Supplementary Material: Functional Plant Biology, 2018, 44(3), 305–314.

Supplementary Material

Aquaporin AtTIP5;1 as an essential target of gibberellins promotes hypocotyl cell elongation in *Arabidopsis thaliana* under excess boron stress

Yongqi Pang^{A,B}, Jintong Li^A, Bishu Qi^{C,D}, Mi Tian^A, Lirong Sun^A, Xuechen Wang^A and Fushun Hao^{A,E}

^AState Key Laboratory of Cotton Biology, Henan Key Laboratory of Plant Stress Biology, College of Life Sciences, Henan University, Kaifeng 475004, P.R. China.

^BInstitute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, P.R. China.

^CNorth China Key Laboratory for Crop Germplasm Resources of Education Ministry, Agricultural University of Hebei, Baoding 071000, P.R. China.

^DThe National Police University for Criminal Justice, Baoding 071000, P.R. China.

^ECorresponding authors. Email: haofsh@henu.edu.cn

Table S1. Primers used to identify homozygous T-DNA insertion lines by PCR

Primer name	Sequence (5'-3')
m1 LP	ACCAAACAAACGAAGCACAC
m1 RP	TGAGGTTGATGGGAATTTCAG
m2 LP	CATACGTGGGGACATTTATG
m2 RP	TACAGGGACCACCACGTTATC

Table S2. Primers used for reverse transcription-PCR

Primer name	Sequence (5'-3')
AtTIP5;1 LP	CGTCATGGAACAGCACGTACCGAT
AtTIP5;1 RP	CCACTGCTAAAGGTAGCCCACGTCT
Actin 2 LP	TGTGCCAATCTACGAGGGTT'
Actin 2 RP	TCATTCGTGCGTGATGCCAA

 $\label{thm:conditional} \textbf{Table S3. Primers used for quantitative real time-PCR} \\$

Primer name	Sequence (5'-3')
AtTIP5;1 QLP	CGTCATGGAACAGCACGTACCGAT
AtTIP5;1 QRP	CCACTGCTAAAGGTAGCCCACGTCT
Actin 2 QLP	ATTACCCGATGGGCAAGTCA
Actin 2 QRP	CACAAACGAGGCTGGAACA
GA3ox1 QLP	CCCCAACATCACCTCAACTACTGC
GA3ox1 QRP	CAAACCGGGTAGTGATTTAGCTGGA
GA20ox1 QLP	CAGCCATTTGGGAAGGTGTATC
GA20ox1 QRP	CAAGCAGCTCTTGTATCTATCGT
GA20ox2 QLP	GAGCAGTTTGGGAAGGTGTATC
GA20ox2 QRP	TAACGTGAGATCTGGTGTTTTGG
Elongation factor 1α QLP	CCTTGGTGTCAAGCAGATGA
Elongation factor 1α QRP	TGAAGACACCTCCTTGATGATTT
Ubiquitin 10 QLP	GGAGGTGGAGAGTTCTGACA
Ubiquitin 10 QRP	AGACCAAGTGAAGTGTGGAC

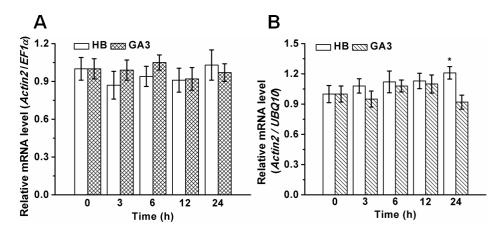


Fig. S1. Relative expression of *Actin2* in response to high concentrations of boron (HB) or GA3 normalized by gene *Elongation factor* 1α and *Ubiquitin* 10.

Ten-day-old WT seedlings grown on MS medium were treated with 3 mM boron or 5 μ M GA3 for different periods of time. Quantitative real time-PCR analysis of *Actin2* was carried out. Gene *Elongation factor 1a* (*EF1a*) and *Ubiquitin 10* (*UBQ10*) were used as controls in A and B, respectively. The asterisk indicate the data derived from the treatments were significantly different from the untreated control at P<0.05 level by Student's t-test.

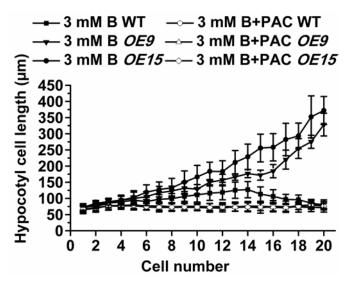


Fig. S2. Effects of paclobatrazol (PAC) on the hypocotyl cell elongation of *OE9* and *OE15* under high-B stress.

Cell length was measured in the hypocotyls of *AtTIP5;1* overexpressors in the presence of 3 mM $_{3}BO_{3}$ (B) and 0.1 $_{4}M$ PAC for 15 d. Error bars are SE, $_{n}\geq 20$.

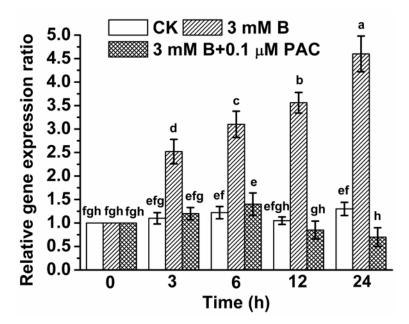


Fig. S3. Effects of paclobatrazol (PAC) on the expression of *AtTIP5;1* in WT under boron stress. Ten-day-old WT seedlings grown in MS medium with 3 mM H₃BO₃ (B) were treated by 0.1 μM PAC for the indicated times. QPCR analysis of *AtTIP5;1* was conducted. *Actin2* used as a control. Different lowercase letters indicate values from the seedlings significantly differ from each other (*P*<0.05).

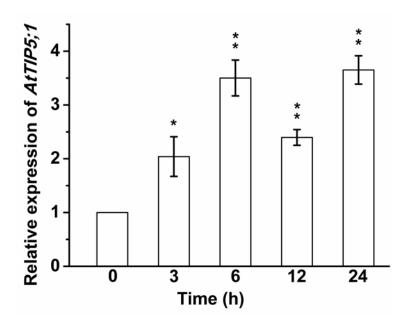


Fig. S4. Effects of GA3 on changes in *AtTIP5;1* expression of WT seedlings.

Ten-day-old WT seedlings grown on MS medium were treated with 5 μM GA3 for different periods of time. Quantitative real time-PCR analysis of *AtTIP5;1* was carried out. *Actin2* was as a control. Asterisk and double asterisk indicate the data derived

from the treatments were significantly different from the untreated control at P<0.05 and P<0.01 levels respectively by Student's t-test.

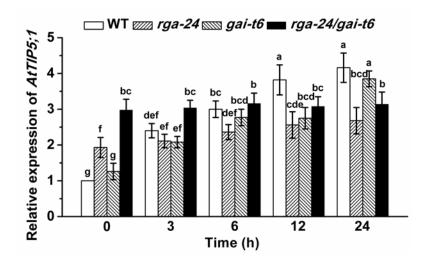


Fig. S5. Expression patterns of *AtTIP5;1* in *rga-24*, *gai-t6* and *rga-24/gai-t6* under boron stress. Ten-day-old seedlings of WT and the mutant *rga-24*, *gai-t6* and *rga-24/gai-t6* grown in MS medium were transferred to MS medium containing 3 mM H₃BO₃ for indicated hours. Quantitative real time-PCR analysis of *AtTIP5;1* was performed. *Actin2* acted as a control. Different lowercase letters represent values from the seedlings significantly differ from each other (*P*<0.05).