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Original article

Identification and Bioinformatic Analysis of CAMTA Genes in Olive (*Olea europaea* L.)

Aslıhan Özbilen [*]

Department of Biology, Canakkale Onsekiz Mart University, Canakkale, Turkey

Abstract

Calcium is a secondary messenger which involves in stress response, adaptation, development and signaling pathways in plants. In cells, calcium is captured by calmodulin and after a conformation change, calmodulin becomes able to target calmodulin binding transcription factors. The calmodulin-binding transcriptional activators (CAMTAs) are one of these calmodulin binding transcription factors and they have CG-1, TIG, ANK, CaMBD and IQ conserved domains. CAMTAs are studied well in lots of plant species, and they are found to be involved in stress responses like drought, cold, salt and hormone responses like ethylene, abscisic acid, auxin, and gibberellin. In this study, CAMTA genes and proteins are characterized in olive. Olive (*Olea europaea* L.) is a Mediterranean commercially important crop, and this is the first study on olive CAMTAs. 7 CAMTA genes are found in olive in total in this study. Then, the cis-actin regulatory elements in the promoter regions of these genes are analyzed. Stress and hormone response related elements in the promoter regions are found, suggesting possible stress and hormone response roles of CAMTAs in olive. Also, protein characteristics, conserved domains, and subcellular localizations are investigated. According to the results, all olive CAMTA proteins are mainly localized in the nucleus as consistent with their roles, and all of them have 2 to 6 conserved domains which are also found in other plant CAMTAs. Additionally, a phylogenetic tree with 109 CAMTA proteins from well-knows plant species is constructed, and this tree showed that olive CAMTA proteins are highly conserved in plant kingdom.

Keywords: Calmodulin, CAMTA, Transcription Factor, Olive.

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[*] **Corresponding author:**

Özbilen Aslıhan is an post-Doc researcher in the Department of Biology at Çanakkale Onsekiz Mart University in Çanakkale, Turkey. Her research interests include the Plant Biotechnology, Plant Molecular Biology and Molecular Biology and Genetics. She has lived, worked, and studied in Çanakkale, Turkey.
Email: aozbilen@comu.edu.tr

INTRODUCTION

The divalent ion of calcium (Ca^{2+}) plays crucial roles in stress response and developmental processes in eukaryotes as a universal secondary messenger. Ca^{2+} -mediated signal transduction is also a key mechanism in plants (Du et al., 2011). Recently, plants have been found to have three different types of Ca^{2+} sensors: calmodulins and calmodulin-likes (CaMs - CMLs), calcium-dependent protein kinases (CAPK), calcineurin B-like proteins (CBLs) (Kim et al., 2009). Calmodulin (CaM) is one of the best characterized small protein among these. CaM is the primary intracellular receptor for Ca^{2+} and a multifunctional regulator of protein activity (Zielinski, 1998). CaM is inactive of its own, however when Ca^{2+} binds to CaM, this complex becomes active for targeting other proteins for metabolic processes (Snedden and Fromm, 2001). To date, lots of transcription factors have been characterized as CaM-binding proteins which belong to CAMTA, MYB, WRKY, bZIP, MADS-box, Scarecrow, NAM, AUX/IAA, SAUR_B families (Popescu et al., 2007; Reddy et al., 2011).

Plant transcription factors (TFs) have been the subject of an increasing number of research in recent years due to their crucial roles in plant development and stress responses. TFs are proteins that binds to DNA and interacts with promoter cis-acting regions to regulate the gene expression (on or off). The calmodulin-binding transcriptional activators (CAMTAs) are first discovered in tobacco (NtER1) (Yang and Poovaiah, 2000). CAMTAs are members of a conserved TFs family which is regulated by calmodulin. CAMTAs contain a DNA binding domain CG-1 in the N terminus, followed by a non-specific DNA binding TIG domain, an ankyrin (ANK) repeats domain, a Ca^{2+} dependent CaM binding domain (CaMBD) which is located between the N- and C terminus and a varying number of IQ calmodulin binding motifs that interact with CaM in a Ca^{2+} independent manner (Bouche et al., 2002; Finkler et al., 2007).

Since CAMTAs are first discovered in tobacco (Yang and Poovaiah, 2000), this gene family is studied widely in numerous plant species such as: *Arabidopsis thaliana* (Yang and Poovaiah, 2002), *Vitis vinifera* (Shangguan et al., 2014), *Glycine max* (Wang et al., 2015), *Populus trichocarpa* (Wei et al., 2017), *Citrus* species (Zhang et al., 2019), *Phaseolus vulgaris* (Büyük et al., 2019), *Linum usitatissimum* (Ali et al., 2020), *Oryza sativa* (Gain et al., 2022) and *Solanum lycopersicum* (Fang et al., 2022). CAMTAs are found to be involved in lots of biological process like drought (Pandey et al., 2013; Kakar et al., 2018; Zhang et al., 2019), cold (Doherty et al., 2009; Kim et al., 2013; Wei et al., 2017; Kakar et al., 2018; Primo-Capella et al., 2021), salt stress (Wei et al., 2017; Büyük et al., 2019; Zhang et al., 2019; Yuan et al., 2021), wounding or pathogenic invasions (Nie et al., 2012; Wei et al., 2017; Kakar et al., 2018), hormone signaling (Wei et al., 2017; Zhang et al., 2019), ethylene signaling and fruit ripening (Yang et al., 2012). However, there are no studies on olive CAMTA proteins in literature.

Olive (*Olea europaea* L.), from the Oleaceae family, is one of the oldest cultivated trees in Mediterranean area (Rugini 1986). In Mediterranean diet, olive is consumed widely for table and oil.

Therefore, olive is commercially important and has a high-income potential. Since the studies on the calmodulin binding transcription activators of olive are not available in the literature, unravelling the phenomenon of how CAMTAs work and understanding the underlying molecular mechanisms in olive will provide us lot of benefits for woody perennials. Thus, this study is focused on identifying the CAMTA genes in olive genome, evaluating the protein characteristics and conserved domains, and assessing the phylogenetic relationship of CAMTAs among well-known angiosperms.

MATERIALS and METHODS

Obtaining Olive CAMTA sequences

The CAMTA protein sequences of *Arabidopsis thaliana* were obtained from The Arabidopsis Information Resource (TAIR) based on the ID numbers (AtCAMTA1: AT5G09410; AtCAMTA2: At5G64220; AtCAMTA3:AT2G22300; AtCAMTA4: AT1G67310; AtCAMTA5: AT4G16150; and AtCAMTA6: AT3G16940) used in previous studies (Doherty et al., 2009; Zhang et al., 2019; Yuan et., 2021). *Olea europaea* L. cv. Sylvestris genomic, transcript and protein sequence reads (Oeuropeae_451_v1.0) were downloaded from Phytozome v13 database (Goodstein et al., 2012). Those sequences were uploaded to Geneious software (Kearse et al., 2012) and a local olive database was constructed. Then, the *A. thaliana* protein sequences were used as query and blastp analysis is performed to obtain the CAMTA protein sequences in olive. These olive protein sequences then used as query in the local database and with tblastn analysis and the olive CAMTA transcript sequences were acquired. And following this, olive transcript sequences were used as query and the genomic sequences of CAMTA genes in olive were obtained with blastn analysis. Blastn analysis for the genomic sequences was performed with "extended regions with annotations" option in Geneious R8 and promoter regions, -2000bp, were also obtained for each genomic sequence for further analysis.

Phylogenetic Analysis

6 CAMTA genes were identified in *A. thaliana* so far (Reddy et al., 2000; Yang and Poovaiah, 2000, 2002). Thanks to genome sequencing and bioinformatic tools, many CAMTAs were identified in various plant species. For the phylogenetic analysis, CAMTA protein sequences of *A. thalian*a (Yang and Poovaiah, 2002), Citrus species (Zhang et al., 2019), *Glycine max* (Wang et al., 2015), *Linum usitatissimum* (Ali et al., 2020), *Nicotiana tabacum* (Kakar et al.,2018), *Oryza sativa* (Gain et al., 2022), *Phaseolus vulgaris* (Büyük et al., 2019), *Populus trichocarpa* (Wei et al., 2017), *Solanum lycopersicum* (Fang et al., 2022) and *Vitis vinifera* (Shangguan et al., 2014) were downloaded from NCBI (National Center for Biotechnology Information, https://www.ncbi.nlm.nih.gov/) and from Phytozome v13 (https://phytozome-next.jgi.doe.gov/) databases. These sequences were aligned with olive sequences using MUSCLE algorithm (Edgar, 2004) in Geneious software with default parameters. The

phylogenetic tree of CAMTA proteins is constructed with this alignment file using Geneious tree builder with Jukes-Cantor genetic distance model and UPGMA tree building method.

Determining the OeCAMTA Protein Characteristics

Also, molecular weights and isoelectric points (pI) of the olive CAMTA proteins were estimated with ProtParam tool in ExPASy portal (https://web.expasy.org/protparam/; Gasteiger et al. 2005), subcellular localizations were estimated with CELLO v.2.5 (http://cello.life.nctu.edu.tw/; Yu et al. 2006) and conserved domain analysis is carried out with NCBI Conserved Domain Search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

RESULTS and DISCUSSION

Olive specific CAMTA protein sequences were obtained from the local database in Geneious R8 by using *A. thaliana* CAMTA protein sequences as query. When AtCAMTA1 (Accession number: AT5G09410) is used as query, 2 olive specific protein sequences are found which has 53,7% and 53,6% pairwise identity. Oeu001794.2 is designated as OeCAMTA1a and Oeu056612.1 is designated as OeCAMTA1b. The accession numbers of all the transcript sequences are same with their proteins in olive genome database. And it is noticed that the Oeu001794.2 transcript is transcribed by chromosome 2 (chr2) and the Oeu056612.1 is transcribed by chr18. When AtCAMTA2 (Accession number: AT5G64220) is used as query, 1 olive specific protein sequence is found with 55,8 % pairwise identity. Oeu019230.1 is designated as OeCAMTA2 and Oeu019230.1 is transcribed by scaffold1815. After using AtCAMTA3 (Accession number: AT2G22300) as query, 1 olive specific protein sequence is found with 49,4% pairwise identity. Oeu002409.2 is designated as OeCAMTA3 and Oeu002409.2 is transcribed by chr10. Similarly, when AtCAMTA4 (Accession number: AT1G67310) is used as query, 2 olive specific protein sequences are found which has 61,5% and 58,3% pairwise identity. Oeu056668.1 is designated as OeCAMTA4a and Oeu049145.2 is designated as OeCAMTA4b. Oeu056668.1 and Oeu049145.2 transcripts are transcribed by chr11 and chr20, respectively. Lastly, after using AtCAMTA5 (Accession number: AT4G16150) and AtCAMTA6 (Accession number: AT3G16940) as query the same protein sequence is found for both queries (57,2% pairwise identity), and therefore Oeu016483.1 is designated as OeCAMTA5 and Oeu016483.1 is transcribed by chr22 in olive. Thus, 7 CAMTA proteins have been identified in total in olive.

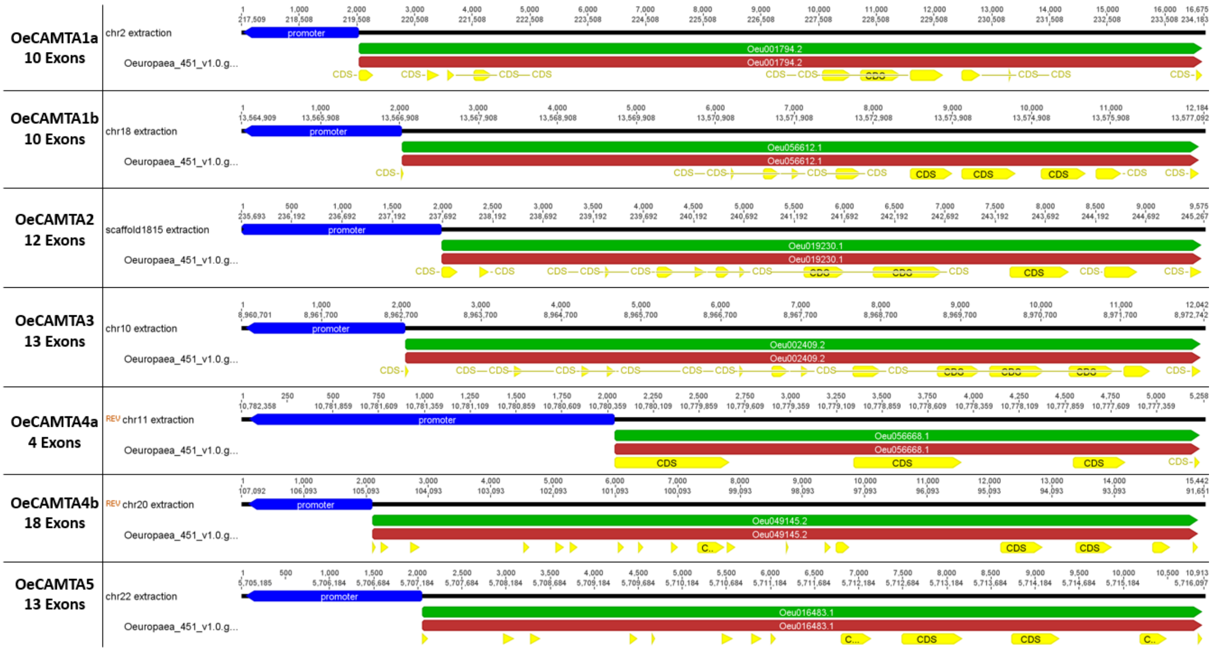

Figure 1. Genomic localization of 7 olive CAMTA genes. Green lines represent the gene ID (exp: Oeu056668.1), blue lines represent -2000bp promoter region of each gene, yellow lines represent the exons and coding sequences

In olive genome, OeCAMTA1a, OeCAMTA1b, OeCAMTA2, OeCAMTA3, OeCAMTA4a, OeCAMTA4b and OeCAMTA5 genes consist of 10, 10, 12, 13, 4, 18 and 13 exons, respectively (Figure 1). Additionally, -2000bp promoter sequences of each gene were saved and analyzed for cis-acting regulatory elements with (Plant Cis-Actin Regulatory Element; http://bioinformatics.psb.ugent.be/webtools/plantcare/html/; Lescot ve ark., 2002). As a result, 71 different cis-acting elements were found in total (Figure 2). 3 of them are core promoter elements like TATA boxes. There are also 16 light response elements, 14 hormone response elements such as auxin, abscisic acid, gibberellin, and ethylene response elements, 6 stress response elements such as for heat or drought, 4 elements for pathogen and wounding response, and other elements for enhancing, binding or for other metabolic responses. For CAMTA1a, only 2 cis-acting elements has been found in the promoter region due to the weak sequencing quality of the downloaded reads (Figure 2).

Figure 2. cis-acting elements found in olive CAMTA gene promoter regions

Some well-known plant CAMTA protein sequences are used with olive CAMTAs (109 protein sequences in total) and aligned them with MUSCLE (Figure 3). Then, that alignment file is used to construct a phylogenetic tree to evaluate the relationship between olive CAMTAs and other plant's CAMTAs. That tree is divided into 4 main branches. OeCAMTA1a, OeCAMTA1b, and OeCAMTA3 are in the first branch (blue), OeCAMTA2 is in the second branch (pink), OeCAMTA4a and OeCAMTA4b are in the third branch (green) and OeCAMTA5 is in the fourth branch (red) (Figure 4). First branch consists of 30 proteins and includes *A. thaliana* CAMTA3 with OeCAMTA1a, OeCAMTA1b, and OeCAMTA3, second branch consists of 14 proteins and includes *A. thaliana* CAMTA1 and CAMTA2 with OeCAMTA2, third branch consists of 29 proteins and includes *A. thaliana* CAMTA4 with OeCAMTA4a and OeCAMTA4b, lastly fourth branch consists of 25 proteins and includes *A. thaliana* CAMTA5 and CAMTA6. In addition, there are 2 out groups which are not clustered with *A. thaliana* or olive CAMTAs.

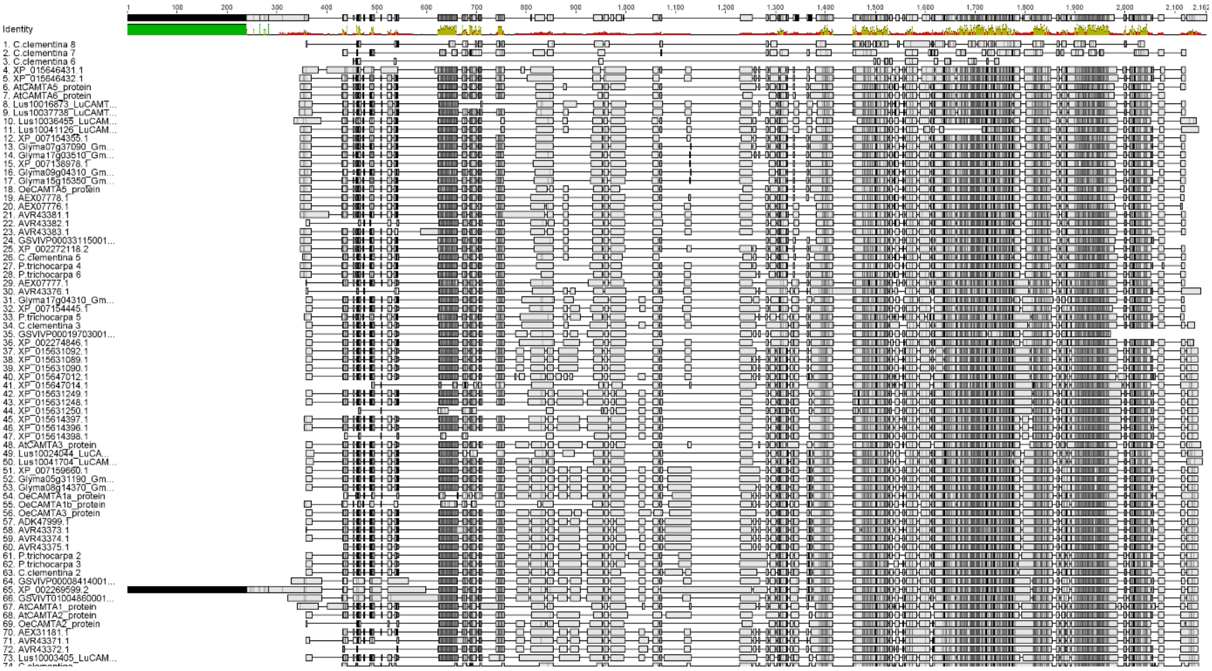

Figure 3. Alignment of well-known angiosperm and olive CAMTA proteins

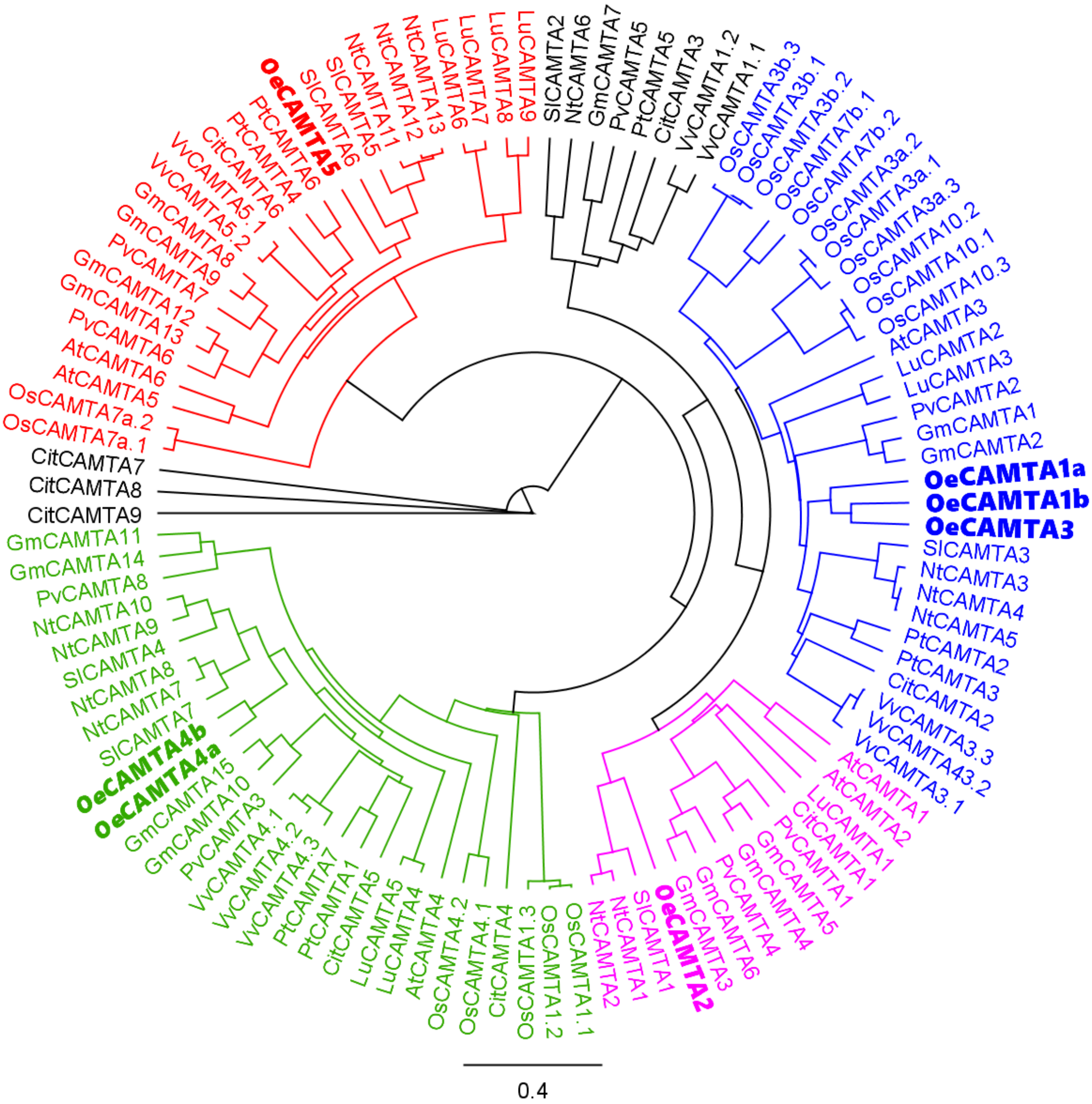

Figure 4. Phylogenetic tree of CAMTA proteins (Olive CAMTA proteins are shown as bold)

*CAMTA gene/protein ID numbers were listed as following: *Arabidopsis thaliana*: AtCAMTA1 (AT5G09410), AtCAMTA2 (AT5G64220), AtCAMTA3 (AT2G22300), AtCAMTA4 (AT1G67310), AtCAMTA5 (AT4G16150), AtCAMTA6 (AT3G16940); *Glycine max*: GmCAMTA1 (Glyma05g31190), GmCAMTA2 (Glyma08g14370), GmCAMTA3 (Glyma15g05900), GmCAMTA4 (Glyma08g07680), GmCAMTA5 (Glyma05g24430), GmCAMTA6 (Glyma08g19101), GmCAMTA7 (Glyma17g04310), GmCAMTA8 (Glyma15g15350), GmCAMTA9 (Glyma09g04310), GmCAMTA10 (Glyma05g28090), GmCAMTA11 (Glyma18g00840), GmCAMTA12 (Glyma17g03510), GmCAMTA13 (Glyma07g37090), GmCAMTA14 (Glyma11g36930), GmCAMTA15 (Glyma08g11080); *Citrus spp*.: CitCAMTA1 (Ciclev10027833m), CitCAMTA2 (Ciclev10024764m), CitCAMTA3 (Ciclev10004234m), CitCAMTA4 (orange1.1g024223m), CitCAMTA5 (Ciclev10030636m), CitCAMTA6 (Ciclev10004273m), CitCAMTA7 (Ciclev10029792m), CitCAMTA8 (Ciclev10008000m), CitCAMTA9 (Ciclev10019990m); *Linum usitatissimum*: LuCAMTA1 (Lus10003405), LuCAMTA2 (Lus10024044), LuCAMTA3 (Lus10041704), LuCAMTA4 (Lus10003119), LuCAMTA5 (Lus10011352), LuCAMTA6 (Lus10016873), LuCAMTA7 (Lus10037738), LuCAMTA8 (Lus10036455), LuCAMTA9(Lus10041126); *Nicotiana tabacum:* NtabCAMTA01 (Ntab0183780), NtabCAMTA02 (Ntab0553680), NtabCAMTA03 (Ntab0473890), NtabCAMTA04 (Ntab0695330), NtabCAMTA05 (Ntab0695280), NtabCAMTA06 (Ntab0114010), NtabCAMTA07 (Ntab0794220), NtabCAMTA08 (Ntab0019010); NtabCAMTA09 (Ntab0852870), NtabCAMTA10 (Ntab0354250), NtabCAMTA11 (Ntab0045050), NtabCAMTA12 (Ntab0797190), NtabCAMTA13 (Ntab0368180); *Olea europaea*: OeCAMTA1a (Oeu001794.2), OeCAMTA1b (Oeu056612.1), OeCAMTA2 (Oeu019230.1), OeCAMTA3 (Oeu002409.2), OeCAMTA4a (Oeu056668.1), OeCAMTA4b (Oeu049145.2), OeCAMTA5 (Oeu016483.1); *Oryza sativa*: OsCAMTA1.1 (XP_015649554.1), OsCAMTA1.2 (XP_015649561.1), OsCAMTA1.3 (XP_015649568.1), OsCAMTA3a.1 (XP_015631248.1), OsCAMTA3a.2 (XP_015631249.1), OsCAMTA3a.3 (XP_015631250.1), OsCAMTA3b.1 (XP_015631089.1), OsCAMTA3b.2 (XP_015631090.1), OsCAMTA3b.3 (XP_015631092.1), OsCAMTA4.1 (XP_015635911.1), OsCAMTA4.2 (XP_025880404.1), OsCAMTA7a.1 (XP_015646431.1), OsCAMTA7a.2 (XP_015646432.1), OsCAMTA7b.1 (XP_015647012.1), OsCAMTA7b.2 (XP_015647014.1), OsCAMTA10.1 (XP_015614396.1), OsCAMTA10.2 (XP_015614397.1), OsCAMTA10.3 (XP_015614398.1); *Phaseolus vulgaris:* PvCAMTA1 (XP_007159108.1), PvCAMTA2 (XP_007159660.1), PvCAMTA3 (XP_007158673.1), PvCAMTA4 (XP_007148412.1), PvCAMTA5 (XP_007154445.1), PvCAMTA6 (XP_007154355.1), PvCAMTA7 (XP_007138978.1), PvCAMTA8 (XP_007163775.1); *Populus trichocarpa:* PtCAMTA1 (Potri.001G057800.1), PtCAMTA2 (Potri.005G075100.1), PtCAMTA3 (Potri.007G093400.1), PtCAMTA4 (Potri.010G141700.1), PtCAMTA5 (Potri.010G153100.1), PtCAMTA6 (Potri.008G107900.1), PtCAMTA7 (Potri.003G170600.1); *Solanum lycopersicum*: SlCAMTA1 (AEX31181.1), SlCAMTA2 (AEX07777.1), SlCAMTA3 (ADK47999.1), SlCAMTA4 (AEX07774.1), SlCAMTA5 (AEX07776.1), SlCAMTA6 (AEX07778.1), SlCAMTA7 (AEX07775.1); *Vitis vinifera*: VvCAMTA1.1 (XP_002274846.1), VvCAMTA1.2 (GSVIVP00019703001), VvCAMTA3.1 (XP_002269599.2), VvCAMTA3.2 (GSVIVT01004860001), VvCAMTA3.3 (GSVIVP00008414001), VvCAMTA4.1 (XP_002270865.2), VvCAMTA4.2 (GSVIVT01010510001), VvCAMTA4.3 (GSVIVP00002786001), VvCAMTA5.1 (XP_002272118.2), VvCAMTA5.2 (GSVIVP00033115001).

The putative biochemical properties (molecular weight, isoelectric points, and subcellular localizations) of the olive CAMTA proteins are determined by bioinformatic approaches. According to the results, subcellular localization of olive CAMTAs are mostly nuclear (Table 1). The conserved domain analysis is performed with NCBI Conserved Domain Search tool. DNA-binding is found domain CG-1 in OeCAMTA2, OeCAMTA3, OeCAMTA4b and OeCAMTA5 (Table 2). Another DNA-binding domain TIG is found in OeCAMTA2 and OeCAMTA4b. Ankyrin repeats domains are detected in all olive CAMTA proteins except OeCAMTA1a. Additionally, IQ calmodulin binding motifs are only lacking in OeCAMTA2 and OeCAMTA4a. Lastly, CBD_MYO6-like super family calmodulin-binding domains exists in OeCAMTA2 and OeCAMTA4a (Table 2).

Table 1. Features of olive CAMTA proteins

Designated ID	Accession Number	aa number	Molecular Weight (Da)	Isoelectric point	Subcellular Localization
OeCAMTA1a	Oeu001794.2	1041	116052.94	5.31	Nuclear
OeCAMTA1b	Oeu056612.1	971	108641.73	5.38	Nuclear
OeCAMTA2	Oeu019230.1	964	108404.29	5.53	Nuclear
OeCAMTA3	Oeu002409.2	1104	124383.19	5.51	Nuclear
OeCAMTA4a	Oeu056668.1	508	56665.64	9.37	Nuclear & Mitochondrial
OeCAMTA4b	Oeu049145.2	1177	131605.01	6.54	Nuclear & Plasma Membrane
OeCAMTA5	Oeu016483.1	891	100792.59	8.00	Nuclear

Table 2. Conserved Domains (CCD) of olive CAMTA proteins and their superfamilies

Protein	CCD Name	Accession	Description	Superfamily
OeCAMTA1a	PHA03095 super family	cl33707	ankyrin-like protein	cl33707
	IQ	smart00015	Calmodulin-binding motif	cl29555
	CBD_MYO6-like super family	cl41207	calmodulin binding domain	cl41207
OeCAMTA1b	Ank_2	pfam12796	Ankyrin repeats	cl39094
	IQ	smart00015	Calmodulin-binding motif	cl29555
	COG5022 super family	cl34868	Myosin heavy chain	cl34868
OeCAMTA2	CG-1	pfam03859	CG-1 domain	cl04295
	Ank_2	pfam12796	Ankyrin repeats	cl39094
	ANKYR	COG0666	Ankyrin repeat	cl34000
	CBD_MYO6-like super family	cl41207	calmodulin binding domain	cl41207
	TIG	pfam01833	IPT/TIG domain	cl15674
OeCAMTA3	CG-1	pfam03859	CG-1 domain	cl04295
	Ank_2	pfam12796	Ankyrin repeats	cl39094
	IQ	smart00015	Calmodulin-binding motif	cl29555
	COG5022 super family	cl34868	Myosin heavy chain	cl34868
OeCAMTA4a	Ank_2	pfam12796	Ankyrin repeats	cl39094
	CBD_MYO6-like super family	cl41207	calmodulin binding domain	cl41207
OeCAMTA4b	CG-1	pfam03859	CG-1 domain	cl04295
	Ank_2	pfam12796	Ankyrin repeats	cl39094
	PLN03192 super family	cl33658	Voltage-dependent potassium channel	cl33658
	IQ	smart00015	Calmodulin-binding motif	cl29555
	TIG	pfam01833	IPT/TIG domain	cl15674
	cytochrome_P450 super family	cl41757	cytochrome P450 (CYP) superfamily	cl41757
OeCAMTA5	CG-1	pfam03859	CG-1 domain	cl04295
	Ank_2	pfam12796	Ankyrin repeats	cl39094
	ANKYR	COG0666	Ankyrin repeat	cl34000
	IQ	smart00015	Calmodulin-binding motif	cl29555

CAMTA TFs regulate the gene the expression in response to numerous biotic and abiotic stresses in plants in a Ca^{2+}- CaM dependent way (Galon et al., 2010; Wei et al., 2017). In previous studies, lots of CAMTA proteins were identified ,including the alternative sliced variants, in various plant species: 6 CAMTA proteins for *Arabidopsis thaliana* (Yang and Poovaiah, 2002), 10 for *Vitis vinifera* (Shangguan et al., 2014), 15 for *Glycine max* (Wang et al., 2015), 7 for *Populus trichocarpa* (Wei et al., 2017), 9 for *Citrus* species (Zhang et al., 2019), 8 for *Phaseolus vulgaris* (Büyük et al., 2019), 9 for

Linum usitatissimum (Ali et al., 2020), 18 for *Oryza sativa* (Gain et al., 2022) and 7 for *Solanum lycopersicum* (Fang et al., 2022).

In some genome-wide identification studies, CAMTA genes are grouped due to their existence on specific chromosomes (Shangguan et al., 2014; Yuan et al., 2021; Gain et al., 2022) however in olive they all are located at different chromosomes. While in *A. thaliana* CAMTA genes from 1 to 6 have 12, 13, 14, 13, 13 and 10 exons in their number one splice variant, respectively, olive OeCAMTA1a has 10, OeCAMTA2a has 10, OeCAMTA2 has 12, OeCAMTA3 has 13, OeCAMTA4a has 4, OeCAMTA4b has 18 and OeCAMTA5 has 13 exons.

In promoter regions of olive CAMTAs, 71 different cis-acting elements have been identified. Among these there are hormone response elements like ethylene abscisic acid, gibberellin, or auxin. Yang et al. (2012) showed that CAMTA gene expressions are regulated by ethylene application in *Solanum lycopersicum* and they suggest that CAMTAs act as a signal node molecules and regulate fruit development and ripening. Additionally, Zhang et al. (2019) applied salicylic acid, ethrel, indole-3-acetic acid, 6-benzylaminopurine, abscisic acid, methyl jasmonate and gibberellin to Citrus and they found that all genes respond to at least one hormone treatment except Citrus CAMTA4. While Citrus CAMTA 5, 7 and 9 showed positive response to all applied hormones, other CAMTA genes are both up- and down-regulated due to the hormone type (Zhang et al., 2019). Thus, the hormone response elements which found in promoter regions of olive CAMTAs indicates that these calmodulin-dependent TFs might be involved in hormone response in olive.

Also, stress response elements are found for heat, drought, and wounding responses in olive CAMTA promoters. There are lots of studies related with plant stress responses and CAMTAs. *A.thaliana* CAMTA1 regulates drought responses (Pandey et al., 2013), *A.thaliana* CAMTA3 provides freezing tolerance for plants and involved in cold-regulated gene expression (Doherty et al., 2009), *P. trichocarpa* CAMTAs gave different reactions to wounding and cold stress, indicating that CAMTAs have both biotic and abiotic stress responses (Wei et al., 2017), differential expression of CAMTA genes in *P. vulgaris* (Büyük et al., 2019) and their key role in *Cucurbita* species (Yuan et al., 2021) are also reported under salt stress, *Citrus* CAMTA expressions are notably varied under salt and drought stress (Zhang et al., 2019) and Primo-Capella et al . (2021) concluded that CAMTA genes also responds to short-term low temperatures in *Citrus* Rootstocks. Our promoter cis-acting element analysis results also show that olive might have a stress response mechanism via CAMTA genes.

There are a few studies about calcium response in olive. One study indicates that supplementary calcium improves tolerance against salinity (Larbi et al., 2020), another one suggests that salicylic acid and calcium pretreatments lighten the toxic effects of salinity (Methenni et al., 2018) and D'Angeli et al. (2003) suggest a long-term adaptation to cold when the Ca^{2+} response is involved. However, there are no studies focused on olive CAMTAs so far and, much more studies is needed to better understand the effect of calcium and its activators on the olive plant.

To understand the phylogenetic relationship of olive CAMTAs, an unrooted phylogenetic tree is drawn and it is noticed that CAMTA proteins are grouped in 4 main branches and every branch contained *A. thaliana* and olive CAMTAs. There results suggest that CAMTAs are highly conserved among angiosperms. In previous studies Shen et al. (2015) grouped different CAMTAs is 6 phylogenetic groups while Kakar et al. (2018) and Zhang et al. (2019) grouped them in 5, Büyük et al. (2019), Yang et al. (2020) and Yuan et al. (2021) grouped them in 3. 109 proteins in total are used from 11 different plant species for our phylogenetic tree and they are grouped in 4 different clades. Those group numbers may differ due to the variety of plant species used in the tree establishment.

Most CAMTAs contain a DNA binding domain CG-1, a non-specific DNA binding TIG domain, an ankyrin repeats domain, a Ca^{2+} dependent CaM binding domain and a varying number of IQ calmodulin binding motifs that interact with calmodulin. DNA-binding domain is found CG-1 in OeCAMTA2, OeCAMTA3, OeCAMTA4b and OeCAMTA5, TIG in OeCAMTA2 and OeCAMTA4b, ANK repeats are found in all olive CAMTA proteins except OeCAMTA1a, IQ motifs are only lacking in OeCAMTA2 and OeCAMTA4a and CBD_MYO6-like super family calmodulin-binding domains in OeCAMTA2 and OeCAMTA4a. These results indicates that conserved domains in CAMTA proteins are highly conserved.

Lastly, the molecular weight, isoelectric points, and subcellular localizations of the olive CAMTAs are analyzed with bioinformatic tools. It is noticed that subcellular localization of olive CAMTAs are mostly nuclear which is consistent with their TFs roles. It was shown experimentally that *A. thaliana* CAMTA1 is present predominantly in the nuclei (Bouche et al., 2002). Also, in *Fragaria × ananassa* (Leng et al., 2015), *P. trichocarpa* (Wei et al., 2017), *Gossypium* species (Pant et al., 2018), in *Triticum aestivum* (Yang et al., 2020), in *Linum usitatissimum* (Ali et al., 2020), *Oryza sativa* (Gain et al., 2022) it was bioinformatically shown that CAMTAs are mostly localized in the nucleus.

Conclusion

In conclusion, this study is focused on olive CAMTA genes and proteins. a genome-wide identification of CAMTA genes in olive is performed and 7 CAMTA genes are found in total. Promoter analysis suggests that CAMTA genes might have a role in hormone and stress responses in olive. This study also showed that olive CAMTA domains and proteins are highly conserved among plants and their subcellular localization is also conserved. To better understand the roles of CAMTAs in olive, further experimental studies are needed.

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