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IDENTIFICATION OF A SMALL HEAT SHOCK PROTEIN GENE FROM PISTACHIO

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Abstract

High temperature and water scarcity drastically influence adversely growth and production of pistachio (Pistacia vera). In this case, breeders have been faced this challenge between resistant to abiotic stress factors and high yield of varieties development. Understanding the responses of plants to abiotic stress factors will make significant contributions to solve this problem. Pistachio is among the important agricultural products for Turkey, which is grown in hot and dry regions during summer months. High temperature stress caused protein dysfunctions in plants. Small Heat Shock Proteins (sHSP) play an important role due to its function to protect the structures of denatured proteins against these stresses. In this study, we determined partial gene sequence of Small Heat Shock Protein from P. vera via degenerate primers prepared from National Center for Biotechnology Information. Herewith, it can be used for gene expression in abiotic stress studies in pistachio plants.

Key words: *abiotic stress, chaperone, phylogeny, Pistacia vera, sHSP.*

INTRODUCTION

Pistacia genus (Anacardiaceae family) has been reported to consist of at least eleven species. Of those species, *P. mexicana* and *P. texana* are of USA and Mexico originated. The other species are mainly distributed within the Mediterranean region, Western and Central Asia and the Middle East (Esmail-Pour, 2001). Turkey is of the important pistachio producer and suppliers in the world and possesses many wild species of pistachio nut. Of those species, *P. vera, P. terebinthus, P. khinjuk, P. atlantica, P. mutica, P. palaestina* and *P. lentiscus* exhibit distribution in different regions of Turkey. The main pistachio rootstock used in Turkey is *P. vera*, and followed by *P. khinjuk, P. terebinthus* and *P. atlantica* (Acar et al., 2017). Of those species, pistachio (*Pistacia vera*) is the most important commercial agricultural products. *Pistacia* is the only commercially grown *Pistacia* species compared to other *Pistacia* species. Other *Pistacia* species are commonly evaluated as rootstock in addition to different purposes as soap, coffee and medical purposes in Turkey (Ertürk et al., 2015).

Production is concentrated especially in the south-eastern Anatolia region of Turkey. This region is classified as a dry and semi-dry area. For this reason, cultivation is done almost without irrigation.

Pistachio is an economically long-lasting fruit species. Therefore, selecting rootstock is a very important factor in new pistachio orchard layout. Although pistachios are relatively tolerant to abiotic stress factors such as drought and salt, drought stress adversely affects growth, dry matter, and yield (Esmaeilpour et al., 2015).

In addition to heat stress, plant sHSPs are also produced under other stress conditions such as drought and salinity. Small heat shock proteins (sHSPs) play an important role against abiotic stress factors. sHSPs are known to protect cells in response to stress from the detrimental effect of stress. However, the mechanisms of cell protection by sHSPs are largely unknown (Sun et al., 2002). In recent years, molecular studies related to abiotic stresses such as drought, heat stress have increased with rapid and sharp changes in environmental conditions. However, the molecular studies of pistachio have remained extremely limited.

There are only a few genome sequences in The National Center for Biotechnology Information (NCBI) about *Pistacia* species (Jazi et al., 2016).

This study was designed to identify a partial sHSPs gene sequence for used gene expression study in *Pistacia* species.

For this reason, along with the study, the present results and identifications are considered to contribute to the forthcoming studies regarding gene expression profiles in response to the abiotic stress conditions.

MATERIALS AND METHODS

Plant Materials

Leaf samples were collected from the collection orchard of the Kilis 7 Aralik University Agricultural Research and Practice Center (TUAM). Leaves of plants were collected in triplicate from pistachio trees and immediately frozen in liquid nitrogen for stored at –80°C.

DNA Extraction

DNA was extracted from young leaf tissue using CTAB DNA isolation method (Untergasser, 2008). Subsequently, an RNase treatment was performed on the eluted DNA samples. Purity and concentration of the DNA were checked both on 1% (w/v) agarose gels and by µDrop Plate spectrophotometer. (Thermo Scientific Multiskan GO).

PCR analysis

Two degenerated primers Forward 5'- CGCYTCYTCAACACCAACGC – 3' and Reverse 5'- GGCGGAGATGAAGAACGG – 3'were designed based on the conserved sequence of known sHSPs in NCBİ. PCR reaction was performed in a 20 ml reaction volume. The PCR temperature profile was 94°C for 5 min followed by 35 cycles of 94°C for 45 sec., 55°C for 45 sec., 72°C for 1 min and a final extension step at 72°C for 10 min. The PCR products were separated on 1.5% agarose gel and purified DNA bands from agarose gel by the PCR Gel purification kit (The Vivantis Gel DNA Recovery Kit). Positive bands were sequenced on an ABI377 Automated Sequencer (Applied Biosystem), and the resulting sequences were verified and subjected to cluster analysis.

Sequence analysis of sHSPs: The searches for nucleotide and amino acid sequence similarities were conducted with BLAST programs at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/).

Phylogenetic analysis of sHSPs gene

A phylogenetic tree was constructed by using the MEGA 7.0 software by the maximum-likelihood (ML) and neighbor-joining (NJ). For statistical reliability, the nodes of the tree were evaluated by boot- strap analysis with 1000 replicates.

RESULTS AND DISCUSSIONS

Plant sHSPs are all encoded by nuclear genes and are divided into six classes. sHSPs are localized in cell of cytosol, nucleus, plastids, endoplasmic. In eukaryotes, sHSPs are abundant and different in high plants compared to other eukaryotes (Waters et al., 1996; Lee & Vierling, 2000). Heat shock proteins as a molecular chaperone are not only triggered by heat stress but they are also expressed in response to the effect of osmotic stress. In this context, there are some studies reporting the sHSP-encoding genes induced by water stress (Coca et al., 1996), cold stress (Pla et al., 1998), heavy metal (Györgyey et al., 1991), UV radiation (Murakami et al., 2002).

In this study, the partial sequence of HSP gene from *Pistacia vera* was reported for the first time. Based on sequences, a phylogenetic tree was constructed by using MEGA7.0.

Plants were separated and formed two distinct branches in the phylogenetic tree.

The relationship in the tree was generally displayed a good agreement by being taxonomy (Figure 1). Pistachio, a member of commonly known as the cashew family has shown a 100% similarity with mango from the same family in comparison to other species. Moreover, sHSPs gene sequence of *Pistacia vera* exhibited 66 % similarity with *Citrus sinensis*.

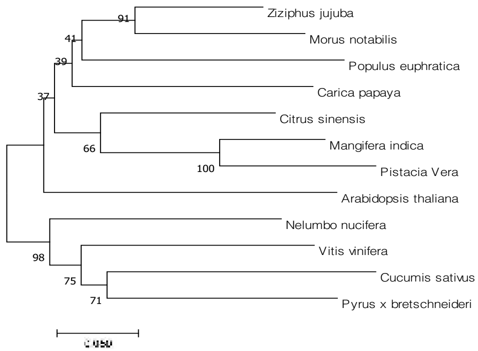

Figure 1. A phylogenetic tree of HSP family members constructed with the neighbor-joining method

The common names, species names and the GenBank accession numbers are the same as those in Table 1. Numbers at each branch indicate the percentage of times a node is supported in 1000 bootstraps pseudo-replication by neighbor-joining.

Table 1 Sequences used in the phylogenetic tree

Sequence ID	Plant Species	Localization
EU513278.1	*Mangifera indica*	--------------
XM_006490055.3	*Citrus sinensis*	mitochondrial
XM_016022420.2	*Ziziphus jujuba*	mitochondrial
XM_010265959.2	*Nelumbo nucifera*	chloroplastic
XM_022053869.1	*Carica papaya*	mitochondrial
XM_010091379.2	*Morus notabilis*	mitochondrial
XM_011012544.1	*Populus euphratica*	mitochondrial
XR_002031999.1	*Vitis vinifera*	mitochondrial
EU289284.1	*Arabidopsis thaliana*	mitochondrial
XM_004147155.2	*Cucumis sativus*	chloroplastic
XM_009350311.2	*Pyrus x bretschneideri*	chloroplastic

CONCLUSIONS

Along with the present study, we-for the first time-isolated and identified a small heat shock protein gene from pistachio. Moreover, we can underline and note that there a few molecular studies for *Pistacia* species hitherto, highlighting the present first report to be fundamental for the ahead studies related to possible gene expression profiles against abiotic stress factors.

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Additional Information

For access partial gene sequence of Small Heat Shock Protein of *P. vera* by being FASTA format at www.orobanche.net/P.vera.shsp.fasta.

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