Genome-wide identification of calcium-dependent protein kinase (CDPK) family members in Phaseolus vulgaris L. and expression analysis during abiotic stresses (#119361)

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Genome-wide identification of calcium-dependent protein kinase (CDPK) family members in Phaseolus vulgaris L. and expression analysis during abiotic stresses

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Background: Calcium-dependent protein kinases (CDPKs) constitute a family of enzymes that play crucial roles in plant signaling pathways. These kinases are activated in response to changes in calcium ions (Ca +2) concentration under stress conditions. The objective of this research, is to perform a genome- wide analysis of the *CDPK* gene family in *Phaseolus vulgaris* and evaluate the expression patterns of these genes under salt and drought stress conditions.
Methods: In this study, comprehensive bioinformatics analyses were conducted on the CDPK gene family members in *P. vulgaris* to reveal the phylogenetic relationships, chromosomal locations, structural features, motif patterns, regulatory elements in promoter regions and expression profiles of the genes in the salt and drought stresses.
Results: Within this research, 25 *PvCDPK* genes were identified in the bean genome. The lengths of proteins vary between 298 and 582 amino acids, and their molecular weights range from 33.43 kDa to 65.13 kDa. The majority of the *PvCDPKs* located on a total of 8 chromosomes have 6 introns. Phylogenetic analysis indicates that PvCDPK proteins cluster in three main groups with *Arabidopsis thaliana* and *Glycine max* species. The divergence times for 6 pairs of segmental duplicated genes ranged from 48.94 million years ago (MYA) to 65.57 MYA, while tandem duplicates ranged from 32.09 to 84.95 MYA.
Conclusions: Comparative expression analysis of *PvCDPK* genes revealed varying expression levels depending on the two bean cultivars. Furthermore, these observations suggest that *PvCDPK* genes could be essential for the growth and development of bean in reaction to abiotic stresses such as drought and salt. This is the first study to investigate the CDPK gene family in P. vulgaris, and these identified genes obtained can be directly evaluated as candidate genes for marker-assisted selection or gene editing approaches. In addition, the findings are expected to contribute to the development of resilient cultivars capable of withstanding climate change.

1

2 Genome-Wide Identification of Calcium-Dependent 3 Protein Kinase (CDPK) Family Members in Phaseolus 4 vulgaris L. and Expression Analysis During Abiotic 5 Stresses

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16

17 Abstract

18 **Background:** Calcium-dependent protein kinases (CDPKs) constitute a family of enzymes that
19 play crucial roles in plant signaling pathways. These kinases are activated in response to changes
20 in calcium ions (Ca^{+2}) concentration under stress conditions. The objective of this research, is to
21 perform a genome- wide analysis of the *CDPK* gene family in *Phaseolus vulgaris* and evaluate
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24 gene family members in *P. vulgaris* to reveal the phylogenetic relationships, chromosomal
25 locations, structural features, motif patterns, regulatory elements in promoter regions and
26 expression profiles of the genes in the salt and drought stresses.
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28 lengths of proteins vary between 298 and 582 amino acids, and their molecular weights range
29 from 33.43 kDa to 65.13 kDa. The majority of the *PvCDPKs* located on a total of 8
30 chromosomes have 6 introns. Phylogenetic analysis indicates that PvCDPK proteins cluster in
31 three main groups with *Arabidopsis thaliana* and *Glycine max* species. The divergence times for
32 6 pairs of segmental duplicated genes ranged from 48.94 million years ago (MYA) to 65.57
33 MYA, while tandem duplicates ranged from 32.09 to 84.95 MYA.
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35 levels depending on the two bean cultivars. Furthermore, these observations suggest that
36 *PvCDPK* genes could be essential for the growth and development of bean in reaction to abiotic
37 stresses such as drought and salt. This is the first study to investigate the CDPK gene family in P.
38 vulgaris, and these identified genes obtained can be directly evaluated as candidate genes for

39 marker-assisted selection or gene editing approaches. In addition, the findings are expected to
40 contribute to the development of resilient cultivars capable of withstanding climate change.
41

42 Introduction

43
44 Plants are continuously subjected to biotic and abiotic stresses during their developmental
45 process. Highly complex mechanisms are activated to respond to the effects of abiotic stresses
46 like salinity, temperature, drought, and heavy metal *(Krasensky & Jonak, 2012; Takahashi et al.,*
47 *2020)*. In this way, plant adaptation develops rapidly and the negative effects are minimized.
48 Secondary messengers play important roles in providing this adaptation. Ca^{+2}, one of these
49 secondary messengers, is an ion that undergoes alterations in plants during stress and is crucial to
50 plant growth *(Ray, 2017)*. Proteins that detect changes in the cytoplasmic Ca^{+2} content inducing a
51 phosphorylation process in the plant, which starts the reaction of signaling pathways (*Boudsocq*
52 *et al., 2010; Valmonte et al., 2014; Luan & Wang, 2021)*. Ca^{+2}-dependent protein kinases
53 (CDPKs), calmodulin (CaM), calcineurin B-like proteins and CAM-like proteins (CMLs) are
54 some of the classes of Ca^{+2}-binding proteins in plants (*Ranty et al., 2016; Mohanta et al., 2017)*.
55 Particularly, CDPKs are crucial calcium-binding proteins that are only present in protists, green
56 algae, and plants—not in fungi or animals (*Hamel et al., 2014; Wang et al., 2015b)*.
57 Ca^{+2}, whose concentration varies under stress, binds to the CaM-like domain to activate CDPKs,
58 which allow the plant to react appropriately throughout growth and development (*Wang et al.,*
59 *2015a)*. Researchs are showing that *CDPKs* are effective under stress conditions. In rice (*Oryza*
60 *sativa*), *OsCDPK4* has been reported to protect the cell membrane from oxidative damage and
61 thus increase salt and drought tolerance (*Campo et al., 2014)*. *AtCPK28* was revealed to decode
62 cold-affected Ca^{+2} signals in *Arabidopsis thaliana* and increase plant resistance to cold by
63 phosphorylating Nin-Like Protein 7 (NPL7) (*Shi et al., 2018; Ding et al., 2022)*. In another
64 study, it was reported that the transcription of *TaCDPK25-U-AS1* and *TaCDPK25-U-AS2*
65 increased under drought stress in wheat (*Triticum aestivum*) and this increased the drought
66 resistance of the plant (*Linghu et al., 2023)*. It has been revealed that *FaCDPK1* and *FaCDPK3*,
67 *FaCDPK4* and *FaCDPK11*, which are among the *CDPKs* in strawberry (*Fragaria x ananassa*),
68 form a strong response to salt, while *FaCDPK4* and *FaCDPK11* form a strong response to
69 drought, and that drought-related genes are significantly affected by ABA treatment. It was also
70 claimed that this may affect drought-related proteins *(Crizel et al., 2020)*.
71 *Phaselous vulgaris* (common bean) belongs to the family Fabaceae, which has 640 genera and is
72 a member of the genus *Phaselous*, which is known to have 240 species (*Broughton et al., 2003)*.
73 Common bean is a plant of high nutritional value and economic importance that is widely grown
74 and consumed worldwide. Among the common causes of crop loss in *P. vulgaris*, abiotic stress
75 factors such as salinity and drought play a significant role.
76 The present study utilized bioinformatics data to identify and characterize the *CDPK* genes in
77 beans. Additionally, the qRT-PCR technique was utilized to clarify the functions of these genes
78 in response to drought and salt stressors. Furthermore, this study provides molecular targets for

79 the development of stress-tolerant bean varieties. The characterization of these genes provides a
80 integrative understanding of bean metabolism.
81
82 ## Materials & Methods
83
84 ### Identification of *PvCDPK* gene family and analysis of basic parameters
85 Amino acid sequences of the *P. vulgaris* *CDPK* gene family were retrieved from the Phytozome
86 v12.1 database (https://phytozome-next.jgi.doe.gov/) under accession number PF03492
87 (http://pfam.xfam.org). The genomes of *A. thaliana* and *Glycine max* were examined in the same
88 database to identify potential CDPK proteins (*Lamesch et al., 2012; Valliyodan et al., 2019*). The
89 default configurations of the Hidden Markov Model (HMM) validated the CDPK protein
90 sequences. Table S1 enumerates the CDPK protein sequences of various plants. The HMMER
91 database (http://www.ebi.ac.uk) was utilized to examine the CDPK domains within the
92 sequences. The amino acid count, molecular weight, and other properties of the CDPK proteins
93 were assessed utilizing the "ProtParam tool" (https://www.expasy.org/protparam/). The
94 phylogenetic studies employed the neighbor-joining (NJ) technique with a bootstrap value of
95 1000 replicates. The ClustalW algorithm was employed to align the PvCDPK protein sequences
96 (*Thompson et al., 1997*). Evolutionary diagrams were produced via MEGA v7 (*Tamura et al.,*
97 *2011*). The iTOL database was utilised to construct the phylogenetic tree (*Letunic & Bork,*
98 *2011*).
99
100 ### The discovery of *PvCDPK* members, their structures, chromosome locations,
101 ### and gene duplications; Comparative mapping with *A. thaliana* and *G. max*;
102 ### and the conserved motif
103 The coding and non-coding sections of the *PvCDPK* gene were retrieved utilizing the Gene
104 Structure Display v2.0 web tool using the genomic and CDS sequences (http://gsds.gao-lab.org/)
105 (*Hu et al., 2015*). The positions of *PvCDPK* genes on the chromosome were derived from the
106 Phytozome v12.1 database (https://phytozome-next.jgi.doe.gov/). *PvCDPK* genes were
107 delineated on each chromosome of *P. vulgaris* utilizing MapChart (*Voorrips, 2002*). MCScanX
108 (The Multiple Collinearity Scan Toolkit) (*Wang et al., 2012*), utilizing default settings, determine
109 the orthologous relationship between *P. vulgaris* and *G. max CDPK* gene.
110 The substitution ratios (Ka, Ks, and Ka/Ks) between duplicate pairs of *PvCDPK* genes were
111 estimated using PAL2NAL (http://www.bork.embl.de/pal2nal/#Ref) (*Suyama et al., 2006*) and
112 AML interface tool (http://abacus.gene.ucl.ac.uk/software/paml.html) (*Yang, 2007*). Synteny
113 maps were made with TBtools (Chen et al., 2020). $T = Ks/2\lambda$ (λ =6.56E-9) was used to estimate
114 *CDPK* gene duplication and divergence time (Mya) (*Yang & Nielsen, 2000; Lynch & Conery*
115 *2003*).
116 To uncover more conserved PvCDPK protein motifs, the "MEME Tool" (https://meme-
117 suite.org/meme/index.html) was used (*Bailey et al., 2006*). The parameters 2, 50, and 10 were

118 used for minimum and maximum width and maximum number of motifs, respectively. There are
119 200–300 theme zones. Area distribution repetitions might be any number. Motifs were analyzed
120 with the InterPro database as outlined by Quevillon et al., (*2005*). The WEBLOGO online web
121 tool (http://weblogo.berkeley.edu/logo.cgi) produced CDPK domain sequence logos for
122 conserved area sequence analysis (*Crooks et al., 2004*).

123 Subcellular Localization and Analysis of *cis*-acting Elements of *PvCDPK* Gene 124 Family

125 The upstream sections (Table S1) containing 2-kb DNA segments of each *PvCDPK* gene family
126 member were analysed using the PlantCARE
127 (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) database for cis-acting element
128 analysis (*Lescot et al., 2002*). A figure showing cis-acting elements was generated using
129 TBTools (*Chen et al., 2020*). WoLF PSORT (https://wolfpsort.hgc.jp) predictor was used to
130 predict the subcellular localization of PvCDPK proteins (*Horton et al., 2007*).
131

132 Bean Homology Modeling for CDPK Proteins

133 The Phyre2 database (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index) was used to
134 acquire the 3D structures, and protein homology modeling was obtained using previously
135 identified CDPK protein sequences (*Kelley et al., 2015*). The best 3D image was obtained by
136 comparing the protein models' reliability rates.
137

138 An analysis of the ontology of genes and the links between CDPK proteins in 139 *P. vulgaris*

140 Protein–protein interactions were examined to ascertain their functional and physical
141 relationships using the STRING (https://string-db.org) database. The obtained information was
142 categorized and integrated with the confidence level for every interaction between proteins. The
143 Cytoscape program changed the way that proteins interact with one another (*Shannon et al.,*
144 *2003*). An essential prerequisite for the functional annotation of novel sequence data in plant
145 biotechnology research is the deployment of functional genomics techniques. Ontology data for
146 *PvCDPK* genes were acquired using the Blast2GO program, and this information was utilized to
147 access the functional characteristics of PvCDPK proteins (*Conesa et al., 2005*).
148

149 *In Silico* Gene Expression Analysis

150 RNA-seq data of *P. vulgaris* under salt and drought stress was taken from NCBI's SRA
151 collection. The used accession numbers were SRR957668 (leaf subjected to salt stress),
152 SRR958469 (leaf salt control) (*Hiz et al., 2014*), SRR8284481 (leaf subjected to drought stress),
153 and SRR8284480 (leaf drought control). Gene expression data were normalized utilising reads
154 per kilobase of transcript per million mapped reads (RPKM) (*Mortazavi et al., 2008*). The
155 Orange software (*Demsar et al., 2013*) was employed to transform the RPKM data to log2 and
156 generate a heatmap.

157

158 **Experimental Plant Materials and Treatments**

159 The *P. vulgaris* cultivars "Elkoca-05" and "Serra" employed in this study were obtained from the
160 Molecular Biology and Genetics Department, Erzurum Technical University. The genotype-
161 specific seeds underwent surface sterilization for 5-7 minutes with a 1% (v/v) NaOCl solution.
162 Subsequently, perlite was utilized for the germination process. The seedlings were relocated to a
163 hydroponic medium comprising 0.2 L of modified 1/10 Hoagland solution upon attaining the
164 developmental stage specified by Büyük et al., (*2019*). *P.vulgaris* seedlings were grown at 25 °C
165 and 70% relative humidity in a controlled cultivation room with light and a photosynthetic
166 photon flux of 250 mmol m^{-2} s^{-1}. After bean seedlings reached the first trifoliate stage in the
167 growth chamber, the control group was treated with 0 mM NaCl and the stress treatment group
168 was subjected to salt stress for nine days using Hoaglands solution and 150 mM NaCl (for
169 medium salinity stress). Concurrently, drought-stressed bean plants grown in the same conditions
170 were kept for 24 hours in Hoagland solution that was treated with either 0 (control) or 20%
171 PEG6000 (*Aygören et al., 2023*). Two different common bean cultivars' root and leaf tissues
172 were taken after the ninth day of stress treatment. Following the specified duration, the leaf
173 tissue of the bean genotypes was stored in liquid nitrogen and preserved at −80 °C until the
174 analysis was performed. Three biological replicates of the bean genotypes utilized in the study
175 were cultivated, and these replicates were employed for qRT-PCR analysis. The root and leaf
176 tissues were subjected to distinct qPCR analyses.

177

178 ***In vitro* qRT-PCR Analysis**

179 Trizol Reagent (Invitrogen Life Technologies, ABD) was used to extract total RNAs. The
180 Multiskan Go spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland) was employed to
181 quantify RNA, while a 1.5% agarose gel was utilized to evaluate the quality of the sample. In
182 order to carry out complementary DNA synthesis, the SensiFAST cDNA Synthesis Kit (Cat No:
183 Bio-65053, UK) was utilized, following the instructions provided by the manufacturer. The qRT-
184 PCR study was focused on five *PvCDPK* genes that were selected from the RNAseq data. The
185 qRT-PCR reactions were conducted using the RotorGene Q Real-Time PCR System (Corbett
186 Research, Qiagen GmbH, Germany) and ABT SYBR Green Mix (Cat. No.: Q03-02-01, Ankara,
187 Turkey). A total of 20 µL of qRT-PCR mix was used, including 10 µL of ABT SYBR Green Mix
188 (2x), 0.4 µL of each primer (1 µM forward and reverse), and 200 ng cDNA. The reaction was
189 carried out as follows; 10 min. at 95°C to be 1 cycle;15 sec. at 94°C, 30 sec. at 60 °C, 30 sec. at
190 72°C, to be 40 cycles.
191 The housekeeping gene used was the β-actin gene from *P. vulgaris*. The $2^{-\Delta\Delta CT}$ technique for
192 relative quantification was used to standardize the qRT-PCR data (*Livak & Schmittgen, 2001*).
193 Information on the primers used in this study is presented in Table S2. Two-way analysis of
194 variance (ANOVA) with Dunnett's test at the 0.05 significant level was utilized to conduct
195 statistical studies in GraphPad Prism 7.

196

197 ## Results

198

199 **Identification and Physicochemical Characteristics of CDPK Gene Family in *P. vulgaris***
200 Here, 25 CDPKs were identified in *P.vulgaris* genome through bioinformatics tools and renamed
201 *PvCDPK1.1* to *PvCDPK32* according to their locations on chromosomes. The number of amino
202 acids, protein molecular weight and theoretical pI (isoelectric point) of the proteins were
203 identified (Table 1). The number of amino acid sequence lengths of *PvCDPK*s ranged from 298
204 (*PvCDPK4.2*) to 582 (*PvCDPK2*). The molecular weights ranged from 33.43 kDa (*PvCDPK4.2*)
205 to 65.13 kDa (*PvCDPK2*) and the pI values ranged from 4.82 (*PvCDPK4.2*) to 9.21
206 (*PvCDPK16*).
207

208 **Chromosomal location and duplication events**
209 The 25 *PvCDPK* genes were distributed unevenly on eight chromosomes: Chr1 (4 genes), Chr2
210 (4 genes), Chr3 (3 genes), Chr6 (1 gene), Chr7 (6 genes), Chr8 (4 genes), Chr9 (2 genes), and
211 Chr11 (1 gene) but not on the other chromosomes (4,5,6 and 10) of common bean (Table 2).
212 The investigation of gene duplication revealed that; *PvCDPK1.1/ PvCDPK2* and *PvCDPK3.1/*
213 *PvCDPK3.2* genes had a Ks value of 0.64, *PvCDPK8/ PvCDPK32* genes had a Ks value of 0.81,
214 *PvCDPK10.1/ PvCDPK10.2* genes with 0.86 Ks, *PvCDPK11.1/ PvCDPK11.2* genes with 0.74
215 Ks and *PvCDPK17.1/ PvCDPK17.2* genes with 0.69 Ks. *PvCDPK4.1 / PvCDPK4.2* and
216 *PvCDPK29.1/ PvCDPK29.2* genes were found to be tandemly duplicated with Ks values of 0.42
217 and 1.11, respectively (Table 3). These genes had Ks/Ks ratios ranging from 0.08 to 0.28.
218 Natural selection during duplication events is represented by values equal to 1, purifying
219 selection is indicated by values less than 1, and positive selection in the evolutionary process is
220 shown by Ka/Ks values greater than 1 (*Juretic et al., 2005*). Accordingly, it can be said that all
221 *PvCDPK* genes are subjected to purifying selection (Fig. 1). The fact that *PvCDPK* genes were
222 subjected to purifying selection suggests that this gene family was effective in the expansion of
223 this plant genome.
224 In addition, the differentiation time of 6 pairs of segmental duplicated genes ranged from 48.94
225 million years ago (MYA) to 65.57, while tandem pairs ranged from 32.09 to 84.95 MYA.
226

227 **Phylogenetic Relationships and synteny analysis of *PvCDPK*s in *P.vulgaris* and different**
228 **plants**
229 Using the protein sequences from the common bean, *A. thaliana*, and *G. max*, a phylogenetic tree
230 was created to ascertain the phylogenetic relationships for the bean's *CDPK* gene family. A total
231 of 98 protein sequences, 25 of which belong to *P.vulgaris*, 34 of which belong to *Arabidopsis*
232 and 39 of which belong to *G.max*, were used in the phylogenetic analysis. As illustrated in Fig.1,
233 the genes were clustered into three major subfamilies: A, B and C. Group A is the largest with 69
234 genes while Group C is the smallest with 9 genes. *PvCDPK4.2* was noticed relatively
235 independently of other *PvCDPKs*. Within the three groups formed in this phylogenetic tree,

236 which is important in explaining the molecular evolutionary process, it is seen that *PvCDPK*
237 genes are homologously distributed, especially with *AtCDPK* genes (Fig. 2).
238 Synteny analysis was performed to examine shared structural changes in the genome, including
239 chromosomal fission and fusion. The analyses revealed 57 syntenic relationships between
240 *P.vulgaris* and *G.max* and 23 syntenic relationships between *P.vulgaris* and *A.thaliana*. While a
241 syntenic relationship was found between all *PvCDPK* genes and *G.max* genes, no syntenic
242 relationship was found between *AtCDPK* genes and *PvCDPK* genes only in PvChr-6. This
243 indicates a strong evolutionary similarity between *G.max* and *P.vulgaris*. It can also be said that
244 there is a strong syntenic relationship between *A. thaliana* and *P. vulgaris* in terms of
245 chromosomal significance. In addition, *CDPK* genes are equally distributed in these genomes,
246 indicating that these gene pairs are widely distributed within the genomes (Fig. 3).
247
248 **Gene structure and motif analysis of PvCDPK gene family**
249 The gene structure analysis indicated that all 25 *PvCDPK* gene family members contain total 169
250 introns and 195 exons. *PvCDPK16* and *PvCDPK28* have 11 introns, *PvCDPK3.1*, *PvCDPK3.2,*
251 *PvCDPK8, PvCDPK9, PvCDPK17.1, PvCDPK17.2, PvCDPK21, PvCDPK24*, *PvCDPK29.1,*
252 *PvCDPK29.2* and *PvCDPK32* have seven introns, *PvCDPK1.1, PvCDPK1.2, PvCDPK2,*
253 *PvCDPK4.1, PvCDPK6, PvCDPK10.1, PvCDPK10.2, PvCDPK11.1, PvCDPK11.2, PvCDPK13*
254 and *PvCDPK20* have six introns and *PvCDPK4.2* has five introns (Fig.4).
255 A total of 10 conserved motifs were identified with lengths ranging in length from 8 to 50 amino
256 acids using MEME (Fig 5; Table S1). All proteins were discovered to include the motifs 2,5 and
257 7 whereas other motifs were identified only in specific subgroups (Fig.4). As an example, motif
258 8 only found in one subgroup (Fig.4). Except PvCDPK16 and PvCDPK28 all PvCDPKs have
259 four motif 2.
260
261 **Analysis of *PvCDPK*s promoter cis-elements**
262 The regulatory mechanisms of *PvCDPK* genes were explored by analyzing the 2 kb upstream
263 sequences from their start codons for cis-regulatory element composition.
264 As a direct result, a total of 341 cis-acting regulatory elements were identified in the promoters
265 of *PvCDPK* genes (Fig.6; Table S1.) In addition, a total of 14 cis-acting regulatory elements
266 were identified in the promoters of *PvCDPK* genes. The cis-acting elements were divided into
267 four main categories: abiotic/biotic stress-responsive including 11 elements (MYB, MBS, LTR,
268 etc.), development including elements (CCAAT-box) core elements and binding sites (W box)
269 and hormonal-responsive (as-1) including only one elements.
270 The greatest number of cis acting elements were determined in MYC and MYB and these
271 elements together with MBS element are associated with drought stress defense. While MYC
272 and MYB were found in all genes, MBS was found to be associated with *PvCDPK* -1.2, -3.1, -
273 4.1, -4.2, -9, -10, -11.1, -13, -17.1, -20, -29.1 and -29.2 genes. Biotic and abiotic stress
274 represented by the highest number of elements indicating *PvCDPK* genes have important roles in

275 response to biotic and abiotic stress (Fig.6; Table S1.). Considering this viewpoint, it is likely
276 that these *PvCDPKs* participate in multiple biological functions.

277 **Three-dimensional homology modelling, Protein Interaction Network of PvCDPKs' and**
278 **GO analysis**

279 Proteins interact with each other to achieve their roles, thus understanding the linkages and how
280 intricate biological processes work is crucial. PvCDPK protein sequences were used to identify
281 their PPIs using the STRING interface. Here, it was found that 25 proteins interacted with five
282 different common bean proteins. These proteins were V7BRL4_PHAVU-Phvul.006G142500
283 and V7BS13_PHAVU-Phvul.006G157600 (calcium binding protein 39), V7BH71_PHAVU-
284 Phvul.007G22340 (heat stress transcription factor A-3), V7CME5_PHAVU-Phvul.002G160700
285 (respiratory burst oxidase homolog protein F-related) and V7CPP5_PHAVU-Phvul.002G293700
286 (PTHR11972//PTHR11972:SF81 - NADPH oxidase) (Fig. 7; Table S1). All PvCDPK proteins
287 interacted with V7CME5_PHAVU-Phvul.002G160700. PvCDPK17.1 and PvCDPK17.2
288 interacted with PvCDPK24. Remaining proteins showed no interaction within each other.
289 Gene ontology helps to understand gene function by comparing it to known function genes in
290 other species. In the biological process, *PvCDPK* genes are enriched in the peptidyl-serine
291 phosphorylation, protein autophosphorylation and intracellular signal transduction. Cellular
292 component category included nucleus, cytoplasm and intracellular anatomical structure. Protein
293 serine kinase activity, calcium ion binding activity, calmodulin-dependent protein kinase
294 activity, calmodulin binding activity and calcium-dependent protein serine/threonine kinase
295 activity were categorized in molecular function (Fig. 8; Table S1).
296

297 **Homology Modeling of PvCDPK Proteins**

298 CDPK proteins were identified through Phyre2 database and homology modelling was visualized
299 by 3D modelling method. The study's identified proteins' 3D homology models are shown in Fig.
300 9.
301

302 ***In silico* Expression Profiles of *PvCDPK* gene family drought and salt stress**

303 Throughout their period of development and growth, plants are greatly impacted by a wide
304 variety of environmental conditions, including low temperatures, high salt, and drought.
305 Expression pattern analysis can help understand the biological functions of *PvCDPK* in tissue
306 specific or abiotic stresses such as salt and drought. To comprehensively analyze the mRNA
307 expressions of *PvCDPK* genes, RNA-Seq data from four normal and treatment samples from the
308 NCBI SRA database were obtained and FPKM values of 25 *PvCDPK* genes were evaluated.
309 Five different tissues were taken for evaluation in this study. All the *PvCDPK* genes expressed at
310 least in one tissue. Different *PvCDPK* genes revealed differential expression patterns. For
311 example, only *PvCDPK17.1* and *PvCDPK24* displayed expression in flowers but no other
312 tissues. *PvCDPK11.2* was the most expressed *PvCDPK* in leaves and stem whereas *PvCDPK6*
313 expressed significantly in flowers, nodules, root and stem (Fig.10a; Table S1). With these
314 findings, it can be said that CDPK genes actively contribute to bean organ development.

315 In this study, *in silico* gene expression analysis under drought and salt stresses were determined.
316 *PvCDPK16* and *PvCDPK6* expressions were higher than control plants compared to drought and
317 salt treated plants. *PvCDPK11.2, PvCDPK10.2, PvCDPK32, PvCDPK21, PvCDPK13* and
318 *PvCDPK10.1* genes expressed higher than control under drought stress however these genes
319 expressions showed lower expression under salt stress (Fig 10b; Table S1). *PvCDPK28* and
320 *PvCDPK3.2* induced after salt treatments but their expressions reduced after drought treatment.
321 No important change was determined in the expression patterns of other gene. On the other hand,
322 among these genes only *PvCDPK10.2* expression displayed the same expression level between
323 control and treated plants. These findings suggest that *PvCDPKs* may be involved in the
324 response to a range of abiotic stresses, with different genes displaying distinct responses to
325 stress.
326
327 **qRT-PCR analyses**
328 In this study, common bean seedlings were treated with drought and salt to examine the function
329 of *PvCDPK* gene members. Here, two cultivars Elkoca-05 and Serra were used. qRT-PCR
330 analyses were performed for 5 specific primers (*PvCDPK1, PvCDPK4, PvCDPK10, PvCDPK20*
331 and *PvCDPK29*) designed using RNAseq data and the results are shown in Figure 11. Firstly, it
332 was determined that no non-specific results were obtained in the negative control analyses
333 performed in qPCR. Under drought stress *PvCDPK1*, *PvCDPK4*, *PvCDPK10* and *PvCDPK29*
334 genes expressions were increased in Elkoca-05. However, there was no significant change in the
335 expressions of *PvCDPK* analyzed, *PvCDPK* genes in Serra neither in leave nor in root.
336 Under salt stress different expression patterns was observed compared to drought stress.
337 *PvCDPK1* and *PvCDPK29* induced in leaf while *PvCDPK4* induced in root in Serra cultivar.
338 Besides *PvCDPK4* expression also increased in Elkoca-05 root. No significant change was
339 observed for *PvCDPK4* expression in leaf under salt stress both in Serra and Elkoca-05.
340 As a result, while gene expression levels of *PvCDPK* gene family members differed according to
341 cultivars, the same genes examined in drought stress did not differ in Elkoca-05 cultivar
342 according to tissues. Although the expression levels of *PvCDPK1* and *PvCDPK29* in Elkoca-05
343 cultivar increased in drought stress treatment their expression decreased in salt treatment in all
344 tissues. *PvCDPK4* induced both under drought and salt stress in both two cultivars. These
345 findings are in agreement with *in silico* analyses.
346

347 Discussion

348
349 *Phaseolus vulgaris* (common bean), a legume species of high economic importance, serves as a
350 major dietary protein and nutrient source globally. However, abiotic stressors—particularly
351 salinity and drought—pose significant threats to its yield and productivity. While previous
352 genome-wide studies in *P. vulgaris* have identified various gene families (*Büyük et al., 2019;*
353 *Akbulut et al., 2022; Aygören et al., 2022; de Souza Resende et al., 2022; Muslu et al., 2023;*
354 *Aygören et al., 2023; Chakraborty et al., 2023; Kasapoğlu et al., 2024*), comprehensive

355 investigations on Ca^{2+}-related gene families remain limited. The present study provides the first
356 genome-wide identification and systematic characterization of the *CDPK* gene family in *P.*
357 *vulgaris*. The findings provide important clues to the potential functions of the gene family in
358 both evolutionary conservation and stress responses.
359 *CDPKs*, similar to transcription factors, are key regulators of gene expression and mediate
360 diverse physiological responses through calcium signaling. These kinases are activated by
361 intracellular Ca^{2+} fluctuations and play essential roles in the perception and transduction of
362 abiotic stress signals. Previous reports have documented varying numbers of *CDPK* genes across
363 plant species, ranging from 11 to 85 (*Cheng et al., 2002; Ray et al., 2007; Wang et al., 2016;*
364 *Crizel et al., 2020; Fan et al., 2023; Linghu et al., 2023; Yang et al., 2023; Burra et al., 2023;*
365 *Xiong et al., 2024)*. In this study, 25 *PvCDPK* genes were identified, a number consistent with
366 findings in related species.
367 Phylogenetic analysis revealed that the PvCDPK proteins clustered into three main clades
368 together with their *Arabidopsis thaliana* homologs, indicating a high degree of evolutionary
369 conservation. However, the independent clustering of some members, such as PvCDPK4.2,
370 suggests that these genes may have acquired species-specific functions. This allows the
371 development of new hypotheses that PvCDPK4.2 may be involved in specific developmental
372 processes (e.g. flowering or symbiotic nitrogen fixation) in P. *vulgaris* other than abiotic stress
373 response. Moreover, structural analyses showed that the *PvCDPK* genes collectively contained
374 169 introns and 195 exons. The diversity in exon-intron architecture suggests functional
375 divergence and evolutionary adaptation within the gene family.
376 Subcellular localization predictions indicated that most PvCDPK proteins are cytosolic, although
377 several members are also localized to the nucleus, chloroplast, peroxisome, and mitochondria.
378 These findings are partially consistent with previous reports in *Fragaria* × *ananassa*
379 (strawberry), where CDPKs localize to the plasma membrane, cytoplasm, nucleus, and
380 chloroplast (*Crizel et al., 2020*). In contrast, CDPKs in *Gossypium hirsutum* (cotton) were
381 predominantly localized in the nucleus (*Lv et al., 2024*), highlighting species-specific differences
382 in subcellular distribution and potentially distinct physiological roles.
383 Genomic mapping revealed widely distribution of 25 *PvCDPK* genes across eight chromosomes.
384 Notably, chromosomes Chr1, Chr2, Chr7, and Chr8 harbored a higher density of CDPK loci,
385 whereas others such as Chr4, Chr5, Chr6, and Chr10 lacked any *PvCDPK* genes. Similarly, in a
386 study in *G. barbadense,* 84 *CDPK* genes were reported to be widely distributed on 26
387 chromosomes, and in tomato, 29 *CDPK* genes were reported to be widely distributed on 12
388 chromosomes (*Hu et al., 2016; Shi & Zhu, 2022*). Such distribution patterns likely reflect
389 chromosomal rearrangements and duplications that contributed to the diversification of this gene
390 family during evolution.
391 Promoter analysis revealed that PvCDPK genes contain a large number of stress-related
392 regulatory elements such as MYB, MBS and LTR. The presence of a large number of these
393 elements suggests that CDPK genes have the potential to respond not only to salt and drought,
394 but also to other environmental influences such as cold, oxidative stress (*Han et al., 2024*). MYC

395 and MYB motifs were detected in the promoter regions of all *PvCDPK* genes, while the MBS
396 element was found in 12 genes. These findings are consistent with prior studies in *Ipomoea*
397 *batatas* (sweet potato) and wheat, where these elements have been linked to abiotic stress
398 tolerance (*Li et al., 2022; Linghu et al., 2023*), suggesting that *PvCDPKs* may act as upstream
399 regulators in stress-responsive transcriptional networks.
400 Gene ontology (GO) enrichment analysis provided additional insights into the functional roles of
401 *PvCDPK* genes. Biological process annotations included peptidyl-serine phosphorylation,
402 autophosphorylation, and intracellular signal transduction. Molecular function categories were
403 dominated by protein serine/threonine kinase activity, calcium ion binding, and calmodulin-
404 dependent protein kinase activity. Cellular component classifications indicated nuclear,
405 cytoplasmic, and organelle-associated localizations. These functional predictions are consistent
406 with recent findings in other plant systems (*Li et al., 2022*).
407 Expression profiling using RNA-Seq datasets demonstrated that *PvCDPK* genes exhibit distinct
408 tissue-specific expression patterns. For instance, *PvCDPK17.1* and *PvCDPK24* were exclusively
409 expressed in floral tissues, whereas *PvCDPK11.2* showed high expression in leaves and stems.
410 *PvCDPK6* displayed elevated expression in flowers, nodules, roots, and stems. These results
411 support the hypothesis that CDPKs function in organ development, tissue differentiation, and
412 stress adaptation. This is in agreement with previous reports implicating CDPKs in root
413 development, pollen maturation, and phytohormone signaling pathways (*Li et al., 2018; Wen et*
414 *al., 2020; Li et al., 2022*). Particularly, the high expression of *PvCDPK6* in flower, tuber, root
415 and shoot raises the hypothesis that this gene may be associated with developmental transitions
416 and hormone signaling. This suggests that *PvCDPK6* may be a regulator that responds to growth
417 regulators such as jasmonate or gibberellin (*Xu and Huang, 2017*)
418 To further validate these findings, qRT-PCR analyses were conducted to assess *PvCDPK* gene
419 expression under drought and salinity stress conditions in two *P. vulgaris* cultivars. The results
420 revealed cultivar-specific and stress-dependent expression dynamics. In particular, *PvCDPK1*
421 and *PvCDPK29* were significantly upregulated in Elkoca-05 under drought stress but were
422 downregulated in all tissues under salinity stress. For example, *PvCDPK1* and *PvCDPK29* were
423 up-regulated only in Elkoca-05 in response to drought, indicating that these genes are regulated
424 by different mechanisms in response to genetic background. It is thought that these differences
425 may constitute the molecular basis of the variation in abiotic stress tolerance among cultivars.
426 This finding supports the idea that *PvCDPK* genes can be used as target gene candidates in the
427 development of lines with high stress tolerance through biotechnological applications. Notably,
428 *PvCDPK4* was induced under both drought and salt treatments in both cultivars. These results
429 corroborate the RNA-Seq data and emphasize the regulatory role of *PvCDPKs* in abiotic stress
430 responses.
431 Collectively, this study presents the first comprehensive characterization of the *CDPK* gene
432 family in *P. vulgaris*, offering novel insights into their structure, evolution, regulatory potential,
433 and functional relevance under stress conditions. The findings provide a valuable foundation for

434 future functional genomics and molecular breeding efforts aimed at enhancing stress tolerance in
435 common bean.
436

437 Conclusion

438 Genome-wide bioinformatics analysis, characterization and identification of *CDPK* genes of *P.*
439 *vulgaris* were performed using up-to-date databases and programs. 25 *CDPK* genes were found
440 in the bean genome based on the results of these investigations. Eight bean chromosomes were
441 identified to contain these genes. Using in silico analysis using various plant tissues, variations in
442 *PvCDPK* gene expression levels were identified. In addition, in order to strengthen *in silico*
443 analyses, two different bean cultivars were treated with salt and drought stress and their gene
444 expression levels were analyzed under *in vitro* conditions. These investigations revealed an
445 important correlation between *PvCDPK* genes and drought and salt stress. For the first time, the
446 function of *CDPK* genes—which are known to be crucial for key metabolic activities like
447 blooming, root growth, and fruit ripening in plants—has been studied in bean plants.
448 It is hoped that this comprehensive study using *P. vulgaris* species will make significant
449 contributions to the breeding research of this plant, clarify the metabolic processes and reactions
450 of the plant under stress, and benefit the scientists involved. Based on this study, *PvCDPK4.2*
451 may be involved in species-specific developmental regulation, and silencing this gene with
452 functional genetic approaches such as CRISPR/Cas9 may indicate whether it alters
453 developmental phenotypes. Furthermore, *PvCDPK6* may be a node of hormone signaling
454 pathways (e.g. ABA, JA). It should be investigated how the expression of this gene changes with
455 hormone treatments.
456

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459

460 Conflicts of interest

461 The author declare no conflict of interest.
462

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Figure 1

Distribution of *PvCDPK* genes on *P. vulgaris* chromosomes.

Black lines represent segmental duplicated genes and red lines represent tandem duplicated genes.

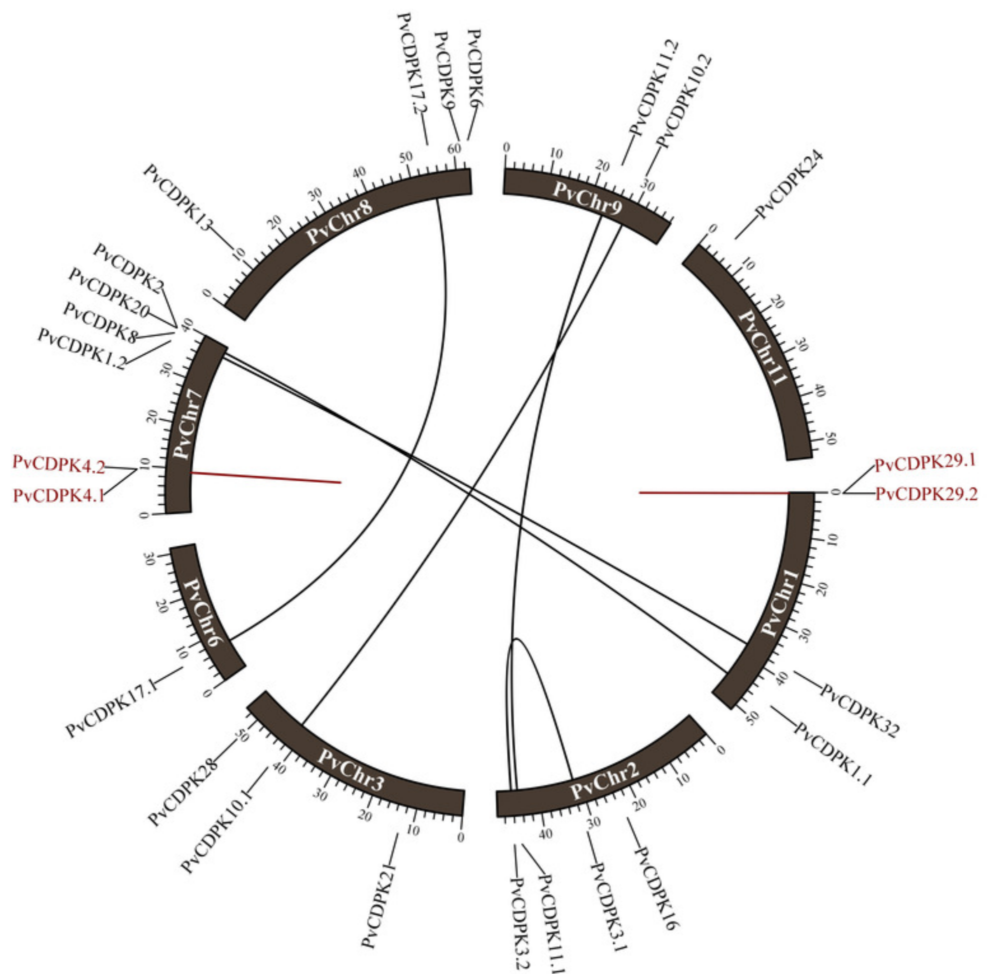
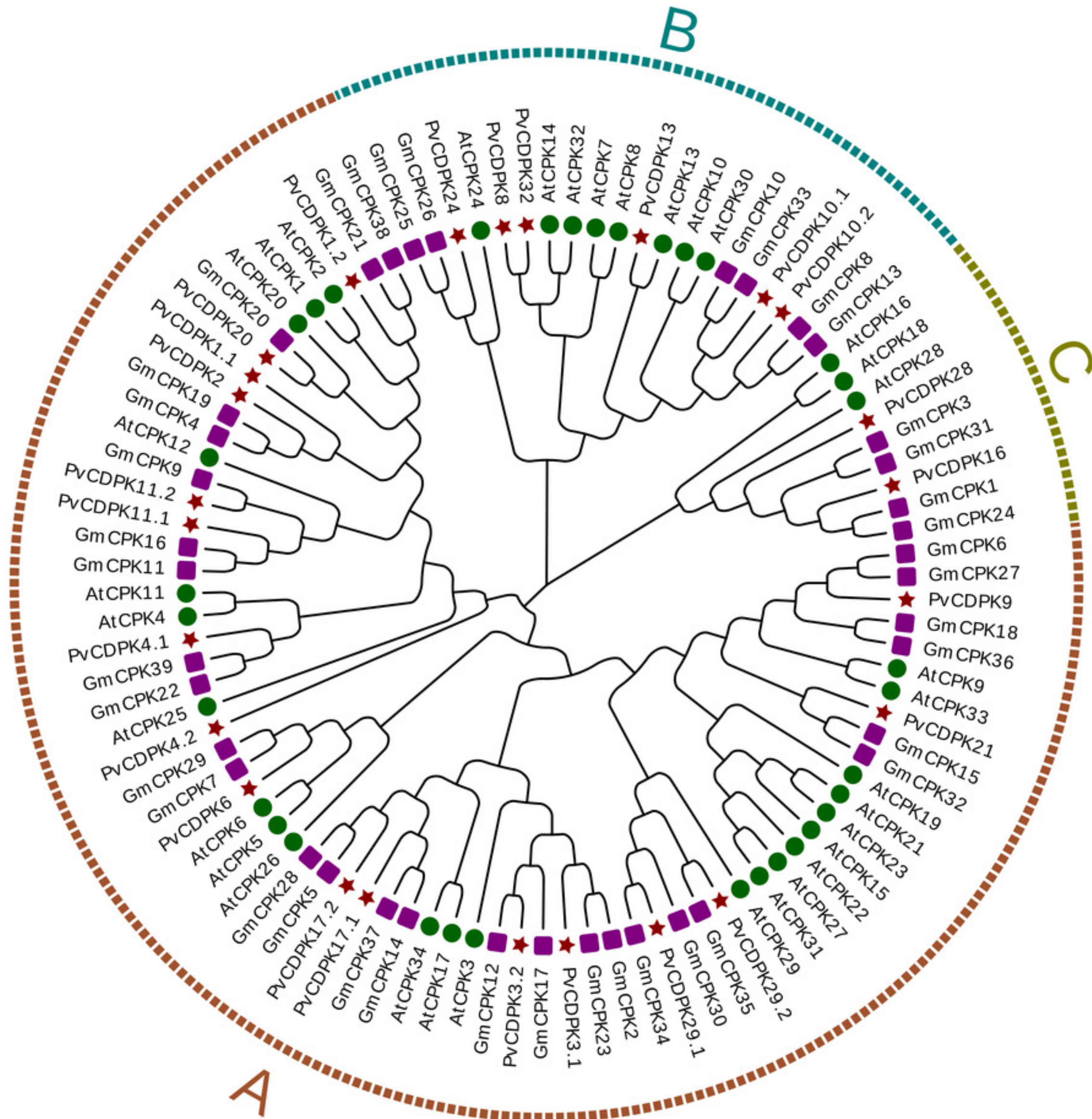


Figure 2

Phylogenetic analysis of CDPK proteins from *A. thaliana* (34), *G. max* (39) and *P. vulgaris* (25).

A. thaliana *is represented by a green circle,* G. max *by a purple square and* P. vulgaris *by a maroon star. G. max* sequences were obtained from Liu et al. (2016) and used. The locations of 69 genes in group (A), 20 genes in group (B) and 9 genes in group (C) are shown.



A
B
C

Figure 3

Synteny analysis between *A. thaliana, G. max* and *P. vulgaris CDPK* genes.

The red lines highlight the syntenic gene pairs between bean and Arabidopsis, while blue lines highlight the syntenic gene pairs with G.max.

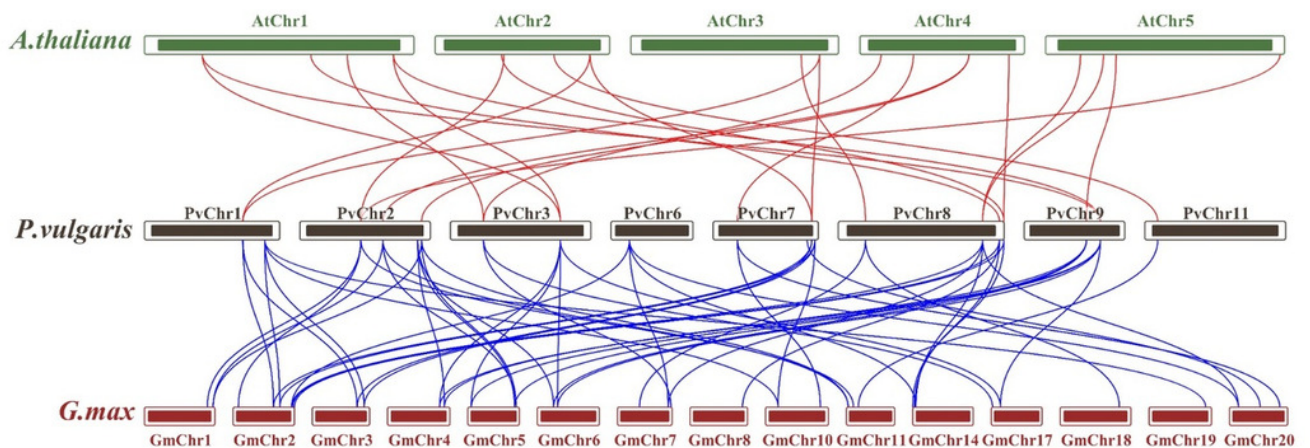


Figure 4

Structural representation of *PvCDPK* genes.

Maroon color represents exon and black lines represent intron regions. Sand colored parts represent 5'and 3' UTR regions. The scale bar indicates 10kb.

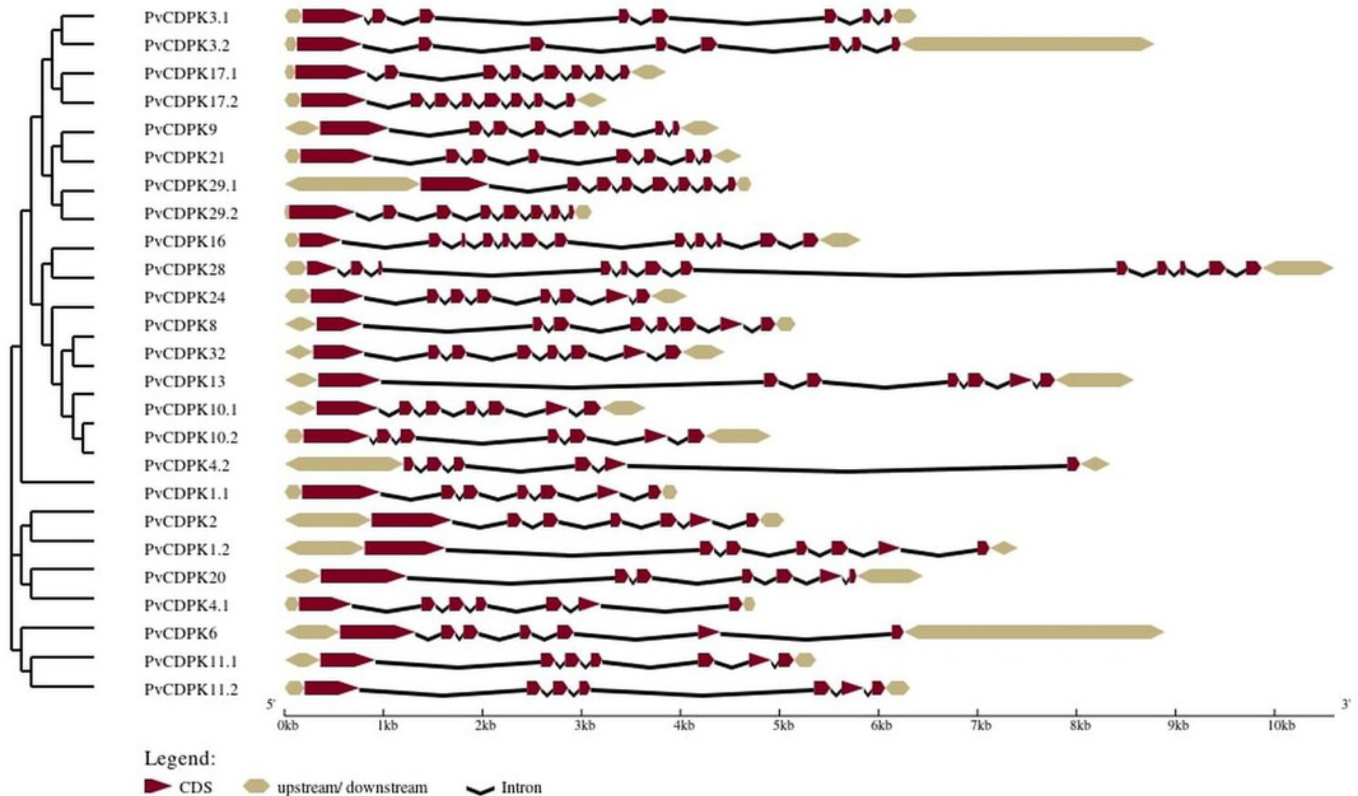


Figure 5

Conserved motif analysis in PvCDPK proteins.

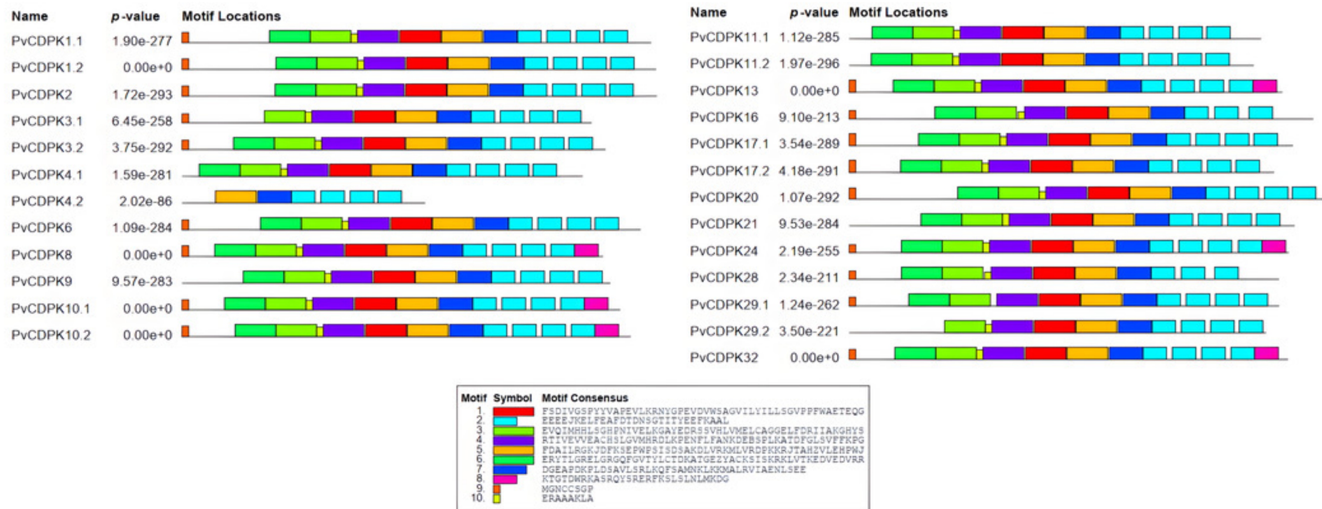


Figure 6

Cis-acting element analysis of *PvCDPK* genes

The elements on the genes represent those that play a role during plant stress. The different colors of the lines indicate the various cis-acting elements within the 2 kb promoter region located upstream of the *PvCDPK* gene.

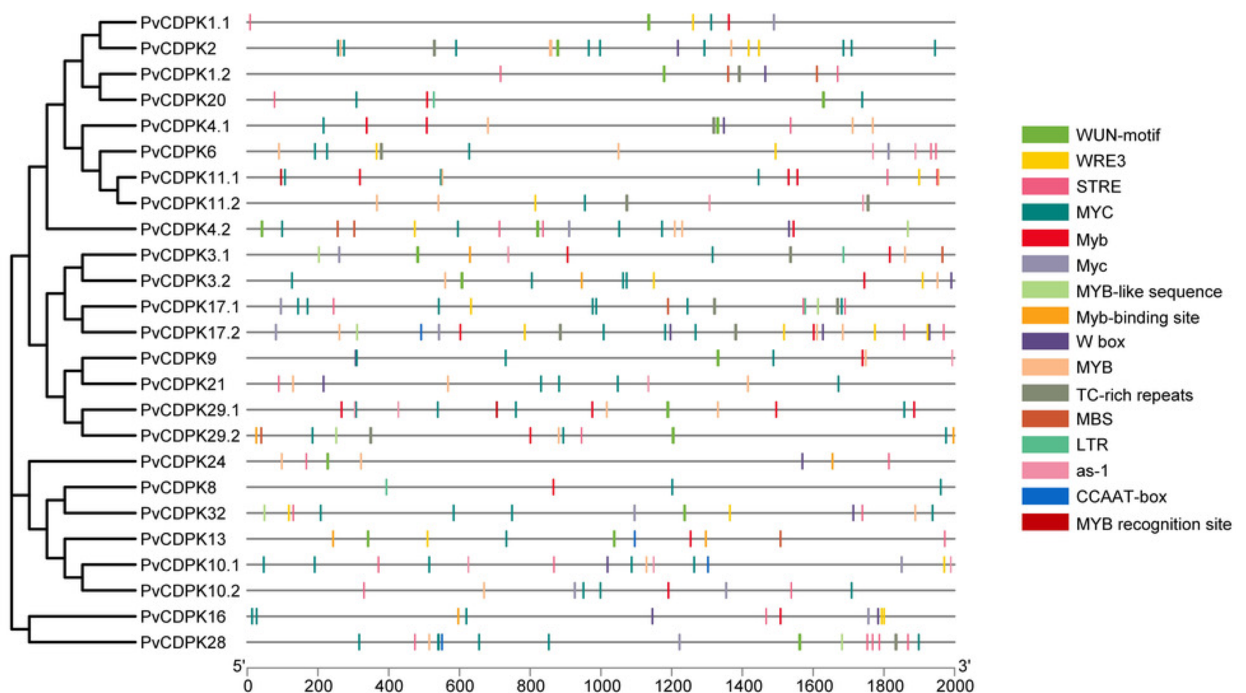


Figure 7

Interaction analysis of PvCDPK proteins both among themselves and with other proteins.

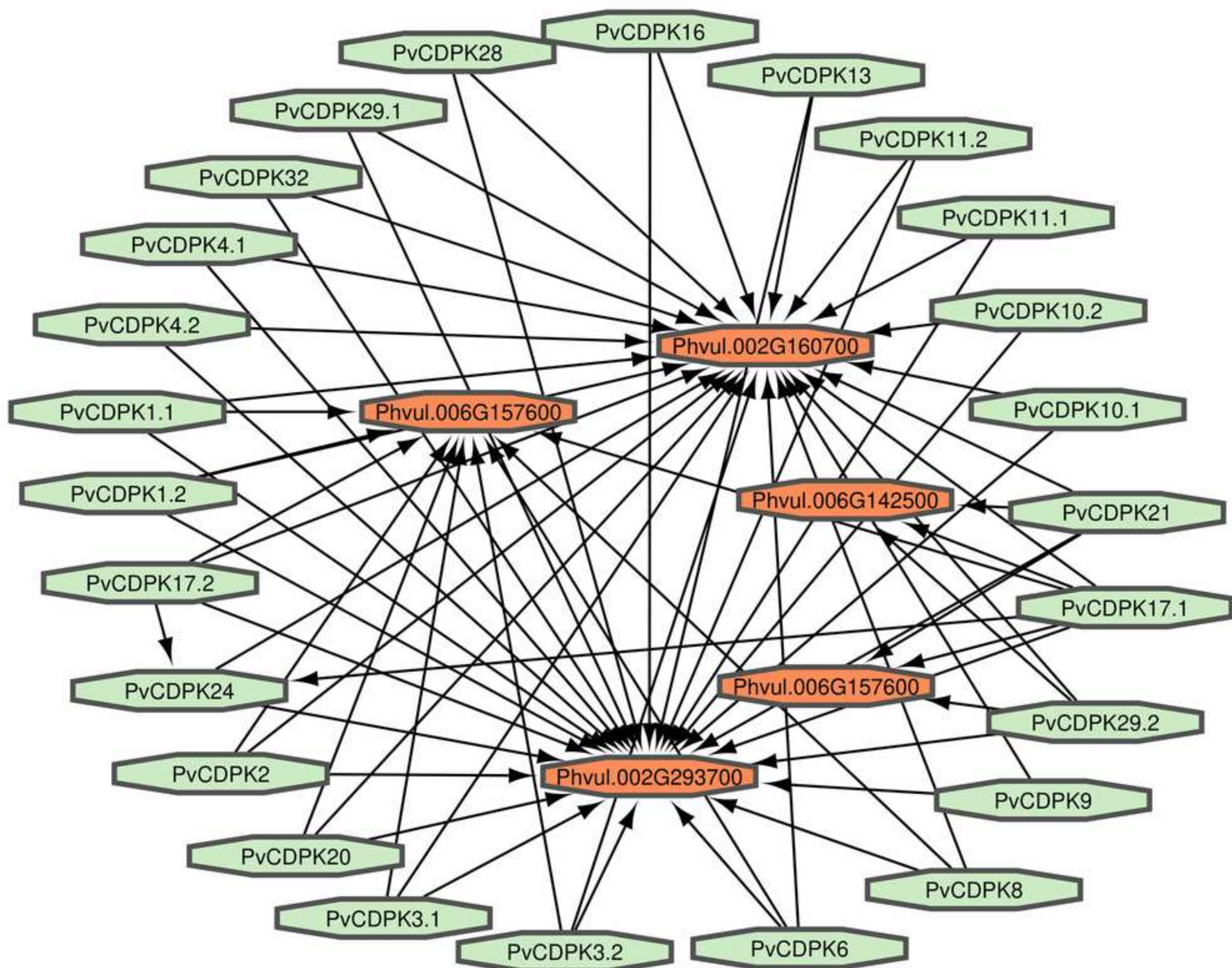


Figure 8

Gene ontology analysis of PvCDPK proteins.

The cellular component in which it is found, the biological process in which it is involved and the molecular functions it shows are included.

Figure 9

3D homology models of PvCDPK proteins using Phyre2 database and by 3D modelling.

Models were visualized using rainbow colors from N to C terminus.

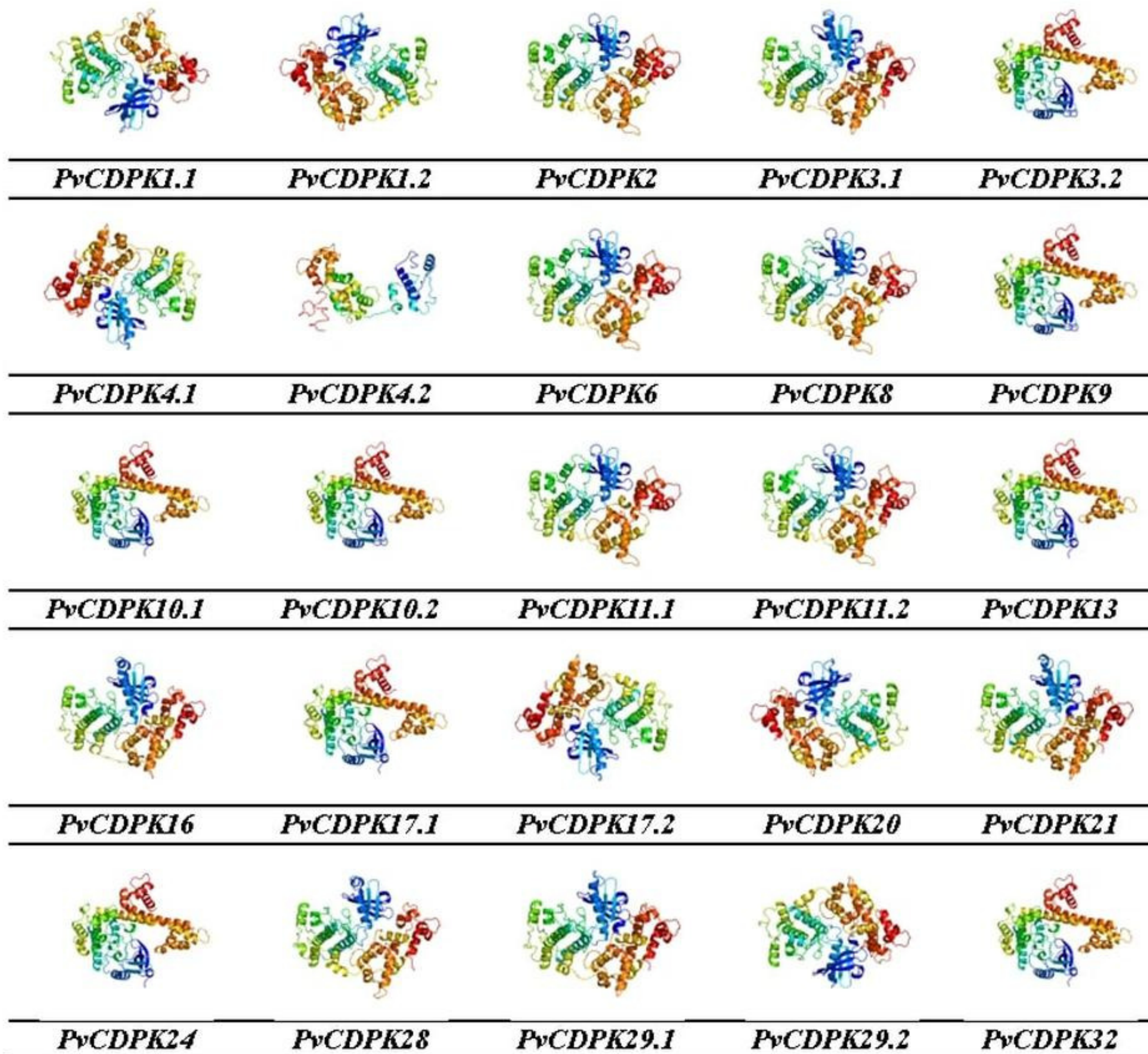


Figure 10

In silico analysis of the expression status of *PvCDPK* genes under drought and salt stress with control groups.

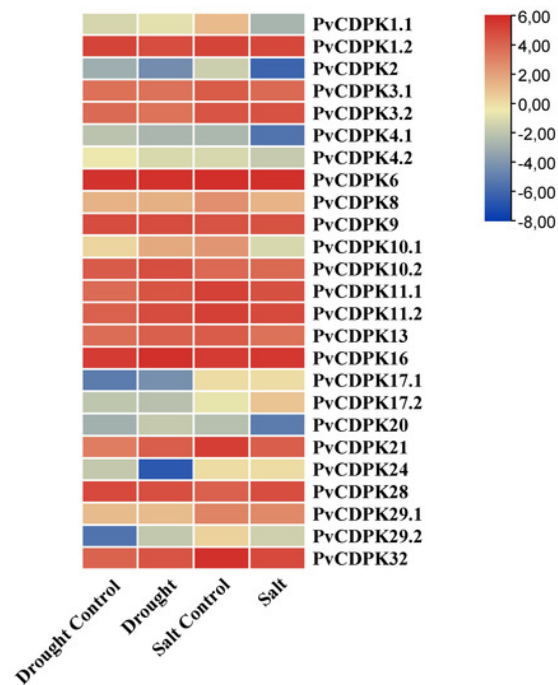


Figure 11

In silico analysis of the expression of *PvCDPK* genes in root, stem, nodule, leaf and flower tissues

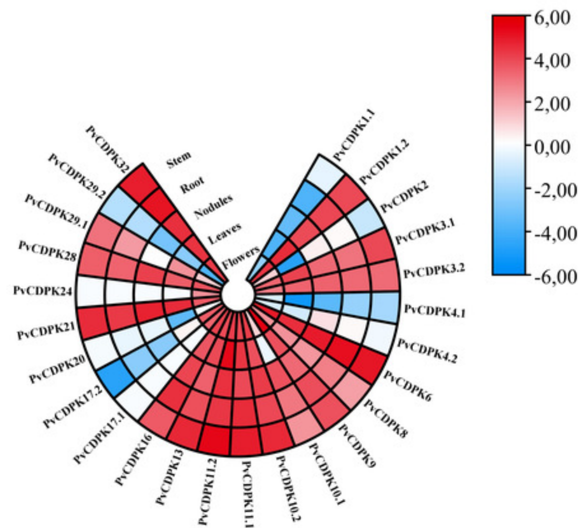


Figure 12

Expression analysis of *PvCDPK* genes under salt an drought stresses in two cultivars' root and leaf (Serra and Elkoca-05) using qRT–PCR method .

*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns: non-significant.

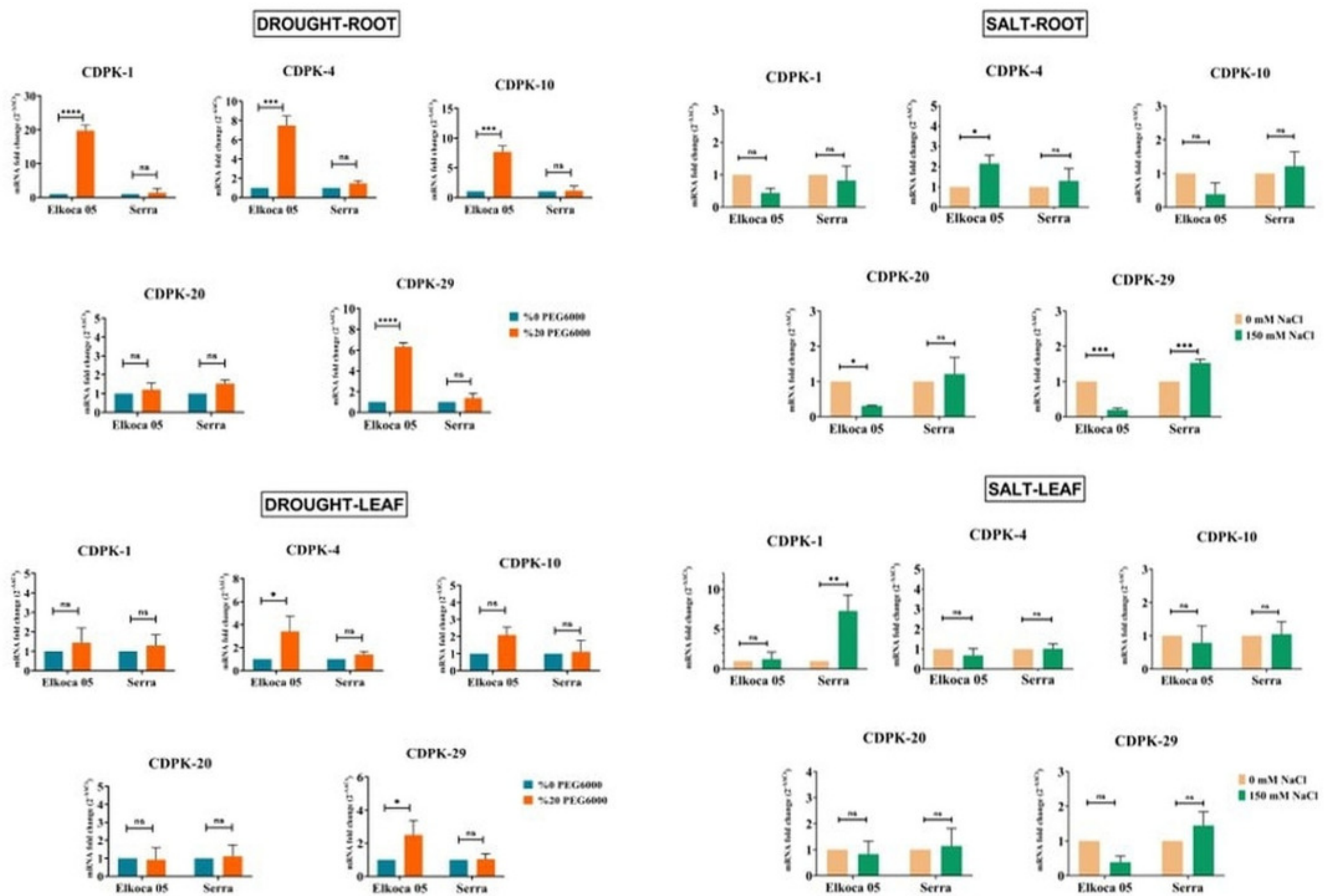


Table 1(on next page)

Characteristics of CDPK proteins in the *P. vulgaris* genome

pI: Theoretical isoelectric point, EF-hand: EF-hand calcium-binding domain, N-myrist: myristoylation, N-palmit: palmitoylation, chlo: chloroplast, mito: mitochondrion, cyto: cytosol, nucl: nuclear, pero: peroxysome

1 **Table 1. Characteristics of CDPK proteins in the *P. vulgaris* genome (**pI: Theoretical
2 isoelectric point, EF-hand: EF-hand calcium-binding domain, N-myrist: myristoylation, N-
3 palmit: palmitoylation, chlo: chloroplast, mito: mitochondrion, cyto: cytosol, nucl: nuclear, pero:
4 peroxysome)

PvGene Name	Amino Acid	MW (kDa)	pI	EF-hand No	N- myrist	N- palmit	Localization
PvCDPK1.1	575	64.55	5.09	3	No	Yes	chlo
PvCDPK1.2	581	64.99	5.28	4	No	Yes	chlo
PvCDPK2	582	65.13	5.8	4	No	Yes	chlo
PvCDPK3.1	502	56.71	6.05	4	Yes	Yes	chlo
PvCDPK3.2	519	58.28	5.9	4	Yes	Yes	mito
PvCDPK4.1	491	55.16	5.43	4	No	Yes	mito
PvCDPK4.2	298	33.43	4.82	4	No	Yes	cyto
PvCDPK6	562	63.01	5.57	4	No	Yes	nucl
PvCDPK8	516	58.68	6.5	2	No	Yes	chlo, mito
PvCDPK9	525	59.10	6.3	4	Yes	Yes	cyto
PvCDPK10.1	537	60.89	5.96	4	No	Yes	cyto
PvCDPK10.2	550	61.99	6.09	4	No	Yes	cyto
PvCDPK11.1	505	56.93	5.24	4	No	Yes	chlo
PvCDPK11.2	496	55.76	5.32	4	No	Yes	pero
PvCDPK13	531	59.76	5.86	3	No	Yes	cyto
PvCDPK16	569	64.69	9.21	4	Yes	Yes	chlo
PvCDPK17.1	544	60.54	5.09	4	Yes	Yes	cyto
PvCDPK17.2	521	58.49	5.58	4	Yes	Yes	cyto
PvCDPK20	582	64.79	5.35	4	No	Yes	chlo
PvCDPK21	546	60.72	5.76	4	Yes	Yes	cyto
PvCDPK24	539	61.07	6.46	4	Yes	Yes	chlo
PvCDPK28	527	59.72	8.92	4	No	Yes	chlo
PvCDPK29.1	527	60.18	6.11	4	Yes	Yes	cyto_nucl
PvCDPK29.2	511	57.77	5.64	2	No	Yes	cyto
PvCDPK32	538	60.98	6.29	3	No	Yes	cyto

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Table 2(on next page)

CDPK gene family members of *P.vulgaris* with *Arabidopsis* orthologs, chromosome locations, gene start and end point.

1 **Table 2.** ***CDPK*** **gene family members of** ***P.vulgaris*** **with** ***Arabidopsis*** **orthologs, chromosome**
2 **locations, gene start and end point.**

PvGene Name	Arabidopsis ortholog locus	Phytozome ID	Chr Name	Strand	Gene Start (bp)	Gene End (bp)
PvCDPK1.1	AT5G04870 (AtCPK1)	Phvul.001G197700.1	PvChr1	forward	45693570	45697541
PvCDPK1.2	AT5G04870 (AtCPK1)	Phvul.007G233900.1	PvChr7	forward	35767288	35774697
PvCDPK2	AT3G10660 (AtCPK2)	Phvul.007G266100.1	PvChr7	reverse	38724008	38729058
PvCDPK3.1	AT4G23650 (AtCPK3)	Phvul.002G161200.1	PvChr2	reverse	31597834	31604224
PvCDPK3.2	AT4G23650 (AtCPK3)	Phvul.002G294500.1	PvChr2	reverse	46343902	46352694
PvCDPK4.1	AT4G09570 (AtCPK4)	Phvul.007G089200.1	PvChr7	forward	9217140	9221899
PvCDPK4.2	AT4G09570 (AtCPK4)	Phvul.007G089301.1	PvChr7	forward	9223593	9231935
PvCDPK6	AT2G17290 (AtCPK6)	Phvul.008G292500.1	PvChr8	forward	62964859	62973752
PvCDPK8	AT5G19450 (AtCPK8)	Phvul.007G253300.1	PvChr7	reverse	37518913	37524077
PvCDPK9	AT3G20410 (AtCPK9)	Phvul.008G266600.1	PvChr8	reverse	61203175	61207570
PvCDPK10.1	AT1G18890 (AtCPK10)	Phvul.003G194100.1	PvChr3	reverse	41784590	41788241
PvCDPK10.2	AT1G18890 (AtCPK10)	Phvul.009G190566.1	PvChr9	reverse	28940689	28945605
PvCDPK11.1	AT1G35670 (AtCPK11)	Phvul.002G279300.1	PvChr2	forward	44866006	44871378
PvCDPK11.2	AT1G35670 (AtCPK11)	Phvul.009G160100.1	PvChr9	reverse	23666550	23672870
PvCDPK13	AT3G51850 (AtCPK13)	Phvul.008G098400.1	PvChr8	forward	10295962	10304546
PvCDPK16	AT2G17890 (AtCPK16)	Phvul.002G108700.1	PvChr2	reverse	23210281	23216106
PvCDPK17.1	AT5G12180 (AtCPK17)	Phvul.006G015300.1	PvChr6	forward	7024909	7028768
PvCDPK17.2	AT5G12180 (AtCPK17)	Phvul.008G201900.1	PvChr8	forward	54865627	54868891
PvCDPK20	AT2G38910 (AtCPK20)	Phvul.007G265100.1	PvChr7	reverse	38623002	38629456
PvCDPK21	AT4G04720 (AtCPK21)	Phvul.003G078400.1	PvChr3	forward	12570425	12575047
PvCDPK24	AT2G31500 (AtCPK24)	Phvul.011G055400.1	PvChr11	forward	4877733	4881802
PvCDPK28	AT5G66210 (AtCPK28)	Phvul.003G261700.1	PvChr3	reverse	50115856	50126462
PvCDPK29.1	AT1G76040 (AtCPK29)	Phvul.001G002800.2	PvChr1	forward	165997	170716
PvCDPK29.2	AT1G76040 (AtCPK29)	Phvul.001G002900.1	PvChr1	forward	171533	174640
PvCDPK32	AT3G57530	Phvul.001G135300.	PvChr1	reverse	37388614	37393061

(AtCPK32) 1

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Table 3 (on next page)

Duplication events and evolutionary analysis of *PvCDPK* genes

(MYA: million years ago)

1 **Table 3. Duplication events and evolutionary analysis of *PvCDPK* genes (**MYA: million
2 years ago)

Gene1	Gene2	Ka	Ks	Ka/Ks	MYA	Duplication Type
PvCDPK1.1	*PvCDPK2*	0.17	0.64	0.27	49.11	Segmental
PvCDPK3.1	*PvCDPK3.2*	0.09	0.64	0.14	48.94	Segmental
PvCDPK8	*PvCDPK32*	0.10	0.81	0.12	61.57	Segmental
PvCDPK10.1	*PvCDPK10.2*	0.07	0.86	0.08	65.30	Segmental
PvCDPK11.1	*PvCDPK11.2*	0.08	0.74	0.11	56.19	Segmental
PvCDPK17.1	*PvCDPK17.2*	0.09	0.69	0.13	52.52	Segmental
PvCDPK4.1	*PvCDPK4.2*	0.12	0.42	0.28	32.09	Tandem
PvCDPK29.1	*PvCDPK29.2*	0.25	1.11	0.22	84.95	Tandem

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