



## Genome wide identification of calcium dependent protein kinase and related kinase gene families in *Solanum melongena* L.

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### ABSTRACT

In plants, Calcium-Dependent Protein Kinases (CDPKs) play multiple roles with a variety of functions in physiological processes, biotic and abiotic stress together with plant hormone signalling. We conducted a genome-wide analysis of CDPK gene family in eggplant. Total 28 CDPKs and 2 CDPK-related kinases (CRKs) genes were identified in eggplant genome. Structural organisation of *Solanum melongena* CDPKs and CRK genes as well as different conserved domains and motifs were studied. Both *SmeCDPKs* and *SmeCRK* proteins harbour STKc\_CAMK type protein kinase domain, while only *SmeCDPKs* contained EF-hand type Ca<sup>2+</sup> binding domain(s). Phylogenetic analysis suggested the conserved basic structure of *SmeCDPKs* and classified into four clades. The amino acid residue patterns of sub-domains including the conserved EF hands were identified for *SmeCDPKs*. An elaborate search of eggplant ESTs available in public domain shows presence of *SmeCDPK* in different tissue specific libraries and most of them were expressed in mixture of flower bud, young fruit, leaf and ovary tissues. *SmeCDPK* 28/18 and 34 were specific to ovary and stamen libraries, respectively. Further, expression of *SmeCDPKs* was checked making use of eggplant transcriptome available in public domain. The information generated will significantly imparts the basic step for further functional study of CDPKs gene family in eggplant.

**Key words:** Brinjal, Calcium-Dependent Protein Kinases, CD PK-related kinases, eggplant, CRK, ESTs, gene expression.

### INTRODUCTION

In eukaryotes; calcium is known to act as universal second messenger that plays key role in signal transduction pathways (Reddy *et al.*, 13). It mediates signaling through Ca<sup>2+</sup> sensors or Ca<sup>2+</sup> binding proteins and regulates gene expression pattern by reversible phosphorylation. There are three major classes of Ca<sup>2+</sup> binding proteins have been identified in plants, calcium dependent protein kinase (CDPKs), calmodulins (CaM) and calcineurin B-like proteins (CBL). CDPKs are unique owing as it possesses both CaM-like domain and protein kinase domain. However, other Ca<sup>2+</sup> sensors characteristically consisted of N-terminus, an auto-inhibitory junction domains followed by the regulatory domain (Klimecka and Muszynska, 8).

The CDPKs are known to play important role in various physiological processes and also in generating response to several biotic or abiotic stresses. CDPKs are activated upon binding of Ca<sup>2+</sup> to their calmodulin-like domain, which is composed of one to four globular EF-hand motifs (Klimecka and Muszynska, 8). A genome-wide study in several plants demonstrated presence of CDPKs (Reddy *et al.*, 13; Asano *et al.*, 1; Li *et al.*, 10). Among horticultural

crops, genome wide CDPKs gene family has been identified in pepper (Cai *et al.*, 2) and cucumber (Xu *et al.*, 14). Biotic and abiotic stress responsive expressions of CDPKs have been reported in several crop plants (Cai *et al.*, 2; Dubrovina *et al.*, 3; Li *et al.*, 10). Hitherto, there has been limited information about CDPKs at genome wide level in eggplant. Recently, draft eggplant genome sequence has been completed by Hirakawa *et al.* (5). In the present study, the genome-wide identification of *SmeCDPKs* and its characterization is reported.

### MATERIALS AND METHODS

The amino acid sequences of 34 *Arabidopsis* CDPKs and its homologous CDPKs were retrieved from the Arabidopsis Information Resource and NCBI GenBank, respectively. Total 176 CDPK sequences were retrieved from 22 divergent plant species including *Arabidopsis thaliana*. Eggplant whole genome protein and CDS sequences were obtained from <http://eggplant.kazusa.or.jp/>. The 176 CDPK protein sequences were used as query against protein sequences database using standalone BLAST with e values 0.001. Hits with more than 50% identity were retrieved and used for subsequent analysis as reported earlier (Wankhede *et al.*, 12). Briefly, the sequences were manually verified for presence of protein kinase domain and EF-hands. Additionally,

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HMMER3.0 (<http://hmmer.org/>) was used to search the local protein database (total protein sequences of eggplant) on the basis of the HMM profile. Results from BLAST and HMMER hits were matched and parsed manually. The deduced protein sequences of eggplant were aligned with Clustal Omega. A phylogenetic tree was constructed among CDPKs proteins, by employing the Neighbor-joining (NJ) method and bootstrap test with 1,000 replicates wrapped in MEGA6 software suite. Prediction of myristoylation and palmitoylation sites were done by Myristoylator (<http://web.expasy.org/myristoylator/>) and CSS-Palm software version 3.0 (<http://csspalm.biocuckoo.org/>), respectively. The coding sequences corresponding to each predicted gene were analyzed for exon-intron distribution pattern. Multiple sequence alignments of CDPKs proteins were executed by Multalin software (<http://bioinfo.genotoul.fr/multalin/multalin>).

All the available eggplant ESTs were downloaded from dbEST, NCBI. A standalone blast search was performed using *SmeCDPKs* against total eggplant ESTs. Hits with more than 90% identity were considered significant. Eggplant transcriptome sequence data available in Sequence Read Archives (SRA), NCBI with accession number (SRR1104129) (Yang *et al.*, 15) were used for expression study. For expression analysis 'RNAseq protocol' of CLC genomics workbench was followed. A maximum number of two mismatches were allowed for alignment. Unique read counts were normalized by calculating RPKM (Reads per kilobase of transcript per million mapped reads) RPKM values were  $\log_2$  transformed showing expression level of *SmeCDPKs*.

## RESULTS AND DISCUSSION

A genome-wide search for CDPK gene family in eggplant identified 28 putative CDPKs and two CDPK-related kinases (Table 1). Orthologous nomenclature was adopted with the basis of *Arabidopsis* CDPKs. It exhibits the useful information pertaining to functional similarities of CDPKs (Mohanta *et al.*, 11) with *Arabidopsis* or eudicots. The number of identified 28 *SmeCDPKs* is comparable to other member of Solanaceae family as pepper and tomato have 31 and 29 CDPK genes, respectively (Cai *et al.*, 2; Hu *et al.*, 8).

The identified *SmeCDPKs* were also studied for biochemical and structural attributes (Table 1). Most of the *SmeCDPKs* showed four EF-hand motifs, however, two *SmeCDPKs* (*SmeCDPK13-1* and 25) were found to have only three EF-hands in the CaM-like domain (Hrabak *et al.*, 6). Variations in number of EF-hands in functional domain have been

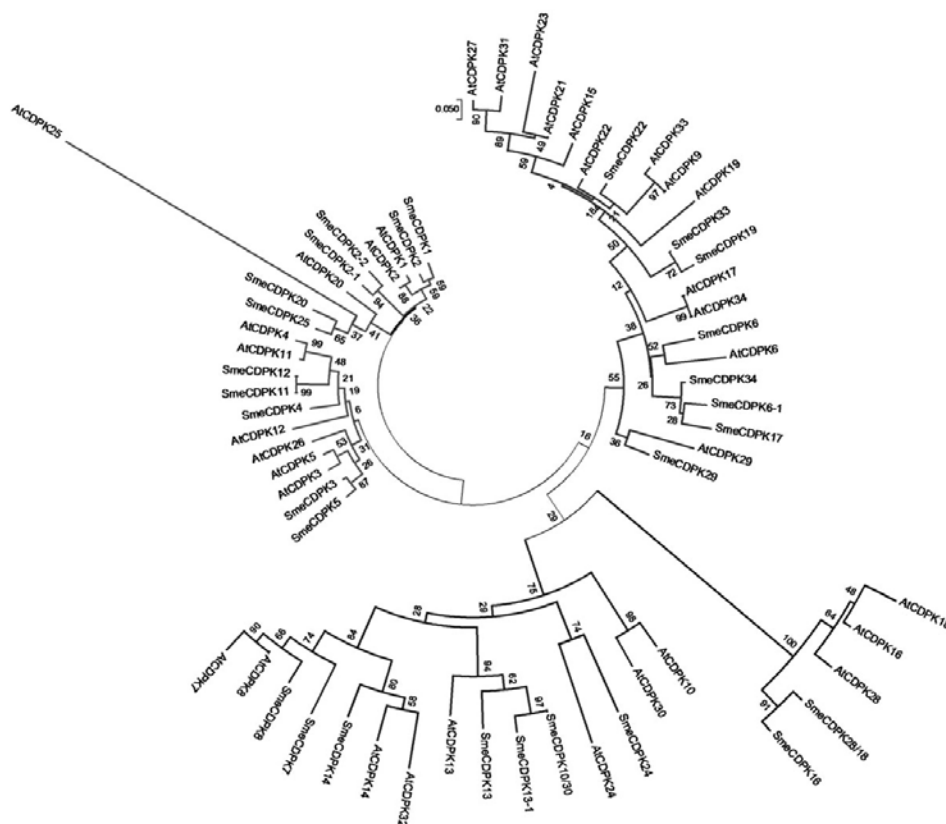
reported in tomato, cucumber (Xu *et al.*, 14). The 28 *SmeCDPKs* proteins range in molecular weight from 29.9 to 133.1 kDa, which were equivalent with CDPK genes from other plant species. The length of open reading frame ranged from 808 (*SmeCDPK12*) to 3619 (*SmeCDPK2-2*), which corresponded polypeptides in the range of 268 to 1205 amino acids. *SmeCDPK* had low GC content nucleotide sequences and it varied from 35.5% (*SmeCDPK34*) to 45.2% (*SmeCDPK13-1*). A lipid modification, N-myristoylation motif that tend to localize in the plasma membrane through protein-membrane and protein-protein interactions (Hrabak *et al.*, 6) reported to be present in 18 of the *SmeCDPKs* at N-terminus of subset of CDPK proteins. Also palmitoylation, which secure the anchoring between protein and membrane, is seen in all the identified *SmeCDPKs*. These lipid modification shows evidence of *SmeCDPKs* involvement for physiological processes through membrane association and this have been confirmed in other eudicots (Cai *et al.*, 2; Xu *et al.*, 14). Additionally, in present work, two CDPK-related kinase genes (CRKs) are identified in eggplant genome. In pepper and rice, the number of CRKs are five, whereas in *Arabidopsis* there are eight CRKs reported (Cai *et al.*, 2; Asano *et al.*, 1; Hrabak *et al.*, 6). Molecular weight of *SmeCRKs* was predicted to be in the range of 36.3 to 38.8 kDa, and their structure is considered to be similar to that of CDPKs except for the EF-hand domains. As observed in earlier studies of *Arabidopsis*, rice, tomato, pepper; *SmeCDPKs* are also categorized into four groups (Fig. 1). Group I and II in eggplant consisted large number of CDPKs, which is near similar to that of tomato and pepper (Cai *et al.*, 2; Hu *et al.*, 7). The presence of characteristic four groups of CDPKs also has been studied from monocot, dicot and lower eukaryotic plants of different species (Hrabak *et al.*, 6; Mohanta *et al.*, 11).

The most important implication of genome-wide evolutionary classification based on orthologous clusters is for functional annotation of newly sequenced genomes. Various studies on divergent functions of *AtCDPKs* helped to predict that the orthologs of *AtCPK1/4* and *AtCPK11* in eggplant, *SmeCDPK1/4* and 11 may be associated with defense components, salt, drought and cold stresses (Reddy *et al.*, 13). Recently, involvement of *AtCPK28* for plant immunity through BIK1 phosphorylation has been revealed. The *AtCPK5* orthologous to *SmeCDPK5* could activate defense gene by exhibiting cytoplasmic calcium ion elevations (Knight and Knight, 9). *AtCPK17* and 34, have been shown to

**Table 1.** Characteristics of eggplant calcium-dependent protein kinases (CDPKs).

Group	Name	Gene identifier	CDS	Amino acids (No.)	MW (kDa)	pI	GRAVY	GC	N-terminal	N-Myr	N-Pal	EF hands No.	Localization	
I	SmeCDPK1	Sme2.5_11161.1_g00001.1	3315	1104	1244	8.21	-0.411	40.7	MGNTCVGP	Y	Y	4	Ptx	
	SmeCDPK2	Sme2.5_03408.1_g00001.1	1824	607	672	5.53	-0.390	42.7	MGNTCVGP	Y	Y	4	ER	
	SmeCDPK2-1	Sme2.5_01206.1_g00007.1	1788	595	671	5.42	-0.410	42.3	MGNNCVHA	Y	Y	4	Chl	
	SmeCDPK2-2	Sme2.5_01118.1_g00001.1	3619	1205	1331	6.00	-0.591	42.0	PSEIVESN	N	Y	4	N	
	SmeCDPK3	Sme2.5_07017.1_g00004.1	1662	553	619	5.50	-0.370	41.3	MGNTRGGS	Y	Y	4	ER	
	SmeCDPK4	Sme2.5_10930.1_g00001.1	1503	500	560	5.09	-0.298	40.2	MDSSKAKT	N	Y	4	ER	
	SmeCDPK5	Sme2.5_12109.1_g00002.1	1686	561	625	5.60	-0.262	44.1	MGNACRGS	Y	Y	4	ER	
	SmeCDPK11	Sme2.5_03825.1_g00001.1	1491	496	559	5.71	-0.386	40.8	MENSKPSS	N	Y	4	ER	
	SmeCDPK12	Sme2.5_29532.1_g00001.1	808	268	299	4.97	-0.469	41.2	AETDNGIF	N	Y	4	N	
	SmeCDPK20	Sme2.5_00746.1_g00009.1	1929	642	718	5.77	-0.411	41.9	MGNTCIGP	Y	Y	4	Cyt	
	SmeCDPK25	Sme2.5_01484.1_g00010.1	1731	576	637	4.99	-0.318	42.1	MGNNCVGP	N	Y	3	Ptx	
	SmeCDPK6	Sme2.5_00013.1_g00035.1	1563	520	584	5.73	-0.453	44.8	MGNCSLS	Y	Y	4	Ptx	
II	SmeCDPK6-1	Sme2.5_02002.1_g00003.1	1092	363	407	5.04	-0.667	37.1	MGNCCCSR	Y	Y	4	PM	
	SmeCDPK17	Sme2.5_00779.1_g00002.1	1566	520	583	5.45	-0.442	38.6	MCDDISFL	N	Y	4	ER	
	SmeCDPK19	Sme2.5_00420.1_g00013.1	1383	460	510	6.14	-0.371	42.0	MGICASK	Y	Y	4	ER	
	SmeCDPK22	Sme2.5_25256.1_g00001.1	884	293	332	5.25	-0.432	43.2	KVYRDIVG	N	Y	4	ER	
	SmeCDPK29	Sme2.5_01233.1_g00004.1	1605	534	601	5.45	-0.410	43.2	MGLCFSKA	Y	Y	4	Cyt	
	SmeCDPK33	Sme2.5_00959.1_g00002.1	1431	476	534	6.23	-0.385	40.6	MRPPASPK	N	Y	4	ER	
	SmeCDPK34	Sme2.5_00158.1_g00007.1	1503	500	566	5.32	-0.623	35.5	MGSCCSKE	Y	Y	4	N	
	SmeCDPK7	Sme2.5_02438.1_g00002.1	1704	567	634	7.20	-0.350	41.7	MGNCCMMK	Y	Y	4	N	
	SmeCDPK8	Sme2.5_00853.1_g00003.1	1605	534	597	6.35	-0.474	42.4	MGNCCGTP	Y	Y	4	N	
	SmeCDPK10/30	Sme2.5_04239.1_g00002.1	1299	432	486	5.23	-0.251	41.6	MIGRILFA	N	Y	4	ER	
	SmeCDPK13	Sme2.5_04540.1_g00001.1	1446	481	542	6.24	-0.528	44.0	MGNCCRSP	Y	Y	4	Mt	
	IV	SmeCDPK13-1	Sme2.5_10488.1_g00001.1	2598	865	981	9.36	-0.260	45.2	MGNFFRYP	Y	Y	3	Plst, Cyt, Nc
SmeCDPK14		Sme2.5_00011.1_g00046.1	1575	524	593	6.03	-0.510	41.5	MGNCCAVP	Y	Y	4	PM	
SmeCDPK24		Sme2.5_00163.1_g00019.1	1560	519	591	5.62	-0.387	37.6	MKPTPIDQ	N	Y	4	N	
SmeCDPK16		Sme2.5_00015.1_g00021.1	1596	531	603	8.99	-0.418	42.8	MGFEMRVL	Y	Y	4	Chl	
SmeCDPK28/18		Sme2.5_00714.1_g00013.1	1728	574	650	9.14	-0.561	40.6	MGSCFSSS	Y	Y	4	Cyt	
SmeCRK1		Sme2.5_30108.1_g00001.1	969	322	363	9.26	-0.386	40.7	MGNACI	Y	Y	0	Chl, Cyt, Nc	
SmeCRK2		Sme2.5_01660.1_g00015.1	1032	343	388	6.84	-0.253	42.7	MASSASRT	N	Y	0	Chl, Mt, Nc	

N = Nucleus, ER = Endoplasmic reticulum, Ptx = Peroxisome, PM = Plasma membrane, Chl = Chloroplast, Mt = Mitochondria, Plst = Plastid, Cyt = cytoplasm



**Fig. 1.** Evolutionary relationships of *Solanum melongena* CDPK genes.

have role in for pollen tube tip growth (Estruch *et al.*, 4), it is interesting to see if its orthologs in eggplant have similar function.

All the eggplant CDPKs were further analyzed for the presence of specific signatures for kinase domains, EF-hand domains and auto-inhibitory domain. Characteristic sequences of kinase domains of CDPKs of dicot plants such as C-x-G-G-E-L-x-D-R-I, H-R-D-L-K-P-E-N-F-L, D-x-V-G-S-x-Y-Y, A-P-E-V-L, D-V/I-W-S, G-V-I-x-Y-I-L-L, G-x-P-P-F-W, P-W-P-x-I-S, A-K-D-L-V and H-P-W (Mohanta *et al.*, 11) were also found to be conserved in eggplant CDPKs (Fig. 2). Whereas, EF-hand motifs of CPKs of dicots and monocots share common sequences (E-E-I/x, D-x-D, D/E-E-L, D-Y-x-E-F, F-D-x-D, E-E-L, D-G-x-I and Y-x-E-F-x-x-M-M), which are throughout conserved in nature, were present as signature motif in eggplant. Members of the same group had diverse exon-intron structure (Fig. 3) in contrast to other Solanaceous crop CDPKs, where close relationship obtained (Hu *et al.*, 7). Members of sub-family I possessed most complicated pattern, where number of introns ranged from four to eighteen. Clustering the intron-exon structure of *SmeCDPKs* suggests a

close relationship between gene organization and evolutionary relationship (Cai *et al.*, 2).

In order to get an insight into expression of CDPKs in different tissue and stress conditions, possible ESTs of eggplant were searched. Most of the identified *SmeCDPKs* found significant hits to ESTs from various reproductive tissues and developmental stages such as in fruit, shoot, leaf, mixture of flower bud, sepal, peduncle, callus and other developmental stages (Table 2). It is evident from the analysis that *SmeCDPK1* and 16 along with others has particularly functional significance for growth and development of vegetative organs (shoot and leaf system) and metabolic function likewise *NtCDPK1* of *Nicotiana tabacum* (Klimecka and Muszynska, 8). Ovary specific CDPKs (28/18) indicate its unique involvement for germination and placental tissues formation, though *SmeCDPK34* might be related to pollen development similar to maize CDPKs, which was specific for pollen tube growth (Estruch *et al.*, 4).

Further, gene expression of *SmeCDPKs* was studied making use of SRA data available in public domain. As shown in Fig. 4, most of the *SmeCDPKs* showed expression except *SmeCDPK2-1*. Since, the

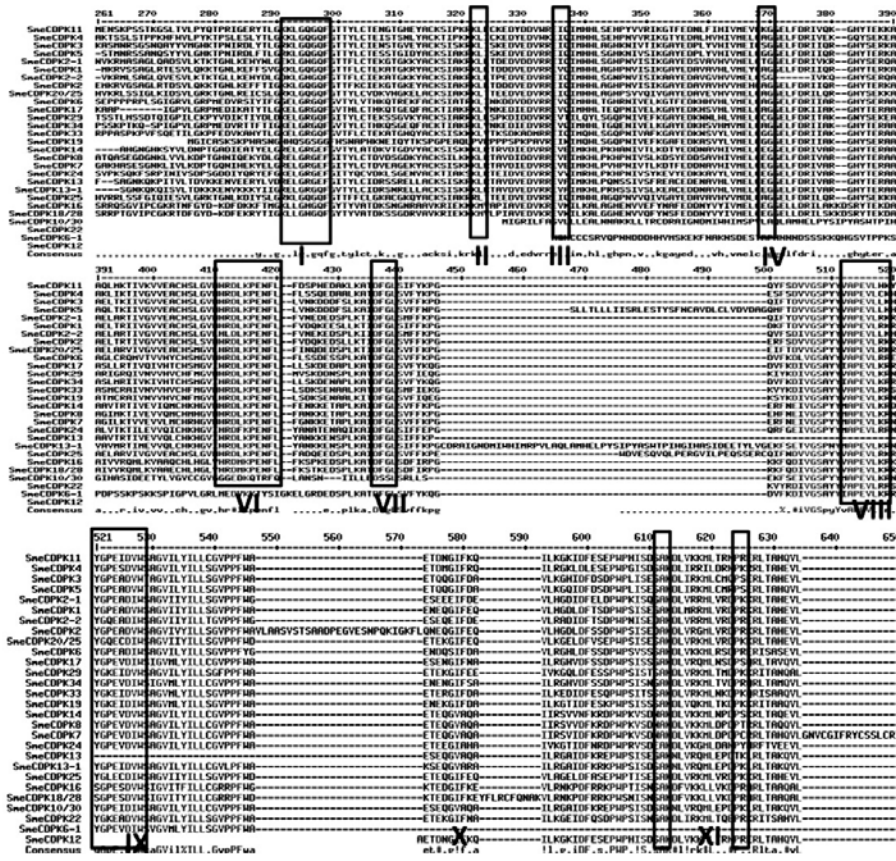


Fig. 2. Alignment of CDPKs family of *Solanum melongena*. The highlighted part shows the conserved motifs from 11 sub-domains of CDPK.

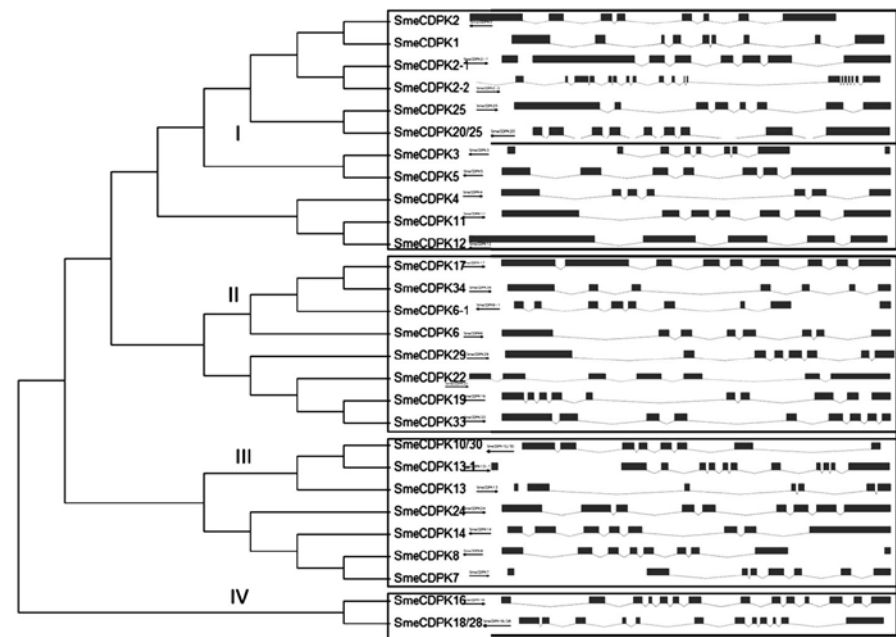
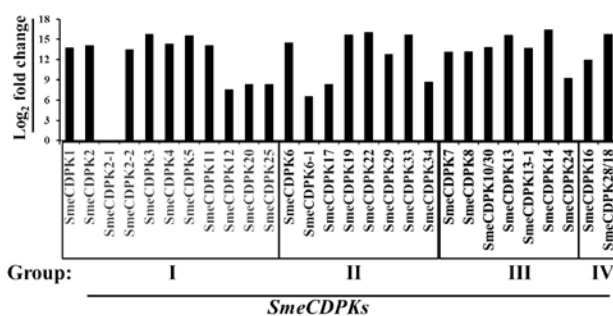


Fig. 3. Exon-intron organization of eggplant CDPK genes. The four sub-groups are marked by square boxes and numbered with roman numerals. The sizes of exons are proportional to their sequence lengths.

**Table 2.** BLAST hits of *SmeCDPKs* to ESTs (dbEST) from plant tissues at different development stages of eggplant.

Group	Name	Plant tissue
I	<i>SmeCDPK1</i>	Shoot
	<i>SmeCDPK2</i>	Shoot, mixture of flower bud leaf and other development stage
	<i>SmeCDPK2-1</i>	Mixture of flower bud leaf and other development stage
	<i>SmeCDPK2-2</i>	Fruit, leaf, mixture of placenta and immature seed, root, flower, fruit and various development stage
	<i>SmeCDPK3</i>	Ovary (anthesis), placenta, mixture of flower bud leaf and other development stage
	<i>SmeCDPK4</i>	mixture of flower bud leaf and other development stage
	<i>SmeCDPK5</i>	Ovary (anthesis), placenta, mixture of flower bud leaf and other development stage
	<i>SmeCDPK11</i>	Fruit, placenta, ovary (2 hr after anthesis), root, mixture of flower bud leaf and other development stage
	<i>SmeCDPK12</i>	Fruit, placenta, ovary (2 hr after anthesis), root, mixture of flower bud leaf and other development stage
	<i>SmeCDPK20</i>	-
	<i>SmeCDPK25</i>	Placenta, root, sepal, leaf, mixture of flower bud leaf and other development stage
	II	<i>SmeCDPK6</i>
<i>SmeCDPK6-1</i>		mixture of flower bud leaf and other development stage, mixture of petal and stamen
<i>SmeCDPK17</i>		mixture of flower bud leaf and other development stage
<i>SmeCDPK19</i>		flower bud leaf and other development stage, callus, placenta
<i>SmeCDPK22</i>		Pericarp, callus, placenta, leaf, flower bud leaf and other development stage
<i>SmeCDPK29</i>		-
<i>SmeCDPK33</i>		Placenta, callus, pericarp, mixture of flower bud leaf and other development stage
<i>SmeCDPK34</i>		Mixture of petal and stamen
III	<i>SmeCDPK7</i>	Mixture of flower bud leaf and other development stage, ovary (5 days pre-anthesis)
	<i>SmeCDPK8</i>	Mixture of flower bud leaf and other development stage, ovary (24 hr after anthesis), ovary
	<i>SmeCDPK10/30</i>	Peduncle, fruit, ovary (5 days pre-anthesis), flower bud leaf and other development stage
	<i>SmeCDPK13</i>	Mixture of flower bud leaf and other development stage, ovary, peduncle, placenta, fruit
	<i>SmeCDPK13-1</i>	Peduncle, fruit, shoot, ovary (5 days pre-anthesis)
	<i>SmeCDPK14</i>	-
	<i>SmeCDPK24</i>	-
IV	<i>SmeCDPK16</i>	Leaf
	<i>SmeCDPK28/18</i>	Ovary (5 days post-anthesis)

eggplant transcriptome represented root, stem and young leaves, the expression of *SmeCDPKs* shows from any of the root, stem or leaves tissues. Maximum expression is recorded for *SmeCDPK14*, whereas the lowest expression is recorded for *SmeCDPK6-1*. *SmeCDPK2-1* showed no mapped reads suggesting no expression in root, leaves and stem at four leaves stage plants under normal condition. It could be possible that *SmeCDPK2-1* has some role to play in specific tissue type or conditions such as biotic or abiotic stress. These expression results show that the identified CDPKs are genuine and show active gene expression.

**Fig. 4.** Expression levels of eggplant CDPKs. Expression of *SmeCDPKs* is represented as log<sub>2</sub> value of RPKM on Y axis.

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