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GHEXXGXXXXXGXXV Zn1 binding





Figure S1. Alignment of amino acid sequences of OsCADs and other plant CADs. The shaded amino acids denoted identical or similar amino acids. The blue and green boxes indicate the Zn1 and Zn2 binding motives, respectively. The red boxes indicate NADPH binding motives. The consensus sequences are indicated below the aliments (x indicates any amino acid). Pink and blue circles indicate the conserved amino acids coordinated with the catalytic Zn²⁺ ion and the structural Zn²⁺ ion, respectively. Red triangles indicate the residues constituting the substrate binding pocket. Numbering of amino acids were follows AtCAD5. Amino acid sequences used are AtCAD1-9 from *A. thaliana*, EgCAD2 from *E. gunnii*, NtCAD1 and 2 from *N. tabacum*, PtaCAD1 from *P. taeda*, SbCAD2 and 4 from *S. bicolor*, ZmCAD1 and 2 from *Z. mays*, BdCAD3 and 5 from *B. distachyon*, TaCAD12 from *T. aestivum*, PtoCAD12 from *P. tomentosa*, and PtrSAD from *P. tremuloides*.



Figure S2. Phylogenetic analysis of OsCADs and other plant CADs. The phylogenetic tree was conducted using neighbor-joining criteria with the bootstrap values at 1,000 replicates using MEGA6. AtCAD1-9 from *A. thaliana*, EgCAD2 from *E. gunnii*, NtCAD1 and 2 from *N. tabacum*, PtaCAD1 from *P. taeda*, SbCAD2 and 4 from *S. bicolor*, ZmCAD1 and 2 from *Z. mays*, BdCAD3 and 5 from *B. distachyon*, TaCAD12 from *T. aestivum*, PtoCAD12 from *P. tomentosa*, and PtrSAD from *P. tremuloides*.



Figure S3. Quantitative real-time PCR analysis of *OsCAD* gene expression in rice tissues from different developmental stages. A ubiquitin gene (*OsUBQ5*) was amplified using specific primers and used as an internal control. Expression levels of each *OsCAD* gene are presented as the relative expression compared to *OsUBQ5*. The qRT-PCR analysis was performed on the triplicate biological samples.



Figure S4. *In silico* transcriptomic analysis of *OsCAD* gene expression in response to biotic stress. The public microarray data of OsCAD genes were downloaded from the Genevestigator plant biology database (<u>https://genevestigator.com/gv/doc/intro plant.jsp</u>). The expression patterns of *OsCADs* were analyzed by 3 and 4 days after infection of *Magnaporthe grisea*. The expression patterns of *OsCADs* in rice infected with different pathogenic lines of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *X. oryzae* pv. *oryzicola* (*Xoc*) are shown. The color scale represents the log₂ fold changes of gene expression in response to *M. grisea*, *Xoo* and *Xoc* infections. Heatmaps were generated using the Multi Experiment Viewer program (http://www.tm4.org/mev.html).

(A)





Figure S5. In silico transcriptomic analysis of OsCAD expression in response to abiotic stress. (A) The expression patterns of OsCADs in UV-treated rice leaves. Microarray data previously obtained from rice leaves 1, 24 and 48 h after UV treatment (Park et al., 2013) were used for the analysis. (B) The expression patterns of OsCCRs in cold- and wounding-treated rice samples. Microarray data of coldand wounding-treated rice samples were obtained from the Genevestigator plant biology database. The color scale represents the log₂ fold changes of gene expression in response to abiotic stress conditions. Heatmaps were generated using the Multi Experiment Viewer program (http://www.tm4.org/mev.html).

Table S1. Sequence homologies between the deduced amino acid sequences of the OsCADs and *bona fide* CADs from other plants. The values show the percentage of amino acid identities and similarities. Left and right numbers indicate the identities and similarities, respectively.

	AtCAD4	AtCAD5	EgCAD2	NtCAD1	NtCAD2	PtaCAD1	SbCAD2	ZmCAD1	ZmCAD2
OsCAD1	44/64	45/64	46/65	46/65	46/65	47/68	42/61	42/61	42/61
OsCAD2	71/83	72/84	74/83	75/85	73/84	65/81	86/93	87/92	87/93
OsCAD3	45/64	46/63	47/64	46/65	46/64	49/67	43/62	44/63	44/63
OsCAD4	40/59	44/60	42/61	42/60	42/60	45/64	39/57	39/57	39/57
OsCAD5	42/57	44/60	44/59	45/60	45/60	46/65	42/58	42/59	42/59
OsCAD6	45/64	45/65	46/65	48/66	47/65	48/66	44/61	44/61	44/61
OsCAD7	43/62	43/62	44/60	43/62	44/61	45/65	41/59	41/59	42/59
OsCAD8A	48/63	47/63	47/63	49/65	50/66	49/67	50/63	50/63	51/64
OsCAD8B	40/56	39/55	42/54	42/57	42/58	40/57	41/54	40/54	41/54
OsCAD8C	40/56	40/55	41/54	43/57	43/58	40/56	41/54	40/54	41/54
OsCAD8D	48/63	47/62	47/62	49/64	50/65	49/67	50/63	51/64	51/64
OsCAD9	42/62	44/63	45/63	45/64	46/63	48/67	46/63	46/63	47/63

Gene	Primer sequence ^a	Annealing Temp. (°C)
OsCAD1	5′- <u>CATATG</u> GCTGCTGAATGTGGAAG-3′ 5′- <u>GTCGAC</u> CTACTTGAACGAGTTCTCAA-3′	50
OsCAD2	5'- <u>CATATG</u> GGCAGCCTCGCCGCCGA-3' 5'- <u>GAATTC</u> TCAGGCGGGCGGCGCGTCGG-3'	50
OsCAD6	5'- <u>CATATG</u> GAGGTCACCCCCAACCA-3' 5'- <u>GAATTC</u> CTAGAGCTTGGAGTCGCCCC-3'	50
OsCAD7	5'- <u>CATATG</u> GCGCCGACGACGACGGC-3' 5'- <u>GAATTC</u> TCAATCCGACCTGAGGGTGT-3'	50

Table S2. Primer sequences and PCR conditions for the cloning of OsCADs.

Gene	Primer sequence	Annealing Temp. (°C)	
OsCAD1	5'- GGCGGAGTTTATCTGGTAGT -3'	55	
	5'- AAATCTGACATCCGGTCAA -3'		
$O_{C}AD^{2}$	5'- TGTGTGAGACTCTGACGACTTGTC -3'	55	
USCADZ	5'- CATATATTGCGAGGCCGAATTT -3'	55	
$O_{2}CAD^{2}$	5'- CTCTGGCAGGGAGCAACATC -3'	(0	
USCAD3	5'- AAGCGATACCTGACATCCGC -3'	60	
0.04D4	5'- GCGACCATGACTGCGAGAAC -3'	(0	
USCAD4	5'- CCTTTGTTACGGTCCCGTCT -3'	60	
	5'- CAGCATCAAGAACGAGTGGA -3'	FF	
USCAD5	5'- GAGTGGCACGAGTTCACCAT -3'	55	
$O_{2}CADC$	5'- ATGACGAGGAGCCTTGACTA -3'	EE	
USCAD6	5'- CTCTTACCGAAGATGAGAGGG -3'	55	
OsCAD7	5'- CATGCATGGCATCATCAACA -3'	60	
	5'- TAGTATCTTGCCCCCTCCAA -3'	60	
$O_{2}CAD^{2}$	5'- TGGGGCAACGCCATGTACCC -3'	60	
USCAD8	5'- AGCCCACGCCACCGTGTCG -3'	60	
$O_{2}CAD0$	5'- ATCGGCGGGATGAGGGATA -3'	60	
USCAD9	5'- GTACCTCACGTCTCCCTTCTG -3'	60	
LIPOE	5'-ACCACTTCGACCGCCACTACT-3'	FF/60	
UBQO	5'-ACGCCTAAGCCTGCTGGTT-3'	00/00	

Table S3. Primer sequences and PCR conditions for quantitative real-time PCR analysis of *OsCADs*.