

Title: *Arabidopsis* YL1/BPG2 Is Involved in Seedling Shoot Response to Salt Stress through ABI4

Authors: Peng-Cheng Li^{1,2†}, Jin-Guang Huang^{1†}, Shao-Wei Yu¹, Yuan-Yuan Li¹, Peng Sun¹, Chang-Ai Wu¹, and Cheng-Chao Zheng^{1*}

Affiliations:

¹State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University, Tai'an, Shandong 271018, PR China

²High-Tech Research Center, Shandong Academy of Agricultural Sciences, Shandong Provincial Key Laboratory of Crop Genetic Improvement, Ecology and Physiology, Jinan, Shandong, PR China

†These authors contributed equally to this work.

*Correspondence should be addressed to C-C.Z. (e-mail: cczheng@sdaau.edu.cn)

Supplementary information

Supplementary Figure 1. Root phenotype of *yll-1*.

Supplementary Figure 2. Mature plant phenotypes of wild type, *yll-1*, *yll-2*, *yll-1com*, and *yll-1 yll-2* (cross F1 line).

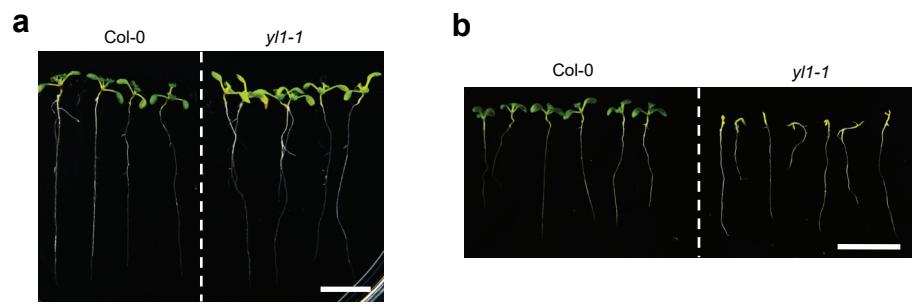
Supplementary Figure 3. GUS activity analysis of the *pYLI-GUS* transgenic plants under salinity treatment.

Supplementary Figure 4. Transcriptional analysis of *YLI* in roots under salt-treatment.

Supplementary Figure 5. Transcriptional analysis of SOS pathway genes, *NHXI*, and ABA biosynthesis pathway genes in seedling shoots.

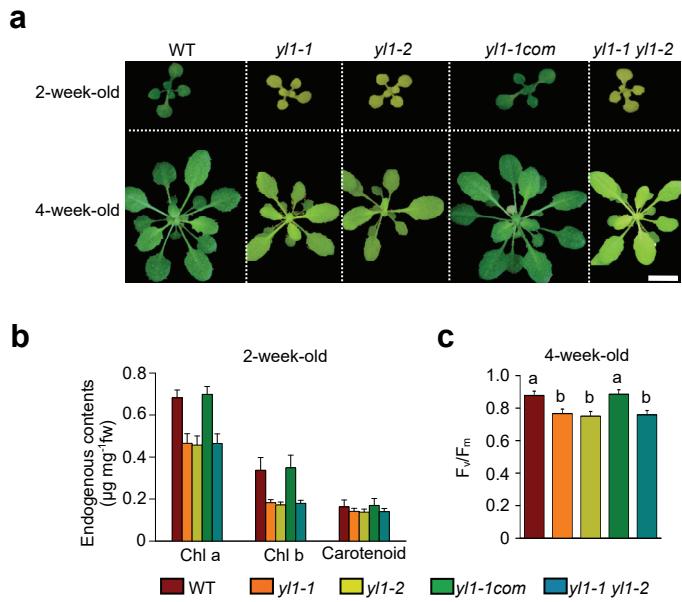
Supplementary Figure 6. Transcriptional analysis of SOS pathway genes, *NHXI*, and ABA biosynthesis pathway genes in seedling roots.

Supplementary Table 1. Key primers used in this study.



Supplementary Figure 1. Root phenotype of *yl1-1*.

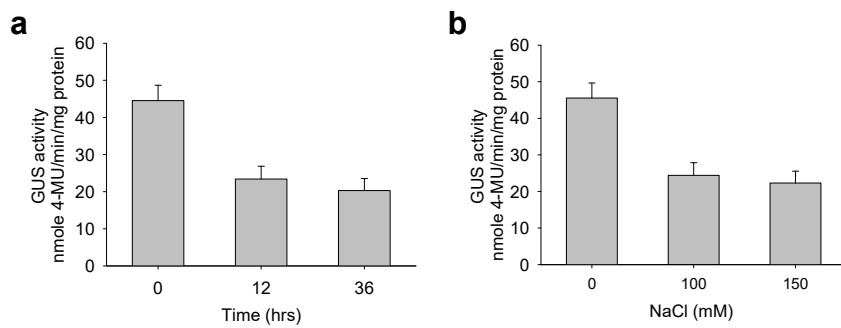
- (a) Phenotype of 10-d-old seedling roots under control conditions. Scale bars = 5 mm.
- (b) Phenotype of 10-d-old seedling roots under 150 mM NaCl conditions. Scale bars = 5 mm.



Supplementary Figure 2. Mature plant phenotypes of wild type, *yll-1*, *yll-2*, *yll-1com*, and *yll-1 yll-2* (cross F1 line).

- (a) Phenotypes of 2/4-week-old wild type, *yll-1*, *yll-2*, *yll-1com*, and *yll-1 yll-2* (F1) grown under normal growth conditions. Scale bars = 10 mm.
- (b) Endogenous contents of chlorophyll a, chlorophyll b, and carotenoid in 2-week-old wild type, *yll-1*, *yll-2*, *yll-1com*, and *yll-1 yll-2* (F1).
- (c) F_v/F_m of 4-week-old wild type, *yll-1*, *yll-2*, *yll-1com*, and *yll-1 yll-2* (F1).

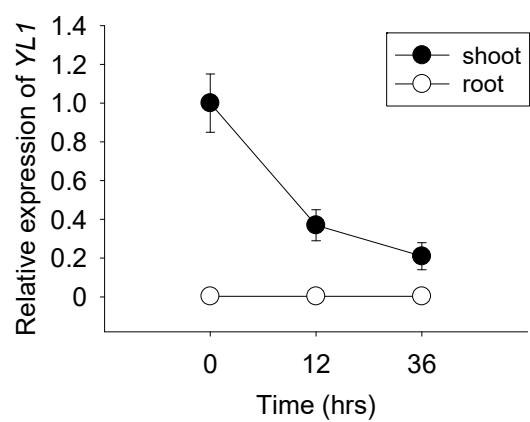
Data are mean values of three replicates \pm SE. Statistical significant differences are indicated by different lowercase letters (P < 0.01).



Supplementary Figure 3. GUS activity analysis of the *pYL1-GUS* transgenic plants under salinity treatment.

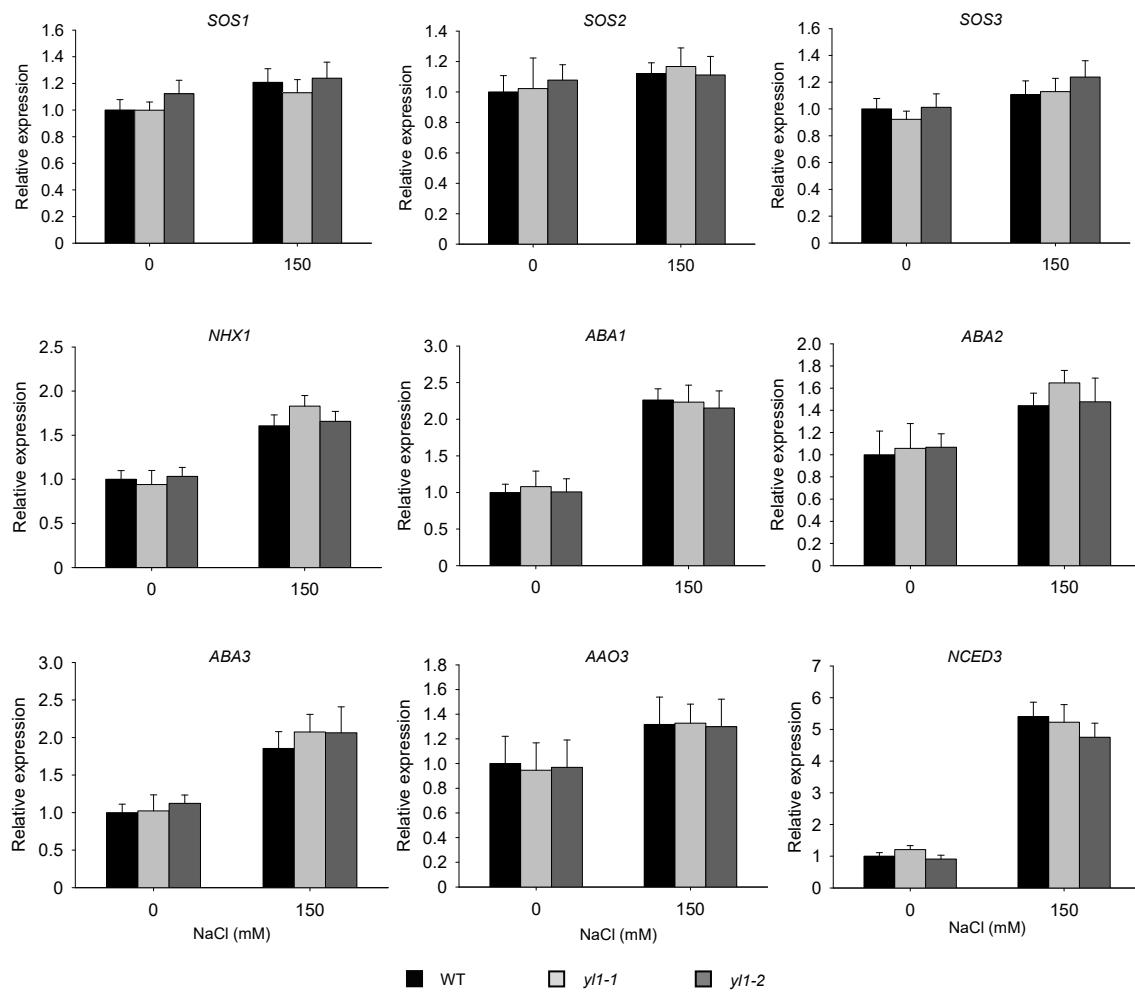
(a) GUS activity analysis of 5-d-old seedlings treated with 150 mM NaCl for 0, 12, or 36 h.

(b) GUS activity analysis of 5-d-old seedlings treated with 0, 100, or 150 mM NaCl for 36 h.



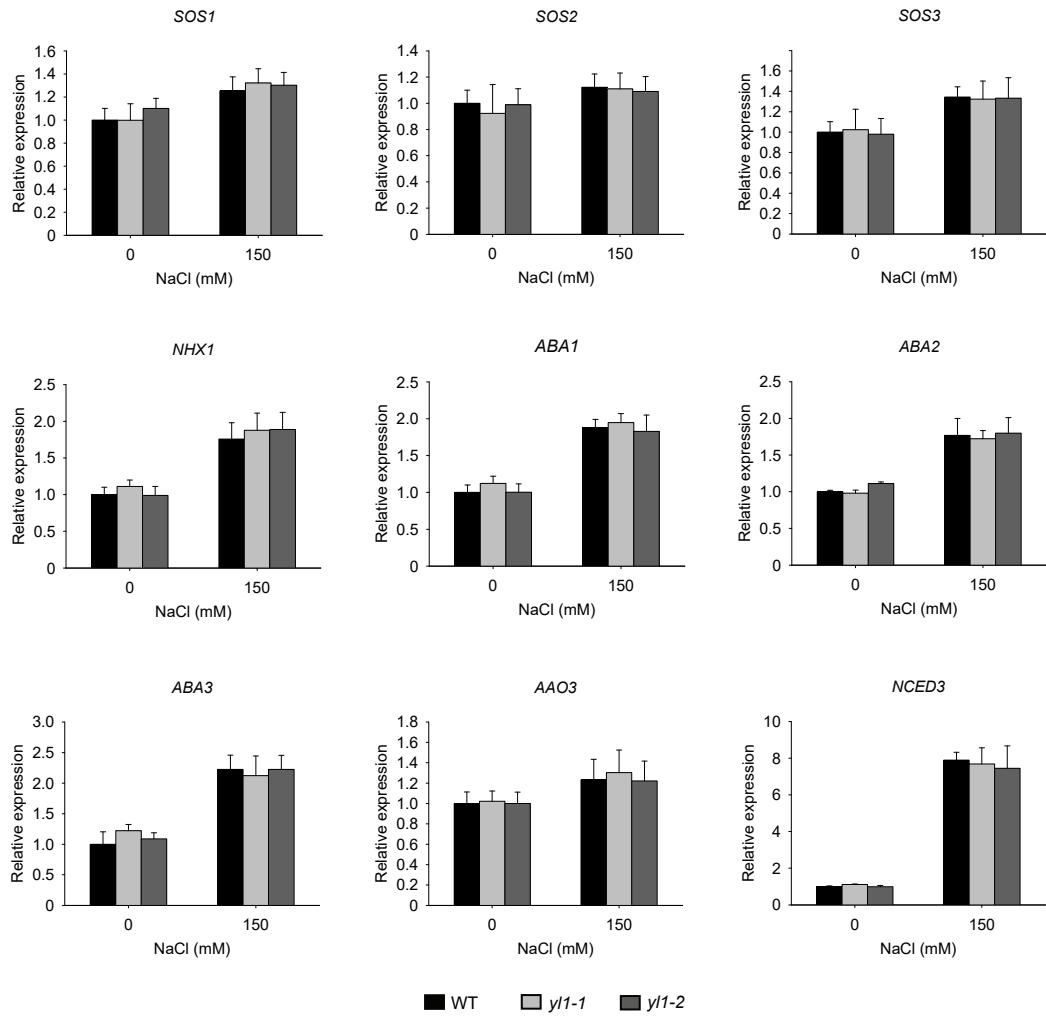
Supplementary Figure 4. Transcriptional analysis of *YL1* in roots under salt-treatment.

Total RNA was isolated from roots of 5-d-seedlings treated with control or 150 mM NaCl for 0, 12, or 36 h. Values are means \pm SE of three independent replicates.



Supplementary Figure 5. Transcriptional analysis of SOS pathway genes, *NHX1*, and ABA biosynthesis pathway genes in seedling shoots.

SOS1, *SOS2*, and *SOS3* are SOS pathway genes; *ABA1*, *ABA2*, *ABA3*, *AAO3*, and *NCED3* are ABA biosynthesis associated genes. Total RNA was isolated from shoots of 5-d-seedlings treated with control or 150 mM NaCl for 12 h. Values are means \pm SE of three independent replicates.



Supplementary Figure 6. Transcriptional analysis of SOS pathway genes, *NHX1*, and ABA biosynthesis pathway genes in seedling roots.

SOS1, *SOS2*, and *SOS3* are SOS pathway genes; *ABA1*, *ABA2*, *ABA3*, *AAO3*, and *NCED3* are ABA biosynthesis associated genes. Total RNA was isolated from roots of 5-d-seedlings treated with control or 150 mM NaCl for 12 h. Values are means \pm SE of three independent replicates.

Supplementary Table 1. Primers used in this study.

Purpose	Primer name	Sequence (5'-3')
Complementation and overexpression constructs	YL1-forward (P1)	GGATCCATGGTGGTTTGATTCAAGTACAG
	YL1-reverse (P2)	TGCGACCTAACACTATCAGAGAGAAAATC
GFP expression constructs	YL1-forward	TCTAGAATGGTGGTTTGATTCAAGTACAGTG
	YL1-reverse	GGATCCAGCAACACTATCAGAGAGAAAATCTGT
	GFP-forward	GGATCCGGTGGTGGTAGTAGTGGTGGTGG- -TATGAAGACTAATCTTTCTCTTC
	GFP-reverse	GTCGACTAAAGCTCATCATGTTGTAGTTC
GUS expression constructs	YL1-promoter-forward	GTCGACATGGTGGTTGATTCAAGTACAG
	YL1-promoter-reverse	GGATCCAGCAACACTATCAGAGAGAAAATC
qRT- PCR	ABA1-forward	TGCTACTCTATCAATCCATCTCC
	ABA1-reverse	TCTTCTCCTCCTTCTCAACTAAC
	ABA2-forward	AGGAGCCACAGGGATAGG
	ABA2-reverse	ACTCACACGAAGCAGAC
	ABA3-forward	CCGAGGAGAGTGGTGTGAC
	ABA3-reverse	AACCGCCAATGATGTGAAATG
	AAO3-forward	TGCTTATGGTCTCGGTATGG
	AAO3-reverse	CCTAACGGCTTCACAACGT
	NCED3-forward	GGTCGTGTGAGTTCTATGTG
	NCED3-reverse	ACTGGTAATCTCGCTCTCTC
	ABI5-forward	AACCTAACCAACCCGAACC
	ABI5-reverse	ACCCCTCCTCCTGTCC
	ABI4-forward	CTCTTCTCCTCCGTCTC
	ABI4-reverse	GTTGGCTCCTCCTCCTAC
	HKT1-forward	TCTGGTCTCATCTGGCTCTTAATC
	HKT1-reverse	CACCGTCACTCCAAGAAGAACAC
	SOS1-forward	TGGAGGAAGCGACCGATT
	SOS1-reverse	CGATAACGAGAAAGAGCGACAG
	SOS2-forward	CTGTTGCTCATTGTCACTG
	SOS2-reverse	CGTAACCCTGTCCACTAAG
	SOS3-forward	GTCACGCCATTACGGTAG
	SOS3-reverse	CACTCCATTCGCTTCACATC
	NHX1-forward	AGGTGCTGTATCTATGGCTCTTG
	NHX1-reverse	GCTCGTGGTGGCGTTCTG
	YL1-forward (qP1)	GGATTGATGTAACCTTGAGAG
	YL1-reverse (qP2)	GCTGTCTTGTGCTTCTTC
	Actin2-forward	CTTCGTCTTCCACTTCAG
	Actin2-reverse	ATCATAACCAGTCTAACAC