

OXS2 is Required for Salt Tolerance Mainly through Associating with Salt Inducible Genes: *CA1* and *Araport11* in *Arabidopsis*

Ying Jing^{1,2,3}, Lin Shi^{1,3}, Xin Li¹, Han Zheng¹, Jianwei Gao^{1,2}, Mei Wang^{1,2}, Lilong He^{1,2*} and Wei Zhang^{1,2*}

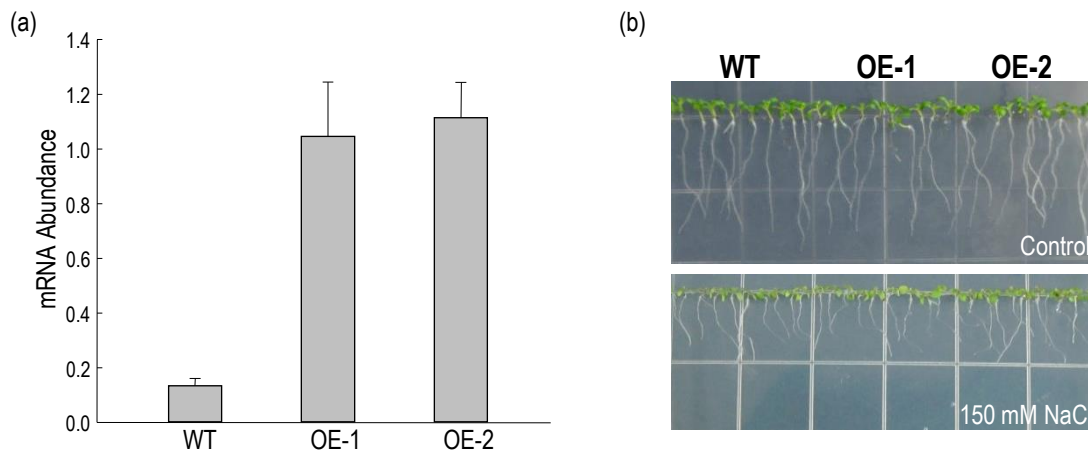
¹Institute of Vegetables and Flowers, Shandong Key Laboratory of Greenhouse Vegetable Biology, Shandong Branch of National Vegetable Improvement Center, Huanghuai Region Vegetable Scientific Station of Ministry of Agriculture (Shandong), Shandong Academy of Agricultural Sciences, Jinan 250100, China

²Key Laboratory of Plant Development and Environment Adaptation Biology, Ministry of Education; School of Life Science, Shandong University, Qingdao, 266237, China

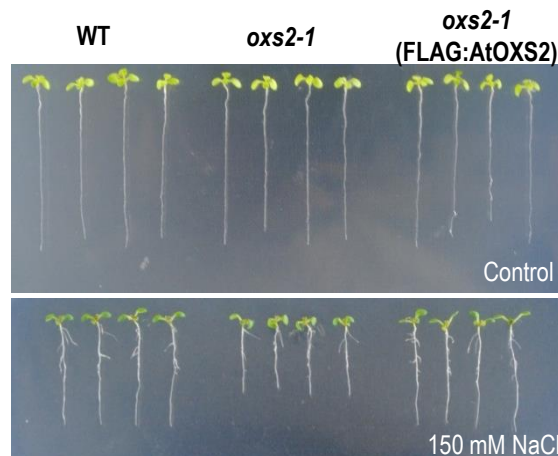
³These authors contributed equally to this work.

* Correspondence: hllong1984@163.com (L.H.); weizhang@sdu.edu.cn (W. Z.)

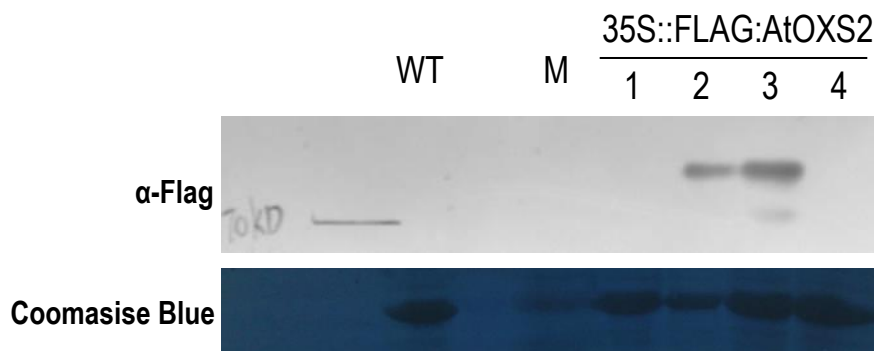
Tel.: +86-531-6665-9060 (L. H.); +86-13188933448 (W. Z.)



Supplementary Figure 1. Phenotyping analysis of *OXS2* overexpressing (OE) lines and wild-type plants. (a) Transcript abundance identification. Expression of *OXS2* (relative to *ACT1* control) in the leaves of 4-w-old wild-type and overexpressing lines. Error bars indicate $\pm$ SD of three independent experiments (three leaves from one plant). (b) Arabidopsis plants germinated on $\frac{1}{2}$ MS plates vertically for 3 d were transferred to plates without or with 150 mM NaCl for another 10 d.



Supplementary Figure 2: Phenotyping analysis of *oxs2-1*, and wild-type plants without or with salt stress. Arabidopsis plants germinated on $\frac{1}{2}$ MS plates vertically for 3 d were transferred to plates without or with 150 mM NaCl for another 10 d. Representative result from three reproducible experiments was shown.



Supplementary Figure 3. Western blot of *oxs2-1* expressing FLAG:AtOXS2 lines and wild type plants. Total protein extracted from 4-week-old leaves of wild-type plant and independent transgenic plants which identified by genomic PCR. The anti-FLAG antibody which described before²⁵ was used to detect FLAG::AtOXS2. Total protein stained by coomassie blue was used as control. The blots were cut from the same images separately. Representative results from three reproducible experiments are shown. WT, Wild type.

Supplementary Table S1. Down-regulated differentially expressed genes in WT VS oxs2-1 comparison group discovered by RNA-seq

DEG Number	GeneID	Log ₂ Ratio (WT VS oxs2-1)	Fold Change WT VS oxs2-1
DEG1	AT3G49230	-5.7398	0.018712572
DEG2	AT3G44006	-5.2148	0.026927463
DEG3	AT4G29200	-4.8655	0.034304627
DEG4	AT4G34790	-3.6286	0.080848939
DEG5	AT3G05945	-2.5701	0.168388441
DEG6	AT3G30720	-1.5808	0.334298315
DEG7	AT2G42380	-1.373	0.386080525
DEG8	AT5G38930	-1.3383	0.395480201
DEG9 (AtOXS2)	AT2G41900	-1.3144	0.402094314
DEG10	AT3G01500	-1.3002	0.406068198
DEG11	AT2G34430	-1.2206	0.429100856
DEG12	AT5G14740	-1.1985	0.435715601
DEG13	AT3G27690	-1.1934	0.437262295
DEG14	AT2G29290	-1.1781	0.441918368
DEG15	AT5G48490	-1.1652	0.445907935
DEG16	AT4G14400	-1.1086	0.463747396
DEG17	AT1G10657	-1.1065	0.464432893
DEG18	AT4G12980	-1.1046	0.465026703
DEG19	AT5G25980	-1.0972	0.467436341
DEG20	AT4G02850	-1.0783	0.473578589
DEG21	AT2G34170	-1.0651	0.477941415
DEG22	AT4G26530	-1.0584	0.480156926
DEG23	AT1G11120	-1.0418	0.485716427
DEG24	AT5G38420	-1.0376	0.487144309
DEG25	AT1G29395	-1.0314	0.489218757
DEG26	AT1G73600	-1.0281	0.490368845
DEG27	AT1G62500	-1.0109	0.49623995
DEG28	AT3G05727	-1.0069	0.497612635

Supplementary Table S3. Primer Sequences. Note: lowercase means sequences from vectors and capital letter means sequences from genes.

primer name	Sequence	primer name	Sequence
AtOXS2 -1F	5' gtaccggggatcctGCGTCGACATGTGCTGTGGATCAGACC 3'	DEG21 q R	5'-GGCTGTGCTTCCAAGAATA-3'
AtOXS2 -1R	5' gcaggtcgactctagCGGGATCCTCAATTCTGCTGAGCCACA 3'	DEG22 q F	5'-GAAGCCATGGACTCTCACTTT-3'
AtOXS2 -2F	5' GGGGTACCATGTGCTGTGGATCAGACCG 3'	DEG22 q R	5'-CCTGAGCTTTGGCTACATTCT-3'
AtOXS2 -2R	5' AACTGCAGATTCTGCTGAGCCACAAGCTGATC 3'	DEG23 q F	5'-GCAACGGCTATTCTCATCTCT-3'
qRT-PCR analysis		DEG23 q R	5'-CCCGACTTGAAGAGGAATCTG-3'
DEG1 q F	5'-ACGGTCACTTGGTTCATCAC-3'	DEG24 q F	5'-CAAGCAACGGAGGAAGAGTTAG-3'
DEG1 q R	5'-TTGGTTGTATCTCCTTCCCTTTC-3'	DEG24 q R	5'-GTCACAAAGGTCAGGGAGGTAA-3'
DEG2 q F	5'-ATGCTGGCACTTACCAA-3'	DEG25 q F	5'-GCCTGGATTAAGGGAGAGTATG-3'
DEG2 q R	5'-CTATCCACCGTTTCAGGACTAAC-3'	DEG25 q R	5'-GCCACAATCACAAGAGTAATG-3'
DEG3 q F	5'-CTGGATCTTTGCCACCTACTT-3'	DEG26 q F	5'-TCTCCAACCTGGCTGCTAATG-3'
DEG3 q R	5'-CCAAGAAAGGCGAGATGATTG-3'	DEG26 q R	5'-GGAAACAAGACTCCCGAAAGA-3'
DEG4 q F	5'-AAGGTCACGTAGCGTTTAC-3'	DEG27 q F	5'-GTTAGACCTCCTCCCGTAGT-3'
DEG4 q R	5'-GCCACCCATAGAGTGATGAAA-3'	DEG27 q R	5'-TGATGGGAGGTCTTGATGTTG-3'
DEG5 q F	5'-AGCTCATAGACACATGTCTTAACAA-3'	DEG28 q F	5'-CCATCTGCTTCACTACTCTT-3'
DEG5 q R	5'-CCCGACGACAATGGAGTTAAT-3'	DEG28 q R	5'-TTTCCTTACATGGGTACTG-3'
DEG6 q F	5'-GTTGAAAGAAGCTTCAAACCAAC-3'	Actin2 qF	5'-GGTAACATTGTGCTCAGTGGTGG-3'
DEG6 q R	5'-GACCCATGATATGACCTCATT-3'	Actin2 qR	5'-AACGACCTTAATCTTCATGCTGC-3'
DEG7 q F	5'-CAGAGATCAAGGGTGAGGAAAC-3'	ChIP-qPCR analysis	
DEG7 q R	5'-GAATGCAACTCTTGGCGATAAC-3'	actin2 F	5' GTTAGCAACTGGGATGATATGG 3'
DEG8 q F	5'-TTGTGGAAGTAGGAACACTTCTC-3'	actin2 R	5' CAGCACCACATCGTGACTTGCCC 3'
DEG8 q R	5'-GAGTCCCTCTGGAACACAAA-3'	DEG2 F1 F	5' ATACAATTCATTTTCTCTCTTT 3'
DEG10 q F	5'-TTCCGTTCCAACGCTTATCC-3'	DEG2 F1 R	5' ACTATATAAGGTAGGCATGGCT 3'
DEG10 q R	5'-GTATGCTTCGGTCCCATCTC-3'	DEG3 F2 F	5' TTGCTCTGTTCTTCTCTCT 3'
DEG11 q F	5'-CATAGCCAACCTCCGTTCT-3'	DEG3 F2 R	5' ATTGACTCTTTTCTCTATCTCT 3'
DEG11 q R	5'-GATGCCTTCGTGGTTCGTAATA-3'	DEG4 F3 F	5' AGATATTTGTGGAGAATTTTGC 3'
DEG12 q F	5'-GATGCCTTCGTGGTTCGTAATA-3'	DEG4 F3 R	5' ACATTATATTGTTTCTGTTAGTGA 3'
DEG12 q R	5'-AGGTGCAAGACAGCGTATTC-3'	DEG18 F4 F	5' TATCTAAAAACATGCATCATACTA 3'
DEG13 q F	5'-CACACGTACTAGACTTCCATCC-3'	DEG18 F4 R	5' TACAATAAAATCTAAATCACAACA 3'
DEG13 q R	5'-GGCTCCAACCTCCTGCATATT-3'	DEG10 F5 F	5' TGCCTTACTGGTCTCTGATTTCTT 3'
DEG14 q F	5'-CGTGAGCAACGAGAGAACT-3'	DEG10 F5 R	5' CTCAGACCTCCATGGCCATA 3'
DEG14 q R	5'-GTGGTCGGCTTGAGCATT-3'	DEG10 F6 F	5' TGATGATTATGGCCATGGAA 3'
DEG15 q F	5'-GGCTAGTTTAGTCGTTGAGAGG-3'	DEG10 F6 R	5' ATCGATCATGGACTGTTCTACTCTA 3'
DEG15 q R	5'-CTCGTCGGGTATTCTTGCT-3'	DEG10 F7 F	5' CTTTCTGAGACCTTTATTTTTTTA 3'
DEG16 q F	5'-TGAAGAGAGTGAGGGACTGAA-3'	DEG10 F7 R	5' ATAGCTCGGGTGAATAACAAAC 3'
DEG16 q R	5'-GACAGGTAGCCATCTCCAAATAG-3'	DEG24 F8 F	5' ATTAGATAGTTTACGATACATTCAA 3'
DEG17 q F	5'-CACAAACACAATCTCCCTTG-3'	DEG24 F8 R	5' CACGTAAAAGCTCATATATG 3'
DEG17 q R	5'-CGCGTCATACGGAGTAGAATG-3'	DEG6 F9 F	5' TCTTAAATAAATGTCCAAGCTTG 3'
DEG18 q F	5'-AAGGTTCCGGCGGATTAG-3'	DEG6 F9 R	5' TTTTGTGATCGAAAGAAGTGAATA 3'
DEG18 q R	5'-CGAGTGATCCCACTGAGTTAAG-3'	DEG13 F10 F	5' TTTAATTATCCAATCATATACCTT 3'
DEG19 q F	5'-ATTCTTCCACCGAACCTATATC-3'	DEG13 F10 R	5' ATAATGGAGCTGATTGGGAT 3'
DEG19 q R	5'-CAATCTTCCCTCCTTGTAATT-3'	DEG12 F11 F	5' GGGATCTTCGATTTCGAC 3'
DEG20 q F	5'-CTGGAGCCTCAAGATGAACAA-3'	DEG12 F11 R	5' GACTTCTCTCTTATCCACATCA 3'
DEG20 q R	5'-CAAGACTCTCTGCTTCTCTTATC-3'	DEG28 F12 F	5' ATATGTTGTTAGAGAATCGA 3'
DEG21 q F	5'-GTGGTGTGAGGCAATGAAC-3'	DEG28 F12 R	5' AATTCATTATTTAGTTACCTTTG 3'