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Effect of plant hormones on callus induction of explant types in endangered medicinal herb Tashnedari

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Abstract

Tashnedari endangered medicinal with *Scrophularia striata* scientific name is native to Iran and its main habitat is Ilam province. In order to callus induction in this plant species two separate factorial experiment as completely randomized design with three replications was performed in Sari agricultural sciences and natural resources university biotechnology laboratory in 2012. Explants were prepared as stem and leaf. And effect of BAP and 2, 4-D hormones in MS medium was studied at four levels on its callus induction. According to the results, combined of hormones containing 1.5 mg.L^{-1} BAP and 1.5 mg.L^{-1} 2-4-D were introduced as the best treatment for callus induction ($\bar{X}$ = 52.22 %) using stem explants, and 1.5 mg.L^{-1} BAP and 2.5 mg.L^{-1} 2-4 D as the best treatment for callus induction ($\bar{X}$ = 76.66 %) using leaf explants. Callus fresh weight was measured for stem explant And the freshest weight was observed in 3.5 mg.L^{-1} BAP and 1.5 mg.L^{-1} 2, 4-D hormone compound.

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Introduction

Medicinal and aromatic plants take a very small cultivation area in comparison to other groups of cultivated plants. On the contrary, they comprise huge number of used plant species with most diverse biological characteristics (Pank, 2006).

Among the herbs, scrophulariaceae family members are considered. Tashnedari with *Scrophularia striata* scientific name is one of the member of this family and native to Iran that grows as wild in meadows, hillsides and impassable areas of Ilam province. It has been used traditionally to treat ulcers (Ardeshiri lajimi, 2009), kidney disease (Bahramian and Valadi, 2010), reduce inflammation and infection of eye and ear (Shoohani *et al.*, 2010) and ... for many years. Also there are reports that scrophulariaceae family has compounds with antioxidant and anti-inflammatory properties (Ahmed *et al*, 2003). Tashnedari (*S.striata*) is a small and many branch perennial herb. Leaves are alternate and serrated and the dimensions are about 7.5 × 2 cm. Length of their stems are about 30 to 90 cm (Monsef Esfahani, 2010). Fruits are usually in the form of capsules containing numerous seeds (Azadbakht, 2000).Indiscriminate and non-normative harvesting, that is with coming out the roots from soil, puts Tashnedari on Iran list of endangered plants.

Plant breeding is the most important way to improve medicinal and aromatic plants that is created the opportunity for adapted different genotypes for obviate consumers need in production cycle and has a major role to produce high quality, reliable use and sustainable product (Pank, 2006). Plant tissue culture is a widely used technology that has been provided new ways to solve many problems of breeders (Galiba, 1994; Slabbert *et al.*, 2004). An important advantage of using tissue culture techniques compared to conventional method is that in limit time and space, can obtain a large population from one sample (Farsi and Zolali, 2011). Indiscriminate and non-normative harvesting, that is with coming out the roots from soil, puts Tashnedari on Iran list of endangered plants. To prevent the extinction of this species using tissue culture and micro-propagation techniques are appropriate. Callus induction is one one of the sensitive stages of micro-propagation.

The aim of this study was investigate the effect of different concentrations of plant hormones (BAP and 2, 4-D) and explant types (stems and leaves) on Tashnedari callus induction in vitro.

Materials and methods

Materials

This study was performed as two separate factorial experiments as completely randomized design with three replications in biotechnology laboratory of Sari agricultural sciences and Natural Resources University.

Methods

Plants were identified from heights of Chavar located in Ilam province in September 2012 and transferred to pots. After kept in the shade for two weeks, were transferred to the laboratory.

In first experiment, young and tender stems selected and washed with water. After putting in 70% (v/v) alcohol for two minutes, were washed with sterile distilled water. Then they were put in 40% (v/v) sodium hypochlorite solution for 20 minutes and were washed with sterile distilled water two times for 15 minutes. MS medium containing different combinations of hormone concentrations, including BAP at 4 levels (0.0, 1.5, 2.5, 3.5 $mg.L^{-1}$) and 2, 4-D at 4 levels (0.0, 1.5, 2, 2.5 $mg.L^{-1}$) were prepared for callus production. To facilitate callus induction and stimulate before planting, surface of stems were scratched with scalpel. All sterilization and disinfection processes and also explants cultured were done in growth room. Petri dishes were maintained in growth room at 25 ± 2 °c under a photoperiod of 16 h light and 8 h dark, and 75% relative humidity. First signs of callus formation were observed after two weeks. Regeneration was performed two weeks

interval time and data were recorded four weeks after start the experiment.

In second experiment, considering that leaves of plant were useless at medium and seeds germination were difficult, first, seeds were cultured in hormone-free MS medium for produced plantlet (fig 1 [A]). For this, Tashnedari seeds were sterilized such as the methods used in first experiment and were cultured in hormone-free medium (MS). After 45 days leaves of seedlings were used for transfer to medium containing hormones (fig 1[B]). MS medium containing different combinations hormone concentrations, including BAP at four levels (0.0, 1.5, 2.5, 3.5 $mg.L^{-1}$) and 2, 4-D at four levels (0.0, 1.5, 2, 2.5 $mg.L^{-1}$) was prepared for callus induction. To facilitate callus induction, before planting, surface of leaves was scratched with scalpel. All sterilization and disinfection processes and also explants cultured were done as well as first experiment in growth room. Cultured explants for callus induction were maintained in incubator at 25 ± 2° c under dark condition and 75% relative humidity. First signs of callus formation were observed after one week. Regeneration was performed with two weeks interval time and data were recorded after two weeks of start the experiment.

In addition to hormones, all of medium contains 30g sucrose and 6g agar per liter. Also PH was set in range 5.6 to 5.8. Normalize the data for callus induction percentage analysis was performed using Arcsin $\sqrt{X+0.01}$ formula. Data analysis using statistical software SPSS18 and MSTSTC and comparison of means were performed using Duncan test.

Result

Stem explant

Results of variance analysis of first experiment (table 1) showed that simple effects of BAP and 2, 4-D hormones and their interaction were significant (α = 0.01). results of comparison mean of interaction of BAP and 2,4-D hormones on callus induction and callus fresh weight of Tashnedari plant using stem explants showed that in different concentrations, callus induction percentage and callus fresh weight were different and hormonal treatment containing BAP and 2,4-D each 1.5 $mg.L^{-1}$ was the best treatment for callus induction ($\bar{X}$ = 52.22 %) and also hormonal treatment containing 2.5 $mg.L^{-1}$ BAP and 2 $mg.L^{-1}$ 2,4-D had lowest callus induction ($\bar{X}$ = 8.88 %) using stem explant (figure 1). Also callus fresh weight were measured and in hormonal treatment containing 3.5 $mg.L^{-1}$ BAP and 1.5 $mg.L^{-1}$ 2,4-D were observed the most callus fresh weight ($\bar{X}$ = 1.09 gr).

Table 1. Analysis of variance of stem and leaf explants traits in different concentration hormones (BAP and 2, 4-D).

MS			df	Source of variation
Leaf	Stem			
(%) Callus induction	(gr) Callus fresh weight	(%) Callus induction		
0.353**	0.104**	0.024**	3	BAP
0.671**	0.820**	0.143**	3	2,4-D
0.082**	0.186**	0.074**	9	BAP × 2,4-D
0.001	0.0001	0.001	32	Error

**Significant in 1% level.

Leaf explant

Results of variance analysis of callus induction for leaf explant (table 1) showed that simple effects of BAP and 2, 4-D hormones and their interaction were significant (α = 0.01). Figure 2 showed comparison mean results of hormonal treatments containing 1.5 $mg.L^{-1}$ BAP and 1.5 $mg.L^{-1}$ 2,4-D as the best treatment for callus induction ($\bar{X}$ = 76.66%) and hormonal

treatment containing 2.5 mg.L^{-1} BAP and 2.5 mg.L^{-1} 2,4-D had the lowest callus induction using leaf explant.

Figure 3 showed two levels of BAP hormone (1.5 and 2.5 mg.L^{-1}) had a similar effect on callus induction and callus induction were reduced by increasing amount of this hormone to 3.5 mg.L^{-1}.

Discussion

The highest percentage of callus induction for stem explants was by BAP and 2, 4-D hormonal treatment each 1.5 mg.L^{-1}, which is consistent with results obtained by Farsi and Bagheri, (2007). They stated that the same ratio of Auxin to Cytokinin causes continued cell division and callus induction. Sundarasekar *et al* (2012), was observed best callus induction for *Hymenocallis littoralis* by 13.50 µM of 2, 4-D and 4.50 µM of BAP concentration. Results of callus fresh weight using stem explants demonstrated that high ratio of BAP to 2; 4-D causes weight gain, which was inconsistent with results of Peyvandi *et al*., (2010). They reported that highest growth and callus fresh weight of chrysanthemum is achieved from treatment containing 4 mg.L^{-1} 2, 4-D and 2 mg.L^{-1} BAP. Hormonal treatment containing 1.5 mg.L^{-1} BAP and 2.5 mg.L^{-1} 2, 4-D had the greatest effect on callus induction using leaf explant which had been inconsistent with the results obtained by Arekhy *et al*., (2012). They expressed that highest callus induction percentage of herbs was achieved from 1 mg.L^{-1} 2, 4-D and 1.5 mg.L^{-1} Kni. . Also Jaberian *et al* (2012), obtained the highest callus for *Falcaria vulgaris* in medium containing (0.5 and 1.0 mg/L) 2, 4-D in combination with BA. These callus and leaf segments were transferred to MS medium supplemented with different combination of NAA and BA for indirect and direct regeneration respectively. The medium containing (1.0 mg/L) NAA in combination with (0.5 and 1.0 mg/L) BAshowed the highest number of shoot and root formation in plant regeneration through the callus. According to the same results of 1 and 1.5 mg.L^{-1} BAP levels on callus induction of leaf explant and negative effect of 3.5 mg.L^{-1} on callus induction, can say callus induction decreased by increasing BAP percentage. According to the results can say callus induction percentage using leaf explant is more than callus induction using stem explants in the same hormonal treatment (fig 4 [C], [D]). Also start of callus induction of leaf explant is one week after cultured, that is time for shoot explants is two weeks. Recommended that if good plant leaf is exist, use leaf explant for callus induction.

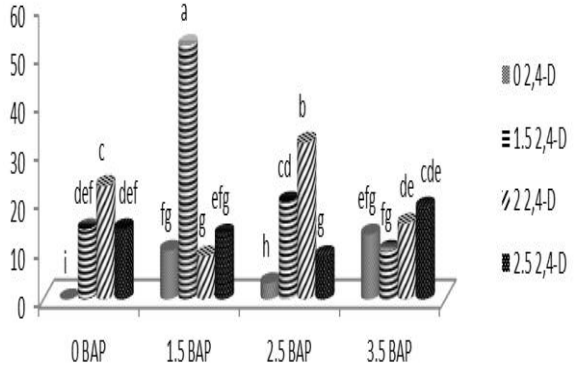

Fig. 1. Interaction of different concentrations of BAP and 2, 4-D on callus induction using stem explants.

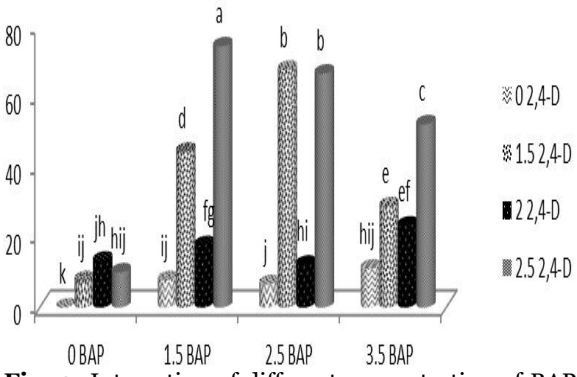

Fig. 2. Interaction of different concentration of BAP and 2, 4-D on callus induction using leaf explants.

Fig. 3 showed two levels of BAP hormone (1.5 and 2.5 mg.L^{-1}) had a similar effect on callus induction and callus induction were reduced by increasing amount of this hormone to 3.5 mg.L^{-1}.

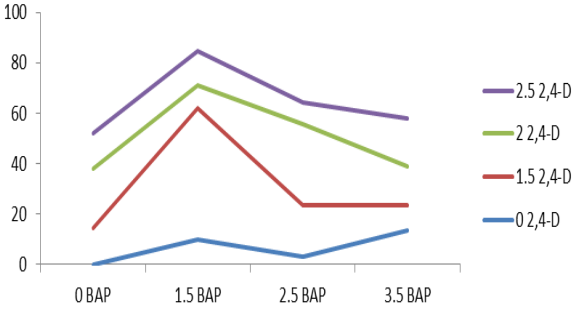

Fig. 3. Effects of different levels of BAP hormone on callus induction using leaf explants.

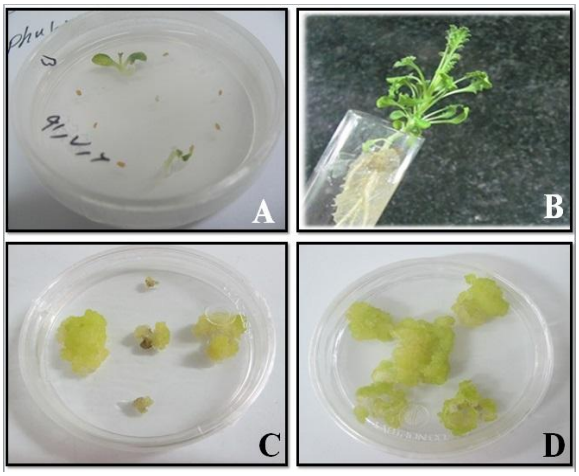

Fig. 4. [A] germinated seeds on MS medium without hormones, **[B]** seedling from seeds planted on MS medium without hormones, **[C]** stem callus in medium containing 1.5 mg.L^{-1} BAP and 2.5 mg.L^{-1} 2, 4-D, **[D]** leaf callus in medium containing 1.5 mg.L^{-1} BAP and 2.5 mg.L-1 2, 4-D.

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