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Original Contribution

PLANT REGENERATION IN *EUSTOMA GRANDIFLORUM* FROM AXILLARIES BUDS (GENTINACEAE)

E. S. Mousavi[1*], M. Behbahani [2], E. Hadavi[1], S. M. Miri[1], N. Karimi[1]

[1]Department of Horticulture, Karaj Branch Islamic Azad University, Karaj, Iran
[2]Faculty of Biotechnology, University of Isfahan, Iran

ABSTACT
Plant regeneration in *Eustoma grandiflorum*, an ornamental plant, was achieved through *in vitro* culture. For direct organogenesis axillaries buds were transferred to a MS and B5 medium containing different concentration GA_3 and BAP. The results show, that B5 medium supplemented with 1 mg/l GA_3 +1 mg/l BAP was most effective. As result has shown rootlets formation occurred in the treatment using B5 medium with 1.5 mg/l NAA. The plantlets acclimatized and transferred to soil.

Key words: Lisianthus, axillaries buds, *in vitro* culture, organogenesis

INTRODUCTION

Eustoma grandiflorum (family *Gentinaceae*) is an ornamental plant. It is commonly used as cut and pot flower for rose-like flower and vase life (1). This plant generally propagated by vegetative methods which extremely time consuming. It is conventionally propagated through seed but is hampered by cross pollination (2-3). *In vitro* propagation of *Eustoma grandiflorum* has played a very important role in rapid multiplication with desirable traits and production of healthy and disease-free plants. Therefore, in this study we attempted to develop an efficient protocol for the rapid regeneration.

MATERIAL AND METHODS

Plant material

The explants used for the *in vitro* propagations of Lisianthus were axillaries buds from 3 mounts-old seedlings. Experiments were performed using Lisanthus cultivated in a controlled condition in the green house at Isfahan University of Iran.

Shoot regeneration

Axillaries buds with at least 2 leaves were sterilized in several methods and then the best were selected. They were surface sterilized for 1 min in a 70% (v/v) solution then in 0.5% sodium hypochlorite (10% colorox) and then were rinsed 3 times with sterile distilled water, MS (Murashig-Skoog) and B5 (Gamborg), 2% sucrose and different concentration of GA_3 and BAP. The media was adjusted to 5.7 before autoclaving at 1.05 kg/cm², 121°c for 20 min. The culture was incubated at 25±1 °c under an illumination of 1200 Lux during 16/8 h photoperiod obtained from Gro-Lux fluorescent lamps.

Rooting

Shoots excised at axillaries buds transferred in rooting medium consisting of MS or B5 media with f 2,4- D (0 - 1.5 mg/L) and NAA (0 - 1.5 mg/L). Number of root per explants and root length were recorded after 4 weeks.

Statistical analysis

The experiment consisted of factorial arrangements of treatments based on completely randomized design (CRD). Data were analyzed using MSTATC and means were evaluated by Duncan Test (5%, 1% level).

RESULTS AND DISCUSSION

Proliferation from axillaries buds

Both the concentration and type of cytokinins used markedly influenced number and length of shoots. Also cytokinins make cell division,

***Correspondence to**: *Elham sadat Mousavi, Department of Horticulture, Karaj Branch Islamic Azad University, Karaj, Iran, E-mail msvlhm@yahoo.com, Tel:00989133083014*

shoot formation and morphogenesis. The result showed that cytokinine is essential to induce shooting in Lisanthus micro cutting. As shown in **table 1** between two media tested, the average rate of plantlet regeneration was the highest on B5 medium containing 1 mg/L BAP + 1 mg/L GA_3 **(Figure 1 A)**.

According to the result, maximum number of shoots per explants observed in medium supplemented with 1 mg/LBAP + 1 mg/L GA_3, and the highest length of shoots were appeared in medium containing 1 mg/L GA_3. Increasing the concentration of BAP and decreasing of apical dominant, was the reason for decreasing the length of shoots. On the other hands, GA_3 increases the length of shoots (4). Treatment, which supplemented with two types of cytokinine was more effective. These results are in agreement with the previous reports on Alestromeria by Khaleghi (5). These results were similar to Rout who reported the same conclusion on multiplication in Rose (6).

In addition, the type of medium had influence on the number and length of shoots. B5 is better than MS medium; it seemed that the slower growth obtained with the MS medium could be due to the high contact of ammonium salts, which have been reported as growth inhibitors for some plant tissue cultures (7).

Rooting of the regenerated shoots

Result in the rootlets induction of *in vitro* plantlets derived from shoot explants cultured in B5 medium supplemented with different levels of NAA (0 - 1.5 mg/L) and 2,4-D (0 - 1.5 mg/L) were summarized in table 2. The data obtained reveled that only treatments with NAA managed to trigger the formation of rootlets from *in vitro* plantlets within 20 - 21days **(Figure 1 B)**. Other treatments tested with 2, 4-D didn't show any sign of rootlets formation.

The best development of root was observed on medium containing 1.5 mg/l NAA. Presence of 0.5 mg/l NAA improved root length as compared to the control. Explants response for rooting was higher than control in presence of 1mg/l NAA. Concentration and type of auxins used markedly influenced number of root and length of them. The result showed that auxin is essential to induce rooting in Lisanthus as a few rooting was observed in the absence of auxin. Further, it has been reported by Blackseley Caldecott (1997) that differentiating cell requires the most appropriate auxin to become competent to respond to organgenic signal (8). It is also clear from result that rooting percentage showed the tendency to increase with increasing the concentration of NAA. If the concentrations of NAA were raised from 0.5 mg/l to 1.5mg/l, there was increase in number and length of roots. Also Almaleki 2009 showed increasing of number and length root *in vitro* cultures of *Ficus anastasia* which was due to the increasing of NAA concentration and higher concentration more than 1.8 was leading to decrease of them (9). The inferior effect of NAA on the root number may be due to the reason that NAA is more persistent than IBA, remains present in the tissue and may block further development of root meristemoids (10). In the other hand, different media have different effect on plant cell due to presence of type and different amount of nutrient composition. The result showed that root forming ability was higher on B5 medium compared to MS. High level of NH^{4+} ion may be toxic to the cell as uptake of accompanying release of an H^+ equivalent, which lowers pH. High NH^{4+} level could also depress calcium, magnesium and potassium uptake which in turn restrict the nutrient flow from medium into cell and may cause low cell yield (11)

Treatments containing differences concentration of 2,4-D has no ability to organogenesis **(Figure 1 C).** According to Juliani *et al*., (1999), shoot contains high levels of endogenous auxins and the addition of strong exogenous auxin may cause the inhibition of rootlets formation and development (12). Plant tissue culture of *Rosa hybrid* tested with 2,4-D did not show any sign of rootlets formation (13).

CONCLUSION

This study indicated shoot regeneration from axillaries buds in *Eustoma grandiflorum.* Different concentration of cytokinins and composition of culture medium, were tested. Significant increase in length and number of shoot, observed in medium containing 1 mg/l GA_3 +1 mg/l BAP. It was the best in all characteristics in B5 medium comparison with MS.

Rootless was obtained by the treatment of microshoot in NAA and B5 medium as compared to the MS medium. The data revealed that only treatments with NAA lead to root formation. The maximum rooting appeared in 1.5 mg/lit NAA. In contrast, 2.4-D was not effective for root induction positively. Therefore, we proposed to develop variety treatment including hormones and media. This method maybe successful for the production.

A B C

Figure 1. (A): Shoot regeneration on axillaries buds
(B): Root formation on leaf explants on the B5 medium containing 1.5 mg/L NAA
(C): Explants in different concentration of 2, 4-D have no ability to organogenesis

Table 1. *Percentage shoot regeneration, shoot length, No. of shoot, days of shoot to form, from Eustoma grandiflorum in medium supplemented with different levels of BAP and GA3 after 4 weeks of culture*

Basal medium	Hormonal combination(mg/l) BAP	GA3	Shoot regeneration	Days of shoot to form	Shoot length) cm)	No. of shoot
B5	1	0	100	17±2	1.060 [a]	0.564 [d]
B5	1	0.5	100	16±2	1.178 [b]	1.247 [a]
B5	1	1	100	16±1	1.560 [b]	1.492 [a]
B5	0	1	100	16±2	0.406 [c]	0.731 [c]
MS	1	0	100	19±1	1.577 [a]	0.562 [d]
MS	1	0.5	100	18±2	0.584 [c]	0.935 [b]
MS	1	1	100	18±2	0.999 [b]	0.935 [b]
MS	0	1	100	18±2	0.440 [c]	0.464 [d]

Table 2. *Percentage root regeneration, root length, No. of root, days of root to form, from Eustoma grandiflorum in medium supplemented with different levels of 2.4-D and NAA after 4 weeks of culture*

Basal medium	Hormone combination		Root regeneration%	Days of root to form	Root length	No. of root
MS	0	NAA	100	21±1	0.250 [e]	11.330 [f]
MS	0.5	NAA	100	21±1	2.00 [d]	31.000 [d]
MS	1	NAA	100	20±1	3.400 [b]	44.333 [b]
MS	1.5	NAA	100	21±2	5.550 [a]	60.335 [a]
B5	0	NAA	100	21±2	0.450 [e]	15.231 [e]
B5	0.5	NAA	100	21±1	1.800 [d]	33.661 [c]
B5	1	NAA	100	21±1	2.975 [c]	41.665 [b]
B5	1.5	NAA	100	20±1	5.775 [a]	62.000 [a]
MS	0.5	2,4-D	-	-	-	-
MS	1	2,4-D	-	-	-	-
MS	1.5	2,4-D	-	-	-	-
B5	0.5	2,4-D	-	-	-	-
B5	1	2,4-D	-	-	-	
B5	1.5	2,4-D	-	-	-	

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