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MICROPROPAGATION OF TEN WEEKS (*Matthiola incana*) AND LISIANTHUS (*Eustoma grandiflorum*) (TWO ORNAMENTAL PLANTS) BY USING KINETIN (KIN), NAPHTHALENE ACETIC ACID (NAA) AND 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

Behzad Kaviani

Rasht Branch, Islamic Azad University, Rasht, Iran

Abstract. Micropropagation is a powerful tool for large-scale propagation of pathogen-free ornamental plants. Studies on micropropagation of ten weeks and lisianthus is relatively low, thus, here we present a reliable method for *in vitro* propagation of these two ornamental plants. The shoot tips explants from *Eustoma grandiflorum* and *Matthiola incana* were cultured on MS medium supplemented with concentrations of 0, 0.5, 1 and 2 mg·L of naphthalene acetic acid (NAA) and kinetin (KIN). In *Eustoma grandiflorum*, 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 (2.158 cm) and shoot number (2.68). Also, the most nodes (8.75) were obtained in medium containing 0.5 mg·L KIN without NAA. The highest root number (2.55) 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 highest content of fresh weight, dry weight and chlorophyll content were calculated in plantlets grown on media containing 0.5 mg·L KIN without NAA, 0.5 mg·L KIN + 1 mg·L NAA and control, respectively. In *Matthiola incana*, four-week-old *in vitro* plants obtained from micro-cuttings showed successful shooting and rooting. MS medium supplemented with 2 mg·L KIN without NAA resulted in the best shoot length (1.166 cm) and largest number of node (4.64). When the shoot tips were inoculated in the medium containing 2 mg·L NAA without KIN and medium containing the combination of 1 mg·L NAA + 2 mg·L KIN, the best result was observed for root number (1.85) and root length (5.2 cm).

Key words: *in vitro*, organogenesis, pathogen-free plants, 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), and *Matthiola incana* (Brassicaceae) are two important ornamental plant. Micropropagation has been extensively used for the rapid production of many plant species and cultivars. 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]. Plant growth regulators act like signals to stimulate, inhibit or regulate growth in the developmental programs of plants [Mercier et al. 1997]. 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].

Cytokinins are usually used on the micropropagation media to stimulate axillary shoot proliferation [Chawla 2009]. However, the ideal concentrations differ from species to species and need to be established accurately to obtain the effective rates of multiplication. Rooting is a crucial step to the success of micropropagation. Auxins enhance the germination, root induction and seedling growth of many species [Gautam et al. 1983, Jain and Ochatt 2010, Hashemabadi and Kaviani 2010].

The number of papers dealing with the *in vitro* cloning of *Matthiola incana* and *Eustoma grandiflorum* is scarce. 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]. Gautam et al. [1983] reported on differentiation of multiple shoot buds from cotyledon explants of *Matthiola incana*, cultured on medium containing BAP and NAA. Hosoki and Ando [1989] reported on protoplast culture of *Matthiola incana* in medium supplemented with BAP, 2,4-D and NAA. Different organs of *Matthiola incana* exhibit differential morphogenic potential. Probably, the change in response depends on the exogenous and endogenous plant growth regulators [Gautam et al. 1983].

Eustoma grandiflorum is commonly propagated by seed or cutting. A large number of seedlings can be produced by seed propagation but they are not uniform due to variations in flowering time, plant height and the number of flowers. In some cultivars, such as those with marginal variegation, 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. Nowadays, studies generally analyze the effect that a plant growth regulator exercises on the explants after a short period of time, and not its influence on later development [Feito et al. 1994, Moncaleán et al. 2003]. 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* and *Matthiola incana*.

MATERIAL AND METHODS

***Lisianthus* (*Eustoma grandiflorum*).** Mother plants of lisianthus (*Eustoma grandiflorum*) were prepared from a commercial greenhouse in 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). 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). 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 $\mu mol \cdot m^2 \cdot s$ with a photoperiod of 16 h per day. Characteristics including shoot length, shoot number, node number, and root number were evaluated 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. The experimental design was the Randomized Complete Block Design (R.C.B.D.) Each experiment was carried out in five replicates and each replicate includes five observations (totally; 25 observations 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.

***Ten weeks* (*Matthiola incana*).** Seeds of *Matthiola incana* were prepared from Mohaghegh-e-Ardabili University, Iran. The seeds were washed thoroughly under running tap water and a few drops of hand washing for 10 min. After three times rinses with distilled water, seeds were disinfected with a 20% NaOCl aqueous solution and Tween-20 for 10 min then rinsed three times in sterile distilled water (10 min each). At the end, seeds were sterilized for 2 min in 70% ethanol followed by three times rinses with sterile distilled water (15 min each). Seeds had gelatinous state, thus they were put on the filter paper for drying and withdrawing the gel. Five seeds were cultivated in culture flasks on MS [Murashige and Skoog 1962] basal medium without growth regulators. Shoot tips were isolated from 4-week-old plants and after removing the extra leaves, they were cultivated on MS medium supplemented with 0.5, 1 and 2 mg·L of KIN and 0.5, 1 and 2 mg·L of NAA (16 treatments). Also, the concentrations of 0.5, 1 and 2 mg·L of 2,4-D were evaluated. The media were adjusted to pH 5.7–5.8 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 25 ±2°C and 75–80% relative humidity, under a photosynthetic photon density flux 50 $\mu mol \cdot m^2 \cdot s$ with a photoperiod of 14 h per day. Characters including shoot length, node number, root number, root length, fresh weight, dry weight and chlorophyll content were evaluated after 30 days. The experimental design was

R.C.B.D. Each experiment was carried out in five replicates and each replicate includes five observations (totally; 25 observations for each treatment). Data were subjected to ANOVA (analysis of variance) and significant differences between treatments means were determined by LSD test.

RESULTS AND DISCUSSION

***Lisianthus* (*Eustoma grandiflorum*).** We studied the effect of different concentrations of KIN and NAA on micropropagation of Lisianthus (*Eustoma grandiflorum*). Studied characteristics were shoot length, shoot number, node number, and root number. 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 (tabs 1 and 2).

Table 1. The effect of different concentrations of KIN and NAA on shoot length, shoot number, and shoot and root number of *Eustoma grandiflorum* (mean ±SD)

Treatments (mg·L)	Shoot length (cm)	Shoot No.	Node No.	Root No.
KIN 0 + NAA 0	1.140 ±0.13^{bd}	1.10 ±0.12^{cd}	2.72 ±0.13^{d}	0.60 ±0.02^{c}
KIN 0.5 + NAA 0	1.434 ±0.14^{bc}	2.61 ±0.13^{a}	8.75 ±0.33^{a}	1.60 ±0.13^{bc}
KIN 1 + NAA 0	2.158 ±0.23^{a}	2.68 ±0.09^{a}	4.28 ±0.23^{bc}	0.00 ±0.00^{c}
KIN 2 + NAA 0	1.554 ±0.13^{b}	2.20 ±0.13^{ab}	4.70 ±0.25^{b}	0.00 ±0.00^{c}
KIN 0 + NAA 0.5	1.270 ±0.10^{bd}	0.40 ±0.06^{de}	3.04 ± 0.13^{cd}	0.00 ±0.00^{c}
KIN 0.5 + NAA 0.5	1.160 ±0.16^{bd}	1.40 ±0.08^{bd}	2.80 ±0.11^{cd}	0.00 ±0.00^{c}
KIN 1 + NAA 0.5	1.178 ±0.14^{bd}	1.20 ±0.10^{bd}	2.88 ±0.13^{cd}	0.60 ±0.03^{c}
KIN 2 + NAA 0.5	1.150 ±0.09^{bd}	2.00 ±0.13^{ac}	2.96 ±0.17^{cd}	2.55 ±0.08^{a}
KIN 0 + NAA 1	0.974 ±0.03^{d}	0.00 ±0.00^{e}	2.19 ±0.14^{de}	0.00 ±0.00^{c}
KIN 0.5 + NAA 1	1.080 ±0.11^{cd}	2.00 ±0.13^{ac}	3.08 ±0.17^{cd}	0.00 ±0.00^{c}
KIN 1 + NAA 1	0.976 ±0.09^{d}	1.18 ±0.04^{bd}	2.72 ±0.13^{d}	2.20 ±0.08^{b}
KIN 2 + NAA 1	0.860 ±0.07^{d}	2.19 ±0.15^{ab}	1.94 ±0.11^{de}	0.00 ±0.00^{c}
KIN 0 + NAA 2	1.202 ±0.13^{bd}	0.40 ±0.04^{de}	2.50 ±0.12^{de}	0.00 ±0.00^{c}
KIN 0.5 + NAA 2	1.016 ±0.10^{cd}	0.40 ±0.04^{de}	2.14 ±0.09^{de}	0.00 ±0.00^{c}
KIN 1 + NAA 2	0.858 ±0.08^{d}	0.00 ±0.00^{e}	1.32 ±0.06^{e}	0.00 ±0.00^{c}
KIN 2 + NAA 2	1.130 ±0.13^{cd}	1.40 ±0.08^{bd}	3.15 ±0.09^{cd}	0.00 ±0.00^{c}

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

Shoot tips were excised and transferred on MS medium containing KIN (0–2 mg·L) and NAA (0–2 mg·L) (fig. 1). 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 (2.158 cm) and shoot number (2.68) (tab. 1, fig. 1). MS medium enriched with 0.5 mg·L KIN induced 2.68 shoots per plantlets which was a proper medium. This result was comparatively better than for the control medium, where the average shoot length was 1.14 cm with 1.10 of shoots (tab. 1). The shortest shoots (0.856 cm) were obtained from plantlets grown in medium containing 2 mg·L NAA without KIN (tab. 1), where also the greatest amount of callus was observed in the base of shoots. In the medium supplemented with 1 and 2 mg·L NAA without KIN there was no shoots obtained.

Data analysis showed that different concentrations of KIN and NAA had significant effect on the number of shoots ($p \leq 0.01$). NAA had significant effect on shoot length ($p \leq 0.01$), but the effect of KIN on shoot length was no significant. The effect of KIN × NAA on the shoot length and shoot number was significant ($p \leq 0.05$) (tab. 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 (8.75) (tab. 1, fig. 1). The minimum node number (1.32) was calculated in medium supplemented with 2 mg·L NAA + 1 mg·L KIN (tab. 1). Analysis of variance showed that the effect of KIN, NAA and KIN × NAA on the node number were significant ($p \leq 0.01$) (tab. 2). The largest number of roots was found when we used 2 mg·L KIN + 0.5 mg·L NAA (2.55) and 0.5 mg·L KIN without NAA (1.60) (tab. 1, fig. 1). Data analysis showed that the effect of KIN, NAA and KIN × NAA were no significant on the root number (tab. 2). Rooted plantlets were successfully transferred to the soil. The results of acclimatization showed that the 100% of plantlets were survived under greenhouse conditions and were morphologically similar to mother plants (fig. 1). A mixture of light soil with good drainage is suitable for acclimatization of Lisianthus (*Eustoma grandiflorum*).

Table 2. Analysis of variance (ANOVA) for the effect of different concentrations of KIN and NAA on the root length, root number, shoot number and node number of *Eustoma grandiflorum*

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

** – significant at α = 1%, * – significant at α = 5%, ns = not significant

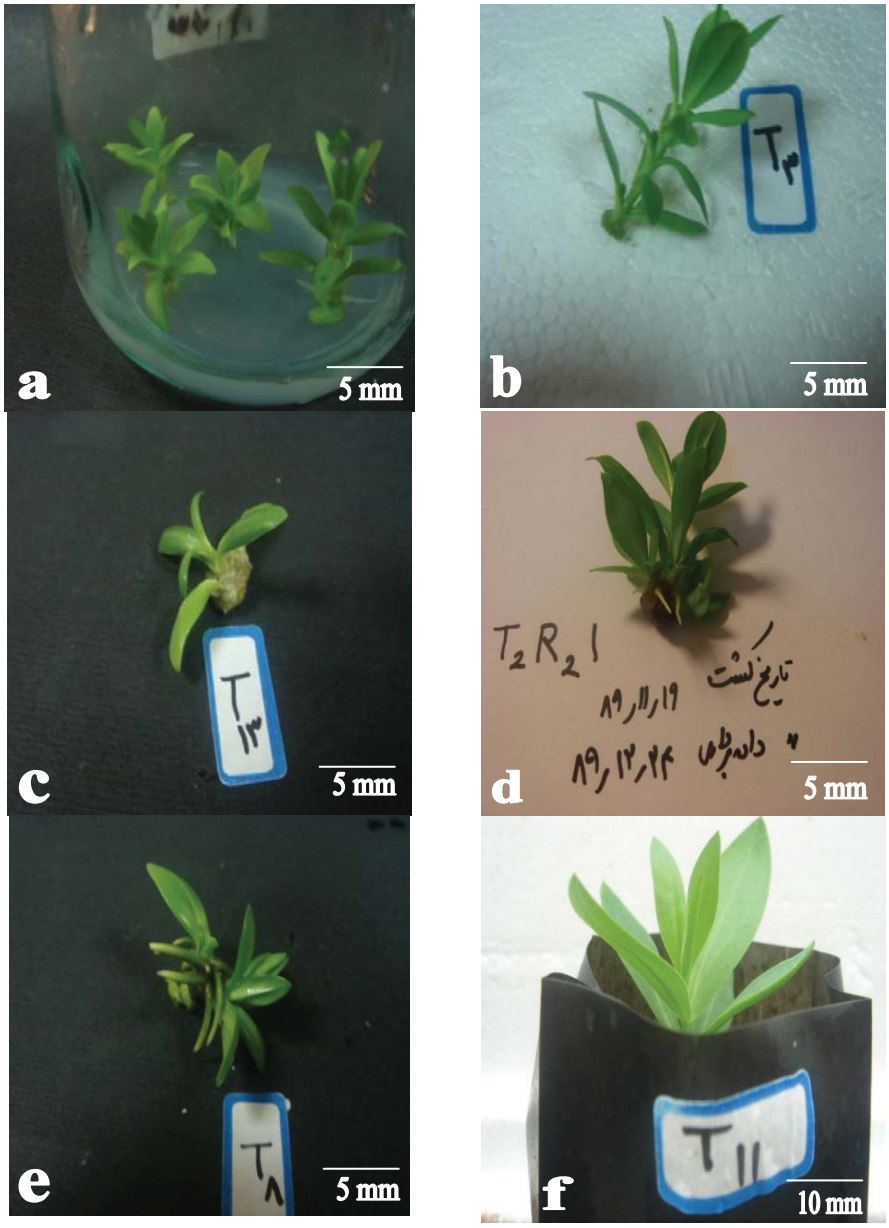

Fig. 1. Micropropagation process of *Eustoma grandiflorum*: a) seedlings of *Eustoma grandiflorum* in *in vitro* conditions, and shoot proliferation in solid MS medium, b) highest shoot length obtained in medium containing 1 mg·L KIN without NAA, c) lowest shoot length with callus formation on shoot base obtained in medium containing 2 mg·L NAA without KIN, d) largest shoot number and root formation obtained in medium containing 1 mg·L KIN without NAA, e) roots on the stem cut end, and f) hardening of plantlets. Plantlets were transferred to plastic pots containing a mixture of peat and perlite (1:1)

Cytokinins are 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. 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 (vitrification) 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 ½ MS medium supplemented with 2 mg·L IAA. The addition of NAA to the medium containing BA did not increase the number of shoots produced. 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 of *Eustoma grandiflorum* and many other plants [Gautam et al. 1983, Jain and Ochatt 2010, Kaviani et al. 2011]. Rooting is a crucial step to the success of micropropagation. Gomes et al. [2010] showed the positive effect of cytokinins on rooting of *Arbutus unedo* L.

Ten weeks (*Matthiola incana*). Current study was evaluated the effect of different concentrations of KIN, NAA and 2,4-D on micropropagation of *Matthiola incana*, an ornamental plant. Studied characteristics were shoot length, node number, root number, root length, fresh weight, dry weight and chlorophyll content (tab. 3, figs 2 and 3). 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. But, the effect of different concentrations of 2,4-D on shoot length and node number were not significant (fig. 3). 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 (fig. 2). The medium supplemented with 2 mg·L KIN without NAA resulted in the best shoot length (1.166 cm) and largest number of node (4.64) (tab. 3). Data analysis showed that the effect of KIN, NAA and KIN × NAA were

Table 3. Effect of different concentrations of KIN and NAA on some traits of *Matthiola incana* (Mean ±SD)

Treatments (mg·L)	Traits						
	chlorophyll content	dry weight	fresh weight	root length	root No.	node No.	shoot length
KIN 0	33.706 ±0.95^{a}	0.09335 ±0.01^{a}	0.9275 ±0.08^{a}	1.30 ±0.15^{ab}	0.85 ±0.04^{a}	2.53 ±0.15^{b}	0.836 ±0.07^{a}
KIN 0.5	25.804 ±0.90^{b}	0.05325 ±0.009^{c}	0.5105 ±0.04^{b}	0.94 ±0.10^{b}	0.42 ±0.04^{a}	2.28 ±0.15^{b}	0.657 ±0.05^{b}
KIN 1	34.212 ±1.80^{a}	0.06965 ±0.01^{b}	0.9505 ±0.09^{a}	1.162 ±0.16^{ab}	0.75 ±0.06^{a}	2.49 ±0.15^{b}	0.737 ±0.07^{ab}
KIN 2	30.658 ±0.95^{ab}	0.0681 ±0.008^{b}	0.678 ±0.06^{ab}	1.91 ±0.15^{a}	0.81 ±0.08^{a}	2.97 ± 0.14^{a}	0.8595 ±0.04^{a}
NAA 0	27.883 ±0.99^{b}	0.0645 ±0.01^{b}	0.6235 ±0.07^{ab}	0.80 ±0.08^{b}	0.51 ±0.03^{b}	3.39 ±0.19^{a}	0.9165 ±0.04^{a}
NAA 0.5	31.791 ±0.98^{ab}	0.0764 ±0.01^{a}	1.017 ±0.15^{a}	0.89 ±0.05^{b}	0.54 ±0.08^{b}	2.12 ±0.15^{c}	0.725 ±0.08^{b}
NAA 1	27.146 ±0.80^{b}	0.0596 ±0.004^{b}	0.584 ±0.03^{b}	1.64 ±0.15^{a}	0.76 ±0.15^{ab}	1.87 ±0.10^{c}	0.576 ±0.03^{c}
NAA 2	37.56 ±1.95^{a}	0.08385 ±0.01^{a}	0.842 ±0.05^{ab}	1.98 ±0.15^{a}	1.02 ±0.15^{a}	2.89 ±0.09^{b}	0.872 ±0.03^{a}
NAA 0 × KIN 0	34.16 ±0.97^{abcd}	0.0772 ±0.01^{c}	0.74 ±0.04^{b}	0.41 ±0.02^{d}	0.36 ±0.02^{cd}	2.16 ±0.07^{efg}	0.692 ±0.03^{cdefg}
NAA 0.5 × KIN 0	35.65 ±0.95^{abc}	0.094 ±0.01^{b}	0.95 ±0.02^{ab}	0.53 ±0.05^{d}	0.21 ±0.01^{d}	2.24 ±0.09^{defg}	0.764 ±0.04^{bcdef}
NAA 1 × KIN 0	19.34 ±0.09^{ef}	0.1024 ±0.02^{a}	1.01 ±0.08^{ab}	1.36 ±0.11^{cd}	1.24 ±0.16^{abc}	2.64 ±0.20^{cde}	0.940 ±0.09^{abc}
NAA 2 × KIN 0	45.68 ±0.89^{a}	0.0998 ±0.01^{ab}	1.01 ± 0.15^{ab}	3.24 ±0.15^{b}	1.85 ±0.17^{a}	3.08 ±0.15^{bc}	0.948 ±0.09^{abc}
NAA 0 × KIN 0.5	26.22 ±0.95^{cde}	0.0548 ±0.005^{h}	0.52 ±0.04^{b}	0.72 ±0.07^{cd}	0.56 ±0.05^{cd}	3.20 ±0.15^{bc}	0.916 ±0.07^{abcd}
NAA 0.5 × KIN 0.5	28.03 ± 0.77^{cde}	0.074 ±0.01^{cd}	0.73 ±0.04^{b}	1.04 ±0.10^{cd}	0.36 ±0.04^{cd}	2.00 ±0.15^{efgh}	0.700 ±0.05^{cdefg}
NAA 1 × KIN 0.5	10.08 ±0.08^{f}	0.0216 ±0.002^{i}	0.19 ±0.02^{b}	0.50 ±0.03^{d}	0.40 ±0.02^{d}	1.36 ± 0.12^{h}	0.364 ±0.06^{h}
NAA 2 × KIN 0.5	38.88 ±0.95^{abc}	0.0626 ±0.01^{fg}	0.61 ±0.02^{b}	2.00 ±0.19^{bc}	0.76 ±0.09^{bcd}	2.56 ±0.15^{cdef}	0.648 ±0.10^{defg}
NAA 0 × KIN 1	29.86 ±0.95^{cde}	0.0572 ±0.003^{gh}	0.57 ±0.01^{b}	1.08 ±0.08^{cd}	0.68 ±0.06^{cd}	3.56 ±0.18^{b}	0.892 ±0.11^{abcd}
NAA 0.5 × KIN 1	43.46 ±1.00^{ab}	0.0658 ±0.01^{ef}	1.69 ±0.15^{a}	2.288 ±0.15^{bc}	1.6 ±0.08^{ab}	1.80 ±0.15^{fgh}	0.584 ±0.08^{efgh}
NAA 1 × KIN 1	32.33 ±0.98^{bcde}	0.0584 ±0.01^{gh}	0.58 ±0.03^{b}	1.20 ±0.15^{d}	0.40 ±0.04^{d}	1.64 ±0.15^{gh}	0.472 ±0.07^{gh}
NAA 2 × KIN 1	31.19 ±0.95^{bcde}	0.0972 ±0.01^{ab}	0.96 ±0.04^{ab}	1.28 ±0.15^{cd}	0.72 ±0.07^{bcd}	2.96 ±0.21^{bcd}	1.000 ±0.11^{ab}
NAA 0 × KIN 2	21.28 ±0.55^{def}	0.0688 ±0.01^{def}	0.67 ±0.03^{b}	0.80 ±0.06^{cd}	0.44 ±0.04^{cd}	4.64 ±0.30^{a}	1.166 ±0.15^{a}
NAA 0.5 × KIN 2	20.02 ±0.85^{ef}	0.0718 ±0.007^{cde}	0.70 ±0.05^{b}	0.54 ±0.04^{d}	0.20 ±0.02^{d}	2.44 ±0.23^{cdef}	0.852 ±0.09^{bcde}
NAA 1 × KIN 2	46.83 ±1.30^{a}	0.056 ±0.007^{gh}	0.55 ±0.05^{b}	5.20 ±0.35^{a}	1.80 ±0.15^{a}	1.84 ±0.17^{fgh}	0.528 ±0.05^{fgh}
NAA 2 × KIN 2	34.49 ±0.95^{abc}	0.0758 ±0.01^{cd}	0.79 ±0.05^{b}	1.40 ±0.15^{cd}	0.80 ±0.08^{bcd}	2.96 ±0.17^{bcd}	0.892 ±0.08^{abcd}

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

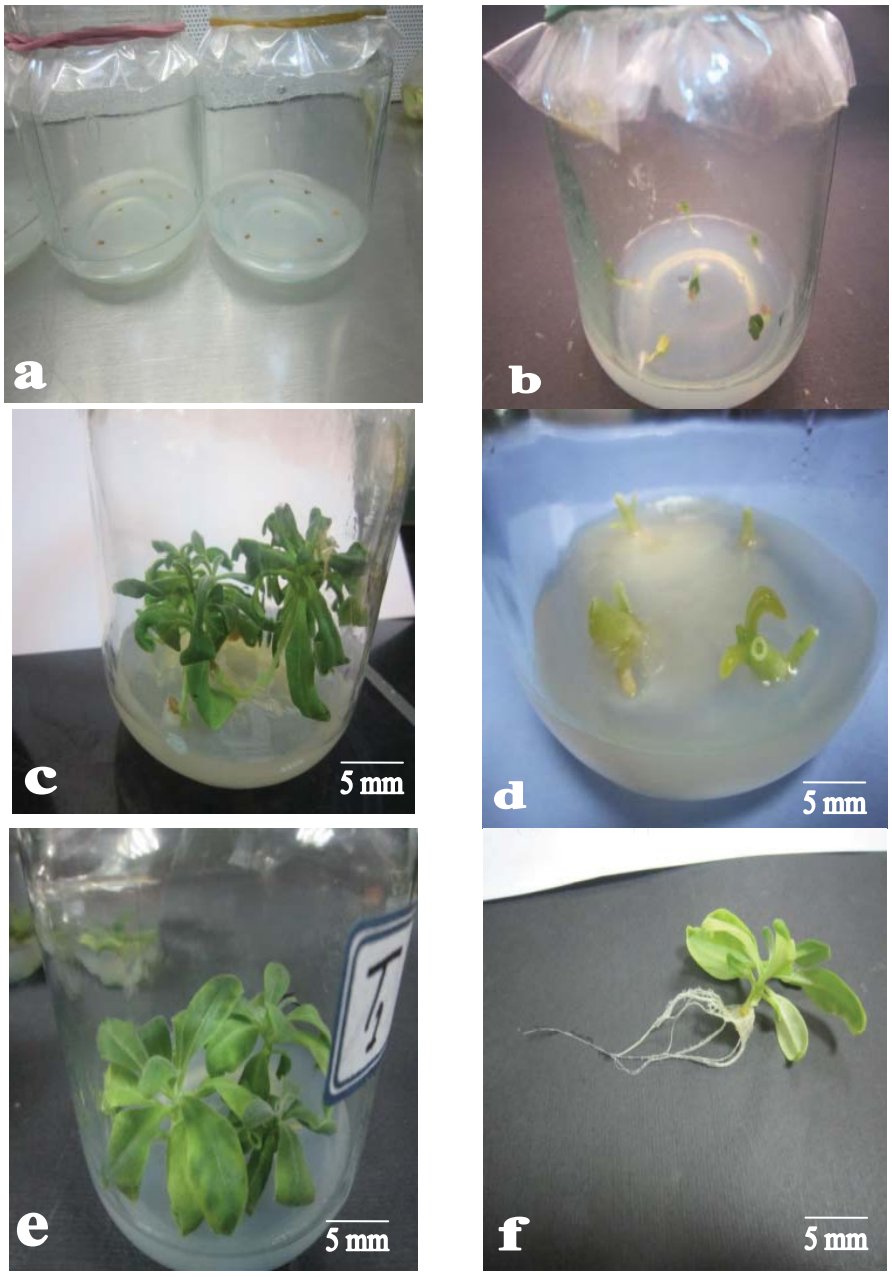

Fig. 2. Micropropagation process of *Matthiola incana* using KIN and NAA: a) seeds cultured in MS medium without plant growth regulators, b) germination of cultured seeds after a week, c) produced seedling 4 weeks after cultivation, d) excised buds as explants and their cultivation, e) regeneration of explants 4 weeks after culturing on medium containing NAA and KIN, and f) produced plantlets

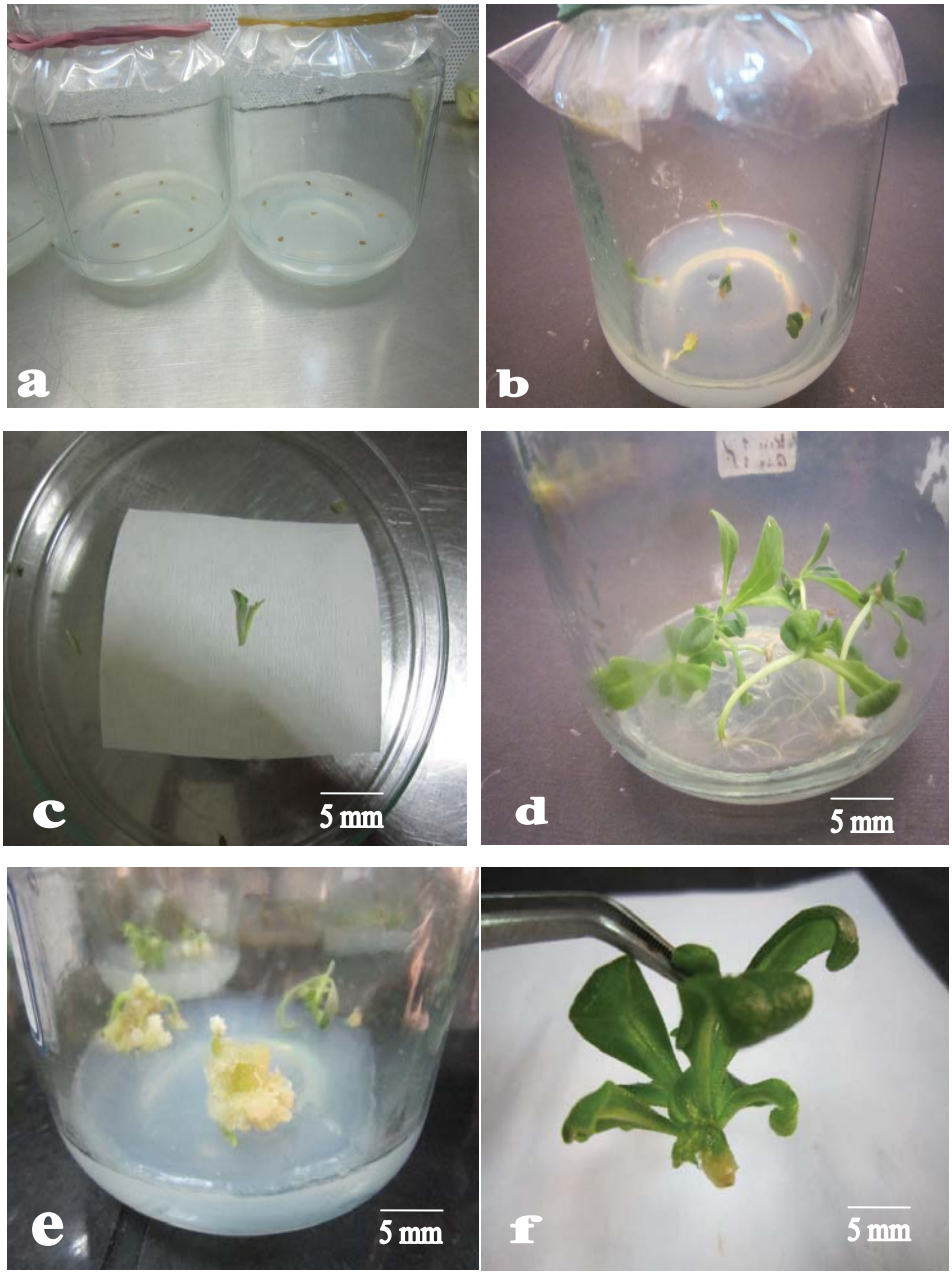

Fig. 3. Micropropagation process of *Matthiola incana*: a) seeds cultured in MS medium without plant growth regulators, b) germination of cultured seeds after a week, c) a shoot tip excised from seedling as an explant, d) regeneration of explants after culturing on medium containing 2,4-D and KIN, e) callus induction on the basis of explants grown on medium containing 2,4-D and KIN, and f) produced plantlets without roots

significant on the length of shoot and the number of node ($p \leq 0.01$) (tab. 4). When the shoot tips were inoculated in the medium containing 2 mg·L NAA without KIN and medium containing the combination of 1 mg·L NAA + 2 mg·L KIN, the best result was observed for root number (1.85) and root length (5.2 cm), respectively (tab. 3). This result was comparatively better than root number (0.36) and root length (0.41 cm) of control. Analysis of variance showed that the effect of KIN was no significant on the root number, while the effect of NAA and KIN × NAA on the root number and root length were significant ($p \leq 0.05$ and $p \leq 0.01$, respectively) (tab. 4). The highest fresh weight (1.69 g) and dry weight (0.102 g) were found when we used 1 mg·L KIN + 0.5 mg·L NAA and 1 mg·L NAA without KIN, respectively (tab. 3). The highest amount of chlorophyll (46.83) was determined in medium supplemented with 1 mg·L NAA + 2 mg·L KIN. This result was comparatively better than the growth of control (tab. 3). Data analysis showed that the effect of KIN, NAA and KIN × NAA were no significant on the fresh weight but on the dry weight was significant ($p \leq 0.01$). The effect of KIN on the chlorophyll content was significant at the probability level of 5%, but the effect of NAA and KIN × NAA on that were significant at the probability level of 1% (tab. 4).

Table 4. Analysis of variance (ANOVA) for the effect of different concentrations of KIN and NAA on some traits of *Matthiola incana*

Source of variations	Mean of squares							df
	chlorophyll content	dry weight	fresh weight	root length	root No.	node No.	shoot length	
KIN	298.12^{*}	0.00549^{**}	0.88763^{ns}	3.450^{**}	0.7685^{ns}	1.68^{**}	0.174^{**}	3
NAA	454.59^{**}	0.00244^{**}	0.81470^{ns}	6.608^{**}	1.116^{*}	9.781^{**}	0.476^{**}	3
KIN × NAA	610.38^{**}	0.00095^{**}	0.32161^{ns}	12.106^{**}	2.468^{**}	1.904^{**}	0.172^{**}	9
Error	100.54304	0.0003473	0.3845981	1.33592	0.402	0.298	0.0378225	64
c.v. (%)	32.2471	26.21485	20.8948	7.03464	9.6	21.26	25.17949	

** – significant at $\alpha = 1\%$, * – significant at $\alpha = 5\%$, ^{ns} = non sense

Our results indicated that there are differences in the effect of the different concentrations of KIN for shoot length and node number. Similar to our findings, many researchers showed that cytokinin KIN induced multiple shoot formation and shoot length [Luo et al. 2009, Gomes et al. 2010]. In current study the highest rates of shoot production were obtained when shoot tips were cultured on the medium supplemented with 2 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 2008]. Contrary to our results, studies of Fuller and Fuller [1995] on the micropropagation of *Brassica* spp. showed that the maximum shoots (88.3%) obtained in medium containing 2 mg·L IBA + 4 mg·L KIN. Our findings demonstrated that the combination of

1 mg·L NAA + 2 mg·L KIN in culture media was effective for increasing the number of root and root length. Without effective root system plant acclimatization will be difficult and the rate of plant propagation may be severely affected. Current study showed the positive effect of KIN on root induction and root length. The largest number of root obtained in media containing 2 mg·L NAA and 1 mg·L NAA + 2 mg·L KIN, respectively. Also, the highest root length was obtained in medium supplemented with 1 mg·L NAA + 2 mg·L KIN. 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 maximum of explants regeneration including the highest root (65.0%) in *Brassica* spp. obtained in culture medium supplemented with 2 mg·L IBA without KIN. In accordance with our results, the lowest rooting of *Bambusa arundinacea* was observed in medium without KIN [Nayak et al. 2010]. Studies of Gautam et al. [1983] on micropropagation of *Matthiola incana* by cotyledon explants revealed that a combination of auxin-cytokinin is antagonistic to the individual response of both and produced only a callus mass. A review of the literature clearly points out the negative effect of cytokinins on shoot rooting [Van Staden 2008], although a positive role has been occasionally referred [Nemeth 1979]. Our study showed positive role of KIN on rooting. The studies of Gautam et al. [1983] on *in vitro* regeneration of plantlets from somatic explants of *Matthiola incana* showed only a few shoots developed on explants reared on MS medium supplemented with 0.1 mg·L KIN. 1 and 4 mg·L of NAA induced profuse rooting in explants. In a study on *in vitro* micropropagation of orchid [Kalimuthu et al. 2007] NAA stimulated root growth. Hartmann et al. [1997] have recommended brief exposure to auxins for root induction and not for prolonged growth. Our studies demonstrated the positive effect of NAA on both root induction and root length.

CONCLUSIONS

1. It is concluded that *Matthiola incana* and *Eustoma grandiflorum* can be well-multiplied, rooted and grown in MS medium enriched by suitable concentrations of KIN and NAA for each step.

2. KIN has an important role on shoot induction in both *Matthiola incana* and *Eustoma grandiflorum*.

3. The highest rate of root induction was obtained on medium containing KIN and NAA for both species *Matthiola incana* and *Eustoma grandiflorum*.

4. In *Eustoma grandiflorum*, shoot and root induction were produced simultaneously on medium supplemented with 0.5–1 mg·L KIN.

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MIKROPROPAGACJA LEWKONII (*Matthiola incana*) I EUSTOMY WIELKOKWIATOWEJ (*Eustoma grandiflorum*) PRZY UŻYCIU KINETYNY (KIN), KWASU NAFTYLOOCTOWEGO (NAA) ORAZ KWASU 2,4-DICHLOROFENOKSYOCTOWEGO (2,4-D)

Streszczenie. Mikropropagacja jest silnym narzędziem rozmnażania wolnych od patogenów roślin ozdobnych na dużą skalę. Badania nad mikropropagacją lewkonii i eustomy są nieliczne. W pracy przedstawiono rzetelną metodę propagacji *in vitro* dwóch gatunków roślin ozdobnych. Eksplantaty wierzchołków pędów *Eustoma grandiflorum* oraz *Matthiola incana* były hodowane na podłożu MS uzupełnionej stężeniami 0; 0,5; 1 i 2 mg·L kwasu naftylooctowego NAA i kinetyny KIN. W przypadku *Eustoma grandiflorum* liczne pędy otrzymywano równocześnie na podłożu MS uzupełnionym tylko 0,5–1 mg·L KIN. Wierzchołki łodyg na pożywkach uzupełnionych 1 mg·L KIN bez NAA miały najlepszą długość (2,158 cm) i największą liczbę (2,68) pędów. Najwięcej kolanek (8,75) uzyskano na podłożu zawierającym 0,5 mg·L KIN bez NAA. Największą liczbę korzeni (2,55) uzyskano na podłożu uzupełnionym 2 mg·L KIN + 0,5 mg·L NAA. Wierzchołki łodyg wyrosłych na podłożu zwierającym 2 mg·L NAA bez KIN tworzyły największą liczbę narośli. Największą zawartość świeżej masy, suchej masy oraz chlorofilu wyliczono dla roślin wyrosłych na podłożach zawierających odpowiednio 0,5 mg·L KIN bez NAA, 0,5 mg·L KIN + 1 mg·L NAA. 4-tygodniowe rośliny *in vitro Matthiola incana* uzyskane z mikrowycinków miały udane kiełki i ukorzenienie. Podłoże MS uzupełnione 2 mg·L KIN bez NAA dało najlepszą długość łodyg (1,166 cm) i największą liczbę kolanek (4,64). Po inokulacji wierzchołków łodyg w podłożu zawierającym 2 mg·L NAA bez KIN oraz w podłożu zawierającym kombinację 1 mg·L NAA + 2 mg·L KIN najlepsze wyniki to liczba korzeni (1,85) oraz ich długość (5.2 cm).

Słowa kluczowe: *in vitro*, organogeneza, rośliny bez patogenów, hodowla tkanek

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