

ID pH7GWIWG2(I),0 standard; circular DNA; ; 13986 BP.

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FH Key Location/Qualifiers

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FT intron complement(2035..2678)

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FT misc\_marker complement(12176..13962)

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FT promoter complement(4425..5462)

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FT /label=p35S

SQ Sequence 13986 BP; 3509 A; 3559 C; 3558 G; 3360 t;

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actttattgc caaatgtttg aacgatctgc ttgactctag ggaattaatt cctgaatcac 12480  
tgcgaccggc cctcccgca cccagccgag cgagcttagc gaactgtgga cgagaactgt 12540  
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aatgggccga acccgctcgt ctggctaaga tcggccgcag cgatagcatc catagcctcc 13320  
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cggcggggtc ataactgac tcccttaatt ctcatgtatg ataattcgag ggtaccggg 13980  
gatcct 13986

**Supplementary Figure S1. Vector pH7GWIWG2(I) used for transformation of *P. tremula* x *tremuloides*.**

Vector pH7GWIWG2(I)

LSD1

>gi|224115559|ref|XM\_002317030.1| *Populus trichocarpa* predicted protein, mRNA (540 bp)

ATGCAGAGCCAGGTGGTGTGTAGAGGGTGTAGAAGTTTATTGTTATATCCAAGTGGAGCTACT  
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ACTCATATGTCGAGGTTGCAGGACATTGCTAATGTATGCACGGGGGGCAACAACTGTGAGAT  
GCTCCTGCTGCCACATAGTGAACCTTGCACCAGTAGGACCTAACCAGGTTGCTCATGTCAACT  
GTGGGAACTGCCAGACTACACTTATGTATCCGAATGGTGCTCCATCTGTCAAGTGTGCGGTCT  
GTCACTATGTTACTAACATTAGTACGGCTAATGTGAGAGTCCGCTTCCAGCAAACAGACCTA  
GTGGTATAGGTGGAACAATGCCGTCTACTTCAACAGTAAGTAACTGGACTTACTTTTCTTTCTTCT

GACTCAAACGTGTCGTTGTGGAACCCCTATGTCTGTTGATGAAAGTGGCAAATTGGTCAGCAA  
TGTGTTGTTGGTGTCACTACAGAAAAAAATAA (insert 86 bp)

EDS1

>gi|224121609|ref|XM\_002318590.1| Populus trichocarpa disease resistance protein, mRNA (2084 bp)

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TTGAGGTGGAAAAAGCTGCGACAGACAGGAGGCAAATTGTATTTACAGGCCACTCTTCAGGA  
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AACTTTTCTCAGGATCTTGACCTAAGGAAGGAGTTGCATGTGCAGGGATTTGCAGATGCGTAA  
CTAATGTTATTCCGAGCCTTGTACACACCTGAGCAAGAACCTTTATCTGTATTAGATATTTTATT  
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AGAAAGGGAA (insert 151 bp)

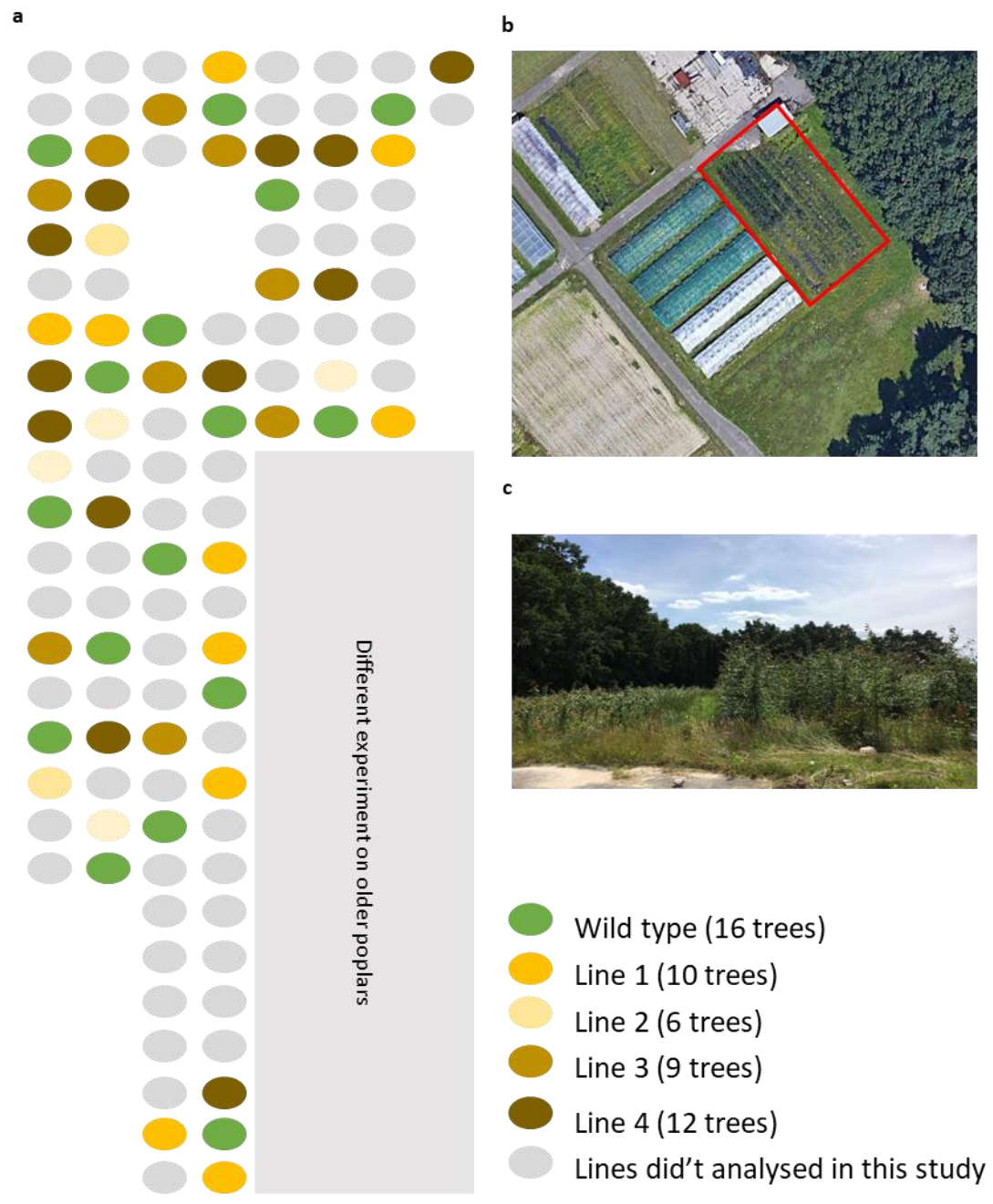
PAD4

>gi|224083495|ref|XM\_002307015.1| Populus trichocarpa PAD4, mRNA (1509 bp)

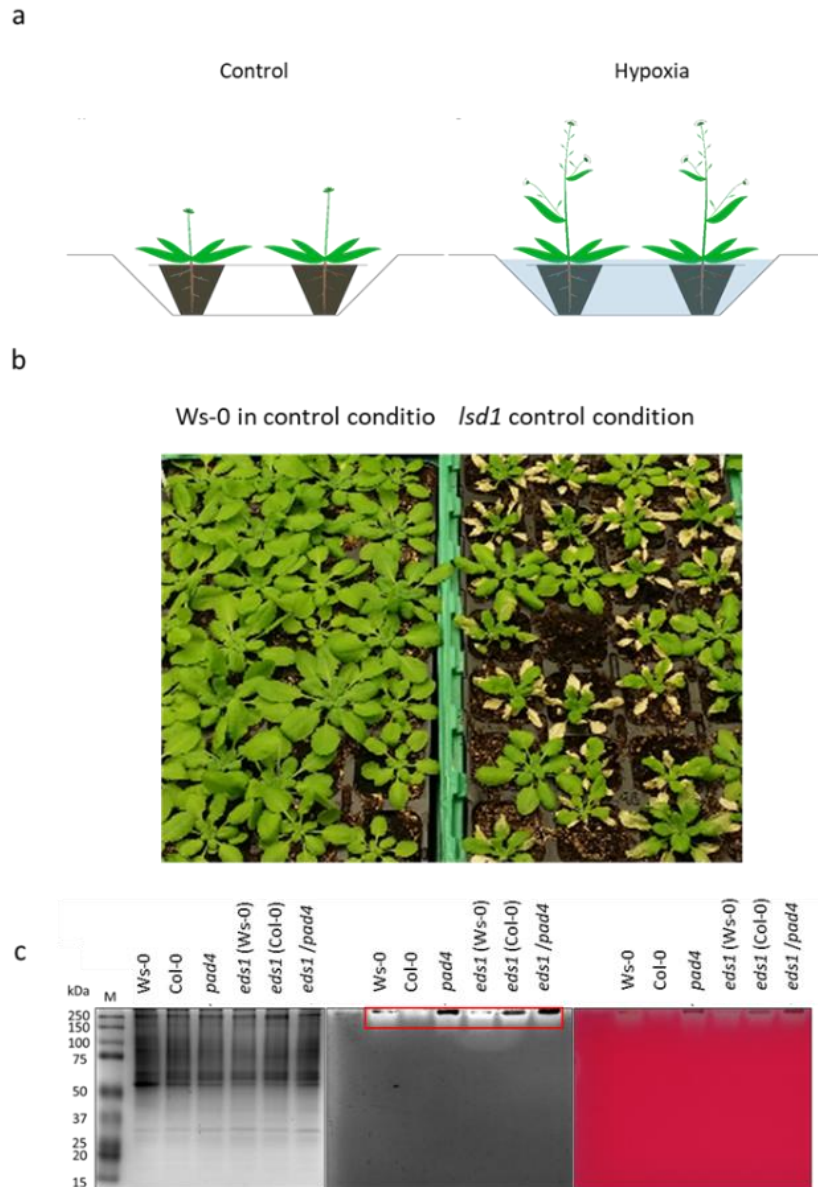
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CAGACAAGGAAAATGGGTCAATGCTTCCCAGTTCTATAAGCTCCTTGTGGAGCCATTGGATAT  
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AAACGGAGCAAGTTTGCAAGTTTGACCCAAGACACATGCTTCTGGGCAAGAGTGGAGGAAGC  
CAGGGATTTGTTAGATGCTCTTAGAAGCACTAGTGATCCGAGTCATTTGGCTTTGCTTTGGCAG  
AAGATAGATAGTTTTGCGAGCGATGCAAACGCGTTGGTTGAAACTAAGGAGGTGTCTATAGA  
TGTAGTGGCAAAGAATTCAACCTATTCTTTGTGGCTAAAAGATTATAATGAATTGAAATCACA  
AAAGGTGCAATTCCGTCCCTTGTTTTTGAGTTTTGTGAATGAGGAAATGGTTCCATAG (insert 79  
bp)

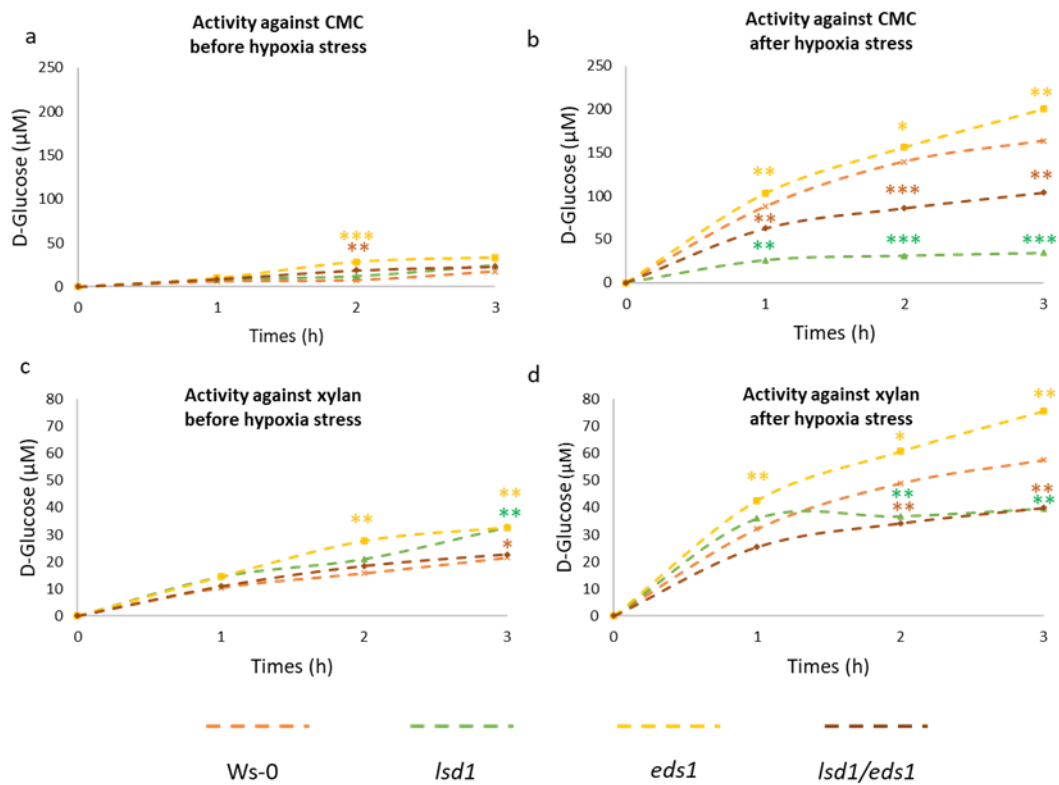
**Supplementary Figure S2. cDNA sequences of LSD1, EDS1 and PAD4.** Short cDNA sequences inserted into silencing vector are highlighted in yellow, primers are highlighted in grey and underlined.



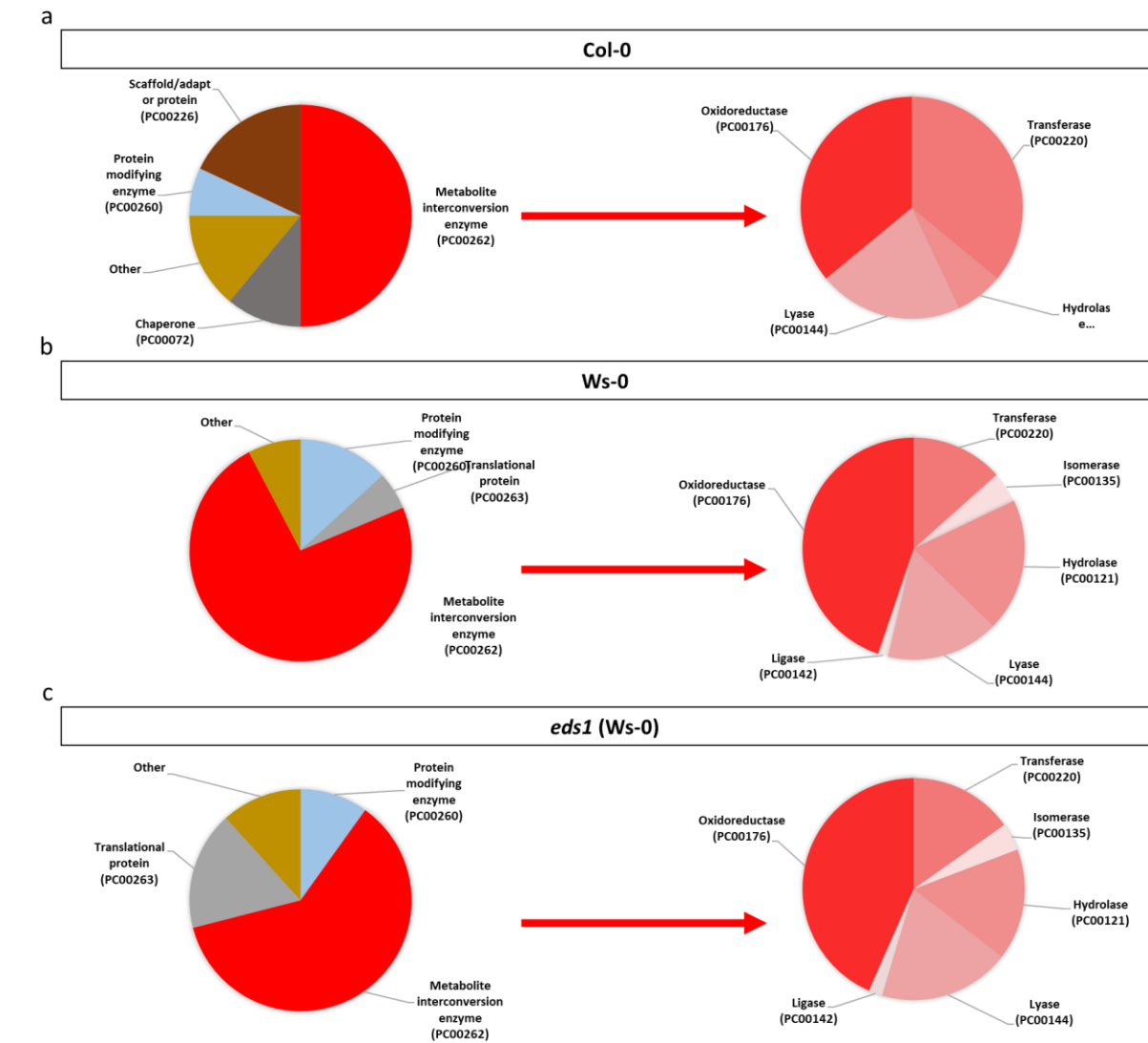
**Supplementary Figure S3. Scheme of aspen cultivation and field experiment.** Schematic presentation of experimental objects randomization in the field (a). Satellite image of the experimental field (GoogleMaps, USA), the field is marked with a red frame (b). Picture of the field experiment in the second year of the experiment (c).



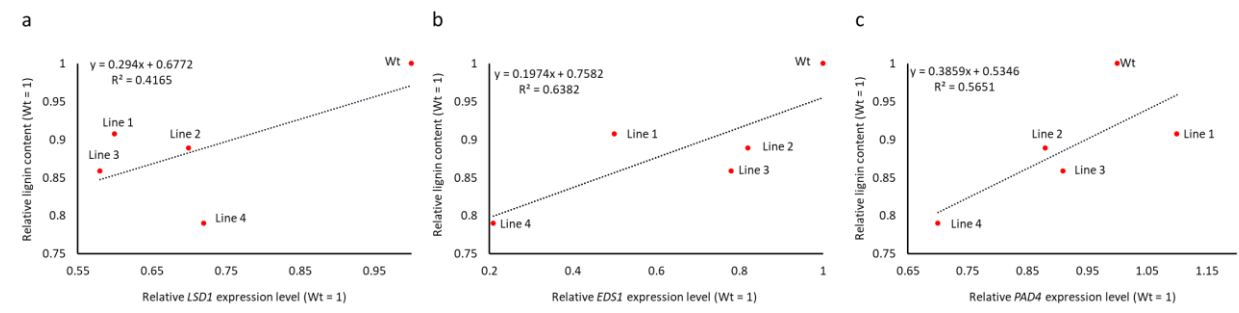
**Supplementary Figure S4. Technical details of experiments.** (a) Model of experimental setup for lysigenous aerenchyma formation in response to hypoxia stress. (b). Higher reducing sugar content in a *lsd1* mutant before and after hypoxia stress was detected. It is easily explainable because the *lsd1* mutant induces PCD during the 8-week growth in short photoperiod (8 hours of light and 16 hours of night) in controlled conditions and cannot survive the hypoxia stress. By contrast, a *eds1* mutant increased its reducing sugar content during the hypoxia stress. We did not observe decrease in reducing sugar content during the hypoxia stress in any other genotype. Comparison of a 8-week-old *Arabidopsis thaliana* plant, Ws-0 (left) and *lsd1* (right) (c). The *eds1* and *eds1/lsd1* mutants did not visually differ from Ws-0. Gel stained with Coomassie Brilliant Blue showing proteins of roots and



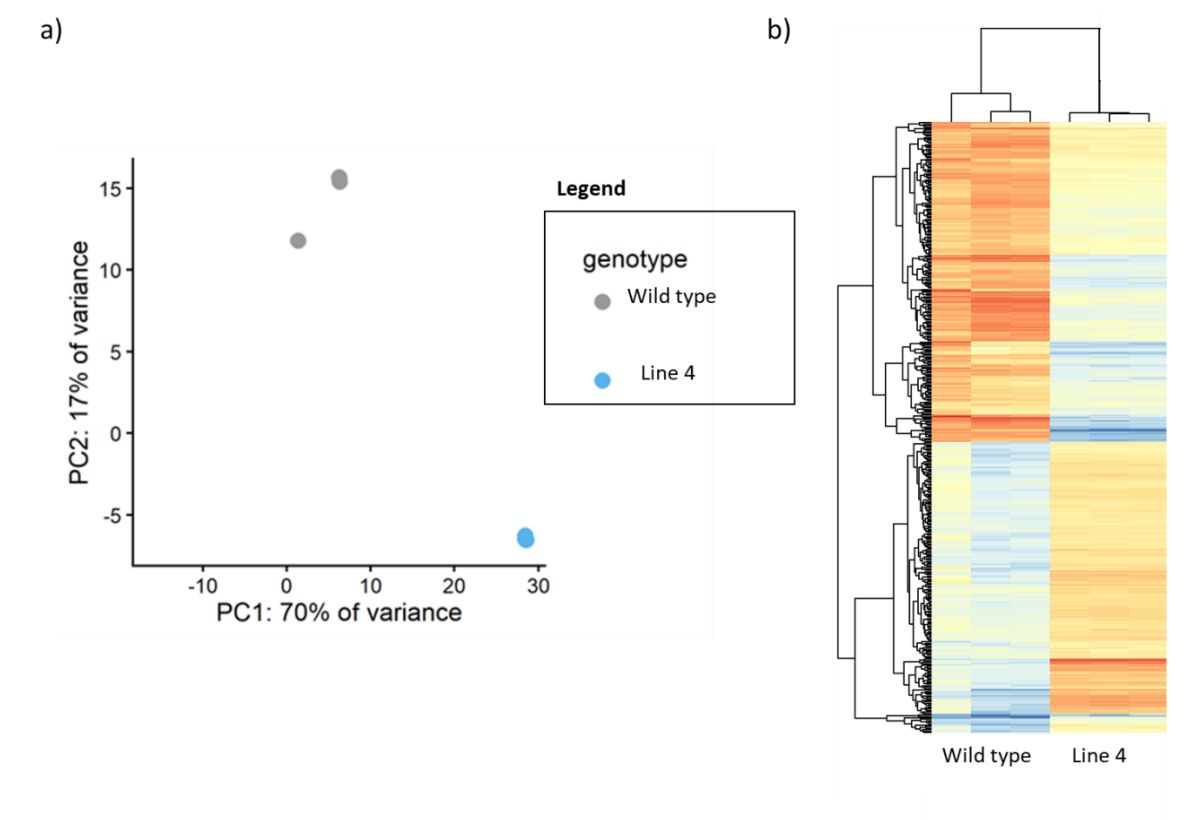
**Supplementary Figure S5 Glucanase and xylanase activities in roots and hypocotyls measured after hypoxia stress.** Protein extracts isolated before hypoxia stress exhibited lower activity against carboxymethyl cellulose (a) and xylan (c), compared to extracts isolated after hypoxia stress (b, d). Means values ( $\pm$ SD) are derived from separate biological replicates ( $n = 3$  to  $6$ ). Stars above the bars indicate statistically significant differences in comparison to the WT plants, according to the Tukey HSD at level  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.001$  (\*\*\*).



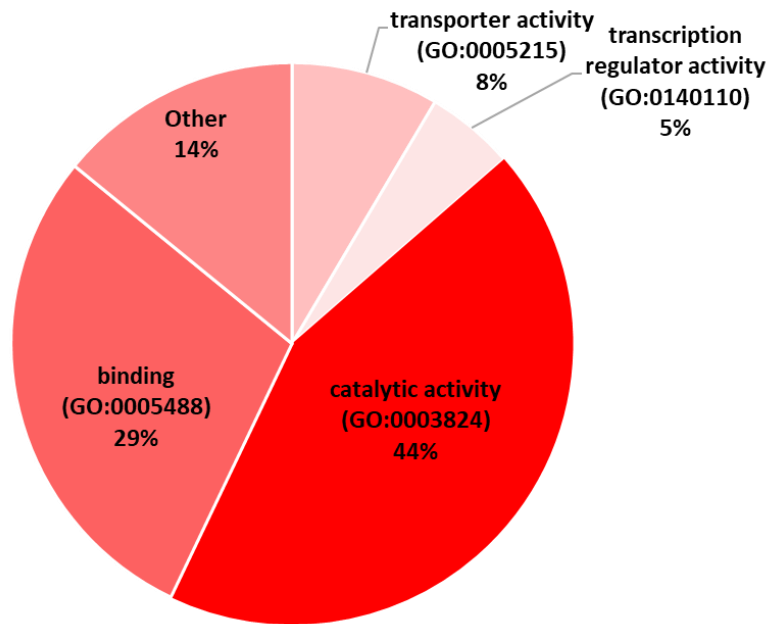
**Supplementary Figure S6. Protein classes analysis by Panther.org.** Pie chart presenting different protein classes taking part in hypoxia stress response and their abundance among identified proteins in tested genotypes; Col-0 (a), Ws-0 (b) and eds1 (Ws-0 background) (c) and pie chart presenting division of categories metabolite interconversion enzyme (PC00262) into subcategories (Right site). In order to obtain material for Maldi-TOF analysis, 6 separate experiments lasting one year in total were carried out. Then the Maldi-TOF analysis was performed and Data were based on 6 separate experiments (biological replicates) and two technical repetitions per sample. The results were analyzed using the Mascot software and trimmed so there would be not more than 1% of false positives in each sample.



**Supplementary Figure S7.** Correlation of wood lignin content and deregulation of PtLSD1, PtEDS1 and PtPAD4 gene expression in leaf of tested lines. Correlation of lignin content (percent) and relative expression of LSD1 (a), EDS1 (b) and PAD4 (c).

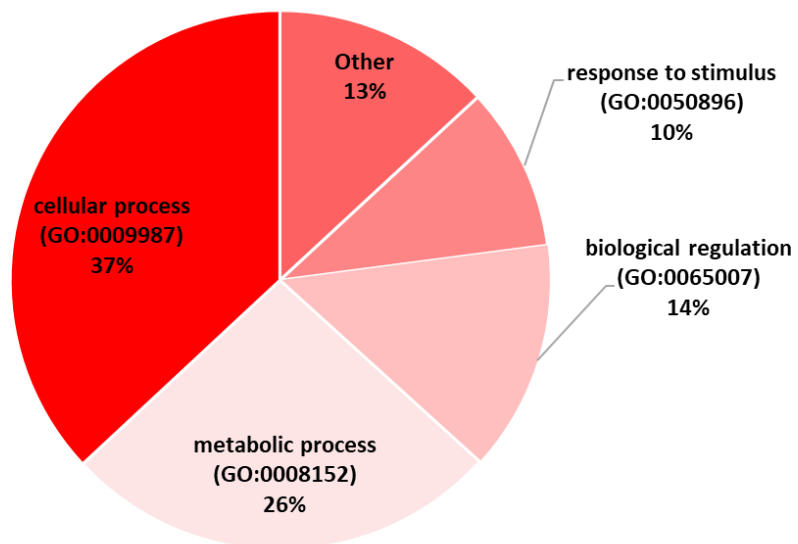


**Supplementary Figure S8.** RNAseq analysis of RNA isolated from cambium and differentiating xylem of wild type and transgenic Line 4 with the lowest relative lignin content (see Figure 3). The PCA analysis is showing huge differences between RNA samples isolated from cambium and differentiating xylem tissue of wood of wild type and Line 4 (a) and heat map of differently deregulated genes in wild type and Line 4 (b). The analysis was made on three independent biological repeats ( $n = 3$ ).



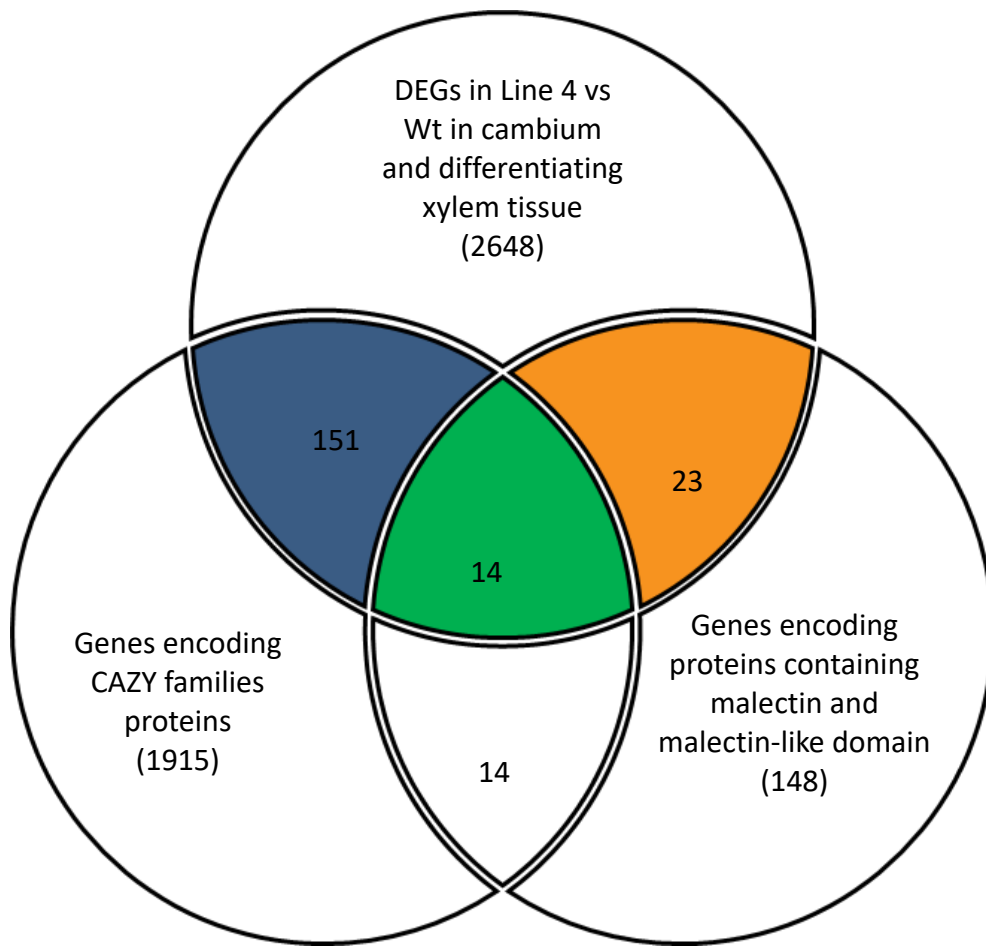
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**Supplementary Figure S9.** Gene ontology analysis of deregulated genes in Line 4 in terms of their molecular function.

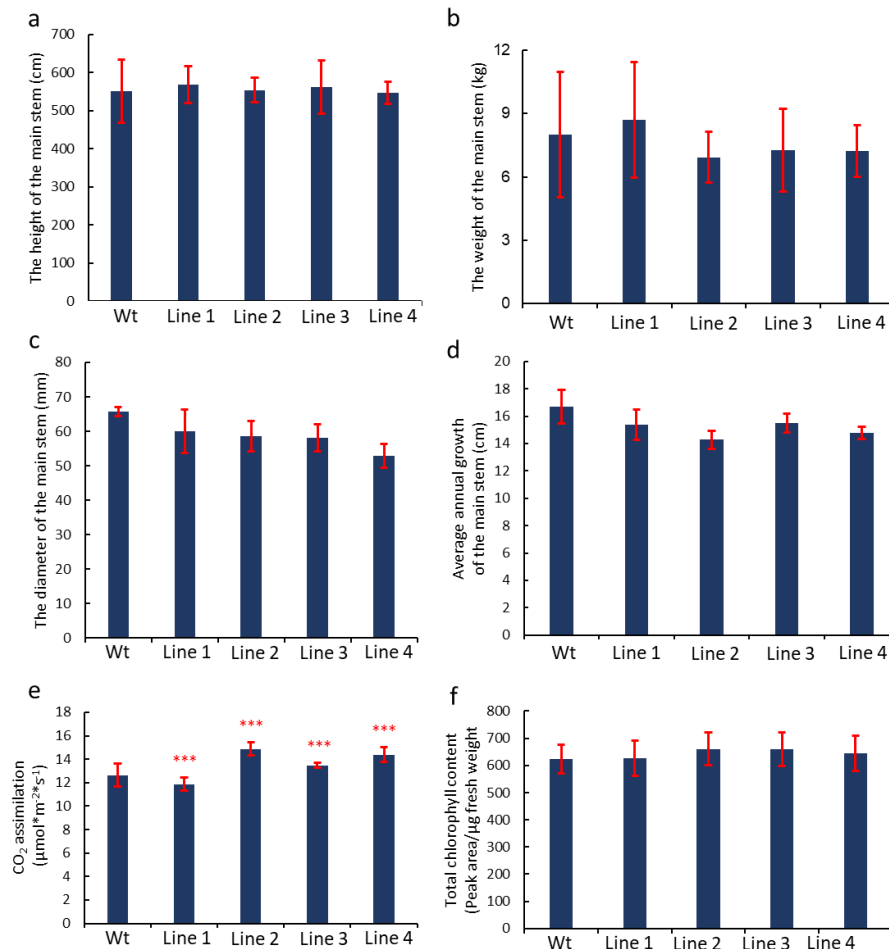


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**Supplementary Figure S10.** Gene ontology analysis of deregulated genes in Line 4 in terms of biological processes.



**Supplementary Figure S11.** Results of Line 4 DEGs and its comparison to genes encoding proteins from CAZY families and genes encoding malectin and malectin-like domain-containing proteins.



**Supplementary Figure S12. Biometric parameters, CO<sub>2</sub> assimilation, and chlorophyll content in transgenic lines.** Height of the main stem (**a**), fresh weight of the main stem (**b**), diameter of the main stem (**c**), average annual growth of the main stem (**c**), CO<sub>2</sub> assimilation (**d**), total chlorophyll content in leaves (**f**). Stars above the bars indicate statistically significant differences in comparison to the WT plants, according to Tukey HSD at level  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.001$  (\*\*\*). Mean values ( $\pm$ SD) for five to eleven different biological replications for data on graphs A and B ( $n = 5$  to 11), for three different biological replications on graph C ( $n=3$ ), for four different biological replications on graph D ( $n = 4$ ), for 30 different leaves *per* genotype on graph E ( $n = 30$ ) and from nine different technical replications of pooled leaf sample *per* genotype on graph F ( $n = 9$ ).