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Isolation and Characterization of Some Genes Involved in Essential Oil Synthesis in Endemic Sütçüler Thyme (*Origanum minutiflorum*)

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Abstract

Origanum minutiflorum, is an endemic species found in the Sütçüler district of Isparta province, Turkey. This study aimed to isolate and characterize the 1-deoxy-D-xylulose-5-phosphate reductoisomerase (OmDXR) and terpene synthase 1 (OmTPS-1) genes, which play roles in essential oil biosynthesis in O. minutiflorum. For this purpose, total RNA was isolated from thyme leaves, and the sequence information of the target genes was obtained using gene-specific primers. The sequences were compared with those from other species in the NCBI database using BLAST programs. The nucleotide sequences were translated into protein sequences using ExPASy Tools, the three-dimensional structures of the encoded proteins were constructed with 3D SWISS-MODEL, and phylogenetic relationships of nucleotide and protein sequences were analyzed using the MEGA X version 11 software package. The results showed that the full-length cDNA nucleotide sequence of the OmDXR gene is 1,700 base pairs (bp), and its nucleotide sequence displayed 74.22%-81.07% similarity to nucleotide sequences from other species in previous studies, while the protein sequence showed 70.92%-72.95% similarity. The full-length cDNA nucleotide sequence of the OmTPS-1 gene was determined to be 1.836 bp, and its nucleotide sequence exhibited 75.99%-89.58% similarity to nucleotide sequences from other species, while the protein sequence showed 51.66%-69.88% similarity. This study represents the first molecular-based research on the O. minutiflorum species. Therefore, it will provide a valuable resource for future studies.

Article Info

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Keywords

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Gen klonlama
Origanum minutiflorum
Terpen sentaz
Uçucu yağ

Endemik Sütçüler Kekiğinde (*Origanum minutiflorum*) Uçucu Yağ Sentezinde Rol Oynayan Bazı Genlerin İzolasyonu ve Karakterizasyonu

Öz

Origanum minutiflorum, Türkiye'de Isparta ilinin Sütçüler ilçesinde yetişen endemik bir türdür. Bu çalışmada O. minutiflorum bitkisinden uçucu yağ sentezinde görev alan 1-deoksi-D-ksilüloz fosfat redüktoizomeraz (OmDXR) ve terpen sentaz 1 (OmTPS-1) genlerinin izolasyonu ve karakterizasyonu amaçlanmıştır. Bu amaç doğrultusunda kekik yapraklarından toplam RNA izole edilmiş ve hedef genlere özgü primerler ile genlerin sekans bilgileri elde edilmiştir. Sekans bilgileri BLAST programları kullanılarak, NCBI veri bankasında bulunan ve farklı türlerden elde edilen gen sekansları ile karşılaştırılmıştır. Nükleotid dizilerin protein dizisine dönüştürülmesinde ExPASy Tools, kodlanan proteinlerin 3 boyutlu yapısının oluşturulmasında 3D SWISS MODEL, nükleotid ve protein dizilerinin filogenetik ilişkileri, MEGAX 11 paket programı kullanılarak oluşturulmuştur. Çalışma sonucunda OmDXR genine ait tam uzunluktaki cDNA nükleotid uzunluğunun 1 700 baz çifti ve gene ait nükleotid dizisinin önceki çalışmalarda farklı türlerden elde edilen nükleotid dizileri ile %74.22-81.07, protein dizisinin ise %70.92-72.95 arasında benzerlik gösterdiği belirlenmiştir. OmTPS-1 genine ait tam uzunluktaki cDNA nükleotid uzunluğunun ise 1.836 baz çifti ve gene ait nükleotid dizisinin önceki çalışmalarda farklı türlerden elde edilen nükleotid dizileri ile %75.99-89.58, protein dizisinin ise %51.66-69.88 arasında benzerlik olduğu tespit edilmiştir. Söz konusu çalışma O. minutiflorum türünde yapılan ilk moleküler temelli çalışma olmuştur. Dolayısıyla konu ile ilgili gelecekte yapılacak olan çalışmalar için bir kaynak özelliği taşıyacaktır.

Anahtar Kelimeler

DXR
Essential oil
Gene cloning
Origanum minutiflorum
Terpene synthase

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Introduction

Owing to its geographical location, Türkiye possesses a rich flora in terms of genetic diversity and endemism, and many plants hold a genetic center status. Türkiye's flora includes over 12,000 flowering plants, with more than 30% of them being medicinal and aromatic plants (Güner et al., 2012).

Origanum minutiflorum L., also known as "Sütçüler Thyme," is an endemic species native to the Eastern Mediterranean and Southwestern Anatolia regions of Türkiye, especially in the provinces of Antalya and Isparta. It is a perennial plant with small, needle-like leaves. Its leaves are green, hairy along the edges, and oval in shape. The flowers are pink or purple and grow densely in clusters. The plant typically thrives in mountainous areas and dry soils. It has an essential oil yield ranging from 2% to 5%. It is rich in carvacrol, comprising around 40-80% of the volatile oil, along with 25% thymol, 13-8% terpinene, and 6% p-cymene (Oke and Aslım, 2010; Kılıçgün et al., 2014; Bozdemir, 2019). Numerous studies have been conducted on the volatile oil components and biological effects of Origanum species. This is because the volatile oils obtained from Origanum species are highly valuable and possess many biological and pharmacological properties, such as antifungal, antimicrobial, antioxidant, anticancer, and antidiabetic properties (Koutsoumanis et al., 1998; Skandamis and Nychas, 2000; Tsigarida et al., 2000; Dadalıoglu and Evrendilek, 2004; Vardar-Ünlü et al., 2007; Bower et al., 2014; Fakir et al., 2016; Marchese et al., 2016; Soliman et al., 2016; Elmastas et al., 2018; Sevindik et al., 2023)

Terpenes (also known as terpenoids or isoprenoids), one of the gene families responsible for the synthesis of volatile oils and various chemical compounds in medicinal aromatic plants, participate in the synthesis of over 30,000 different structures with various carbon skeletons, including hundreds of different monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), and triterpenes (C30) (Buckingham, 1998; Degenhardt et al., 2010). To understand the structural features and functions of terpenes, numerous studies have been conducted on their isolation from different plant species (Yang et al. 2013; Din et al. 2014; Ashaari et al., 2020; Wang et al., 2020). Terpenes play significant roles in various fundamental plant processes, including growth, development, reproduction, and defense (Gershenzon and Kreis, 1999). However, despite the importance of these products in medicine, agriculture, and industry, there is not enough information available about the true role of most terpenes in nature (Gershenzon and Dudareva, 2007).

Therefore, in this study work, the isolation and characterization of 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) and terpene synthase 1 (TPS-1) genes, which play a role in the quality of essential oil in *O. minutiflorum*. Upon reviewing the literature, it was found that there had been no prior studies on gene cloning in *O. minutiflorum* (Sütçüler Thyme), which is an endemic species. In line with this general objective,

- Primers specific to the DXR and TPS1 genes isolated from different species were designed and synthesized.
- Rapid amplification of the 5' and 3' ends of the target genes with gene-specific primers and sequencing of these gene fragments were achieved.
- Full-length cDNA nucleotide sequences were determined by assembling the gene fragments.
- Nucleotide and amino acid sequences were analyzed using software programs. The nucleotide and amino acid sequences of the target genes were compared with homologs available in gene banks to analyze phylogenetic and molecular evolutionary relationships.

Endemic species are often restricted to specific geographical regions, which creates limitations in collecting plant material and conducting research. Most of these plant species have limited or no genomic information available, making gene identification and sequencing more challenging. The same applies to *O. minutiflorum*, as it is an endemic plant species. A review of the literature reveals that molecular studies

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on this species are very limited. This study represents the first gene cloning research conducted on *O. minutiflorum*. Therefore, it serves as a valuable resource for future studies or breeding programs aimed at improving essential oil quality or yield after the domestication of this plant.

Material and Method

Plant material

In this study, young leaves of the plant "Sütçüler Thyme" (*O. minutiflorum*) were used as the plant material. Plant materials were collected from the Sütçüler district of the Isparta province (Figure 1), and after freezing with liquid nitrogen, they were stored at -80°C in the laboratory. Necessary permits for the collection of materials used in the study were obtained from TAGEM (General Directorate of Agricultural Research and Policies) and the Provincial Directorate of Agriculture and Forestry in Isparta.

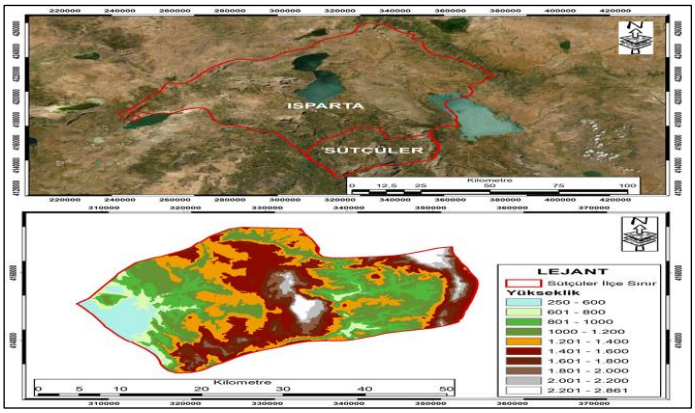


Figure 1. Coordinates where the plant materials used in the study were collected.

RNA extraction and cDNA synthesis

Total RNA was isolated according to the manufacturer's protocol using the RNeasy Plant Mini Kit (Catalog No: 74904). To eliminate potential DNA contamination, total RNA isolates were treated with DNase using a DNase treatment kit (Ambion) (Catalog No: AM2222). The quality of the isolated RNA samples was assessed using a nanodrop, and RNAs with purity values ranging from 1.98 to 2.00 were used.

Following DNase treatment, mRNA was converted to cDNA using the protocol provided for the "ProtoScript II First cDNA Synthesis" kit (Catalog no: E6560L). For the partial isolation of 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) and terpene synthase 1 genes (TPS1)' cDNA, the synthesized thyme cDNA, as mentioned above, was subjected to PCR amplification using primers commonly used in different species (Crocoll, 2011; Majdi et al., 2017) (Table 1).

Table 1. Primers used in the study.

Gene Name	Primer Name	Forward and reverse primers	Primer Length	Tm (°C)	%GC
TPS1	Tps1-F	5'-TTCTCAAAGACCGCGGCATCAACACTATCCT-3'	31	68	48
	Tps1-R	5'-CTGCAGCCCCCGCCCCTTGTTC-3'	22	70	73
DXR	DXR-F	5'-GCCTTTTGTCCTTCCTCTTGC-3'	21	60	52
	DXR-R	5'-TCCGCTCGATGCTTGTCGC-3'	19	61	63

PCR samples consisted of 5 µl cDNA, 2 µl degenerate primers, 5 µl 10X PCR buffer solution, 1 µl dNTP mix (10 µM), and 1 µl Taq polymerase, with the reaction volume completed to 50 µl with distilled water. The PCR steps used to generate partial cDNA with gene-specific primers for target genes are given in Table 2.

The PCR products were extracted from the agarose gel using the "Nucleo Trap" gel extraction kit (Clontech) (Catalog No: 740584), and the obtained pure DNAs were cloned using the "NEB® PCR Cloning Kit" (Catalog no: E1203S). Subsequently, chemical transformation was performed on Escherichia coli strain DH5α competent cells according to the established procedure. The recombinant plasmids were verified

using “Monarch® Plasmid Miniprep” (Catalog no: T1010S) kit, and the positive DNA clones were subjected to sequencing using the dideoxy method with an ABI3730 automated sequencer (Iontek Company, Türkiye).

Table 2. PCR stages used to generate partial cDNA with gene-specific primers for target genes

Step	Phase	Time	Heat	Cycle
I. Step	Denaturation	1.5 min	95°C	5
	Annealing	2 min	57°C	
	Extension	1 min	72°C	
II. Step	Denaturation	30 sec	95°C	15
	Annealing	1 min	57°C	
	Extension	30 sec	72°C	
III. Step	Denaturation	30 sec	95°C	20
	Annealing	1 min	57°C	
	Extension	2 min	72°C	
7 min 72°C final extension				

Amplification of DXR and TPS-1 genes using RACE sequencing and gene cloning

For RACE Ready cDNA synthesis, the protocol of the "SMARTer® RACE 3'5' amplification kit (Takara) (Catalog no: 634859)" was used. For 5'-RACE cDNA, 3.0 µL of DNase-treated RNA sample, 1 µL of 5’–CDS primer A, and 7.0 µL of sterile H2O were mixed in separate tubes. For 3'-RACE cDNA, 3.0 µL of RNA sample, 1 µL of 3’–CDS primer A, and 7.0 µL of sterile H2O were mixed in separate tubes. To obtain the entire coding regions of the target genes, 3'- and 5'-RACE Ready cDNA samples and the protocol of the "SMARTer® RACE 5'/3" amplification kit (Takara, Catalog no: 634859) were used. Next, PCR was conducted as follows: 6 cycles of 30 s at 95°C followed by 60 s at 72°C, 6 cycles of 30 s at 95°C, 30 s at 68°C, and 1 min at 72°C; 25 cycles of 30 s at 95°C, 30 s at 66°C, and 120 s at 72°C. The amplification products were run on an agarose gel containing 1 tris/borate/ EDTA buffer solution. PCR products were extracted from the gel and cloned into TOPO-TA cloning vectors, and the recombinant plasmids were sequenced (Iontek Company, Turkey).

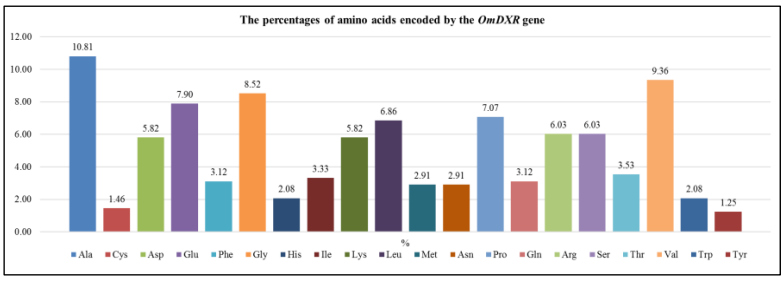
Bioinformatics analyses

The nucleic acid fragments obtained in this study were sequenced by Iontek Company. Nucleotide sequencing was performed using the enzymatic Sanger’s dideoxy method. These sequences were compared with gene sequences obtained from other species and available in the NCBI database using the BLAST program (BLASTn and BLASTp) (Altschul et al. 1997). To convert nucleotide sequences into protein sequences, ExPASy was employed. In sequence analysis, ExPASy Tools were used, and for constructing the three-dimensional structure of the protein, the 3D SWISS MODEL program was used. The phylogenetic relationships between nucleotide and protein sequences and sequences of previously isolated species from the gene bank were established using the MEGA 11 software package (Tamura et al., 2021). The open reading frame (ORF) of the obtained gene was determined using the "ORF Finder" database.

Results and Discussion

The full-length cDNA nucleotide length of the *OmDXR* gene (GenBank Accession No: OR652277) was determined to be 1,700 base pairs (bp). Of the 1,700-bp-long gene, 138 bp corresponded to the 5'-UTR and 116 bp corresponded to the 3'-UTR regions. The amino acid sequence of *OmDXR* was analyzed using the ExPASy Tools database. It has been determined that the ORF region of *OmDXR* encodes a protein that is 481 amino acids in length. In this study, secondary structure predictions of the amino acid sequence were analyzed using the Predict Protein database. According to structural classification analysis, the secondary structure composition of the chain was calculated as follows: helix 41.79%, strand 10.39%, and coil 47.81%. Because of the bioinformatics analysis, the molecular weight of the *OmDXR* protein was calculated to be 52.36 kDa, and the theoretical isoelectric point (PI) was determined to be 5.71. The GRAVY (Grand average of hydropathicity index) value for the protein synthesized by the *OmDXR* gene was calculated as -0.334. Kyte and Doolittle (1982) reported that a protein with a GRAVY value of less than zero is hydrophilic. Therefore, it is possible to say that the protein synthesized by the *OmDXR* gene exhibits a

hydrophilic character. The amino acid counts and their percentages encoded by *OmDXR* are presented in Figure 2. According to this, it was determined that the alanine (Ala) amino acid had the highest occurrence in the amino acid sequence with a percentage of 10.81%. This was followed by Valine (Val) with 9.36% and Glycine (Gly) with 8.52%. The least abundant amino acids were Tyrosine (Tyr) with 1.25% and Cysteine (Cys) with 1.46%. In addition, the conserved motifs and phosphorylation regions of the *OmDXR* protein were identified. The MEME program was used to determine the motifs, and phosphorylation regions were identified based on a score of 0.8 or higher using NetPhos 3.1. Three conserved motifs were identified in the *OmDXR* protein sequences. It was found that the most abundant phosphorylation region contained serine residues. Helices: Structures formed by a specific arrangement of a sequence of amino acids in the primary structure of a protein. They come in different types, such as alpha-helices and beta-helices. An alpha-helix is a structure in which the polypeptide chain is coiled in a spiral. Straight Chain: A section of the protein's primary structure where consecutive amino acids are linked together in a straight line. There is no specific structural folding in this region. Coil: A part of the protein's secondary structure. It is a region where the straight chain is loosely coiled and the polypeptide chain is folded into a more compact three-dimensional structure (Zhu et al., 1993). Therefore, it is possible to say that the predicted three-dimensional structure of the *OmDXR* protein generated using the SWISS-MODEL interface is in the form of an alpha-helix (Figure 3).



Ala: Alanine, Arg: Arginine, Asn: Asparagine, Asp: Aspartic Acid, Cys: Cysteine, Glu: Glutamic Acid, Gln: Glutamine, Gly: Glycine, His: Histidine, Ile: Isoleucine, Leu: Leucine, Lys: Lysine, Met: Methionine, Phe: Phenylalanine, Pro: Proline, Ser: Serine, Thr: Threonine, Trp: Tryptophan, Tyr: Tyrosine, Val: Valine

Figure 2. Percentage of amino acids encoded by the *OmDXR* gene.

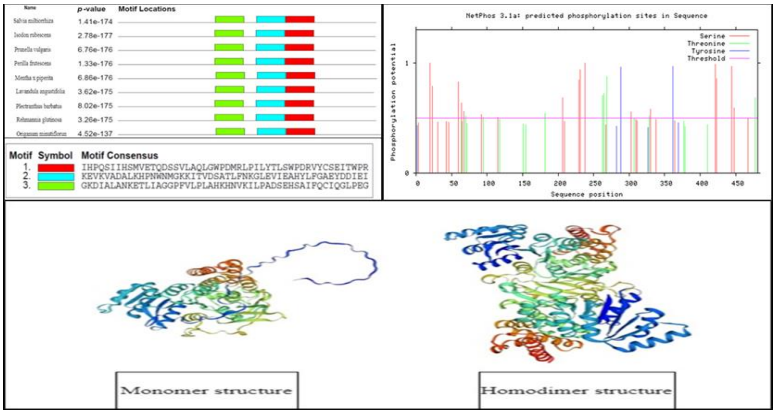


Figure 3. Conserved motifs of the *OmDXR* protein, putative phosphorylation sites of *OmDXR* proteins, and 3D structure of the *OmDXR* protein

The relationship between the nucleotide sequence obtained from *OmDXR* and previously identified DXR sequences in the NCBI database was investigated (Figure 4). The MEGAX 11 (Molecular Evolutionary Genetics Analysis) program was used to establish this relationship. The *OmDXR* nucleotide sequence was compared with sequences of 10 different plant species with similar nucleotide sequences using BLASTn analysis in the NCBI database (https://blast.ncbi.nlm.nih.gov). Similarities were determined using the Bootstrap 1000 and neighbor-joining methods (Felsenstein, 1985). The constructed dendrogram was divided into two main groups (I and II), and the second main group (II) was further subdivided into

subgroups. The highest similarity was observed between Salvia miltiorrhiza (DXR) and S. splendens (DXR). The *OmDXR* gene was found to belong to the second main group (II).

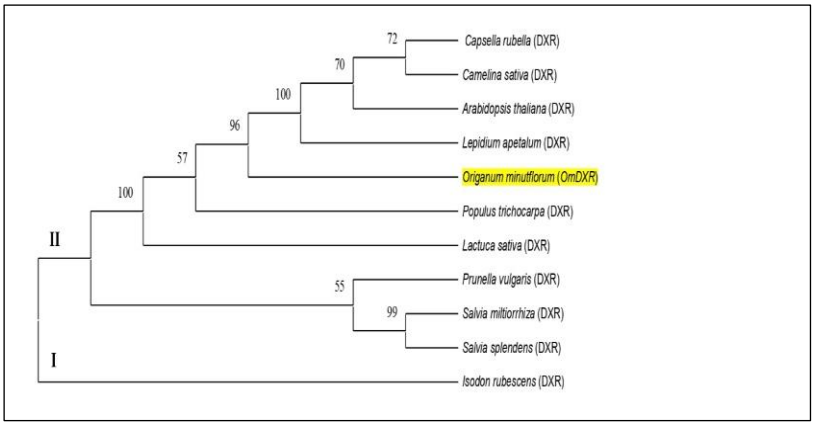
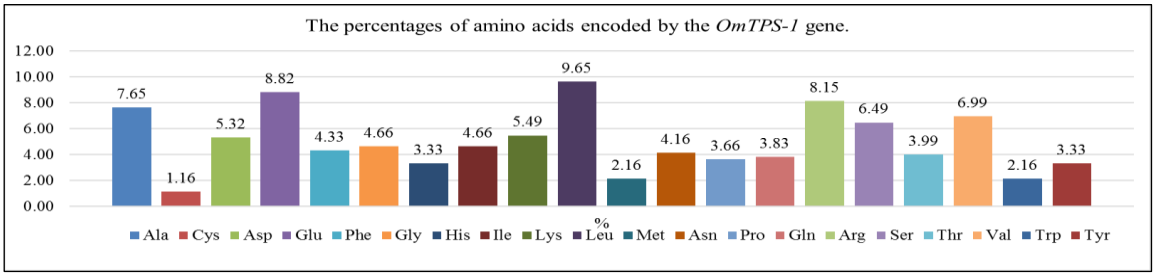


Figure 4. Phylogenetic relationship of the *OmDXR* nucleotide sequence

The full-length cDNA nucleotide sequence of the *OmTPS-1* gene (GenBank Accession No: OR652278) was determined to be 1.836 base pairs (bp). Of the 1.836-bp length of the gene, 1.806 bp comprise the ORF region of the gene. The remaining 7 bp (highlighted in blue) constitute the poly(A) tail. The amino acid sequence of *OmTPS*-1 was analyzed using the ExPASy Tools database. It was determined that the *OmTPS-1* gene's ORF region encodes a protein that is 601 amino acids long. In this study, secondary structure predictions of the *OmTPS-1* amino acid sequence were analyzed using the "Predict Protein" database. According to the structural classification analysis of *OmTPS-1*, the secondary structure composition of the chain was calculated as follows: helix 65.56%, extended strand 0.33%, and coil 34.61%. Because of the bioinformatics analysis, the molecular weight of the *OmTPS-1* protein was calculated to be 69.64 kDa, and its theoretical isoelectric point (PI) was determined to be 6.72. The GRAVY (Grand average of hydropathicity index) value for the protein synthesized by the *OmTPS-1* gene was calculated to be -0.482. It was found that similar to the *OmDXR* protein, the protein synthesized by the *OmTPS-1* gene also has a hydrophilic character. The amino acid sequence of *OmTPS-1* and the corresponding amino acid counts and percentages are shown in Figure 5. According to this analysis, the amino acid Leucine (Leu) has the highest occurrence in the sequence, accounting for 9.65% of the total amino acids. This was followed by Glutamine (Gln) at 8.82% and Arginine (Arg) at 8.15%. The least abundant amino acids in the sequence are Cysteine (Cys) at 1.16% and Tryptophan (Trp) and Methionine (Met) at 2.16%. In addition, the conserved motifs and phosphorylation regions of the *OmTPS-1* protein were identified. The MEME program was used to determine the motifs, and phosphorylation regions were identified based on a score of 0.8 or higher using the NetPhos 3.1 program. Three conserved motifs were identified in the *OmTPS-1* protein sequences. It was found that the most abundant phosphorylation region contained serine residues. The predicted 3D structure of the *OmTPS-1* protein, generated using the SWISS-MODEL interface, was determined to exhibit an alpha-helix conformation (Figure 6).



Ala: Alanine, Arg: Arginine, Asn: Asparagine, Asp: Aspartic Acid, Cys: Cysteine, Glu: Glutamic Acid, Gln: Glutamine, Gly: Glycine, His: Histidine, Ile: Isoleucine, Leu: Leucine, Lys: Lysine, Met: Methionine, Phe: Phenylalanine, Pro: Proline, Ser: Serine, Thr: Threonine, Trp: Tryptophan, Tyr: Tyrosine, Val: Valine

Figure 5. Percentage of amino acids encoded by the *OmTPS-1* gene.

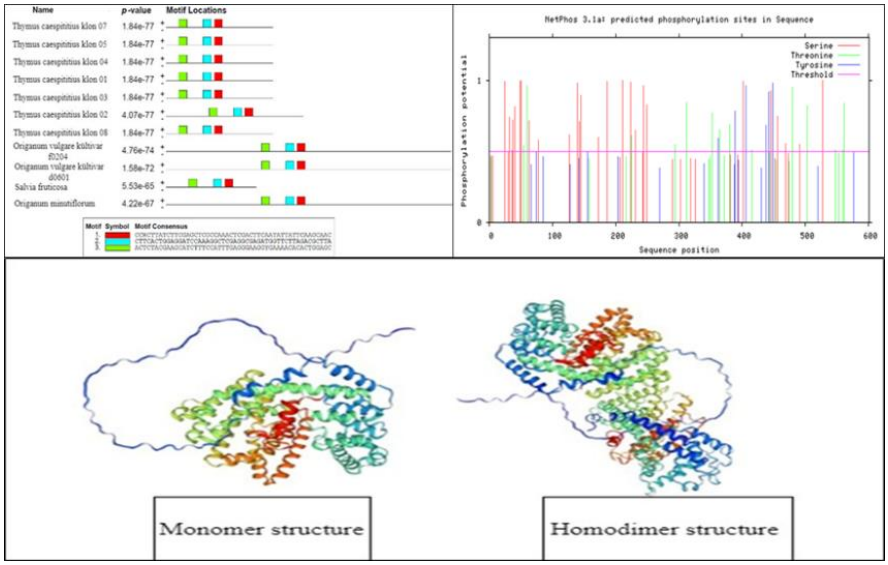


Figure 6. Conserved motifs of *OmTPS-1* protein, putative phosphorylation sites of *OmTPS-1* proteins, and 3D structure of *OmTPS-1* protein

The relationship between the nucleotide sequence of *OmTPS-1* and previously identified TPS1 sequences in the NCBI database was also investigated (Figure 7). The establishment of this relationship used the MEGAX.11 program. The *OmTPS-1* nucleotide sequence was subjected to BLASTn analysis in the NCBI database to identify 10 different plant species with similar nucleotide sequences. Similarities were determined using the Bootstrap 1000 and neighbor-joining methods. The generated dendrogram was divided into two main groups (I and II), with the second main group (II) further subdivided into subgroups. The highest similarity was observed in the *O. vulgare* cultivar f0204 - *O. vulgare* cultivar d0601 and *Thymus caespititius* clone 01 - T. *caespititius* clone 02 species. It was found that the *OmTPS-1* nucleotide sequence exhibited high similarity with the TPS nucleotide sequence isolated from *O. vulgare* species and that both belonged to the same main group within the same subgroup.

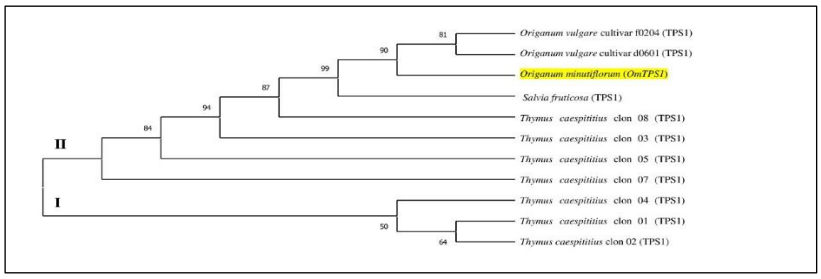


Figure 7. Phylogenetic relationship of the *OmTPS-1* nucleotide sequence

Terpene synthase genes in aromatic plants encode enzymes responsible for the synthesis of plant essential oils (Bayrak 2006). Therefore, terpene synthases play a crucial role in the synthesis of volatile oils (Tholl, 2006). Terpene synthases are categorized into several classes. One of these is the first-class terpene synthases, which are classified as mono-, sesqui-, and diterpene synthases based on their substrates geranyl diphosphate, farnesyl diphosphate, and geranylgeranyl diphosphate, respectively. Terpene synthase 1 (TPS-1) is a type of sesquiterpene and is an enzyme involved in terpene synthesis in plants (Schnee et al. 2002). A enzyme contributing to isoprenoid synthesis is 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), which converts 1-deoxy-D-xylulose-5-phosphate to 2-C-methyl-D-erythritol 4-phosphate. DXR genes have been isolated and characterized in E. *coli* (Takahashi et al., 1998; Kuzuyama et al. 2000; Radykewicz et al. 2000), Eubacterium (Jomaa et al. 1999; Grolle et al. 2000), and several plant species (Lange and Croteau 1999; Schwender et al. 1999; Veau et al. 2000; Rodriguez-Concepcion et al. 2001; Kim et al. 2006). However, no previous study has investigated the isolation of the DXR gene in *O.*

minutiflorum species. Based on this information, homologs of previously characterized DXR and TPS1 genes from different plant species were cloned into *O. minutiflorum*, and their full-length DNA sequences were determined. In addition, these characterized genes were compared with their homologs in the literature. Yao et al. (2008) reported that the full-length cDNA of *CaDXR* isolated from *Camptotheca acuminata* is 1.823 base pairs (bp) in length, encoding a 472 amino acid polypeptide with a molecular weight of 56 kDa and an isoelectric point (PI) of 6.58. Gong et al. (2005) isolated *GbDXR* from *Ginkgo biloba* and found that its full-length cDNA is 1.720 bp, encoding a 477 amino acid protein with a molecular weight of 52 kDa and a PI of 6.58. Similarly, Engprasert et al. (2005) isolated a DXR gene from *Coleus forskohlii*, and its full-length cDNA was found to be 1.407 bp, encoding a 469 amino acid protein with an estimated molecular weight of 50.8 kDa. Wu et al. (2009) reported that *SmDXR* isolated from *Salvia miltiorrhiza* has a full-length sequence of 1.756 bp and encodes a 474-amino acid protein with a molecular weight of 51.859 kDa. Gupta et al. (2013) isolated *WsDXR* from *W. somnifera*, which has a full-length cDNA of 1.428 bp, encoding a 475 amino acid protein. Liu et al. (2015) isolated *AsDXR* from *Aquilaria sinensis*, and its full-length sequence is 1.768 bp, encoding a 478 amino acid protein with a molecular weight of 51.859 kDa. Tong et al. (2015) isolated a DXR gene from *Tripterygium wilfordii*, and its full-length sequence is 1.816 bp, encoding a 496-amino acid protein. Cheng et al. (2019) isolated *DzDXR* from *Dioscorea zingiberensis*, which has a full-length cDNA of 1.643 bp, encoding a 470 amino acid protein. Kwon et al. (2003) reported the full-length cDNA of *ZrTPS1* from *Zygosaccharomyces rouxii*, which encodes a 492-amino acid protein with a molecular weight of 56 kDa. Kim et al. (2008) isolated PgTPS from *Panax ginseng*, with a 1.883-bp cDNA encoding a 568-amino acid protein. Crocoll et al. (2010) isolated multiple TPS genes from O. *vulgare*, ranging from 1.659 to 1.803 bp in length. Li and Fan (2011) reported *HcTPS2* from *Hedychium coronarium* with a 1.788-bp cDNA encoding a 596-amino acid protein. Hsu et al. (2012) isolated *TsTPS1* and *TsTPS2* from *Toona sinensis*, with lengths of 1.788 and 1.671 bp, encoding 595 and 556 amino acid proteins, respectively. Vezzaro et al. (2012) reported *OeTPS1* from *Olea europaea*, with a 1.749-bp cDNA encoding a 583-amino acid protein. Lima et al. (2013) isolated *TcTPS2* from T. *caespititius*, with a 2.308-bp cDNA encoding a 598-amino acid protein. Nawade et al. (2019) reported *PdTPS1* from *Prunus dulcis* with a 1.671-bp cDNA encoding a 557-amino acid protein. Xie et al. (2020) conducted the cloning and analysis of the DXR gene in *Morinda officinalis*. The researchers determined through sequence comparisons using BlastP in the NCBI database that *MoDXR* exhibited high sequence similarity with several other DXR genes, such as *Coffea arabica* DXR (*CaDXR*) and *Rauvolfia verticillata* DXR (*RvDXR*). Wu et al. (2009) conducted the cloning and characterization of the DXR gene in *Salvia miltiorrhiza*. They reported that the amino acid sequence of the gene, which they named *SmDXR*, exhibited 94% homology with *Mentha* × *piperita*, 82% with *Oryza sativa*, and 80% with *Arabidopsis thaliana*. Yan et al. (2009) determined the molecular characterization of the DXR gene in *Salvia miltiorrhiza*. Bioinformatics analyses revealed that DXR showed homology with DXR genes isolated from other plant species. Phylogenetic analyses indicated that the DXR gene isolated from S. *miltiorrhiza* is most closely related to the DXR gene isolated from *Lycopersicon esculentum*. Seetang-Nun et al. (2008) conducted the molecular cloning and characterization of the DXR1 and DXR2 genes from *Hevea brasiliensis*. They found that the genes, which they named *HbDXR1* and *HbDXR2*, showed 73%-87% similarity to DXR genes isolated from other plant species. Kim et al. (2008) aimed to isolate and characterize the terpene synthase gene from *Panax ginseng*. They reported that the amino acid sequence of the gene, named *PgTPS*, showed 61% similarity with *Actinidia deliciosa* (AAX16121), 61% with *Vitis vinifera* (AAS66357), 55% with *Linepithema hirsutum* (AAG41891), and 52% with *Medicago truncatula* (AAV36464). Li and Fan (2011) isolated the *HcTPS2* gene responsible for fragrance formation in *Hedychium coronarium*. The researchers reported that the amino acid sequence obtained from this gene showed 35%-38% similarity with known monoterpene synthase genes in other species. Vezzaro et al. (2012) conducted the isolation and characterization of terpene synthase genes involved in taste development during the ripening of *Olea europaea* L. fruits. The researchers found that the *OeTPS1* gene showed 68% sequence similarity with *Phyla dulcis* (ADK62524), 62% with the geraniol synthase gene from *Ocimum basilicum* (AAR11765), and 58% with the R-linalool synthase gene isolated from O. *basilicum* (Q5SBP3). Abbas et al. (2019) conducted the functional characterization and expression analysis of two terpene synthase (TPS) genes

(*LoTPS1* and *LoTPS3*) responsible for fragrance formation in *Lilium 'Siberia'* flowers. The researchers reported that the amino acid sequence of *LoTPS1* showed 45.88% similarity with TPS4 isolated from *Populus trichocarpa*, while *LoTPS3* exhibited a high similarity of 40.85% with S-(+)-linalool synthase from *Cinnamomum osmophloeum*. They also found that *LoTPS1* and *LoTPS3* showed 27.41% similarity with each other. In our study, the full-length cDNA of *OmDXR* was determined to be 1.700 bp, with a 138-bp 5'-UTR, a 116-bp 3'-UTR, and a 1.446-bp ORF encoding a 481-amino acid protein. Sequence comparisons with previous studies revealed similarity ranging from 74.22% to 81.07% at the nucleotide level and from 70.92% to 72.95% at the protein level. Additionaly, the full-length cDNA of *OmTPS-1* was determined to be 1.836 bp, with a 1.806-bp ORF encoding a 601-amino acid protein. Sequence comparisons with previous studies revealed similarity ranging from 75.99% to 89.58% at the nucleotide level and from 51.66% to 69.88% at the protein level.

Conclusion

In conclusion, in this study, the nucleotide sequences of *OmDXR* and *OmTPS-1* genes, which are involved in the synthesis of essential oils in the endemic species *O. minutiflorum*, were successfully characterized and compared with homologs. The importance of the DXR and TPS genes examined in this study lies in its critical role in volatile oil formation, and the fact that it has not been previously studied in *O. minutiflorum* makes our research significant. Therefore, we believe that the information obtained from this study will be valuable for future studies aimed at improving the quality of this species when it is professionally cultivated after being harvested from the wild.

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Author Contributions

The authors equally contributed to the preparation of this paper.

Conflict of Interest

As the authors of this study, we declare that we do not have any conflict of interest statement.

Ethics Committee Approval

As the authors of this study, we declare that we do not have any ethics committee approval.

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