***New Phytologist* Supporting Information**

Article title: CsiLAC4 modulates boron flow in *Arabidopsis* and *Citrus* via high-boron-dependent lignification of cell walls
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The following Supporting Information is available for this article:

Table S3 miRNA northern blotting probe

Table S4 Specific and nested primers for 5'-RACE PCR

Table S5 Primers for RT-PCR of LACCASE-like family genes

Table S6 Boron fractions in *Citrus* leaves treated with different boric acid treatments

Fig. S1 Information of the pBI121 vector

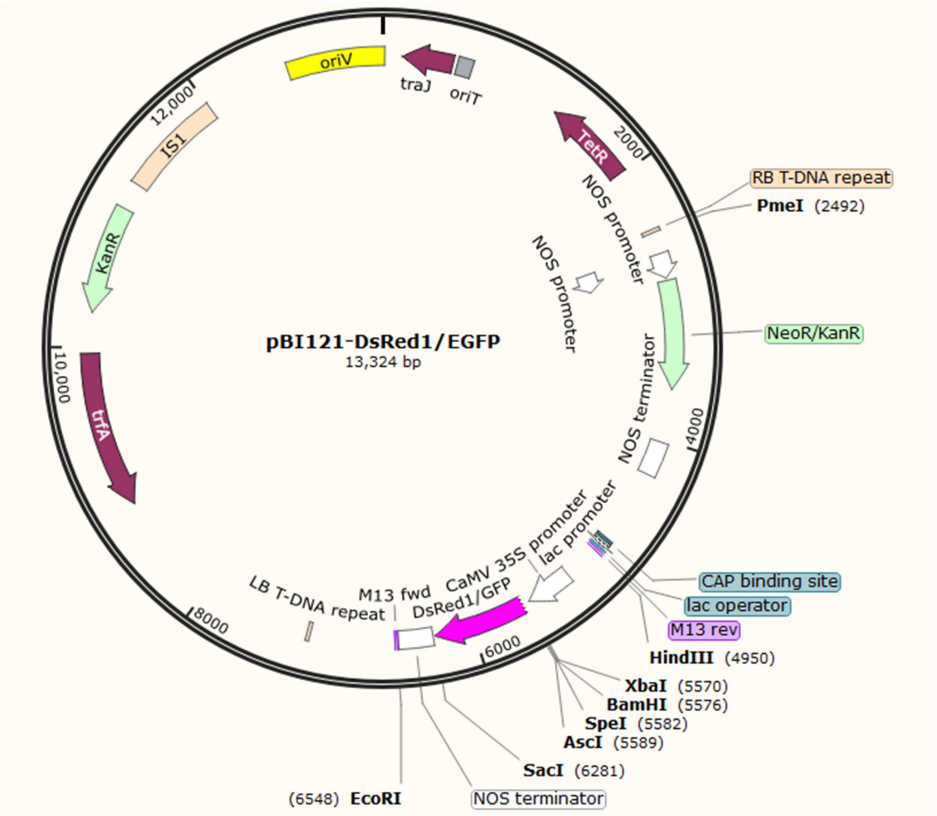

Fig. S2 Alignment of CsiLAC4 and CsiLAC17. A BLAST search of the *Citrus* genomic database (http://citrus.hzau.edu.cn/cgi-bin/orange/blast) displayed that CsiLAC4 (Cs6g07800, from *C. sinensis*) shares 99.94% identity with CgiLAC4 (Cg6g006410.1, from *C. grandis*) and that CsiLAC17 (Cs8g17630) shares 99.77% identity with CgiLAC17 (Cg8g021370.1). Therefore, we used only CsiLAC4 and CsiLAC17 for the subsequent studies. Blue lines indicate signal peptides predicted by the SignalP v5.0 program (http://www.cbs.dtu.dk/services/SignalP).

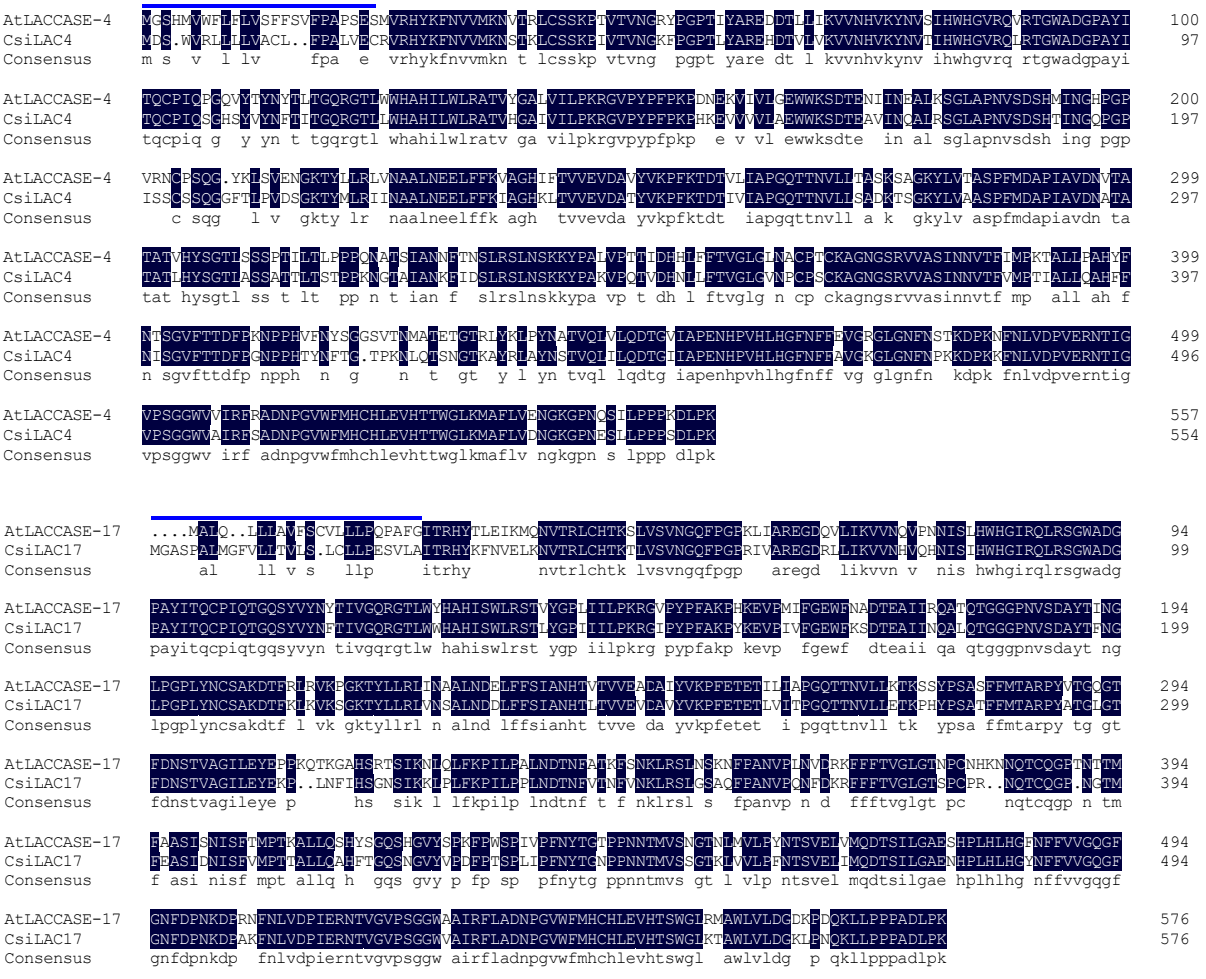

Fig. S3 Topology of CsiLAC4 (a) and CsiLAC17 (b). Signal peptide and transmembrane topology were predicted with online software Pobius (http://phobius.sbc.su.se/index.html). None of the transmembrane domains could be found by the online software SOSUI (http://harrier.nagahama-i-bio.ac.jp/sosui). Red lines indicate signal peptides; blue lines display non-cytoplasmic peptides, while green lines indicate cytoplasmic peptides.

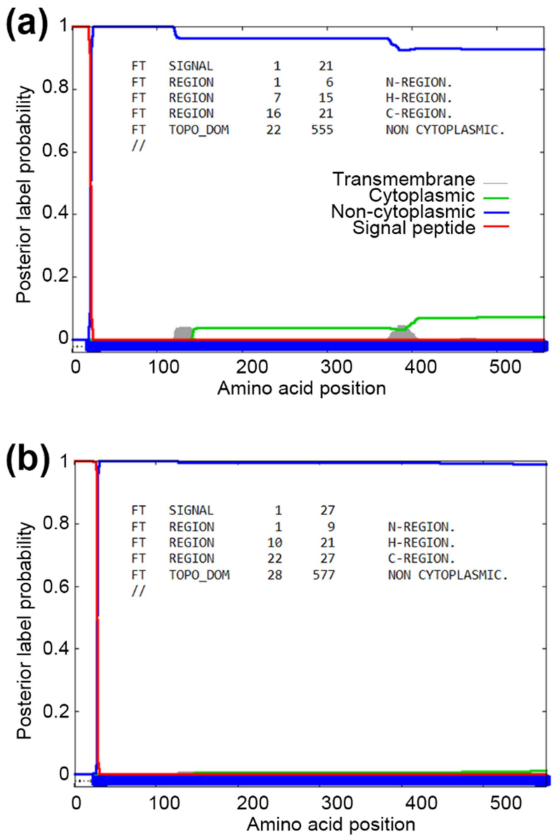

Fig. S4 Subcellular localization of CsiLAC4 and CsiLAC17. CsiLAC4-DsRed (g-l) and CsiLAC17-DsRed (m-r) were transiently introduced into tobacco epidermal cells by Agrobacterium-mediated transfection. GFP was co-transfected and used as a protoplasmic marker. (d-f, j-l and p-r) Leaf blades were plasmolyzed with 0.8 M sorbitol before being subjected to microscopy. The fluorescence signal was detected 72h post-transfection. White arrows indicate the apoplastic signals. Bars, 90 µm.

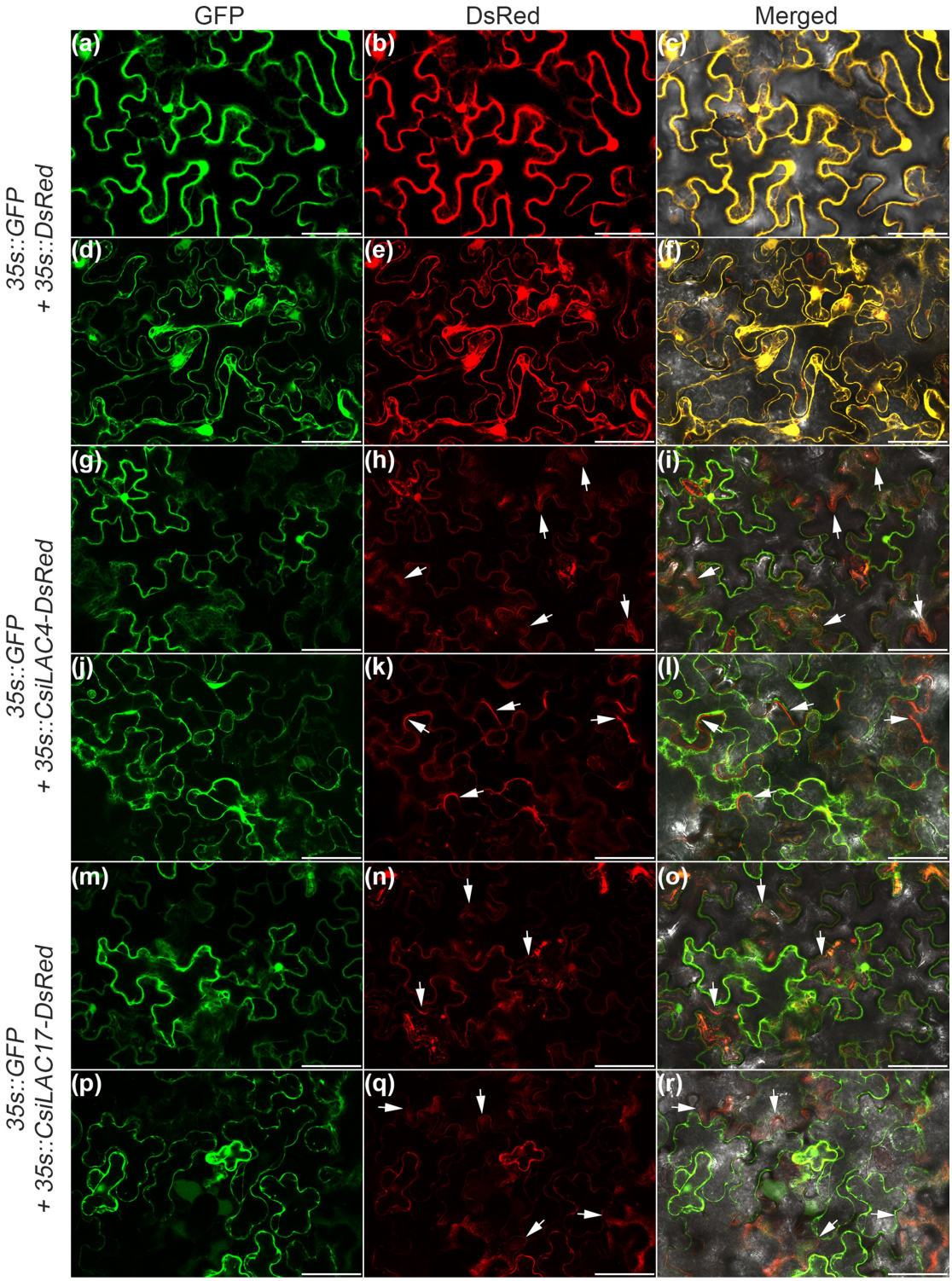

Fig. S5 Phylogenetic analysis of CsiLAC17. Multiple alignments were performed using the ClustalW tool of MEGA7 (https://www.megasoftware.net/). An unrooted phylogenetic tree was then constructed using MEGA7 (Neighbor-Joining method with 1000 bootstrap test). The 0.10 scale bar shows substitution distance. LAC17 homologs in woody *C. grandis* and *C. sinensis* and herbaceous plants (*A. thaliana* and *N. tabacum*) are shown.

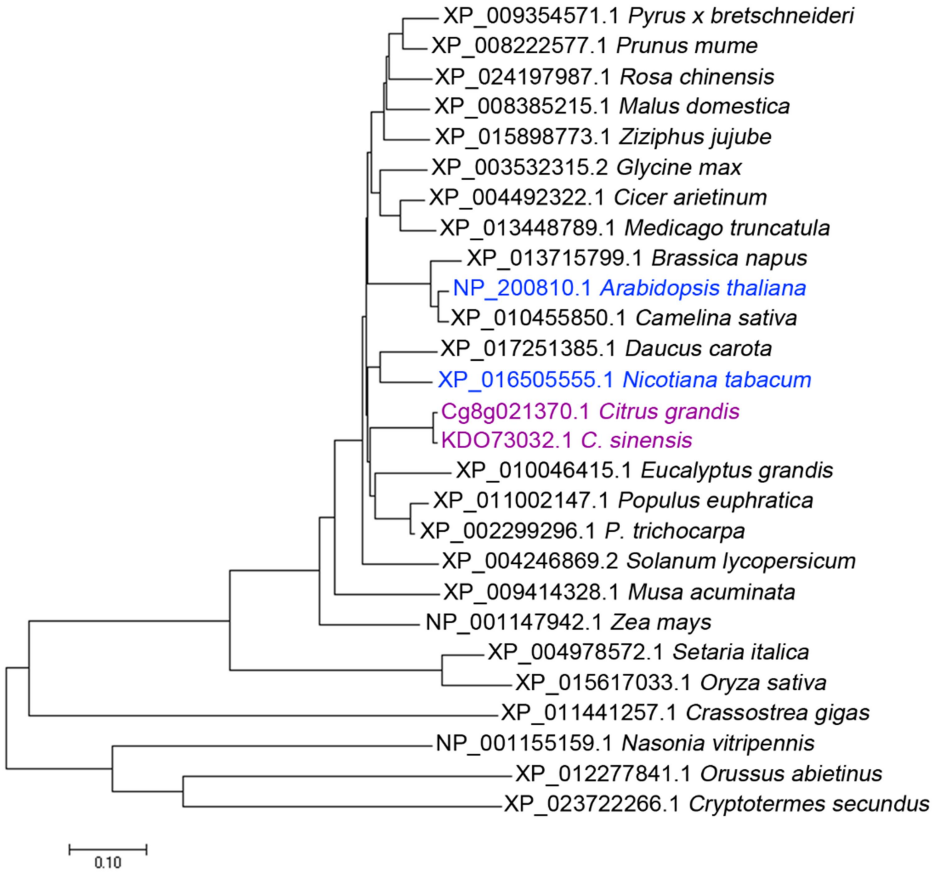

Fig. S6 RT-PCR detection of *CsiLAC4* (a) and *CsiLAC17* (b) and lignin contents (c) in the corresponding transgenic *Arabidopsis* lines. (a-b) Gene expression in shoots was detected from two-week-old T3 seedlings. (c) Rosette leaves and inflorescence stems were dissected from two-month-old T3 plants. Ranges of lignin content are 145.8-155.1, 142.3-150.3, 126.3-157.5 and 126.8-141.3 mg per grand cell-wall material (CWM) in rosette leaf, and 232.6-253.4, 178.9-262.2, 275.5-296.0 and 255.3-295.3 in inflorescence stem for Col-0, miR397^{OE}, CsiLAC4^{OE} and CsiLAC17^{OE} line, respectively. Significant difference are shown at $P < 0.05$ level by One-way ANOVA (S-N-K method, n = 3, except for n = 5 for miR397^{OE} line)

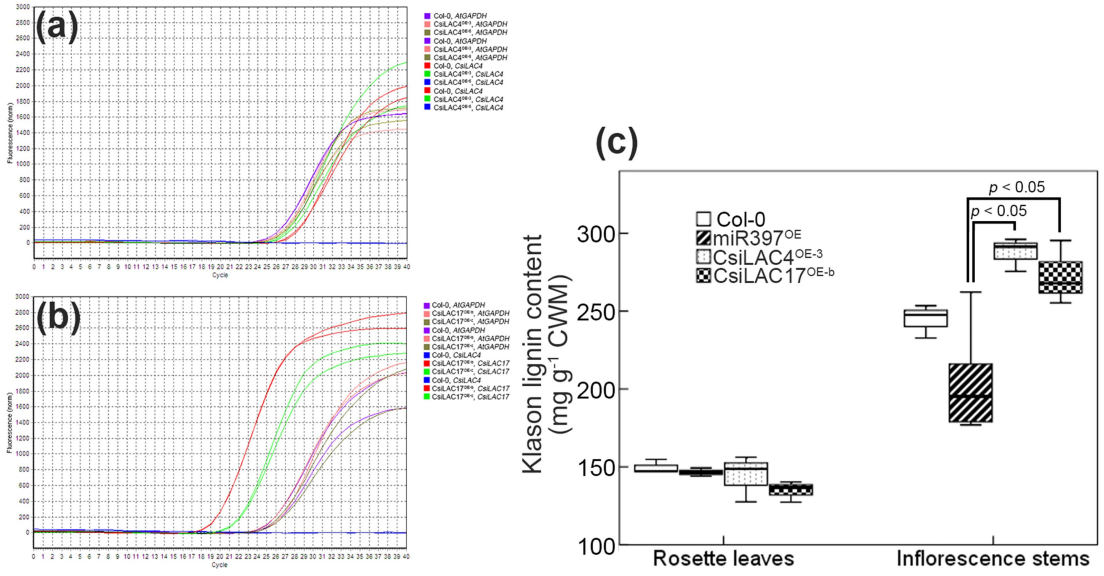

Fig. S7 Supplementation of boric acid (a and b) or NaCl (c and d) significantly increases the hypocotyl length of CsiLAC4^{OE} lines. Vernalized seeds sown on 1/2 MS medium were kept in a chamber under standard culture conditions for 14 days. Hypocotyl length was measured by Image J 1.48 (NIH, USA). (b and d) Results were mean +/- SD. '*' and '**' indicate significant difference at $P < 0.05$ and 0.01 level respectively by One-way ANOVA (S-N-K method, n = 6). Bars, 1 cm.

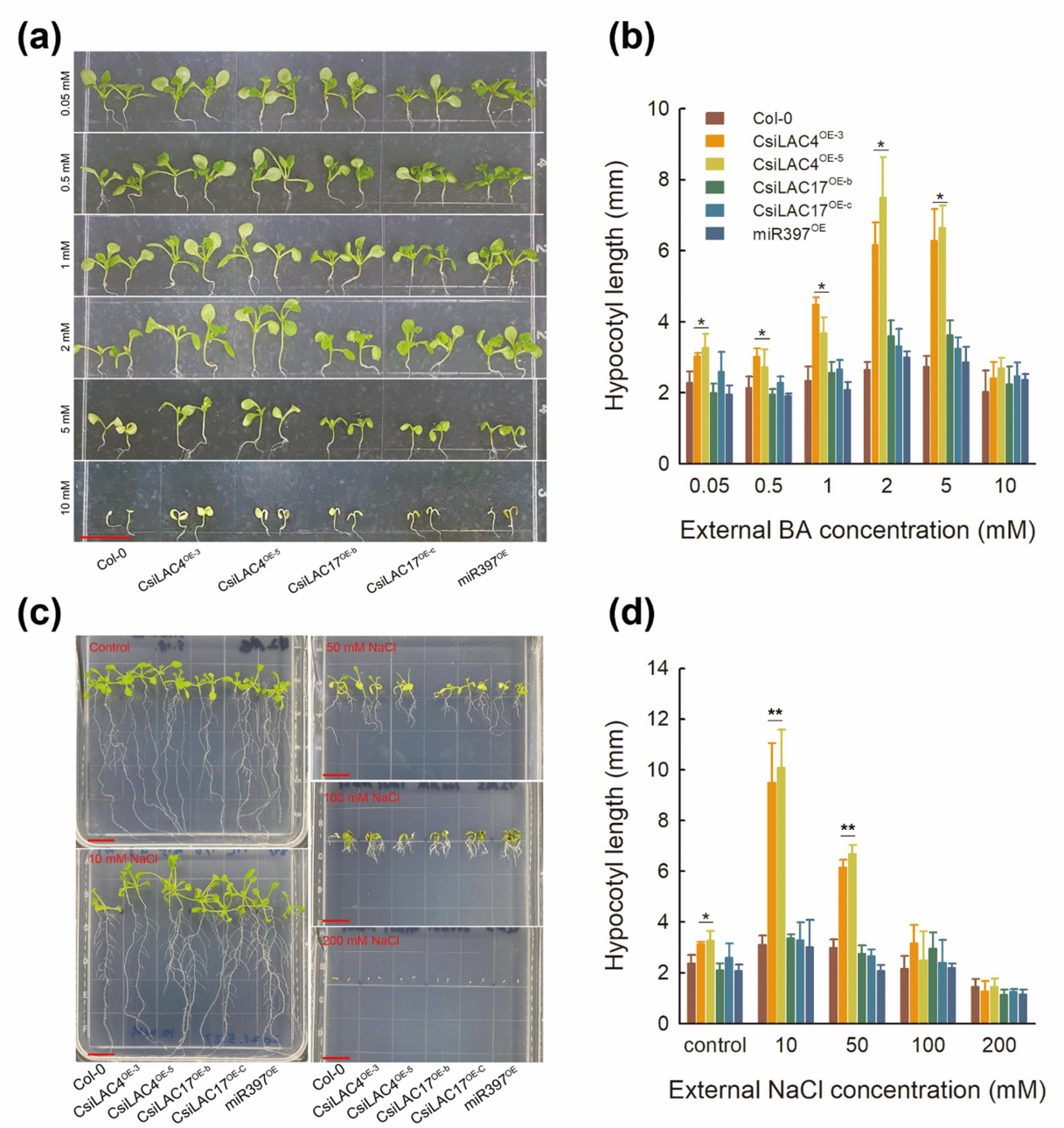

Fig. S8 Boric acid excess advances flowering of the CsiLAC4^{OE} lines. Three-week-old seedlings were irrigated with 1/2 MS liquid medium supplemented with boric acid every other day. Pictures were taken 7 d after the boric acid treatment application.

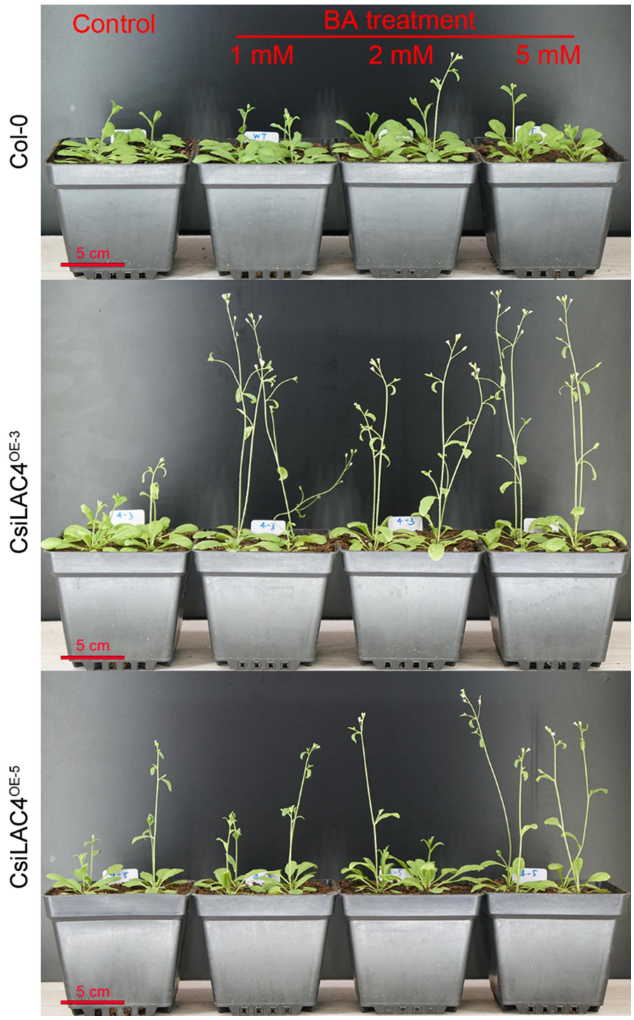

Fig. S9 Boric acid supplementation increased the diameter of metaxylem vessels in the hypocotyl of the CsiLAC4^{OE} line. (a) Transverse paraffin sections from middle hypocotyls were stained with phloroglucinol-HCl. Red arrows indicated the Casparian strip, and double red arrowheads displayed enlarged metaxylem vessels. The number of xylem vessels (b) were calculated and the diameter of metaxylem vessels (c) were determined with the Image J software. Results were mean +/- SD. '*' and '**' indicate significant difference at $P < 0.05$ and 0.01 level respectively by One-way ANOVA (S-N-K method, n = 4). Bars, 50 μm.

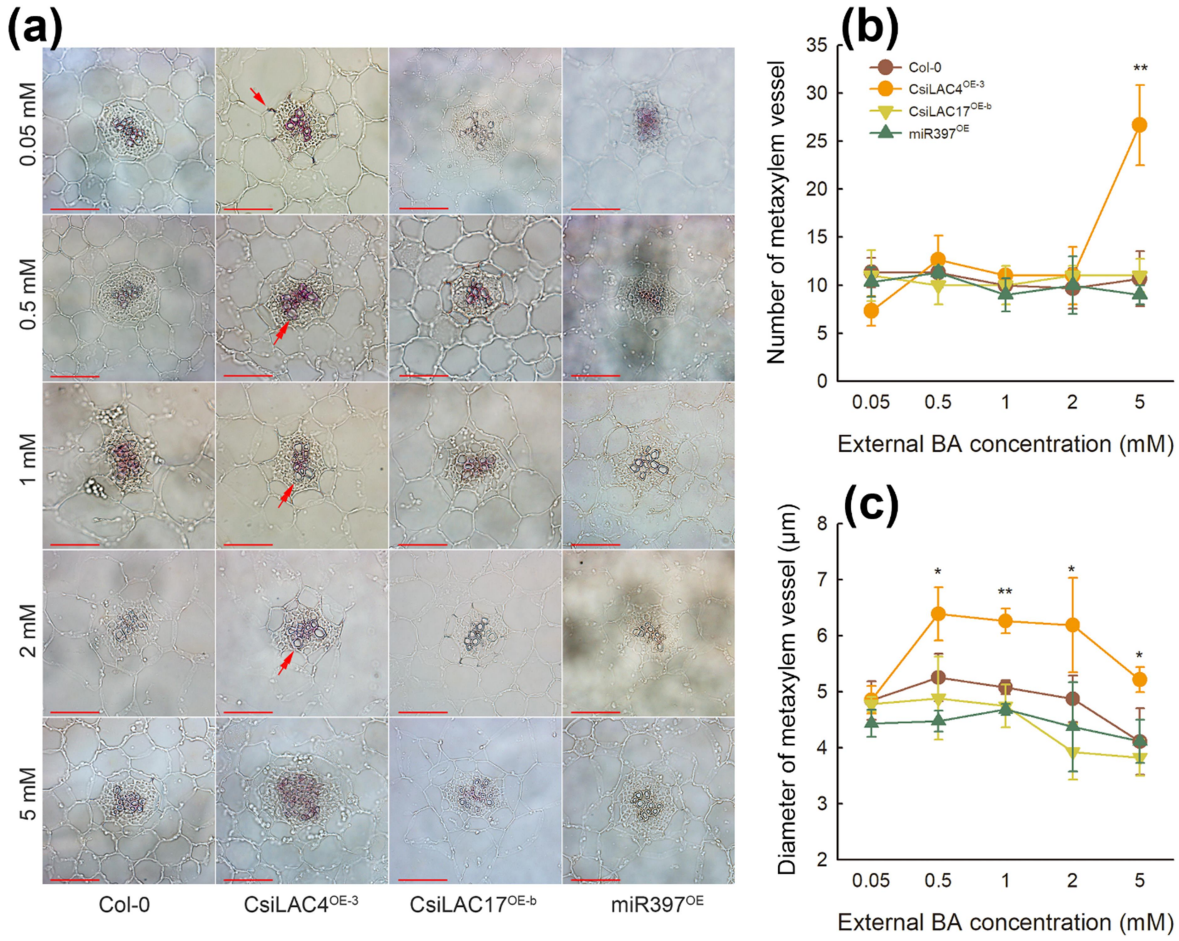

Fig. S10 Relative expressions of *AtLAC4*, *AtLAC17*, *CsiLAC4*, *CsiLAC17* and miR397 in the wild type (Col-0), CsiLAC4^{OE}, and CsiLAC17^{OE} lines (a and b) and alignment of miR397 of different plant species (c). (a-b) low boric acid level promotes *AtLAC4* and *AtLAC17* expression in all the plant lines; however, both *AtLAC4* and *AtLAC17* expressions are suppressed as external boric acid increases. *AtGAPDH* (a) and *AtUBQ5* (b) were used as internal references. (c) Matured miR397 sequences were obtained from miRBase 22.1 (http://www.mirbase.org). hvu, *Hordeum vulgare*; bdi, *Brachypodium distachyon*; tcc, *Theobroma cacao*; vvi, *Vitis vinifera*; csi, *C. sinensis*; ath, *Arabidopsis thaliana*; nta, *N. tabacum*.

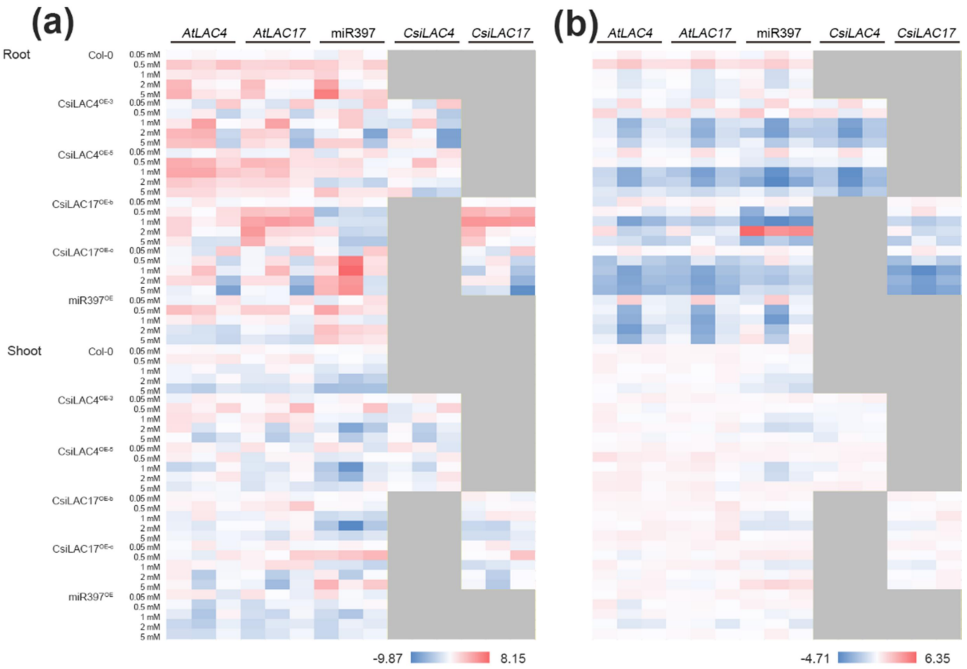

Fig. S11 Melting curves of the laccase-like family members and internal reference genes tested in Fig. S10 and S13.

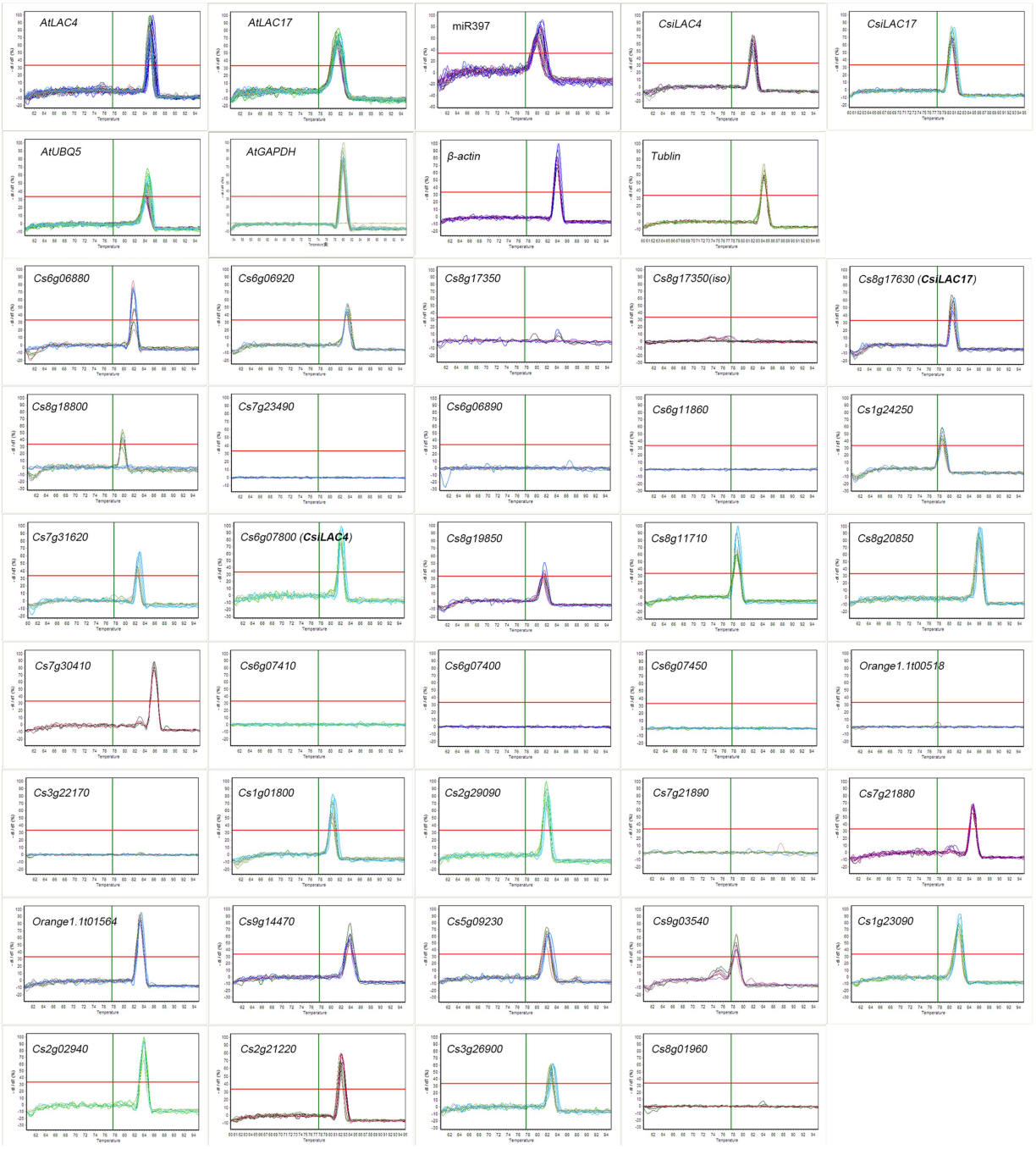

Fig. S12 Boric acid excess increases the thickness of interfascicular fiber (yellow double-arrowheads) in the inflorescence stem of CsiLAC4^{OE} lines. Hand sections were cut from mature inflorescence stem (5 cm above the rosette) 14 d post the boric acid treatment and stained with phloroglucinol-HCl. Bars, 100 µm.

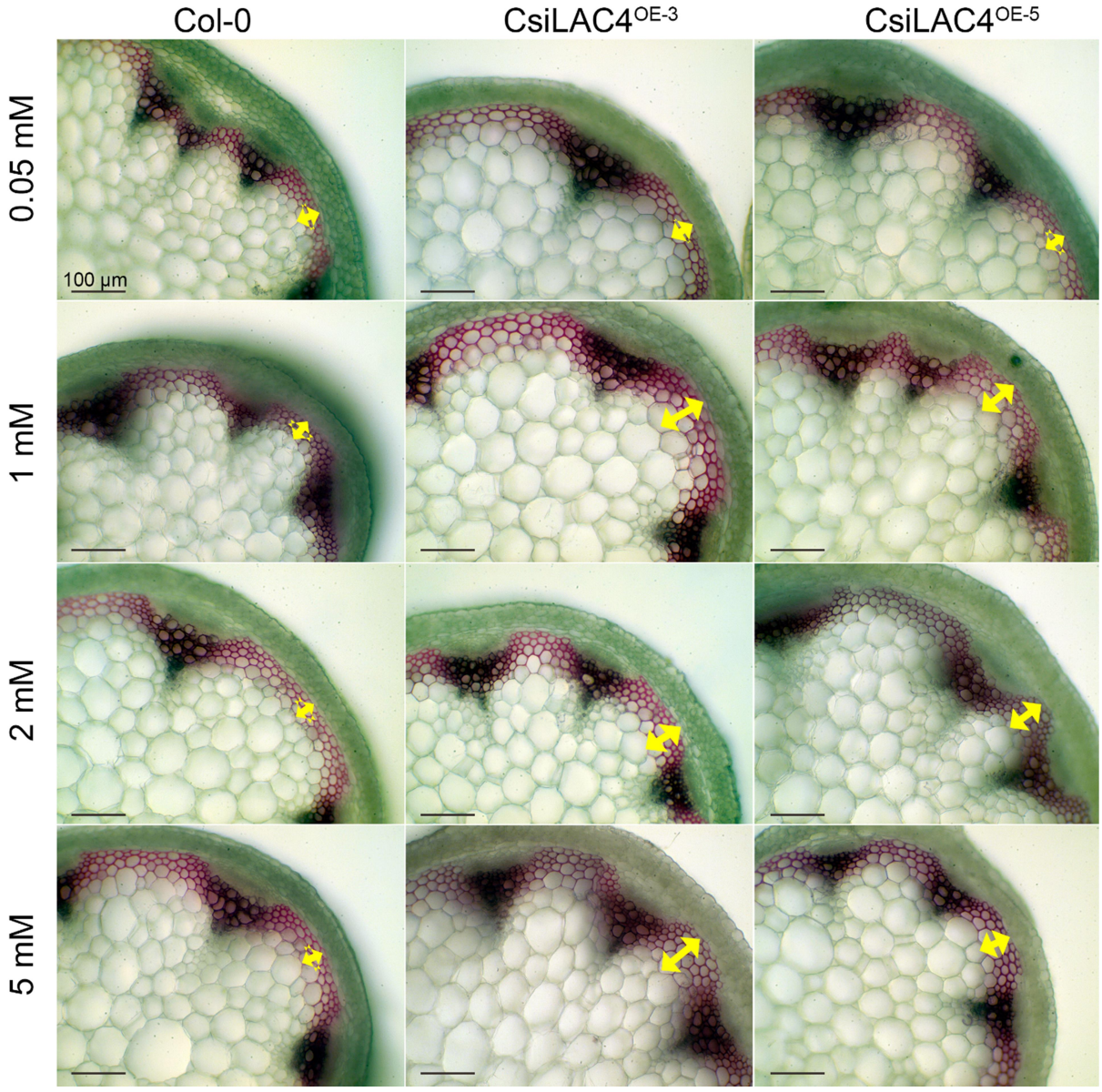

Fig. S13 Bioinformatics analysis of LACCASE-like family members in *Citrus* and their expressions in boric acid-treated leaves. (a) The phylogenetic tree was constructed with MEGA7 software (Maximum-Likelihood method with 1000 bootstrap test). The 0.20 scale bar shows substitution distance. (b-c) Relative levels of genes were expressed as 2-ΔΔCt using β-*actin* (b) or *Tubulin* (c) as internal control. Results were normalized with Log2 transformation and represented in heatmap using excel software.

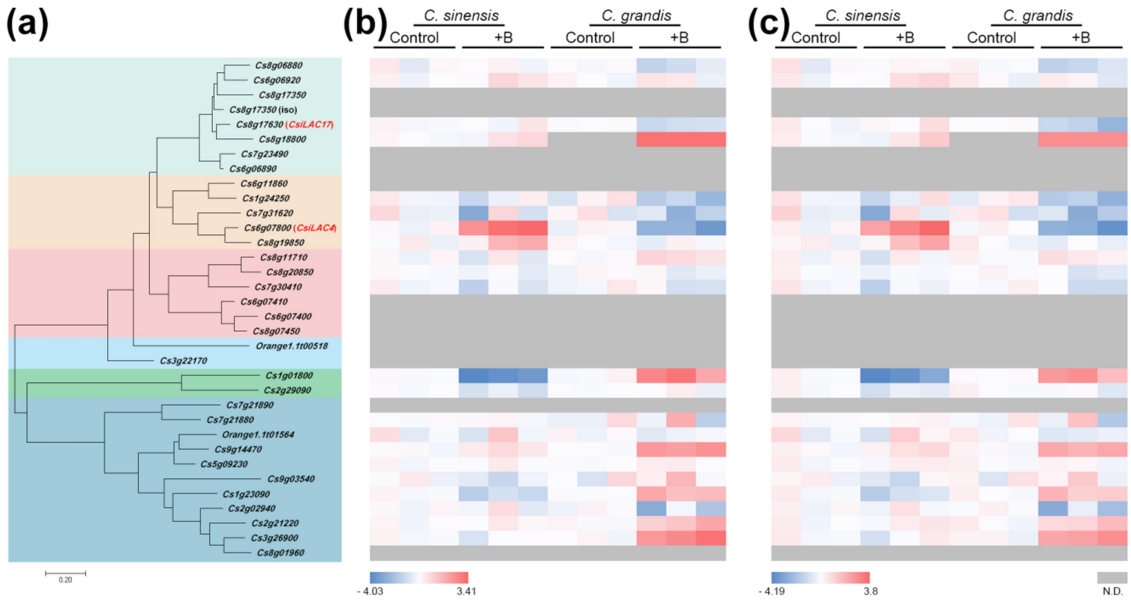

Fig. S14 Nutrient concentration in rosette leaves (a-e, p), roots (f-j, q) and root cell walls (k-o) of *Arabidopsis*. Boric acid treatment decreases Mg (a), Mn (c), Zn (e) and B (p) content but does not affect Ca (b) and Cu (d) contents in rosette leaves of CsiLAC4^{OE} lines. In the roots, boric acid treatment decreases Mg (f) and B (q) contents, but it results in the binding of more Ca (l), Mn (m), Cu (n) and Zn (o) in the cell walls of CsiLAC4^{OE} lines. Nutrient elements were determined by ICP-MS 14 d post the boric acid treatment. Results were mean +/- SD. '*' indicates significant difference at $P < 0.05$ level by One-way ANOVA (S-N-K method, n = 4).

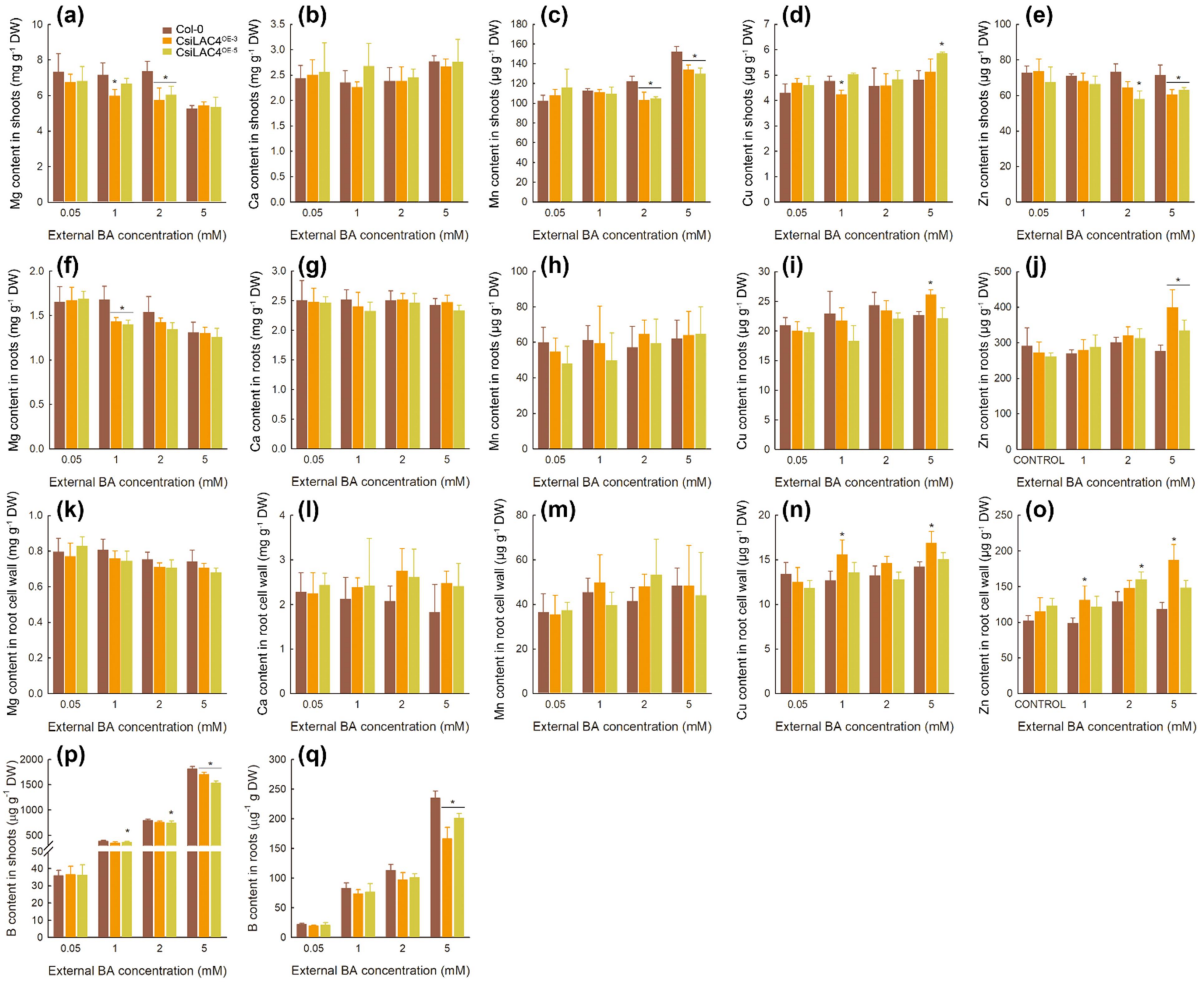

Table S1 Primers for PCR cloning of *pre-miR397*, *CsiLAC4*, *CsiLAC17*, and their corresponding probe template for *in situ* mRNA hybridization (ISH)

Genes	Primers	Sequence (5' - 3')
Pre-miR397	Forward	GGATCCATGGATCTGTTGCAGTTGAAACAAG
	Reverse	GAGCTCTTATTGACGATTGAGTCACAGAGGG
CsiLAC4	Forward	GGATCCATGGACTCCTGGGTTCGGCTTCT
	Reverse	GAGCTCTTAACACTTTGGAAGATCACTTGGAGGT
CsiLAC17	Forward	GGATCCATGGGTGCTTCTCCGGCATTAAT
	Reverse	GAGCTCTCAACACTTTGGAAGATCTGCCGGTG
CsiLAC4-DsRed	Forward	GGGACTCTTGCCTGCAGGTATGGACTCCTGGGTTCGGCTTCT
	Reverse	CGCGCCACTAGTGGATCCTACACTTTGGAAGATCACTTGGAGGT
CsiLAC17-DsRed	Forward	GGGACTCTTGCCTGCAGGTATGGGTGCTTCTCCGGCATTAAT
	Reverse	CGCGCCACTAGTGGATCCTACACTTTGGAAGATCTGCCGGTG
miR397-ISH	Forward	ATTTAGGTGACACTATAGAGCACTTGAAGATTCCCAGATTG
	Reverse	TAATACGACTCACTATAGGGAAAGAAACCTGTCAACCGTCAT
CsiLAC4-ISH	Forward	ATTTAGGTGACACTATAGCAACTCTCACCAGCACTCCT
	Reverse	TAATACGACTCACTATAGGGAGCCTGAAGCAACGCAATC
CsiLAC17-ISH	Forward	ATTTAGGTGACACTATAGGTTGTCGAAGTGGATGCTGTT
	Reverse	TAATACGACTCACTATAGGGTTGAGAGGTGGTAGGATTGGTT

Table S2 Primers for site-directed mutagenesis PCR of *CsiLAC4* and *CsiLAC17*

Primers	Sequence (5'-3')
*CsiLAC4**-F	CCTACATGCTACGAATAATCAATGCTACCCTAACTGAAGAGCTA
*CsiLAC4**-R	TTGATTATTCGTAGCATGTAGGTTTTGCCGCTGTCCACAGGCAATG
*CsiLAC17**-F	AATTCTACCCTAACTGATGACCTCTTCTTCAGTATAGCAAAT
*CsiLAC17**-R	GTCATCAGTTAGGGTAGAATTGACTAATCGGAGAAGGTAAGTTTT

Table S3 miRNA northern blotting probe

Probe	Sequence (5' - 3')
miR397	ATCAACGCTGCACTCAATGA
U6	AGGGGCCATGCTAATCTTCTC

Table S4 Specific and nested PCR primers for cleavage site validation

Primers	Sequence (5' - 3')
*CsiLAC17*_GSP	ATTGTAGCCATGCAAATGAAGAGG
Nested *CsiLAC17*_GSP	GTTTCTCATACTCTAAGATGCCAGC
*CsiLAC4*_GSP	TCATTAGGGCCTTTGCCATTGTCAACC
Nested *CsiLAC4*_GSP	TTGAATCCGTGTAAATGGACTGG

Table S5 Primers for qPCR of LACCASE-like family genes in *Citrus* and *Arabidopsis*

Primer	Sequence (5' - 3')
β-actin-F(*Citrus*)	AGAACTATGAACTGCCTGATGGC
β-actin-R(*Citrus*)	GCTTGGAGCAAGTGCTGTGATT
Tubulin-F	GCATCTTGAACCCGGTAC
Tubulin-R	ATCAATTCGGCGCCTTCAG
Cs6g06880-F	TGCTCAGCCAAAGATACATTCA
Cs6g06880-R	AGAAAGTGGCATTTGGGTAAGA
Cs6g06920-F	CAGGAAAGACATACCTCTTGAGAC
Cs6g06920-R	AGTGTCGAAAGGCTTGACG
Cs8g 17350-F2	ATTTATCGCCCTGTGTCTGCT
Cs8g 17350-R2	GACCTCCGTTGAACCACTCTC
Cs8g17350-F (iso)	CTTCAGCATAGCAAACCACACC
Cs8g17350-R (iso)	TTTCATACTCTAAGATACCAGCAACG
Cs8g17630-F (*CsiLAC17*)	CTTCTCCGATTAGTCAATTCTGC
Cs8g17630-R (*CsiLAC17*)	ATGACTAGCGTTTCGGTCTCAA
Cs8g18800-F	GAGGCAATTATCAATCAGTCTTTAC
Cs8g18800-R	GCTTCAACAACAGTGAGCGTA
Cs7g23490-F	AAACAGGAGGAGCACCGAATA
Cs7g23490-R	GTTGGCGTTAGGAGCCTTAGAT
Cs6g06890-F	TGGAGAATGGTGGAAAGCAGACA
Cs6g06890-R	GCAATTATACAAGGGACCAGGAAGA
Cs6g11860-F	CTCTTCCCTTGCTCTGAGAAA
Cs6g11860-R	CAAGAACATTTGTAGTTTGACCTG
Cs1g24250-F	CTTTCTCTTTCTGCTACTTGGACTT
Cs1g24250-R	GCTAACATTCTTCACTTGAACATCG
Cs7g31620-F	TCAGGGCAGATAATCCAGGTGTT
Cs7g31620-R	CGTCGGAAGGTCACTAGGAGGAG
Cs6g07800-F (*CsiLAC4*)	CGTCACAGGGAGGCTTTACATT
Cs6g07800-R (*CsiLAC4*)	TGGTTTAACATAAGTGGCATCA
Cs8g19850-F	CGGGAAGACGTACATGCTGAG

Cs8g19850-R	TCAACGACTGTGAGTTTATGGC
Cs8g11710-F	TTGCTTTCGTGGCTTCTCTTTCT
Cs8g11710-R	CTCTTCACTGGCTTGGCTTCA
Cs8g20850-T	AAATGCTGCCTTTGACAATACC
Cs8g20850-R	CCATAGACCTCGGCTTACATTAC
Cs7g30410-F	CAAGCACATCACCACGGCAT
Cs7g30410-R	CTCCAACAGGCACACCAACG
Cs6g07410-F	GCTCTCAAAATCAAACCTACACGC
Cs6g07410-R	GGTCCACCCTTTAATCCAGTCA
Cs6g07400-F	ATCCCTGCTCGGAAAATCAAA
Cs6g07400-R	CGTGTAGGCTGTAGGCGTATCA
Cs6g07450-F	GGAAGGGAAGACATATCTGCTAC
Cs6g07450-R	GACGTAGGGATCAGTGTAGGAA
Orange1.1t00518-F	TATGGCTATGCTTGTTATTCTGTG
Orange1.1t00518-R	GACTGTGACGATGTCTTTGGC
Cs3g22170-F	GGCTTACTACGAACGCATCA
Cs3g22170-R	TTCCGAACCCAAATCCAAC
Cs1g01800-F	TTGAGTAACAAGACGAGTGAGACA
Cs1g01800-R	ATCCATAACGATGAACAGGCA
Cs2g29090-F	GGAAGAAAACCTGAAACCCCT
Cs2g29090-R	TGTTGGAGAATGATTTGCTGTG
Cs7g21890-F	TAGTAGTATTTACGGTGTTGCGG
Cs7g21890-R	CCTCTAATGTCCTTGGCTTGA
Cs7g21880-F	ATCGCATAGTCATTTCTGTCCC
Cs7g21880-R	TTGTAAGCCGTAAGTGAAGTGTT
Orange1.1t01564-F	GGCAGTTTCCAGGACCGAT
Orange1.1t01564-R	CCTTAGTTTCTTATGACTCTTCGT
Cs9g14470-F	ACCAAGTGACATTTACAACCAA
Cs9g14470-R	GAACCATAATGAAAAGAACCCT
Cs5g09230-F	AGAGAGCATCGGGCGGTTTCG
Cs5g09230-R	GAGCCGTGTGGTTCCTCGTGT
Cs9g03540-F	CTTTAGGATACAGGGTCACACG
Cs9g03540-R	CCATAATACTCCGCAAACTTCA
Cs1g23090-F	GGTCCTAGATTGGATGTGGTAA
Cs1g23090-R	TCAGAGTTGATGGGAAGTAAAAGT
Cs2g02940-F	TGCTGGGGATTTTACTGTGCT
Cs2g02940-R	CAAGATGAATGTCAAGGGCGG
Cs2g21220-F	ACTTATTCCTGTGCCCTTTGA
Cs2g21220-R	GTGTTAGCATTTGAACCCCGA
Cs3g26900-F	TTGGGTTGTCGGTATGGATGGAG
Cs3g26900-R	ATGACTTGGGATACACCTGAACG

Cs8g01960-F	CAGCCCCCACAGGATTTCTAC
Cs8g01960-R	GTTTGGTCTTGGTCCACTTGC
AtGAPDH2-F	GTTTGTGTGTGGTTGAGTTC
AtGAPDH2-R	TTCAATGTTCCAGTTGCCAG
AtUBQ5-F	CTCCTTCTTTCTGGTAAACGT
AtUBQ5-R	GGTGCTAAGAAGAGGAAGAAT
AthLAC17-F	GTGCCAAACAACATCTCTCTCCA
AthLAC17-R	CCATCAGCCCAACCACTTCGTAA
AthLAC4-F	CTACGCACGAGAAGATGACAC
AthLAC4-R	ATGTGTAGACTTGACCAGGCT
stl-mi397-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACATCAAC
miR397-Forward	GGTCTCATTGAGTGCAGCGTTG
Universal Reverse	GTGCAGGGTCCGAGGT

Table S6 Boron fractions in *Citrus* leaves treated with different B treatments. Results were mean +/- SD. Different small letters in the same column indicated significant differences at $P < 0.05$ level (One-way ANOVA).

		Water-soluble B (μg g^{-1}, FW)	Organic-bound B (μg g^{-1}, FW)	Cell wall-bound B (μg g^{-1}, FW)
C. sinensis	Control	4.66 ± 1.42 [b]	4.33 ± 0.81	5.06 ± 0.61
	B-toxicity	180.65 ± 12.52 [a]	4.51 ± 0.65	5.62 ± 1.08
C. grandis	Control	4.13 ± 1.01 [b]	3.65 ± 0.97	5.33 ± 0.83
	B-toxicity	174 ± 10.18 [a]	4.30 ± 0.45	4.98 ± 0.77