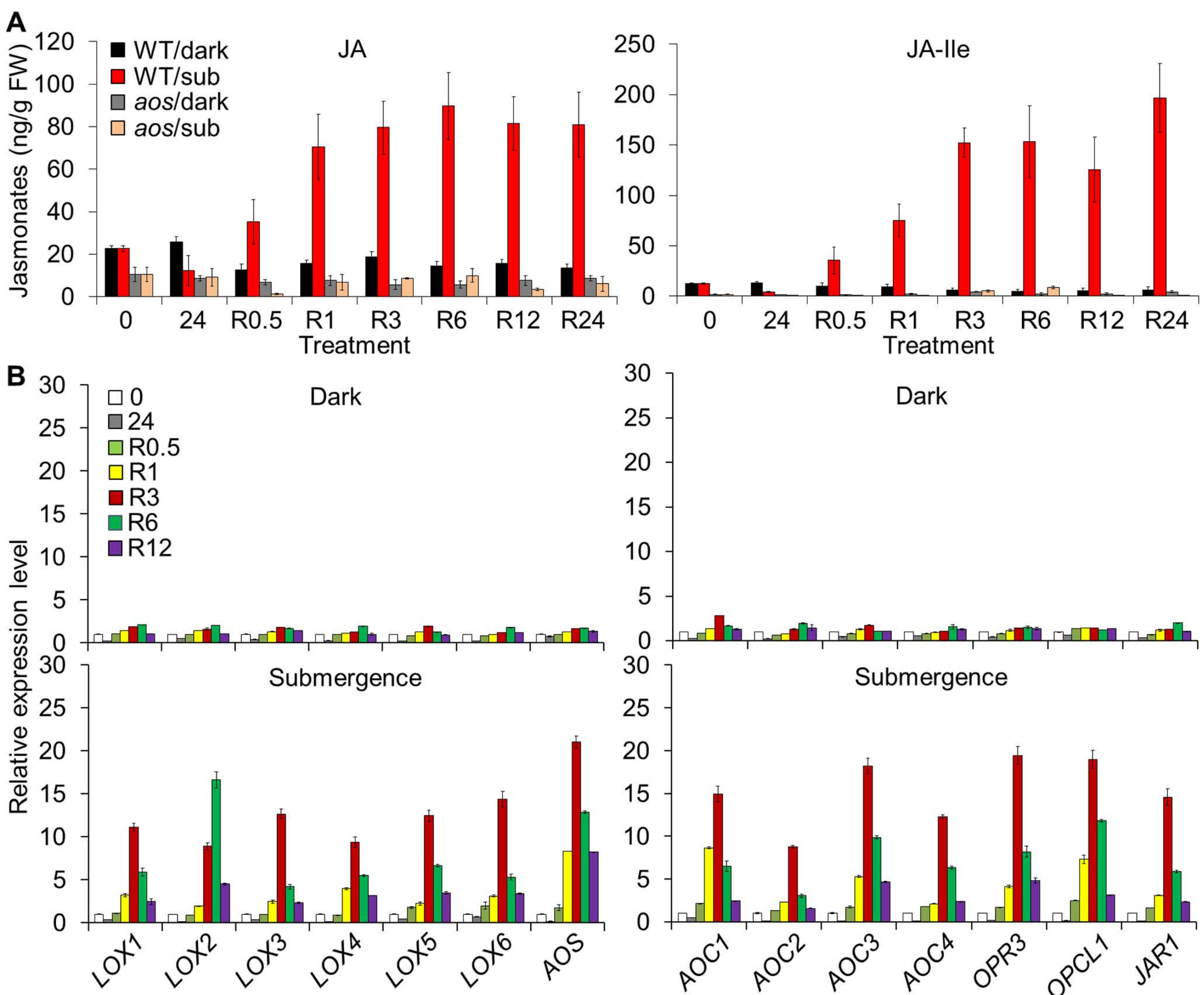
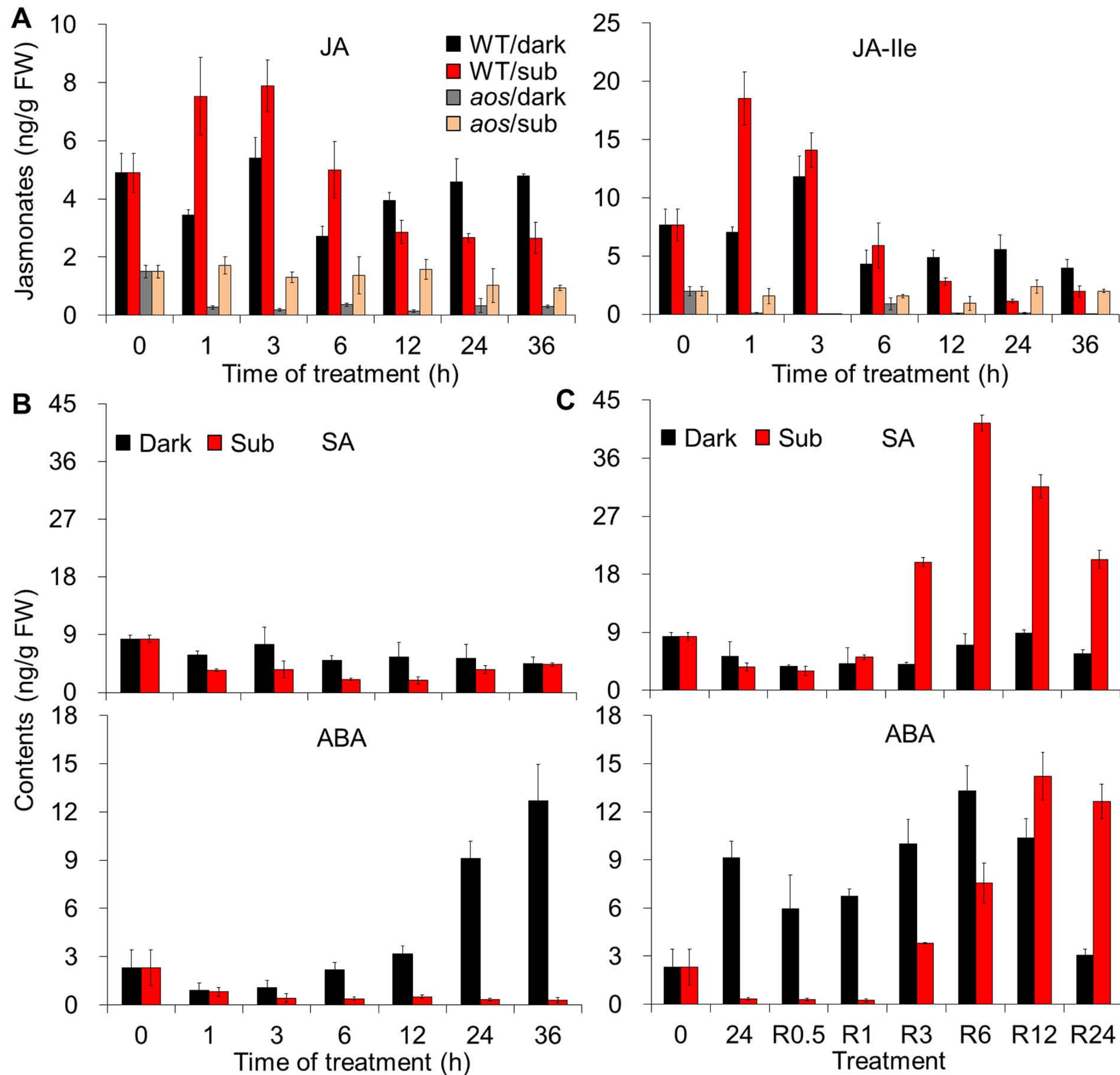
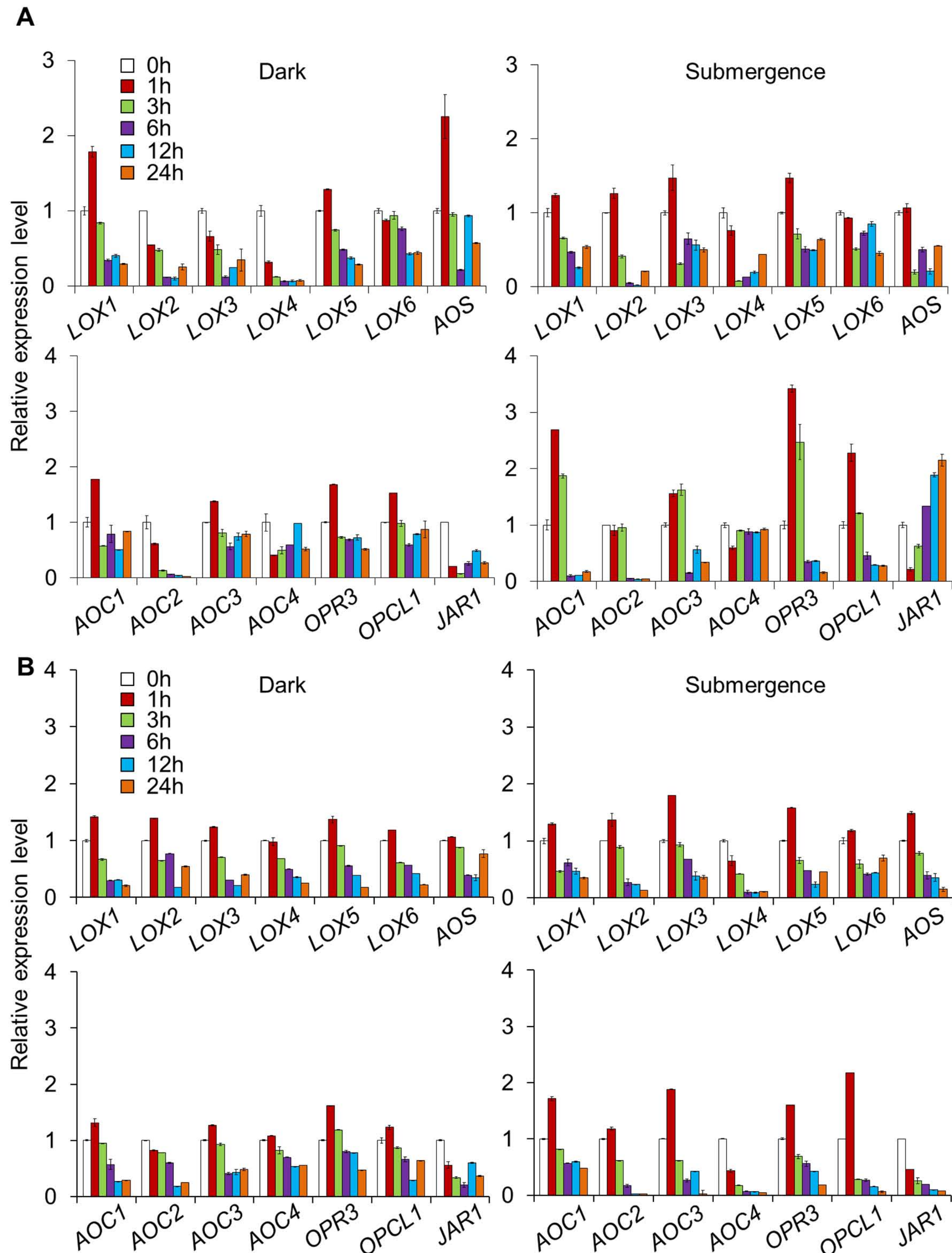


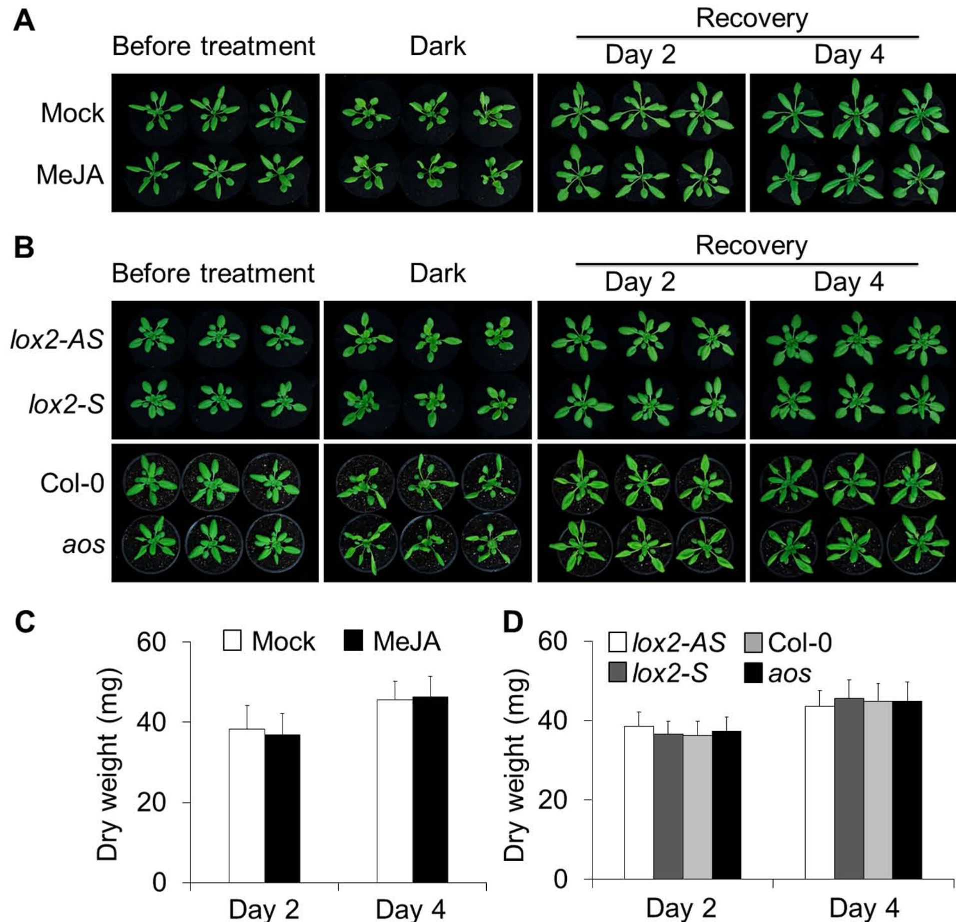
Supplementary Figure S1. A second biological repeat showing the induction of JA biosynthesis in wild-type leaves during recovery after dark or dark submergence. **A**, The endogenous JA and JA-Ile levels in the wild type (WT) and *aos* mutant in response to submersion and reoxygenation. Three-week-old WT and *aos* mutant plants were treated with dark only or dark submergence for 24 h, followed by reoxygenation. Rosette leaf samples were collected for JA extraction at 0 and 24 h of dark or dark submergence and after 0.5, 1, 3, 6, 12, and 24 h of reoxygenation and then analyzed by LC/MS. The data are means ± SD (*n* = 8 technical replicates) (200 mg of leaves harvested from three independent plants were pooled for each technical replicate). **B**, Quantitative PCR analyses showing the expression levels of JA biosynthetic genes (*LOX1*, *LOX2*, *LOX3*, *LOX4*, *LOX5*, *LOX6*, *AOS*, *AOC1*, *AOC2*, *AOC3*, *AOC4*, *OPR3*, *OPCL1*, and *JAR1*). Total RNA was isolated from rosettes of four-week-old soil-grown plants at 0 and 24 h of dark or dark treatment and recovery at 0.5, 1, 3, 6, and 12 h under light conditions. Transcript levels relative to 0 h (prior to treatment) for each time point were normalized to the levels of *TUB3*. Three biological replicates were conducted with similar results and the representative data from one replicate are shown. The data are means ± SD (*n* = 3 technical replicates).



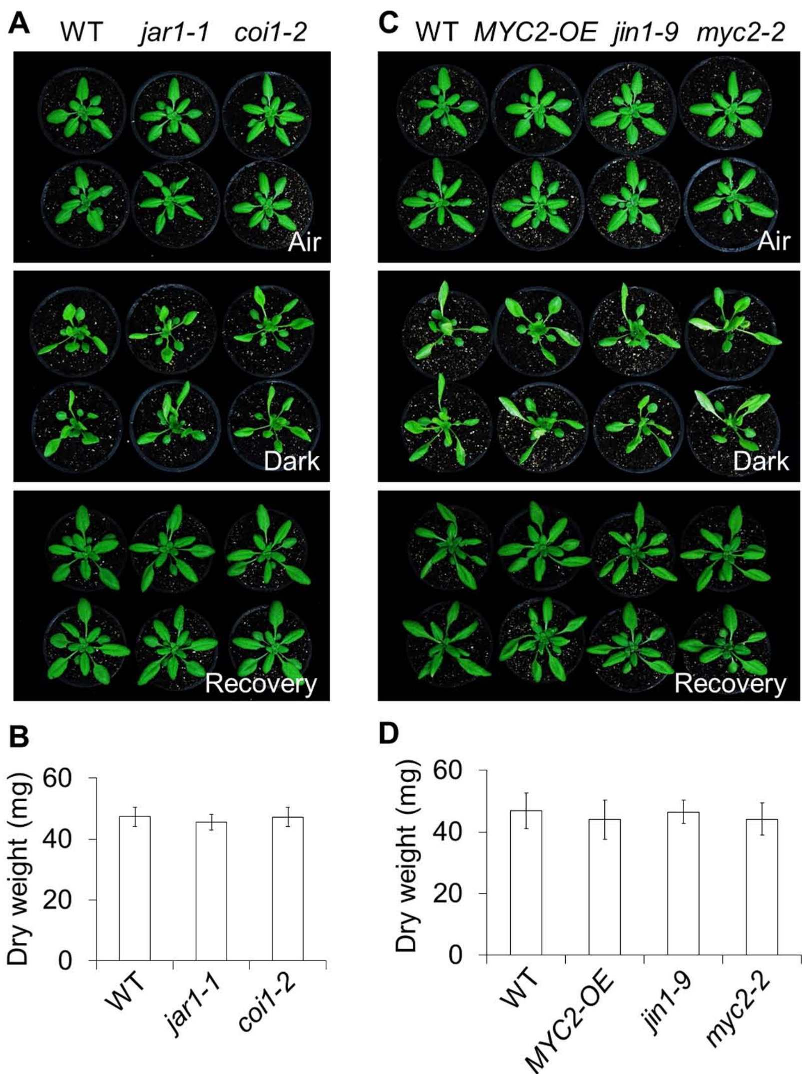
Supplemental Figure S2. Measurement of JA, JA-Ile, SA, and ABA during submergence or post-submergence reoxygenation. **A**, The endogenous JA and JA-Ile levels in the wild type (WT) and *aos* mutant during submergence. Four-week-old WT and *aos* mutant plants were treated with dark or dark submergence. **B** and **C**, The levels of SA and ABA in the WT leaves during submergence (**B**) and post-submergence reoxygenation (**C**). Four-week-old WT plants were treated with dark (Dark) or dark submergence (Sub) for 24 h, followed by reoxygenation. Rosette samples were collected at 0, 1, 3, 6, 12, 24, and 36 h of dark or dark submergence (**A** and **B**) as well as at 0, 0.5, 1, 3, 6, 12, and 24 h of reoxygenation (**C**) for JA, SA, and ABA extraction and then analyzed by LC/MS. H_2-JA, D_4-SA, and D_6-ABA were added as internal quantitative standards. The data are means $\pm$ SD (n = 8 technical replicates).



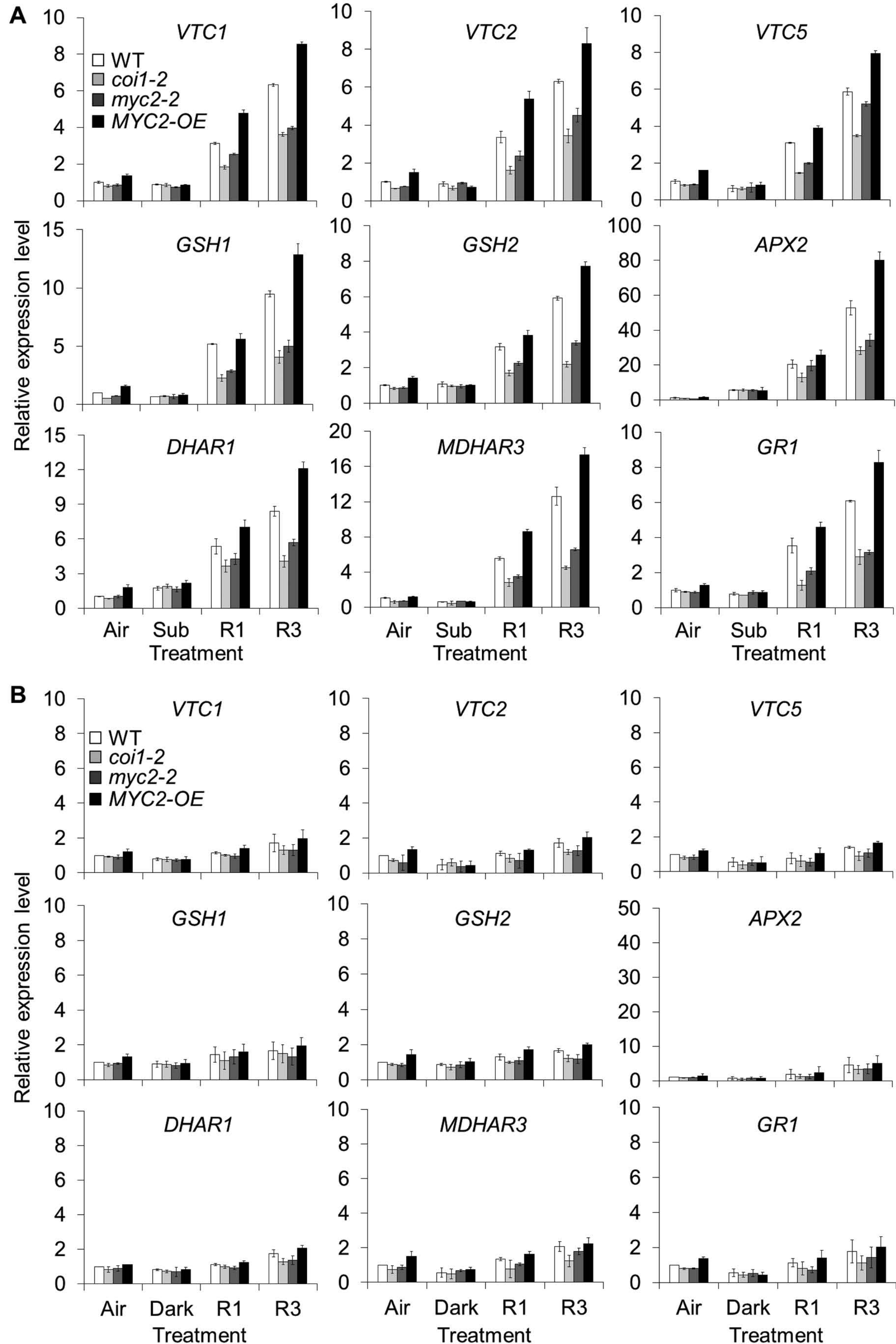
Supplemental Figure S3. The expression of JA biosynthetic genes in wild-type leaves during submergence treatment. Quantitative PCR analyses showing the submergence-downregulated expression of JA biosynthetic genes (*LOX1*, *LOX2*, *LOX3*, *LOX4*, *LOX5*, *LOX6*, *AOS*, *AOC1*, *AOC2*, *AOC3*, *AOC4*, *OPR3*, *OPCL1*, and *JAR1*). Total RNA was isolated from rosette leaves of four-week-old soil-grown plants during dark submergence at the indicated time points. Transcript levels relative to 0 h (prior to submergence) for each time point were normalized to the levels of *TUB3*. Two biological replicates (**A** and **B**) were conducted with similar results. The data are means ± SD (n = 3 technical replicates).



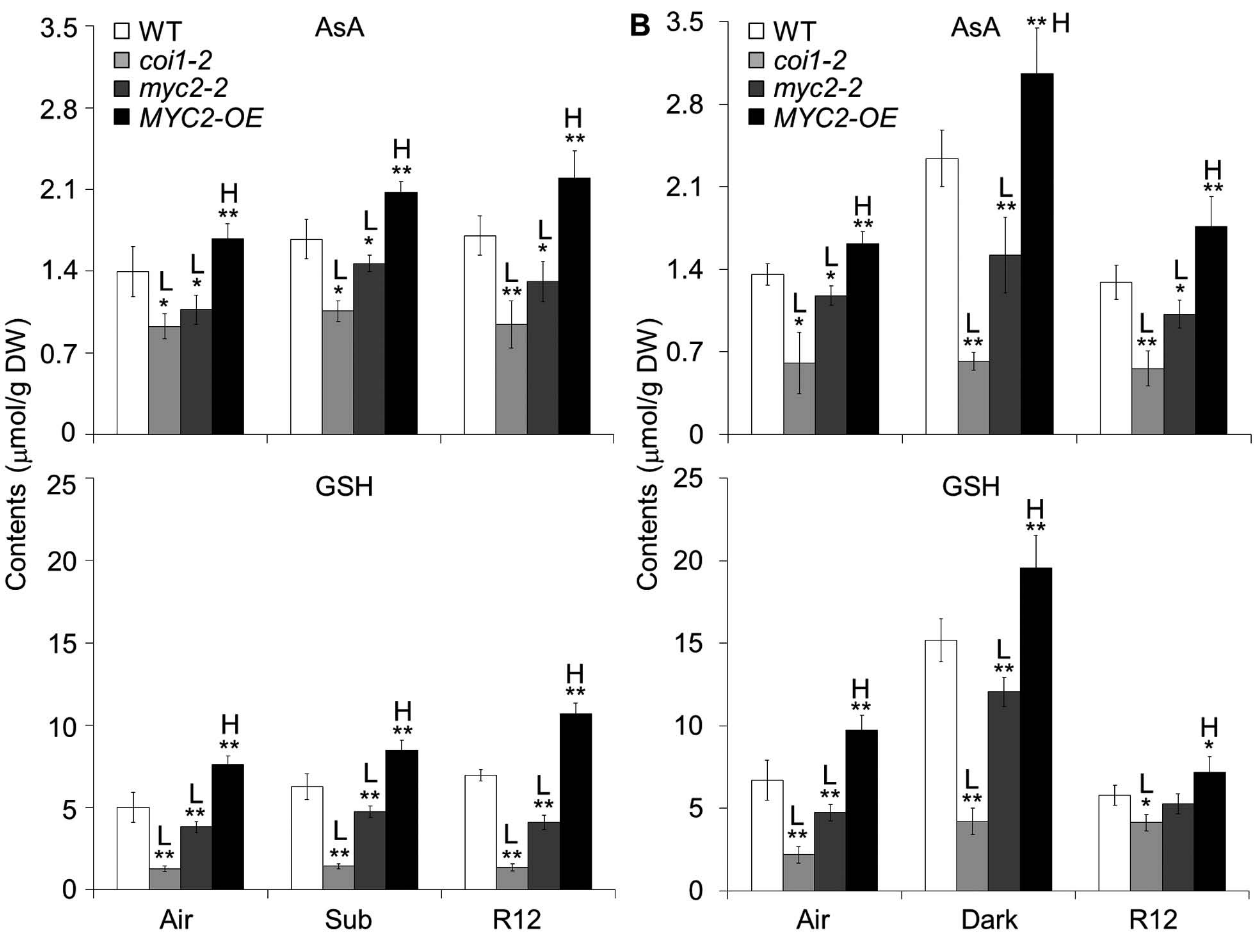
Supplemental Figure S4. The control plants for JA biosynthesis showing no difference between each group before and after 48-h dark treatment followed by recovery. **A** and **C**, phenotypes (**A**) and dry weights (**C**) of wild-type (WT) plants treated with exogenous MeJA and then subjected to dark treatment and recovery. Four-week-old WT plants were pre-treated with 100 μM MeJA or mock solution (0.1% ethanol in water) for 24 h. Plants were photographed before treatment, and after 48-h dark treatment followed by recovery for 2 and 4 d. **B** and **D**, Phenotypes (**B**) and dry weights (**D**) of *lox-S* and *aos* mutants and their corresponding controls (*lox2-AS* and Col-0; four-week-old) before treatment and after 48-h dark treatment followed by reoxygenation for 2 and 4 d. The data in **C** and **D** are means ± SD (*n* = 3 independent experiments). For each experiment, at least 15 plants were used for each genotype. All genotypes completely recovered from the dark treatment and no significant difference was detected for the dry weight measurements.



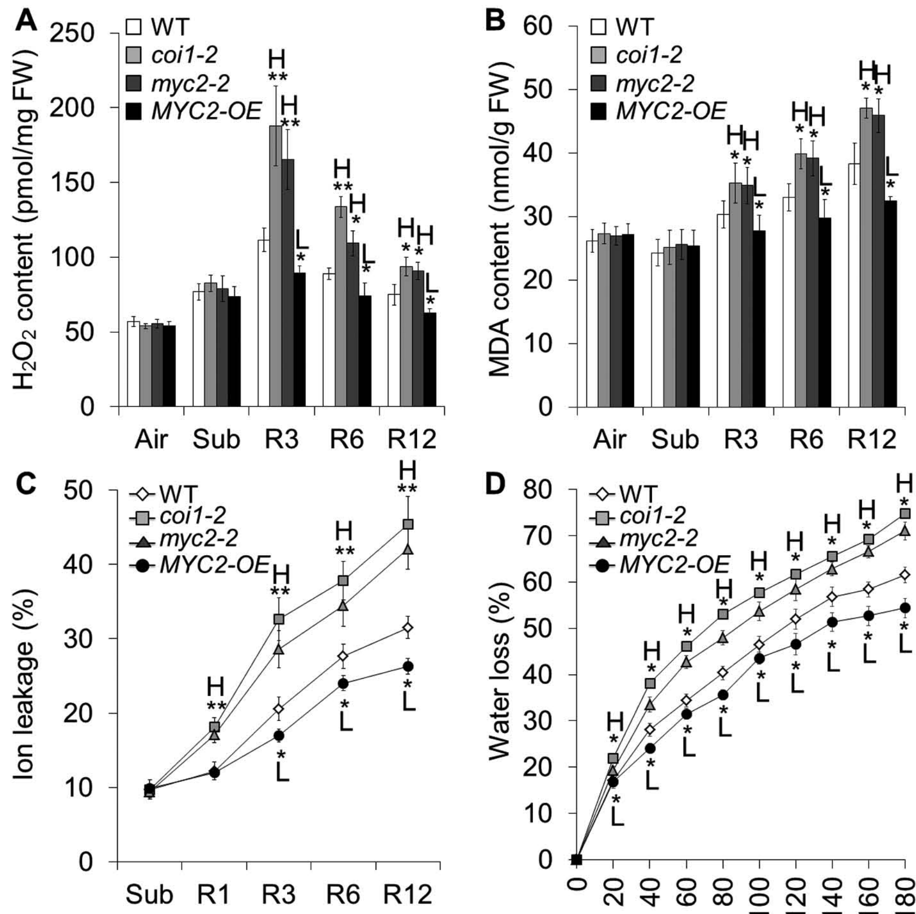
Supplemental Figure S5. The control plants for JA signaling showing no difference between each group before and after 48-h dark treatment followed by recovery. **A** and **B**, phenotypes (**A**) and dry weights (**B**) of wild-type (WT), *jar1-1*, and *coi1-2* plants after dark treatment and recovery. Plants were photographed before treatment, and after 48-h dark treatment followed by recovery for 4 d. **C** and **D**, Phenotypes (**C**) and dry weights (**D**) of *jin1-9* and *myc2-2* mutants and *MYC2-OE* line before treatment and after 48-h dark treatment followed by reoxygenation for 4 d. The data in **B** and **D** are means ± SD (*n* = 3 independent experiments). For each experiment, at least 15 plants were used for each genotype. All genotypes completely recovered from the dark treatment and no significant difference was detected for the dry weight measurements.



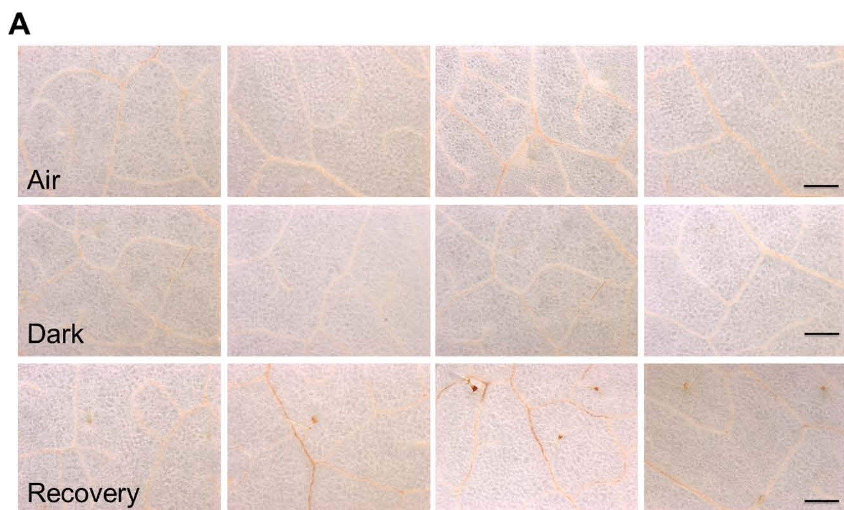
Supplemental Figure S6. Expression of antioxidant defense-related genes in response to rexoygenation or dark recovery. **A,** The second biological repeat showing the relative transcript levels of antioxidant defense-related genes in the wild type (WT), *coi1-2*, *myc2-2*, and *MYC2-OE* lines during reoxygenation. **B,** The relative transcript levels of antioxidant defense-related genes in the WT, *coi1-2*, *myc2-2*, and *MYC2-OE* lines in response to dark followed by recovery. Total RNA was extracted from four-week-old plants before treatment (Air) and after 24-h dark submergence (Sub) or dark (Dark) followed by 1 and 3 h reoxygenation/recovery (R1 and R3). The relative expression levels of antioxidant synthesis-related genes (*VTC1*, *VTC2*, *VTC5*, *GSH1*, and *GSH2*) and genes encoding enzymes involved in ROS detoxification (*APX2*, *DHAR1*, *MDHAR3*, and *GR1*) were determined by qPCR. Transcript levels relative to the WT at 0 h were normalized to that of *TUB3*. The data are means ± SD (*n* = 3 technical replicates).

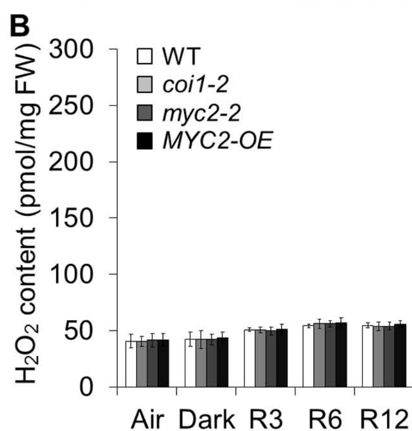


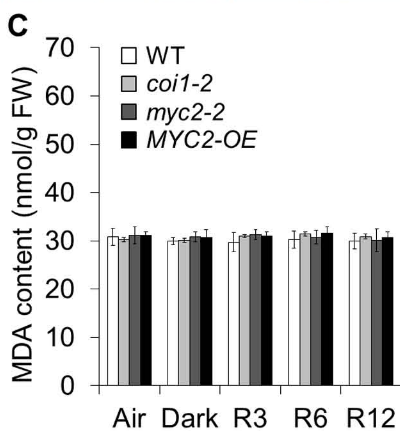
Supplemental Figure S7. Measurements of AsA and GSH in response to rexoygenation or dark recovery. **A**, a second biological repeat showing the AsA and GSH contents in the wild type (WT), *coi1-2*, *myc2-2*, and *MYC2-OE* lines during reoxygenation. **B**, the levels of antioxidants (AsA and GSH) in the WT, *coi1-2*, *myc2-2*, and *MYC2-OE* lines in response to dark followed by recovery. Total AsA and GSH contents were measured in four-week-old WT, *coi1-2*, *myc2-2*, and *MYC2-OE* plants before treatment (Air) and after 24 h dark submergence (Sub) or dark (Dark) followed by 12 h reoxygenation/recovery (R12). B, The data are means ± SD (n = 5 technical replicates). Asterisks indicate significant differences from WT (*, $P < 0.05$; **, $P < 0.01$ by Student's t-test). "H" and "L" indicate significantly higher or lower contents, respectively, of AsA and GSH in the mutants or transgenic lines than in the WT.

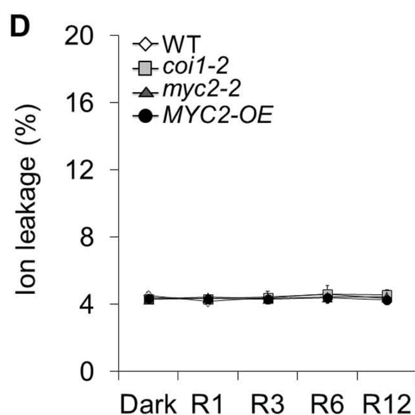


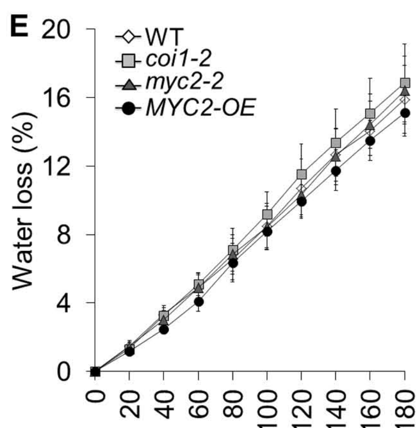
Supplemental Figure S8. A second biological repeat showing the accumulation of ROS in wild-type (WT), *coi1-2*, *myc2-2*, and *MYC2-OE* plants in response to reoxygenation. **A** and **B**, Contents of H_2O_2 (**A**) and MDA (**B**) in the four-week-old WT, *coi1-2*, *myc2-2*, and *MYC2-OE* plants before submergence (Air) and after 24 h dark submergence (Sub) followed by 3, 6, and 12 h reoxygenation (R3, R6, and R12). **C**, Electrolyte leakage in the four-week-old WT, *coi1-2*, *myc2-2*, and *MYC2-OE* plants after 24-h dark submergence (Sub) and reoxygenation for 1, 3, 6, and 12 h (R1, R3, R6, and R12). **D**, Water loss in the four-week-old WT, *coi1-2*, *myc2-2*, and *MYC2-OE* leaves immediately after 24 h dark submergence (which was set to 0 min) and reoxygenation for 20, 40, 60, 80, 100, 120, 140, 160, and 180 min. The data are means ± SD (n = 6 technical replicates). Asterisks indicate significant differences from WT (*, $P < 0.05$; **, $P < 0.01$ by the Student's t-test). "H" and "L" indicate values that are significantly higher or lower, respectively, in the mutants or transgenic lines than in the WT.



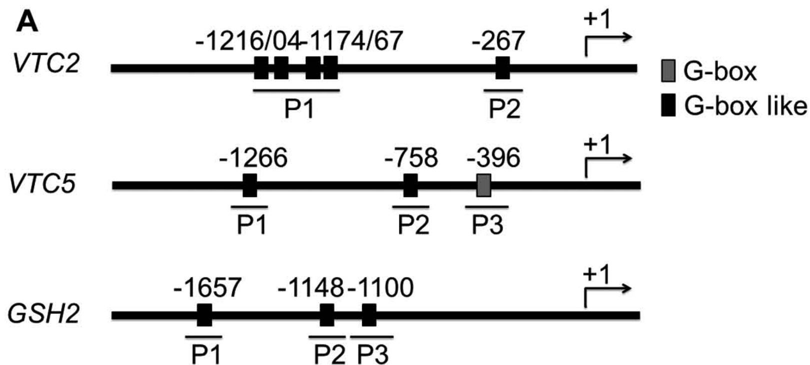




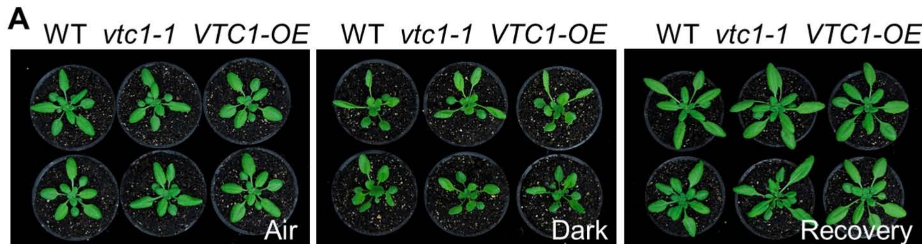


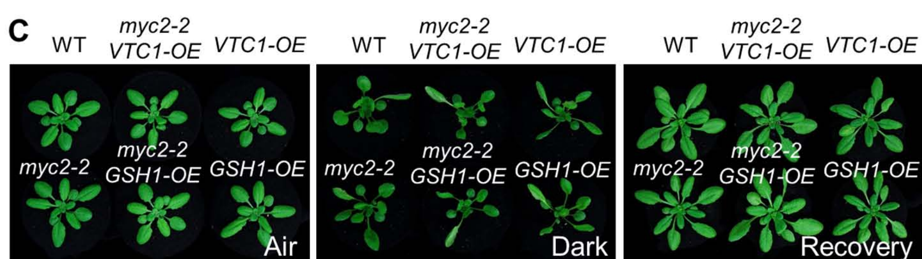


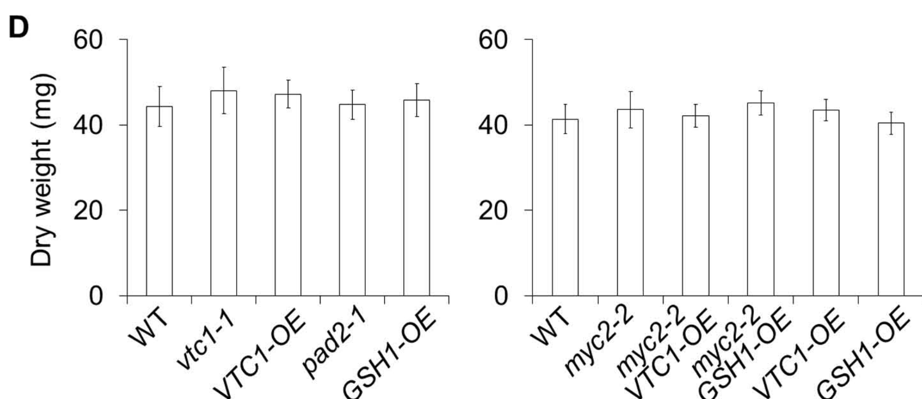
Supplemental Figure S9. ROS contents in wild-type (WT), *coi1-2*, *myc2-2*, and *MYC2-OE* plants in response to dark recovery. **A**, DAB staining showing the accumulation of ROS in the leaves of four-week-old WT, *coi1-2*, *myc2-2*, and *MYC2-OE* plants before treatment (Air) and after 24-h dark (Dark) followed by 6 h recovery (Recovery). Bars = 500 μm. **B** and **C**, Contents of H_2O_2 (**B**) and MDA (**C**) in the four-week-old WT, *coi1-2*, *myc2-2*, and *MYC2-OE* plants before treatment (Air) and after 24 h dark (Dark) followed by 3, 6, and 12 h recovery (R3, R6, and R12). **D**, Electrolyte leakage in the four-week-old WT, *coi1-2*, *myc2-2*, and *MYC2-OE* plants after 24 h dark (Dark) and recovery for 1, 3, 6, and 12 h (R1, R3, R6, and R12). **E**, Water loss in the four-week-old WT, *coi1-2*, *myc2-2*, and *MYC2-OE* leaves immediately after 24 h dark (which was set to 0 min) and recovery for 20, 40, 60, 80, 100, 120, 140, 160, and 180 min. The data are means ± SD (*n* = 6 technical replicates).



Supplemental Figure S10. MYC2 interacts with the *VTC2*, *VTC5*, and *GSH2* promoters *in vivo*. **A**, Schematic diagram of the potential G-box and G-box-like motifs in the promoter fragments of *VTC2*, *VTC5*, and *GSH2* genes. Numbers indicate the nucleotide positions relative to their corresponding translational start sites (ATG, +1). **B**, ChIP-qPCR analyses showing the *in vivo* interaction of MYC2 with the DNA fragments in the promoters of *VTC2*, *VTC5*, and *GSH2.* The protein/DNA complexes isolated from two-week-old *MYC2-OE* plants were immunoprecipitated with or without the anti-MYC2 antibodies. For each promoter, the DNA fragments were used to determine the enrichment of the DNA fragment containing the G-box and G-box-like motifs. The *ACTIN2* promoter fragment was used as a negative control. The data are means $\pm$ SD (n = 3 independent experiments).







Supplemental Figure S11. The control plants showing no significant difference among wild type (WT), *VTC1-*, *GSH1*-deficient mutants (*vtc1-1* and *pad2-1*), *VTC1-*, *GSH1*-overexpressors (*VTC1-OE* and *GSH1-OE*), as well as *myc2-2 VTC1-OE* and *myc2-2 GSH1-OE* lines in response to dark treatment and dark recovery. **A** and **B**, phenotypes of WT, *vtc1-1*, *VTC1-OE* (**A**), *pad2-1* and *GSH1-OE* (**B**) plants before treatment (Air), and after 48 h dark treatment (Dark) followed by recovery for 4 d (Recovery). **C**, Phenotypes of WT, *myc2-2*, *myc2-2 VTC1-OE*, *myc2-2 GSH1-OE*, VTC1-OE, and *GSH1-OE* plants before treatment (Air), and after 48 h dark treatment (Dark) followed by recovery for 4 d (Recovery). **D**, dry weights of plants described in **A** and **B** (left graph) and **C** (right graph) after dark recovery. For each experiment, at least 15 plants were used for each genotype. All genotypes completely recovered from the dark treatment and no significant difference was detected for the dry weight measurements.