***New Phytologist* Supporting Information**

Article title: **Catechol, a major component of smoke, influences primary root growth and root hair elongation through ROS-mediated redox signaling**
Authors: Ming Wang, Matthias Schoettner, Shuqing Xu, Christian Paetz, Julia Wilde, Ian T. Baldwin, Karin Groten
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The following Supporting Information is available for this article:

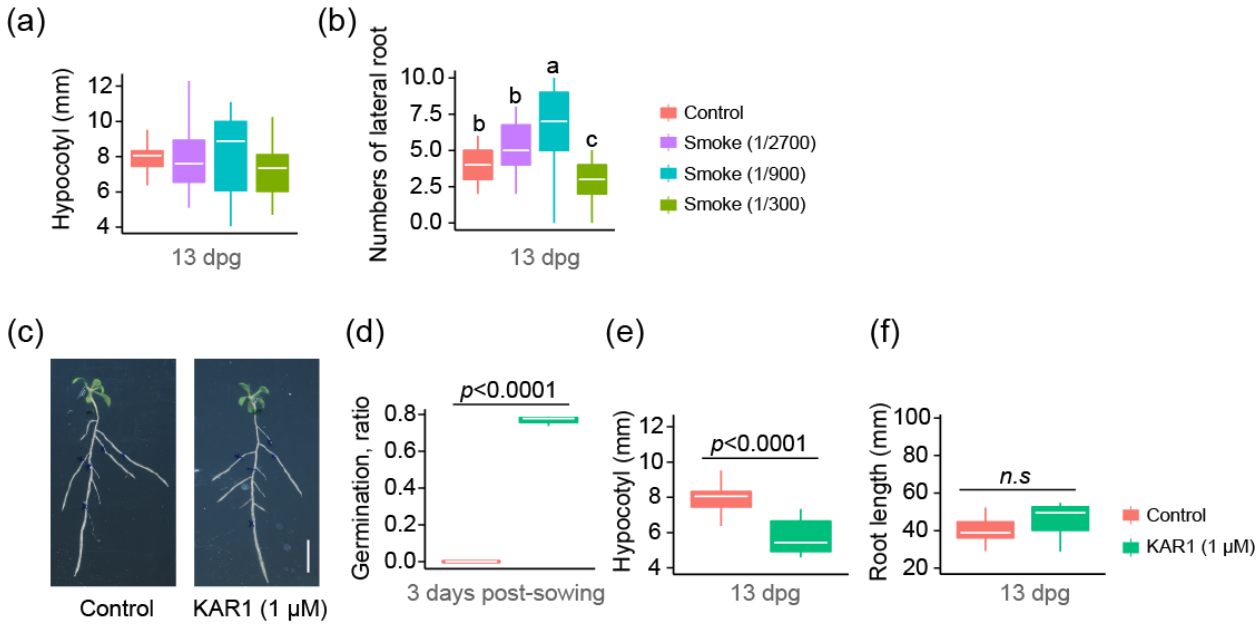


Fig. S1 Treatment with karrikin1 (KAR1) increases *N. attenuata* seed germination and decreases hypocotyl elongation, but does not induce changes in primary root length.
(a)-(b) The number of lateral roots was significantly increased by smoke treatment, while hypocotyl length did not change. Seedlings for measurements were the same as those described in Fig. 1. Different letters indicate significant differences, $p \leq 0.05$, one-way ANOVA followed by Tukey's HSD. Cross-bars in the box plots represent the median ($n=6$, for each biological replicate 25-30 seedlings per Petri dish were measured). (c) Morphology of seedlings treated by KAR1 (1 µM), scale bar, 0.5 mm. (d) Positive effect of KAR1 treatment on seed germination ($n=3$, for each biological replicate 30 seeds per pot were analyzed 3 days after sowing). (e) Negative effect of KAR1 treatment on hypocotyl elongation. (f) No significant differences were observed in primary root elongation after KAR1 treatment compared to controls. (d)-(f) Student's *t*-test. *N. attenuata* seedlings were germinated on GB5 media containing KAR1 (1 µM) or different doses of liquid smoke. Scans were taken 13 dpg for measurements.

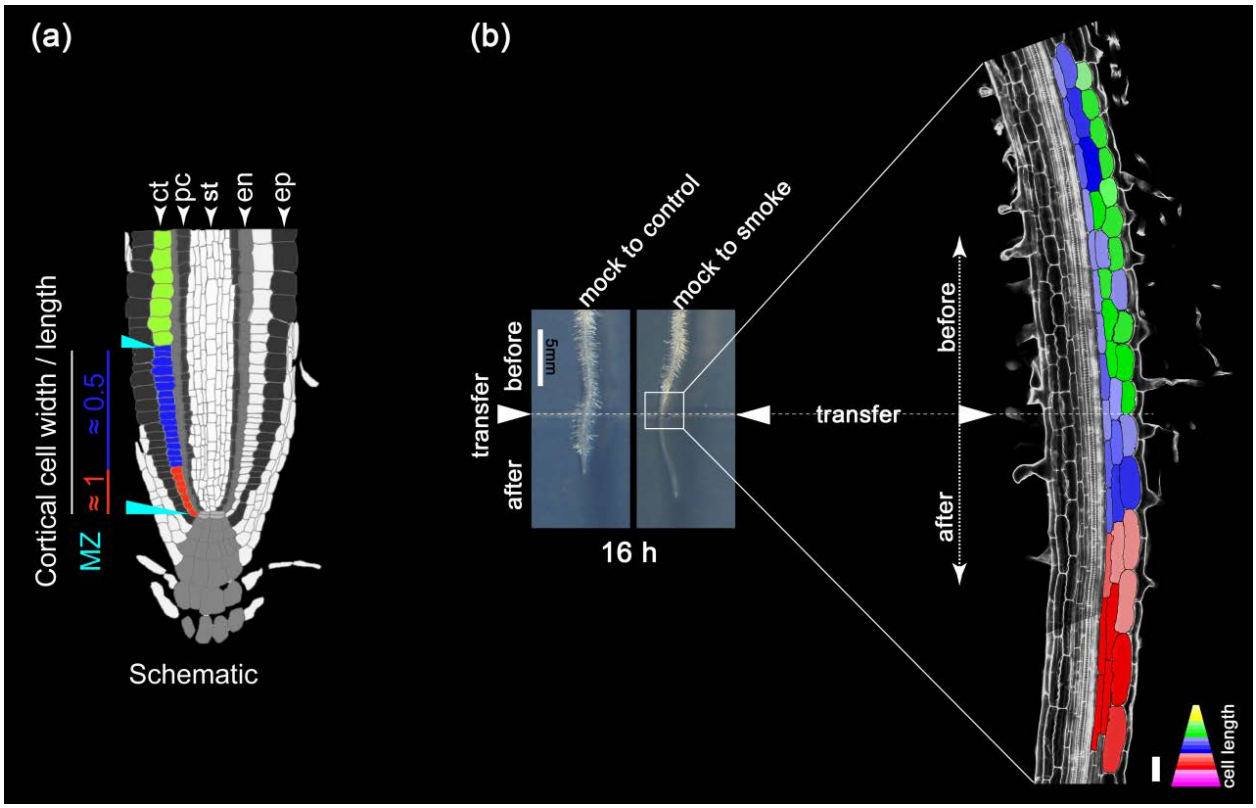


Fig. S2 Scheme of the meristem zone of *N. attenuata* roots and longitudinal elongation of cells in the elongation zone induced by smoke.
(a) Schematic presentation of width to length (w/l) ratio progression of cortex cell development in an *N. attenuata* root meristem zone (MZ), with the developing stem cell daughters in red (w/l $\approx$ 1.0) and intensive division zone in blue (w/l $\approx$ 0.5). Cells in both red and blue as MZ cells were counted (ep: epidermis, ct: cortex, en: endodermis, pc: pericycle, st: stele). (b) Root morphology after short-term application of smoke. Seedlings (5 dpg) were transferred from mock GB5 media to media containing liquid smoke (V:V = 1:900) for 16 h. Scale bar, 5 mm. Imaging by confocal LSM 510 (ZEISS) of roots before and after smoke-treatment stained with propidium iodide to visualize the cell walls. The color codes indicate the lengths of cells. 12-15 seedlings were tested, the experiment was repeated twice. Scale bar, 50 µm.

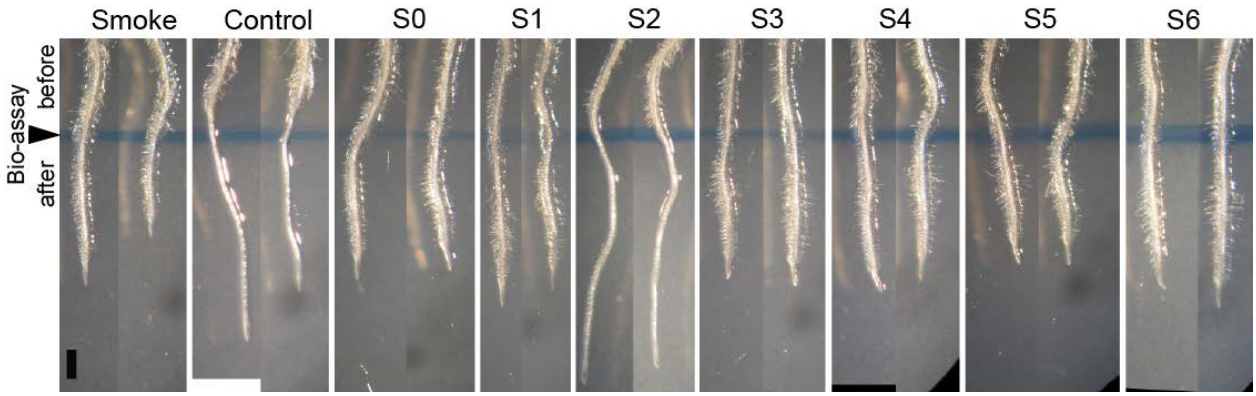
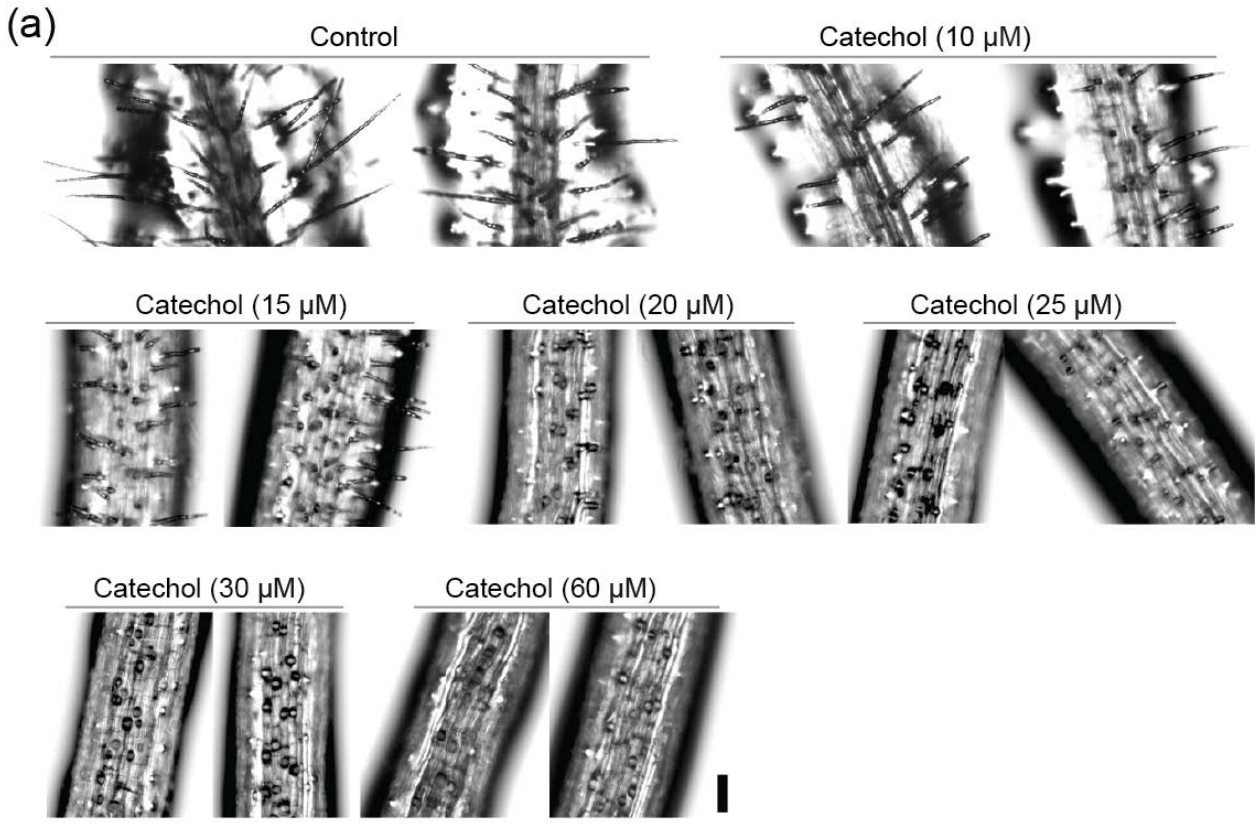


Fig. S3 Root hair phenotype induced by SPE elutions.
Same set-up as described in Fig. 2(a), but additional replicates are shown. Scale bar, 1 mm.



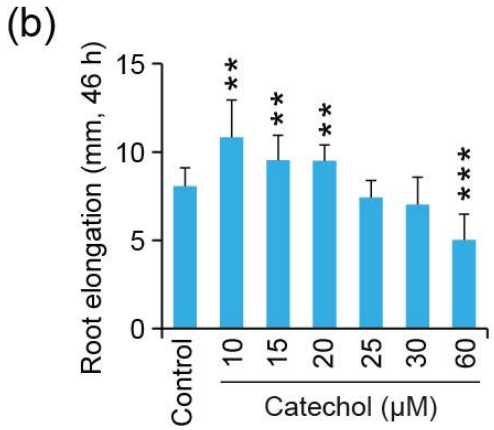


Fig. S4 Root phenotyping in response to different amounts of catechol.
(a) Root hair phenotype 46 h after catechol incubation. At concentrations higher than 15 µM, root hair elongation was strongly impaired. Scale bar, 100 µm. (b) Primary root elongation induced by catechol treatment is concentration dependent. Seedlings (5 dpg) were transferred to catechol supplemented media for 46 h. Images were taken by ApoTome microscopy (ZEISS) for root hair phenotyping (8-10 seedlings were observed) and for root elongation measurements by desktop scanner (data are means ± SD (n=3, for each biological replicate, 6-8 seedlings per Petri

dish were measured). Significant differences relative to the control group are indicated by asterisks (Student's *t*-test, **$p \leq 0.01$, ***$p \leq 0.001$).

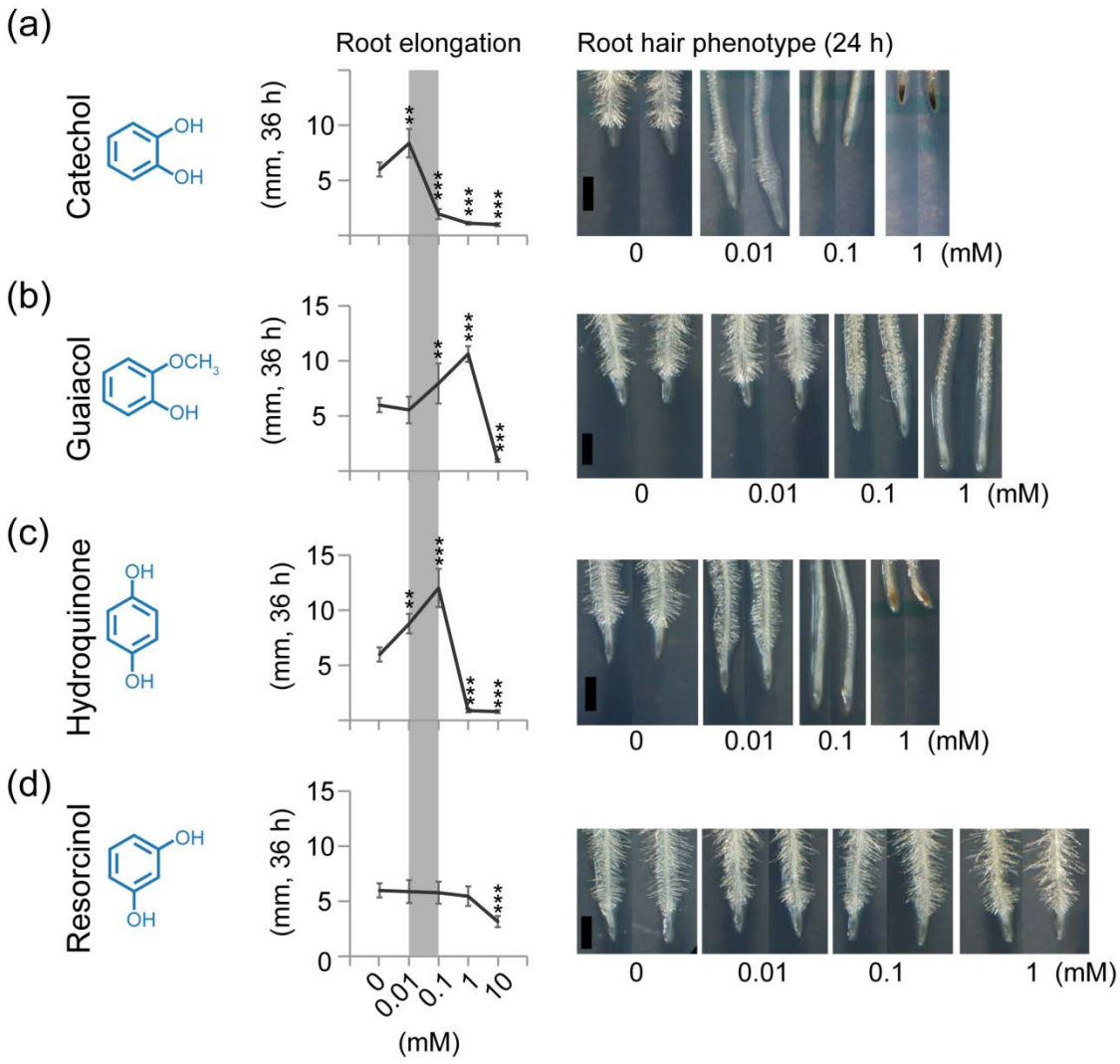


Fig. S5 Comparison of the catechol-induced root phenotype induced by structurally similar di-hydroxy/-methoxy phenolics.
(a)-(d) Structurally related compounds of catechol, including resorcinol, guaiacol and hydroquinone, were tested for root elongation measurements (36 h) and root hair phenotyping (24 h). Catechol is the most active compound used to decrease root hair elongation (0.01 mM) and abolish root elongation (0.1 mM) among tested substances. Seedlings (5 dpg) were transferred from mock media to media supplemented with different concentrations of catechol (a), guaiacol (b), hydroquinone (c) and resorcinol (d). For root elongation measurements, data are means ± SD (n=6, for each replicate 6-10 seedlings per Petri dish were measured). Significant differences relative to the control group are indicated by asterisks (Student's *t*-test, **$p \leq 0.01$, ***$p \leq 0.001$). Scale bars, 1 mm.

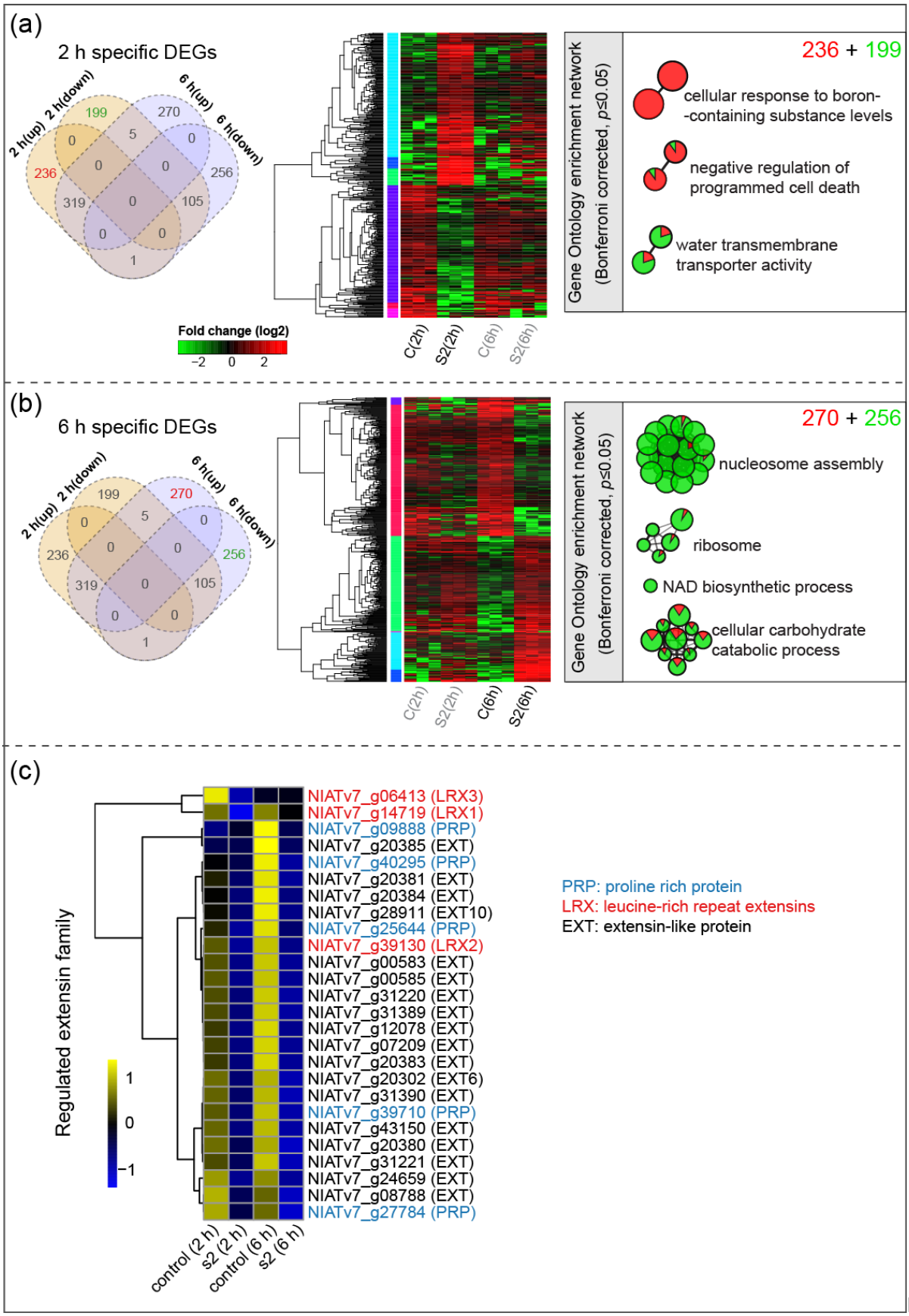

(a)
2 h specific DEGs
2 h(up)
2 h(down)
6 h(up)
6 h(down)
199
270
5
0
0
236
0
0
256
319
0
105
0
0
1
Fold change (log2)
−2 0 2
C(2h)
S2(2h)
C(6h)
S2(6h)
Gene Ontology enrichment network
(Bonferroni corrected, p≤0.05)
236 + 199
cellular response to boron-containing substance levels
negative regulation of programmed cell death
water transmembrane transporter activity
(b)
6 h specific DEGs
2 h(up)
2 h(down)
6 h(up)
6 h(down)
199
270
5
0
0
236
0
0
256
319
0
105
0
0
1
C(2h)
S2(2h)
C(6h)
S2(6h)
Gene Ontology enrichment network
(Bonferroni corrected, p≤0.05)
270 + 256
nucleosome assembly
ribosome
NAD biosynthetic process
cellular carbohydrate catabolic process
(c)
Regulated extensin family
1
0
−1
NIATv7_g06413 (LRX3)
NIATv7_g14719 (LRX1)
NIATv7_g09888 (PRP)
NIATv7_g20385 (EXT)
NIATv7_g40295 (PRP)
NIATv7_g20381 (EXT)
NIATv7_g20384 (EXT)
NIATv7_g28911 (EXT10)
NIATv7_g25644 (PRP)
NIATv7_g39130 (LRX2)
NIATv7_g00583 (EXT)
NIATv7_g00585 (EXT)
NIATv7_g31220 (EXT)
NIATv7_g31389 (EXT)
NIATv7_g12078 (EXT)
NIATv7_g07209 (EXT)
NIATv7_g20383 (EXT)
NIATv7_g20302 (EXT6)
NIATv7_g31390 (EXT)
NIATv7_g39710 (PRP)
NIATv7_g43150 (EXT)
NIATv7_g20380 (EXT)
NIATv7_g31221 (EXT)
NIATv7_g24659 (EXT)
NIATv7_g08788 (EXT)
NIATv7_g27784 (PRP)
control (2 h)
s2 (2 h)
control (6 h)
s2 (6 h)
PRP: proline rich protein
LRX: leucine-rich repeat extensins
EXT: extensin-like protein

Fig.

S6 Hierarchical clustering and functional enrichment analysis of differentially expressed genes (DEGs) in *N. attenuata* roots after treatment with SPE-fraction S2.
The same analysis was performed as described in Fig. 4 ($|\log_2 FC| > 1$, FDR ≤ 0.05), but only the DEGs specifically regulated at either 2 h (a) or 6 h (b) are shown. Gene ontology (GO) enrichment analysis of the 1391 DEGs was computed with CluoGO according to the GO categories "Biological Process" and "Molecular Function" (two-sided hypergeometric test, Bonferroni corrected, $p \leq 0.05$). DEGs of 2 h with functions in programmed cell death were specifically enriched. Only 6 h after treatment, DEGs were mainly down-regulated and related to general cell functions.

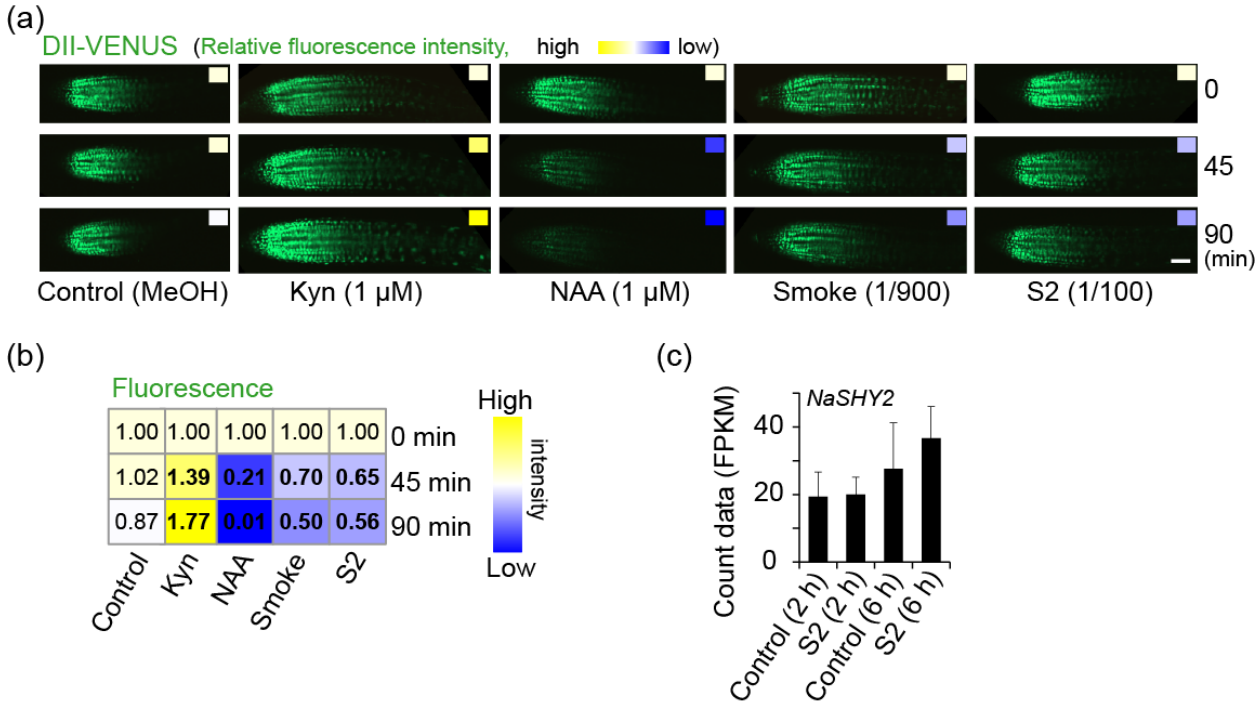


Fig. S7 Real-time auxin response monitoring (DII-VENUS, *Arabidopsis*) indicates increased auxin level after smoke and active fraction S2 incubation within 90 min.
DII-VENUS reporter line (*Arabidopsis*) was tested to monitor auxin response in a real time course after different treatments. (a) After the application of auxin biosynthesis inhibitor (L-kynurenine) and synthetic auxin (NAA), fluorescence strongly increased and decreased, respectively. Both liquid smoke and active SPE elution S2 slightly increased auxin level shown by decreased fluorescence intensity. Seedlings were scanned every 45 min for 90 min to follow the DII-VENUS signal, immediately after the beginning of treatment. Imagining was conducted by LSM-510 laser-scanning confocal microscope (Zeiss). Scale bar, 50 µm. To quantify fluorescence in the root tips, the average fluorescence intensity over the identical scanned portion of the root was extracted by ImageJ software. (b) All of the exported values of fluorescence intensity were visualized by pheatmap using R. The values in the boxes and filled color codes indicate relative fluorescence intensities. The values in black bold indicate significant differences relative to the first time-point (0 min) (Student's *t*-test, $p \leq 0.05$). For each treatment, 4-6 seedlings were analyzed and the experiment was repeated twice. (c) *NaSHY2* expression profile

in *N. attenuata* seedlings. Data are normalized FPKM (Fragments per Kilobase Million) extracted from RNA-seq (means ± SD, *n*=3).

Table S1 Provided as separate excel file

Methods S1 Extraction, fractionation of liquid smoke and quantification of catechol

Solid phase extraction (SPE, Multi 96 HR-XC (96 x 25 mg) column, Macherey-Nagel, http://www.mn-net.com/) was used as the first step of liquid smoke fractionation to isolate the active compound. In brief, 0.8 mL undiluted liquid smoke was loaded onto an activated column and flow-through was collected as S0, then successively eluted with 1 mL each of 1 M HCOOH, 80% methanol in HCOOH, 100% methanol, 0.35 M NH_4OH, 0.35 M NH_4OH in 60% methanol and 2 M NH_4OH in acetone. At each step the eluted flow-through was collected and desiccated as S0-S6 fractions and dissolved in methanol, and the obtained fractions were used for further bioassays and HPLC fractionation. The concentrated active S2 fraction was subjected to further fractionation using a reverse-phase HPLC (Luna C-18 (2), 250 × 10 mm; Phenomenex) at a 3-mL min^{-1} flow rate. The following binary gradient was used: Solvent A: millipore water (from Millipore model Milli-Q Advantage A10) and solvent B: HPLC grade methanol (Fluka, www.sigmaaldrich.com/germany.html). The details of Chromatographic solvent gradients were, from 0 to 5 min isocratic 15% of B, from 5 to 40 min linear gradient to 30% of B and from 40 to 45 min linear gradient to100% of B. “d3” and”d4” were collected from fraction “d” (26 min and 26.5 min, respectively). These purified fractions were further profiled using a Dionex UltiMate 3000 RS (U)-HPLC system (Thermo Scientific, Waltham, MA, USA) equipped with a Thermo Scientific Acclaim RP-18 column 2.2µm, 120Å, 2.1x150 mm. The following binary gradient was applied for HPLC fractionation: 0 to 0.5 min isocratic 1% A (millipore water, 0.1% [v/v] aceto-nitrile [HPLC LC-MS grade, VWR, https://de.vwr.com/, Germany], and 0.05% formic acid), 5% B (acetonitrile and 0.05% formic acid); 0.5 to 3.5 min linear gradient to 15% B; 3.5 to 30.5 min linear gradient to 30% B; 30.5 to 36min linear gradient to 99% B with a flow rate of 400 µL/min. Eluted compounds were detected by a Micro-ToF (Time of Flight) mass spectrometer (Bruker Daltonik, Bremen, Germany) equipped with an electrospray ionization source in a negative ionization mode. Instrument settings were as follows: capillary voltage, 4500 V; capillary exit, 130 V; dry gas temperature, 200°C; dry gas flow, 10 L/min. Ions were detected from m/z 50 to

1400 at a repetition rate of 2 Hz. Mass calibration was performed using sodium formate clusters (10 mM solution of NaOH in 50/50% v/v isopropanol/water containing 0.2% formic acid).

To quantify catechol levels in liquid smoke, S2 fraction and burnt soil, compounds were detected by UHPLC-ESI/qTOF mass spectrometer (Bruker Daltonik, Bremen, Germany) and analyzed using the method described above. Different concentrations of catechol were measured for a standard curve, and the content of catechol calculated was based on a comparison with the peak areas of the standard curve.