



## Phylogenetic Analysis and Lipoxygenase (LOX) Gene Family Variation in The Pistachio

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### ABSTRACT

Lipoxygenases (LOX) gene family is a type of nonheme iron-containing dioxygenases, which has a very important aspect in plant development and fruit quality. LOX gene, which is responsible for lipid oxidation, the main role for the biosynthesis pathway of unsaturated fatty acids. Although some studies have investigated the LOX gene family in several species including arabidopsis, soybean, peanut and apple, there is no information from Pistachio; and the phylogeny of this gene family in the *Pistacia* genus is still not determined. In this study, *Arabidopsis thaliana* LOX1 gene (NCBI Reference Sequence: NM\_104376.3) was selected and used as a query sequence for performing a BLASTN search. Among all sequence query which was found by NCBI platform, 9 sequences were selected for further analysis. Phylogenetic tree of full-length LOX gene sequences from the *Pistacia* genus was constructed using the Maximum Likelihood method with MEGA software. By using phylogenetic analysis, we identified variations in gene structure and revealed the phylogenetic evolutionary relationship of this gene family. Additionally, this may serve as a reference value for assessing the genetic relationships among various LOX genes in *Pistacia* genus species. This variations provides us the possibility the design the primer to achieve us to find the exact LOX gene in *Pistacia* genus and future research on the evolutionary history and transgenic research on LOX genes.

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## Introduction

Pistachio belongs to *Pistacia* genus, which is the most important genus of flowering plants from Anacardiaceae family. (Zohary, 1952; Motalebipour et al., 2018; Kafkas et al., 2023). Pistachios owe their economic significance to being highly favored nuts due to their rich content of unsaturated fatty acids and potassium. These elements possess both antioxidant and anti-inflammatory properties. Nevertheless, Pistachio (*Pistacia vera*) crops face substantial losses each year worldwide due to a range of factors, including drought, salt, herbivorous insects, and viruses. (Christensen et al., 2015). In plants, the production of oxylipins and related compounds, such as jasmonic acid (JA), green leaf volatiles, divinyl ethers, and traumatic acid, is facilitated by enzymes known as lipoxygenases (LOXs) within the LOX pathway (Christensen et al., 2015).

Lipoxygenases (LOXs), a type of non-heme iron-containing dioxygenase, are widely found in plants and contribute significantly to the development of fruit aroma,

a crucial aspect of fruit quality (Needleman et al., 1986). These enzymes are also prevalent in the animal and plant kingdoms and play essential roles in resisting both biotic and abiotic stresses (Song et al., 2016). The biosynthesis of Lipoxygenases has been extensively studied in various plant species, including *Arabidopsis thaliana* (Melan et al., 1994), *Triticum aestivum* (Feng et al., 2012), *Glycine max* (González-Gordo et al., 2023), *Arachis hypogaea* (Mou et al., 2022) and *Malus × domestica* (Vogt et al., 2013). According to NCBI platform, 9 full length genes were recorded for Lipoxygenases (LOXs): 3 gene in *Arabidopsis thaliana*, 3 gene in *Homo sapiens*, 3 gene in *Rattus norvegicus* and one gene in *Triticum aestivum*. Among 3 gene in *Arabidopsis thaliana* (LOX1, LOX3, LOX5), LOX1 were used as a reference sequence in the current study. The aim of the study is to characterize the main Lipoxygenases gen in *Pistacia vera* and showed the distance between genes.

**Material and Methods**

Specific information on the LOX gene was obtained using the NCBI-Gene. A total of 9 species gene information was retrieved, including 9 known species information LOX gene of *Arabidopsis thaliana* with Reference Sequence: NM\_104376.3 were selected for further analysis.

Phylogenetic trees of full-length sequences of different LOX genes of *Pistacia* genus were constructed by the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993) using Molecular Evolutionary Genetics Analysis Version 11 (MEGA version 11 <http://www.megasoftware.net>, Tamura et al., 2021).

**Results and Discussion**

The present study provided basic information to understand the genetic diversity of LOX gene in the

*Pistacia* genus. LOX gene has been studied in various plant species, such as *Arabidopsis thaliana* (Melan et al., 1994) and *Triticum aestivum* (Feng et al., 2012). Among 9 LOX genes, three genes in *A. thaliana*, were define in NCBI platform. NCBI's RefSeq of LOX1 gene of *A. thaliana* (NM\_104376.3) was selected and used as a query sequence for performing a BLASTN search. BLASTN was done against all nucleotide database in *Pistacia* (taxid: 55512) organism. A Total of 11 putative non-redundant LOX gene were identified by performing BLASTN search against all database. Among 11 sequence query, 9 sequences with high coverage were selected for further analysis (Table 1). The sequence with zero E-value sequence represented the query sequence that matched the database sequence and was more significant. Nine sequence queries showed the predicted LOX family gene of *Pistacia* genus.

According to MEGA software, 9 different genes were analyzed and the genetic distance was calculated using the highest log likelihood method constructed a phylogenetic tree of the *Pistacia* genus (Figure 1).

Table 1. Information of all 9 gene sequence information of LOX in *Pistacia vera*.

No	Accession	Predicted name	Query Cover	E value	Accession length
1	XM_031404813.1	linoleate 9S-lipoxygenase 5	0.96%	0.0	2696
2	XM_031425379.1	linoleate 13S-lipoxygenase 3-1	0.96%	0.0	2976
3	XM_031406178.1	linoleate 13S-lipoxygenase 3-1	0.96%	0.0	2987
4	XM_031416247.1	linoleate 9S-lipoxygenase 5	0.96%	0.0	2992
5	XM_031426686.1	linoleate 13S-lipoxygenase 2-1	0.96%	0.0	2820
6	XM_031426696.1	linoleate 13S-lipoxygenase 2-1	0.95%	0.0	2864
7	XM_031398663.1	lipoxygenase 6	0.94%	0.0	2908
8	XM_031412135.1	lipoxygenase 6	0.94%	0.0	3062
9	XM_031392570.1	linoleate 9S-lipoxygenase 5	0.94%	0.0	2717

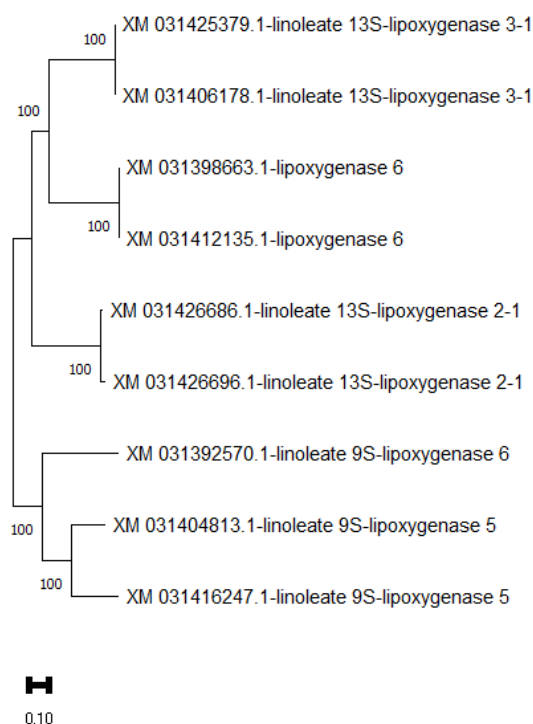


Figure 1, Phylogeny analysis for 9 predicted LOX gene sequences of *Pistacia vera* by using the Maximum Likelihood method and Tamura-Nei mode in MEGA software. The tree with the highest log likelihood (-20334.16) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches.

The results showed that the nine different LOX gene sequences from *Pistacia vera* were divided into two main groups. Each group was divided to 2 subgroups. The first group contained four sequences from two predicted gene (linoleate 13S-lipoxygenase 3-1, lipoxygenase 6), which separated in two branches. It means two predicted genes were very similar to each other. Furthermore, two gene of linoleate 13S-lipoxygenase 2-1 genes were placed in one branch and very close to the first group. Linoleate 9S-lipoxygenase 5 and linoleate 9S-lipoxygenase 6, were placed in different branches which means the differences of these genes. Two genes of linoleate 9S-lipoxygenase 5, and one gene of linoleate 9S-lipoxygenase 6 were placed very close and located in the same branch.

According to the results, the variation predicted by LOX genes in *Pistacia vera* showed the differences among the sequence and also protein followed by mechanism of a LOX gene. These variations are useful for primer design and finding the level of gene expression in other *Pistacia* species. The high level of gene expression can be concluded by the high amount of LOX gene in species for special purposes in breeding programs. Furthermore, some of the above species could be used directly as a breeding program.

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