Establishment of long-term methyl jasmonate-induced resistance in Norway spruce

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Abstract

Norway spruce (Picea abies) is an economically and ecologically important tree species that grows across northern and central Europe. Treating Norway spruce with jasmonate has long-lasting beneficial effects on tree resistance to damaging pests, such as the European spruce bark beetle Ips typographus and its fungal associates. The potential involvement of (epi)genetic mechanisms in this long-lasting jasmonate-induced resistance (IR) has gained much recent interest, but remains largely unknown. In this study, we treated 2-year-old spruce seedlings with methyl jasmonate (MeJA) and challenged them with the I. typographus vectored necrotrophic fungus Grosmannia penicillata. MeJA treatment reduced the extent of necrotic lesions in the bark and thus elicited IR to the fungus. The transcriptional response of spruce bark to MeJA treatment was analyzed over a 4-week time course using mRNA-seq. This analysis provided evidence that MeJA treatment induced a transient upregulation of jasmonic acid, salicylic acid and ethylene biosynthesis and downstream signaling genes. Additionally, genes encoding components of the RNA-directed DNA methylation pathway showed long-term repression, suggesting a possible role of DNA demethylation in the maintenance of MeJA-IR. These results provide new clues about the potential mechanisms underpinning long-term MeJA-IR in Norway spruce.

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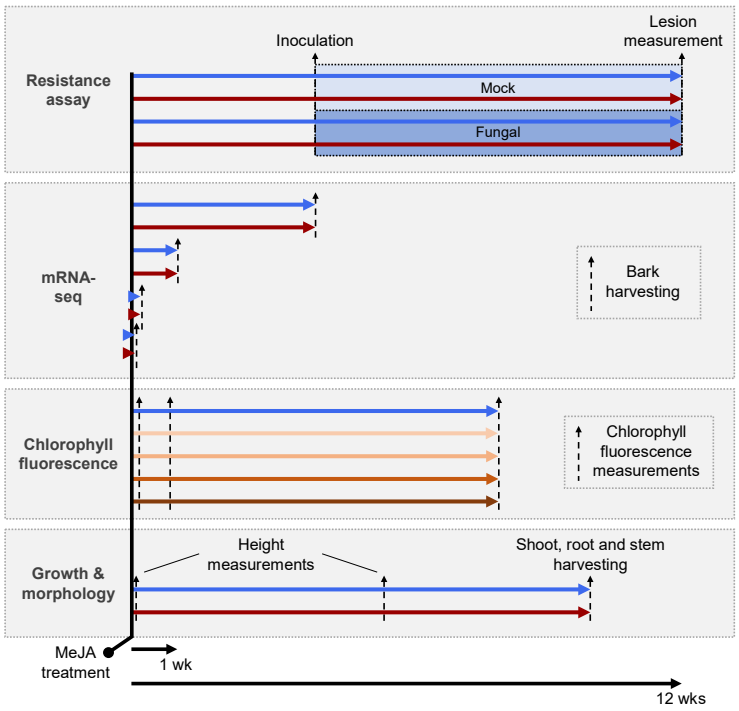

Figure 1. Experimental setup to study establishment and maintenance of methyl jasmonate (MeJA)-induced resistance in Norway spruce seedlings.

Two-year-old seedlings were treated with either water (blue lines), 10 mM MeJA (dark red lines) or other concentrations of MeJA (orange lines; details below). For the resistance assay, plants were inoculated with a necrotrophic fungal pathogen or mock 4 weeks (wks) after treatment. Eight weeks later, necrotic lesions were measured. mRNA-seq was conducted on mRNA extracted from bark tissue harvested from plants at 3 hours (hrs), 24 hrs, 1 wk and 4 wks after treatment. Chlorophyll fluorescence was measured 1 day, 6 days and 8 wks after treatment with 0, 5, 25, 50, or 100 mM MeJA. Plant height growth was measured twice over a period of 5.5 wks. Shoots, roots and stems were then harvested at 10 wks to explore the impact of MeJA on plant growth and morphology.

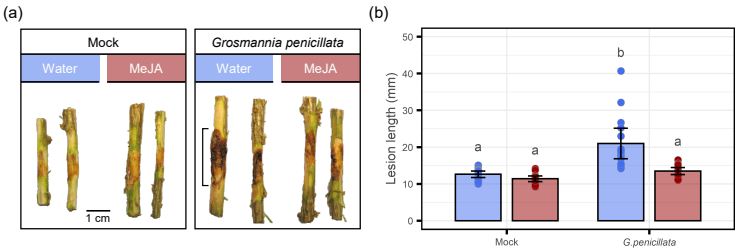

Figure 2. MeJA elicits induced resistance against *Grosmannia penicillata*.

Two-year-old Norway spruce seedlings were treated with either water (blue) or 10 mM MeJA (red) four weeks prior to inoculation with either a *G. penicillata* or sterile malt agar inoculum. Symptoms were assessed 8 weeks post inoculation. (a) The first stem internode, with wound and/or fungal induced cell death, from all plants of one representative experimental block. (b) The full axial length of wounds or necrotic lesions (indicated in (a)) were measured on all stems. Results are presented as mean ± 95% confidence interval of the mean. Points show individual replicates (n = 15). Bars with different letters are significantly different (ANOVA followed by Tukey post hoc test, $p < 0.05$).

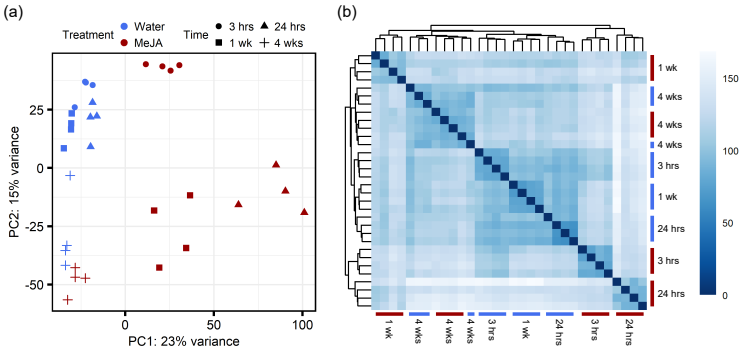

Figure 3. Treatment with methyl jasmonate (MeJA) induces a transient shift in the transcriptome of Norway spruce bark.

Principle component analysis (PCA) (a) and hierarchical clustering analysis (HCA) (b) plots displaying how treatment of 2-year-old spruce seedlings with water (blue) or 10 mM MeJA (red) impacts on the bark transcriptome over the subsequent 4 weeks. All genes with a total mRNA-seq read count of ≥ 100 across the 32 samples (n = 4 per treatment and time point) were included in the analyses. Both the PCA and the HCA utilised counts normalised with a variance-stabilizing transformation. Samples in the HCA were clustered using the Euclidean distances between samples (darker blue for lower distances) and the complete-linkage method.

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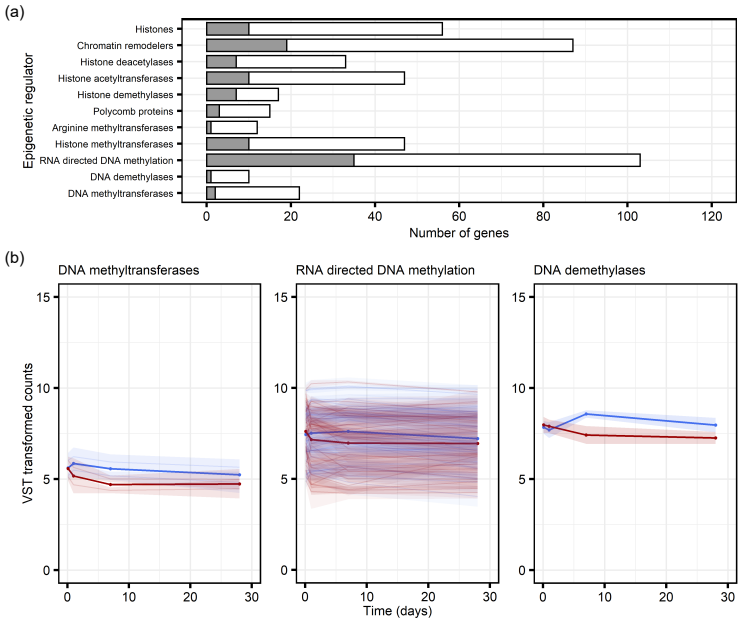

Figure 8. Methyl jasmonate (MeJA) treatment alters the expression of regulators of multiple epigenetic modifications in Norway spruce bark, including DNA methylation.

(a) Bars show the number of annotated genes in different epigenetic regulator categories. Genes showing a significantly (adjusted p-value < 0.001) altered expression pattern across time as a result of treatment are indicated in grey. (b) The individual transcripts (faint lines with 95% confidence intervals) and category means (solid lines) for differentially expressed regulators of DNA methylation homeostasis in water (blue) and MeJA (red) treated bark (See Supplemental Data Set 6). Read counts were normalised using the variance stabilizing transformation (VST) in DEseq2. Epigenetic regulator categories are taken from Mageroy et al (2020b).

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