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Evaluation of the Genetic Fidelity of *in vitro* Raised Plants of *Origanum majorana* L. Using Random Amplified Polymorphic DNA

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Abstract

Determination of the genetic characters of the plants obtained using plant tissue culture methods is important. In this study, the genetic fidelity of the plants, which are obtained by the micropropagation of the shoot tip explants of the *Origanum majorana* L. plant of medicinal and economical value in Murashige and Skoog (MS) medium which contains 1.0 mg/L Benzil Amino Purin (BAP), 0.1 mg/L naphthaleneacetic acid (NAA), 3% sucrose and 0.7% agar, has been investigated by using random amplified polymorphic DNA (RAPD-PCR) technique. Monomorphic bands were obtained as a result of all of the RAPD-PCR analyses performed. According to the results obtained, no polymorphism was detected among the micropropagated plants.
Keywords: Genetic fidelity, Medicinal herb, Micropropagation, *Origanum majorana* L., RAPD.

1. Introduction

Turkey holds approximately 70% of world thyme trade. One of the major thyme species exported is *Origanum majorana* L.' (Lamiaceae) [1]. *O. majorana*, characterised by its strong, sweet, spicy and pleasant smell, contains borneol, camphor, pinene, thymol, carvacrol, and its non-volatile components contain several metabolites, such as phenolic terpenoids (thymol and carvacrol), flavonoids (diosmetin, luteolin, and apigenin), tannins, hydroquinone, phenolic glycosides (arbutin, methyl arbutin, vitexin, orientin, and thymonin) and triterpenoids (ursolic acid and oleanolic acid) [2].

The tea made with *O. majorana* leaves and flowers is used in traditional medicine to alleviate symptoms of hay fever, sinus obstruction, indigestion, asthma, abdominal pain, headache, dizziness, colds, cough, and nervous system disorders [3]. Because of its pharmacological properties as well as its traditional use, it has been found that it has many medical effects, such as anticancer, antioxidant, antibacterial, antifungal, antiprotozoal, antiulcerogenic, anticoagulant, antiplatelet, antihyperglycemic, antigut, sedative, and diuretic [4].

Vegetative propagation is preferred over generative multiplication in origanum species. Studies on the production of tissues, organs or cells of many plants with vegetative production ability with plant tissue culture methods under *in vitro* conditions in artificial nutrient media, regardless of climatic factors, are reported in literatures [5, 6]. In the optimization studies performed in plant tissue culture applications, the general aim is primarily to obtain a large number of plants. However, studies have shown that tissue culture conditions may cause stress in plants and consequently genetic stability of the cloned genotype may change [7]. Genetic characters can be determined by phenotypic, cytological, and molecular examinations. It has been reported after several investigations that the most suitable of these methods are the molecular techniques [8, 9]. The principal molecular techniques used in the determination of the genetic differences are RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequence Repeats), ISSR (Inter Simple Sequence Repeats), and SCAR (Sequence Characterised Amplified Region) [10].

The RAPD technique, among these techniques, is one of the most preferred methods because of the diversity of genetic resources, the determination of relationships among individuals in plant populations, and the cheap, short-term use of genetic mapping studies [11]. In recent years, there have been many studies in literature on the use of the RAPD method in the analysis of genetic characterisation of plants produced by plant tissue culture [12-14].

In this study, the analysis of the genetic fidelity of the plants obtained by *in vitro* micropropagation of the *Origanum majonara* L. plant, which has medicinal, aromatic, and economical significance, using the RAPD-PCR method was investigated.

2. Materials and Methods

2.1. Micropropagation studies

Origanum majorana seeds have been supplied from Kütahya Hekim Sinan Medicinal Herbs Research Centre. In order to obtain explants to be used in micropropagation studies, *Origanum majorana* L. seeds were germinated under *in vitro* conditions in Murashige and Skoog (MS) medium [15]. Seeds were surface

disinfested with 70% ethanol for 4 min followed by immersion in 2.5% NaOCl for 6 min and finally rinsed with sterilised distilled water 3 times for 3 min in flow chamber. The shoot tip explants isolated from the plantlets obtained in sterile conditions were transferred to MS medium containing 1.0 mg/L 6-benzylaminopurine (BAP) and 0.1 mg/L naphthaleneacetic acid (NAA), 3% sucrose and 0.7% agar. For rooting studies, shoots with well-developed leaves were selected and transferred to MS medium containing 1 mg/L IBA. Approximately 2 weeks later, rooted plantlets were transferred to pots containing sterile humus and garden soil for acclimatisation studies.

2.2. Culture media and culture conditions

MS medium containing 0.1 mg/L myo-inositol, 3% (*v/v*) sucrose, 0.7% (*w/v*) agar was used in all studies. The pH of the medium was adjusted to 5.8 with 1N NaOH and 1N HCl prior to agar loading. Sterilisation of all equipment and media was carried out at autoclave under 1.1 atm pressure at 121°C for 15 min. All the cultures were incubated in a culture chamber at a temperature of 25±2°C and a light intensity of 3000-lux in a 16/8 hour (light/dark) photoperiod. Cultures were transferred to fresh media every 2 weeks.

Table 1. Nucleotide sequence of primers used in RAPD studies.

Primer code	Primer Sequence (5'-3')
OPF-16	GGAGTACTGG
OPO-15	TGGCGTCCTT
OPO-11	GACAGGAGGT
OPN-12	CACAGACACC
OPK-06	CACCTTTCCC
OPT-16	GGTGAACGCT
OPP-06	GTGGGCTGAC
OPM-06	CTGGGCAACT

2.3. Genetic fidelity analyses

The genetic fidelity of the plants obtained from 14-week-old plants by the shoot tip culture method was investigated using the RAPD-PCR method. tic Genomic DNA isolation was performed according to the protocol of the firm using GeneJET plant Genomic DNA isolation Mini Kit (Thermo Sci.). The amounts of DNA isolated from eight plants were measured with the Nanodrop™ instrument. For RAPD-PCR studies, 8 primers consisting of 10 bases produced by Operon (Almeda, Canada) firm were used (Table 1). The PCR amplification mixture (10 µl) contains 1 U Taq DNA polymerase (Fermentas), 1.2 µl dNTP mix (Promega), 1.5 µl 10 X PCR reaction buffer, 2.0 µl $MgS0_4$, 1.0 µl primer (Operon) and 20 ng of genomic DNA. The amplification reaction consisted of an initial denaturation step at 94 °C for 30 s, followed by 35 cycles of 25 s denaturation at 94 °C, 45 s annealing at 35 °C, 1 min extension at 72 °C with a final extension of 72 °C for 5 min using at as the last step. The PCR products were resolved by electrophoresis on a 1.0% agarose gel. The gel was stained with ethidium bromide (0.5 µg/ml) and visualized under UV light.

3. Results and Discussion

Plant tissue culture methods allow the production of plant and herbal metabolites in a fixed quantity and quality independent of environmental conditions. Raw material can be produced by plant tissue culture studies and use for the food, drug, and cosmetic industries [16]. For this reason, studies on the production of medicinal herbs with plant tissue culture methods have gained importance in recent years. The *O. majorana* plant has medicinal and economic importance owing to the metabolites it contains. Previous studies on the micropropagation of the plant have demonstrated that shoot tip explants show the best reproducibility in MS medium containing 1.0 mg/L BAP and 0.1 mg/L NAA plant growth regulators (Figure 2.) [17].

Figure 2. *In vitro* micropropagation of *Origanum majorana* L. in MS.

After the random amplification procedure, 6 of the 8 primers amplified and gave a visable band in agarose gel electrophoresis. It was observed that all banding profiles from micropropagated plants were monomorphic in RAPD-PCR. Figure 2. presents the monomorphic RAPD-PCR amplification bands and OPM-06 primer of the micropropagated plants.

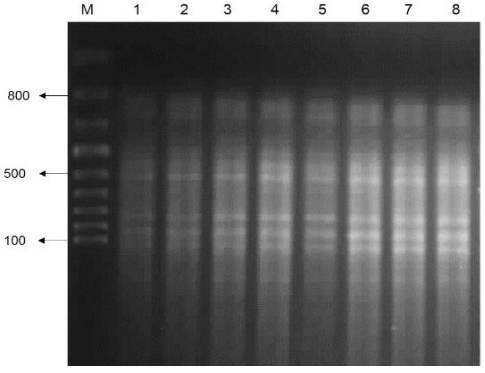

Figure 1. Polymerase chain reaction (PCR) amplification products obtained with random amplified polymorphic DNA, Primer (OPM-06), Lane M-molecular marker (1kbp), Lanes 1-8 represent *in vitro*-raised hardened plants.

In the investigations carried out by Sing et al. [8], Asthana et al. [18] and Chavan et al. [19] no genetic differences were identified during the RAPD analyses applied to micropropagated plants. In the study, shoot tips were used as explants, and no callus formation was observed during the performance of micropropagation. It has been reported by Joshi and Dhawan [20] that the use of meristematic tissues in micropropagation studies results in a lower-rate genetic difference in comparison to regeneration through callus. The study can be continued as more detailed by increasing the number of primers or by using different molecular characterisation techniques. While protection of the genetic structure is an important issue in commercial micropropagation studies, formation of genotypic differences are valuable in cases that natural variation shrinks in terms of breeding practices, or where variation is difficult to be generated. Plant tissue culture applications are the preferred method among the breeding trials in which different genotypes are aimed at. For these reasons, it is highly significant to determine the genetic characterisation of the plants obtained by plant tissue culture processes.

It has been designated in this study that, as a result of the RAPD-PCR analysis made with 6 different primers applied on the plants obtained by culture procedures in which the shoot tips of the *Origanum majorana* L. plant were exposed to the temperature of 24±2°C and 16/8 photoperiod in MS medium containing 1.0 mg/L BAP and 0.1 mg/L NAA plant growth regulators, no genetic differences were formed between them.

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