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Investigation of Plant Growth Regulators Effects on Callus Induction and Shoot Regeneration of *Bunium persicum* (Boiss.) B. Fedtsch.

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ABSTRACT

Parsi Zira, *Bunium persicum* (Boiss.) B. Fedtsch., or Black Zira, a member of the Umbellifera family, is one of the most important medicinal species with high economic value. Generally, there is limited information about in vitro culture of *Bunium persicum*. In this study, the effect of various media (MS, B5) and combinations of plant growth regulators, NAA and 2,4-D alone or together with Kin, on callus induction and shoot regeneration from hypocotyl explant were investigated. This experiment has been carried out in a completely randomized design with 30 treatments and 10 replications per treatment. The results showed significant effects of treatments on regeneration and callus induction. All the measured traits for MS medium were superior. The highest callus frequency was observed on the medium containing 0.1 mg L^{-1} or 1 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) as well as 2 mg L^{-1} 2,4-D and 0.5 mg L^{-1} kinetin. The best treatment for somatic embryogenesis was the medium containing 2 mg L^{-1} 2,4-D. The best response for shoot regeneration was observed on the medium supplemented with 1 mg L^{-1} 2,4-D. The medium containing 1 mg L^{-1} 2,4-D was the best for callus induction and shoot regeneration simultaneously. The regenerated shoots were rooted on basal medium.

Keywords: *Bunium persicum* (Boiss.) B. Fedtsch., Hypocotyls explants, Shoot regeneration, Tissue culture.

INTRODUCTION

Parsi Zira, *Bunium persicum* (Boiss.) B. Fedtsch., is a native plant of limited zones of the West Asia and grows to the Northareas of Khorasan and Kerman, East of the Zagros range to Bandar Abbas and South of the Alborz range in Iran (Khosravi, 1994). Production of this plant is limited due to seed dormancy and several biotic stresses of which wilt diseases are the most serious. It seems that only cold treatments are effective in seed germination and other treatments such as gibberellic acid, cytokinin, potassium nitrate, washing and light treatments are not useful (Sharifi and Pouresmael, 2006). Umbellifera species, including *Bunium persicum*, generally have antimicrobial properties (Shetty *et al*., 1994). Potential genetic variability for conventional breeding is limited in *Bunium persicum* (Hunault *et al.,* 1989). Therefore, genetic transformation may enable the development of transgenic plants with enhanced resistance to wilt diseases. However, an efficient micropropagation system with high shoot regeneration frequency is required. Wakhlu (1990) obtained callus from mericarps of *Bunium persicum* on MS medium supplemented with 2 mg L^{-1} 2,4-D and 4 mg L^{-1} Kin. Small white clumps of compactly packed cells developed on the callus on a medium containing 1.0 mg L^{-1} 2,4-D. These cell clumps divided into numerous globular embryos on the same medium. Embryo maturation was achieved on the basal medium

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as well as on 1 mg L^{-1} Kin supplemented medium. Sharifi (1995) used hypocotyl and cotyledon explants in *Bunium persicum* tissue culture. The calli grew faster on B5 medium containing 2 mg L^{-1} NAA and 2 mg L^{-1} Kin. Axillary buds and shoot formation from hypocotyls and somatic embryogenesis were higher on the medium supplemented with 0.1 mg L^{-1} NAA and 2 mg L^{-1} Kin and MS medium containing 0.5 mg L^{-1} 2,4-D, respectively. Ebrahimi *et al.* (2003) used embryo explants for cumin (*Cuminum cyminum*) tissue culture, yielding a large number of shoots within a short period of time without any sub culturing. The best treatments were B5 medium containing 0.2 mg L^{-1} IAA and 1 mg L^{-1} BAP or 0.2 mg L^{-1} NAA and 0.2 mg L^{-1} BAP. The objective of this study was to investigate the effect of different plant growth regulators on callus induction and shoot regeneration and to present an optimal medium for shoot regeneration of *Bunium persicum*.

MATERIALS AND METHODS

Parsi Zira seeds were collected from Kalat IN Khorasan Province in Iran. Seeds were surface sterilized in 1.5% (w/v) sodium hypochlorite solution for 15 minutes and rinsed three times with sterile distilled water. Seeds were then cultured on basal B5 and MS media supplemented with full-strength of macro- and micro-elements, vitamins and sucrose (30 g L^{-1} in MS and 20 g L^{-1} in B5 media) for 80 days at 4°C in the dark to germinate. After 3-4 days, hypocotyls were cut into segments with the same lengths (5 mm). The different plant growth regulators, NAA (0, 0.1, 1, 2 mg L^{-1}), 2,4-D (0, 0.1, 1, 2 mg L^{-1}) were used alone or together with Kin (0, 0.5, 1 ,2 ,4 mg L^{-1}). Prior to autoclaving at 121°C for 15 minutes, pH was adjusted to 5.7. The medium were solidified with 8% (w/v) agar (Sigma). This experiment has been carried out in a completely randomized design with 30 treatments and 10 replications per treatment.

The explants were cultured (one explant per each dish or replication) in sterile dishes (7×12 mm) each containing 15 ml of culture medium, sealed with Parafilm and maintained at 25±2°C under 16-hour photoperiod (30 µmoles m^{-2} s^{-1}). After 4-6 weeks, the calli were subcultured and then the number of explants producing callus and regeneration and the number of shoots regenerated from each replication were counted two weeks later. The frequency of callus induction and shoot regeneration were calculated by dividing the number of calli and shoots to the original number of plated explants. Regenerated shoots were transferred to basal medium to be rooted. Completely randomized design was used to analyze the frequency of callus formation and

Table1. Analysis of variance for shoot regeneration and callus induction frequency on B5 and MS medium.

			Mean squares	
Source of variation		Degrees of freedom	Callus induction frequency	Shoot regeneration frequency
Treatment	B5	19	0.33**	0.021 n.s.
	MS	26	0.77**	0.15*
Error	B5	168	0.18	0.027
	MS	206	0.17	0.08

*, **: Significant difference at 0.05% and 0.0.1% probability level.

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Table 2. Effect of different PGRs on shoot regeneration and callus induction frequency on B5 and MS medium.

PGRs Treatments (mg L^{-1})	Callus induction frequency		Shoot regeneration frequency	
	B5	MS	B5	MS
2 NAA	0.33 ab [a]	0.43 bcdef	0.0 b	0.0 c
2 NAA+0.5 KIN	0.1 b	0.2 ef	0.0 b	0.0 c
2 NAA+1 KIN	0.25 ab	0.4 cdef	0.13 a	0.2 abc
2 NAA+2 KIN	0.14 b	0.5 bcde	0.0 b	0.13 abc
2 NAA+4 KIN	0.2 ab	1.0 a	0.0 b	0.3 abc
0.1 2,4-D	0.3 ab	0.71 abcd	0.1 a	0.14 abc
0.1 2,4-D+0.5 KIN	0.22 ab	0.22 ef	0.0 b	0.0 c
0.1 2,4-D+1 KIN	0.4 ab	0.29 def	0.1 a	0.14 abc
0.1 2,4-D+2 KIN	0.22 ab	0.11 ef	0.11 a	0.0 c
0.1 2,4-D+4 KIN	0.0 b	0.33 def	0.0 b	0.0 c
1 2,4-D	0.4 ab	1.0 a	0.0 b	0.43 a
1 2,4-D+0.5 KIN	0.64 a	0.86 abc	0.09 a	0.29 abc
1 2,4-D+1 KIN	0.63 a	0.43 bcdef	0.0 b	0.14 abc
1 2,4-D+2 KIN	0.44 ab	0.1 ef	0.0 b	0.0 c
1 2,4-D+4 KIN	0.0 b	0.25 def	0.0 b	0.0 c
2 2,4-D	0.09 b	0.89 ab	0.0 b	0.33 abc
2 2,4-D+0.5 KIN	0.0 b	1.0 a	0.0 b	0.4 ab
2 2,4-D +1 KIN	0.33 ab	0.29 def	0.0 b	0.14 abc
2 2,4-D +2 KIN	0.14 b	0.55 abcde	0.0 b	0.18 abc
2 2,4-D +4 KIN	0.27 ab	0.27 def	0.0 b	0.09 bc

[a] Different letters within each column indicate significant differences at P= 0.05.

shoot regeneration data. The mean separation was made using Duncan's multiple range test at 0.05 probability**.** Mean of replications was used for statistical analysis.

RESULTS

Callus initiation and shoot regeneration were observed on B5 medium, two and 8 weeks after transferring explants to medium, respectively [Figures 1(A) and (B)]. Callus initiation and shoot regeneration were observed on MS medium, three and 8 weeks after explant transfer to medium, respectively. The results showed significant effects of treatments on callus induction and shoot regeneration (Table 1). Due to PGR treatments the size of callus was different. Generally, the amount of callus in treatments without cytokinin was low. All the measured traits for MS medium were superior. The highest callus frequency was observed on the medium containing 0.1 mg L^{-1} 2,4-D or 1 mg L^{-1} 2,4-D as well as 2 mg L^{-1} 2,4-D and 0.5 mg L^{-1} Kin (Table 2) but the difference among these treatments was not significant. The best response for shoot regeneration was observed on the medium containing 1 mg L^{-1} 2,4-D. The best treatment for somatic embryogenesis was the medium containing 2 mg L^{-1} 2,4-D. The medium supplemented with 1 mg L^{-1} 2,4-D was the best for callus induction and shoot regeneration simultaneously (Table 2).

The above results were obtained eight weeks after explant transfer to media. Callus proliferation and stem elongation significantly increased by sub culturing the calli that are being regenerated. The regenerated shoots were rooted after transfer to the basal medium without PGRs [Figure1(C)].

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Figure 1. Effect of PGRs on callus and root induction and shoot regeneration of *Bunium persicum*: (A) Callus initiation on hypocotyls explant of *Bunium persicum* after three weeks, (B) Early shoot regeneration from hypocotyls explant of *Bunium persicum* after 8 weeks and, (C) Root induction after transfer of *Bunium persicum* shoot to basal medium without PGRs.

DISCUSSION

Somatic embryogenesis during callus induction is not unusual in the Umbellifera family specially carrot and fennel (Hunault *et al.*, 1989). 2,4-D and NAA alone or in combination with Kin are essential for continuity of callus induction. Auxin reduction leads to oraganogenesis and adventitious embryo formation in Parsi Zira. Shoot regeneration occurred in some treatments without kinetin, showing that kinetin is not essential for Parsi Zira regeneration like other Umbellifera species such as carrot and fennel (Hunault *et al.*, 1989), but it is essential for cumin regeneration (Ebrahimie *et al.*, 2003). The previous studies suggest that callus transfer to media supplemented with 1 mg L^{-1} 2,4-D (Wakhlu, 1990) or 0.5 mg L^{-1} 2,4-D (Bonianpoor, 1995) leads to embryogenesis. However, a suitable combination of auxins and cytokinins is important for embryogenesis and organogenesis (Guohua, 1998). In some other species, induced somatic embryos might need a little cytokinin or other plant growth regulators in order to grow (Kumar *et al.*, 1988). Subsequent callus proliferation and differentiation on a basal medium without PGRs depend on 2,4-D and NAA concentration in the callus induction medium. In previous studies which were conducted on cumin, callus proliferation and stem elongation also occurred after transfer to basal medium without PGRs (Tawfik and Noga, 2002; Wakhlu *et al.*, 1990). Such an embryogenesis development is not unusual in other Umbellifera species (Hunault *et al.*, 1989). However 2,4-D restricted germination in the induced somatic embryos. Embryonic callus transfer to basal medium decreases the 2,4-D content and causes somatic embryos to germinate.

As mentioned above, Parsi Zira production is very limited because of seed dormancy and fungal diseases. By application of this method and transformation of disease resistant genes, a large number of desirable and pathogen free genotypes can be propagated.

Abbreviations

B5= Gamborg medium;
BAP= 6-benzylaminopurine;
2,4-D= 2,4-dichlorophenoxyacetic acid;
IAA= Indol-3-acetic acid;
Kin= Kinetin;
MS= Murashige and Skoog ;
NAA= α-Naphthalene acetic acid;
PGRs= Plant growth regulators.

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بررسی اثرات تنظیم کننده های رشد گیاهی بر روی القاء کالوس و باززایی شاخساره زیره پارسی *Bunium persicum* (Boiss.) B. Fedtsch.

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چکیده

زیره پارسی، *Bunium persicum* (Boiss.) B. Fedtsch. یا زیره سیاه از تیره کرفس بوده و یکی از مهمترین گونه های دارویی با ارزش اقتصادی بالا می باشد. بطور کلی اطلاعات محدودی در مورد کشت درون شیشه ای زیره سیاه وجود دارد. در این مطالعه اثر محیط کشت های مختلف (B5 و MS) و ترکیبات مختلف هورمونی، NAA (نفتالن استیک اسید)، 2,4-D (۲و۴- دی کلروفنوکسی استیک اسید) به تنهایی یا همراه با کینتین، بر روی القاء کالوس و باززایی در ریز نمونه هیپوکوتیل مورد بررسی قرار گرفت. آزمایش در قالب طرح کاملا تصادفی با ۳۰ تیمار مختلف و ۱۰ تکرار در هر تیمار انجام شد. نتایج، اثرات معنی دار تیمارها بر روی باززایی و القاء کالوس را نشان داد. محیط کشت MS در تمامی صفات اندازه گیری شده نتایج بهتری نشان داد. بیشترین کالوس در تیمار با ترکیب هورمونی ۰/۱ یا ۱ میلی گرم در لیتر 2,4-Dو یا ۲ میلی گرم در لیتر2,4-D و ۰/۵میلی گرم در لیتر کینتین مشاهده شد. بهترین تیمار از جهت القاء جنین سوماتیکی، تیمار با ترکیب هورمونی ۲ میلی گرم در لیتر2,4-D بود. بیشترین مقدار باززایی در تیمار با ترکیب هورمونی ۱ میلی گرم در لیتر2,4-D مشاهده شد که این یافته نشان می دهد برای باززایی زیره سیاه وجود هورمون سیتوکینین الزامی نیست. محیط کشت حاوی ۱ میلی گرم در لیتر2,4-D بهترین تیمار برای القاء کالوس و باززایی ساقه بطور همزمان بود. گیاهچه های باززایی شده به محیط کشت پایه جهت تولید ریشه منتقل شدند.

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