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In vitro Propagation of *Alstroemeria* cv. 'Fuego'

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Abstract: *Alstroemeria* (Alstroemeriaceae) is one of the most important cut flower in the world that is commonly propagated by rhizome splitting. Because of its characteristics such as low multiplication rates, time-consuming process and high risk of carrying viral disease, *in vitro* propagation techniques based on rhizome meristems culture have been developing nowadays. In this experiment, lateral and terminal buds of rhizomes (4-6mm) were cultured on solidified MS medium containing 30 g/l agar supplemented with different concentration of BAP and NAA after surface disinfection and were subcultured every three weeks. Some factors such as number of rhizomes, shoots and roots, length of shoots and roots were studied. Explants started to growth after three weeks. The greatest number of shoots was obtained from the medium supplemented with 1.5 mg/l BAP and 0.2 mg/l NAA. Furthermore, the results showed that increasing of BAP concentration caused a reducing length of shoots due to decrease apical dominant; also presence of low NAA concentration in the medium has been necessary for shoots primordial and rhizomes growth. Consequently, the medium included by 0.5 mg/l BAP and 0.2 mg/l NAA, in the average, 4.1 rhizomes and 2.62 shoots per explant is the best hormonal treatment for micropropagation of *Alstroemeria* cv. "Fuego".

Key words: *Alstroemeria* cv. fuego . *In vitro* propagation . rhizome . BAP . NAA

INTRODUCTION

Alstroemeria is a monocotyledon plant from Alstroemeriaceae family. It is a perennial rhizomed plant with flowers in different colors [1]. *Alstroemeria* hybrids are mostly cultured to produce cut flowers in greenhouses [2]. Presently among cut flowers, *Alstroemeria* is one of the most important flowers in international market [3]. This plant is propagated vegetatively by rhizome division [4]. Micropropagation of horticultural crops has been developed in recent years [5-7]. The proportion of low propagation of *Alstroemeria* becomes a time-consuming process causing to spread virus diseases [2], in addition, it faces seasonal time limits [3]. To remove this limitation, presently *in vitro* culture systems based on bud culture and rhizome meristems are developed [8, 9]. Some researchers reported that using rhizome apical bud as an explant is more suitable than peduncle and vegetative stems [9]. Zygote embryos are also used successfully to prepare embryogenic calli with high efficiency [10, 11]. Since *Alstroemeria* is a highly heterozygous crop, embryogenic callus derived from zygotic embryos can not be used to propagate present cultivars [12]. Pierik *et al*. [13] reported that in rhizome branchining, BA is the most effective hormone among cytokinins and also for rooting, a medium contained 4-3% sucrose with nearly 2µm NAA would be effective [14]. Nevertheless, through *in vitro* rooting, rhizome branchining does not happen, so rooting stage has made the propagation an expensive method [15]. Therefore, a propagation method in which both rooting and rhizome branching processes happen at the same time decreases the expenses of seedling production. So in order to identify the best medium for *in vitro* propagation of this flower in away that both rooting and rhizome processes happen in a unique medium a research accomplished in National Research Center of Ornamental Plants, Mahallat, Iran.

MATERIALS AND METHODS

Preparation of plant materials: In order to accomplish this research, Fuego variety of Alstroemeria rhizomes were collected from greenhouses and used as explants.

Rhizome sterilization: For plant sterilization, at first rhizomes rinsed in alcohol 70% for 30s. then transferred to 2% Hypochlorite sodium solution for 30 min. and so rinsed three times with sterile distilled water.

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Propagation medium: In order to study the measure of propagation, MS medium [16] supplemented with 3% sucrose, 7 g/l Agar and different concentration of growth regulators, benzyl amino purine (BAP) 0.0, 1.0, 1.5 and 2.5 mg/l alone or combined with 0.2 mg /l naphthalene acetic acid (NAA) were used. After preparing medium, media was adjusted to pH 5.8 and then autoclaved at 121°C for 20 minutes.

Explants preparation: Auxiliary and apical buds (4-6 mm length) were excised from sterilized rhizome segments and each explant was cultured in tub singularly contains 15 ml medium. Cultures incubated under conditions with 16/8 (light/dark) and temperature was 24°C. Explants were subcultured for 3 times (3 weeks intervals) and evaluating was done in regular intervals. The measurement indexes contained: the number of shoots, roots, rhizomes and buds and also roots and shoots length.

Hardening and transferring to the greenhouse: After 9 weeks, Plantlets with developed roots and rhizomes were transferred into plastic pots contained Peat Moss and Perlite (2:1). After that, pots were transferred to the baskets covered with plastic and were sprayed with water for two times a day and were allowed to grow at 24°C with 16-h photoperiod. On the second week, small holes were made on the plastic cover and from third week, the covers were removed gradually. Finally, plantlets were transferred to the greenhouse after adaptation processes.

Statistical analysis: The factorial experiment was arranged in a complete randomized design (CRD) with two factors (levels of NAA at 2 levels and BAP at 5 levels) in eight replications and each replicate was one explant. The main value of data was compared at ($p<0.01$, $P<0.05$) level using Duncan's test.

RESULTS AND DISCUSSION

Effect of different levels of BAP and NAA on bud breaking and shoot proliferation: Based on analysis of variance shown in Table 1, effect of different levels of BAP and NAA as well as their interaction effect on bud breaking were not found to be significant whereas BAP had significant effect on shoot proliferation ($p<0.05$). Maximum shoot number was obtained from culture medium contains 1.5 mg/l of BAP (2.25 shoots per explant) and the medium contains 0.0 mg/ l BAP showed the lowest proliferation (Table 2). Effect of presence or lack of NAA in the culture medium on shoot regeneration was found to be significant at 1% level of probability.

As shown in Table 3, media contains 0.2 and 0 mg/l NAA had means of 2.12 and 1.5 shoot, respectively. Interaction effect of NAA and BAP on shoot proliferation was non-significant.

Table 1: Analysis of variance of BAP and NAA effects on the parameters considered

		Mean square					
Source	df	Number of bud	Number of shoot	Shoot length (cm)	Number of root	Root length (cm)	Number of Rhizome
BAP	4	0.023^{ns}	0.09^{ns}	0.193**	0.093^{ns}	0.44**	0.434**
NAA	4	0.008^{ns}	0.783**	0.167**	0.352*	0.335^{ns}	0.281**
BAP*NAA	4	0.026^{ns}	0.104^{ns}	0.087**	0.06^{ns}	0.013^{ns}	0.031^{ns}
Error	70	0.035	0.090	0.016	0.07	0.101	0.029
CV%		17.600	20.240	9.330	24.09	16.800	14.000

Table 2: Comparisons of means of BAP effect on the parameters measured

BAP (mg/l)	number of bud	number of shoot	shoot length (cm)	number of root	root length (cm)	number of Rhizome
0.0	1.18a	1.31c	5.51a	2.75a	3.63a	0.56c
0.5	0.93a	1.56bc	3.69b	1.62ab	3.90a	3.37a
1.0	1.18a	1.87abc	2.77c	1.00b	2.00a	3.93a
1.5	0.75a	2.25a	2.76c	1.37ab	2.77a	2.62ab
2.5	1.06a	2.06ab	2.22d	1.18b	0.44b	1.81b

Table 3: Comparisons of means of NAA effect on the parameters measured

NAA (mg/l)	number of bud	number of shoot	shoot length (cm)	number of root	root length (cm)	number of Rhizome
1.80b	1.98b	1.00b	2.87b	1.50b	1.05a	0.0
2.72a	3.11a	2.17a	4.07a	2.12a	1.00a	0.2

Table 4: Comparisons of means of BAP and NAA interaction effect on the parameters measured

BAP (mg/l)	NAA (mg/l)	Number of bud	Number of shoot	Shoot length (cm)	Number of root	Root length (cm)	Number of Rhizome
0.0	0.0	1.25a	1.25c	2.99bcd	1.12b	3.06abc	0.00e
0.5	0.0	1.12a	1.37bc	4.10b	0.62b	3.75ab	2.62abcd
1.0	0.0	0.87a	1.62bc	2.66cd	1.00b	1.23abc	2.37bcd
1.5	0.0	1.00a	1.50bc	2.41d	1.12b	1.80abc	2.12bcd
2.5	0.0	1.00a	1.75bc	2.18d	1.12b	0.07c	1.87cd
0.0	0.2	1.12a	1.37bc	8.24a	5.37a	4.19a	1.12de
0.5	0.2	0.75a	1.75bc	3.83bc	2.62b	4.06ab	4.12a
1.0	0.2	1.50a	2.12abc	2.88bcd	1.00b	2.76abc	3.50ab
1.5	0.2	0.50a	3.00a	3.11bcd	1.62b	3.73ab	3.12abc
2.5	0.2	1.12a	2.37ab	2.27d	1.25b	0.80bc	1.75cd

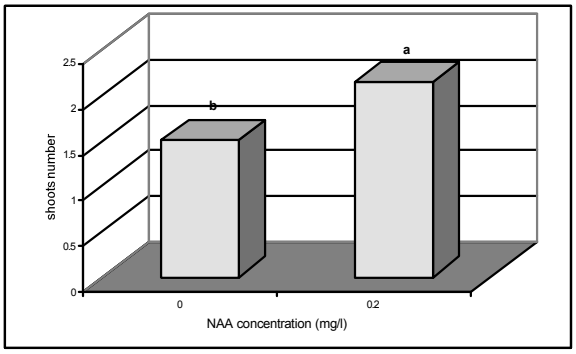

Fig. 1: Effect of NAA on the number of shoot

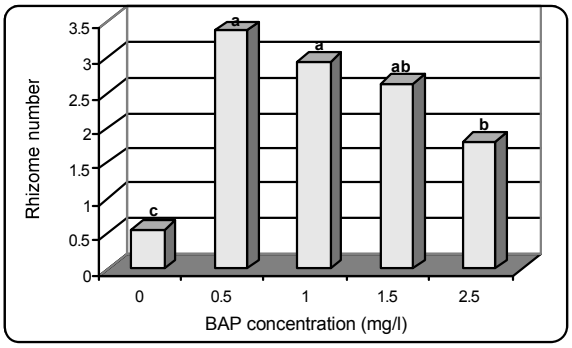

Fig. 3: Effect of BAP on the number of rhizome

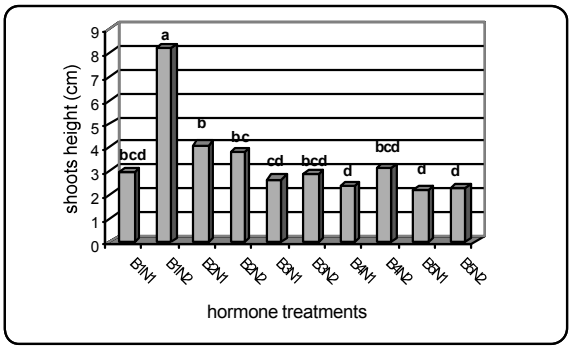

Fig. 2: NAA & BAP interaction effects on the average shoot length
BAP levels including B_1: 0.0mg/l, B_2: 0.5mg/l, B_3: 1.0 mg/l, B_4: 1.5 mg/l, B5: 2.5 mg/l.
NAA levels including N_1 0.0mg/l. N_2: 0.2 mg/l

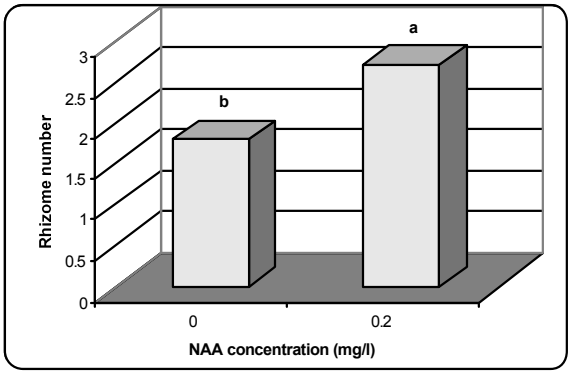

Fig. 4: Effect of NAA on the number of rhizome

Considering Table 4, it can be concluded that increasing in BAP concentration within 0-2.5 mg/l causes increase in shoot number and any concentration of BAP along with 0.2 mg/l NAA could be led to increase shoot number per explant in such a way the highest proliferation rate (with average of 3 shoot from each bud) was observed in a culture medium supplemented by 1.5 mg/l BAP combined with 0.2 mg/l NAA. After that, the greatest shoot number was obtained from BAP 2.5 mg/l in combined with NAA 0.2 mg/l. The lowest shoot number was found in a medium without any PGR.

Cytokinins as a plant growth regulator causes shoot induction by stimu lating cell division and decreasing apical dominance [17].

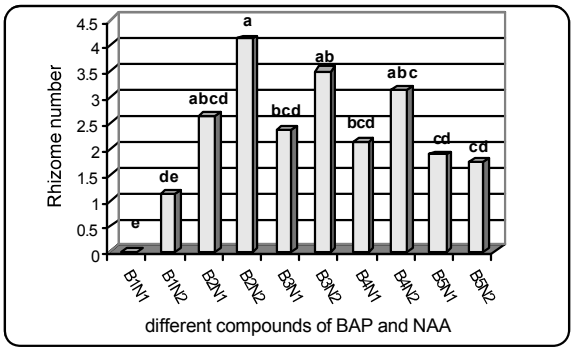

Fig. 5: BAP and NAA interaction effect on the average number of rhizome
BAP levels including B_1: 0.0mg/l, B_2: 0.5mg/l, B_3: 1.0 mg/l, B_4: 1.5 mg/l, B5: 2.5 mg/l.
NAA levels including N_1 0.0mg/l. N_2: 0.2 mg/l

A. Expanding and beginning to growth of cultured apical bud after second week

B. Shoot growth from cultured bud on fifth week

C. Axillaries buds and roots formation in explant.

D. Rhizome primordial formation and growth.

E. Rhizome and developed roots before transplanting.

F. Transplanted plantlets to pot after hardening.

Based on obtained results, it can be suggested that BAP promote cell division in meristem region. Also, using a low concentration of NAA increase shoot regeneration. Considering the latter fact, it could be useful for growth and elongation of produced shoots. This is in agreement with findings of Han *et al*. [18] who reported that using BA accompanied by low concentration of NAA have more effect on rhizome bud formation compared with only using BA. The results found in the present study are not consistent to Pierik *et al*. [13] who suggested increasing of auxin levels had no means effect on shoot proliferation.

Effect of NAA and BAP levels on shoot length: According to Table 1, different levels of BAP had a significant effect on shoot elongation ($p<0.01$). Considering comparison of means (Table 1), culture medium without BAP causes induction of Shoots with

maximum length. As stated earlier, Cytokinins (such as BAP) cause apical dominant. In a medium without BAP, internally natural auxins stimulated shoot growth and showed apical dominant. By increasing in BAP (Table 2), concentration from 0 to 2.5mg/l, the shoot length decreased. In addition, the shortest shoot was observed in the culture medium containing 2.5 mg/l of BAP with mean of 2.22 that this result is in line with what reported by Podwysznska *et al*. [19].

Considering (Table 3), 0.2 and 0.0 mg/l NAA showed 4.07 and 2.87 cm shoot length, respectively. These results can be attributed to auxin's role in cell elongation [17].

Furthermore, the interaction effect was found to be significant for shoot length ($p< 0.01$). The longest shoot (8.24cm) was observed in the medium contains 0.2 mg/l NAA without BAP, whereas the shortest shoot (2.18 cm) was found in the culture medium contains 2.5 mg/l BAP as well as the culture medium contains 2.5 mg/l BAP combined with 0.2 mg/l of NAA (B_5 N_1). This results support the finding of Podwysznska [19] for other cultivars.

Effect of BAP and NAA on rooting stage: According to the analysis of variance (Table 1), BAP had no significant effect on root formation, whereas effect of NAA was found to be significant at 5% level of probability. Culture medium contains 0.2 mg/l NAA (with mean of 2.17 root per explant) and culture medium without any PGR (with mean of 1 root per explant) ranked statistically as *a* and *b* categories, respectively. It also was indicated that NAA and BAP interaction effect was not significant for root number. Considering (Table 4) the greatest root number was found in the culture medium contains 0.2 mg/ l NAA without BAP. This result showed that NAA is a suitable for root induction that is consistent to Lin *et al*. [9] reports who suggested that NAA is an effective growth regulator for rooting. Similarly, Kristiansen *et al*. [15] reported that NAA promote root induction, whereas BA inhibits root formation and NAA is not capable to confront the negative effect of BA on rooting.

Effect of BAP and NAA on root elongation: Effect of BAP on root elongation was found to be significant ($p< 0.01$). Considering (Table 2) it can be suggested that low concentration of BAP, showed longest roots but with increasing of BAP concentration, root length also reduced. The maximum of root length was obtained in the medium contains 0 and 0.5 mg/l BAP, respectively with average root length of 3.63 and 3.9 cm. The lowest root length (0.44 cm) was observed in the medium contains 2.5 mg/l BAP.

Base on the analysis of variance, presence or absence of NAA had no significant effect on root length.

Effect of BAP and NAA on number of Rhizome: As saw in Table 1, effect of BAP on produced rhizome was found to be significant at 1% level of probability. The greatest number of rhizome (3.37 per explant) was obtained in BAP 0.5 mg/l while the lowest of that was observed in the culture medium contains 0.0 mg/l BAP (0.56 par explant) (Table 2). The results indicated that effect of presence or absence of NAA on the number of produced rhizome was significant ($p<0.01$). The culture media contains 0.2 mg/l NAA had average production of 2.72 rhizomes and the media without NAA had average regeneration of rhizomes (1.8 per explant) (Table 3). It also found that BAP and NAA interaction was no significant effect on rhizome formation.

Gabryszewska and Hampel [20] reported that increasing of BA could stimulate the proliferation of rhizome. Similar results were reported by Pierik *et al*. [13] who suggested that among Cytokinins, BAP stimulates rhizome formation but NAA and IBA have no effect on that. The results obtained from the current research are in accordance with the results reported by Gabryszewska and Hampel [20] and Pierik *et al*. [13] but our results are not consistent to this report that NAA has no positive effect on rhizome production reported by Pierik *et al*. [13]. Han *et al.* [18] reported that the medium supplemented with 1-2 mg/l BA and 0.2 NAA mg/l showed the greatest number of rhizome. In the current study, high rate of rhizome formation was found in the culture medium contains 0.2 mg/l NAA. This is in concordance with the results of other researchers.

As shown in Table 4, the greatest number of rhizome (4.12 per explant) obtained from the medium contains 0.5 mg/l BAP combined with 0.2 mg/l NAA (B_2 N_2). This corresponds with the results that were observed by Chiari *et al*. [21] who reported that BAP 0.5 mg/l is suitable for rhizome proliferation while the concentrations higher than that has no positive effect on the number of produced rhizome. Based on the results obtained, in the presence of BAP, low concentration of NAA caused the number of rhizome to be increased. Considering (Table 4). In all concentrations of BAP, when 0.2 mg/l of NAA is added, the number of rhizome increases and this fact is governing on all BAP concentrations.

CONCLUSIONS

Since in Alestroemeria propagation, regeneration of rhizome is more important than shoot proliferation,

hence this factor, the number of rhizome formed from each explant is the most important factor in Alestroemeria micropropagation and shoots without rhizome are failed to grow. If we can omit the rooting stage from the latter propagation method, based on the obtained results in this study, the best culture medium for Fuego cultivar is the medium contains 0.5 mg/l BAP and 0.2 mg/l NAA with the average production of 4.1 rhizomes and 2.62 roots from per explant.

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