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Full Length Article

Micro-propagation of Tashnedari Endangered Medicinal Plant *in vitro* culture

Meysam Azimi Lalabadi*[1], Nad Ali Babaeian Jelodar[1], Nad Ali Bagheri[1]
[1]Sari Agricultural Sciences and Natural Resources University
E-mail: meysamazimi24@yahoo.com

ABSTRACT
Tashnedari (*Scrophulariastriata*) is native to Iran and its main habitat is Illam province, that indiscriminate harvesting is placed it on the list of endangered medicinal plants. In order to micro-propagation and resolve its endangered experiment was done in biotechnology laboratory of sari agricultural and natural resources university in September 2012. Effects of BAP, 2, 4-D, BA and IAA hormone were evaluated in MS medium on callus induction and regeneration of this plant. Explants were prepared from stem. Factorial experiment was done as completely randomized design with three replications. Results showed that the combination of BAP (1.5 mg/l) and 2, 4-D (1.5 mg/l) was the best treatment for callus induction ($\bar{x}$= 52.22) and BA (0.5 mg/l) and NAA (1 mg/l) was recorded as the best treatment for regeneration ($\bar{x}$= 46.66). Callus fresh weight was measured for shoot explant. And hormonal combination of BAP (3.5mg/l) and 2, 4-D (1.5 mg/l) had the highest increase in fresh weight of callus (1.09 g).
Key word: tashnedari, callus induction, explant, regeneration

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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 [8]. Among the herbs, scrophulariaceae family members are considered. Tashnedari with *Scrophulariastriata* 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 [4], kidney disease [6], reduce inflammation and infection of eye and ear [10], and for many years. Also there are reports that scrophulariaceae family has compounds with antioxidant and anti-inflammatory properties [3].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 [14]. Fruits are usually in the form of capsules containing numerous seeds [15]. These species is placed on the list of endangered plants of Iran because of Non-principle and indiscriminate harvesting of it, that is associated with exit the plant root out of soil. Plant breeding is the most important methods to improve medicinal and aromatic plants. That has created the opportunity to adapt the genotypes to meet the needs of individual consumers in production cycle. And has a major role in produce high, reliable and sustainable products [8].An important advantage of tissue culture in compared the conventional methods is in limited time and space can be achieved to a large population [2].The aim of this study was to investigate the effects of different concentrations of plant hormones (2, 4-D, BAP, BA and IAA) on callus induction and regeneration of tashnedari on in-vitro.

MATERIAL AND METHODS

This study was done in biotechnology laboratory of agricultural and natural resources university of Sari. 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% (w/v) alcohol for two minutes, were washed with sterile distilled water. Then they were put in 40% (w/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 (zero, 1.5, 2.5, 3.5 mg/l) and 2,4-D at 4 levels (zero, 1.5, 2, 2.5 mg/l) were prepared for callus induction and hormonal combination containing BA at three levels (0.5, 1 and 4 mg/l) and IAA at three levels (0.05, 0.5 and 1 mg/l) were prepared for regeneration. 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. Experiments were performed with three replicates and each replicate consisted of 6 petri dishes that had been cultured explants per petri dish of 5 pcs. 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 after producing suitable callus, were transferred to regeneration medium for regenerations. Medium culture contains 30g sucrose and 6g agar per liter. Also PH was set in range 5.8 to 6.5. Factorial experiments were done as completely randomized design. 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.

RESULTS

Callus induction

Results of variance analysis (table 1) in first experiments, showed that simple effects of BAP and 2,4-D hormones and their interactions were significant ($\alpha = 0.01$). results of comparison mean of interaction of BAP and 2,4-D hormones on callus induction and callus fresh weight of tashnedari using stem plants 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 was the best treatment for callus induction ($\bar{X}$=52.22) and also hormonal treatment containing 2.5 mg/L BAP and 2 mg/L 2,4-D had lowest callus induction ($\bar{X}$=8.88) using stem explant (chart 1). Also callus fresh weight were measured and in hormonal treatment containing 3.5 mg/L BAP and 1.5 mg/L 2,4-D were observed the most callus fresh weight ($\bar{X}$=1.09 gr).

Table 1: Variance analysis of stem explants trait in different concentration of BAP and 2,4-D

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

**Significant in 1% level

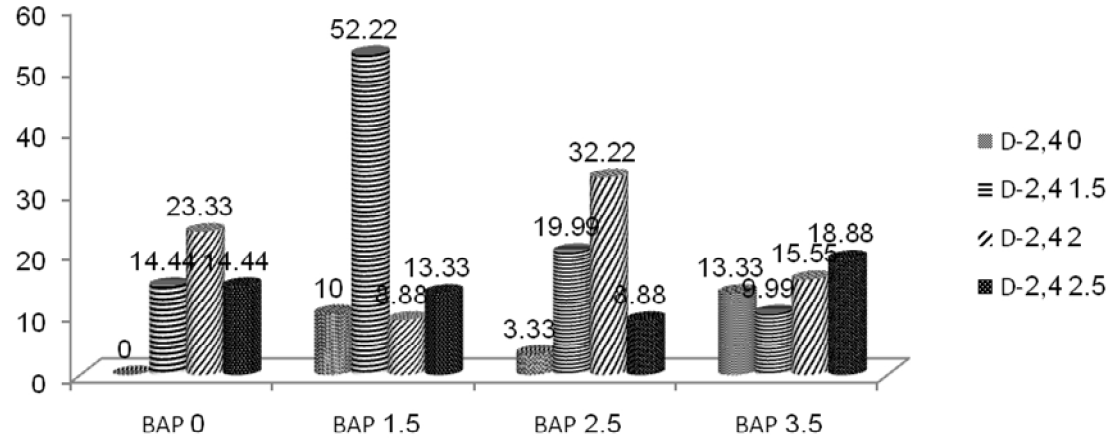

Figure 1: Interaction of different concentrations of BAP and 2, 4-D on callus induction using stem explants

Regeneration

According to figure 2, results showed that the highest rate of regeneration ($\bar{X}$ = 46.66 %) was recorded for BA (0.5 mg/l) and IAA (1 mg/l) hormonal treatment. The lowest percentage (($\bar{X}$ = 16.66 %) was observed

for BA (1 mg/l) and IAA (0.05 mg/l) hormonal treatment. Also regeneration ($\bar{X}$ = 40 %) was seen in BA (1mg/l) and IAA (1 mg/l) hormonal treatment. In other hormonal treatment not observed any regeneration. Most root produce was observed in BA (0.5 mg/l) and IAA (1 mg/l) hormonal treatment (figure 1).

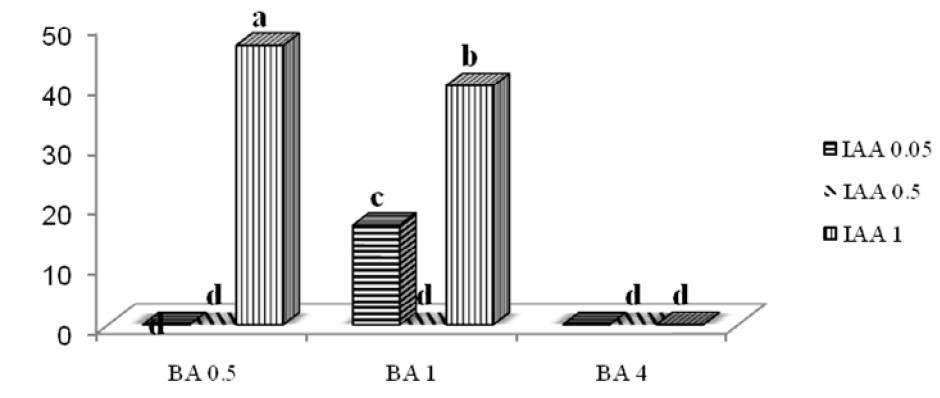

Figure 2: effects of different concentrations of BA and IAA hormones on tashnedari regeneration

Figure 3: tashnedari regenerated seedlings on hormonal treatment (0.5 BA + 1 IAA mg/l)

DISCUSSION

The highest percentage of callus induction was for BAP and 2, 4-D hormonal treatment each 1.5 mg/L, which is consistent with results obtained by [4]. They stated that the same ratio of Auxin to Cytokinin causes continued cell division and callus induction. 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). That reported that highest growth and callus fresh weight of chrysanthemum is achieved from treatment containing 4 mg/L 2, 4-D and 2 mg/L BAP. The highest rate of regeneration was recorded in BA (0.5 mg/l) and IAA (1 mg/l) hormonal treatment that is similar with Behrooznam*et al*[1] results. They reported that the most regeneration and stem produce in Maryam goli plant was in BAP (0.5 mg/l) and IAA (1 mg/l) hormonal treatment. Also results of this study is similar with results of [3]. They said the most regeneration of razak was recorded in MS culture containing (BA 0.5 mg/l + IAA 1 mg/l). Result of this study was similar with [5] report. They said the most regeneration and stem produce from behlimo calls was observed in (BA 0.5 mg/l + IAA 1.5 mg/l) hormonal treatment. The lowest regeneration percentage
($\bar{X}$ = 16.66 %) was recorded for BA (1 mg/l) and IAA (0.5 mg/l) that is inconsistent with Rahpeima and Moini [2] results. They said that the most regeneration of golmohamadi was in hormonal treatment containing BAP (2 mg/l) and IAA (0.5 mg/l), also regeneration was observed in hormonal treatment BA (1 mg/l) and NAA (1 mg/l), and not observed any regeneration in other hormonal concentration. Most root produce was observed in BA (0.5 mg/l) and IAA (1 mg/l) hormonal treatment that is similar with [3], results. [5] said the most rooting in behlimo was observed in MS medium containing BA (1.5 mg/l0 and IAA (0.5 mg/l) that is inconsistent with this study.

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