Journal of Medicinal Plants Biotechnology

Vol 5, No 1, Spring & Summer 2019

Effects of explant type and growth regulators on callus induction in four ecotypes of Persian shallot

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Abstract

Allium hirtifolium commonly known as Persian shallot is an important wild medicinal plant distributed from North West to central and South West of Iran. To establish an efficient protocol for callus induction, the effects of explant type and growth regulators on callus induction in four ecotypes of Persian shallot were evaluated. Two explants types included basal plates and young leaves were cultured on MS media supplemented with 1.5 mg l^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D) or 1.5 mg l^{-1} of 1-naphthalene acetic acid (NAA) in combination with 0, 0.5 or 1 mg l^{-1} of 6-benzylaminopurine (BAP). All the cultures were maintained at 25±1°C in the dark. The results showed that basal plate was the best explant for callus induction when cultured on medium supplemented with 1.5 mg l^{-1} 2,4-D and 0.5 mg l^{-1} BAP. This optimized protocol will be useful for any future breeding improvement programs of Persian shallot using biotechnological means.

Keywords: Persian shallot, callus, growth regulators, growth index

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Introduction

Allium hirtifolium commonly known as Persian shallot is an important wild medicinal plant distributed from North West to central and South West of Iran. For many years, fresh and dry bulbs of *A. hirtifolium* are used in herbal medicine to treat rheumatic, inflammatory, arthritis, diarrhea and stomach pains (Asili et al., 2010). The antimicrobial, antifungal, antiparasitic and antioxidant activities of the bulb extract have also been reported. In Iran, the bulbs of *A. hirtifolium*, added to a variety of foods such as salads, pickles, yogurt and different sauces (Mahboubi and Kazempour, 2015). Conventionally, *A. hirtifolium* propagates through bulbs and seeds but these two methods are not efficient due to low growth rate of bulbs and deep dormancy, low viability and germination rate of seeds (Ebrahimi et al., 2014).

Therefore, to meet its demand in herbal medicine, there is an urgent need for applying non-conventional method of propagation for the conservation of this species. In vitro tissue culture technique is a feasible alternative method for improving the efficiency of *A. hirtifolium* propagation. Recently, micro propagation via tissue culture techniques has been reported in a number of *Allium* species from a range of explant sources (Hailekidan et al., 2013). There are numerous reports on callus induction and shoot regeneration of some *Allium* species (Yan et al., 2009). Therefore, the objective of this study was to determine optimum explant and plant growth regulators for callus production in four ecotypes of *A. hirtifolium*.

Materials and Methods

The bulbs of *A. hirtifolium* were collected from natural habitats in four regions of Iran included Isfahan, Hamadan, Lorestan and Zanjan. After washing with running tap water for 2 h, and removing outer dry scales, they were washed again for 15 min in detergent, sterilized with 70% ethanol for 5 min and with 5% sodium hypochlorite for 15 min. Then, these bulbs were rinsed three times with sterile distilled water. For callus induction the sterilized bulbs were trimmed, and the basal plates, approximately 3-5 mm thick, were excised and were cultured on MS (Murashige and Skoog, 1962) media supplemented with 1.5 mg l^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D) or 1.5 mg l^{-1} of 1-naphthalene acetic acid (NAA) in combination with 0, 0.5 or 1 mg l^{-1} of 6-benzylaminopurine (BAP). All the cultures were maintained at 25±1°C in the dark.

The rest of sterilized bulbs were cultured on MS media with 1.5 mg l^{-1} BAP and 0.5 mg l^{-1} NAA for shoot induction. Cultures were maintained at temperature of 25±1°C under a 16/8 h light regime provided by white fluorescent tubes at 40 μmol m^{-2} s^{-1}. From these explants, leaves were well developed within 8 weeks, and young leaves of 8-10 mm long were excised and placed horizontally on the media that mentioned for callus induction. The percentage of explants forming callus was recorded after 12 weeks. This experiment was done as a factorial in the base of completely randomized design.

All experiments had four replications with four explants in each replication. Results were subjected to analysis of variance using the Statistical Analysis Program (SPSS ver.16.0). The mean values were calculated

and compared by Duncan's multiple range tests ($P < 0.05$).

Results and Discussion

In the present study callus formation significantly influenced by explant type, growth regulators and ecotype, as well as their interactions (Table 1). Scotton et al. (2013) reported that the explant type, genotype, concentrations and combinations of growth regulators are the most important factors affecting callus induction. Basal plates showed the earliest signs of callus formation after 2 weeks of culture, but leaves started to initiate callus from cut surfaces after 3 weeks. The explants of basal plate showed up to 60.06% callus formation after 12 weeks and leaves exhibited a significantly lower callus induction up to 43.68% (Table 1). Therefore, basal plate was better explant for callus production in *A. hirtifolium.* Also basal plates were reported as best explants for callus production in garlic (Luciani et al., 2006; Haider et al., 2015) and *Allium chinense* (Yan et al., 2009).

Callus induction from both explants varied significantly depending on growth regulators. Although successful callus formation has been achieved in all plant growth regulator treatments but the combination of auxin with cytokinin accelerated the callus initiation and increased the percent of callus induction. Addition of BAP increased callus induction in both auxin treatments. In this experiment, the highest callus induction rate was achieved when explants were cultured on medium supplemented with 1.5 mg l^{-1} 2,4-D and 0.5 mg l^{-1} BAP. Results showed that callus formation were decreased as BAP concentration increased to 1 mg l^{-1} in combination with 2,4-D and NAA (Table 1). These data are similar to the results reported by Yan et al. (2009) who used combinations of 2,4-D with BAP for the callus induction from basal plate cultures of *Allium chinense*. Luciani et al. (2006) reported that combination of 2,4-D and BAP caused to best callus induction in different explants of garlic. Also the addition of BAP along with 2,4-D was the best condition for callus induction of *A. ampeloprasum* (Toaima et al., 2003) and *A. cepa* (Tiwari et al., 2004). In agreement with Nair and Seo (1993) our results showed that the callus induction efficiency of NAA was lower than 2,4-D in all treatments.

The highest callus induction of basal plate and leaf explants cultured in media contain 1.5 mg l^{-1} 2,4-D and 0.5 mg l^{-1} BAP were obtained in Zanjan, followed by Isfahan ecotype (Table 1). Fig. 1 shows the induced callus from basal plates of Zanjan ecotype.

Table 1
The effects of different explant types, growth regulators and ecotypes on callus induction of *A. hirtifolium.*

Treatments		Characters			
Growth regulators (mg l^{-1})	Ecotypes	Days to callus initiation	Callus induction (%)	Days to callus initiation	Callus induction (%)
		Leaf		Basal plate	
1.5 2,4-D	Isfahan	25.48^{def*}	37.86^{bc}	14.10^{g}	49.06^{d}
1.5 2,4-D + 0.5 BAP		22.43^{g}	43.68^{a}	12.10^{j}	56.08^{bc}
1.5 2,4-D + 1 BAP		27.13^{abc}	38.84^{b}	16.30^{cd}	47.03^{def}
1.5 NAA		27.47^{ab}	33.11^{d}	16.10^{cde}	45.13^{efg}
1.5 NAA + 0.5 BAP		25.69^{de}	35.66^{bcd}	15.10^{f}	47.42^{de}
1.5 NAA + 1 BAP		27.40^{ab}	33.16^{d}	16.95^{b}	43.76^{gh}
1.5 2,4-D	Hamadan	24.44^{ef}	22.92^{fg}	13.55^{gh}	42.10^{hi}
1.5 2,4-D + 0.5 BAP		21.41^{g}	33.87^{d}	12.55^{ij}	45.04^{fg}
1.5 2,4-D + 1 BAP		25.30^{def}	22.44^{fg}	16.73^{bc}	44.05^{gh}
1.5 NAA		28.49^{a}	25.18^{ef}	17.30^{b}	39.39^{j}
1.5 NAA + 0.5 BAP		24.41^{ef}	27.19^{e}	13.80^{g}	41.81^{hi}
1.5 NAA + 1 BAP		27.44^{ab}	20.76^{g}	17.30^{b}	40.09^{ij}
1.5 2,4-D	Lorestan	25.04^{def}	27.19^{e}	14.10^{g}	38.13^{j}
1.5 2,4-D + 0.5 BAP		22.04^{g}	28.45^{e}	13.10^{hi}	40.04^{ij}
1.5 2,4-D + 1 BAP		25.87^{cde}	20.73^{g}	15.55^{ef}	35.06^{k}
1.5 NAA		28.04^{a}	20.86^{g}	18.10^{a}	34.16^{k}
1.5 NAA + 0.5 BAP		24.99^{def}	22.15^{fg}	14.10^{g}	39.05^{j}
1.5 NAA + 1 BAP		28.07^{a}	24.91^{ef}	15.10^{f}	33.78^{k}
1.5 2,4-D	Zanjan	24.13^{f}	37.74^{bc}	16.05^{de}	58.05^{ab}
1.5 2,4-D + 0.5 BAP		21.16^{g}	39.16^{b}	13.10^{hi}	60.06^{a}
1.5 2,4-D + 1 BAP		26.18^{bcd}	34.66^{cd}	14.10^{g}	55.09^{c}
1.5 NAA		27.16^{abc}	34.53^{cd}	17.10^{b}	43.05^{gh}
1.5 NAA + 0.5 BAP		26.16^{bcd}	35.57^{bcd}	14.10^{g}	46.63^{ef}
1.5 NAA + 1 BAP		28.15^{a}	32.76^{d}	15.10^{f}	44.06^{gh}

* Values followed by the same letter within a column indicate they are not significantly different ($p < 0.05$).

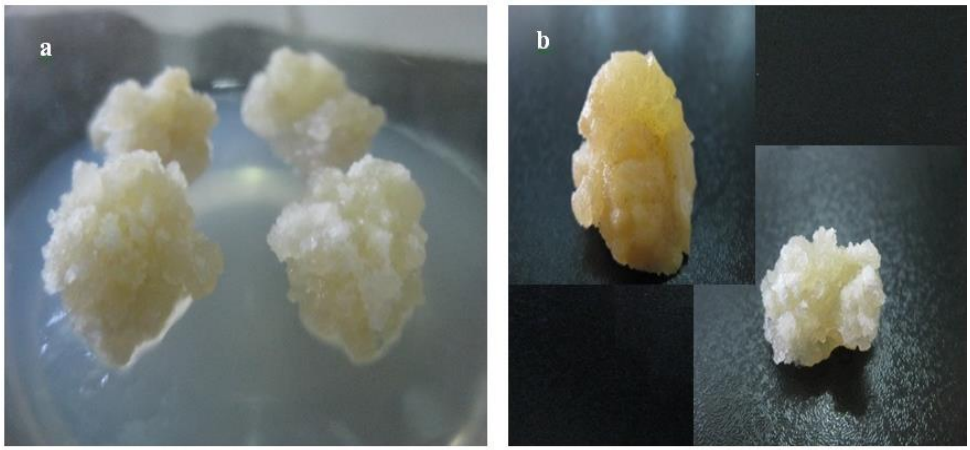

Figure 1. (a) Callus derived from in vitro cultured basal plates of *Allium hirtifolium* on induction medium with 1.5 mg l^{-1} 2,4-D after 12 weeks of culture. (b) Two types of basal plate derived callus.

In summary, we concluded that the selection of best initial explants with the correct growth regulators is necessary for achievement the highest callus induction and plant regeneration. In *Allium hirtifolium* callus induction was improved by selecting basal plates as initial explants when cultured on medium with 1.5 mg l^{-1} 2,4-D and 0.5 mg l^{-1} BAP.

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