Long-term Exposure to Methyl Jasmonate Increases Myrosinases TGG1 and TGG2 in Arabidopsis coi1 and myc2,3,4 Mutants

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April 18, 2024

Abstract

Myrosinase is an enzyme that activates a group of metabolites, namely glucosinolates, which are involved in the defense against herbivores and pathogens. In Arabidopsis thaliana, THIOGLUCOSIDE GLUCOHYDROLASE 1 (TGG1) and TGG2 are two myrosinases that accumulate in specialized myrosin cells of rosette leaves. Here, we show that prolonged exposure to the wounding hormone methyl jasmonate (MeJA) enhances TGG1 and TGG2 expression independent of the canonical jasmonic acid (JA) signaling pathway. We found that airborne MeJA treatment for up to 5-days enhanced both TGG1 and TGG2 gene expression and their protein levels in Arabidopsis leaves. TGG1 and TGG2 gene expression did not stop in two JA signaling pathway mutants, namely coi1-16 and myc2,3,4, after 5-days of MeJA treatment, although other typical JA responses were completely stopped in these two mutants. FAMA is a transcription factor that participates in the specification of myrosin cell development, but it does not appear to be involved in TGG1 and TGG2 gene expression regulation in the 5-day MeJA treatment, as its expression did not increase. Taken together, our results suggest the existence of an alternative JA signaling pathway that is activated by long-term exposure to MeJA.

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Figure 1. Long-term MeJA treatment increases the expression of TGG1 and TGG2 in wild-type rosette leaves.

a) The schemes show the experimental setup of mock and MeJA treatment. MeJA treatment was started 7 d after germination, and plants were sampled 12 d after germination. b, c) The relative expression levels of TGG1 and TGG2 (b), and the protein amount of TGG1 and TGG2 (c) in the shoots of mock or long-term MeJA treated Arabidopsis wild type (Col-0) plants. Error bars denote the standard error of three biological replications. Double asterisks denote p < 0.01 based on the student's *t*-test. d) Confocal microscopic images of the first leaves of 12-d-old transgenic plants harboring pTGG2:Venus-2sc. Myrosin cells (arrows) and stomata guard cells (arrowheads) are recognized with Venus fluorescence but have different shapes. e, f) The ratio of myrosin cell areas per leaf area in the first and second (e) or fourth (f) leaves, which is calculated from confocal images. Error bars denote the standard error of seven biological replications. *** denotes p < 0.001 and ns denotes no significance based on the student's t-test. g) Confocal microscopic images of the emerging first leaves of 6-dold transgenic plants harboring pTGG2: Venus-2sc. MeJA treatment was started 2 d after germination. Green shows Venus fluorescence in myrosin cells, and red shows propidium iodide fluorescence in cell walls and nuclei.



Figure 2. Long-term MeJA treatment increases the expression of TGG1 and TGG2 in *coi1-16* and *myc2,3,4* mutants.

a) The schemes show the experimental setup of mock, short-term and long-term MeJA treatments. MeJA treatment was started 7 (for the long-term MeJA treatment) or 11 d (for the short-term MeJA treatment) after germination, and plants were sampled 12 d after germination. **b-e**) The relative expression levels of TGG1 (b) and TGG2 (c), and the protein amount of TGG1 (d) and TGG2 (e) in the shoots of mock, short- and long-term MeJA treated Arabidopsis wild type (Col-0), *coil-16* and *myc2,3,4* mutants. Error bars denote the standard error of three biological replications. Double asterisks denote p < 0.01 based on the student's *t*-test.



Figure 3. Long-term MeJA treatment does not change the expression of FAMA.

a) The relative expression levels of FAMA in the rosette leaves of 12-d-old Arabidopsis wild-type (Col-0) plants. Error bars denote the standard error of three biological replications. ns, no significance based on the student's *t*-test. b) Epifluorescence microscope images of the first leaves of 10-d-old transgenic plants harboring pFAMA:GFP. Long-term MeJA treatment was started 5 d after germination.



Figure 4. MeJA treatment does not reduce plant growth in *coi1-16* and *myc2,3,4* mutants.

a) Images of mock or long-term MeJA treated 12-d-old-plants. Arrows show root growth toward the MeJA source (asterisks). b) Shoot fresh weight of plants used in (a). Error bars denote the standard error of ten plants. Asterisk denotes p < 0.05, and ns denotes no significance based on the student's *t*-test.



Figure 5. Canonical MeJA-responsive genes do not respond in *coi1-16* and *myc2,3,4* mutants.

a, **b**) The relative expression levels of *VSP2* (a) and *BGLU18* (b) in the shoots of mock, short- and long-term MeJA-treated Arabidopsis wild type (Col-0), *coi1-16* and *myc2,3,4* mutants. Error bars denote the standard error of three biological replications. Double asterisks denote p < 0.01 based on the student's *t*-test.