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Supplementary Information for

Suberin plasticity to developmental and exogenous cues is regulated by a set of MYB transcription factors

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This PDF file includes:

Supplementary Information Text

Supplementary material and methods

Gene numbers

The corresponding gene numbers are as follow: *CASP1*, At2g36100; *CASP3*, At2g27370; *ESB1*, At2g28670; *SGN3*, At4g20140; *CDEF1*, At4g30140; *ELTP*, At2g48140; *ABI1*, At4g26080; *GPAT5*, At3g11430; *SYP122*, At3g52400, *MYB41*, At4g28110; *MYB53*, At5g65230; *MYB92*, At5g10280; *MYB93*, At1g34670.

Constructs

Plasmids were constructed using Multisite Gateway cloning (Thermo Fisher Scientific). The list of primers used for cloning are presented in Table S1. *MYB* promoter sequences upstream of ATG - *MYB41* (2167bp), *MYB53* (4117bp), *MYB92* (4098bp), *MYB93* (2873bp) were amplified from Col-0 genomic DNA and cloned into *pDONRP4-P1R* (Thermo Fisher Scientific). For promoter-reporter expression clones, *PROMOTER::NLS-3xmVenus or PROMOTER::NLS-3xmScarlet,* the entry plasmids containing the promoter region, along with *pDONRL1-NLS-3xmVenus-L2* (1) or *pDONRL1-NLS-3xmScarlet-L2* and the pEN-R2-*tNOS*-L3 containing the terminator *tNOS* (2) were recombined into the destination vectors pFR7m34GW or pFG7m34GW. The destination vectors pFR7m34GW or pFG7m34GW were obtained by substitution of the Hygromycin sequence in pH7m34GW by the FastRed and FastGreen sequences respectively (3). For endodermal specific expression of MYBs using *CASP1* and *ELTP* promoters (4, 5), MYB coding sequences were amplified from wild-type Arabidopsis cDNA and cloned into pDONR221_L1-ORF-L2 vector were recombined with pDONR-P3-*tNOS*-P2R in the destination plasmid pFR7m34GW. Except for *MYB41cDNA* that was obtained from (6) and recloned into pDONR221_L1-CDDB-CAM-L2. For endodermal specific estradiol inducible *MYB41* expression (*CASP1xve::MYB41-mVenus)*, the entry vectors containing the inducible *CASP1* promoter pEN-L4-CASP1xve-R1 (7) was recombined with pDONR221_L1-MYB41nostop-L2 and pEN-R2-mVenus +stop-L3 into the destination vector pFG7m34GW. Cloning of vectors for CRISPR/Cas9 was done as previously described in (8-10). sgRNA for spCas9 were designed using webtools – CRISPR-P 2.0 design tool (http://crispr.hzau.edu.cn/CRISPR2/) (11) and Benchling (https://www.benchling.com). Pairs of annealed oligos of the sgRNA were cloned into the Bbs-I linearized entry vector (8) and recombined into the destination vector containing Cas9 expression cassette and a FastRed or FastGreen selection marker cassette. For large deletion of genomic regions in *myb41_c1* or multiplex targeting of *MYB53*, *MYB92* and *MYB93*, multiple entry vectors were used to clone different sgRNAs. Thereafter, recombined into the destination vector containing Cas9 expression cassette and FastRed or FastGreen selection marker cassette. After fluorescent seed selection in T1, non-fluorescent seeds in the T2 generation (indicating a segregation of the vector backbone containing the Cas9 cassette) were used to

identify the mutations. Primary screening of mutants was done using High-Resolution Melting (HRM) curve analysis as previously described in (9). Candidates from HRM analysis were further confirmed for the mutations by sequencing of PCR-amplified genomic regions. Absence of off-target effects were controlled by sequencing the closest *MYB* homologues in the final mutant. To test the loss of function for *myb41_c2, myb53_c1, myb92*_c1 and *myb93_c1*, the corresponding cDNA were cloned from the mutated plants into pDONR221 and recombined with pEN-L4-CASP1-R1 and pDONR-P3-*tNOS*-P2R in the destination plasmid pFRm34GW. All constructs were transformed into Agrobacterium strain GV3101 by electroporation and used for transformation of Arabidopsis plants by the floral-dip method (12).

Growth conditions

Seedlings for staining and live-imagine were grown vertically on square plates containing half-strength MS with 0.8% agar (Duchefa), without sucrose. Seeds were surface sterilized before sowing on plates and were incubated 2 to 4 days at 4°C and put to grow in growth chambers under continuous light (~100 µE) at 22 °C. All histological and live-microscopy analysis were performed on 5-day old seedlings. For other experiments the age of the plant is specified in the figure legends. In soil, for amplifications and experiments in pots, plants were grown in long-day conditions (16 h day, 8 h night) with light intensity of 150-180 µE with 60-70% humidity and at 20 ± 2 °C.

Image analyisis

For suberin pattern quantifications, tiled images covering the whole seedlings in single images were captured with an epifluorescence stereomicroscope- ZEISS Axio Zoom.V16. For imaging of large field of view ath high-resolution, multiple small images were captured as tiles and stitched. Region of interest of the root was defined by marking the 'tile-region' after a quick scan of the sample at lower resolution. Adequate number of focus points were used to adjust the focus of the sample along the region of interest. 10% area of overlap was defined for alignment and stitching of tiles. Fiji (http://fiji.sc/Fiji) (13) was used on Zen2.3 blue exported stitched tile images for quantification of suberin patterns (in mm) along the root: suberized for the fully suberized zone, patchy for the partially suberized zone and non-suberized –for the zone prior to suberization. Results are presented as percentage of the root as previously done (14, 15).

For analysis of promoter reporter lines, imaging of large field of view at high-resolution, were captured as tiles and stitched together for a larger view of roots. Region of interest of the root was defined by marking the 'tile-region' after a quick scan of the sample at lower resolution. Adequate number of focus points were used to adjust the focus of the sample along the region of interest. Acquisition of tiled images was combined with Z-stacking and in certain cases with time series as well. 10% area of overlap

was defined for alignment and stitching of tiles and tiled Z-stacks were used for orthogonal projection and subsequently exported. For time-course experiments, 25-30 min time interval in between the scans was defined for 10-12 cycles. Scanner and detector settings were kept unchanged for every experiment. Images were analyzed with Zen2.3 blue (LSM 800) or Zen2.3 black (LSM 780) software and Fiji (http://fiji.sc/Fiji) (13). Fluorescence intensities were calculated nucleus by nucleus along one cell file from the onset of nuclear signal, considering the maximum intensity detected in each individual nucleus as an estimate the difference of intensity between nuclei.

Lignin staining

CearSee-adapted cell wall staining was performed as described (16). Briefly, 5-day-old seedlings were fixed in 1 × PBS containing 4% paraformaldehyde, 1 h at room temperature and washed twice with 1 × PBS. Following fixation, the seedlings were cleared overnight in ClearSee solution after which the solution was exchanged to 0.2% Basic Fuchsin in ClearSee solution lignin staining. After overnight staining, the dye solution was removed and rinsed once with ClearSee solution, the seedlings were subsequently washed in ClearSee solution for 30 min and washed again in another ClearSee solution for at least one overnight before observation with a Leica SP8 confocal. All clearing, staining and washing steps were performed in 12 well plates, covered with aluminum foil and under gentle agitation.

Propidium iodide test

Propidium iodide (PI) was used as an apoplastic tracer to assess Casparian strip functionality as previously described (7, 17). Seedlings were live-stained with 15 µM PI; kept in the dark for 10 min and then rinsed twice with water. The apoplastic barrier was determined under a fluorescent Leica DM6 B microscope with I3 filter and 20x magnification, as the number of endodermal cells after the onset of elongation where PI uptake is blocked at the endodermis. The onset of elongation was defined as the first endodermal cell for which the length was at least three times its width.

Chemical suberin analysis

5-day-old roots were shaved off after flash freezing and extracted in isopropanol/0.01% butylated hydroxytoluene (BHT). They were then delipidized two times (16h, 8h) in each of the following solvents, i.e., chloroform-methanol (2:1), chloroform-methanol (1:1), methanol each with 0.01% BHT, under agitation before being dried for 3 days under vacuum. Depolymerization was performed by base catalysis (18). Briefly, dried plant samples were trans-esterified in 2 mL of reaction medium. 20 mL reaction medium was composed of 3 mL methyl acetate, 5 mL of 25% sodium methoxide in dry methanol and 12 mL dry methanol. The equivalents of 5 mg of methyl heptadecanoate and 10 mg of ω-pentadeca-lactone/sample were added as internal standards. After incubation of the samples at 60°C for 2h 3.5 mL dichloromethane, 0.7 mL glacial acetic acid and 1 mL 0.9% NaCl (w/v) /100 mM Tris-HCl (pH 8.0) were added to each sample and subsequently vortexed for 20 s. After centrifugation (1500g for

2 min), the organic phase was collected, washed with 2 mL of 0.9% NaCl, and dried over sodium sulfate. The organic phase was then recovered and concentrated under a stream of nitrogen. The resulting suberin monomer fraction was derivatized with BFTSA/pyridine (1:1) at 70°C for 1 h and injected out of hexane on a HP-5MS column (J&W Scientific) in a gas chromatograph coupled to a mass spectrometer and a flame ionization detector (Agilent 6890N GC Network systems). The temperature cycle of the oven was the following: 2 min at 50°C, increment of 20°C/min to 160°C, of 2°C/min to 250°C and 10°C/min to 310°C, held for 15 min. 3 independent experiments were performed with 4 replicates for each genotype, respectively, and a representative dataset is presented. The amounts of unsubstituted C16 and C18 fatty acids were not evaluated because of their omnipresence in the plant and in the environment.

Ionomic analysis

Dried leaves were transferred into the Pyrex test tubes, weighted, and digested with 1 ml of concentrated trace metal grade nitric acid Primar Plus (Fisher Chemicals) containing an indium internal standard, in the dry block heaters (SCP Science; QMX Laboratories) at 115˚C for 4 h. After cooling, digested samples were diluted to 10mL with 18.2 MΩcm Milli-Q Direct water (Merck Millipore) and elemental analysis was performed using an ICP-MS (PerkinElmer NexION 2000 equipped with Elemental Scientific Inc autosampler) in the collision mode (He). A matrix-matched liquid reference material composed of pooled digested samples was prepared before the beginning of the sample run and used every ninth sample to correct for variation within ICP-MS analysis runs. The calibration standards were prepared from single element standards solutions (Inorganic Ventures; Essex Scientific Laboratory Supplies Ltd, Essex, UK). Samples concentrations were calculated using external calibration method within the instrument software. The final concentrations were obtained by normalizing the element concentrations to the sample dry weight.

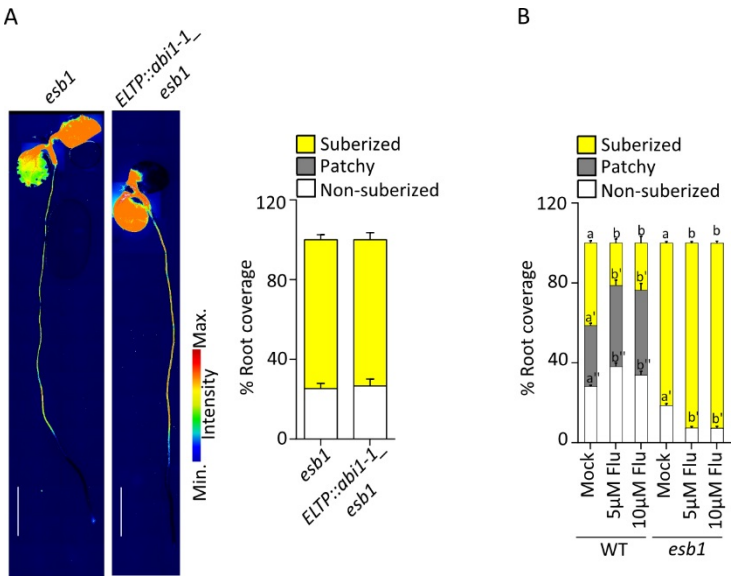

Figure S1. Enhanced suberin phenotypes in CS mutants are ABA-independent. (*A*) Fluorol Yellow (FY) staining for suberin in *esb1* and *ELTP::abi1-1_esb1* plants. Whole-mount staining in full seedlings (*Left panels*) and quantifications of suberin pattern along the root (*Right panel*), n ≥ 10, error bars, standard deviation. No significant difference observed between genotypes. Scale bars, 2 mm. (B) FY staining in WT and *esb1* plants in mock conditions or treated with 5 or 10 µM Fluridone (Flu.) for 16 h. Different letters indicate significant differences between conditions for a given genotype ($P < 0.05$).

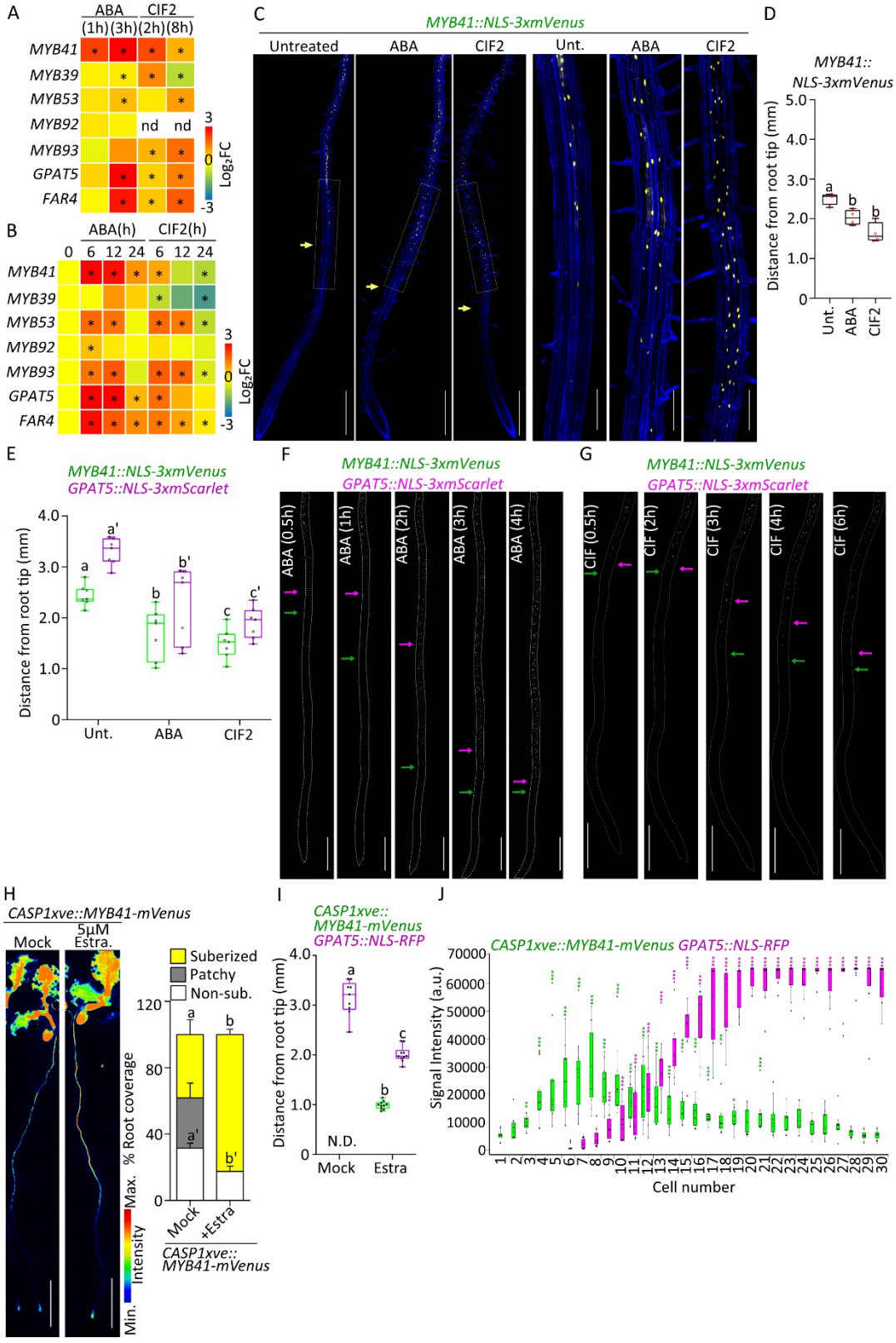

Figure S2. *MYB41* regulation and function in suberin regulation. (*A*) Comparative expression profiles of *MYB* candidate genes upon 1 h and 3 h ABA treatments from a whole seedling microarray dataset (19) and upon 2 h and 8 h CIF2 treatment in a root RNA-seq dataset (20). Asterisks indicate statistically significant differences ($P < 0.05$). (*B*) Relative expression levels of the candidate *MYBs* and two suberin biosynthesis genes in WT roots treated with 1 µM ABA or 1 µM CIF2 for 6, 12 and 24 h (n = 4 pools of 25-30 roots). Results are presented as fold changes compared to the mock condition. Numeric values are presented in Table S3. Asterisks indicate statistically significant differences ($P < 0.05$). *(C) MYB41::NLS-3xmVenus* expression (in yellow) untreated or treated with 1

µM ABA or 1 µM CIF2 for 16 h. Pictures are presented as maximum intensity Z projections taken from the root tip to 4 - 5 mm (*Left panels*) with closer views in the zone of patchy suberization in untreated condition. Propidium iodide (PI, in blue) was used to highlight cells. Scale bars, 500 µm (*Left*), 125 µm (*Right*). (*D*) Distance from the root tip to the first endodermal cell with NLS-3xmVenus signal in *MYB41::NLS-3xmVenus* background (mm). Data are presented as box plots with individual values overlaid, n ≥ 5, different letters indicate significant differences between conditions ($P < 0.05$). (*E*) Distance from the root tip to the first endodermal cell with NLS-3xmScarlet and NLS-3xmVenus signals in *GPAT5::NLS-3xmScarlet* x *MYB41::NLS-3xmVenus* background. Data are presented as box plots with individual values overlaid, n ≥ 7, different letters indicate significant differences between conditions ($P < 0.05$). (*F-G*) Live imaging of the dual reporter for *GPAT5::NLS-3xmScarlet* and *MYB41::NLS-3xmVenus* upon treatment with 1 µM ABA (*F*) or 1 µM CIF2 (*G*). Pictures are presented as maximum intensity Z projections from the root tip to 4-5 mm. Time course after ABA and CIF2 treatments (0.5 to 4/6 h). Arrows highlight the onset of *MYB41* (green) and *GPAT5* (magenta) expression. Scale bars, 500 µm. (*H*) Fluorol Yellow staining for suberin of *CASP1xve::MYB41* after 16 h of mock or 5 µM Estradiol treatment. Whole-mount staining (*Left panels*) and quantifications of suberin pattern are presented (*Right panel*), n ≥ 10, error bars, standard deviation, different letters indicate significant differences between conditions *(*$P < 0.05$). Scale bars, 2 mm. (*I*) Distance from the root tip to the first endodermal cell with MYB41-mVenus and NLS-RFP signals in *CASP1xve::MYB41-mVenus* x *GPAT5::NLS-RFP* background. Data are presented as box plots with individual values overlaid, n ≥ 7, different letters indicate significant differences between conditions ($P < 0.05$). *(J)* Signal intensity for MYB41-mVenus (green) and NLS-RFP (magenta) along 30 endodermal cells from the onset of *MYB41-mVenus* signal in the background *CASP1xve::MYB41-mVenus* x *GPAT5::NLS-RFP* after 5 µM Estradiol (Estra.) treatment for 16 h. Data are presented as box plot with individual values overlaid, n = 10, significant differences to the first cell with signal (cell 1 for MYB41-mVenus and cell 6 for NLS-RFP*)*, * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$.

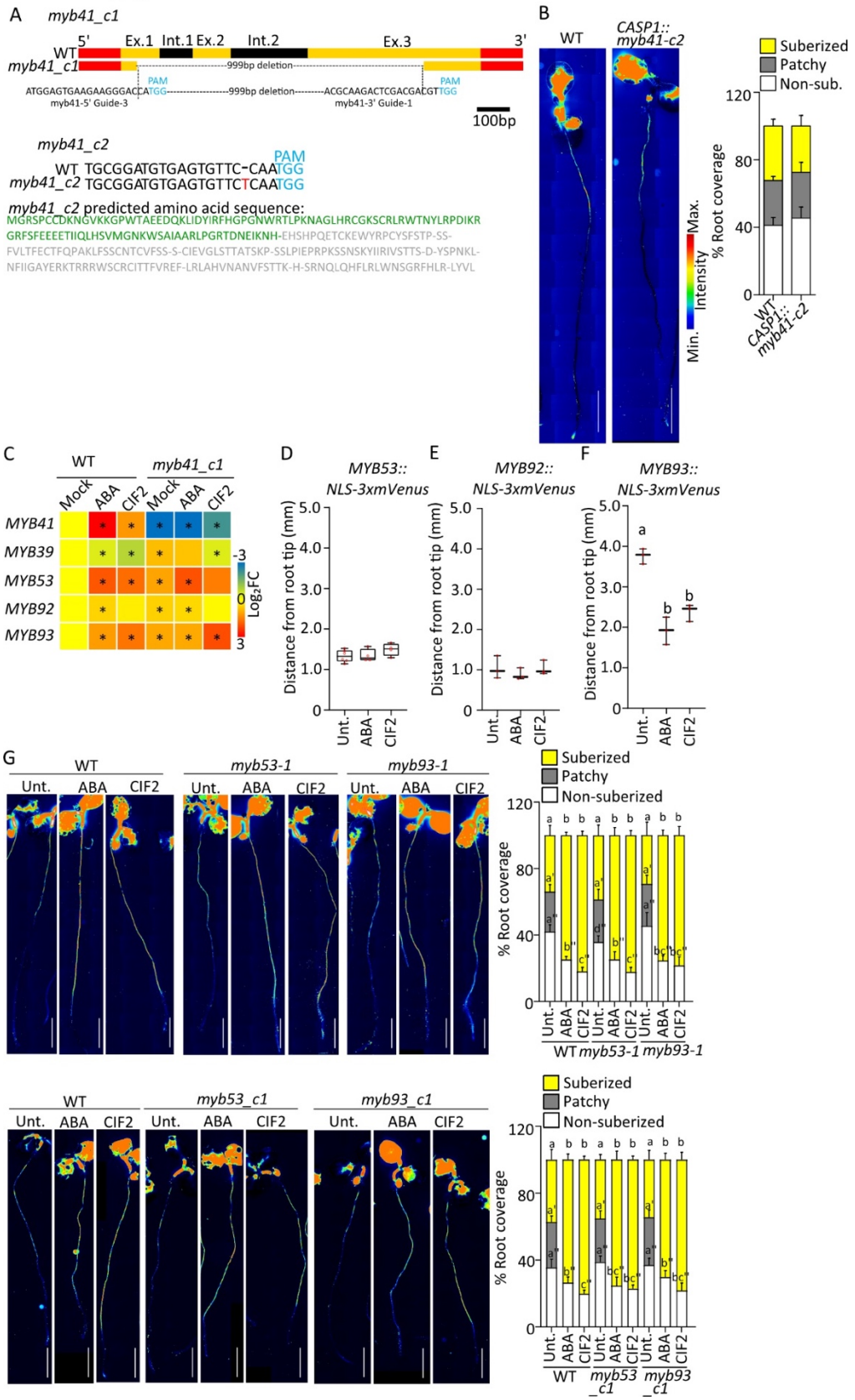

Figure S3. Characterization of candidate MYBs - *MYB41, MYB53, MYB92* and *MYB93*. (*A*) Schematic representation of CRISPR mutations in *myb41_c1* and *myb41_c2* mutants. (*B*) Fluorol Yellow staining for suberin of *CASP1::myb41_c2*. Whole-mount staining (*Left panels*) and quantifications of suberin pattern are presented (*Right panel*), n ≥ 10, error bars, standard deviation. No significant difference observed between genotypes. Scale bars, 2 mm. (C) Relative expression levels of the candidate *MYB* genes in the roots of *myb41_c1* compared to WT

after 8 h treatment with 1 µM ABA or 1 µM CIF2 (n = 4 pools of 25-30 roots). Results are presented as fold changes compared to the WT in mock condition. Numeric values are presented in Table S3. Asterisks indicate statistically significant differences ($P < 0.05$). *(D-F)* Distance from the root tip to the first endodermal cell with NLS-3xmVenus in (*D*) MYB53*::NLS-3xmVenus,* (*E*) *MYB92::NLS-3xmVenus* and (*F*) *MYB93::NLS-3xmVenus* backgrounds. Data are presented as box plots with individual values overlaid, $n \geq 3$, different letters indicate significant differences between conditions ($P < 0.05$). *(G)* FY staining of WT, *myb53* and *myb93* mutant alleles untreated or treated with 1 µM ABA or 1 µM CIF2 for 16 h. Different letters indicate significant differences between conditions for a given genotype *(*$P < 0.05$). The WT control in upper and bottom panels are also shown in Figure 3 F, as they are extracted from the same experiments. Scale bars, 2 mm.

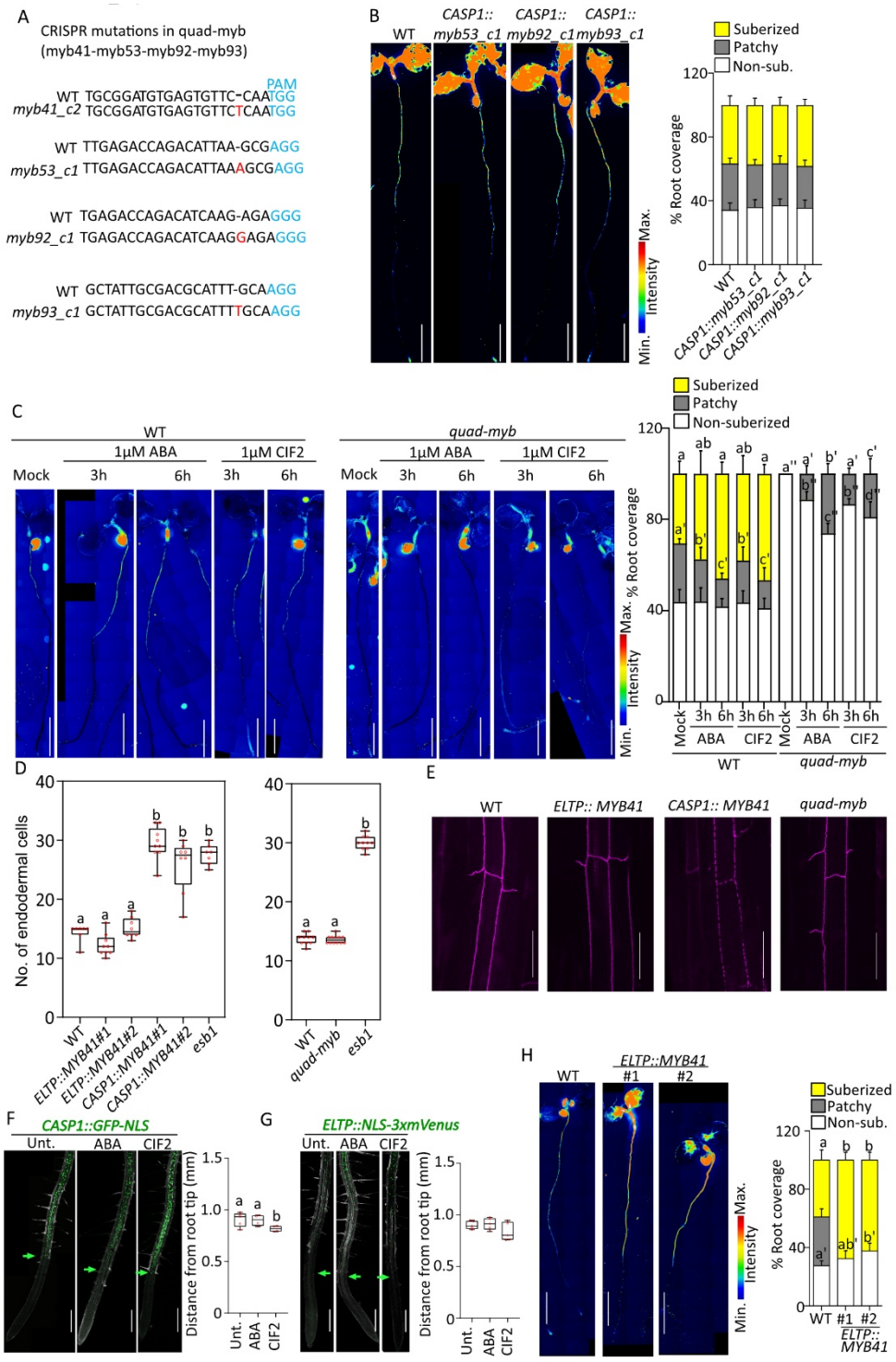

Figure S4. *quad-myb* mutant and *CASP1::MYB41* and *ELTP::MYB41* characterization. (*A*) Schematic representation of CRISPR mutations introduced in *MYB41*, *MYB52*, *MYB92* and *MYB93* to generate a quadruple *myb41-mab53-mab92-myb93* (*quad-myb*) mutant. (*B, C, H*) Fluorol Yellow (FY) staining for suberin. Whole-mount staining (*Left panels*) and quantifications of suberin pattern along the root (*Right panels*), n ≥ 10, error bars, standard deviation, different letters indicate significant differences between conditions or genotypes ($P < 0.05$). Scale bars, 2 mm. (*B*) FY staining of WT and *CASP1::myb53_c1, CASP1::myb92_c1* and *CASP1::myb93_c1*. (*C*) FY staining of WT and *quad-myb* mutant untreated or treated with 1 µM ABA or 1 µM CIF2 treatment for 3 or 6 h. Different letters indicate significant differences between conditions for a given genotype. (*D*) Establishment of a functional apoplastic barrier in different lines and mutants generated in this study compared to WT and *esb1*. Apoplastic barrier function of the endodermis was evaluated with PI diffusion assay. Numbers indicate the average number of endodermal cells from the onset of endodermal cell elongation, where PI uptake is blocked at the level of the endodermis. Data are presented as box plots with individual values overlaid, n ≥ 7. Different letters indicate

significant differences between genotypes ($P < 0.05$). (*E*) Lignin staining for visualization of Casparian strips in lines and mutants generated in this study compared to WT. Pictures were taken around 20-25 cells after the onset of elongation, scale bars, 50µm. (*F*) *CASP1::NLS-GFP* expression (in Green) untreated or treated with 1 µM ABA or 1 µM CIF2 for 16 h. (*G*) *ELTP::NLS-3xmVenus* expression (in Green) untreated or treated with 1 µM ABA or 1 µM CIF2 for 16 h. (*F-G*) Pictures (*Left panels*) are presented as maximum intensity Z projections taken from the root tip to 4 - 5 mm. Propidium iodide (PI, in grey) was used to highlight cells. Scale bars, 500 µm Distance from the root tip to the first endodermal cell with GFP-NLS or NLS-3xmVenus signals in *CASP1::NLS-GFP* and *ELTP::NLS-3xmVenus* backgrounds respectively. Data are presented as box plots with individual values overlaid, $n \geq 5$, different letters indicate significant differences between conditions ($P < 0.05$) *(Right panels)*. (*H*) FY staining of *ELTP::MYB41* compared to WT seedlings.

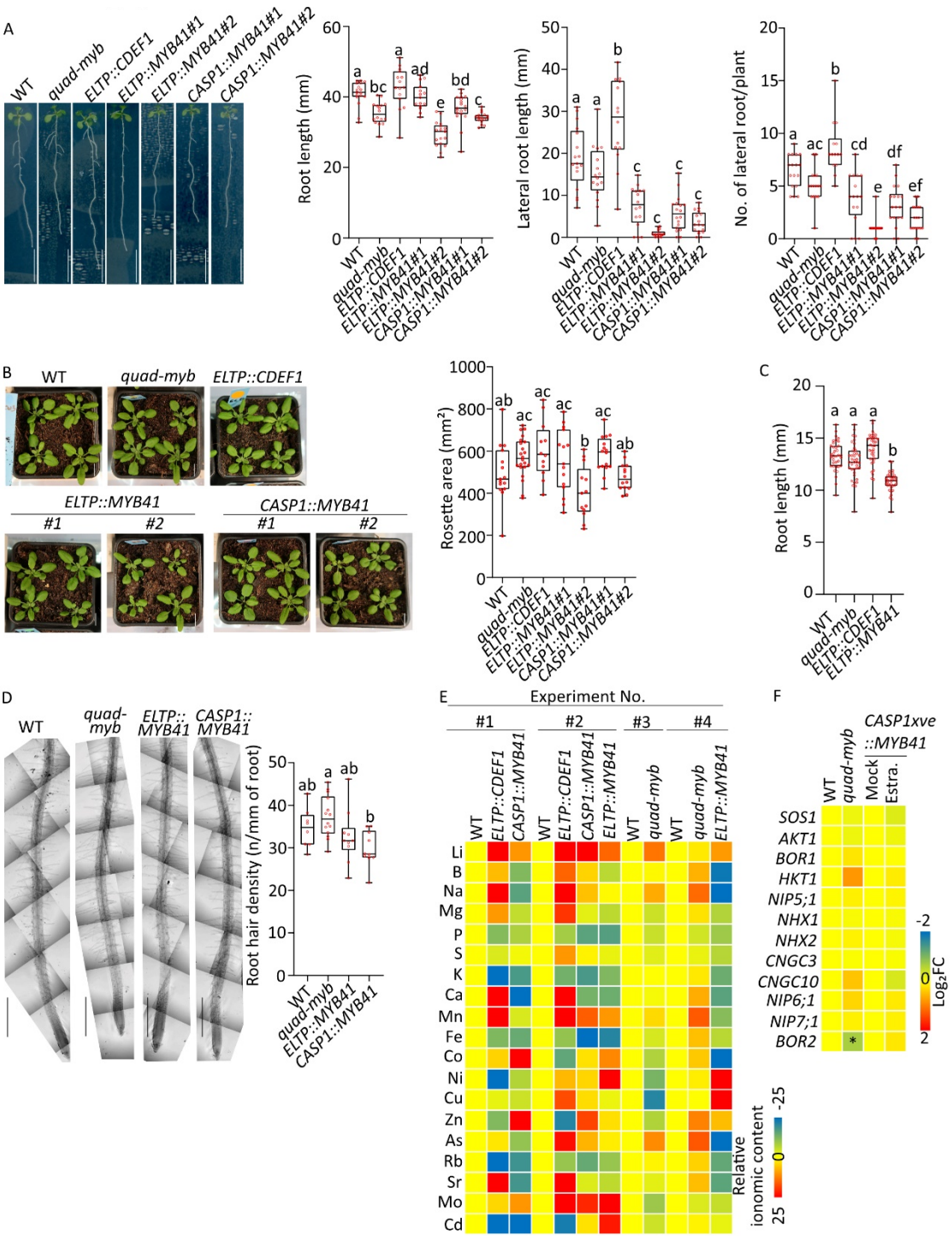

Figure S5 Phenotypic characterization of *quad-myb* mutant and *CASP1::MYB41* and *ELTP::MYB41* lines. (*A*) Root phenotype of 9-day-old WT, *quad-myb*, *ELTP::CDEF1, ELTP::MYB41* and *CASP::MYB41* lines. Pictures (*Left panels)* and quantifications of primary root length, lateral root length and lateral root numbers are presented (*Right panels*). Data are presented as box plots with individual values overlaid, n ≥ 15, different letters indicate significant differences between lines ($P < 0.05$). Scale bars, 10 mm. (*B*) Pictures of 21-day-old WT, *quad-myb*, *ELTP::CDEF1, ELTP::MYB41* and *CASP::MYB41* lines grown in soil (*Left panels*) and quantification of rosette area (*Right panel*). Data are presented as box plots with individual values overlaid, n ≥ 8, different letters indicate significant differences between lines ($P < 0.05$). Scale bars, 10 mm. (*C*) Quantification of primary root length of 5-day-old WT, *quad-myb*, *ELTP::CDEF1, and ELTP::MYB41* lines. Data are presented as box plots with individual values overlaid, n ≥ 15, different letters indicate significant differences between lines ($P < 0.05$). (*D*) Root hair density of seven-day-old WT, *quad-myb*, *ELTP::MYB41* and *CASP::MYB41* (*Left panels*) and quantification (*Right panel*). Data are presented as box plots with individual values overlaid (n ≥ 10), different

letters indicate significant differences between lines ($P < 0.05$). Scale bars, 500 μm. (*E)* Ionomic profiling of leaves of WT, *ELTP::CDEF1, quad-myb, ELTP::MYB41* and *CASP1::MYB41* plants from 4 independent experiments. Elements were determined by ICP-MS. Results are presented as fold changes compared to the WT. Numeric values are presented in Table S4. Experiment 1, and 2: 7-day old plants (n=3 pools of 50); Experiments 3 and 4: 5-day old plants (n=3 pools of 100). (*F*) Relative expression levels of genes encoding nutrient transporters involved in B, Na, Ca, As, and/or Sr acquisition, in the roots of *quad-myb* mutant compared to WT and *CASP1xve::MYB41-mVenus* treated with 5 μM Estradiol for 6 h (n = 4 pools of 25-30 roots). Results are presented as fold changes compared to the WT. Numeric values are presented in Table S3. Asterisks indicate statistical significance ($P < 0.05$).

Supplemental Table 1. Nucleotide sequence of primers used for cloning in this study

Cloning primers		
promMYB41	promMYB41_attB_fw	GGGGACAACTTTGTATAGAAAAGTTGTATCACATATCAACACTCATCA AGTATTCATATTTAAGATTAATCAAATAAC
	promMYB41_attB_rv	GGGGACTGCTTTTTTGTACAAACTTGTCTTTGTTTTGTTTCGCACAACTT TAAATTTCGA
promMYB53	promMYB53_attB_fw	GGGGACAACTTTGTATAGAAAAGTTGTATTATGCAGGTATTTTGGATTT TCTTTTTG
	promMYB53_attB_rv	GGGGACTGCTTTTTTGTACAAACTTGTCCGTTGTTAGATACAGAAGTTG ATCAAACATG
promMYB92	promMYB92_attB_fw	GGGGACAACTTTGTATAGAAAAGTTGTATGTACAACATGCTATCCTAT AGTAACC
	promMYB92_attB_rv	GGGGACTGCTTTTTTGTACAAACTTGTGTTAGATGATCTTCTCTTCTCT GTATC
promMYB93	promMYB93_attB_fw	GGGGACAACTTTGTATAGAAAAGTTGTAAATTAAACAGTATGTAAGTG C
	promMYB93_attB_rv	GGGGACTGCTTTTTTGTACAAACTTGTCTCTCTCTGATTAGGTTGTC
MYB41_CDS	MYB41_attB_fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGGAAGATCACC
	MYB41_attB_rv	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAAAACATAAAGTCATC TAAGATG
MYB41nostop _ CDS	MYB41ns_attB_fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGGAAGATCACCTT GTTGTGATAAAAATG
	MYB41ns_attB_rv	GGGGACCACTTTGTACAAGAAAGCTGGGTTAAACATAAAGTCATCTAA GATGAAATCTTCCGGAA
MYB53_CDS	MYB53_attB_fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGGAAGATCTCCTA GCTC
	MYB53_attB_rv	GGGGACCACTTTGTACAAGAAAGCTGGGTCCAGACATTTCTTATCAAT CTTAA
MYB92_CDS	MYB92_attB_fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGGAAGATCTCCTAT CTCTGATG
	MYB92_attB_rv	GGGGACCACTTTGTACAAGAAAGCTGGGTGATTCTATATTTTCCGACA TTCCTTAG
MYB93_CDS	MYB93_attB_fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGGGAGGTCGCCTT GTTGCG
	MYB93_attB_rv	GGGGACCACTTTGTACAAGAAAGCTGGGTGCCTCATGAACGTTATATC TTAG
Oligos for CRISPR guides		
myb41_c1	myb41_c1_5primeG1_fw	ATTGGAAAAAATCGAAGAGCCAAG
	myb41_c1_5primeG1_rv	AAACCTTGGCTCTTCGATTTTTTC
	myb41_c1_5primeG2_fw	GTCAACTCCATTTTTATCACAACA
	myb41_c1_5primeG2_rv	AAACTGTTGTGATAAAAATGGAGT
	myb41_c1_5primeG3_fw	ATTGATGGAGTGAAGAAGGGACCA
	myb41_c1_5primeG3_rv	AAACTGGTCCCTTCTTCACTCCAT
	myb41_c1_3primeG1_fw	ATTGAACGCAAGACTCGACGACGT
	myb41_c1_3primeG1_rv	AAACACGTCGTCGAGTCTTGCGTT
	myb41_c1_3primeG2_fw	GTCATCCAAAGTCGAAGAAAGTGC
	myb41_c1_3primeG2_rv	AAACGCACTTTCTTCGACTTTGGA
	myb41_c1_3primeG3_fw	ATTGTAGTCCATATACACAGTTGA
	myb41_c1_3primeG3_rv	AAACTCAACTGTGTATATGGACTA
myb41_c2	myb41_c2_fw	ATTGTGCGGATGTGAGTGTTCCAA
	myb41_c2_rv	AAACTTGGAACACTCACATCCGCA
myb53_c1	myb53_fw	attgTTGAGACCAGACATTAAGCG
	myb53_rv	aaacCGCTTAATGTCTGGTCTCAA
myb92_c1	myb92_fw	gtcaTGAGACCAGACATCAAGAGA
	myb92_rv	aaacTCTCTTGATGTCTGGTCTCA
myb93_c1	myb93_fw	attgGCTATTGCGACGCATTTGCA
	myb93_rv	aaacTGCAAATGCGTCGCAATAGC
Primers for genotyping T-DNA lines		
myb53	SALK_076713_LP	CATCCTTGTGGTGACATCTGTT
	SALK_076713_RP	ATCACCAGTAACATTTAGGATAAGT
myb92	SM_3_41690_LP	TGGAATTTAGGGTTTTCAGGG
	SM_3_41690_RP	GCAAGCAACCAAATCTCAGAG
myb93	SALK_131752_LP	TGTTGGTTAATGAAGCCGAAG
	SALK_131752_RP	GGCTTCGTCGCTAGCTAGAAG

Supplemental Table 2. Nucleotide sequence of primers used for real-time analysis in this study

Primer name	AGI	Gene name	Primer sequence
ACTIN2/8 _fw	*At3g18780*	*ACTIN2/8*	GGTAACATTGTGCTCAGTGGTGG
ACTIN2/8 _rv	*At3g18780*	*ACTIN2/8*	AACGACCTTAATCTTCATGCTGC
GPAT4_fw	*At1g01610*	*GPAT4*	TGAAAGAGTTCGGCGATGACTCAC
GPAT4_rv	*At1g01610*	*GPAT4*	CATGCACCATGTAACCTTCCTTGC
KCS2_fw	*At1g04220*	*KCS2*	TCGCTAAACAGCTTCTTCAGGTTC
KCS2_rv	*At1g04220*	*KCS2*	TTGATCGGTCGTTGCCTAAATACC
MYB93_fw	*At1g34670*	*MYB93*	GCTCGCAGATTTGAATAGGTGTGG
MYB93_rv	*At1g34670*	*MYB93*	TTCCCGAGAATGGAGTGGAGATGG
LACS2_fw	*At1g49430*	*LACS2*	CGCGTAACGACTGATTCTAAGGTC
LACS2_rv	*At1g49430*	*LACS2*	CAATCCGAATTGCAGCGTCGTG
GELP22_fw	*At1g54000*	*GELP22*	GCTCCGGTGGAATCTATGACTCTG
GELP22_rv	*At1g54000*	*GELP22*	ACTGACTTTGCCACGAAATCATCG
GELP38_fw	*At1g74460*	*GELP38*	ACGGGTTTGATAACTCGGATTCGC
GELP38_rv	*At1g74460*	*GELP38*	ACAATGTCGACGCTGGAATACACG
GELP49_fw	*At2g19050*	*GELP49*	TGAATACCTCGGCCAAAGTCAAC
GELP49_rv	*At2g19050*	*GELP49*	AGCTCAGCGATAATGTCAGGAATG
GELP51_fw	*At2g23540*	*GELP51*	ACATCTACGCGACCAACTAACCAG
GELP51_rv	*At2g23540*	*GELP51*	ATCCAATCGGTCCAACGTTCCC
C4H_fw	*At2g30490*	*C4H*	TGAGAGGAAGCAAATTGCGAGTTC
C4H_rv	*At2g30490*	*C4H*	CGGCGACATTGATGTTCTCGAC
PAL1_fw	*At2g37040*	*PAL1*	GCAGTGCTACCGAAAGAAGTGG
PAL1_rv	*At2g37040*	*PAL1*	TGTTCGGGATAGCCGATGTTCC
PAL4_fw	*At3g10340*	*PAL4*	GAAGCCGCCGCAATTATGGAAC
PAL4_rv	*At3g10340*	*PAL4*	CGTACGTAAAGCGTACCGATCTTG
GPAT5_fw	*At3g11430*	*GPAT5*	ACGGATAGGATTGTTCCGGTTGC
GPAT5_rv	*At3g11430*	*GPAT5*	TGTAGTCGCGTGGAAGAATCCG
FAR4_fw	*At3g44540*	*FAR4*	TCCCGGTTGGATCGAAGGGTTAAG
FAR4_rv	*At3g44540*	*FAR4*	TGCGACCATGTCCACAGGTATAAG
FAR5_fw	*At3g44550*	*FAR5*	TCGGTCTGCGATATGATACCAGTG
FAR5_rv	*At3g44550*	*FAR5*	TGAACCTCCAGCGTGTTTGG
PAL2_fw	*At3g53260*	*PAL2*	ACGTACCCGTTGATGCAGAGAC
PAL2_rv	*At3g53260*	*PAL2*	AGTCTCACCGTTGGACAAAGCG
ABCG20_fw	*At3g53510*	*ABCG20*	AATTCGCCGCTGGGACAAGTTC
ABCG20_rv	*At3g53510*	*ABCG20*	AAACGCCGCTCATGCTCTTCAG
MYB39_fw	*At4g17785*	*MYB39*	CCCTCCTTGGCAACAAATGGTC
MYB39_rv	*At4g17785*	*MYB39*	GATCGTTGGTTCTTGGCTCGTG
MYB41_fw	*At4g28110*	*MYB41*	CCAGGGAGGACCGATAACGA
MYB41_rv	*At4g28110*	*MYB41*	GGCGTGGAGAATGAGTAACAGG
MYB92_fw	*At5g10280*	*MYB92*	GTTCTTGGAAACAAGTGGTCAACG
MYB92_rv	*At5g10280*	*MYB92*	TATCGGTTCTTGGACGGTGAGTC
ABCG6_fw	*At5g13580*	*ABCG6*	AAGAACGTCTTGGATGCTTCGC
ABCG6_rv	*At5g13580*	*ABCG6*	GCATCTGCGCAAGTGTAGAACG
FAR1_fw	*At5g22500*	*FAR1*	TGACCTTATACCGGCAGACATGG
FAR1_rv	*At5g22500*	*FAR1*	ATTGCCTGAATCCCGGTGTCTC
CYP86B1_fw	*At5g23190*	*CYP86B1*	TCCCGTGGATCACAAAGAGGTTC
CYP86B1_rv	*At5g23190*	*CYP86B1*	AGCTTCCATACGACCCATTGCG
GELP96_fw	*At5g37690*	*GELP96*	AACCCTACACAATCAGCTTACGAC
GELP96_rv	*At5g37690*	*GELP96*	CCATTCGTTGACACGGTTCAGG
ASFT_fw	*At5g41040*	*ASFT*	ACGATGTCGTAGACGCCAAGAAC
ASFT_rv	*At5g41040*	*ASFT*	TAGTCACCTGAGCGGTAACAGG
CYP86A1_fw	*At5g58860*	*CYP86A1*	GTTTACCTCAAGGCTGCTTTGGC
CYP86A1_rv	*At5g58860*	*CYP86A1*	TGAAATCCTGAGGCACAGAAGGG
FACT_fw	*At5g63560*	*FACT*	CTCCAGTGGTTGTTCAGGTGAC
FACT_rv	*At5g63560*	*FACT*	ACTCCATGGCTGCGATACCATC
MYB53_fw	*At5g65230*	*MYB53*	TGCGGTTCTAGGCAACAAGTGG
MYB53_rv	*At5g65230*	*MYB53*	TCATCGGTTCTTGGCTGATGGG
SOS1_fw	*AT2G01980*	*SOS1*	GTGGTGTTGTCATTGCTGAAGGC
SOS1_rv	*AT2G01980*	*SOS1*	AGAAATCGCCATGAATTCCCTTGG
AKT1_fw	*AT2G26650*	*AKT1*	TGCTTCGTCTTTGGCGTCTTCG
AKT1_rv	*AT2G26650*	*AKT1*	AGTTGCGGTCTTTCTCTAGTCTGG
BOR1_fw	*AT2G47160*	*BOR1*	TCGCTTCTGCGATTCCTGTCATC
BOR1_rv	*AT2G47160*	*BOR1*	AACAGCCGTGAGAACTCCATCG
HKT1_fw	*AT4G10310*	*HKT1*	TGGTTTCACTACCGGGTACAGC
HKT1_rv	*AT4G10310*	*HKT1*	ACCCATAACTCGCGTCTTTGCAG
NIP5;1_fw	*AT4G10380*	*NIP5;1*	TGTCCGGTGGTGTCACTATTCC
NIP5;1_rv	*AT4G10380*	*NIP5;1*	ATACCTGCCAATTCTCCAACGG
NHX1_fw	*AT5G27150*	*NHX1*	AAGAGCAGCGTTCGTCTTTCCG
NHX1_rv	*AT5G27150*	*NHX1*	ACCAGACCACCAAATCACAACCTG
ATCNGC10_fw	*AT1G01340*	*ATCNGC10*	ACTTTGGCATCTTCACTGATGCTC

ATCNGC10_rv	*AT1G01340*	*ATCNGC10*	TGGAGATTCTGTCCCAATGCACTC
NIP6;1_fw	*AT1G80760*	*NIP6;1*	TGTTCGTTGTCACAGCCGTAGC
NIP6;1_rv	*AT1G80760*	*NIP6;1*	TCGAAGCAGAAGTTGCAGGTCCAG
ATCNGC3_fw	*AT2G46430*	*ATCNGC3*	GGTCTCCGCAATCTTAGTGCTTTG
ATCNGC3_rv	*AT2G46430*	*ATCNGC3*	TCTCTCCTTCAAAGGCACTCGTC
ATNHX2_fw	*AT3G05030*	*ATNHX2*	ACTCGACTGATCGAGAAGTTGCC
ATNHX2_rv	*AT3G05030*	*ATNHX2*	ACCACTCAAGGCGAATAGCTCAG
NIP7;1_fw	*AT3G06100*	*NIP7;1*	AGCTACGAGCATCGTCGTGTTTC
NIP7;1_rv	*AT3G06100*	*NIP7;1*	TCGGTCCGGTAATAAGCACTCC
BOR2_fw	*AT3G62270*	*BOR2*	AAGAAGCACCCGCTTTACCGTTC
BOR2_rv	*AT3G62270*	*BOR2*	CAGTTGATCCCATCTCAGCTTCCG

Supplemental Table 3. Numerical values of the mRNA levels of the candidate *MYBs* and suberin biosynthetic genes shown in differrent figures - Fig.2A, Fig.2G, Fig.S2B, Fig.S3C, Fig.4C and Fig.5E

Fig.2A Relative expression levels of the candidate *MYBs* and suberin biosynthesis and polymerization genes in WT roots treated with 1 µM ABA or 1 µM CIF2 for 3 and 6 h (n = 4 pools of 25-30 roots).

Mock			3h ABA			6h ABA		
Genes	Renorm_ AVG	Renorm_ SD	Renorm_ AVG	Renorm_ SD	p-value	Renorm_ AVG	Renorm_ SD	p-value
MYB41	1.00	0.14	72.66	14.41	0.002	38.61	4.10	0.000
MYB39	1.00	0.16	0.65	0.08	0.015	0.62	0.13	0.011
MYB53	1.00	0.08	2.37	0.49	0.010	4.57	0.66	0.002
MYB92	1.00	0.13	1.04	0.23	0.796	1.38	0.16	0.010
MYB93	1.00	0.04	2.01	0.58	0.040	3.39	0.26	0.000
GPAT5	1.00	0.22	15.20	2.70	0.002	17.45	0.44	0.000
FAR4	1.00	0.09	4.09	0.63	0.002	6.81	0.20	0.000
KCS2	1.00	0.11	12.47	1.57	0.001	22.97	3.85	0.001
LACS2	1.00	0.14	2.28	0.25	0.000	5.17	0.87	0.002
ABCG6	1.00	0.14	23.44	4.45	0.002	39.21	7.78	0.002
86B1	1.00	0.12	7.78	1.65	0.004	12.41	1.53	0.001
FAR5	1.00	0.10	4.44	0.60	0.001	8.60	0.74	0.000
ABCG20	1.00	0.14	3.55	0.61	0.003	8.85	0.99	0.000
FAR1	1.00	0.24	3.01	0.48	0.001	5.05	0.27	0.000
ASFT	1.00	0.15	14.11	3.03	0.003	23.46	3.10	0.001
ABCG2	1.00	0.19	8.55	0.99	0.000	11.59	1.52	0.001
86A1	1.00	0.13	1.59	0.20	0.004	3.92	0.87	0.006
FACT	1.00	0.10	2.85	0.59	0.007	4.74	0.73	0.002
GPAT4	1.00	0.24	4.36	1.06	0.006	6.62	0.94	0.001
PAL1	1.00	0.26	2.41	0.29	0.000	3.63	0.36	0.000
PAL2	1.00	0.19	1.13	0.13	0.297	1.41	0.29	0.065
PAL4	1.00	0.19	11.13	2.77	0.005	22.75	4.38	0.002
C4H	1.00	0.22	2.01	0.19	0.001	2.84	0.38	0.000
GELP22	1.00	0.55	6.10	1.98	0.011	10.55	1.85	0.001
GELP38	1.00	0.16	1.60	0.16	0.002	2.29	0.31	0.001
GELP49	1.00	0.07	28.04	6.77	0.004	35.21	9.10	0.005
GELP51	1.00	0.07	2.67	0.63	0.012	3.90	0.76	0.004
GELP96	1.00	0.18	8.57	2.61	0.010	21.96	4.65	0.003
Mock			3h CIF			6h CIF		
Genes	Renorm_ AVG	Renorm_ SD	Renorm_ AVG	Renorm_ SD	p-value	Renorm_ AVG	Renorm_ SD	p-value
MYB41	1.00	0.14	7.18	1.04	0.001	4.89	3.13	0.089
MYB39	1.00	0.16	1.16	0.28	0.364	0.58	0.40	0.123
MYB53	1.00	0.08	1.93	0.44	0.022	2.51	0.34	0.002
MYB92	1.00	0.13	0.96	0.09	0.605	1.04	0.12	0.658
MYB93	1.00	0.04	3.21	0.39	0.001	3.30	0.40	0.001
GPAT5	1.00	0.22	2.39	0.28	0.000	4.12	2.08	0.057
FAR4	1.00	0.09	1.90	0.25	0.003	2.20	0.33	0.004
KCS2	1.00	0.11	1.64	0.25	0.009	3.04	1.26	0.048
LACS2	1.00	0.14	2.28	0.40	0.005	5.29	1.20	0.005
ABCG6	1.00	0.14	4.24	0.73	0.002	6.80	3.22	0.036
86B1	1.00	0.12	2.59	0.26	0.000	2.67	0.35	0.001
FAR5	1.00	0.10	2.80	0.33	0.001	3.86	0.44	0.001
ABCG20	1.00	0.14	2.76	0.26	0.000	5.14	2.22	0.033
FAR1	1.00	0.24	1.50	0.29	0.042	1.80	0.64	0.084
ASFT	1.00	0.15	2.24	0.21	0.000	1.99	0.66	0.053
ABCG2	1.00	0.19	1.90	0.45	0.022	2.39	0.62	0.016
86A1	1.00	0.13	1.29	0.19	0.050	3.32	0.52	0.002
FACT	1.00	0.10	1.38	0.19	0.019	1.59	0.37	0.047
GPAT4	1.00	0.24	1.93	0.24	0.002	2.71	0.88	0.026
PAL1	1.00	0.26	1.78	0.24	0.005	1.95	0.40	0.010
PAL2	1.00	0.19	2.01	0.15	0.000	2.11	0.26	0.001
PAL4	1.00	0.19	2.93	0.66	0.007	3.61	1.16	0.019
C4H	1.00	0.22	1.62	0.11	0.006	1.51	0.35	0.055
GELP22	1.00	0.55	1.72	0.84	0.211	8.05	12.19	0.332
GELP38	1.00	0.16	1.06	0.16	0.625	2.87	2.59	0.245
GELP49	1.00	0.07	5.69	1.12	0.004	5.39	2.45	0.037
GELP51	1.00	0.07	2.15	0.42	0.011	3.35	0.69	0.006
GELP96	1.00	0.18	4.02	0.23	0.000	5.89	2.28	0.023

Supplemental Table 3. Numerical values of the mRNA levels of the candidate *MYBs* and suberin biosynthetic genes shown in differrent figures - Fig.2A, Fig.2G, Fig.S2B, Fig.S3C, Fig.4C and Fig.5E
Fig.2G Relative expression levels of the *MYBs* candidates and suberin biosynthesis and polymerization genes in the roots of *CASP1xve::MYB41-mVENUS* treated with 5 µM Estradiol for 3 and 6 h (n = 4 pools of 25-30 roots).

	Mock		3h			6h		
Genes	Renorm_ AVG	Renorm_ SD	Renorm_ AVG	Renorm_ SD	p-value	Renorm_ AVG	Renorm_ SD	p-value
MYB41	1.00	0.07	5.81	0.53	0.000	5.54	0.64	0.001
MYB39	1.00	0.10	0.97	0.23	0.820	0.66	0.10	0.003
MYB53	1.00	0.11	1.11	0.18	0.357	0.76	0.10	0.017
MYB92	1.00	0.10	0.88	0.14	0.225	0.69	0.04	0.004
MYB93	1.00	0.16	1.39	0.21	0.025	1.40	0.33	0.085
GPAT5	1.00	0.20	2.39	0.25	0.000	2.36	0.51	0.008
FAR4	1.00	0.26	1.73	0.32	0.013	2.24	0.53	0.011
KCS2	1.00	0.19	2.22	0.16	0.000	2.00	0.16	0.000
LACS2	1.00	0.17	1.89	0.20	0.001	2.68	0.43	0.002
ABCG6	1.00	0.18	3.53	0.19	0.000	3.91	0.39	0.000
86B1	1.00	0.33	2.16	0.22	0.002	2.27	0.51	0.008
FAR5	1.00	0.32	1.34	0.13	0.122	1.61	0.55	0.113
ABCG20	1.00	0.32	1.39	0.11	0.089	1.16	0.24	0.457
FAR1	1.00	0.08	1.12	0.14	0.201	1.23	0.22	0.123
ASFT	1.00	0.22	1.70	0.17	0.003	1.95	0.41	0.012
ABCG2	1.00	0.19	1.37	0.25	0.062	1.59	0.19	0.005
86A1	1.00	0.35	1.20	0.10	0.341	0.94	0.10	0.760
FACT	1.00	0.15	1.21	0.12	0.068	1.25	0.22	0.112
GPAT4	1.00	0.35	2.68	0.56	0.004	2.65	0.15	0.001
PAL1	1.00	0.10	1.62	0.14	0.000	1.60	0.19	0.004
PAL2	1.00	0.12	1.54	0.18	0.004	1.93	0.45	0.021
C4H	1.00	0.26	1.44	0.08	0.035	1.78	0.40	0.021
PAL4	1.00	0.38	1.95	0.40	0.014	2.07	0.30	0.005
GELP22	1.00	0.57	4.14	0.73	0.005	2.38	2.20	0.306
GELP38	1.00	0.20	1.49	0.76	0.292	0.87	0.17	0.351
GELP49	1.00	0.23	4.16	0.63	0.001	4.68	1.01	0.004
GELP51	1.00	0.39	2.07	0.75	0.058	1.65	0.66	0.152
GELP96	1.00	0.56	1.11	0.09	0.728	1.02	0.42	0.954

Supplemental Table 3. Numerical values of the mRNA levels of the candidate *MYBs* and suberin biosynthetic genes shown in different figures - Fig.2A, Fig.2G, Fig.S2B, Fig.S3C, Fig.4C and Fig.5E

Fig.S2B Relative expression levels of the candidate MYBs and suberin biosynthesis and polymerization genes in WT roots treated with 1 μM ABA or 1 μM CIF2 for 6h, 12h and 24h (n = 4 pools of 25-30 roots).

	Oh		6h Mock		6h ABA			6h CIF		
Genes	Renorm_AVG	Renorm_SD	Renorm_AVG	Renorm_SD	Renorm_AVG	Renorm_SD	p-value	Renorm_AVG	Renorm_SD	p-value
MYB41	1.00	0.17	1.00	0.15	20.66	2.72	0.001	2.09	0.21	0.000
MYB39	1.00	0.14	1.00	0.47	0.97	0.14	0.797	0.60	0.08	0.006
MYB53	1.00	0.30	1.00	0.07	3.46	0.31	0.000	3.40	0.66	0.005
MYB92	1.00	0.19	1.00	0.13	1.68	0.18	0.001	1.29	0.21	0.073
MYB93	1.00	0.28	1.00	0.63	3.06	0.17	0.000	3.78	0.55	0.002
GPAT5	1.00	0.22	1.00	0.43	15.73	1.32	0.000	3.24	0.49	0.002
FAR4	1.00	0.27	1.00	0.50	7.23	0.70	0.000	3.02	0.37	0.001

	12h Mock		12h ABA			12h CIF		
Genes	Renorm_AVG	Renorm_SD	Renorm_AVG	Renorm_SD	p-value	Renorm_AVG	Renorm_SD	p-value
MYB41	1.000	0.707	7.05	2.39	0.011	0.62	0.34	0.387
MYB39	1.000	0.815	2.35	2.26	0.331	0.29	0.15	0.235
MYB53	1.000	0.241	3.13	0.49	0.001	3.01	0.36	0.000
MYB92	1.000	0.211	1.21	0.20	0.211	0.94	0.20	0.681
MYB93	1.000	0.791	3.96	0.74	0.002	3.52	0.58	0.001
GPAT5	1.000	0.768	8.03	1.70	0.003	1.90	0.96	0.162
FAR4	1.000	0.893	3.98	0.29	0.000	2.12	0.22	0.000

	24h Mock		24h ABA			24h CIF		
Genes	Renorm_AVG	Renorm_SD	Renorm_AVG	Renorm_SD	p-value	Renorm_AVG	Renorm_SD	p-value
MYB41	1.000	0.240	2.41	0.47	0.004	0.54	0.10	0.025
MYB39	1.000	0.605	1.48	0.78	0.311	0.23	0.07	0.004
MYB53	1.000	0.187	0.91	0.43	0.726	0.59	0.20	0.026
MYB92	1.000	0.145	1.06	0.13	0.557	0.81	0.14	0.115
MYB93	1.000	0.439	0.78	0.15	0.060	0.75	0.09	0.016
GPAT5	1.000	0.476	1.80	0.45	0.032	1.17	0.16	0.158
FAR4	1.000	0.540	2.56	0.19	0.000	1.19	0.07	0.019

Supplemental Table 3. Numerical values of the mRNA levels of the candidate *MYBs* and suberin biosynthetic genes shown in differrent figures - Fig.2A, Fig.2G, Fig.S2B, Fig.S3C, Fig.4C and Fig.5E
Fig.S3C Relative expression levels of the candidate *MYB* genes in the roots of myb41_c1 compared to WT after 8 h treatment with 1 µM ABA or 1 µM CIF2 (n = 4 pools of 25-30 roots).

	WT		WT_ABA			WT_CIF2			
Genes	Renorm_ AVG	Renorm_ SD	Renorm_ AVG	Renorm_ SD	p-value	Renorm_ AVG	Renorm_ SD	p-value	
MYB41	1.00	0.18	14.99	1.74	0.000	2.33	0.33	0.001	
MYB39	1.00	0.07	0.76	0.02	0.005	0.56	0.09	0.000	
MYB53	1.00	0.26	3.96	0.69	0.002	3.32	0.33	0.000	
MYB92	1.00	0.19	1.42	0.14	0.014	1.16	0.17	0.266	
MYB93	1.00	0.20	2.26	0.13	0.000	3.09	0.66	0.006	
GPAT5	1.00	0.14	9.72	1.41	0.001	2.60	0.44	0.003	
FAR4	1.00	0.14	4.24	0.32	0.000	2.40	0.53	0.010	
	myb41_c1			*myb41_c1_ABA*			*myb41_c1_CIF2*		
Genes	Renorm_ AVG	Renorm_ SD	p-value	Renorm_ AVG	Renorm_ SD	p-value	Renorm_ AVG	Renorm_ SD	p-value
MYB41	0.01	0.02	0.002	0.00	0.00	0.298	0.21	0.43	0.410
MYB39	1.73	0.30	0.015	1.64	0.13	0.643	0.77	0.11	0.005
MYB53	2.11	0.42	0.006	4.10	0.43	0.001	2.91	0.70	0.108
MYB92	1.46	0.28	0.039	1.38	0.23	0.660	1.05	0.15	0.052
MYB93	2.03	0.54	0.025	2.02	0.37	0.971	4.29	1.49	0.049
GPAT5	1.94	0.29	0.003	6.00	1.98	0.025	2.04	0.43	0.720
FAR4	1.34	0.19	0.033	3.30	0.47	0.002	2.34	0.19	0.000

Supplemental Table 3. Numerical values of the mRNA levels of the candidate *MYBs* and suberin biosynthetic genes shown in differrent figures - Fig.2A, Fig.2G, Fig.S2B, Fig.S3C, Fig.4C and Fig.5E
Fig.4C Relative expression levels of the suberin biosynthesis and polymerization genes in the roots of *quad-myb* mutant compared to WT (n = 4 pools of 25-30 roots). Results are presented as fold changes compared to the WT.

Genes	Renorm_AVG	Renorm_SD	Renorm_AVG	Renorm_SD	p-value
MYB41	1.00	0.14	0.69	0.06	0.016
MYB39	1.00	0.16	2.89	0.14	0.000
MYB53	1.00	0.08	0.39	0.06	0.000
MYB92	1.00	0.13	1.18	0.24	0.264
MYB93	1.00	0.04	0.48	0.11	0.001
GPAT5	1.00	0.22	0.41	0.12	0.006
FAR4	1.00	0.09	0.31	0.05	0.000
KCS2	1.00	0.11	0.32	0.03	0.001
LACS2	1.00	0.14	0.38	0.06	0.001
ABCG6	1.00	0.14	0.42	0.06	0.002
86B1	1.00	0.12	0.49	0.07	0.001
FAR5	1.00	0.10	0.37	0.03	0.000
ABCG20	1.00	0.14	1.30	0.04	0.016
FAR1	1.00	0.24	0.21	0.03	0.007
ASFT	1.00	0.15	0.92	0.07	0.394
ABCG2	1.00	0.19	0.68	0.08	0.035
86A1	1.00	0.13	0.32	0.02	0.002
FACT	1.00	0.10	0.27	0.06	0.000
GPAT4	1.00	0.24	0.69	0.14	0.073
PAL1	1.00	0.26	0.72	0.08	0.119
PAL2	1.00	0.19	1.91	0.38	0.010
PAL4	1.00	0.19	1.40	0.04	0.019
C4H	1.00	0.22	1.19	0.13	0.211
MYB74	1.00	0.20	1.22	0.16	0.142
GELP22	1.00	0.55	0.70	0.47	0.129
GELP38	1.00	0.16	0.72	0.06	0.062
GELP49	1.00	0.07	0.31	0.03	0.000
GELP51	1.00	0.07	0.73	0.09	0.070
GELP96	1.00	0.18	0.69	0.07	0.029

Supplemental Table 3. Numerical values of the mRNA levels of the candidate *MYBs* and suberin biosynthetic genes shown in differrent figures - Fig.2A, Fig.2G, Fig.S2B, Fig.S3C, Fig.4C and Fig.5E and Fig.5E

Fig.5E Relative expression levels of genes encoding nutrient transporters involved in B, Na, Ca, As, and/or Sr acquisition, in the roots of quad-myb mutant compared to WT and *CASP1xve::MYB41-mVenus* treated with 5 µM Estradiol for 6 h (n = 4 pools of 25-30 roots). Results are presented as fold changes compared to the WT. Numeric values are presented in Table S3. Asterisks indicate statistical significance (P < 0.05).

	WT		*quad-myb (myb41-53-92-93)*		
Genes	Renorm_AVG	Renorm_SD	Renorm_AVG	Renorm_SD	p-value
SOS1	1.00	0.07	0.91	0.13	0.28
AKT1	1.00	0.09	0.91	0.11	0.24
BOR1	1.00	0.17	1.15	0.15	0.23
HKT1	1.00	0.21	1.75	0.72	0.12
NIP5;1	1.00	0.11	1.09	0.42	0.71
NHX1	1.00	0.13	0.96	0.31	0.84
NHX2	1.00	0.14	1.07	0.23	0.61
CNGC3	1.00	0.15	0.98	0.25	0.91
CNGC10	1.00	0.16	1.37	0.38	0.14
NIP6;1	1.00	0.11	1.25	0.50	0.40
NIP7;1	1.00	0.24	1.09	0.57	0.78
BOR2	1.00	0.13	0.63	0.16	0.01
	CASP1xve::MYB41-mVenus				
	Mock		6h		
Genes	Renorm_AVG	Renorm_SD	Renorm_AVG	Renorm_SD	p-value
SOS1	1.00	0.29	0.83	0.15	0.37
AKT1	1.00	0.29	0.92	0.19	0.68
BOR1	1.00	0.27	1.01	0.18	0.95
HKT1	1.00	0.42	1.11	0.09	0.64
NIP5;1	1.00	0.17	1.06	0.10	0.55
NHX1	1.00	0.16	0.96	0.12	0.71
NHX2	1.00	0.24	1.14	0.15	0.36
CNGC3	1.00	0.24	0.96	0.09	0.77
CNGC10	1.00	0.24	0.98	0.16	0.87
NIP6;1	1.00	0.33	0.81	0.14	0.34
NIP7;1	1.00	0.19	1.13	0.09	0.30
BOR2	1.00	0.26	1.08	0.40	0.74

Supplemental Table 4. Numerical values of raw data from different ionomic experiments and normalized ionomic data shown in Fig.4D and Fig.S5C. Ionomic profiling of leaves of WT, *ELTP::CDEF1, quad-myb, ELTP::MYB41* and *CASP1::MYB41* plants. Elements were determined by ICP-MS. Results are presented as average fold changes compared to the WT.

Ionomic analysis 1								
	Average (ppm)				Stdev			
Elements	WT	*ELTP:: CDEF1*	*CASP1:: MYB41#1*	*CASP1:: MYB41#2*	WT	*ELTP:: CDEF1*	*CASP1:: MYB41#1*	*CASP1:: MYB41#2*
Li 7	0.021	0.030	0.022	0.023	0.001	0.001	0.005	0.003
B 11	26.898	28.687	25.072	23.620	1.356	0.749	1.027	1.008
Na 23	488.000	696.844	442.591	409.046	72.174	84.094	15.691	38.588
Mg 24	2865.608	3153.589	2702.741	2695.868	145.932	90.983	75.959	151.522
P 31	19603.33	17285.48	18479.16	18029.81	1987.664	1155.865	1217.271	1152.952
S 34	12808.08	12345.30	12499.97	12658.80	992.690	662.792	791.323	419.699
K 39	78914.07	57580.30	71298.14	64986.02	7700.874	6400.459	4755.106	2400.489
Ca 43	6322.936	8428.005	5923.168	4619.001	508.691	342.674	175.522	497.570
Mn 55	0.135	0.484	208.784	0.131	0.041	0.698	2.254	0.054
Fe 56	208.043	265.099	148.748	200.105	8.307	8.921	6.303	15.953
Co 59	153.613	134.607	0.223	130.906	6.130	8.212	0.018	6.646
Ni 60	0.169	0.177	0.678	0.237	0.013	0.009	0.304	0.022
Cu 63	0.468	0.919	4.009	1.171	0.037	0.273	0.666	0.010
Zn 66	3.616	3.541	562.223	3.434	0.293	0.411	22.407	0.082
As 75	424.885	372.926	3.447	613.655	14.499	9.145	0.580	69.854
Rb 85	64.905	48.444	57.893	52.685	7.429	5.716	3.612	1.181
Sr 88	11.241	14.220	10.680	9.185	0.591	0.372	0.157	0.585
Mo 98	4.135	4.298	4.051	4.562	0.173	0.046	0.213	0.150
Cd 111	0.833	0.422	0.454	0.506	0.233	0.061	0.033	0.085

Supplemental Table 4. Numerical values of raw data from different ionomic experiments and normalized ionomic data shown in Fig.4D and Fig.S5C. Ionomic profiling of leaves of WT, *ELTP::CDEF1, quad-myb, ELTP::MYB41* and *CASP1::MYB41* plants. Elements were determined by ICP-MS. Results are presented as average fold changes compared to the WT.

Ionomic analysis 2						
	Average (ppm)					
Elements	WT	*ELTP:: CDEF1*	*CASP1:: MYB41#1*	*CASP1:: MYB41#2*	*ELTP:: MYB41#1*	*ELTP:: MYB41#2*
Li 7	0.0	0.0	0.0	0.0	0.0	0.0
B 11	31.2	36.0	32.0	32.6	29.2	32.4
Na 23	396.3	770.3	437.2	420.6	398.5	459.6
Mg 24	2278.9	2733.0	2212.6	2217.6	2130.8	2232.9
P 31	14536.6	13244.9	13458.8	12202.7	12117.7	12315.2
S 34	10276.7	11334.5	10466.2	10404.7	10067.8	10329.1
K 39	57460.1	49601.3	54124.3	47288.8	50327.6	49695.4
Ca 43	5120.8	7509.6	4813.8	4437.5	4392.4	4464.6
Mn 55	193.9	277.8	223.0	224.5	213.8	222.6
Fe 56	182.0	163.1	164.3	136.0	143.6	131.0
Co 59	0.2	0.2	0.3	0.2	0.3	0.2
Ni 60	0.5	0.5	0.4	0.5	0.6	0.4
Cu 63	2.9	3.4	2.8	3.0	2.8	3.1
Zn 66	420.7	331.5	507.3	498.5	433.6	478.6
As 75	0.1	0.2	0.1	0.2	0.2	0.2
Rb 85	57.1	51.4	54.9	48.6	51.4	50.3
Sr 88	9.8	16.6	10.5	9.5	9.2	10.2
Mo 98	3.9	5.3	4.7	4.8	5.0	5.3
Cd 111	0.3	0.2	0.3	0.3	0.3	0.3
	Stdev					
Elements	WT	*ELTP:: CDEF1*	*CASP1:: MYB41#1*	*CASP1:: MYB41#2*	*ELTP:: MYB41#1*	*ELTP:: MYB41#2*
Li 7	0.0	0.0	0.0	0.0	0.0	0.0
B 11	1.6	1.2	1.5	0.3	1.4	2.1
Na 23	48.9	151.1	53.8	15.8	23.9	36.4
Mg 24	134.6	68.6	65.9	23.5	50.2	24.1
P 31	547.0	463.2	538.4	547.1	542.1	677.7
S 34	286.9	416.3	418.7	393.7	291.0	88.2
K 39	3142.2	1424.7	2020.7	3051.1	717.0	3007.8
Ca 43	508.2	630.0	297.7	371.5	265.2	39.5
Mn 55	13.6	22.4	8.0	10.0	13.1	6.8
Fe 56	4.6	13.5	10.7	12.1	7.1	2.7
Co 59	0.0	0.0	0.0	0.0	0.0	0.0
Ni 60	0.1	0.1	0.0	0.1	0.1	0.0
Cu 63	0.1	0.1	0.1	0.2	0.1	0.1
Zn 66	46.2	19.3	9.1	30.8	33.1	32.8
As 75	0.0	0.0	0.0	0.0	0.0	0.0
Rb 85	3.9	1.4	2.7	3.7	1.5	2.4
Sr 88	0.6	1.8	0.5	0.4	0.3	0.0
Mo 98	0.2	0.2	0.3	0.3	0.3	0.2
Cd 111	0.0	0.1	0.0	0.0	0.1	0.0

Supplemental Table 4. Numerical values of raw data from different ionomic experiments and normalized ionomic data shown in Fig.4D and Fig.S5C. Ionomic profiling of leaves of WT, *ELTP::CDEF1, quad-myb, ELTP::MYB41* and *CASP1::MYB41* plants. Elements were determined by ICP-MS. Results are presented as average fold changes compared to the WT.

Ionomic analysis 3				
	Average (ppm)		Stdev	
Elements	WT	*quad-myb*	WT	*quad-myb*
Li 7	0.0	0.0	0.0	0.0
B 11	15.9	16.1	0.1	1.6
Na 23	804.7	871.5	39.3	12.0
Mg 24	2436.0	2310.4	30.8	43.8
P 31	12907.6	12215.5	214.4	283.9
S 34	10657.4	10351.8	386.0	217.6
K 39	55819.6	55928.3	796.3	1614.8
Ca 43	4636.0	4600.5	88.3	171.0
Mn 55	168.3	161.6	6.1	4.6
Fe 56	187.9	173.4	17.4	6.9
Co 59	0.2	0.1	0.0	0.0
Ni 60	1.3	1.0	0.3	0.1
Cu 63	4.4	4.0	0.1	0.3
Zn 66	305.2	339.8	7.9	26.6
As 75	0.1	0.2	0.0	0.0
Rb 85	60.7	61.4	0.6	1.5
Sr 88	8.9	8.7	0.1	0.4
Mo 98	6.4	5.8	0.3	0.2
Cd 111	0.4	0.4	0.0	0.0

Supplemental Table 4. Numerical values of raw data from different ionomic experiments and normalized ionomic data shown in Fig.4D and Fig.S5C. Ionomic profiling of leaves of WT, *ELTP::CDEF1, quad-myb, ELTP::MYB41* and *CASP1::MYB41* plants. Elements were determined by ICP-MS. Results are presented as average fold changes compared to the WT.

	Ionomic analysis 4					
	Average (ppm)			Stdev		
Elements	WT	*quad-myb*	*ELTP::MYB41#1*	WT	*quad-myb*	*ELTP::MYB41#1*
Li 7	0.0	0.0	0.0	0.0	0.0	0.0
B 11	20.8	22.4	15.9	0.4	0.6	3.7
Na 23	1304.9	1495.1	284.1	22.0	108.5	15.7
Mg 24	2101.5	2160.1	1943.8	29.5	28.6	398.4
P 31	12488.9	11925.3	11322.2	308.1	163.5	3116.7
S 34	9307.9	9121.9	8643.3	206.6	279.4	2326.2
K 39	57855.0	59140.9	49542.1	482.9	1193.7	11305.2
Ca 43	3736.9	4036.4	3129.3	22.2	123.8	633.0
Mn 55	139.3	148.0	129.9	1.2	6.1	26.2
Fe 56	227.2	239.4	167.1	12.5	4.4	36.9
Co 59	0.1	0.1	0.2	0.0	0.0	0.0
Ni 60	0.6	0.6	0.9	0.0	0.1	0.2
Cu 63	3.9	4.3	4.1	0.5	0.2	1.1
Zn 66	305.2	355.7	222.0	23.1	21.3	60.2
As 75	0.1	0.1	0.1	0.0	0.0	0.0
Rb 85	61.5	64.2	52.1	0.6	1.8	11.2
Sr 88	7.3	7.8	6.1	0.1	0.2	1.2
Mo 98	4.3	4.2	4.1	0.3	0.4	0.7
Cd 111	0.3	0.3	0.3	0.0	0.0	0.1

Supplemental Table 4. Numerical values of raw data from different ionomic experiments and normalized ionomic data shown in Fig.4D and Fig.S5C. Ionomic profiling of leaves of WT, *ELTP::CDEF1, quad-myb, ELTP::MYB41* and *CASP1::MYB41* plants. Elements were determined by ICP-MS. Results are presented as average fold changes compared to the WT.

Normalized data (%) shown in Fig.4D					
Elements	WT	*ELTP::CDEF1*	*quad-myb*	*ELTP::MYB41 #1*	CASP1::MYB41#2
Li 7	0.00	60.85	7.86	12.47	21.01
B 11	0.00	13.82	4.09	-15.07	-3.94
Na 23	0.00	69.21	11.43	-38.84	-5.03
Mg 24	0.00	8.87	-1.18	-7.00	-4.31
P 31	0.00	-9.65	-4.94	-12.99	-12.04
S 34	0.00	0.80	-2.43	-4.59	0.04
K 39	0.00	-18.14	1.21	-13.39	-17.68
Ca 43	0.00	29.06	3.62	-15.24	-20.15
Mn 55	0.00	27.31	9.93	-0.67	6.00
Fe 56	0.00	-3.23	1.12	-13.92	-20.03
Co 59	0.00	1.98	-1.18	-7.39	21.39
Ni 60	0.00	-9.51	-5.66	36.91	-1.67
Cu 63	0.00	5.27	-10.56	16.97	-0.82
Zn 66	0.00	-7.49	2.27	4.79	31.46
As 75	0.00	6.49	13.95	-12.73	-1.52
Rb 85	0.00	-30.54	2.78	-12.66	-16.90
Sr 88	0.00	31.64	1.85	-11.04	-10.88
Mo 98	0.00	7.83	-6.59	10.47	15.80
Cd 111	0.00	-31.14	-1.88	10.28	-18.19

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