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

M. Valizadeh¹, and S. K. Kazemi Tabar*¹

**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.), 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 North areas 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 shoot regeneration.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Callus induction frequency</th>
<th>Shoot regeneration frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B5</td>
<td>19</td>
<td>0.33**</td>
<td>0.021 n.s.</td>
</tr>
<tr>
<td>MS</td>
<td>26</td>
<td>0.77**</td>
<td>0.15*</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B5</td>
<td>168</td>
<td>0.18</td>
<td>0.027</td>
</tr>
<tr>
<td>MS</td>
<td>206</td>
<td>0.17</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* *, **: Significant difference at 0.05% and 0.01% probability level.
Table 2. Effect of different PGRs on shoot regeneration and callus induction frequency on B5 and MS medium.

<table>
<thead>
<tr>
<th>PGRs Treatments (mg L⁻¹)</th>
<th>Callus induction frequency</th>
<th>Shoot regeneration frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B5</td>
<td>MS</td>
</tr>
<tr>
<td>2 NAA</td>
<td>0.33 ab</td>
<td>0.43 bcd</td>
</tr>
<tr>
<td>2 NAA+0.5 KIN</td>
<td>0.1 b</td>
<td>0.2 e</td>
</tr>
<tr>
<td>2 NAA+1 KIN</td>
<td>0.25 ab</td>
<td>0.4 cde</td>
</tr>
<tr>
<td>2 NAA+2 KIN</td>
<td>0.14 b</td>
<td>0.5 bcde</td>
</tr>
<tr>
<td>2 NAA+4 KIN</td>
<td>0.2 ab</td>
<td>1.0 a</td>
</tr>
<tr>
<td>0.1 2,4-D</td>
<td>0.3 ab</td>
<td>0.71 abcd</td>
</tr>
<tr>
<td>0.1 2,4-D+0.5 KIN</td>
<td>0.22 ab</td>
<td>0.22 e</td>
</tr>
<tr>
<td>0.1 2,4-D+1 KIN</td>
<td>0.4 ab</td>
<td>0.29 def</td>
</tr>
<tr>
<td>0.1 2,4-D+2 KIN</td>
<td>0.22 ab</td>
<td>0.11 ef</td>
</tr>
<tr>
<td>0.1 2,4-D+4 KIN</td>
<td>0.0 b</td>
<td>0.33 def</td>
</tr>
<tr>
<td>1 2,4-D</td>
<td>0.4 ab</td>
<td>1.0 a</td>
</tr>
<tr>
<td>1 2,4-D+0.5 KIN</td>
<td>0.64 a</td>
<td>0.86 abc</td>
</tr>
<tr>
<td>1 2,4-D+1 KIN</td>
<td>0.63 a</td>
<td>0.43 bcd</td>
</tr>
<tr>
<td>1 2,4-D+2 KIN</td>
<td>0.44 ab</td>
<td>0.1 ef</td>
</tr>
<tr>
<td>1 2,4-D+4 KIN</td>
<td>0.0 b</td>
<td>0.25 def</td>
</tr>
<tr>
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<td>0.09 b</td>
<td>0.89 ab</td>
</tr>
<tr>
<td>2 2,4-D+0.5 KIN</td>
<td>0.0 b</td>
<td>1.0 a</td>
</tr>
<tr>
<td>2 2,4-D+1 KIN</td>
<td>0.33 ab</td>
<td>0.29 def</td>
</tr>
<tr>
<td>2 2,4-D+2 KIN</td>
<td>0.14 b</td>
<td>0.55 abcde</td>
</tr>
<tr>
<td>2 2,4-D+4 KIN</td>
<td>0.27 ab</td>
<td>0.27 b def</td>
</tr>
</tbody>
</table>

* Different letters within each column indicate significant differences at P= 0.05.

RESULTS

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.

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)].
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 organogenesis 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.

References

Regeneration of Bunium persicum


بررسی اثرات تنظیم کننده های رشد گیاهی ب روی افلاک کالوس و بازازایی شاخه‌ریزه پارسی Bunium persicum (Boiss.) B. Fedtsch.

م. ولی زاده و س. ک. کاظمی تیار

چکیده

زیره پارسی Bunium persicum (Boiss.) B. Fedtsch. یکی از مهم‌ترین گونه‌های دارویی با ارزش اقتصادی بالایی باشید. بطور کلی اطلاعات محدودی در مورد کشت درون شیشه‌ای زیره سیاه وجود دارد. در این مطالعه اثر محیط کشت های مختلف B5 و MS و ترکیبات مختلف هورمونی NAA و 2,4-D روی این گونه به ترتیب با هیوکاپتین و کالیومین و در ریز نمونه هیپوکتیل مورد بررسی قرار گرفت. آزمایش در قالب طرح کامل تصادفی با 30 تیمار مختلف و 10 تکرار در هر تیمار انجام شد. نتایج ارتباط میان داربود روش بر روی بازازایی و افلاک کالوس را نشان داد. محیط کشت در تمامی صفات اندازه گیری شده نتایج بهتری نشان داد. بیشترین کالوس در تیمار با ترکیب هورمونی 1/10 میلی گرم در لیتر داشت. بیشترین کلیس در تیمار با جنین افلاک جنین بود. بیشترین مقدار بازازایی در تیمار با ترکیب هورمونی 1/5 میلی گرم در لیتر بود. بیشترین مقدار بازازایی در تیمار با ترکیب هورمونی 1/10 میلی گرم در لیتر بود. 2,4-D مشاهده شد که این یافته نشان می‌دهد برای بالایی زیره سیاه وجود هورمون سیتوکینین‌های زمینی نیست. محیط کشت حاوی 1 میلی گرم در لیتر 2,4-D بیشترین تیمار برای افلاک کالوس و بازازایی شاخه بهترین بود. گیاهانه های بازازایی شده به محیط کشت با یه گزینه تولید ریشه مستقل شدند.

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