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Callus induction in *Alstroemeria* using NAA and BAP

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ABSTRACT

This study evaluates the effect of explants type and plant growth regulators (NAA and BAP) on callus formation of Alstroemeria cv. Fuego, an ornamental plant. Alstroemeria uses as cut flower as well as pot and garden plants because of their beautiful attractive flowers with wide variations in flower color. Results showed that the explants source and different concentrations of growth regulators influenced on callus production. Segments of nodes and internodes were cultured in MS basal medium with different concentrations of BAP (0.0 and 0.5 mg l^{-1}) and NAA (0.0, 1.0 and 2.0 mg l^{-1}) to produce callus. Based on the results, node was better explant than internode to produce callus. 0.5 mg l^{-1} of BAP and 2.0 mg l^{-1} of NAA induced more callus on the explants.

Keywords: Ornamental plants, Callus induction, Tissue culture, *In vitro*.

INTRODUCTION

Callus is an important source for indirect plant organogenesis and embryogenesis. Over one thousand plant species have been regenerated *in vitro* via organogenesis and embryogenesis [13]. These two methods are among the most striking processes in plant micropropagation [13]. Callus induction is hard and time consuming in many monocotyledons like *Alstroemeria*. The establishment and improvement of micropropagation by *in vitro* culture through callus production is desirable. Thus, the aim of this paper was to obtain the best type of explants and plant growth regulators related to the callus formation of *Alstroemeria* cv. Fuego. Micropropagation of horticultural crops has been developed in recent years. Explants containing meristems like buds have been used for *in vitro* propagation of *Alstroemeria* [3, 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 [7, 14]. The success of the micropropagation method depends on several factors like plant growth regulators and type of explants [8, 9]. Plant growth regulators act like signals to stimulate, inhibit or regulate growth in the developmental programs of plants [5]. In general, three modes of *in vitro* plant regeneration have been in practice: organogenesis, embryogenesis and axillary proliferation. In monocotyledons, embryogenic callus is a suitable target tissue for transformation [12]. In *Alstroemeria*, embryogenic callus was obtained from some tissues like zygotic embryos, ovule, seedling tissue and ovary [2]. Kim et al. [1] showed somatic embryogenesis and plant regeneration from the tissue-cultured plantlets of *Alstroemeria*. In the present study, the effect of different concentrations of NAA and BAP and type of explants on callus formation of *Alstroemeria* cv. Fuego was evaluated.

MATERIALS AND METHODS

Stems of *Alstroemeria* cv. Fuego were prepared from a greenhouse in Mahalat and Pakdasht cities, Iran. Fragments of stem containing node and internode were washed thoroughly under running tap water for 20 min and disinfected with 1.5% NaOCl aqueous solution for 15 min. Disinfected explants were rinsed three times in sterile distilled water (10 min each). Four explants were cultivated in each Petri dish on 1/2MS [6] basal medium supplemented with plant growth regulators. Plant growth regulators were NAA (1 and 2 mg l^{-1}) and BAP (0 and 0.5 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. Ability of callus induction, type of callus, callus volume and callus color were evaluated. 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

Nodes and internodes as explants produced callus. Volume and percent of compact and round-shaped calli in node explants were more than those of internodes explants (Table 1). Two different types of callus were produced from these explants, compact and round-shaped. Highest volume (2.41) and percent (35.33) of compact callus were obtained on node explants (Table 1). Analysis of variance (ANOVA) showed that the effect of BAP on the volume and percent of compact and round-shaped calli were significant ($p \leq 0.01$). Mean comparison of the data showed that the highest volume (2.58) and percent (35.58) of callus were obtained on node explants grown on the medium containing 0.5 mg l^{-1} BAP (Table 2). Analysis of variance showed that the effect of NAA on the volume and percent of compact and round-shaped calli were significant ($p \leq 0.01$). Mean comparison of the data showed that the highest volume (2.33) and percent (32.92) of callus were obtained on node explants grown on the medium containing 2 mg l^{-1} NAA (Table 3). Analysis of variance showed that the interaction effect of NAA and explants, as well BAP and explants on the volume and percent of compact and round-shaped calli were significant ($p \leq 0.01$). Mean comparison of the data showed that the highest volume (2.50) and percent (37.33) of callus were obtained on node explants grown on the medium containing 0.5 mg l^{-1} BAP (Table 4). Also, maximum percent (37.33) of callus was seen in node medium supplemented with 2 mg l^{-1} NAA (Table 5). In related to interaction effect of NAA and BAP on callus formation, our results showed that the best medium was the medium containing 0.5 mg l-1 BAP along with 1 mg l-1 NAA (35.50%) (Table 6). In current study, node explant was proper for callus induction. Hormonal regulation of auxin and cytokinin balance is a key factor in the control of cell division in tissue culture. Reddy et al. [11] obtained callus from leaf explants, when placed on half strength MS media with 2.0 mg/L BAP and 0.5 mg/L 2,4-D. In monocots plants, callus has been used as a useful material for plant regeneration and genetic transformation studies [12]. Induction of callus from tissues of vegetative plant organs has been difficult in monocots [4]. In *Alstroemeria*, Lin et al. [4] reported the induction of callus from stem segments of *Alstroemeria*. Kim et al. [1] also reported the induction of callus in *Alstroemeria* from nodal segments. In a study on *Alstroemeria* cv. Fuego, Khaleghi et al. [2] reported the induction of callus and subsequently the regeneration of plants via somatic embryogenesis from nodes of greenhouse-grown plants, which have been propagated vegetatively. Lin et al. [4] showed that about 1% of the cultured stem segments formed callus on the nodal region. In *Alstroemeria*, auxins most frequently used to initiate embryogenic callus are 2,4-D and NAA [2].

Table 1. Mean comparison of the effect of explant type on volume and percent of compact and round-shaped callus of *Alstroemeria*

Explants	Volume of round-shaped callus	Percent of round-shaped callus	Volume of compact callus	Percent of compact callus
Node	2.41a	35.33a	1.92a	13.33a
Internode	1.92b	27.25b	1.53a	7.83b

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 BAP concentrations on volume and percent of compact and round-shaped callus of *Alstroemeria*

BAP concentrations (mg l^{-1})	Volume of round-shaped callus	Percent of round-shaped callus	Volume of compact callus	Percent of compact callus
0	1.75b	29.00b	1.33b	11.08a
0.5	2.58a	35.58a	2.17a	10.08b

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 NAA concentrations on volume and percent of compact and round-shaped callus of *Alstroemeria*

NAA concentrations (mg l^{-1})	Volume of round-shaped callus	Percent of round-shaped callus	Volume of compact callus	Percent of compact callus
1	2.00a	29.67b	1.50b	9.58b
2	2.33a	32.92a	2.00a	11.58a

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

Table 4. Mean comparison of the effect of explants type and BAP concentrations on volume and percent of compact and round-shaped callus of *Alstroemeria*

Explants × BAP concentrations (mg l^{-1})	Volume of round-shaped callus	Percent of round-shaped callus	Volume of compact callus	Percent of compact callus
Node × 0	1.50b	33.33b	1.00a	14.33a
Node × 0.5	2.50a	37.33a	2.00a	14.17a
Internode × 0	1.67b	29.83b	1.17a	9.67b
Internode × 0.5	1.67b	24.67c	1.67a	6.00c

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

Table 5. Mean comparison of the effect of explants type and NAA concentrations on volume and percent of compact and round-shaped callus of *Alstroemeria*

Explants × NAA concentrations (mg l^{-1})	Volume of round-shaped callus	Percent of round-shaped callus	Volume of compact callus	Percent of compact callus
Node × 1	2.00a	31.83b	1.33a	14.67a
Node × 2	2.00a	38.83a	1.67a	13.83a
Internode × 1	1.33a	27.50c	1.17a	9.33b
Internode × 2	2.00a	27.00c	1.67a	6.33c

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

Table 6. Mean comparison of the effect of NAA and BAP concentrations on volume and percent of compact and round-shaped callus of *Alstroemeria*

NAA × BAP (mg l^{-1})	Volume of round-shaped callus	Percent of round-shaped callus	Volume of compact callus	Percent of compact callus
0 × 1	1.17b	27.67b	0.50b	13.33a
0 × 2	2.00a	30.33ab	1.67a	10.67a
0.5 × 1	2.17a	31.67ab	2.00a	10.67a
0.5 × 2	2.00a	35.50a	1.66a	9.50a

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

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