana*. Plant Sci. Lett., 29, 25–32.

Ghaffari Esizad S., Kaviani B., Tarang A.R., Bohlooli Zanjani S., 2012. Micropropagation of lisianthus, an ornamental plant. Plant Omics J., 5, 314–319.

Gomes F., Simões M., Lopes M.L., Canhoto M., 2010. Effect of plant growth regulators and genotype on the micropropagation of adult trees of *Arbutus unedo* L. (strawberry tree). New Biotech., 27, 882–892.

Hammaudeh H.Y., Suwwan M.A., Abu Quoud H.A., Shibli R.A., 1998. Micropropagation and regeneration of Honeoye strawberry. Dirasat Agric. Sci., 25, 170–178.

Han B.H., Yu H.J., Yae B.W., Peak K.Y., 2004. *In vitro* micropropagation of *Lilium* 'Georgia' by shoot formation as influenced by addition of liquid medium. Sci. Hortic., 103, 39–49.

Harbaugh B.K., 2006. Lisianthus, *Eustoma grandiflorum*. In: flower breeding and genetics, Anderson N.O. (ed.). Springer, Netherlands, 645–663.

Hartmann H.J., Kester D.E., Davies F.T., Geneve R.T., 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.

Huang X., Yang B., Hu C., Yao J., 2009. *In vitro* induction of inflorescence in *Dioscorea zingiberensis*. Plant Cell. Tiss. Org. Cult., 99, 209–215.

Jain S.M., Ochatt S.J., 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.

Kaviani B., Ahmadi Hesar A., Kharabian Masouleh A., 2011. *In vitro* propagation of *Matthiola incana* (Brassicaceae)-an ornamental plant. Plant Omics J., 4(7), 435–440.

Kulpa D., Nowak N., 2011. *In vitro* flowering of *Petunia* × *atkinsiana* D. Don. Folia Hortic. 23/2, 125–129.

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 H.E., Murguia-Gonzalez J., Garcia-Rosas B., Cordova-Contreras A.L., Laguna C., 2008. *In vitro* clonal propagation of vanilla (*Vanilla planifolia* Andrews). HortSci. 43, 454–58.

Lin Ch.S., Liang C.J., Hsaio H.W., Lin M.J., Chang W.C., 2007. *In vitro* flowering of green and albino *Dendrocalamus latiflorus*. New Forests., 34(2), 177–186.

Masmoudi-Alloche F., Meziou B., Kriaâ W., Gargouri-Bouzid R., Drira N., 2010. *In vitro* flowering induction in date palm (*Phoenix dactylifera* L.). J. Plant Growth Reg., 29(1), 35–43.

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

Mousavi E.S., Behbahani M., Hadavi E., Miri S.M., Karimi N., 2012. Plant regeneration in *Eustoma grandiflorum* axillaries buds (Gentinaceae). Trakia J. Sci., 10(2), 75–78.

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

Nhut D.T., Hai N.T., Phan M.X., 2010. A highly efficient protocol for micropropagation of *Begonia tuberous*. In: Protocols for *in vitro* propagation of ornamental plants, Jain S.M., Ochatt S.J. (eds). Springer Protocols, Humana Press, 15–20.

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

Paek K.Y., Hahn E.J., 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 P.K., Rath S.P., Sharma M., Sood A., Ahuja P., 2005. *In vitro* propagation of rose – a review. Biotech. Adv., 24, 94–114.

Semeniuk P., Griesbach R.J., 1987. *In vitro* propagation of prairie gentian. Plant Cell Tiss. Org. Cult., 8, 249–253.

Taylor N.J., Van Staden J., 2006. Towards an understanding of *in vitro* flowering. In: Floriculture, ornamental and plant biotechnology: advances and topical issues, Da Silva J.A.T. (ed.). Global Sci. Book, London, 2, 1–22.

Tee C.S., Maziah M., Tan S.C., 2008. Induction of in vitro flowering in the orchid *Dendrobium* Sonia 17. Biol. Plant., 52(4), 723–726.

Van Staden J., Zazimalova E., George E.F., 2008. Chapter 6: Plant growth regulators, II: Introduction; cytokinins, their analogues and inhibitors. In: Plant propagation by tissue culture, 3rd edition. Vol. 1., George E.F., Hall M.A., De Klerk G.J. (eds). Springer, Dordrecht, The Netherlands, 205–226.

Wang G.Y., Yuan M.F., Hong Y., 2002. *In vitro* flower induction in roses. *In Vitro* Cell. Dev. Biol. Plant. 38, 513–518.

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, 5.

Zhang Z., Leung D., 2002. Factors influencing the growth of micropropagated shoots and *in vitro* flowering of gentian. Plant Growth Reg., 36, 245–250.

KWITNIENIE I MIKROPROPAGACJA *in vitro* LISIANTHUSA (*Eustoma grandiflorum*) W REAKCJI NA REGULATORY WZROSTU ROŚLIN (NAA I BA)

Streszczenie. Kwitnienie i mikropropagacja *in vitro* są użyteczne w programach hodowli roślin oraz produkcji komercyjnej ważnych roślin ozdobnych. Warunki *in vitro*, łącznie ze składnikami pożywek, rodzajem, stężeniem oraz proporcją regulatorów wzrostu roślin, a także warunkami hodowli, w sposób istotny wpływają na kwitnienie i mikropropagację *in vitro*. Nie istnieje żadne badanie dotyczące kwitnienia *in vitro* lisanthiusa (*Eustoma grandiflorum*). W niniejszym badaniu opracowano kwitnienie i wysoką częstotliwość mikropropagacji *in vitro* dla *E. grandiflorum,* który jest rośliną ozdobną. Mikropropagacja jest skutecznym narzędziem rozmnażania roślin ozdobnych na dużą skalę. Celem niniejszego badania była ocena wpływu różnych stężeń NAA i BA na mikropropagację i kwitnienie lisianthiusa *in vitro.* Używana pożywka hodowlana została wzbogacona za pomocą 0; 0,1; 0,2 i 2 mg L^{-1} NAA i BA. Przy powstawaniu eksplantów największa długość łodygi (2,07 cm na roślinę) była uzyskana na pożywce uzupełnionej o 0,1 mg L^{-1} BA (bez NAA). Maksymalna liczba łodyg (5,80 na roślinę) została wytworzona na pożywce zawierającej 0,1 mg L^{-1} BA wraz z 0,2 mg L^{-1} NAA. Eksplanty pączków na pożywce hodowlanej zawierającej 0,2 mg L^{-1} NAA (bez BA) oraz 0,1 mg L^{-1} NAA wraz z 2 mg L^{-1} BA wytworzyły maksymalną liczbę węzłów (3,20 na roślinę). Największą liczbę korzeni (14,53 na roślinę) oraz największą długość korzenia (3,87 na roślinę) zaobserwowano na 0,2 mg L^{-1} NAA bez BA jak również 0,2 mg L^{-1} BA plus 0,2 mg L^{-1} NAA oraz 0,2 mg L^{-1} BA bez NAA. Eksplanty tworzyły kwiat na pożywce zawierającej 0,1 mg L^{-1} BA wraz z 0,1 mg L^{-1} NAA bez przeniesienia kalusa. Kwiat był tworzony z kalusa na pożywce zawierającej 0,1 mg L^{-1} BA wraz z 2 mg L^{-1} NAA. Zregenerowane rośliny wykazały 98% przeżycie w szklarni podczas aklimatyzacji. Zaaklimatyzowane rośliny były morfologicznie podobne to swych roślin macierzystych.

Słowa kluczowe: pączki szczytowe, kalus, rośliny ozdobne, hodowla tkanek roślinnych

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