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Methylation of a MITE insertion in the *MdRFNR1-1*promoter is positively associated with its allelic expression in apple in response to drought stress

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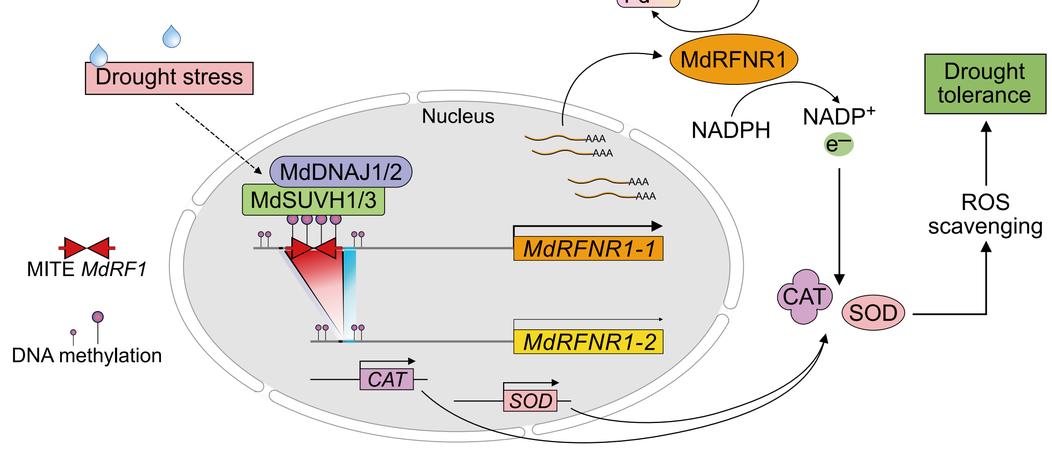
Abstract



Miniature inverted-repeat transposable elements (MITEs) are widely distributed in the plant genome and can be methylated. However, whether DNA methylation of MITEs is associated with induced allelic expression and drought tolerance is unclear. Here, we identified the drought-inducible *MdRFNR1* (root-type ferredoxin-NADP$^+$ oxidoreductase) gene in apple (*Malus domestica*). MdRFNR1 plays a positive role in drought tolerance by regulating the redox system, including increasing NADP$^+$ accumulation and catalase and peroxidase activities and decreasing NADPH levels. Sequence analysis identified a MITE insertion (MITE-MdRF1) in the promoter of *MdRFNR1-1* but not the *MdRFNR1-2* allele. *MdRFNR1-1* but not *MdRFNR1-2* expression was significantly induced by drought stress, which was positively associated with the MITE-MdRF1 insertion and its DNA methylation. The methylated MITE-MdRF1 is recognized by the transcriptional anti-silencing factors MdSUVH1 and MdSUVH3, which recruit the DNAJ domain-containing proteins MdDNAJ1, MdDNAJ2, and MdDNAJ5, thereby activating *MdRFNR1-1* expression under drought stress. Finally, we showed that MdSUVH1 and MdDNAJ1 are positive regulators of drought tolerance. These findings illustrate the molecular roles of methylated MITE-MdRF1 (which is recognized by the MdSUVH–MdDNAJ complex) in induced *MdRFNR1-1* expression as well as the drought response of apple and shed light on the molecular mechanisms of natural variation in perennial trees.

Results

I. Drought-induced MdRFNR1 has in vitro oxidoreductase activi-

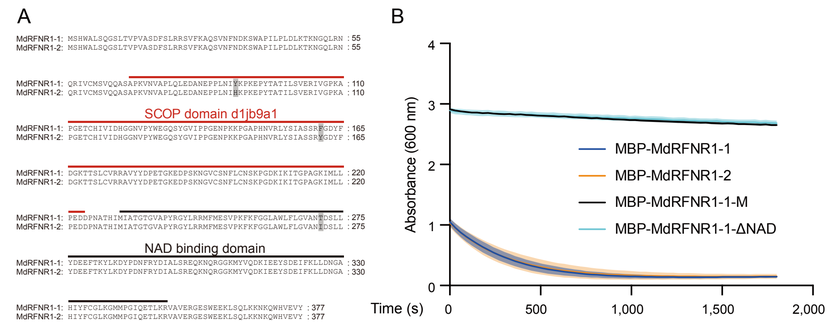


Figure1. NADPH-dependent enzyme activities of the proteins encoded by the two *MdRFNR1* alleles. A, Amino acid sequence alignment of MdRFNR1-1 and MdRFNR1-2. B, NADPH-dependent enzyme activities of the MdRFNR1 proteins with DCPIP.

II. MdRFNR1 confers in vivo oxidoreductase activity and drought tolerance

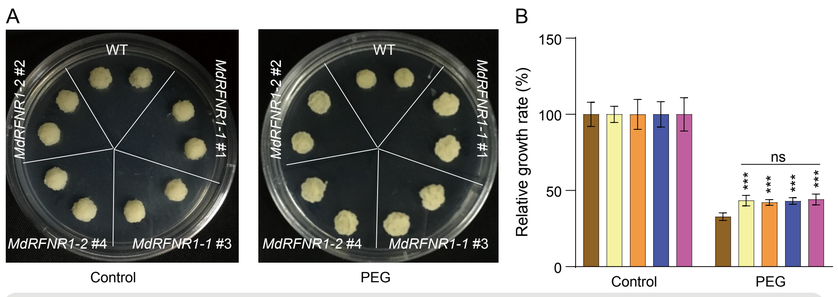


Figure 2. Two MdRFNR1 variants play the same positive role in plant responses to simulated drought stress. A, Morphology of *35S:MdRFNR1-1* OE, *35S:MdRFNR1-2* OE, and wild-type calli in response to PEG treatment. A, Morphology of *35S:MdRFNR1-1* OE, *35S:MdRFNR1-2* OE, and wild-type calli in response to PEG treatment. Calli were cultured on MS medium (left) or MS medium supplemented with PEG (right) for 20 days. B, Relative growth rates of WT, *35S:MdRFNR1-1* OE, and *35S:MdRFNR1-2* OE transgenic calli under control and PEG treatment.

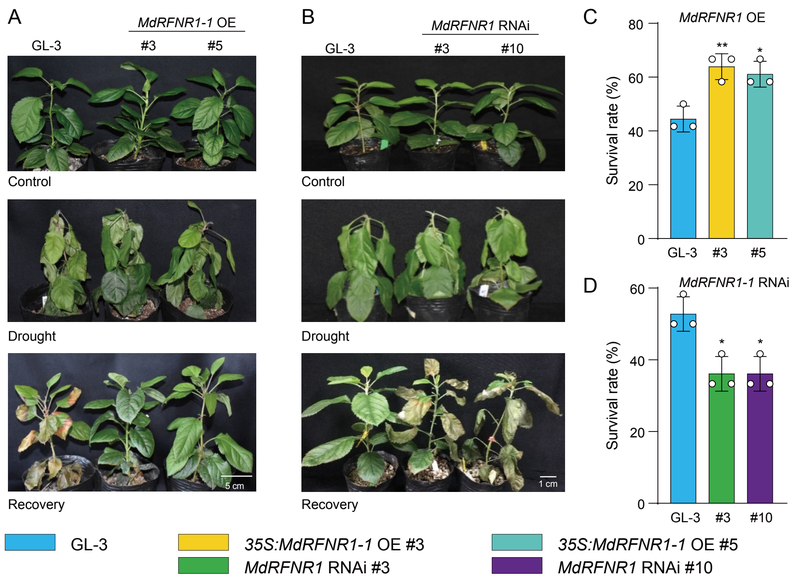


Figure 3. MdRFNR1 positive regulate drought tolerance. (A-B) Morphology of *MdRFNR1-1* OE (A) and *MdRFNR1* RNAi (B) transgenic plants under control and drought conditions. (C-D) The survival rates of plants shown in (A) and (B), respectively.

III. A MITE insertion in the promoter of *MdRFNR1-1* is essential for its induced expression and its positive role under drought

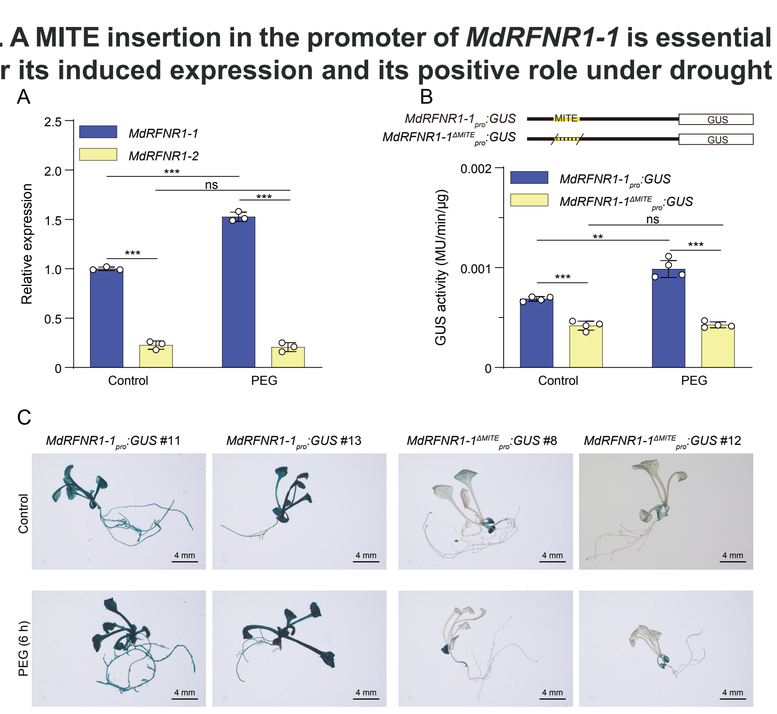


Figure 4. A MITE insertion in the *MdRFNR1-1* promoter is associated with its allelic induction by simulated drought stress. A, Allelic expression of *MdRFNR1* in GL-3 under control and PEG treatment. B, GUS activity of transgenic calli carrying *MdRFNR1-1*$_{pro}$*:GUS* or *MdRFNR1-1*$^{\Delta MITE}_{pro}$*:GUS* under control and PEG treatment. C, GUS staining of Arabidopsis plants carrying *MdRFNR1-1*$_{pro}$*:GUS* or *MdRFNR1-1*$^{\Delta MITE}_{pro}$*:GUS* in response to PEG treatment.

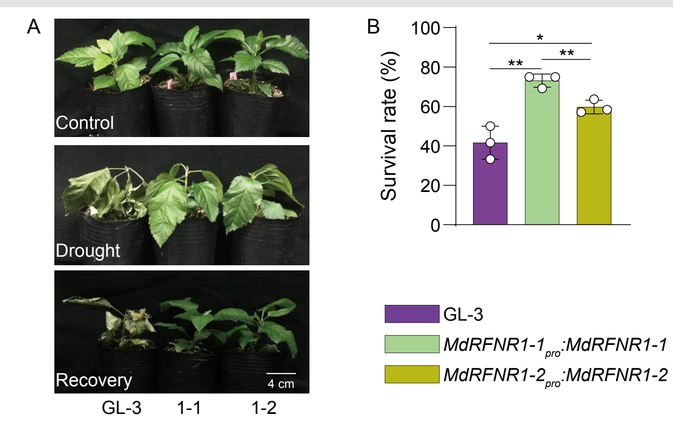


Figure 5. The *MdRFNR1-1* allele plays a more important role than *MdRFNR1-2* in drought stress tolerance. A, Morphology of GL-3 and transgenic plants carrying *MdRFNR1-1*$_{pro}$*:MdRFNR1-1* or *MdRFNR1-2*$_{pro}$*:MdRFNR1-2* under control and drought conditions. B, The survival rates of plants shown in (A).

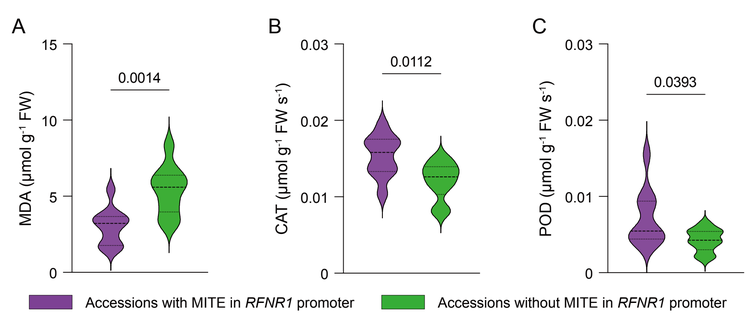


Figure 6. Association of MDA contents (A) and POD (B) and CAT (C) activities with the MITE-MdRF1 insertion in the *RFNR1* promoter in *Malus* accessions under dehydration conditions.

IV. Induced expression of MdRFNR1-1 in response to drought stress is associated with DNA methylation of MITE-MdRF1

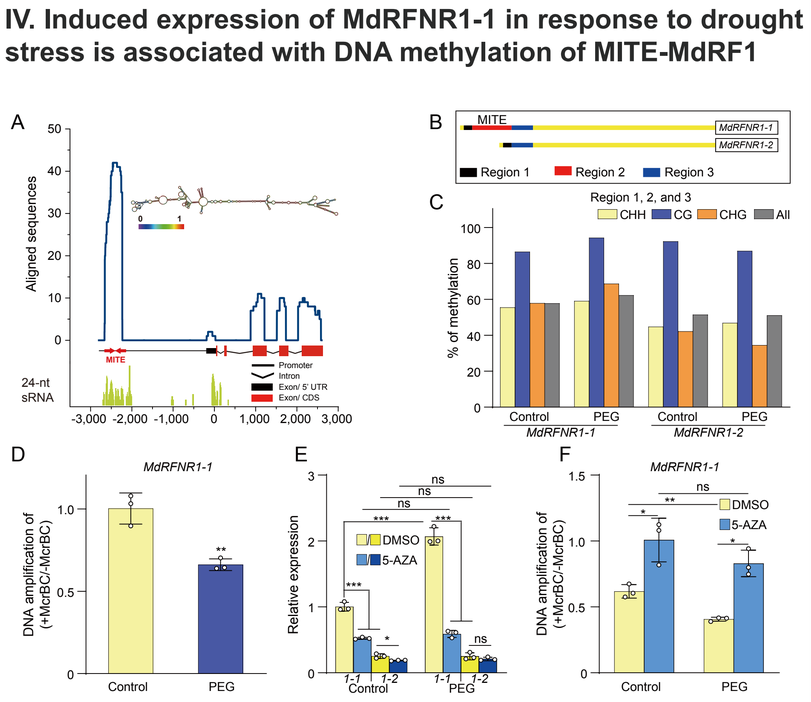


Figure 7. DNA methylation of the MITE-MdRF1 insertion in the *MdRFNR1-1* promoter is positively associated with *MdRFNR1-1* expression in response to simulated drought. A, Enrichment of stem–loop structure RNA and 24-nt sRNAs in the MITE-MdRF1 region of the *MdRFNR1-1* promoter. B, Schematic representation of locus-specific BS-seq analysis in (C). C, The methylation percentage of Regions 1, 2, and 3 of the *MdRFNR1* promoter in GL-3 leaves in response to simulated drought stress. D, McrBC–qPCR showing the DNA methylation level of MITE-MdRF1 in the *MdRFNR1-1* promoter in GL-3 leaves under control and PEG treatment. E and F, Allelic expression of *MdRFNR1-1* and *MdRFNR1-2* (E) and DNA methylation level of MITE-MdRF1 in the *MdRFNR1-1* promoter (F) in leaves of GL-3 plants treated with DMSO or 5-AZA in response to simulated drought.

V. The MdSUVH–MdDNAJ complex recognizes methylated MITE-MdRF1 in the MdRFNR1-1 promoter and facilitates its expression under drought stress

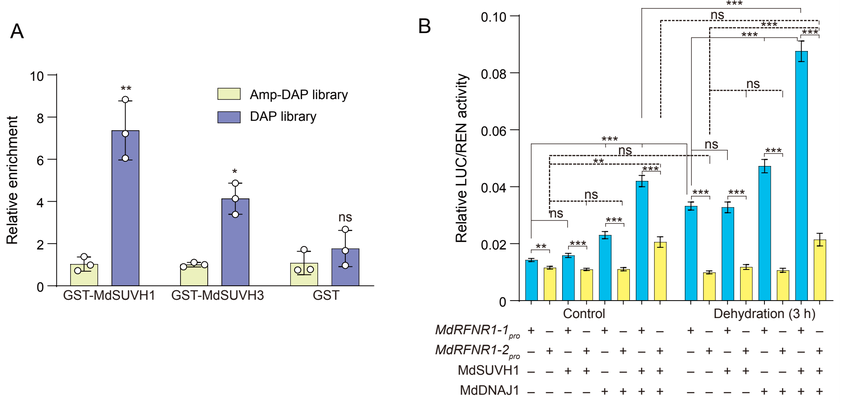


Figure 8. The MdSUVH–MdDNAJ complex binds to methylated MITE-MdRF1 and activates *MdRFNR1-1* expression in response to drought stress. A, Binding of MdSUVH1 and MdSUVH3 to methylated MITE-MdRF1 in the *MdRFNR1-1* promoter, as revealed by DAP-qPCR. DAP, methylated DNA library. Amp-DAP, amplified DNA library, which contained non-methylated DNA. B, Relative luciferase activity from the dual luciferase reporter assays in *N. benthamiana* leaves.

VI. MdSUVH and MdDNAJ are positive regulators of drought stress tolerance

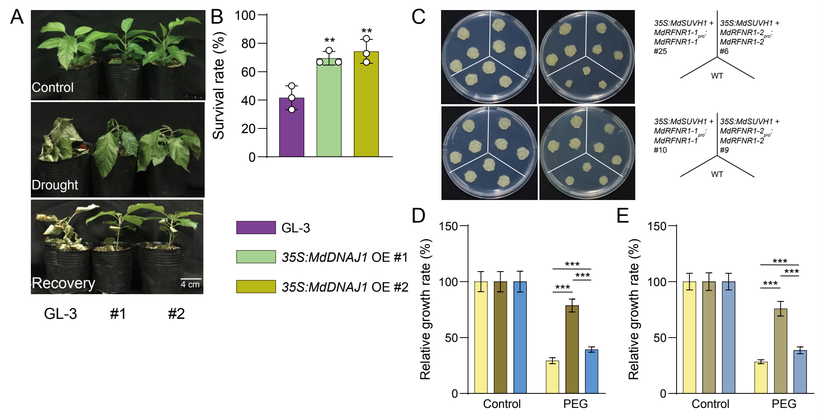


Figure 9. MdDNAJ1 and MdSUVH1 play positive roles in drought stress tolerance. A, Morphology of GL-3 and transgenic *35S:MdDNAJ1* OE plants under control and drought conditions. B, The survival rates of plants shown in (A). C, Morphology of WT and transgenic calli, which were co-transformed with *35S:MdSUVH1* and *MdRFNR1-1*$_{pro}$*:MdRFNR1-1* or *MdRFNR1-2*$_{pro}$*:MdRFNR1-2* and incubated for 20 days on MS medium (left) or MS medium supplemented with PEG (right). D and E, Relative growth rates of WT and transgenic calli in response to PEG treatment.

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