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European Journal of Experimental Biology, 2013, 3(5):133-136

ISSN: 2248 –9215
CODEN (USA): EJEBAU

The effect of different concentrations of NAA and BAP on micropropagation of *Alstroemeria*

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ABSTRACT

Alstroemeria (Alstroemeriaceae) is an important ornamental plant, which use as cut flower as well as pot and garden plants because of their beautiful attractive flowers with wide variations in flower color. Traditionally, it is propagated via rhizome splitting, but multiplication rate is low and time-consuming process. Micropropagation by rhizome meristems culture is an efficient in vitro propagation method for Alstroemeria. Apical and lateral buds on rhizome were used as explants. These explants were cultured in MS medium containing NAA (0, 0.2, 0.5 and 1.0 mg l^{-1}) and BAP (0, 0.5, 1.0, 1.5 and 2.5 mg l^{-1}). MS medium supplemented with 1.0 mg l^{-1} NAA resulted in the highest shoot length (6.30 cm), root length (4.86 cm), maximum root number (5.00) and bud number (3.00). Largest number of shoot (3.00) and rhizome (4.00) were obtained in MS medium containing 0.20 and 0.50 mg l^{-1} NAA.

Keywords: Ornamental plants, Rhizome, Tissue culture, *In vitro*.

INTRODUCTION

Alstroemeria is a monocotyledon and cross-pollinating plant from Alstroemeriaceae family originated in South America [7]. It is a perennial rhizome plant with flowers in different colors. This plant is cultured in greenhouse for cut flower production and is propagated vegetatively by rhizome division [5]. Micropropagation of horticultural crops has been developed in recent years. The proportion of low propagation of *Alstroemeria* becomes a time-consuming process causing to spread virus diseases [20], in addition, it faces seasonal time limits [10]. To remove this limitation, presently *in vitro* culture systems based on bud culture and rhizome meristems are developed [7]. For the rapid propagation of novel cultivars and important breeding materials, it has been tried to establish micropropagation system. Flower pedicels sub-apical, segments from the vegetative stem and rhizome buds of *Alstroemeria* were tested as initial explants. Of these, rhizome buds of *Alstroemeria* gave the best response as initial explants in *in vitro* conditions [11]. *In vitro* culture methods have attracted considerable interest as a means to overcome these limitations and for producing virus-free plants [7]. Apical shoot bud [17], lateral shoot bud [9] and rhizome [10] explants have been used for *in vitro* propagation of *Alstroemeria*. Rhizome-tip (apical meristem) has been reported to feature a higher growth rate compared to other types of explant mentioned above [10]. In the field of ornamental plants, tissue culture has allowed mass propagation of superior genotypes and plant improvement, thus enabling the commercialization of healthy and uniform planting material [14, 21]. 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 [10, 16]. Most important of these parameters are the plant growth regulators included in the culture media [2]. Plant growth regulators act like signals to stimulate, inhibit or regulate growth in the developmental programs of plants [12]. Cytokinins and auxins like BA, BAP, TDZ and 2-iP and NAA were usually used on the micropropagation of *Alstroemeria*. In general, three modes of *in vitro* plant regeneration have been in practice: organogenesis, embryogenesis and axillary proliferation. In tissue culture, cytokinins and auxins play a crucial role as promoters of cell division and act in the induction and development of meristematic centers leading to the formation of organs [18]. In the present study, the effect of different concentrations of NAA and BAP on micropropagation of *Alstroemeria* cv. Fuego was evaluated.

MATERIALS AND METHODS

Rhizomes of *Alstroemeria* cv. Fuego were prepared from a greenhouse in Mahalat and Pakdasht cities, Iran. Fragments of rhizome containing apical and lateral buds were washed thoroughly under running tap water for 30 min and disinfected with 70% ethanol for 30 s and 2% NaOCl aqueous solution for 20 min. Disinfected explants were rinsed three times in sterile distilled water (10 min each). Four explants were cultivated in each Petri dish on MS (Murashige and Skoog, 1962) basal medium supplemented with plant growth regulators. Plant growth regulators were NAA (0, 0.2, 0.5 and 1.0 mg l^{-1}) and BAP (0, 0.5, 1.0, 1.5 and 2.5 mg l^{-1}). The media were adjusted to pH 5.7-5.8 and solidified with 7 g l^{-1} Agar-agar. The media were pH adjusted before autoclaving at 121°C, 1 atm. for 20 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 µmol/m^2/s with a photoperiod of 14 h per day. Bud number, shoot number, shoot length, root number, root length and rhizome number were measured. The experimental design was R.C.B.D. Each experiment was carried out in three replicates and each replicate includes four specimens. Analysis of variance (ANOVA) was done using SPSS and SAS statistical software and means were compared using Duncan's test at 0.05 level of probability.

RESULTS AND DISCUSSION

In this study, the effect of different concentrations of BAP and NAA on micropropagation of *Alstroemeria* cv. Fuego, an ornamental plant, through organogenesis was evaluated. Studied characteristics were bud number, shoot number, shoot length, root number, root length and rhizome number. The results are summarized in Tables 1, 2 and 3. Our data revealed that there are differences in the effect of the different concentrations of BAP, NAA and interaction between these two growth regulators on these characters. MS medium supplemented with 1.0 mg l^{-1} NAA resulted in the highest shoot length (6.30 cm), root length (4.86 cm), maximum root number (5.00) and bud number (3.00). Largest number of shoot (3.00) and rhizome (4.00) were obtained in MS medium containing 0.20 and 0.50 mg l^{-1} NAA (Table 3). MS medium supplemented with 2.50 mg l^{-1} BAP resulted in the lowest shoot length (3.09 cm). Lowest root length (1.30 cm) was seen in medium containing 2.50 mg l^{-1} BAP along with 0.5 mg l^{-1} NAA (Table 3). Minimum root number (0.33) was obtained in medium containing 2.50 mg l^{-1} BAP (Table 3). Least bud number (1.00) was calculated in medium containing 0.50 mg l^{-1} BAP along with 0.2 and 1.0 mg l^{-1} NAA. Lowest number of shoot (1.00) and rhizome were obtained in control medium (without any plant growth regulators (Tables 2 and 3). Analysis of variance (ANOVA) showed that the effect of BAP on the bud number, shoot length, root number, root length and rhizome number root length were significant ($p \leq 0.01$). No the effect of BAP on the shoot number was significant (Table 4). NAA had significant effect ($p \leq 0.01$) on all measured traits (Table 4). Interaction effect of BAP and NAA was significant on bud number, root length and rhizome number (Table 4). Our results indicated that there are differences in the effect of the different concentrations of BAP and NAA for bud number, shoot number, shoot length, root number, root length and rhizome number.

Studies of Khaleghi et al. [7] on *Alstroemeria* cv. "Fuego" showed that the greatest number of shoots was obtained from the medium supplemented with 1.5 mg l^{-1} BAP and 0.2 mg l^{-1} NAA. 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. The medium included by 0.5 mg l^{-1} BAP and 0.2 mg l^{-1} NAA, in the average, 4.1 rhizomes and 2.62 shoots per explant was the best hormonal treatment for micropropagation of *Alstroemeria* cv. "Fuego". Cytokinins as a plant growth regulator causes shoot induction by stimulating cell division and decreasing apical dominance [6]. NAA is a suitable for root induction that is consistent to Lin et al. [10] reports who suggested that NAA is an effective growth regulator for rooting. Similarly, Kristiansen et al. [8] 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. Gabryszewska and Hampel [1] reported that increasing of BA could

stimulate the proliferation of rhizome. Similar results were reported by Pierik et al. [19] who suggested that among Cytokinins, BAP stimulates rhizome formation but NAA and IBA have no effect on that. Han et al. [4] reported that the medium supplemented with 1-2 mg l^{-1} BA and 0.2 NAA mg l^{-1} showed the greatest number of rhizome. Study of Hamidoghli et al. [3] on *Alstroemeria* showed that in vivo rhizome bud produced the largest number of small rhizome and roots on medium containing 0.2 mg l^{-1} NAA with 1 mg l^{-1} BA. In conclusion, the present investigation revealed that the medium supplemented with certain concentrations of BAP and NAA influenced on shoot multiplication and root initiation of *Alstroemeria* cv. "Fuego".

Table 1. Mean comparison of the effect of different concentrations of BAP on some traits of *Alstroemeria*

Treatments (mg l^{-1})	Bud number	Shoot number	Shoot length (cm)	Root number	Root length (cm)	Rhizome number
BAP 0	2.25b	1.83a	5.92a	3.83a	4.44a	2.66a
BAP 0.5	1.33c	1.58a	4.72b	3.42a	3.65b	2.16b
BAP 1	2.16b	1.83a	3.67c	2.42b	3.73b	1.83b
BAP 1.5	2.67a	1.75a	3.81c	2.25b	2.29c	1.41c
BAP 2.5	2.16b	1.75a	3.38d	1.08c	1.53d	1.92b

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

Table 2. Mean comparison of the effect of different concentrations of NAA on some traits of *Alstroemeria*

Treatments (mg l^{-1})	Bud number	Shoot number	Shoot length (cm)	Root number	Root length (cm)	Rhizome number
NAA 0	1.60c	1.06c	4.04c	1.73c	3.20a	0.80c
NAA 0.2	2.00b	2.60a	4.25b	2.47b	3.16a	1.80b
NAA 0.5	2.40a	1.93b	4.33b	2.67b	2.92b	2.66a
NAA 1	2.46a	1.40c	4.57a	3.53a	3.22a	2.73a

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

Table 3. Mean comparison of the effect of different concentrations of BAP and NAA on some traits of *Alstroemeria*

Treatments (mg l^{-1})	Bud number	Shoot number	Shoot length (cm)	Root number	Root length (cm)	Rhizome number
BAP 0 + NAA 0	3.00abc	1.00d	5.68b	2.67cd	4.80a	1.00fgh
BAP 0 + NAA 0.2	1.67cde	3.00a	5.97ab	3.33bc	4.30b	2.67bc
BAP 0 + NAA 0.5	2.67ab	2.00bcd	5.75b	4.33ab	4.30b	4.00a
BAP 0 + NAA 1	3.00a	1.33cd	6.30a	5.00a	4.86a	3.00b
BAP 0.5 + NAA 0	1.33de	1.20d	4.38d	2.67cd	3.56e	1.33efg
BAP 0.5 + NAA 0.2	1.00e	2.33abc	4.72cd	3.33bc	3.67e	2.00cde
BAP 0.5 + NAA 0.5	2.00bcd	1.67cd	4.82c	3.33bc	3.26f	2.33bcd
BAP 0.5 + NAA 1	1.00e	1.33cd	4.97c	4.33ab	4.10bc	3.00b
BAP 1 + NAA 0	1.33de	1.85d	3.39ghi	1.33efg	3.67e	1.67gh
BAP 1 + NAA 0.2	2.67ab	2.67ab	3.58efgh	2.67cd	3.76de	1.67def
BAP 1 + NAA 0.5	2.00bcd	2.33abc	3.76efg	2.33cde	3.56e	2.33bcd
BAP 1 + NAA 1	2.67ab	1.33cd	3.94e	3.33bc	3.93cd	2.67bc
BAP 1.5 + NAA 0	2.33abc	1.10d	3.68efg	1.67def	2.53g	1.33efg
BAP 1.5 + NAA 0.2	3.00a	2.33abc	3.76efg	2.00def	2.26h	1.32efg
BAP 1.5 + NAA 0.5	2.67ab	2.00cd	3.85ef	2.00def	2.20h	2.00cde
BAP 1.5 + NAA 1	2.67ab	1.67cd	3.96e	3.33bc	2.17h	2.00cde
BAP 2.5 + NAA 0	1.33de	1.33cd	3.09i	0.33g	1.40jk	1.67gh
BAP 2.5 + NAA 0.2	1.67cde	2.67ab	3.23hi	1.00fg	1.83i	1.33efg
BAP 2.5 + NAA 0.5	2.67ab	1.67cd	3.48fghi	1.33def	1.30k	2.67bc
BAP 2.5 + NAA 1	3.00a	1.33cd	3.70efg	1.67def	1.60j	3.00b

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

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

		MS					
Source of variations	df	Bud number	Shoot number	Shoot length (cm)	Root number	Root length (cm)	Rhizome number
BAP	4	2.81^{**}	0.125^{ns}	12.94^{**}	13.93^{**}	16.82^{**}	2.54^{**}
NAA	3	2.41^{**}	6.72^{**}	0.718^{**}	8.22^{**}	0.28^{**}	12.31^{**}
BAP × NAA	12	0.75^{**}	0.158^{ns}	0.032^{ns}	0.278^{ns}	0.159^{**}	0.41^{*}
Error		0.21	0.26	0.051	0.37	0.018	0.25
CV (%)		21.99	29.50	5.27	23.28	4.28	25.00

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

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