***New Phytologist* Supporting Information**

Article title: Seedling developmental defects upon blocking CINNAMATE-4-HYDROXYLASE are caused by perturbations in auxin transport

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The following Supporting Information is available for this article:

Methods S1

Statistical analysis

For statistical analysis of primary root length of the c4h-4 mutant, a Student's t-test was performed assuming equal variances (assessed with an F-test for equality of variances). For the effect of a PA concentration range on primary root length and first auxin transport assay, a one-way ANOVA analysis was performed. A post-hoc Dunnett's test was performed after establishing a significant effect (5% significance level) of the treatment with an F-test. Upon a significant interaction at the 5% significance level, Tukey post-hoc tests were performed. The number of adventitious roots were analyzed with General Estimation Equation (GEE) models with the genmod procedure from SAS. A Poisson distribution was specified with the log link function and the log transformed hypocotyl length as offset variable. Fixed factors in the analysis were depending on the experiment. For all analyses, all interaction terms were included in the model. The correlation between measurements taken on the same plate were taken into account by assuming an exchangeable correlation structure. For experiments where the number of adventitious roots was counted in the upper and lower zone of the hypocotyl, the independent blocks were at the plant level. Post-hoc tests were performed with the plm procedure from SAS. All pairwise comparison tests were performed and the familywise error

rate was controlled at the 5 % significance level using Tukey's method. The ANOVA models were performed with SAS Enterprise Guide (version 7.15), the GEE models with the SAS windowing environment (Version 9.4). Both are from SAS Institute Inc. (Cary, NC., USA).

Methods S2

Metabolite profiling

For each repeat 10 etiolated seedlings of each treatment were pooled. Samples were incubated for 15 min at 70 °C while shaking at 1000 rpm. Samples were centrifuged and 800 µL of the supernatant was transferred to a new Eppendorf and dried under vacuum. The pellet was disolved 100 µL cyclohexane and 100 µL milliQ water was added. After centrifugation 70 µL of the water phase was subjected to UHPLC-MS on an ACQUITY UPLC I-Class system (Waters) consisting of a binary pump, a vacuum degasser, an autosampler, and a column oven. Chromatographic separation was performed on an ACQUITY UPLC BEH C18 (150 × 2.1 mm, 1.7 µm) column (Waters), while maintaining the temperature at 40 °C. A gradient of two buffers (A and B) was utilized: buffer A (99:1:0.1 water:acetonitrile:formic acid, pH 3) and buffer B (99:1:0.1 acetonitrile:water:formic acid, pH 3), as follows: 99% A for 0.1 min decreased to 50% A in 30 min, decreased to 0% from 30 to 40 min. The flow rate was 0.35 mL per min, and the injection volume was 10 µL. This UHPLC system was connected to a Vion IMS QTOF hybrid mass spectrometer (Waters). The LockSpray ion source was used in negative electrospray ionization mode under the following specific conditions: capillary voltage, 3 kV; reference capillary voltage, 2.5 kV; cone voltage, 30 V; source offset, 50 V; source temperature, 120 °C; desolvation gas temperature, 550 °C; desolvation gas flow, 800 liter per h; and cone gas flow, 50 liter per h. The collision energy for full MSe was set at 6 eV (low energy) and ramped from 20 to 70 eV (high energy), intelligent data capture intensity threshold was set at 5. For DDA-MSMS, the low mass ramp was ramped between 15 and 30 eV. The high mass ramp was ramped between 30 and 70 eV. Nitrogen (greater than 99.5%) was used as desolvation and cone gas. Leucin-enkephalin (250 pg per µL solubilized in water:acetonitrile 1:1 (v/v), with 0.1% formic acid) was utilized for the lock mass calibration, with scanning every 2 min at a scan time of 0.1 s. Profile data were recorded through a UNIFI Scientific Information System (Waters). Data processing

was performed with Progenesis QI software version 2.4 (Waters). Compound annotation was based on matching m/z, retention time, and tandem mass spectroscopy fragmentation against an in-house library. PCA plots were generated using MetaboAnalyst 4.0.

Methods S3

Auxin binding and docking experiments

Auxin receptor proteins AtTIR1 and AtAFB5 were expressed in insect cells (T. ni High5) and purified as described previously (Lee et al., 2014). The SPR experiments to determine auxin binding and antiauxin activity followed the methods described previously (Lee et al., 2014). Docking was performed using the Vina docking algorithm (Morris et al., 2009; Trott and Olson, 2010) using the TIR1 crystal structure (PDB code 2P1P). In-silico modeling, molecular graphics and analyses were performed with the UCSF Chimera package. Chimera is open source and developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIGMS P41-GM103311) (Pettersen et al., 2004). Marvin calculator Plugins were used for structure property prediction and calculation Marvin v15.10.12.0, 2015, ChemAxon (http://www.chemaxon.com).

Methods S4

GC-MS analysis

For each repeat 10 etiolated seedlings of each treatment were pooled. The material was flash-frozen in liquid N2 and ground in 2-mL Eppendorf tubes using a Retsch mill (1 min, 20 Hz, 5-mm bead). Samples were extracted with 1 mL methanol and the dry weight of the pellet was determined. The obtained extracts were derivatized using 10 µL of pyridine and 50 µL of N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA; Sigma-Aldrich, Saint Louis, MO, USA) for GC-MS analysis and stock solutions for a standard curve (concentration range from 100 to 5000 ng/ml) were prepared in 100% methanol. GC-MS analysis was carried out using a 7890B GC system equipped with a 7693A Automatic Liquid Sampler and a 7250 Accurate-Mass

Quadrupole Time-of-Flight MS system (Agilent Technologies, Santa Clara, CA, USA). 1 µl of the sample was injected in splitless mode with the injector port set to 280°C. Separation was achieved with a VF-5ms column (30 m x 0.25 mm, 0.25 µm; Varian CP9013; Agilent Technologies) with helium carrier gas at a constant flow of 1.2 ml/min. The oven was held at 80°C for 1 min post-injection, ramped to 280°C at 5°C/min, held at 280°C for 5 min, ramped to 320°C at 20°C/min, held at 320°C for 5 min, and finally cooled to 80°C at 50°C/min at the end of the run. The total run time was 63.8 min. The MSD transfer line was set to 280°C and the electron ionization energy was 70 eV. Full EI-MS spectra were recorded between m/z 50-800 at a resolution of >25,000 and with a solvent delay of 7.8 min. The resulting GC-MS chromatograms were converted to TDA format and cinnamic acid peak areas were determined using the MassHunter Quantitative Analysis (for Q-TOF) software package (Agilent Technologies). The fragment ion at m/z 205.0677 was selected as quantifier, with the fragment ions at m/z 161.0778, m/z 145.0466, and m/z 131.0489 as qualifiers.

REFERENCES

Lee, S., Sundaram, S., Armitage, L., Evans, J.P., Hawkes, T., Kepinski, S., Ferro, N., and Napier, R.M. (2014). Defining Binding Efficiency and Specificity of Auxins for SCFTIR1/AFB-Aux/IAA Co-receptor Complex Formation. Acs Chem Biol 9, 673-682.

Morris, G.M., Huey, R., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S., and Olson, A.J. (2009). AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. J Comput Chem 30, 2785-2791.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E. (2004). UCSF chimera - A visualization system for exploratory research and analysis. J Comput Chem 25, 1605-1612.

Trott, O., and Olson, A.J. (2010). Software News and Update AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. J Comput Chem 31, 455-461.