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RESEARCH ARTICLE

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Characterization and expression analysis of calcium-dependent protein kinase genes in rice (*Oryza sativa* L.)

Abstract Under abiotic stress, the calcium-dependent protein kinases (CDPKs) in plant species are activated by the fluctuated Ca^{2+} levels in cytoplasm and thereby provide a mechanism to decode calcium signals. In this paper, twenty-two rice *CDPK* genes were identified based on scanning the rice genome released in National Center for Biotechnology Information (NCBI). It was found that there were dramatic differences on the DNA length, cDNA length, open reading frame (ORF) and the translated amino acids among the rice *CDPK* genes, with the highest diversity on the DNA length. Calculations of the exon/intron numbers and the lengths of exon and intron revealed that all of the rice *CDPK* genes had the longest exon at the position of exon 1, but the lengths of introns in different genes showed different patterns. The gene structure and phylogenetic analysis indicated that the rice *CDPK* genes had derived at least from two different ancestors during the evolution. The expression analysis elucidated that the rice *CDPK* genes showed different patterns under normal growth (CK) and salt stress condition, including constitutively expression (*OsCDPK4*, *OsCDPK18*, *OsCDPK19* and *OsCDPK24*), down- or up-regulated in roots by salt stress (*OsCDPK10* and *OsCDPK16*), up-regulated in leaves by salt stress (*OsCDPK6*, *OsCDPK20* and *OsCDPK13*), and no detected transcripts under CK and salt stress condition. Therefore, the members of rice *CDPK* gene family should be evolutionally divergent and several members could play an important role in transducing the signal of salt stress.

Keywords rice (*Oryza sativa* L.), calcium-dependent protein kinases (CDPKs), characterization, gene expression

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1 Introduction

Calcium is a ubiquitous second messenger in eukaryotic signal transduction cascades. In plants, intracellular Ca^{2+} levels are modulated in response to various signals, including abiotic stress, hormones, light, mechanical disturbances and pathogen elicitors (Sanders et al., 1999; Evans et al., 2001; Rudd and Franklin-Tong, 2001). It is reported that different stimuli elicits specific calcium signatures, generated by altering the kinetics, magnitude and cellular source of the influx (Allen et al., 2000; 2001; Evans et al., 2001; Klimecka and Muszynska, 2007).

As the unique to plant species, the CDPK-related kinases (CRKs) contain several known classes of Ca^{2+}-binding sensory proteins, including calmodulins, calcium-dependent protein kinases (CDPKs) and calcineurin B-like proteins. Among them, CDPKs are the best characterized and are of particular interest. With representing a novel class of Ca^{2+} sensors, the CDPKs have both protein kinases and calmodulin-like domains in a single polypeptide (Klimecka and Muszynska, 2007). It is reported that the kinases of CDPKs are calcium-regulated and are distinguished by a structural arrangement in which a calmodulin-like regulatory domain is located at the C-terminal end of the enzyme. The CDPKs can directly bind calcium, and their calcium-stimulated kinase activities are independent of calmodulins (Roberts and Harmon, 1992). Currently, most of the known calcium-stimulated protein kinase activities in plants are associated with CDPKs (Klimecka and Muszynska, 2007).

Many stress signals, such as wounding, cold, high salinity and drought, are known to elicit fluctuations in cytosolic Ca^{2+} levels, as well as changes in protein phosphorylation (Bush, 1995; Trewavas, 1999; Knight and Knight, 2001, Liu et al., 2006). Several lines of evidence suggest that CDPKs mediate abiotic stress signaling pathways. Transcriptional activation of many different CDPKs by a variety of abiotic stresses has been demonstrated in tissues from diverse species (Urao et al., 1994; Monroy and Dhindsa, 1995; Botella et al., 1996; Yoon et al., 1999; Patharkar and Cushman, 2000; Saijo et al.,

2000; Chico et al., 2002; Liu et al., 2006). The enzymatic activities of CDPKs also increase in response to these stresses.

In rice, there have been several CDPKs characterized. For example, cold treatments enhance the activity of a membrane-bound rice CDPK (Martin and Busconi, 2001). Overexpression of *OsCDPK7* in vascular bundles confers cold, salt and drought tolerance in transgenic rice (Saijo et al., 2000; 2001), demonstrating the usefulness of engineering CDPKs to enhance abiotic stress tolerance in crops. In this paper, twenty-two CDPK members were identified based on a bioinformatics approach by scanning the rice genome released in the NCBI website. The characterizations and the expression patterns of the twenty-two rice CDPK members under salt stress were studied. It is found that several *CDPK* genes of rice have been involved in the signal transduction under the salt stress.

2 Materials and methods

2.1 Plant material

Seeds of rice (subsp. *japonica* cv Nipponbare) were germinated in a growth chamber in darkness at 28°C for 72 h. The germinated seeds were then transferred in a growth room under the temperature of 25°C/20°C with a photoperiod of 12 h, and grown on the steel mesh grid which propped in a plastic pot holding Murashige and Skoog (MS) liquid medium. The MS liquid medium was just contacted with the mesh surface. During the growth of the seedlings, the liquid medium was changed once every three days. In 14 days after seed germination, the seedlings were treated with 100 mmol/L NaCl. Treatments were terminated after 1 h, and leaves and roots were harvested into liquid nitrogen and stored at −80°C for total RNA isolation. For analysis of the expression patterns of *OsCDPK6* and *OsCDPK20*, leaf samples were taken at a time course. Time points of 20 min, 3 and 6 h were included except the time point of 1 h. At each time point, samples of leaves and roots of the seedlings growing in MS medium without NaCl were used as the control.

2.2 Identification and characterization analysis of rice *CDPK* genes

Two rice (*Oryza sativa* L.) cDNAs corresponding to *OsCDPK1* (GenBank Accession No. AP002819) and *OsCDPK2* (GenBank Accession No. AP003073) were obtained at NCBI based on the accession numbers. The *OsCDPK1* and *OsCDPK2* were used as queries to perform Basic Local Alignment Search Tool (BLAST) search of the rice genome. In total, twenty *CDPK* genes in rice (*OsCDPK3* to *OsCDPK24*) were identified, in which the *OsCDPK5* and *OsCDPK22* were not included owing to failed search during the scan of the rice genome. The length of DNA and cDNA, exon/intron organization of above rice *CDPK* genes were detected and calculated from the rice genome sequence (cv Nipponbare) in the GenBank database. The translated amino acids of each member of the rice *CDPK* genes were predicated by ExpASy server on the website (www.expasy.org).

2.3 Phylogenetic tree analysis

The nucleotide sequences of *OsCDPK* genes were used to draw a phylogenetic tree of the rice CDPKs by DNAStar software. The procedure was followed by the recommendations of the software. The identities at the nucleotide level among the *OsCDPKs* were calculated based on the alignment results to compare with *OsCDPK1*.

2.4 Expression analysis of *OsCDPKs* under normal growth (CK) and NaCl stress condition

Total RNA of leaves and roots in the seedlings of control and salt stress were isolated with RNA extraction kit (TRIzol) reagent (Gibco-BRL, Life Technologies), according to the manufacturer's instructions. For the detection of transcripts of rice *OsCDPK* genes, a specific primer pair for every rice *CDPK* gene was designed based on DNAStar software, based on the cDNA sequence of each *CDPK* gene (Table 1). Semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) approach was used to detect the transcripts of rice *CDPK* genes under CK and salt stress condition. The procedure of RT-PCR was carried out by following the manufacturer's recommendations (TaKaRa). As a result, in total the transcripts of eight genes were detected. For the eliminating of unsuitable primer design which resulted in undetectable transcripts of the *CDPK* genes, new specific primers were re-designed for the genes without detected transcripts. The primer pairs designed at the second time were listed in Table 2. During the RT-PCR procedure for detecting the rice *CDPK* genes, extra RT-PCR for *Rac1*, which is a rice gene with a constitutive expression, was concurrently performed to be the positive control showing the same RNA amount loaded in the reactions. The primer pair for Rac1 was follows: 5′-CATGCTATCCCTCGTCTCGACCT-3′ (forward) and 5′-CGCACTTCATGATGGAGTTGTAT-3′ (reverse).

3 Results

3.1 Characterization of the *CDPK* genes in rice

By performing BLAST search of the rice genome with the cDNAs of *OsCDPK1* (GenBank Accession No. AP002819) and *OsCDPK2* (GenBank Accession No. AP003073) to be the queries, 20 other rice *CDPK* genes (Table 3) were identified by scanning the rice genome released in NCBI website. It was found that there were dramatic differences on the DNA length, cDNA length, open reading frame (ORF) and the translated amino acids among the rice *CDPK* genes, with

Table 1 Gene-specific RT-PCR primers were designed for each *OsCDPK* at the first time

Gene name	Primer pair	PCR product/bp
OsCDPK1	Forward: 5′–agcatgactataaaaccccgatag Reverse: 5′–cctcttaaggggtgttcttggaat	2 259
OsCDPK2	Forward: 5′–acacagacgcccaacgtgatcgac Reverse: 5′–agctcctctcctatatcacaacgt	1 649
OsCDPK3	Forward: 5′–aatgcagcacaaaccctagccctc Reverse: 5′–gagaaagggtttctgcttagcgag	1 772
OsCDPK4	Forward: 5′–gcttaactccccaacccaaaccct Reverse: 5′–ttacgacgtctcatctcggttctt	1 636
OsCDPK6	Forward: 5′–tctcttgaggatcgtcgtttcgtt Reverse: 5′–atccgtgagtcggagttgtgaatt	1 739
OsCDPK7	Forward: 5′–tggtttgcataaggagttcttgga Reverse: 5′–tctccaggttttgcaaattgcaac	1 767
OsCDPK8	Forward: 5′–gcctcgcctcctttgttgaattga Reverse: 5′–ataatgtaacagccagcacacagc	1 738
OsCDPK9	Forward: 5′–gaagtttccagaaggttctagaag Reverse: 5′–tgaatcgactgaattcgtcgagct	1 781
OsCDPK10	Forward: 5′–tgtccgagaaagattcggagcggc Reverse: 5′–tacaagtgtgtgactgactctagt	1 861
OsCDPK11	Forward: 5′–cggcgatgggcaacaactgcgt Reverse: 5′–tggttccaagtaaggcaaccgaaa	1 835
OsCDPK12	Forward: 5′–caccccaggagaaggaaaggaaac Reverse: 5′–tctgcttgcagcatatactcgaac	1 706
OsCDPK13	Forward: 5′–tttgatgcgcgtggccgccggttt Reverse: 5′–agacaggaagaagctaaaatccgt	1 738
OsCDPK14	Forward: 5′–accccaatcgcacaaatcggctcc Reverse: 5′–caatccacctctcaatcttgagct	1 703
OsCDPK15	Forward: 5′–aacccccaaaaccaaagccaaaac Reverse: 5′–cacttccctaagatggaaccatat	1 737
OsCDPK16	Forward: 5′–aatgcggaacaaaccctagtagtg Reverse: 5′–gcaacaccagctgcaagtttgcaa	1 704
OsCDPK17	Forward: 5′–atccaaatccaccaaccctcgccg Reverse: 5′–tagggccttccggctgttcacttt	1 882
OsCDPK18	Forward: 5′–cattgatggtaatagttggatccg Reverse: 5′–gccctttgttcacttttgtgaagt	1 629
OsCDPK19	Forward: 5′–tcgtttgcggagtaacaaatttgc Reverse: 5′–acctttttatccgtcgactaccatc	1 756
OsCDPK20	Forward: 5′–actcgccgcagatctttgattcgc Reverse: 5′–gaaattcacaacttgcctattccg	1 748
OsCDPK21	Forward: 5′–gagcgcgacacacacagaggagga Reverse: 5′–agttgatgagattgttgggcatca	1 840
OsCDPK23	Forward: 5′–ttttccggatccctgtgaagtgtt Reverse: 5′–aacaaactgaattcttgggggtgc	1 719
OsCDPK24	Forward: 5′–gtagtccgtacggttcttgatctg Reverse: 5′–agagattcagacaatgatggcatg	1 629

Table 2 Gene-specific RT-PCR primers were re-designed for undetected transcripts of *OsCDKs*

Gene name	Primer pair	PCR product/bp
OsCDPK1	Forward: 5′-tatgtcaactccgctgctacc Reverse: 5′-tggaatataatgaggaaagatgtc	2 277
OsCDPK2	Forward: 5′-cgcgtgcccggctgtcgtc Reverse: 5′-gaagatcatgtttttgttttgttg	1 620
OsCDPK3	Forward: 5′-gcgcgagcatggggaact Reverse: 5′-aaggcctaaactacacgcataaaa	1 705
OsCDPK7	Forward: 5′-tcttggaatggggaatcagtgc Reverse: 5′-aatagtttttctccaggttttg	1 759
OsCDPK9	Forward: 5′-gctgagctgagatgggcgagag Reverse: 5′-ttggacgaaatggaaaacc	1 965
OsCDPK10	Forward: 5′-ggcgtaatccgaaggtgtttgt Reverse: 5′-gatctcgttgtcgtcgcctcta	1 918
OsCDPK11	Forward: 5′-cgtccgcgtagggtaggggcatcc Reverse: 5′-tccaagtaaggcaaccgaaatcat	1 858
OsCDPK12	Forward: 5′-tcggcgccaccccaggagaagg Reverse: 5′-caaaccacaacagccaaagcaact	1 766
OsCDPK13	Forward: 5′-ggtttctccccgtcgtttgat Reverse: 5′-cgttgggcaggaagacaggaagaa	1 731
OsCDPK14	Forward: 5′-acccccaccccaatcgcacaaatc Reverse: 5′-tcgccggccaccctgag	1 732
OsCDPK15	Forward: 5′-cccaatccaaaccccccaaaaccaa Reverse: 5′-tccgccgatacgaactgctctt	1 790
OsCDPK17	Forward: 5′-cggcgcgatggggaacacctg Reverse: 5′-ccttccggctgttcactttat	1 772
OsCDPK21	Forward: 5′-gctgctactccgcctacg Reverse: 5′-ctggtgaaggtgaaagatgtgc	1 768
OsCDPK23	Forward: 5′-tgcaggatatacatttttg Reverse: 5′-tgccaggcgacaggagttcagt	1 646

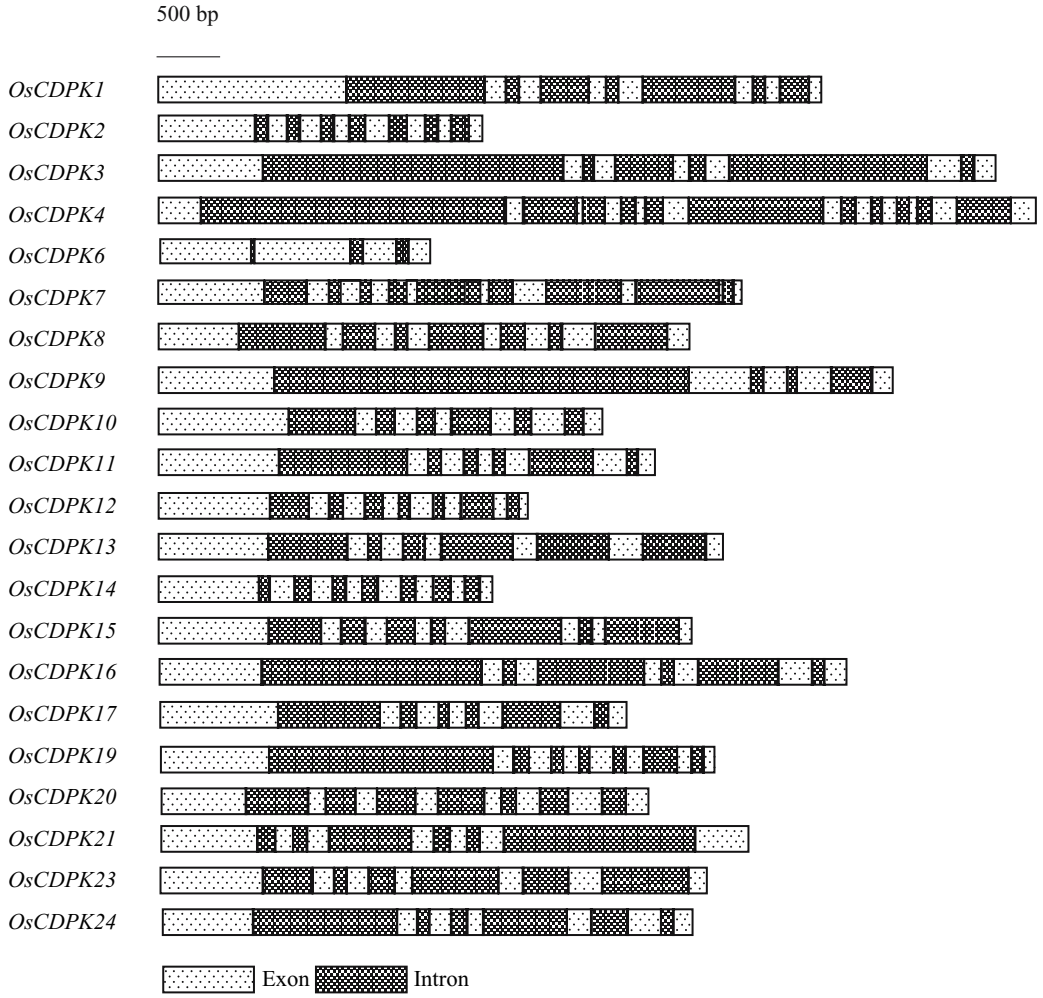
Table 3 Accession numbers, lengths of DNA, cDNA and open reading frame (ORF) and the translated amino acids of rice *CDPK* genes

Gene name	Accession number	DNA length/bp	cDNA length/bp	ORF /bp	Translated amino acid
OsCDPK1	AP002819	4 442	2 259	2 142	713
OsCDPK2	AP003073	2 274	1 649	1 548	515
OsCDPK3	AP004366	5 627	1 772	1 656	551
OsCDPK4	AP005311	5 930	1 636	1 569	522
OsCDPK6	AP004082	1 804	1 739	1 638	545
OsCDPK7	AP000615	3 849	1 767	1 713	570
OsCDPK8	AC135595	3 599	1 738	1 617	538
OsCDPK9	AC097277	4 898	1 781	1 725	574
OsCDPK10	AC084296	2 991	1 861	1 800	599
OsCDPK11	AC087096	3 346	1 835	1 731	576
OsCDPK12	AL606687	2 549	1 706	1 602	533
OsCDPK13	AL662957	3 789	1 738	1 656	551
OsCDPK14	AC129718	2 305	1 703	1 587	528
OsCDPK15	AC098836	3 635	1 737	1 629	542
OsCDPK16	AC108503	4 600	1 740	1 644	547
OsCDPK17	AP003847	3 124	1 882	1 707	568
OsCDPK19	AP003954	3 751	1 764	1 602	533
OsCDPK20	AP003866	3 271	1 748	1 653	550
OsCDPK21	AP003948	3 916	1 840	1 698	565
OsCDPK23	AC073166	3 703	1 719	1 605	534
OsCDPK24	AC128643	3 587	1 629	1 542	513

the highest diversity on the DNA length. Among the tested rice *CDPK* genes, DNA lengths change from 1 804 bp (*OsCDPK6*) to 5 930 bp (*OsCDPK4*), cDNA lengths from 1 629 bp (*OsCDPK24*) to 2 259 bp (*OsCDPK1*), ORFs from 1 542 bp (*OsCDPK24*) to 2 142 bp (*OsCDPK1*), and the translated amino acids from 513-aa (*OsCDPK24*) to 713-aa (*OsCDPK1*) (Table 3).

3.2 Exon/intron organizations of rice *CDPK* genes

The exon/intron organizations of the rice *CDPK* genes are listed in Fig. 1. Calculations of the exon/intron numbers and the lengths of each exon and intron revealed that dramatical diversities existed among the rice *CDPK* genes. The exon and intron numbers changed from four and three (*OsCDPK6*) to twelve and eleven (*OsCDPK4*), respectively, but mostly contained seven exons and six introns (*OsCDPK3*, *OsCDPK10*, *OsCDPK11*, *OsCDPK13*, *OsCDPK16*, *OsCDPK17*,



Note: In the schematic diagram, the exons and introns of each *CDPK* gene were modulated to be proportional to their corresponding lengths.

Fig. 1 Exon/ intron organizations of rice *CDPK* genes

OsCDPK21, *OsCDPK23* and *OsCDPK24*) or eight exons and seven introns (*OsCDPK1*, *OsCDPK2*, *OsCDPK8*, *OsCDPK12*, *OsCDPK14*, *OsCDPK15*, *OsCDPK19* and *OsCDPK20*). It was found that all of the rice *CDPK* genes had the longest exon at the position of exon 1, but the lengths of introns in different genes showed different patterns. The genes of *OsCDPK9*, *OsCDPK3* and *OsCDPK4* had long introns (2 741 bp for *OsCDPK9*, 1 992 bp for *OsCDPK3* and 2 021 bp for *OsCDPK4*, respectively), whereas *OsCDPK2*, *OsCDPK6*, *OsCDPK14* and *OsCDPK12* had short lengths of the introns (changing from 24 bp for intron 1 of *OsCDPK6* to 265 bp for intron 1 of *OsCDPK12*). This result indicated that the evolution of rice *CDPK* genes underwent an obvious different pathway.

3.3 Phylogenetic analysis of the rice *CDPKs* genes

A phylogenetic tree for the rice *CDPK* genes was constructed based on DNAStar software (Fig. 2). It was found that the rice *CDPK* genes could be clustered into two subgroups. Among them, *OsCDPK1* to *OsCDPK4*, *OsCDPK6*, *OsCDPK7*, *OsCDPK10*, *OsCDPK13*, *OsCDPK18* and *OsCDPK23* were clustered into Subgroup I. The *CDPK* genes in this subgroup had relative high similarities with *OsCDPK1*, with the identities to *OsCDPK1* at a nucleotide level changing from 22.8% to 59.0%. *OsCDPK8*, *OsCDPK9*, *OsCDPK11*, *OsCDPK12*, *OsCDPK14* to *OsCDPK17*, *OsCDPK19* to *OsCDPK21* and *OsCDPK24* were clustered into Subgroup II. The genes in this subgroup had very low identities with *OsCDPK1*. This result indicated that the rice *CDPK* genes had at least derived from two different ancestors during the evolution of this superfamily members.

3.4 Expression analysis of rice *CDPK* genes under salt stress condition

3.4.1 Constitutively expression of the *OsCDPK* genes under control and salt stress conditions

Semi-quantitative RT-PCR was carried out to detect the transcripts of the rice *CDPK* genes in roots and leaves under CK and salt stress conditions. The results indicated that several

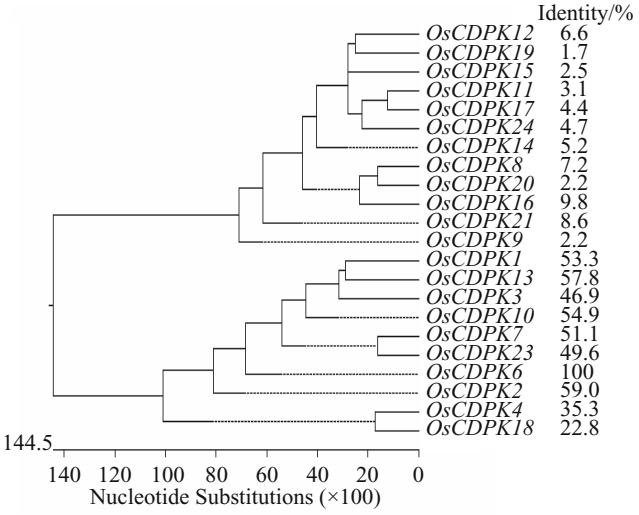
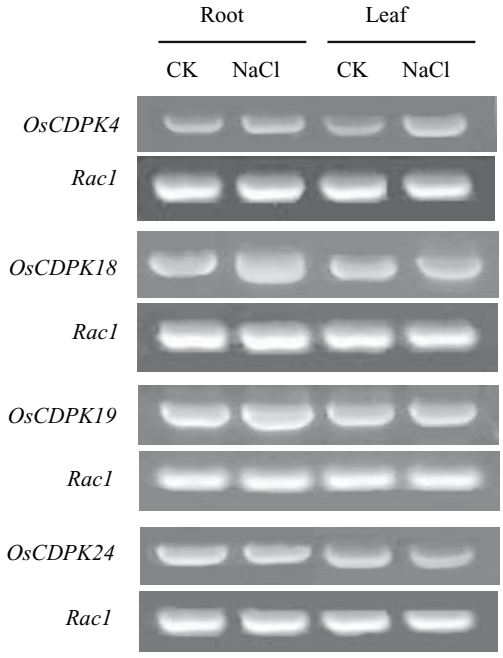


Fig. 2 Phylogenetic analysis of rice *CDPK* genes at the nucleotide level

rice *CDPK* genes had much more transcripts in roots and leaves, and were constitutively expressed. The genes showing this expression pattern included *OsCDPK4*, *OsCDPK18*, *OsCDPK19* and *OsCDPK24*. Although there exists a little difference in the transcript amounts in above genes, all of them had high expression levels in roots and leaves, and similar transcripts were detected in leaves and roots under CK and salt stress conditions for each gene (Fig. 3).

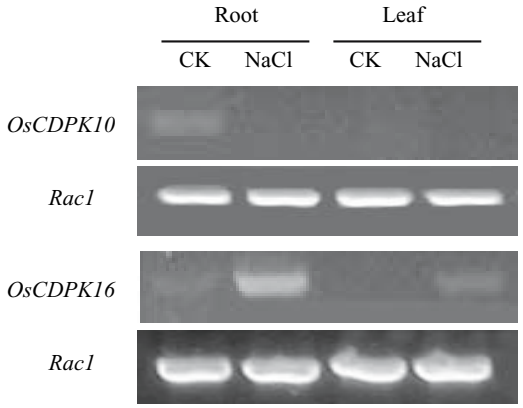


Note: The actin gene *Rac1* was included as control for uniform RT-PCR conditions. The primer sequences and reaction conditions used are presented in Table 1. The samples for detecting transcripts of the rice genes in salt stress were harvested after 1 h treatment.

Fig. 3 Transcripts detected by RT-PCR in roots and leaves under CK and salt stress conditions in *OsCDPK4*, *OsCDPK18*, *OsCDPK19* and *OsCDPK24*

3.4.2 Regulated expression in roots of the *OsCDPK* genes by salt stress

Among the 22 rice *CDPK* genes, *OsCDPK10* and *OsCDPK16* were found that the expression levels in roots to be regulated by salt stress. For *OsCDPK10*, some transcripts in roots under normal growth condition could be detected, but the transcripts become undetectable after 1 h of 100 mmol/L NaCl treatment. This implied *OsCDPK10* was down-regulated by salt stress. The expression pattern of roots in *OsCDPK16* was converse to that in *OsCDPK10*, showing the expression of this gene was up-regulated in root when treated with NaCl. Meanwhile, some transcripts in leaves were also induced by NaCl in *OsCDPK10*, though relatively low compared with those in roots (Fig. 4). This result indicated that *OsCDPK10* and *OsCDPK16* were possibly involved in the signal transduction of the salt cue in rice roots.



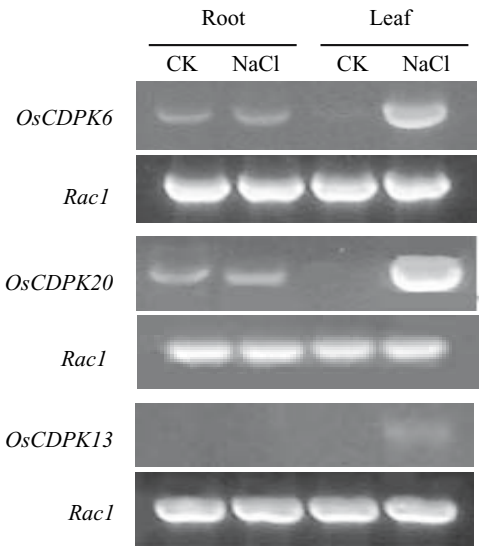
Note: The actin gene *Rac1* was included as control for uniform RT-PCR conditions. The primer sequences and reaction conditions used are presented in Table 1 (*OsCDPK16*) and Table 2 (*OsCDPK10*). The samples for detecting transcripts of the rice genes in salt stress were harvested after 1 h treatment.

Fig. 4 Transcripts detected by RT-PCR in roots and leaves under CK and salt stress condition in *OsCDPK10* and *OsCDPK16*

3.4.3 Up-regulated expression in leaves of the *OsCDPK* genes by salt stress

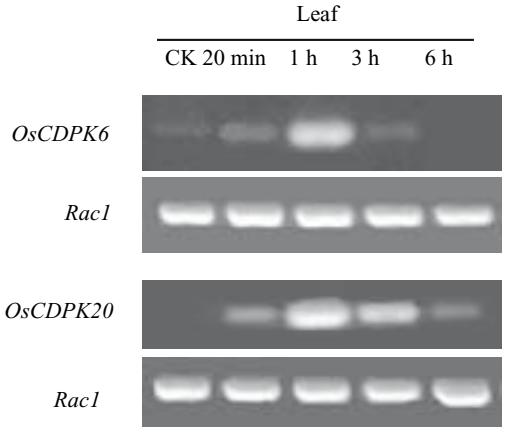
Three rice *CDPK* genes including *OsCDPK6*, *OsCDPK20* and *OsCDPK13* were shown to be up-regulated in leaves under salt stress condition. Among them, the same expression patterns were found between *OsCDPK6* and *OsCDPK20*. For these two genes, the expression levels in roots were low and no differences between CK and salt stress treatment. The transcripts in leaves were not detected in CK, but the transcripts in leaves for above genes were dramatically enhanced in the salt stress treatment (Fig. 5). No transcripts were detected in roots under CK and salt stress condition, and leaves under CK, but some transcripts were detected in leaves in salt stress treatment for *OsCDPK13* (Fig. 5). Therefore, it was speculated that *OsCDPK6*, *OsCDPK20*, *OsCDPK13*

and the gene above mentioned *OsCDPK16* had mediated the signal transduction of salt stress in leaves.



Note: The actin gene Rac1 was included as control for uniform RT-PCR conditions. The primer sequences and reaction conditions used are presented in Table 1 (*OsCDPK6* and *OsCDPK20*) and Table 2 (*OsCDPK13*). The samples for detecting transcripts of the rice genes in salt stress were harvested after 1 h treatment.

Fig. 5 Transcripts detected by RT-PCR in roots and leaves under CK and salt stress conditions in *OsCDPK6*, *OsCDPK20* and *OsCDPK13*



The actin gene *Rac1* was included as control for uniform RT-PCR conditions. The primer sequences and reaction conditions used are presented in Table 1. The samples for detecting transcripts of the rice genes were harvested in a time course including 20 min, 1, 3 and 6 h.

Fig. 6 Leaf transcripts of *OsCDPK6* and *OsCDPK20* in a time course under salt stress condition

3.4.4 Time-course expression patterns in leaves of *OsCDPK6* and *OsCDPK20* under salt stress condition

Based on the results that *OsCDPK6* and *OsCDPK20* were up-regulated in leaves under salt stress condition, the expression patterns in leaves in a time course were evaluated for *OsCDPK6* and *OsCDPK20*. Under CK condition, nearly no transcripts of above genes could be detected. After 20 min of 100 mmol/L NaCl treatment, the transcripts could be detected at a moderate level and the expression levels reached the highest at 1 h of NaCl treatment among all the time points. From 1 to 6 h of salt treatment, the transcripts in leaves of the above genes were all rapidly decreased (Fig. 6).

4 Discussion

A family of CDPKs, which are the most abundant serine/threonine kinases in plants, have been implicated as key elements in signaling processes (Sheen, 1996; Hegeman et al., 2006). In *Arabidopsis*, a genome-wide analysis of CDPKs provides an overview of the diversity of this large multigene family. It appears likely that gene duplication and subsequent evolution generated CDPKs in *Arabidopsis* to be both redundant and distinct functions (Cheng et al., 2002; Hrabak et al., 2003). Up to know, although several CDPKs have been partially purified and cloned from various organs of rice (Saijo et al., 2000), there is still lack of systematic elucidation of rice CDPKs at the genome level.

Based on scanning the rice genome released in NCBI website, twenty-two rice *CDPK* genes were identified. Although all of them have similar basic structural features of CDPKs, which are biochemical distinct from other calcium-regulated kinases, such as within a single polypeptide chain, these kinases contain three functional domains: catalytic, auto-inhibitory and calcium-binding (Harper et al., 1991; Roberts and Harmon, 1992; Hrabak et al., 1996; Harmon et al., 2000). In this study, it was found that there were dramatic differences on the DNA length, cDNA length, ORF and the translated amino acids among the rice *CDPK* genes, phylogenetic analysis showed that the rice *CDPK* genes could be classified into two subgroups, indicating that the rice *CDPK* genes have possibly derived at least two ancestors.

Lots of studies have reported that the expression of *CDPK*s in plants is affected by a variety of stimuli including wounding (Chico et al., 2002), salt or drought stress (Botella et al., 1996; Patharkar and Cushman, 2000; Saijo et al., 2000), cold (Monroy and Dhindsa, 1995; Saijo et al., 2000), hormone treatment (Abo-El-Saad and Wu, 1995; Botella et al., 1996; Davletova et al., 2001), light (Frattini et al., 1999), cold (Martin and Busconi, 2001; Llop-Tous et al., 2002) and pathogens (Murillo et al., 2001; Romeis et al., 2001). Specific expression patterns have been described for *CDPK*s in many plant species including maize (Zea mays L.) pollen (Estruch et al., 1994), rice vascular tissue (Saijo et al., 2001) and seeds (Kawasaki et al., 1993; Frattini et al., 1999), and potato (Solanum tuberosum) stolons (Raices et al., 2001). In *Arabidopsis*, the expression of *Arabidopsis CDPK* genes is also regulated by different stimuli and/or in specific cell types. Using a protoplast transient expression system, specific CDPKs (AtCPK10 and 30 but not AtCPK1 or AtCPK11) have been demonstrated to activate a stress and ABA-inducible

promoter. The above result shows a connection of particular CDPKs to specific signaling pathways *in vivo* (Sheen, 1996). In this study, the expression patterns of twenty-two rice *CDPK* genes could be classified into four categories, including constitutively expression (*OsCDPK4*, OsCDPK18, OsCDPK19 and OsCDPK24), down- or up-regulated in roots by salt stress (*OsCDPK10* and *OsCDPK16*), up-regulated in leaves by salt stress (*OsCDPK6*, *OsCDPK20* and *OsCDPK3*), and no detected transcripts under CK and salt stress condition. Therefore, the members of rice *CDPK* gene family should be evolutional divergence and several members could play an important role in transducing the signal of salt stress cue.

It is reported that the expression of *OsCDPK7* was induced by salt (Saijo et al., 2000; 2001). But we have not detected the transcripts of this *CDPK* member in this study. This was perhaps due to unsuitable design of the primer pairs for this gene, though two times of primer designing for eliminating the fail of the RT-PCR. It may be also related to the different tissue culture conditions or different seedling ages for salt stress treatment. In this study, it was found that the leaf transcripts of *OsCDPK6* and *OsCDPK20* were shown to be induced within 1 h of salt treatment, and then rapidly decreased from 1 to 6 h under the salt stress condition. The mechanisms how these genes were involved in the signal transduction under salt stress need to be confirmed in the future.

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References

Abo-el-Saad M, Wu R (1995). A rice membrane calcium-dependent protein kinase is induced by gibberellin. Plant Physiol, 108: 787–793

Allen G J, Chu S P, Harrington C L, Schumacher K, Hoffmann T, Tang Y Y, Grill E, Schroeder J I (2001). A defined range of guard cell calcium oscillation parameters encodes stomatal movements. Nature, 411: 1053–1057

Allen G J, Chu S P, Schumacher K, Shimazaki C T, Vafeados D, Kemper A, Hawke S D, Tallman G, Tsien R Y, Harper J F (2000). Alteration of stimulus-specific guard cell calcium oscillations and stomatal closing in *Arabidopsis* det3 mutant. Science, 289: 2338–2342

Botella J R, Arteca J M, Somodevilla M, Arteca R N (1996). Calcium-dependent protein kinase gene expression in response to physical and chemical stimuli in mung bean (*Vigna radiata*). Plant Mol Biol, 30: 1129–1137

Bush D S (1995). Calcium regulation in plant cells and its role in signaling. Annu Rev Plant Physiol Plant Mol Biol, 46: 95–122

Cheng S H, Willmann M R, Chen H C, Sheen J (2002). Calcium signaling through protein kinases. The *Arabidopsis* calcium-dependent protein kinase gene family. Plant Physiol, 129: 469–485

Chico J M, Raı´ces M, Te´llez-In˜ o´n M T, Ulloa R M (2002). A calcium-dependent protein kinase is systemically induced upon wounding in tomato plants. Plant Physiol, 128: 256–270

Davletova S, Meszaros T, Miskolczi P, Oberschall A, Torok K, Magyar Z, Dudits S, Deak M (2001). Auxin and heat shock activation of a novel member of the calmodulin-like domain protein kinase gene family in cultured alfalfa cells. J Exp Bot, 52: 215–221

Estruch J J, Kadwell S, Merlin E, Crossland L (1994). Cloning and characterization of a maize pollen-specific calcium-dependent calmodulin-independent protein kinase. Proc Natl Acad Sci USA, 91: 8837–8841

Evans N H, McAinsh M R, Hetherington A M (2001). Calcium oscillations in higher plants. Curr Opin Plant Biol, 4: 415–420

Frattini M, Morello L, Breviario D (1999). Rice calcium-dependent protein kinase isoforms *OsCDPK2* and *OsCDPK11* show different responses to light and different expression patterns during seed development. Plant Mol Biol, 41: 753–764

Harmon A C, Gribskov M, Harper J F (2000). *CDPK*s: A kinase for every Ca^{2+} signal? Trends Plant Sci, 5: 154–159

Harper J F, Sussman M R, Schaller G E, Putnam-Evans C, Charbonneau H, Harmon A C (1991). A calcium-dependent protein kinase with a regulatory domain similar to calmodulin. Science, 252: 951–954

Hegeman A D, Rodriguez M, Han B W, Uno Y, Philips G N, Hrabak E M, Cushman J C, Harper J F, Harmon A C, Sussman M R (2006). A phyloproteomic characterization of in vitro autophosphorylation in calcium-dependent protein kinases. Proteomics, 6: 3649–3664

Hrabak E M, Chan C W M, Gribskov M, Harper J F, Choi J H, Halford N, Kudla J, Luan S, Nimmo H, Sussman M R, Thomas M, Walker-Simmons K, Zhu J K, Harmon A C (2003). The *Arabidopsis CDPK-SnRK* superfamily of protein kinases. Plant Physiol, 132: 666–680

Hrabak E M, Dickmann L J, Satterlee J S, Sussman M R (1996). Characterization of eight new members of the calmodulin-like domain protein kinase gene family of *Arabidopsis thaliana*. Plant Mol Biol, 31: 405–412

Kawasaki T, Hayashida N, Baba T, Shinozaki K, Shimada H (1993). The gene encoding a calcium-dependent protein kinase located near the *sbe1* gene encoding starch branching enzyme I is specifically expressed in developing rice seeds. Gene, 129: 183–189

Klimecka M, Muszynska G (2007). Structure and functions of plant calcium-dependent protein kinases. Acta Biochim Pol, 54: 219–233

Knight H, Knight M R (2001). Abiotic stress signaling pathways: specificity and cross-talk. Trends Plant Sci, 6: 262–267

Liu G, Chen J, Wang X (2006). *VfCPK1*, a gene encoding calcium-dependent protein kinase from *Vicia faba*, is induced by drought and abscisic acid. Plant Cell Environ, 29: 2091–2099

Llop-Tous I, Dominguez-Puigjaner E, Vendrell M (2002). Characterization of a strawberry cDNA clone homologous to calcium-dependent protein kinases that is expressed during fruit ripening and affected by low temperature. J Exp Bot, 53: 2283–2285

Martin M L, Busconi L (2001). A rice membrane-bound calcium-dependent protein kinase is activated in response to low temperature. Plant Physiol, 125: 1442–1449

Monroy A F, Dhindsa R S (1995). Low-temperature signal transduction: Induction of cold acclimation-specific genes of alfalfa by calcium at 25°C. Plant Cell, 7: 321–331

Murillo I, Jaeck E, Cordero M J, Segundo B S (2001). Transcriptional activation of a maize calcium-dependent protein kinase gene in response to fungal elicitors and infection. Plant Mol Biol, 45: 145–158

Patharkar O R, Cushman J C (2000). A stress-induced calcium-dependent protein kinase from *Mesembryanthemum crystallinum* phosphorylates a two-component pseudo-response regulator. Plant Cell, 24: 679–691

Raices M, Chico J M, Telez-Inon M T, Ulloa R M (2001). Molecular characterization of *StCDPK1*, a calcium-dependent protein kinase from *Solanum tuberosum* that is induced at the onset of tuber development. Plant Mol Biol, 46: 591–601

Roberts D M, Harmon A C (1992). Calcium-modulated proteins: targets of intracellular calcium signals in higher plants. Annu Rev Plant Physiol Plant Mol Biol, 43: 375–414

Romeis T, Ludwig A A, Martin R, Jones J D G (2001). Calcium-dependent protein kinases play an essential role in a plant defence response. EMBO J, 20: 5556–5567

Rudd J J, Franklin-Tong V E (2001). Unravelling response specificity in Ca^{2+} signalling pathways in plant cells. New Phytol, 151: 7–33

Saijo Y, Hata S, Kyozuka J, Shimanoto K, Izui K (2000). Over-expression of a single Ca^{2+}-dependent protein kinase confers both cold and salt/drought tolerance on rice plants. Plant J, 23: 319–327

Saijo Y, Kinoshita N, Ishiyama K, Hata S, Kyozuka J, Hayakawa T, Nakamura T, Shimamoto K, Yamaya T, Izui K (2001). A Ca^{2+}-dependent kinase that endows rice plants with cold-and salt-stress tolerance functions in vascular bundles. Plant Cell Physiol, 42: 1228–1233

Sanders D, Brownlee C, Harper J F (1999). Communicating with calcium. Plant Cell, 11: 691–706

Sheen J (1996). Ca^{2+}-dependent protein kinases and stress signal transduction in plants. Science, 274: 1900–1902

Trewavas A (1999). Le calcium, C'est la vie: Calcium makes waves. Plant Physiol, 20: 1–6

Urao T, Katagiri T, Mizoguchi T, Yamaguchi-Shinozaki K, Hayashida N, Shinozaki K (1994). Two genes that encode Ca^{2+}-dependent protein kinases are induced by drought and high-salt stresses in *Arabidopsis thaliana*. Mol Gen Genet, 244: 331–340

Yoon G M, Cho H S, Ha H J, Liu J R, Lee H S P (1999). Characterization of *NtCDPK1*, a calcium-dependent protein kinase gene in *Nicotiana tabacum*, and the activity of its encoded protein. Plant Mol Biol, 39: 991–1001