

Supplementary Materials: Overexpression of *AmRosea1* Gene Confers Drought and Salt Tolerance in Rice

Mingzhu Dou, Sanhong Fan, Suxin Yang, Rongfeng Huang, Huiyun Yu and Xianzhong Feng

Table S1. Summary of mapping results.

Map to Gene	WT-PEG-0 h		WT-PEG-6 h		WT-PEG-24 h		WT-Salt-0 h		WT-Salt-1 h		WT-Salt-6 h	
	Reads Number	Percentage	Reads Number	Percentage	Reads Number	Percentage	Reads Number	Percentage	Reads Number	Percentage	Reads Number	Percentage
clean reads	7,333,513	100%	7,116,841	100%	7,389,213	100%	7,387,204	100%	7,239,102	100%	7,390,469	100%
Total mapped reads	6,596,369	89.95%	6,432,701	90.39%	6,265,857	84.80%	66,69,882	90.29%	6,607,019	91.27%	6,768,117	91.58%
Total unmapped reads	737,144	10.05%	684,140	9.61%	1,123,356	15.20%	717,322	9.71%	632,083	8.73%	622,352	8.42%
Unique_match	3,293,165	44.91%	3,283,291	46.13%	3,168,963	42.89%	3,314,077	44.86%	3,409,482	47.10%	3,656,368	49.47%
Multi-position match	3,303,204	45.04%	3,149,410	44.25%	3,096,894	41.91%	3,355,805	45.43%	3,197,537	44.17%	3,111,749	42.10%
Perfect match	6,040,500	82.37%	5,874,905	82.55%	5,711,530	77.30%	6,129,419	82.97%	6,048,954	83.56%	6,180,542	83.63%
≤5 bp mismatch	525,415	7.16%	530,256	7.45%	522,317	7.07%	505,249	6.84%	526,733	7.28%	548,742	7.42%
Map to Gene	OXR-PEG-0h		OXR-PEG-6h		OXR-PEG-24h		OXR-Salt-0h		OXR-Salt-1 h		OXR-Salt-6 h	
	Reads Number	Percentage	Reads Number	Percentage	Reads Number	Percentage	Reads Number	Percentage	Reads Number	Percentage	Reads Number	Percentage
clean reads	7,245,902	100%	7,321,939	100%	7,401,080	100%	7,221,670	100%	7,336,233	100%	7,344,809	100%
Total mapped reads	6,449,341	89.01%	6,556,674	89.55%	6,598,040	89.15%	6,501,240	90.02%	6,173,666	84.15%	6,639,746	90.40%
Total unmapped reads	796,561	10.99%	765,265	10.45%	803,040	10.85%	720,430	9.98%	1,162,567	15.85%	705,063	9.60%
Unique_match	3,239,357	44.71%	3,309,357	45.20%	3,343,701	45.18%	3,246,232	44.95%	3,208,032	43.73%	3,554,091	48.39%
Multi-position match	3,209,984	44.30%	3,247,317	44.35%	3,254,339	43.97%	3,255,008	45.07%	2,965,634	40.42%	3,085,655	42.01%
Perfect match	5,885,693	81.23%	6,009,162	82.07%	6,075,612	82.09%	5,954,529	82.45%	5,635,346	76.82%	6,034,068	82.15%
≤5 bp mismatch	529,401	7.31%	512,213	7.00%	484,554	6.55%	514,871	7.13%	502,769	6.85%	559,694	7.62%

Table S5. List of DEGs involved in plant hormone pathways under drought stress.

Hormone	EC	Gene ID	log ₂ (RPKM _{OXR} /RPKM _{WT})		
			0 h	6 h	24 h
Cytokinin	A-ARR	Os04t0673300-01	1.238085	3.065659	0.979313
Absciscic acid	PYR/PYL	Os03t0297600-01	0.563933	1.987756	-0.19252
		Os05t0473000-01	1.387322	4.935228	0.705962
	PP2C	Os01t0583100-01	-0.170357	-1.130925	0.161511
	ABF	Os02t0766700-01	-0.064178	-1.153477	0.430554
Ethylene	EBF1/2	Os06t0605900-01	1.120422	2.458976	0.638164
Jasmonic acid	COI1	Os05t0449500-01	0.676641	1.037637	0.484258
Salicylic acid	PR-1	Os07t0129300-00	2.452397	0.953668	1.374756

Table S6. List of DEGs involved in plant hormone pathways under salt stress.

Hormone	EC	Gene_ID	\log_2 (RPKM _{OXR} /RPKM _{WT})		
			0 h	1 h	6 h
Cytokinin	A-ARR	Os04t0673300-01	0.764954	-1.070037	-0.241291
		Os11t0143300-01	-2.214695	-1.208195	2.157301
		Os12t0139400-01	-2.050575	-1.877847	0.362545
Absciscic acid	SnRK2	Os07t0622000-01	-0.437177	-1.439286	0.192399
Ethylene	CTR1	Os02t0527600-01	0.353983	1.167067	0.133855
	EBF1/2	Os06t0605900-01	1.068686	0.48677	0.007147
Jasmonic acid	JAZ	Os03t0402800-01	-0.878805	-1.461285	-0.143723
		Os07t0615200-01	-0.687653	-1.525973	-0.237743
Salicylic acid	PR-1	Os07t0129300-00	-1.130328	-0.249022	-1.460031

Table S7. List of DEGs related stress tolerance in OXR plants compared with WT under drought condition.

Gene ID	log ₂ (RPKM _{OXR} /RPKM _{WT})			Description
	0 h	6 h	24 h	
HSP (heat shock protein)				
Os01t0135900-02	−0.26	1.15	0.36	LMW heat shock protein.
Os01t0184100-01	1.08	2.14	−0.46	Similar to 17.5 kDa class II heat shock protein.
Os06t0650900-02	0.99	−1.25	0.17	Heat shock protein DnaJ family protein.
Os06t0716700-01	−2.92	2.69	0.68	Similar to Heat shock protein 90.
Os07t0517100-03	0.20	1.49	−0.27	Similar to 18.8 kDa class V heat shock protein.
Os11t0216100-01	−0.09	−1.00	−0.89	Similar to Heat shock protein binding protein.
LEA (late embryogenesis abundant)				
Os01t0314800-01	−0.61	−1.21	0.62	Late embryogenesis abundant protein 3 family protein.
Os05t0542500-02	0.21	3.36	2.41	LEA-like protein.
Chaperone protein				
Os08t0452900-01	0.09	1.26	0.12	Similar to Chaperone protein dnaJ.
Os12t0277500-01	−0.65	0.39	1.00	60 kDa chaperonin alpha subunit.
Metallothionein				
Os01t0974200-01	−0.49	−1.19	−1.13	RicMT (Metallothionein-like protein).
Os05t0202800-00	0.39	−4.34	NA	Similar to Metallothionein-like protein 3B.

Table S8. List of DEGs related stress tolerance in OXR plants compared with WT under salt condition.

Gene ID	log ₂ (RPKM _{OXR} /RPKM _{WT})			Description
	0 h	1 h	6 h	
HSP (heat shock protein)				
Os01t0135900-02	-0.36	0.32	1.14	LMW heat shock protein.
Os03t0266300-01	-0.48	1.08	-0.43	Class I low-molecular-weight heat shock protein 17.9.
Os03t0266900-02	-0.63	1.24	-0.13	Low molecular mass heat shock protein Oshsp17.3.
Os05t0579900-02	0.03	-1.36	0.42	Heat shock protein DnaJ.
Os06t0253100-01	-0.86	2.57	-0.65	Heat shock protein Hsp20 domain containing protein.
Os06t0716700-01	-0.57	3.6	2.08	Similar to Heat shock protein 90.
Os08t0500700-01	-0.71	1.03	-0.12	Similar to Heat shock protein 82.
LEA (late embryogenesis abundant)				
Os01t0314800-01	-0.99	-1.44	-0.42	Late embryogenesis abundant protein 3 family protein.
Os01t0705200-01	NA	-2.09	-0.37	Late embryogenesis abundant protein repeat containing protein.
Os03t0322900-00	-1.6	-2.14	-0.32	Late embryogenesis abundant protein repeat containing protein.
Os08t0327700-01	NA	-2.09	-0.8	Late embryogenesis abundant (LEA) group 1 family protein.
Os05t0542500-02	2.02	-0.65	-0.23	LEA-like protein.
Chaperone protein				
Os08t0405700-01	0.66	-1.12	-0.43	Similar to Copper chaperone homolog CCH.
Metallothionein				
Os12t0570700-01	0.49	1.46	0.29	Similar to Metallothionein-like protein type 1.
PIP (plasma membrane intrinsic protein)				
Os02t0629200-02	-0.46	-1.12	-0.49	Similar to HvPIP2;1 protein.

Table S9. Expressions of anthocyanin relating genes in OXR and WT plants under drought stress

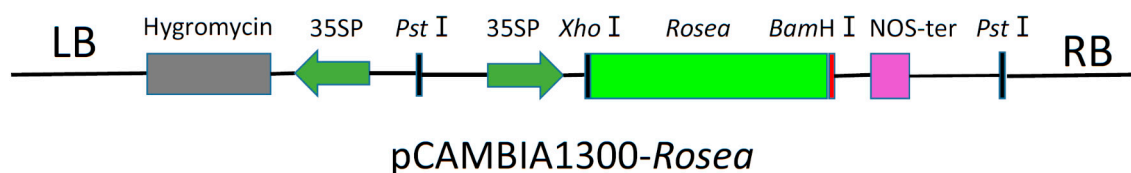
Gene ID	OXR (RPKM)			WT (RPKM)		
	0 h	6 h	24 h	0 h	6 h	24 h
F3H						
OS02T0767300-01	1.406	0.667	1.527	2.659	0.602	0.771
Os03t0122300-01	142.858	188.527	245.626	210.445	156.028	297.271
Os04t0581000-01	4.058	3.429	8.436	1.735	0.648	3.459
F3'H						
Os10t0317900-01	37.962	14.645	17.149	48.349	19.766	26.152
Os10t0320100-01	8.212	0.755	1.481	25.071	0.553	1.937
FLS						
OS02T0767300-01	1.406	0.667	1.527	2.659	0.602	0.771
DFR						
OS01T0633500-00	0	0	0	0	0	0
Os03t0184550-01	15.324	19.105	21.783	3.696	8.042	19.385
OS03T0184600-01	13.05	10.801	14.496	10.672	11.117	13.673
OS08T0183900-01	13.821	11.787	3.293	23.287	27.634	9.186
Os08t0515900-01	0.701	1.16	0.701	0.636	0.461	0.614
Os09t0491868-00	0	0	0	0	0	0
UFGT						
Os06t0192100-00	0.377	0.312	0.063	0.559	0.619	0.264
Os07t0148200-01	6.002	5.865	3.533	8.74	6.007	4.794

Table S10. Expressions of anthocyanin relating genes in OXR and WT plants under salt stress.

Gene ID	OXR (RPKM)			WT (RPKM)		
	0 h	1 h	6 h	0 h	1 h	6 h
F3H						
OS02T0767300-01	1.624	0.129	0	0.962	0.362	0
Os03t0122300-01	122.342	172.764	91.472	174.902	354.6	113.196
Os04t0581000-01	3.723	3.016	0.648	3.99	6.655	0.427
F3'H						
Os10t0317900-01	19.664	7.843	2.046	37.843	7.572	0.649
Os10t0320100-01	1.971	0.847	1.421	5.476	0.791	1.988
FLS						
OS02T0767300-01	1.624	0.129	0	0.962	0.362	0
DFR						
OS01T0633500-00	0	0	0	0	0	0
Os03t0184550-01	48.98	82.765	48.436	27.077	18.01	44.685
OS03T0184600-01	15.193	15.91	11.596	13.312	12.659	14.467
OS08T0183900-01	8.599	10.503	18.592	14.881	10.973	22.537
Os08t0515900-01	0.863	0.865	1.037	0.517	0.577	0.626
Os09t0491868-00	0	0	0	0	0	0
UFGT						
Os06t0192100-00	0.247	0.266	0	0.185	0.186	0
Os07t0148200-01	3.251	2.362	5.685	3.981	6.014	6.883

Table S11. Primers for qRT-PCR.

Gene ID	Primer F (5'-3')	Primer R (5'-3')	Gene Name
Os10t0409400-01	CGGTGCGTCACATCAGCAG	TTTATTCCCTTGACCTTACCAATCA	RD22
Os06T0716700-01	CACAATGACGACAAACAGCATG	CAGGGGTTTCATTCCATGTATCC	Heat shock protein (HSP90)
Os05T0542500-02	CGTCCTCCAAACAGGCGAGT	CTCGTCTTCGGTCATCCCCA	LEA-like protein
Os11T0454300-01	GCTCCAGCTCAAGCTCGTCTG	CTGCTGCTCGCCCTTGTTG	Water-stress inducible protein RAB16A
Os06t0727200-02	GTCGGAGAGCGAGGCCCTA	AGGGATACGCTCCCTGTCAAAC	Catalase (CAT)
Os10t0419400-01	AGCCAGAGGTTACTCTTATGTGGAC	CGGTATGCAGGTGTTCTTCAAAGA	Submergence-induced protein 2 (Sip2)
Os04t0549700-01	GCAGACGCGAGCCATACACC	GAGCTCTTGGGCCGGTCTTG	Dehydration responsive element binding protein 1F (DREB1F)
Os01t0975300-01	GATCAACGCTGGTTGTGGATC	AGCTCTTCCCGCTGCGCT	MYB48
Os12t0156100-01	CGCGAGGATTGTCAGTGATCC	GGAAGGATGGTAGCCGTAGACGT	NAC134
Os12t0113500-01	GGAATCTGGAGTCAAACAGAGTG	GTCTGATCTGCTCCGAAAGCC	CIPK14

**Figure S1.** The sketch of the binary vector pCambia1300-*AmRosea1*.

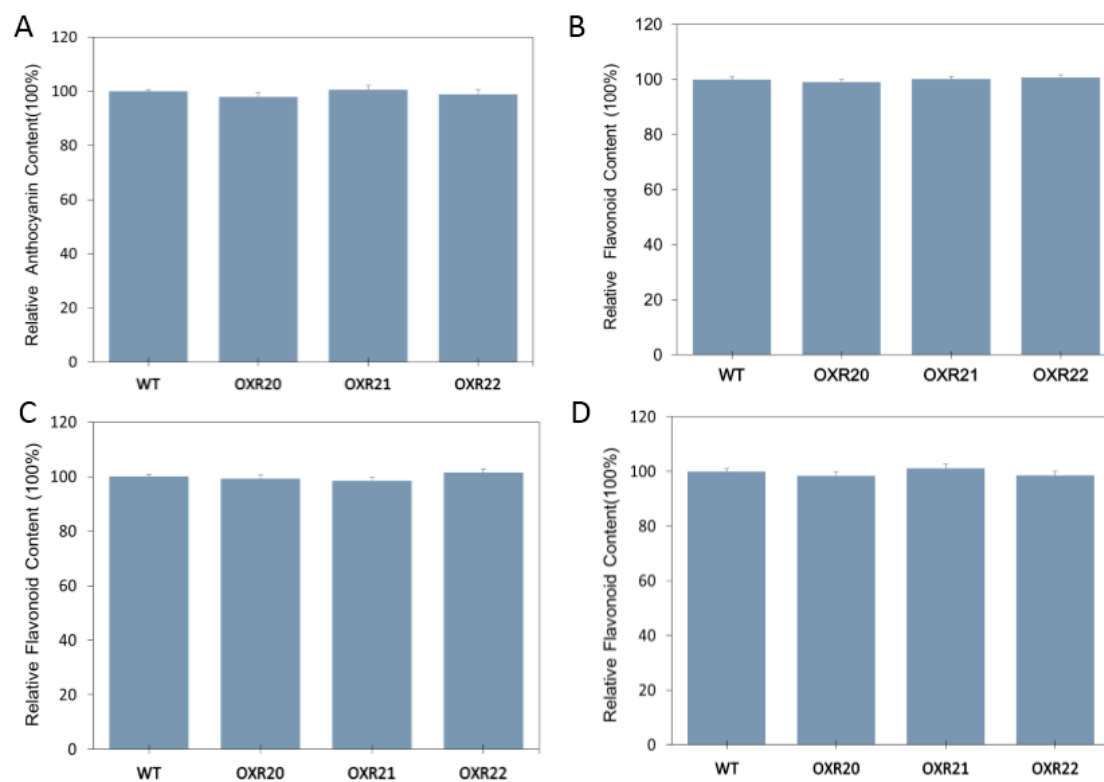


Figure S2. The relative contents of anthocyanin and flavonoids in leaves and seeds of WT and transgenic plants. (A) Anthocyanin of leaves; (B) anthocyanin of seeds; (C) flavonoids of leaves; (D) flavonoids of seeds.