Additional files

A rice calcium-dependent protein kinase OsCPK9 positively regulates drought stress tolerance and spikelet fertility

Short running title: *OsCPK9* gene function in rice

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Table S1. PCR primers used in qRT-PCR analysis

Gene	Forward primer	Reverse primer
OsbZIP23	5'-TTTGCCTTCGGAGGACTCTG-3'	5'-GGCAGATCGATCTGGCGATT-3'
Rab21	5'-CACACCACAGCAAGAGCTAAGTG-3'	5'-TGGTGCTCCATCCTGCTTAAG-3'
OsLEA3	5'- AATGATTTCCCTTTGGGTC-3'	5'- CATCAGTACACATCACCCA-3'
OsP5CS	5'-CAGAGTTGATAGTGCTGCTG-3'	5'-CAGGCTAAAAGGAGCATTTG-3'
OsNAC6	5'-CCATGGCAGCTTCCCCGGAT-3'	5'-CCAATCATCCAACCTGAGGC-3'
OsRSUS	5'-TCAACCTACTGGGACAATATTTCAC-3'	5'-GCGTTTATTTGAGGCAAGCACT-3'
OsbZIP66	5'- AACGTGGAGAAGGTGGTGG -3'	5'–TTCCTCCTGTTTCTTCTG-3'
OsbZIP72	5'- AATGAGGTAGAAGAAATGAT-3'	5'- GCACAGTCGCTGATGAAGG -3'
OsNAC9	5'-CTGAGCTACGACGATATCCA -3'	5'-GAAGAGCGACGAGTAGAAGT -3'
OsNAC45	5'-ATATGCCTCCACCTCCTCCT -3'	5'-CATCTCGTAGGGCTTTCACC -3'
OsCPK9	5'-TTGACGGCAGCGGCTACAT-3'	5'-GCTGTTGCTGAGCGTCTTG-3'
ACTIN	5'-ATGGTGGTGACGGGTGAC-3'	5'-CAGACACTAAAGCGCCCGGTA-3'

Table S2. Primer sequences used in plasmids construction and PCR

Primer	Sequence	Purpose
P1	5'-GCTCTAGAAGATCGCCATGGGCAACAC-3'	OsCPK9 amplification for rice transformation
P2	5'-CGGGATCCAATGCTGAAACAATCCCAGCGTTCA-3'	
P3	5'-GGGGTACCTTGTGCCAGATATTTGCTCC-3'	RNAi intron fragment amplification
P4	5'-GGACTAGTTGATATCCGTTCTGTTTCTA-3'	
P5	5'-GCTCTAGAACCACCGATGAGGTGGGCGC-3'	RNAi sense fragment amplification
P6	5'-GGGGTACCCGAGCTGGTACTTGTCCGTG-3'	
P7	5'-CGGGATCCACCACCGATGAGGTGGGCGC-3'	RNAi antisense fragment amplification
P8	5'-GGACTAGTCGAGCTGGTACTTGTCCGTG-3'	
P9	5'-ACGGTGTCGTCCATCACAGTTTGCC-3'	Positive plants identified
P10	5'-GGAAGTGCTTGACATTGGGGAGT-3'	

Table S3. Growth indices of WT, VC and positive transgenics (mean ±SE) under normal growth conditions or after osmotic treatment followed by 7 days recovery (*p <0.05; **p <0.01)

line	Root length (cm)		Shoot length (cm)		Fresh weight (g)		Wilted leaves (%)	Green leaves (%)
	Control	20%PEG	Control	20%PEG	Control	20%PEG	20%PEG	20%PEG
WT	5.28±0.14	5.80±0.17	21.13±0.45	18.04±1.15	0.125±0.006	0.194±0.025	77.23±5.03	22.67±4.98
VC	5.10±0.12	5.60±0.12	21.02±0.92	16.78±2.60	0.115±0.008	0.199±0.023	77.68±6.13	22.20±6.07
OE28	5.41±0.13	6.90±0.33**	20.26±0.72	22.05±1.41*	0.125±0.004	0.322±0.013**	66.68±2.98*	33.17±2.98*
OE16	5.48±0.34	7.38±0.44**	21.00±0.38	23.67±2.20*	0.110±0.005	0.271±0.049*	65.83±4.36*	30.33±6.91*
Ri16	5.05±0.16	5.57±0.18	19.84±0.50	15.87±1.24	0.108±0.004	0.174±0.015	79.50±6.87	16.67±5.43
Ri2	4.92±0.23	5.00±0.27**	19.98±0.75	13.63±1.43**	0.100±0.001	0.151±0.006*	88.33±5.27*	11.67± 5.27 *

Table S4. Growth indices of WT, VC and positive transgenics (mean ±SE) under normal growth conditions or after dehydration treatment followed by 10 days recovery (*p <0.05; **p <0.01.)

line	Root length (cm)		Shoot length (cm)		Fresh weight (mg)	
	Control	dehydration	Control	dehydration	Control	dehydration
WT	4.04±0.37	4.77±0.09	20.84±0.35	9.67±4.37	170.88±3.33	140.06±39.26
VC	4.34±0.21	5.00±0.19	19.00±0.55	8.15±3.88	165.36±2.52	146.92±20.28
OE28	4.38±0.28	6.53±0.41**	19.70±0.77	19.13±1.26**	178.20±2.27	263.10±28.36**
OE16	4.43±0.37	6.20±0.15**	20.75±1.60	21.78±1.74**	177.90±4.10	249.13±27.64*
Ri16	4.48±0.80	4.80±0.29	21.70±0.41	9.00±4.17	177.96±4.01	146.64±16.54
Ri2	4.45±0.10	4.42±0.12*	20.80±0.20	6.18±4.02	181.46±2.44	130.58±13.68

Table S5. The number of open, closed, and partially open stomata in control plants and transgenic lines under normal conditions or drought treatment.

Stomatal status	Normal(number)						Drought(number)					
	WT	VC	OE28	OE16	Ri16	Ri2	WT	VC	OE28	OE16	Ri16	Ri2
Completely closed	11.33±0.88	12±0.58	13±0.58	12.67±0.33	11±0.58	10±0.58	21±0.58	22±0.58	31±1.15**	29±1.15**	18±0.58*	16±0.58*
Partially open	21±1.53	21±0.58	23±0.58	22±0.58	19±0.58	18.67±0.88	24±1.00	24±1.00	24±0.58	22.67±1.20	21±0.58	19±0.58*
Completely open	27.67±1.45	27±0.58	24±0.58	25.33±0.67	30±0.58	31.33±1.20	15±0.58	14±0.58	5±0.58**	8.33±0.88**	21±0.58**	25±0.58**
Total	60	60	60	60	60	60	60	60	60	60	60	60

*p <0.05; **p <0.01.

T**able S6. Growth indices of WT, VC and positive transgenics (mean ±SE) under normal growth and 1 μM ABA conditions**

line	Root length (cm)		Shoot length (cm)		Root dry weight (mg)		Shoot dry weight (mg)	
	Control	1 μM ABA	Control	1 μM ABA	Control	1 μM ABA	Control	1 μM ABA
WT	6.88±0.17	5.98±0.14	23.46±0.53	18.89±1.46	5.75±0.05	7.80±0.34	12.93±0.69	14.28±1.54
VC	6.92±0.38	5.70±0.23	21.10±0.87	18.68±1.21	5.76±0.71	7.28±0.21	13.22±0.76	13.22±0.80
OE28	6.72±0.20	5.13±0.33**	20.46±2.26	16.11±1.66	5.78±0.29	5.65±0.21**	13.53±0.56	11.94±0.27*
OE16	6.83±0.20	5.38±0.40*	19.86±1.49	14.73±1.05**	5.85±0.28	5.48±0.27**	13.05±0.42	10.20±0.55**
Ri16	6.53±0.25	6.02±0.24	20.13±2.17	20.06±2.12	5.72±0.46	7.32±0.13	13.54±1.47	13.53±1.37
Ri2	6.48±0.39	6.34±0.17*	21.18±2.82	20.17±2.74	5.68±0.07	8.22±0.42	16.30±1.81	16.30±1.81

*p <0.05; **p <0.01.

Table S7. Growth indices of WT, VC and positive transgenics (mean ±SE) under normal growth and 3 μM ABA conditions

line	Root length (cm)		Shoot length (cm)		Root dry weight (mg)		Shoot dry weight (mg)	
	Control	3 μM ABA	Control	3 μM ABA	Control	3 μM ABA	Control	3 μM ABA
WT	5.88±0.14	3.31±0.34	19.76±0.16	7.50±0.66	7.95±0.61	5.53±0.25	19.10±1.39	6.05±0.70
VC	6.00±0.16	3.47±0.29	18.78±0.33	7.00±0.34	7.66±0.72	5.78±0.22	20.03±1.87	5.82±0.62
OE28	5.94±0.55	1.04±0.18**	19.55±0.58	3.18±0.19**	7.56±0.33	1.57±0.25**	19.72±1.05	2.67±0.41**
OE16	6.04±0.22	0.98±0.04**	20.50±0.72	4.26±0.34**	7.73±0.17	1.24±0.16**	18.16±0.59	2.67±0.35**
Ri16	6.03±0.10	3.20±0.11	20.31±0.73	6.80±0.37	7.86±0.16	6.04±0.63	19.75±0.62	6.02±0.23
Ri2	5.83±0.13	3.93±0.51	18.23±0.29	6.60±0.17	7.64±0.21	6.80±0.38**	20.37±0.69	6.13±0.40

*p <0.05; **p <0.01.

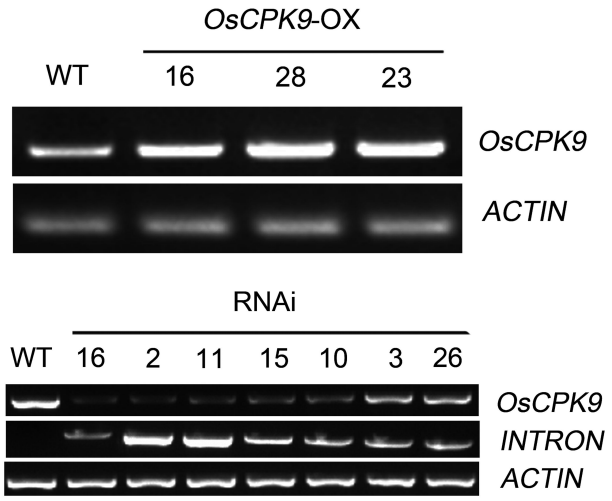

Fig. S1. The expression of *OsCPK9* in transgenic lines.

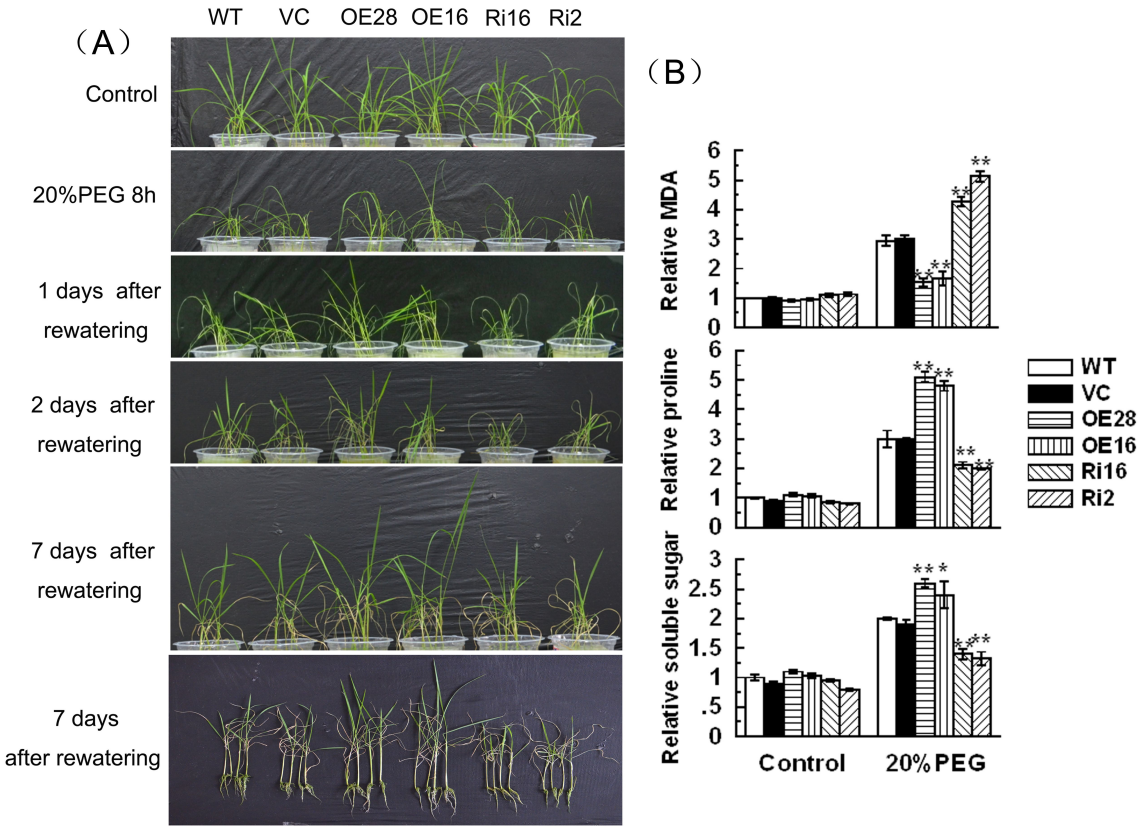

Fig. S2. Analysis of osmotic stress tolerance of *OsCPK9*-OX and *OsCPK9*-RNAi transgenic plants. Two-week-old rice seedlings were exposed to 20% PEG for 8 h and then the seedlings were recovered for 7 d to detect the performance of transgenic lines and WT (A). After PEG treatment for 8 h, leaves of rice seedling were collected

to examine MDA, proline and soluble sugar (B). Data are means ±SE calculated from four independent experiments. Asterisks indicate significant difference between the WT and transgenic lines (*p <0.05; **p <0.01).

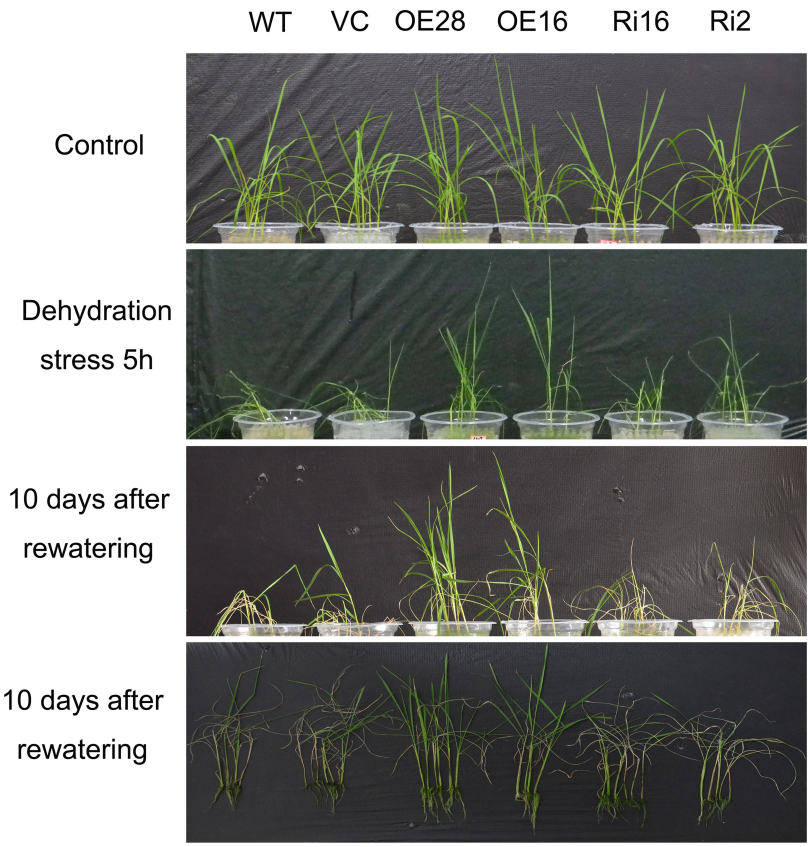

Fig. S3. Analysis of dehydration stress tolerance of *OsCPK9*-OX and *OsCPK9*-RNAi transgenic plants. Two-week-old rice seedlings were exposed to air for 5 h and then the seedlings were recovered for 10 d to detect the performance of transgenic lines and WT. Three biological experiments were conducted, which produced similar results.

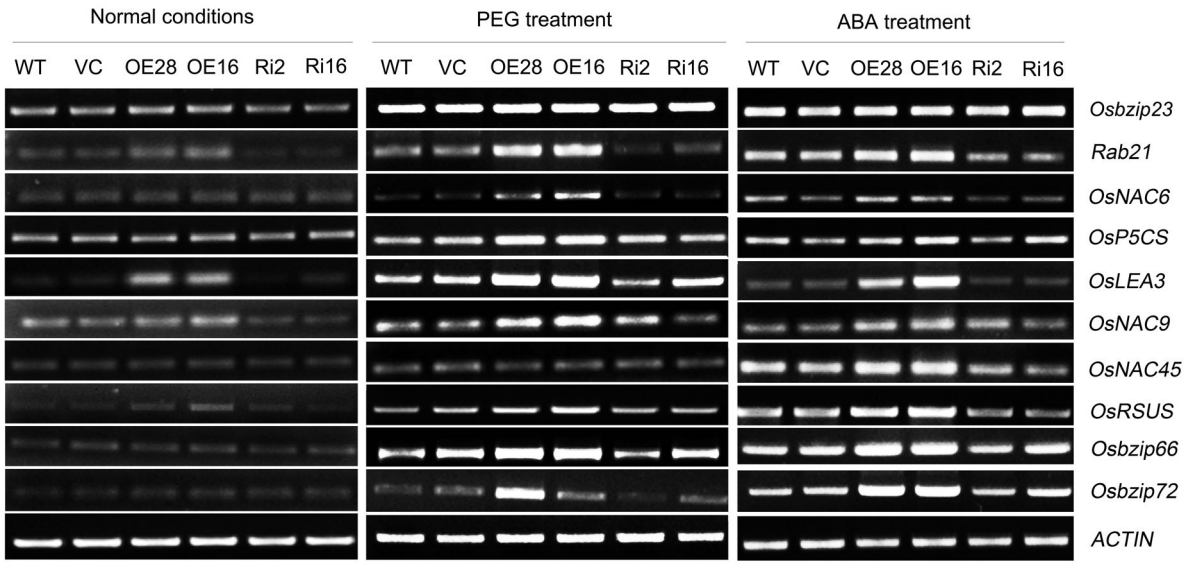

Fig. S4. Expression analysis of some selected ABA- and stress-responsive genes by RT-PCR analysis under no stress, ABA, or PEG6000 treatments in *OsCPK9*-OX, *OsCPK9* RNAi, and control lines. Three-day-old seedlings were exposed to 1 μM ABA for 14 d. Two-week-old rice plants were treated without (normal conditions) or with 20% PEG6000 for 8 h. Rice leaves were collected to detect expression of those ABA- and stress-responsive genes. Two biological experiments were performed, which produced similar results.