

Supporting Information Legend

Supplemental Figure S1. Transcriptome profiling of Arabidopsis after treatment with *E*-2-hexenal. **(A)** Venn diagrams showing the number of transcripts with a change in abundance of at least two-fold ($p < 0.05$; FDR 0.05) following 1h, 3h or 24h exposure of Arabidopsis leaves to *E*-2-hexenal. **(B, C)** Functional categorization of the genes differentially expressed after *E*-2-hexenal treatment using FatiGo ([hQp://babelomics.bioinfo.cipf.es](http://babelomics.bioinfo.cipf.es)). Overrepresented GO terms for the genes either up regulated (B) or down regulated (C) after 1h, 1–3h and 3h treatment with *E*-2-hexenal at biological process levels 3–9 ($p \leq 0.005$). Number of genes for each GO category is indicated as percentage of the total number of genes with an ontology at each time-point. Only categories $\geq 3\%$ of total number of genes are shown. Functional categorizations at the classes Molecular Function and Cellular Compartment are shown in supplemental figure S1 and S2 online.

Supplemental Figure S2. Q-RT-PCR of Arabidopsis Plants Treated with SA, MeJA, ABA or ACC for control marker genes. Arabidopsis plants were treated with 300 μ M SA, 100 μ M MeJA, 50 μ M ABA, 100 μ M ACC or mock treated for 1h and 3h and the transcript levels of *SUMO3*, *JAZ10*, *RD29B* and *ERF1*, respectively, were measured by Q-RT-PCR. Values are normalized to the expression of *SAND* and are means of three independent biological replicates. Error bars indicate SE; asterisks indicate significant differences between hormone and mock treated samples (ANOVA, ** = $p \leq 0.01$, * = $p \leq 0.05$ according to LSD post-hoc analysis).

Supplemental Figure S3. Enrichment of the WRKY-box Motif in the Promoters of the Early-Point (1h) Time and Mid-Point Time (1h and 3h) Genes Up-Regulated by *E*-2-hexenal. **(A)** Motif analysis (The Arabidopsis Information Resource; TAIR), Regulatory Sequence Analysis Tool (RSAT; [hQp://rsat.ulb.ac.be](http://rsat.ulb.ac.be); (van Helden et al., 1998)), ATHENA ([hQp://www.bioinformalcs2.wsu.edu/cgi-bin/Athena/cgi/home.pl](http://www.bioinformalcs2.wsu.edu/cgi-bin/Athena/cgi/home.pl); (O'Connor et al., 2005)) and AlignACE ([hQp://atlas.med.harvard.edu](http://atlas.med.harvard.edu); (Roth et al., 1998)) identified the W-box motif (TTGACY) as the only known *cis*-element statistically enriched in the promoters of the early-point (1h) time and mid-point (1h and 3h) time genes up-regulated by *E*-2-hexenal. Numbers between brackets indicate p-values; * = MAP score, a measure of the motif significance (the higher the MAP score the more significant is the associated motif). ND, not detected; Y: C/T; K: G/T. **(B)** Occurrences of the TTGACY motif in the promoter regions of the early-point time genes induced by *E*-2-hexenal (blue line) compared to a set of random Arabidopsis promoters (red line) analysed with POBO ([hQp://ekhidna.biocenter.helsinki.fi/poxo](http://ekhidna.biocenter.helsinki.fi/poxo); (Kankainen and Holm, 2004)). Different letters indicate statistically significant differences in occurrence of the TTGACY motif (χ^2 test; $\alpha = 0.05$).

Supplemental Figure S4. Root Growth of Wild-Type (Col-0) and *wrky* Mutant Seedlings after Aerial Treatment with *E*-2-hexenal. Three-day old wild-type (Col-0), *wrky40*, *wrky53*, *wrky53 wrky6* and *wrky53* seedlings were exposed for 24h to various concentrations of aerial *E*-2-hexenal. Root growth was measured 3 days after the treatment. Values (representative of three independent experiments) are means of ≥ 15 seedlings for each treatment/genotype combination. Error bars indicate SE.

Supplemental Figure S5. WRKY6 and WRKY40 are non-redundant regulators of gene expression and GABA-shunt induction by *E*-2-hexenal in Arabidopsis. **(A–C)** Transcript levels of early time point (1h) *E*-2-hexenal responsive genes were determined by Q-RT-PCR in Arabidopsis leaves in response to 3 μ M aerial *E*-2-hexenal or to methanol (MeOH) as control treatment. Values are normalized to the expression of *SAND* and are the means of three independent biological replicates (2 pooled plants each). Error bars indicate SE; asterisks indicate significant change by *E*-2-hexenal treatment; different letters indicate significant differences in expression level between different genotypes (generalized linear model with γ error distribution, logarithmic link function and technical replicates nested to biological replicates, ** = $p \leq 0.01$ and different letters = $p \leq 0.05$ according to LSD Post-hoc analysis). **(D)** GABA levels in wild-type and *gad4* Arabidopsis leaves after treatment with 3 μ M aerial *E*-2-hexenal or methanol (MeOH), as control treatment, for 3h. Values are normalized to the expression of *SAND* and are the means of six independent biological replicates (2 or 3 pooled plants each). Error bars indicate SE; letters indicate significant differences in expression level after log transformation of the data (ANOVA, $p \leq 0.01$ according to LSD Post-hoc analysis).

Figure S6. Percentage recovery of GLVs in the gaseous phase in the desiccators. The amount of GLVs present in the gaseous phase in the desiccators after 3h exposure time was determined, with $3 \mu\text{mol l}^{-1}$ (plant assays) for each GLV or $0,3 \mu\text{mol l}^{-1}$ (plate assays) for *E*-2-hexenal. The volatiles in the desiccators were collected as described in the methods section and compared with the initial amount of GLV applied. The percentage recovery of each GLV in the gaseous phase is shown. *E*-2-hexenal (E2AL), *Z*-3-hexenal (Z3AL), *E*-2-hexenol (E2OL), *Z*-3-hexenol (Z3OL), *E*-2-hexenyl acetate (E2Ac), *Z*-3-hexenyl acetate (Z3Ac).

Table S1. Regulation of *E*-2-hexenal induced genes by SA, JA, ABA and ET.

Table S2. Sequences of the primer pairs used for the Q-RT-PCR experiments.

Data Set1. Differentially expressed genes up-regulated by *E*-2-hexenal treatment on at least one time-point in Arabidopsis.

Data Set2. Differentially expressed genes down-regulated by *E*-2-hexenal treatment on at least one time-point in Arabidopsis.

Data Set3. Genes up-regulated by *E*-2-hexenal and not by SA, JA, ABA and ET in Arabidopsis.