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

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Abstract: The effect of various media and combinations of Plant Growth Regulators (PGRs) on callus induction and shoot regeneration from hypocotyl explant were investigated. Simultaneous callus and shoot regeneration were obtained. The experiment was conducted as a completely randomized design. The highest callus frequency was observed on MS medium containing 0.1 mg L^{-1} 2,4-dichlorophenoxyacetic acid (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} Kinetin (Kin). The best response for shoot regeneration was observed on MS medium containing 1 mg L^{-1} 2,4-D. MS medium supplemented with 1 mg L^{-1} 2,4-D was the best for callus induction and shoot regeneration simultaneously. The regenerated plantlets were transferred to basal medium to be rooted. However suitable combination of auxins and cytokinins are important for embryogenesis and organogenesis. For the exploitation of *in vitro* techniques it is essential to optimize the conditions for whole plant regeneration.

Key words: *Bunium persicum* (Boiss.) B. Fedtsch., hypocotyl explant, rsegeneration, tissue culture

INTRODUCTION

Parsi Zira, *Bunium persicum* (Boiss.) B. Fedtsch., is a native plant of limited zones of the west Asia and grows in northern areas of Khorasan, Kerman, and east of Zagros to Bandar Abbas and south of Alborz in Iran. Production of this plant is limited due to seed dormancy and several biotic stresses of which wilt diseases are the most serious (Khosravi, 1994). Only cold treatments are effective in seed germination. Other treatments such as giberellic acid, cytokinin, potassium nitrate, washing and light treatments are not useful (Bonianpoor, 1995).

Generally *Umbeliferae* species including *Bunium persicum* have antimicrobial properties (Shetty *et al*., 1994). Also, potential genetic variability for conventional breeding is limited in *B. persicum* (Hunault *et al*., 1989). Genetic transformation may enable the development of transgenic plants with enhanced resistance to wilt diseases. However, an efficient micro propagation system with high regeneration frequency is required.

Wakhlu *et al*. (1990) obtained callus from mericarps of *Bunium persicum* on MS (Murashige and Skoog, 1962) medium supplemented with 2 mg L^{-1} 2,4-D and 4 mg L^{-1} Kin. In this report, small white clumps of compactly packed cells developed on the callus on a medium containing 1.0 mg L^{-1} 2,4-D and 0 mg L^{-1} Kin. These cell clumps differentiated into numerous globular embryos on the same medium. Embryo maturation was achieved on the basal as well as on 1 mg L^{-1} Kin supplemented medium.

Sharifi (1995) used hypocotyl and cotyledon explants in *Bunium persicum* tissue culture. The callus growth was faster on B5 (Gamborg *et al*., 1968) medium containing 2 mg L^{-1} NAA and

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2 mg L^{-1} Kin. Plumule and shoot formation from hypocotyls and somatic embryogenesis were higher on 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.

Wakhlu and Sharma (1998) obtained callus on MS medium containing 0.5 mg L^{-1} 2,4-D and 0.5 mg L^{-1} BAP from leaf base of *Heracleum candicans* Wall. Many globular embryos differentiated on callus after transfer to MS medium enriched with 2,4-D without cytokinin. Somatic embryos were matured to plantlet after transfer to medium supplemented with 0.01 mg L^{-1} BAP and 0.01 mg L^{-1} IBA.

Ebrahimi *et al*. (2003) used embryo explants for cumin tissue culture yielding a large number of shoots within short period of time without any sub culturing. In this report, 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.

Martin (2004) used stem internode and leaf explants for regeneration of plantlet from *Eryngium foetidum* L. (*Apiaceae*). The callus formation occurred on MS medium supplemented with 5.37-10.74 μM NAA and 2.32 or 4.65 μM Kin. Then, somatic embryogenesis occurred on calli after transfer to half- strength liquid MS with 2.69 μM NAA and 1.16 μM Kin.

The objective of this study was twofold: first, to investigate the effect of different plant growth regulators on callus induction and regeneration; secondly, to present an optimum medium for regeneration of *Bunium persicum*.

MATERIALS AND METHODS

Parsi Zira seeds were collected from Kalat at Khorasan province in Iran. Seeds were surface sterilized in 1.5% (w/v) sodium hypochlorite solution for 15 min and rinsed three times with sterile distilled water. Seeds were then cultured on 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) 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 (0.1, 1, 2 mg L^{-1}), 2,4-D (0.1, 1, 2 mg L^{-1}) only or together with Kin (0, 0.5, 1, 2, 4 mg L^{-1}) were used. Prior to autoclaving at 121°C for 15 min pH was adjusted to 5.7. The medium were solidified with 8% (w/v) agar (Sigma).

The explants were cultured in sterile dishes (7×12 mm) each containing 15 ml of culture medium, which was sealed with Para film and maintained at 25±2°C under 16 h photoperiod (30 μmoles m^{-2} s^{-1}). After 4-6 weeks, the calli were sub cultured. After eight weeks, the number of explants producing callus and regeneration and the number of shoots regenerated from each replication were counted. Then 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 plantlets were transferred to basal medium to be rooted. The experiment was conducted as a completely randomized design with 30 treatments and 10 replications per treatment. Mean of replications was used for statistical analysis. ANOVA was used to analyze the frequency of callus formation and shoot regeneration. Mean separation was conducted using Duncan's Multiple Range test at 0.05 probability. This study carried out in Khorasan Agriculture and Natural Resources Research Center of Iran in 2005.

RESULTS

Callus initiation and regeneration were observed on B5 medium, 2 and 8 weeks after explant transfer to medium, respectively. They were observed on MS medium, 3 and 8 weeks after explant transfer to medium, respectively (Fig. 1 and 2). The results showed significant effects of treatments

Fig. 1: Callus initiation on hypocotyl explant of *Bunium persicum* after 3 weeks

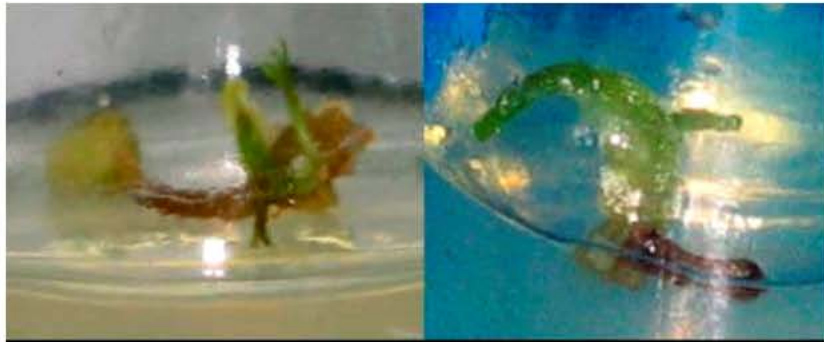

Fig. 2: Early regeneration from hypocotyl explant of *Bunium persicum* after 8 weeks

Fig. 3: Root induction after transfer of *Bunium persicum* plantlet to basal medium without PGRs

Table 1: The effect of PGRs on shoot regeneration and callus induction frequency on B5 and MS media in some treatments

PGRs treatments (mg L^{-1})	Shoot regeneration frequency		Callus induction frequency	
	B5	MS	B5	MS
2NAA	0^{b}	0^{c}	0.33^{ab}	0.43^{b-f}
$2N^{aa}$+0.5KIN	0^{b}	0^{c}	0.1^{b}	0.2^{ef}
2NAA+1KIN	0.13^{a}	0.2^{a-c}	0.25^{ab}	0.4^{c-f}
2NAA+2KIN	0^{b}	0.13^{a-c}	0.14^{b}	0.5^{b-e}
2NAA+4KIN	0^{b}	0.3^{a-c}	0.2^{ab}	1.00^{a}
0.1 2,4-d	0.1^{a}	0.14^{a-c}	0.3^{ab}	0.71^{a-d}
0.1 2,4-D+0.5KIN	0^{b}	0^{c}	0.22^{ab}	0.22^{ef}
0.1 2,4-D+1KIN	0.1^{a}	0.14^{a-c}	0.4^{ab}	0.29^{d-f}
0.1 2,4-D+2KIN	0.11^{a}	0^{c}	0.22^{ab}	0.11^{ef}
0.1 2,4-D+4KIN	0^{b}	0^{c}	0^{b}	0.33^{d-f}
1 2,4-D	0^{b}	0.43^{a}	0.4^{ab}	1.00^{a}
1 2,4-D+0.5KIN	0.09^{a}	0.29^{a-c}	0.64^{a}	0.86^{a-c}
1 2,4-D+1KIN	0^{b}	0.14^{a-c}	0.63^{a}	0.43^{b-f}
1 2,4-D+2KIN	0^{b}	0^{c}	0.44^{ab}	0.1^{ef}
1 2,4-D+4KIN	0^{b}	0^{c}	0^{b}	0.25^{d-f}
2 2,4-D	0^{b}	0.33^{a-c}	0.09^{b}	0.89^{ab}
2 2,4-D+0.5KIN	0^{b}	0.4^{ab}	0^{b}	1.00^{a}
2 2,4-D +1KIN	0^{b}	0.14^{a-c}	0.33^{ab}	0.29^{d-f}
2 2,4-D +2KIN	0^{b}	0.18^{a-c}	0.14^{b}	0.55^{a-e}
2 2,4-D +4KIN	0^{b}	0.09^{bc}	0.27^{ab}	0.27^{d-f}

Different letter(s) within each column indicate significant differences

Table 2: Variance analyze of shoot regeneration and callus induction frequency on B5 and MS media.

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

*Significant difference at 0.05 probability level, **Significant difference at 0.01 probability level

on regeneration and callus induction (Table 2). Due to PGR treatments the size of callus was different. Generally, the amount of callus in treatments without cytokinin was lower. The highest callus frequency was observed on MS 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 1). The best response for shoot regeneration was observed on MS medium containing 1 mg L^{-1} 2,4-D. MS medium supplemented with 1 mg L^{-1} 2,4-D was the best for callus induction and shoot regeneration simultaneously (Table 1). Generally, regeneration and callus induction frequency on MS medium and treatments containing 2,4-D auxin were higher than B5 and treatments with NAA (Table 1).

The above results were obtained eight weeks after explant transfer to media. Callus proliferation and stem elongation significantly increased by sub culturing the regenerated calli. The regenerated plantlets were rooted after transfer to basal medium without PGRs (Fig. 3).

DISCUSSION

Somatic embryogenesis during callus induction is not unusual in *Umbeliferae* family specially carrot and fennel (Hunault *et al*., 1989). 2,4-D and NAA only or in combination with Kin are essential for continuity of callus induction. Auxin reduction leads to organogenesis and adventitious embryo formation in Parsi Zira. Regeneration occurred in some treatments without kinetin, showing that kinetin is not essential for Parsi Zira regeneration like other *Umbeliferae* 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 suitable combination of auxins and cytokinins are important for embryogenesis and organogenesis (Guohua, 1998). In some other species, induced somatic embryos might need a little cytokinin or other plant growth regulators to grow (Kumar *et al*., 1988). Subsequent callus proliferation and differentiation on basal medium without PGRs depend on 2,4-D and NAA amount in callus induction medium. Also in the previous studies about cumin, callus proliferation and stem elongation 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 *Umbeliferae* species (Hunault *et al*., 1989). However 2,4-D prevents the induced somatic embryos to germinate. Embryonic callus transfer to basal medium decreases the 2,4-D and solves this problem. With regard to the results, it seems that MS medium and 2,4-D auxin are more suitable for *Bunium persicum* regeneration and callus induction.

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

ABBREVIATIONS

BAP- 6-benzylaminopurine; 2,4-D - 2,4-dichlorophenoxyacetic a cid; IAA- indol-3-acetic acid; Kin - kinetin; MS - Murashige and Skoog (1962); NAA - α-Naphthalene acetic acid; PGRs - plant growth regulators.

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