



Genome-wide Identification of PME1 Genes in Wild Olives (*Olea europaea sylvestris* L.) by Bioinformatic Analysis

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ABSTRACT

In the present study, 47 PME1 type 1 genes and 57 PME1 type 2 genes were identified with bioinformatic analysis. The PME1 genes were localized separately on chromosomes 1, 2, 3, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21 and 22, but mainly at the level of the scaffold. The biological functions of the PME1 type 1 genes were found to be in the areas of biological regulation, metabolism and cellular functions. Their cellular localization appears to be associated with cell parts. For the PME1 type 2 genes, the biological functions were determined as biological regulation, metabolic and cellular functions. A total of 393 Arabidopsis miRNAs targeting 47 olive PME1 type 1 genes were identified. Two specific miRNAs targeting the OePME11-07 gene were found (ath-miR8168 and ath-miR774b-5p). For the PME1 type 2 genes, 269 Arabidopsis miRNAs were found, including 14 specific miRNAs targeting OPMEI2-02, OPMEI2-03, OPMEI2-27, OPMEI2-28, OPMEI2-29, OPMEI2-30, OPMEI2-40 and OPMEI2-54. These results suggest that PME1 genes in olives may not only play a role in cell development, germ cell formation and plant growth, but also play an important role in abiotic and biotic stress conditions in the olive.

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Introduction

The olive tree (*Olea europaea* L.) is one of the most popular trees widespread throughout the Mediterranean region. The socially, economically and ecologically valuable olive is a long-lived oil plant that belongs to the *Oleaceae* family. In view of the morphological characteristics and geographical distribution of the olive tree, six subspecies are distinguished today: subsp. *cuspidata* in Africa and Asia, subsp. *laperrinei*, subsp. *maroccana*, subsp. *guanchica*, subsp. *cerasiformis* and finally subsp. *europaea*. *Olea europaea* L., which is thought to have been domesticated 6000 years ago, is divided into two types, wild and cultivated. Wild olives (*Olea europaea sylvestris*) are known for having thorny branches, rather small fruit and a lower oil yield (Sesli and Yeğenoğlu, 2017).

The most common type of olive plant, *Olea europaea* L., which is abundant in the Mediterranean region, is a short and firm fruit plant that grows up to 10 meters high. The olive plant has many advantages. The leaves of the olive plant, which are used in the cosmetics industry and alternative medicine and have a high nutritional value, are also used as animal feed in many countries (Guerrero et al., 2016; Ozturk et al., 2021).

DNA markers such as RAPDs, ISSRs, SSRs or AFLPs are widely used for characterization, revealing genetic relationships, genome mapping or population studies (Belaj et al., 2003; De la Rosa et al., 2003; El Aabidine et al., 2010; Brake et al., 2014; Sesli and Yeğenoğlu, 2017). Bioinformatics is the computational study and processing of biological data that can be of great use in molecular biology. It is basically a multidisciplinary field in which

new tools and software are being developed to expand our knowledge of biological data. Bioinformatics tools are also used to study specific gene families, with quantitative trait loci being particularly important as these are economically important traits in agriculture, as well as to understand the functions and expressions of genes under abiotic and biotic stress, to improve production quality and yield, and to understand the mechanism of susceptibility and resistance. Biological data analysis with different software tools is now a useful way for analyzing the genomes of both prokaryotes and eukaryotes such as the olive. It has been used for cultivar discrimination, classification and studies on olive oil (Beiki et al., 2012; Ben Ayed et al., 2016; Ayed and Rebai, 2018; Sevindik, 2019).

Pectin Methyl Esterase Enzyme

Plant cells are surrounded by a wall of polysaccharides, highly glycosylated proteins and various polymers. Plant cell walls, in turn, have a complex and dynamic structure that can adapt and respond to biotic and abiotic stress factors, in addition to the normal processes of development, growth and change. PME (pectin methylesterase) is a type of enzyme found in many different plant species and in bacteria. Pectin methylesterase, a type of pectin enzyme, deesterifies pectin by cleaving hydrogen and oxygen elements into methyl ester groups (Showalter, 1993; Wong, 2008; Keegstra, 2010).

The pectin methylesterase inhibitor, known as PMEI, belongs to a family that includes pectin methylesterase genes in some plant species. There are two types of PMEI proteins. While type I has a pectin esterase domain, type II lacks the pectin esterase domain. PMEIs inhibit pectin methylesterases and invertases by forming a non-covalent complex. It has been observed to be frequently expressed as a large inactive preprotein. It is also located at the N-terminus of PMEs predicted from DNA sequences. This suggests that both PMEs and their inhibitors are expressed and subsequently processed as a single polyprotein. The enzyme pectin methylesterase, which is found in almost all plants, has been detected in citrus fruits, apples, bananas, grapes, cherries, carrots, plums, potatoes, tomatoes and radishes. Pectin methylesterase inhibitor proteins were first found in the cell walls of algae. The pectin methylesterase inhibitor plays an active role in cell wall metabolism, in the ripening stage of the fruit, in cell wall growth and in the stage of pollen shedding (Deytieux-Belleau et al. 2008; Coculo and Lionetti, 2022).

PMEIs are associated with fruit texture and firmness, they affect firmness of fruit by the regulation of methylesterification of pectin. It is important for industry because it influences consumer preferences. The other role of PMEIs is related with disease resistance, the cell wall is a barrier against pathogens, by conducting the resistance of cell wall, they alter the resilience of olive trees.

PMEIs are indisputably active in plant physiological developmental processes such as pollen formation, pollen growth, seed germination, root development, stem modification and fruit ripening. The bioinformatic analysis and gene expression analysis of PMEI genes in different plants such as tea plant, maize, banana, soybean, strawberry and orange carried out recent years, the genes were identified and localized in plant genomes (Wang, 2019; Zhang, 2019; Xue, 2020; Li, 2021; Husna, 2021; Li, 2022).

The aim of the present study is the characterization and identification of PMEI genes which are associated with different functions in plant vegetation and the bioinformatic analysis of these genes in the wild olive tree (*Olea europaea sylvestris*) using bioinformatic tools.

Material and Methods

The olive genome was downloaded from the NCBI database in FASTA format. PFAM domain searches for PMEI genes in the olive genome were performed using the CLC Genomic Workbench 21 (Qiagen, 2022). After screening for PMEI genes, two types of the PMEI gene family were identified in the analyzed genome: PMEI type I and PMEI type II. The genome and CDS sequences were found in NCBI (NCBI, 2022). The chromosomal location of the PMEI genes was determined in the NCBI database and the genes were named according to their chromosomal locations. Molecular weights of the proteins (kDa), lengths, pI points and instability of the proteins (Table 1 a, b) were determined using ExPASy ProtParam tools (Gasteiger et al., 2005). The loci of PMEIs displayed as diagrams using MapChart (WUR, 2022), and introns and exons were identified using GSDS (Hu et al., 2015). The conserved domains and 3-dimensional structure of the proteins were identified using MemeSuite (Bailey et al., 2015) and Phyre2 (Kelley et al., 2015), respectively. In MemeSuite, motif discovery mode was selected as Classic, site distribution expected as Zero or One Occurrence Per Sequence (zoops), number of motifs assigned as 8, default values used for advance options. To construct 3D structure of proteins, intensive mode used in Phyre2, the tool utilizes Hidden Markov Model for prediction of secondary structure. The phylogenetic tree of PMEI genes was constructed using MEGA11 (Tamura et al., 2021). Phylogeny reconstruction in MEGA11 was carried out using default options (substitution type nucleotide, Tamura Nei Model, Rates among sites-uniform rates, ML Heuristic Method-Nearest Neighbor Interchange). Blast2Go (Conesa et al., 2005) was used to analyze the gene ontology and reveal the functions of the proteins.

Results and Discussion

As a result of the bioinformatic analysis, 47 PMEI type1 and 57 PMEI type2 protein family members were identified in the olive genome. The PMEI proteins identified in olives were designated as OePMEI. The isoelectronic effect (pI) value, molecular weights and variability of the olive proteins were determined. The data obtained can be found at Table 1. Thirty-four members of the OePMEI type1 proteins were basic (pH > 7) and 13 members were acidic (pH < 7). Thirty-one members of the OePMEI type2 proteins showed basic (pH > 7) properties and 26 members were acidic (pH < 7).

The smallest member of the OePMEI type1 proteins contained 243 aa, while the largest PMEI type1 protein contained 622 aa. In the OePMEI type 2 proteins, the smallest member has 95 aa and the largest 296 aa. Comparing the molecular weights of the PMEI type 1 and PMEI type 2 proteins, type 1 varies between 69.6 kDa and 49.5 kDa, while type 2 varies between 34 kDa and 16.1 kDa. Three members of the type 1 proteins were unstable and 44 members were stable, while the 24 members of the type 2 proteins were unstable and 33 members were stable.

Table 1a. Characteristics of PMEII Type I and Type II Genes

ID	NCBI Database	Physical position on olives genome			Protein length (aa)	pI	Molecular weight (Da)	Instability index	Stable or unstable
		Chromosome	Start position (bp)	End Position (bp)					
OePMEII-01	XP_022887658.1	1	444,005	447,092	589	8.88	64737.45	33.36	stable
OePMEII-02	XP_022888716.1	1	6,971,845	6,975,216	551	9.36	61332.67	34.04	stable
OePMEII-03	XP_022898195.1	2	14,529,313	14,532,463	514	5.05	56743.62	26.94	stable
OePMEII-04	XP_022851013.1	2	14,533,391	14,535,731	515	6.33	56875.34	26.10	stable
OePMEII-05	XP_022843285.1	2	22,432,006	22,435,244	360	9.50	40555.87	40.98	unstable
OePMEII-06	XP_022862728.1	3	4,208,310	4,211,456	514	9.33	56866.44	32.41	stable
OePMEII-07	XP_022859094.1	3	21,239,762	21,243,181	445	5.32	49496.07	34.72	stable
OePMEII-08	XP_022880392.1	7	425,359	427,772	517	9.00	57975.29	38.49	stable
OePMEII-09	XP_022879513.1	7	13,375,438	13,378,770	582	8.56	63084.99	29.66	stable
OePMEII-10	XP_022882054.1	8	18,401,256	18,404,479	533	8.66	59236.04	38.49	stable
OePMEII-11	XP_022883301.1	9	15,439,744	15,442,459	581	8.81	64843.31	35.44	stable
OePMEII-12	XP_022884987.1	10	21,614,595	21,617,949	576	6.96	63185.21	31.91	stable
OePMEII-13	XP_022884988.1	10	21,614,874	21,617,669	517	6.40	56613.87	32.75	stable
OePMEII-14	XP_022884990.1	10	21,634,311	21,637,462	576	7.50	63203.27	32.04	stable
OePMEII-15	XP_022884991.1	10	21,634,574	21,637,199	517	6.85	56631.93	32.89	stable
OePMEII-16	XP_022886639.1	10	45,074,273	45,077,311	546	8.38	60089.72	23.07	stable
OePMEII-17	XP_022887039.1	11	2,200,120	2,203,494	586	9.28	64698.81	35.52	stable
OePMEII-18	XP_022888116.1	11	21,084,584	21,088,026	579	7.99	63010.12	33.27	stable
OePMEII-19	XP_022888201.1	11	23,694,699	23,697,373	584	9.21	65381.22	35.04	stable
OePMEII-20	XP_022888209.1	11	23,736,112	23,739,162	448	8.68	49459.88	20.61	stable
OePMEII-21	XP_022889233.1	11	30,324,454	30,327,656	540	5.57	59443.76	40.74	unstable
OePMEII-22	XP_022889551.1	12	1,068,208	1,072,379	579	7.56	64300.83	40.58	unstable
OePMEII-23	XP_022892079.1	12	10,234,472	10,239,163	525	9.18	58331.09	37.67	stable
OePMEII-24	XP_022893622.1	13	27,232,953	27,235,529	622	5.86	69608.77	37.52	stable
OePMEII-25	XP_022899057.1	16	18,793,221	18,798,630	567	9.42	63397.28	30.72	stable
OePMEII-26	XP_022842506.1	17	4,183,740	4,186,719	531	5.31	58572.68	37.95	stable
OePMEII-27	XP_022843895.1	18	6,694,160	6,698,114	576	8.23	63335.58	33.43	stable
OePMEII-28	XP_022844638.1	18	22,675,116	22,703,337	569	6.83	62201.36	34.16	stable
OePMEII-29	XP_022844639.1	18	22,677,468	22,700,985	540	6.32	59081.54	33.22	stable
OePMEII-30	XP_022849290.1	22	5,303,712	5,306,505	567	6.90	61209.36	30.98	stable
OePMEII-31	XP_022864004.1	scaffold	1	1.45	243	8.66	25905.22	28.01	stable
OePMEII-32	XP_022852275.1	scaffold	6.689	9.964	569	8.48	62700.85	29.27	stable
OePMEII-33	XP_022852276.1	scaffold	6.962	9.691	569	8.48	62700.85	29.27	stable
OePMEII-34	XP_022866762.1	scaffold	12.878	15.382	538	8.85	60466.43	33.57	stable
OePMEII-35	XP_022861419.1	scaffold	13.053	15.834	517	9.55	57758.87	36.33	stable
OePMEII-36	XP_022867034.1	scaffold	14.916	20.496	590	9.37	64918.80	34.65	stable
OePMEII-37	XP_022852277.1	scaffold	18.539	22.433	526	8.28	57938.57	25.08	stable
OePMEII-38	XP_022867865.1	scaffold	20.408	26.606	573	9.19	63686.98	35.49	stable
OePMEII-39	XP_022852746.1	scaffold	54.671	57.161	564	8.24	62002.57	31.24	stable
OePMEII-40	XP_022861329.1	scaffold	68.331	70.742	570	8.58	62104.93	23.85	stable
OePMEII-41	XP_022852279.1	scaffold	73.422	76.309	528	8.00	57156.41	38.63	stable
OePMEII-42	XP_022852750.1	scaffold	127.447	129.92	563	8.99	62204.03	30.70	stable
OePMEII-43	XP_022852280.1	scaffold	162.966	165.784	528	9.01	57509.98	35.57	stable
OePMEII-44	XP_022851456.1	scaffold	295.743	298.227	563	6.53	61865.31	30.98	stable
OePMEII-45	XP_022866488.1	scaffold	342.698	344.941	571	9.32	62944.85	33.97	stable
OePMEII-46	XP_022854648.1	scaffold	353.648	355.79	534	9.36	60022.85	36.97	stable
OePMEII-47	XP_022866513.1	scaffold	354.135	356.322	507	7.97	55843.55	37.51	stable
OePMEII-01	XP_022851002.1	2	14,511,726	14,512,573	196	4.27	21487.89	27.71	Stable
OePMEII-02	XP_022898323.1	2	14,756,856	14,757,903	194	9.20	21553.70	47.33	unstable
OePMEII-03	XP_022856060.1	3	4,182,082	4,183,249	146	9.64	16064.27	23.73	stable
OePMEII-04	XP_022863066.1	3	4,804,923	4,805,920	186	8.69	20836.05	32.41	stable
OePMEII-05	XP_022872674.1	3	15,077,150	15,078,070	186	8.91	20945.07	35.95	stable
OePMEII-06	XP_022872685.1	3	15,232,742	15,233,629	210	9.98	23604.41	34.02	stable
OePMEII-07	XP_022879523.1	7	13,404,667	13,405,810	177	7.56	19600.60	45.09	unstable
OePMEII-08	XP_022882281.1	8	12,701,674	12,704,566	174	5.11	18990.68	37.75	stable
OePMEII-09	XP_022882055.1	8	18,357,073	18,358,196	215	6.88	23160.32	29.07	stable
OePMEII-10	XP_022883243.1	9	14,529,012	14,545,702	255	4.24	27947.00	46.20	unstable
OePMEII-11	XP_022883514.1	10	15,054,867	15,056,631	148	9.17	16318.06	40.91	unstable
OePMEII-12	XP_022886794.1	10	16,248,912	16,249,627	165	5.58	17236.55	25.35	stable
OePMEII-13	XP_022885214.1	10	25,341,837	25,343,047	244	4.80	26465.32	33.37	stable
OePMEII-14	XP_022886893.1	10	43,358,736	43,359,531	184	4.85	20141.79	32.42	stable
OePMEII-15	XP_022889458.1	11	29,986,493	29,987,484	196	9.08	21554.56	35.67	stable
OePMEII-16	XP_022889489.1	11	37,238,889	37,239,760	197	8.93	21911.30	40.42	unstable

Table 1b. Characteristics of PMEII Type I and Type II Genes

ID	NCBI Database	Physical position on olives genome			Protein length (aa)	pI	Molecular weight (Da)	Instability index	Stable or unstable
		Chromosome	Start position (bp)	End Position (bp)					
OePMEI2-17	XP_022891736.1	12	10,389,893	10,390,496	167	9.08	18672.55	37.41	stable
OePMEI2-18	XP_022892009.1	12	28,970,151	28,971,377	143	4.72	15368.51	34.81	stable
OePMEI2-19	XP_022892164.1	12	28,994,745	28,996,049	175	8.91	18634.50	42.57	unstable
OePMEI2-20	XP_022891568.1	12	30,346,936	30,363,530	271	9.68	30070.74	41.81	unstable
OePMEI2-21	XP_022895199.1	14	2,315,542	2,316,247	195	8.93	21356.61	45.26	unstable
OePMEI2-22	XP_022897425.1	15	31,679,586	31,680,396	186	9.18	20891.06	41.03	unstable
OePMEI2-23	XP_022897426.1	15	31,703,971	31,704,746	186	9.19	21033.30	44.79	unstable
OePMEI2-24	XP_022897428.1	15	31,709,820	31,710,635	186	9.32	21052.35	49.27	unstable
OePMEI2-25	XP_022899415.1	16	18,802,807	18,803,501	192	4.43	20405.34	39.41	stable
OePMEI2-26	XP_022899070.1	16	18,905,865	18,906,873	192	6.94	21341.29	43.36	unstable
OePMEI2-27	XP_022843330.1	18	22,794,106	22,794,798	175	5.34	19481.57	41.08	unstable
OePMEI2-28	XP_022843395.1	18	27,438,908	27,442,757	296	8.17	34040.18	43.82	unstable
OePMEI2-29	XP_022844969.1	18	27,445,536	27,446,727	290	4.97	32869.75	43.90	unstable
OePMEI2-30	XP_022843074.1	18	31,764,620	31,765,426	186	4.50	20487.23	29.32	stable
OePMEI2-31	XP_022846297.1	20	9,639,888	9,640,527	177	5.65	20156.27	25.47	stable
OePMEI2-32	XP_022846298.1	20	9,642,139	9,642,778	177	6.73	20330.56	21.50	stable
OePMEI2-33	XP_022847930.1	21	5,515,492	5,516,670	185	5.03	21150.93	42.38	unstable
OePMEI2-34	XP_022857643.1	scaffold	130	1,229	201	10.12	22268.66	37.45	stable
OePMEI2-35	XP_022872945.1	scaffold	902	1,541	177	8.25	20213.24	29.24	stable
OePMEI2-36	XP_022868032.1	scaffold	23,261	24,964	177	5.65	20190.29	27.95	stable
OePMEI2-37	XP_022864292.1	scaffold	33,388	34,552	211	6.18	22829.91	32.33	stable
OePMEI2-38	XP_022852272.1	scaffold	48,121	49,175	292	5.08	33225.89	47.87	unstable
OePMEI2-39	XP_022855083.1	scaffold	54,133	58,503	174	5.27	18956.65	42.61	unstable
OePMEI2-40	XP_022858987.1	scaffold	55,480	56,5600	200	7.01	21626.69	30.78	stable
OePMEI2-41	XP_022858986.1	scaffold	58,707	59,765	200	6.29	21670.67	25.52	stable
OePMEI2-42	XP_022863249.1	scaffold	59,533	60,504	269	4.61	28594.52	49.57	unstable
OePMEI2-43	XP_022861418.1	scaffold	61,118	61,881	190	9.42	20493.99	29.10	stable
OePMEI2-44	XP_022864296.1	scaffold	62,105	63,301	211	5.92	22852.88	33.15	stable
OePMEI2-45	XP_022861422.1	scaffold	70,747	71,665	196	9.33	21108.69	26.47	stable
OePMEI2-46	XP_022860678.1	scaffold	71,685	72,765	227	6.50	24856.35	39.01	stable
OePMEI2-47	XP_022861420.1	scaffold	90,576	91,538	199	9.60	21768.10	26.89	stable
OePMEI2-48	XP_022860888.1	scaffold	96,025	96,625	166	8.83	18721.97	32.08	stable
OePMEI2-49	XP_022854854.1	scaffold	151,600	153,234	128	7.72	14105.27	47.67	unstable
OePMEI2-50	XP_022851735.1	scaffold	158,433	158,991	154	4.44	16534.90	36.10	stable
OePMEI2-51	XP_022851523.1	scaffold	206,733	209,143	175	4.89	19551.01	44.71	unstable
OePMEI2-52	XP_022856426.1	scaffold	222,803	223,805	201	10.12	22268.66	37.45	stable
OePMEI2-53	XP_022851656.1	scaffold	241,327	242,245	210	9.59	23530.10	35.57	stable
OePMEI2-54	XP_022851657.1	scaffold	250,963	251,418	95	9.26	10446.00	17.85	stable
OePMEI2-55	XP_022873568.1	scaffold	260,575	261,236	183	6.94	21138.44	42.84	unstable
OePMEI2-56	XP_022873569.1	scaffold	274,802	276,081	241	6.30	27860.30	41.43	unstable
OePMEI2-57	XP_022873570.1	scaffold	301,768	303,065	234	7.57	26571.01	47.28	unstable

Genome-wide identification of the PMEII gene family in tea plants and functional analysis of CsPMEI2 and CsPMEI4. A total of 51 CsPMEIs and 2 CsVIF/CIFs were identified in the tea plant. The amino acids lengths were found from 139 to 307, the molecular weights (MW) were determined from 15.48 to 34.12 kD. More than 35 CsPMEIs were estimated to be stable proteins present in the cytoplasm (Li, 2021). Genome-wide identification, bioinformatic characterization and functional analysis of pectin methyltransferase inhibitors associated with low temperature-induced juice sac granulation in navel oranges (*Citrus sinensis* Osbeck). A total of 45 CsPMEI genes were identified from *Citrus sinensis* (Li, 2022).

The Chromosomal Distribution of PMEII Genes in the Olive Tree

The olive tree has 46 chromosomes (2n=46). The results of the localization of the PMEII type I and PMEII type II genes on the chromosomes of the olive (*Olea*

europaea sylvestris), which were determined using the MapChart program, can be found in Figure 1a and b. The PMEII genes were localized separately in chromosomes 1st, 2nd, 3rd, 7th, 8th, 9th, 10th, 11th, 12th, 13th, 14th, 15th, 16th, 17th, 18th, 20th, 21st, and 22nd, however, they are mainly localized at scaffold level.

Genome-wide analyses were performed on the identification, evaluation and expression of PME genes in soybean (*Glycine max* L.). A total of 127 GmPME genes were identified from the soybean genome. The identified GmPME genes are unevenly distributed across 20 soybean chromosomes, possibly due to gene replications or partially fragmented gene copies of the genome during soybean evolution. Introns have been reported to be associated with gene family expansions during plant evolution, which usually occur in the early stages of gene family expansion and are gradually deleted over time (Wang, 2021).

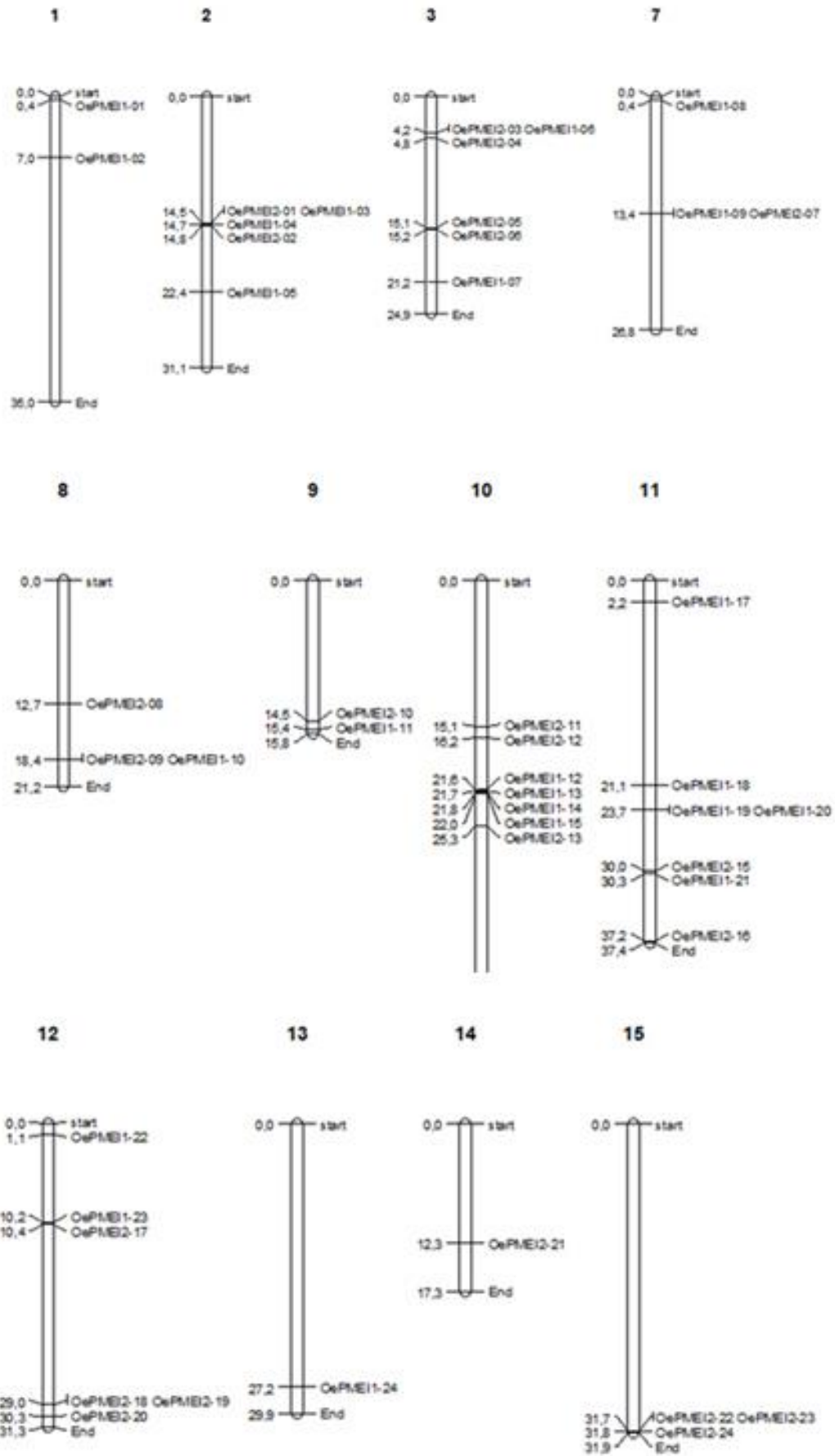


Figure 1a. The localization of the PMEI type I and PMEI type II genes on the chromosomes

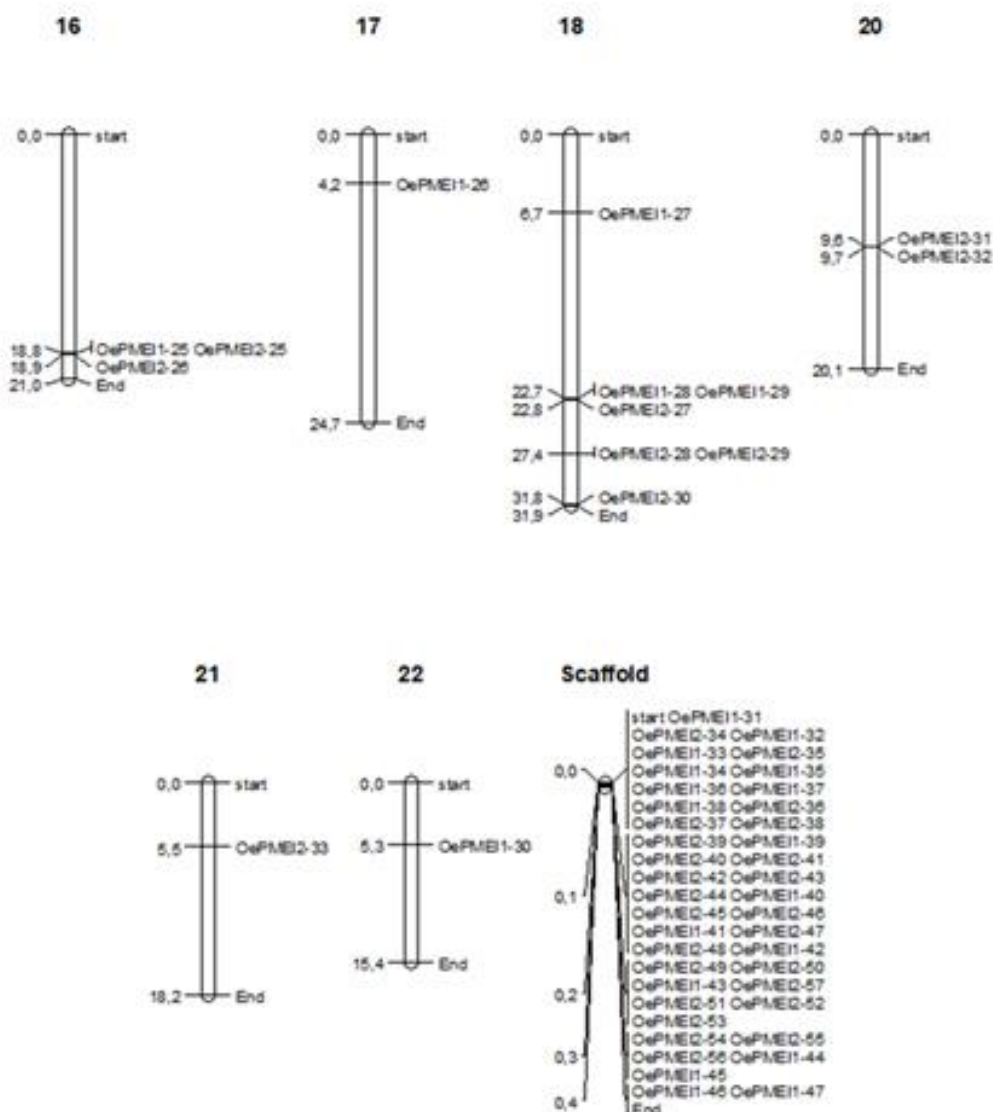


Figure 1b. The localization of the PMEI type I and PMEI type II genes on the chromosomes

Identification of Patterns and Exon-Intron Structures of PMEI Genes in Olives

To obtain conserved motifs, the amino acid sequences of each PMEI class were loaded separately into the MEME suite database and the motif composition of each PMEI family was determined. A total of 8 different motifs were found for each PMEI family. As a result, 6 dominant motif patterns were identified when examining the motifs belonging to PMEI type 1. In contrast, there were 5 dominant motif patterns for PMEI type 2 (Figure 2 and Figure 3). It was found that the genes containing this motif pattern are also in the same class in the phylogenetic tree. This indicates that the proteins in the same group have similar properties. Looking at the exon-intron regions of the PMEI families, the exon and intron regions of PMEI type1 and type2 are approximately the same size and show a similar structure to the motif structure and the phylogenetic tree (Figure 4 and Figure 5). The longest intron length in PMEI type 1 was found in OPMEI2-29. However, the genes in OPMEI type 2 were mainly determined as intronless and the longest intronless gene in OPMEI type 2 group was OPMEI2-20.

Phylogenetic Analysis of the Olive PMEI Genes

The phylogenetic trees constructed using the maximum likelihood method were examined. It was found that the PMEI type 1 proteins are divided into 4 major branches. These are arranged from I to IV. It was found that there are 6 proteins in group I, 17 proteins in group II, 7 proteins in group III and 15 proteins in group IV. The proteins OePMEI1-05 and OePMEI1-31, two of which we identified, were found to separate into different branches from the other sequences. These protein sequences were found to contain different motif sequences than the others (Figure 6).

PMEI Type 2 proteins are divided into 4 main branches. These are arranged from I to IV. It was found that there are 7 proteins in group I, 24 proteins in group II, 11 proteins in group III and 13 proteins in group IV. Proteins OePMEI2-01 and OePMEI2-20, two of which we identified, were found to be distinct from the other sequences. These protein sequences contained different motifs (Figure 7).

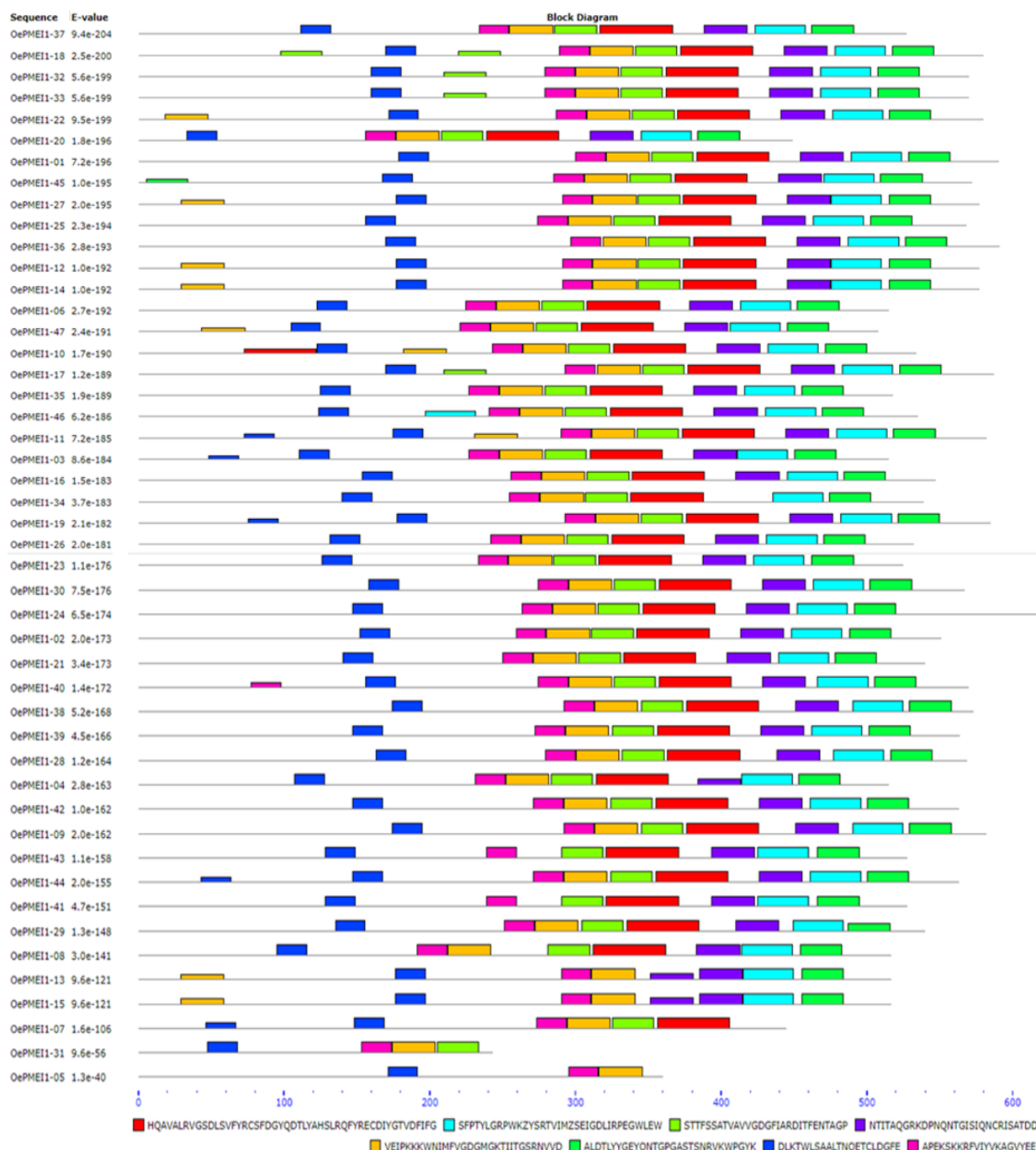


Figure 2. Motifs in PME1 type 1 genes

Ontology Analysis of Olive PME1 Genes

The biological function, cellular localization and molecular function of the PME1 genes were determined. For the PME1 type 1 genes, their biological functions were found to be biological regulation, metabolic and cellular functions. The molecular function was determined as binding and catalytic activity. Their cellular localization appears to be associated with cell parts. For PME1 type 2 genes, the biological functions were determined as biological regulation, metabolic and cellular functions. It is observed that cellular localization is associated with cell parts, with molecular function being binding and catalytic activity (Figure 8 and Figure 9).

Genome-wide identification, phylogeny and expression analysis of PME and PME1 gene families in maize were performed. The e value of GO analysis is 1.0E-6. GO terms

are presented in three main categories: biological process, cellular component, and molecular function. The fact that intragroup ZmPME/PMEIs have retained their gene structure and motif composition indicates that ZmPME/PMEIs in the same group have the same function and may have originated from a common ancestor (Zhang, 2019).

Husna (2021) used bioinformatics for genome-wide identification and characterization of the pectin methylesterase (PME) and pectin methylesterase inhibitor (PMEI) gene family in the A genome (*Musa acuminata*) and B genome (*Musa balbisiana*) of banana. The analysis revealed that the mature type 1 PME protein and the type 2 PME protein have a length of 48 – 1,014 amino acids in both genomes (Husna, 2021).

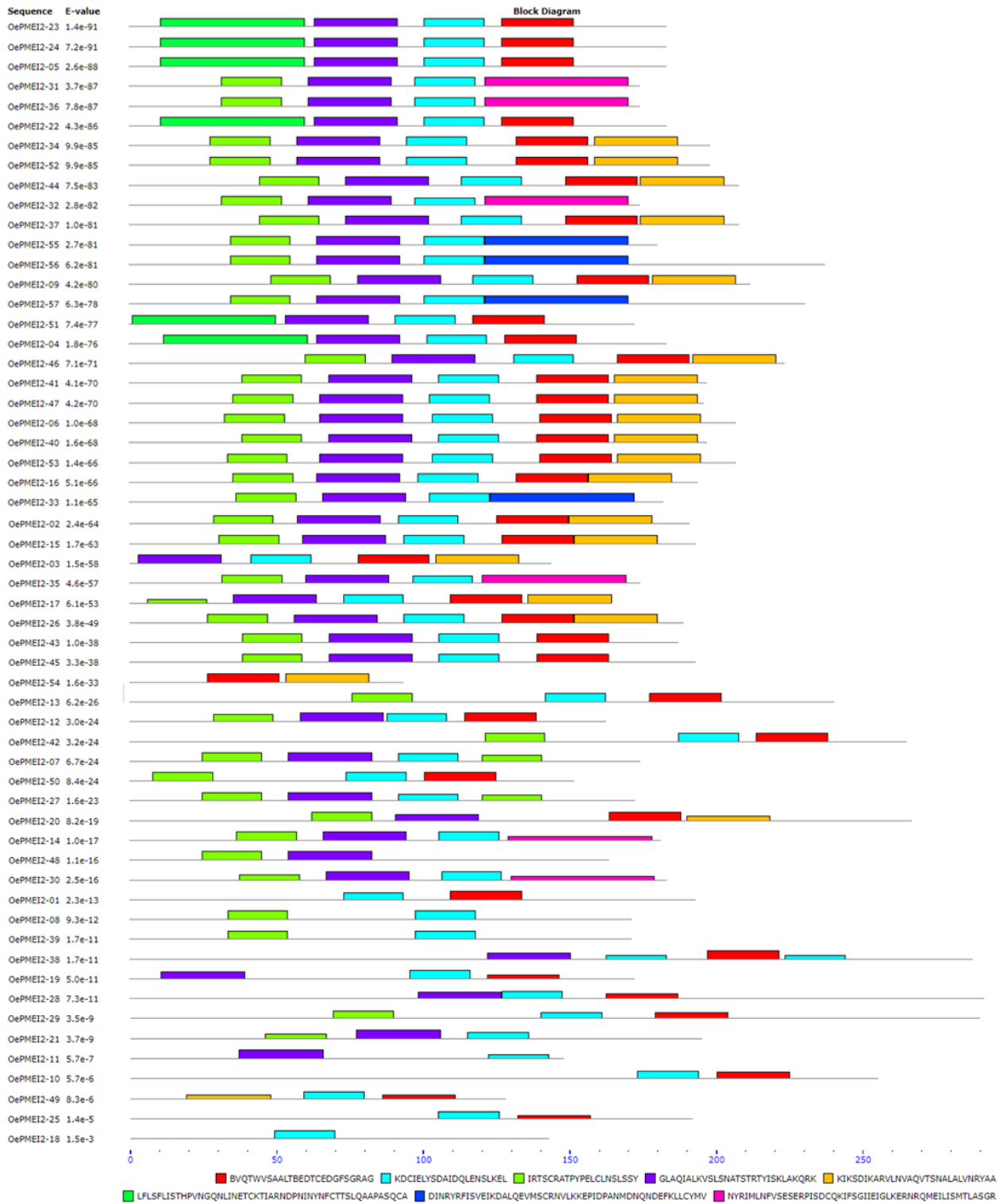


Figure 3. Motifs in PME1 type 2 genes

The genome-wide identification and functional characterization of pectin methyl esterases in strawberry related to fruit softening was also investigated. The study aimed to improve strawberry fruit firmness through genetic manipulation of key cell wall-modifying enzymes, pectin methyl esterases (FvPMEs), during fruit development. By analyzing gene evolution in rosaceous plants, tandem and scattered duplication events were found to play an important role in gene expansion of the FvPME family. Further genetic manipulations of fruit-specific FvPME38 and FvPME39 by overexpression and RNAi silencing

showed that the FvPMEs significantly affect fruit firmness, pectin content and cell wall structure, suggesting that PME are functionally required for strawberry fruit softening (Xue, 2020).

In *Diospyros kaki* (Japanese persimmon) 28 DkPMEs and 29 DkPMEIs were found (Zhang et al., 2022). Motif analysis indicated that DkPMEs and DkPMEIs were highly conserved, it's suggested that functions of Japanese persimmon PME and PMEIs were similar. The results of expression analysis propose that these genes may have distinct functions Zhang et al. (2022).

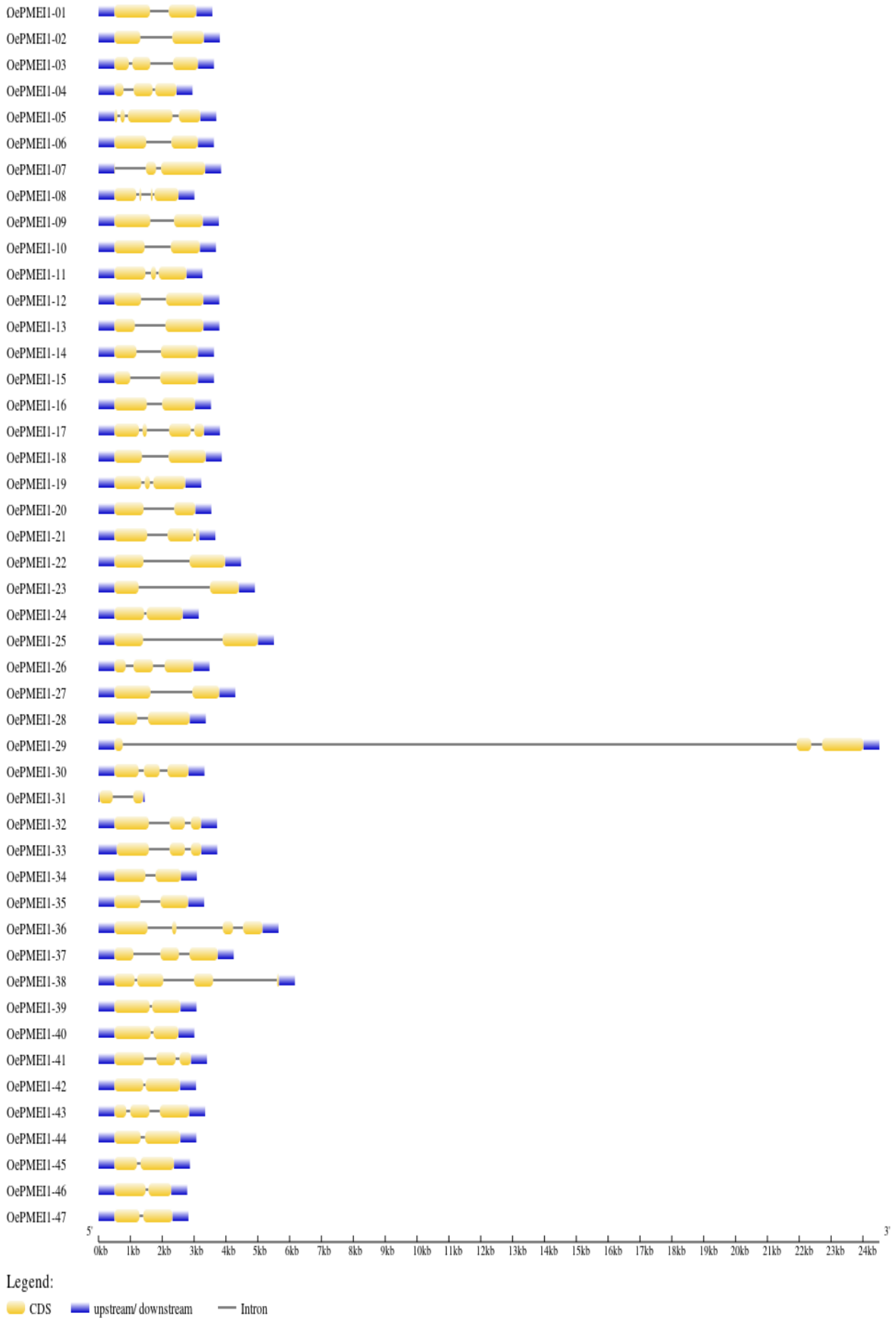


Figure 4. Exons and introns in PMEI Type 1 genes

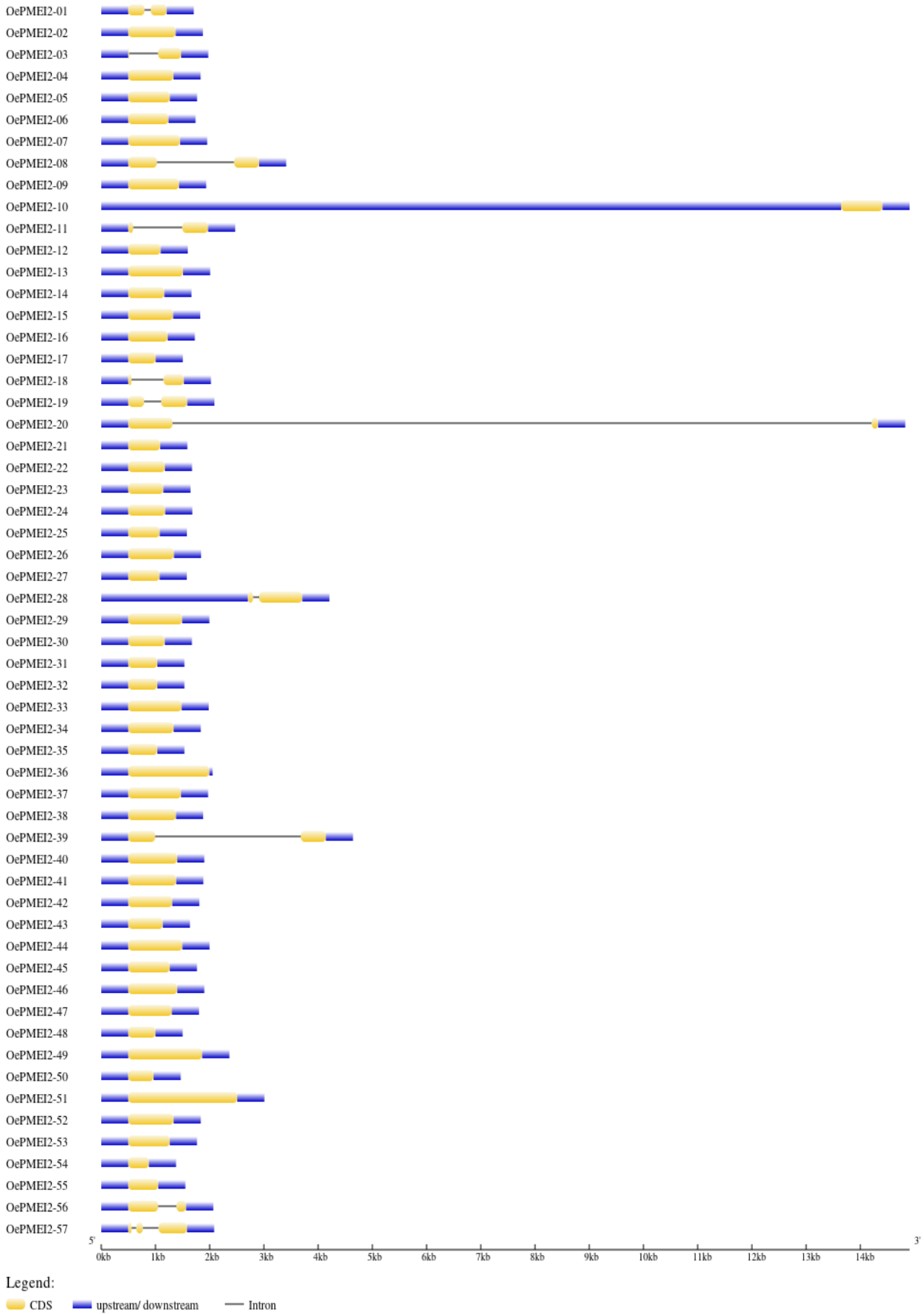


Figure 5. Exons and introns in PMEII Type 2 genes

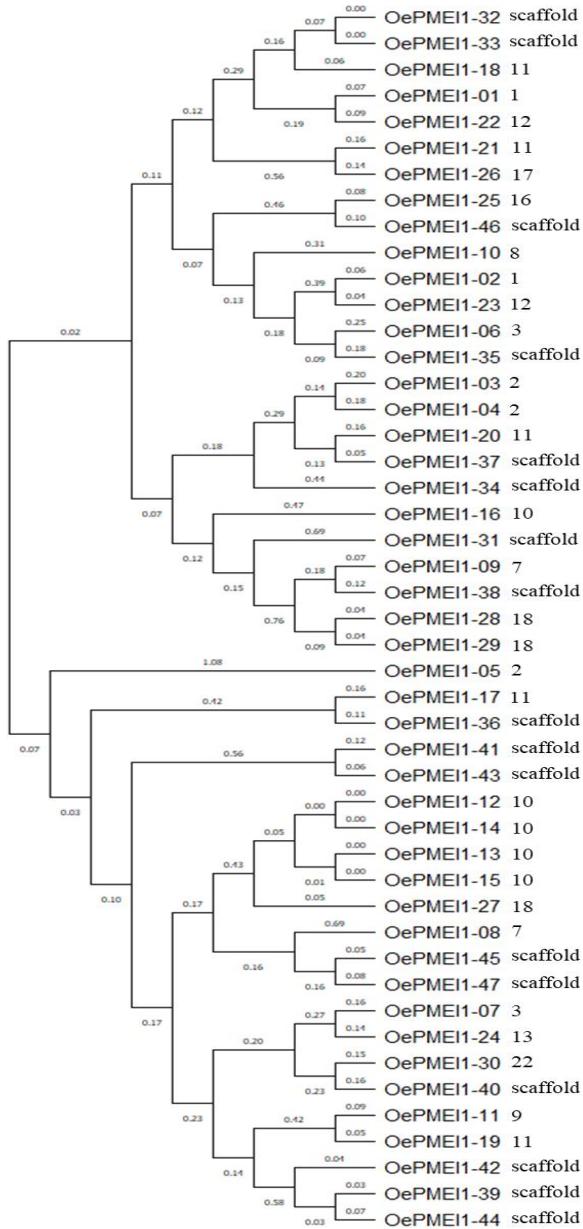


Figure 6. Dendrogram of PME1 type 1 genes in olive

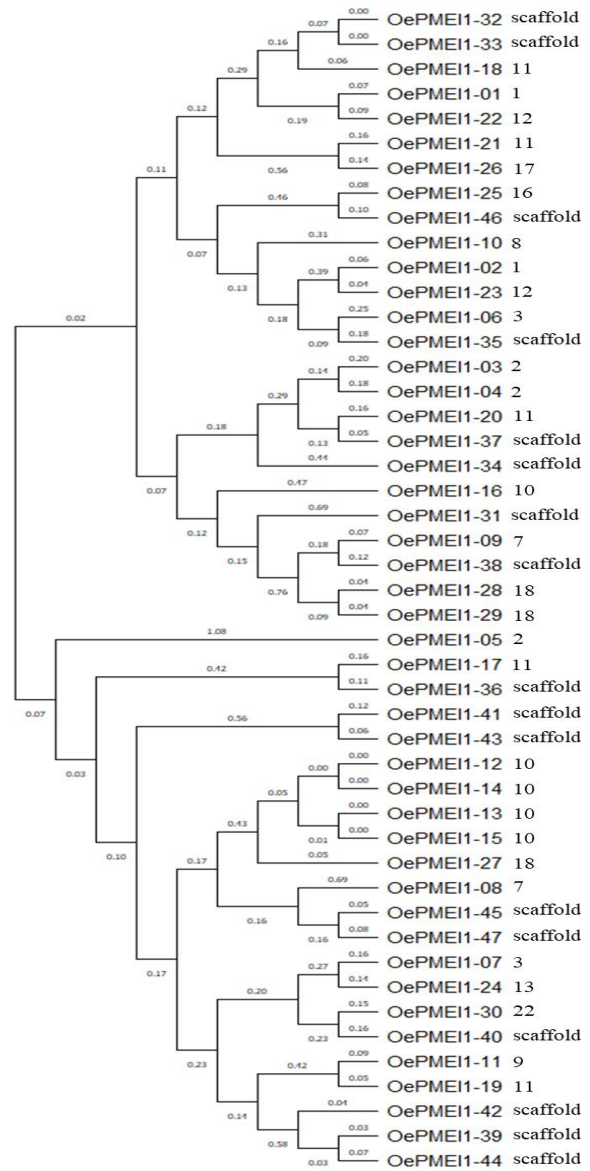


Figure 7. Dendrogram of PME1 type 2 genes in olive

Homology Modeling of the PME1 Proteins

As a result of homology modeling using the Protein Data Bank (PDB), the PME1 type1 and PME1 type2 proteins, OePMEI1 and OePMEI2, respectively, showed high similarity. The similarity rate was determined by selecting the intensive mode from the Phyre2 database. The reliability was determined to be 90% and the similarity ranged from 90% to 100%. It was observed that the β -sheet and α -helix structure was superimposed on the Ca protein trace skeleton in the structures. The examples of PME1 proteins are given in Figure 10.

miRNA Analysis

For the comparison between all plant miRNAs and olive target genes, all known plant miRNAs were first downloaded from miRbase (Kozomara and Griffith-Jones, 2013) and olive PME1 genes were evaluated using the Plant Small RNA Target Analysis Server, psRNATarget (Dai et

al. 2018). Since *Arabidopsis thaliana* is a model organism, the miRNAs from *Arabidopsis* that interact with olive PME1 genes were selected and used for analysis.

As a result, a total of 393 miRNAs from *Arabidopsis* targeting 47 olive PME1 type 1 genes were identified. Two specific miRNAs targeting the OePMEI1-07 gene were found (ath-miR8168 and ath-miR774b-5p). ath-miR8168 was identified as one of the novel genes for nitrate regulation in *Arabidopsis thaliana* (Vidal et al., 2013) and ath-miR774b-5p is most likely related to the ethylene response factor (ERF) gene family. Rakhmetullina et al (2021) reported that miR774b-3p targets the ERF family genes of *A. thaliana* and *O. Sativa* genes.

For PME1 type 2 genes, 269 *Arabidopsis* miRNAs were found, 14 specific miRNAs targeting OPMEI2-02, OPMEI2-03, OPMEI2-27, OPMEI2-28, OPMEI2-29, OPMEI2-30, OPMEI2-40 and OPMEI2-54 genes.

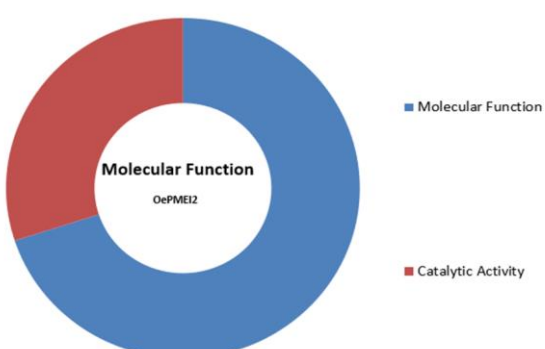
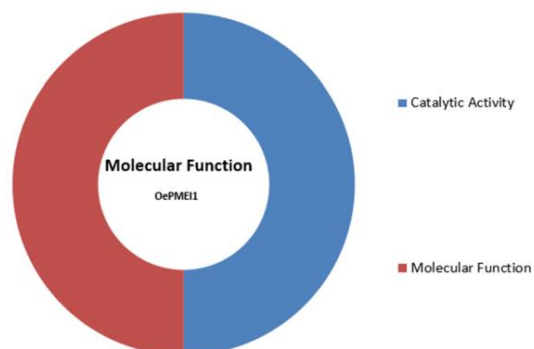
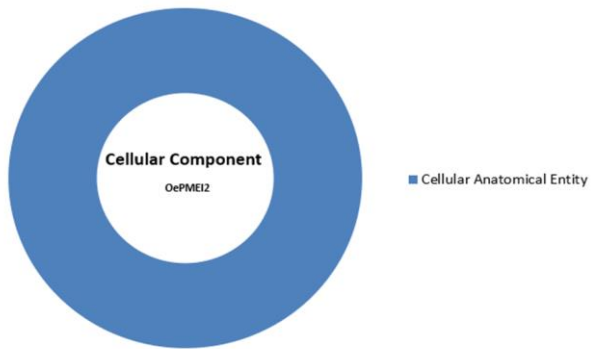
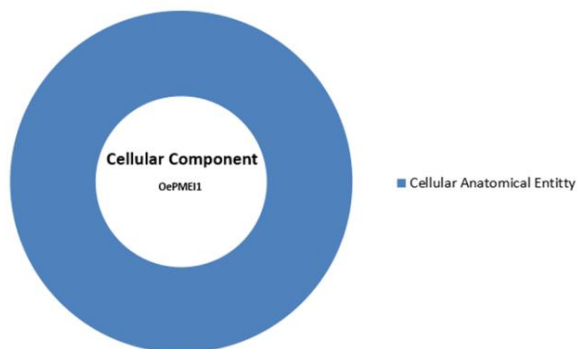
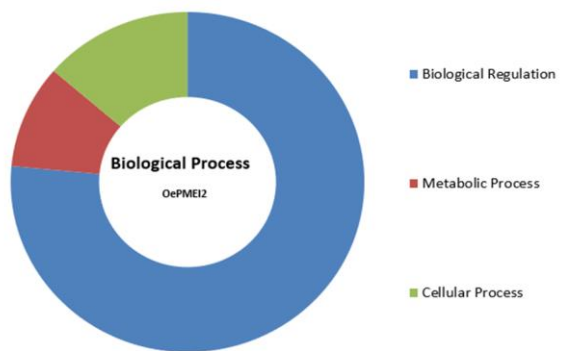
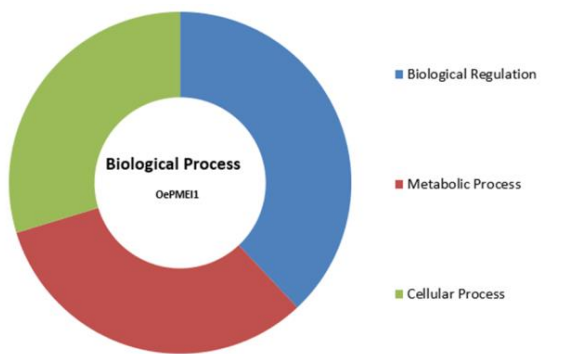
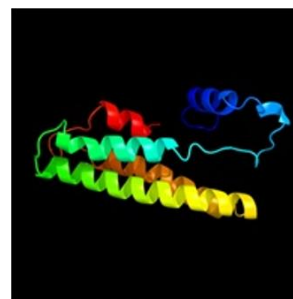


Figure 8. Ontology analysis of PME1 type 1 genes in olive

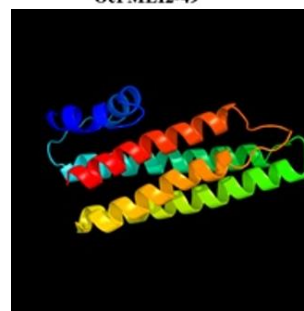
Figure 9. Ontology analysis of PME1 type 2 genes in olive



OePMEI1-20



OePMEI2-49



OePMEI2-50

Figure 10. The examples of PME1 proteins

These specific miRNAs were ath-miR158a-5p, ath-miR1888b, ath-miR834, ath-miR773a, ath-miR861-3p, ath-miR173-3p, ath-miR847, ath-miR5656, ath-miR390a-5p, ath-miR390b-5p, ath-miR5020a, ath-miR393a-3p and ath-miR5641, respectively. ath-miR5641. miR393 expression in *Arabidopsis thaliana* has been associated with moderate and severe boron toxicity (Kayihan, 2020). The study suggests that the developmental problems under boron toxicity are related to miR393 expression, which targets auxin regulation and transcription factors. miRNAs may also play a role in biotic stress response. Cao et al (2020) suggested that ath-miR158a-5p has a potential role in AGO2-dependent resistance to *S. Sclerotiorum* in *Arabidopsis thaliana*. Therefore, miRNAs target the abiotic response genes such as hsp70. Tannriseven (2020) found that ath-miR173-3p is associated with hsp70 genes in *Phaseolus vulgaris* L.

In conclusion, Type 1 and type 2 PME1 genes were identified and analysed in wild olives. It was found that the PME1s in the olive genome have a variety of functions, such as response to stress or plant growth. The role of PME1 genes in plant and fruit development seems evident, therefore, these genes contribute to disease resistance and post-harvest quality. The results suggest that further analysis of PME1 genes could contribute to the understanding of stress response and plant and fruit development.

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