PhERF2, an ethylene-responsive element binding factor, Improves waterlogging tolerance in Petunia

Dongmei Yin[1], Daoyang Sun[2], Ayla Norris[3], Cai-Zhong Jiang[3,4*]

[1]School of Ecology, Shanghai Institute of Technology, Shanghai 201418, China;
[2]Landscape Architecture and Arts, Northwest A&F University,Yangling, Shaanxi ,712100, China;
[3] Department of Plant Sciences, University of California Davis, Davis, CA 95616, USA;
[4] USDA-ARS, Crops Pathology & Genetic Research Unit, Davis, CA 95616, USA

Abstract Ethylene-responsive element binding factors (ERFs) are involved in regulation of various stress responses in plants, but their biological functions in waterlogging stress are largely unclear. In this study, we identified a petunia (Petunia × hybrida) ERF gene, *PhERF2*, that was significantly induced by waterlogging in wild-type (WT). To study the regulatory role of *PhERF2* in waterlogging responses, transgenic petunia plants with RNAi silencing and overexpression of *PhERF2* were generated. Compared with WT plants, *PhERF2* silencing compromised the tolerance of petunia seedlings to waterlogging, shown as 96% mortality after 4 days waterlogging and 14 days recovery, while overexpression of *PhERF2* improved the survival of seedlings subjected to waterlogging. *PhERF2*-RNAi lines exhibited earlier and more severe leaf chlorosis and necrosis than WT, whereas plants overexpressing *PhERF2* showed promoted growth vigor under waterlogging. Chlorophyll content was dramatically lower in *PhERF2*-silenced plants than WT or overexpression plants. Typical characteristics of programmed cell death (PCD), DNA condensation, and moon-shaped nuclei were only observed in *PhERF2*-overexpressing lines but not in *PhERF2*-RNAi or control lines. Furthermore, transcript abundances of the alcoholic fermentation-related genes *ADH1-1, ADH1-2, ADH1-3, PDC1*, and *PDC2* were reduced in *PhERF2*- silenced plants, but increased in *PhERF2*-overexpressing plants following exposure to 12-h waterlogging. In contrast, expression of the lactate fermentation-related gene *LDH* was up-regulated in *PhERF2*-silenced plants, but downregulated in its overexpressing plants. Moreover, *PhERF2* was observed to directly bind to the *ADH1-2* promoter bearing ATCTA motifs. Our results demonstrate that *PhERF2* contributes to petunia waterlogging tolerance through modulation of PCD and alcoholic fermentation system.

Introduction

Waterlogging becomes to one of the major abiotic stresses constraining its'plantation in the landscape. Petunia, an important horticultural plant that is highly sensitive to submergence, is an excellent model system for studies of waterlogging responses. We have recently reported a critical role of *PhERF2* in antiviral RNA silencing and also observed that expression levels of *PhERF2* were significantly induced by stress-related hormones including ethylene, abscisic acid, salicylic acid, and methyl jasmonate as well as abiotic stress such as cold, NaCl, and water stress . The fact that roles of *PhERF2* homologs in tolerance to abiotic stresses, such as salt and cold, are characterized in other species prompts us to hypothesize that *PhERF2* is involved in the stress regulation. Here, we report an additional function of *PhERF2* in petunia waterlogging tolerance. *PhERF2* silencing reduced petunia tolerance to waterlogging, and its overexpression increased the tolerance. Our results support an important role of *PhERF2* in the regulation of waterlogging resistance in petunia.

Materials and Methods

Waterlogging treatment for 5-week-old petunia 'Mitchell Diploid' (WT, *PhERF2* silencing and overexpression plants):Waterlogging treatment for 0h, 24h; Waterlogging treatment 4 d + recovery 14 d; All treatments are repeated 5 times, and 16 plants are used each time; the relative water content of the control (CT) soil is maintained at 70-80%). Determination of chlorophyll content; Programmed cell death assay; After extracting leaf RNA, perform reverse transcription, design primers and perform quantitative real-time PCR analysis; Electrophoretic mobility transfer analysis; Detect the binding signal of *PhERF2* and biotin-labeled probe; Dual luciferase detection.

Results

After waterlogging for 4 days and recovery for 14 days, the *PhERF2* -silenced plants were severely damaged and suffered 96% mortality, whereas nearly all *PhERF2* -overexpressing plants survived and displayed a quicker and stronger recovery than WT plants (Fig. 1).

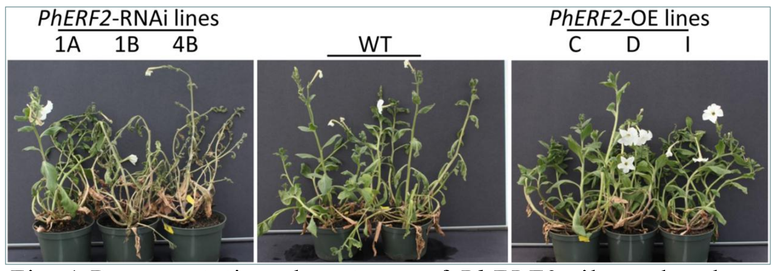

Fig. 1 Representative phenotypes of *PhERF2*-silenced and -overexpressing seedlings exposed to waterlogging and recovery. Five-week-old seedlings of wild type (WT), *PhERF2*-RNAi (1A, 1B, and 4B), and *PhERF2*-overexpressing (OE) lines (C, D, and I) were subjected to flooding treatment for 4 days, and then recovery for 14 days. Photographs were taken at 14 days post recovery.

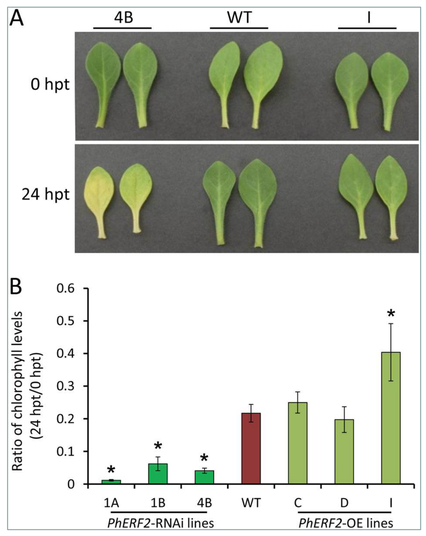

Fig.2 Chlorophyll levels of leaves from *PhERF2*-silenced and-overexpressing seedlings under waterlogging conditions.

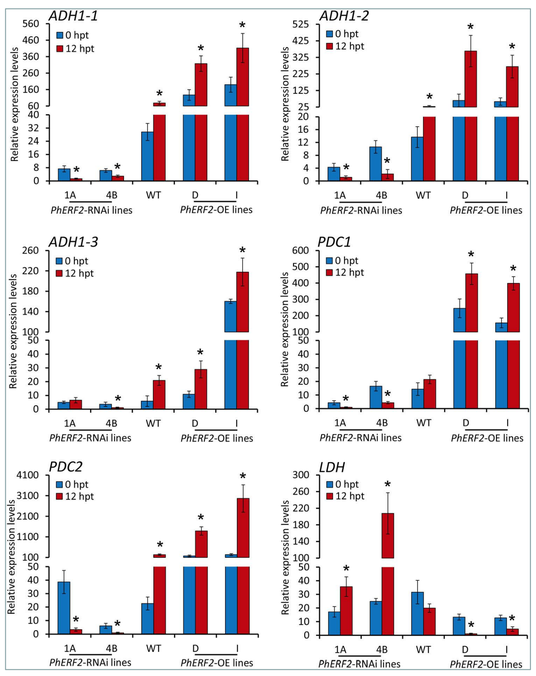

Fig. 3 Expression of lactate and alcoholic fermentation-related genes in *PhERF2*-silenced and -overexpressing seedlings under waterlogging conditions.

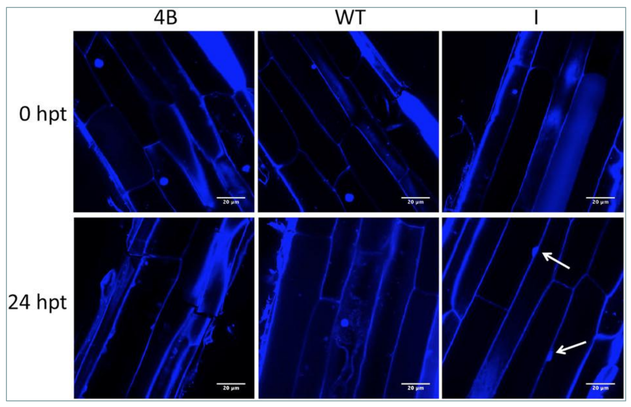

Fig. 4 The programmed cell death in the roots of *PhERF2*-overexpressing lines subjected to waterlogging. DAPI staining was used to identify cell death at 0 h and 24 h post treatment (hpt) with waterlogging. Arrows denote the moon-shaped nuclei.

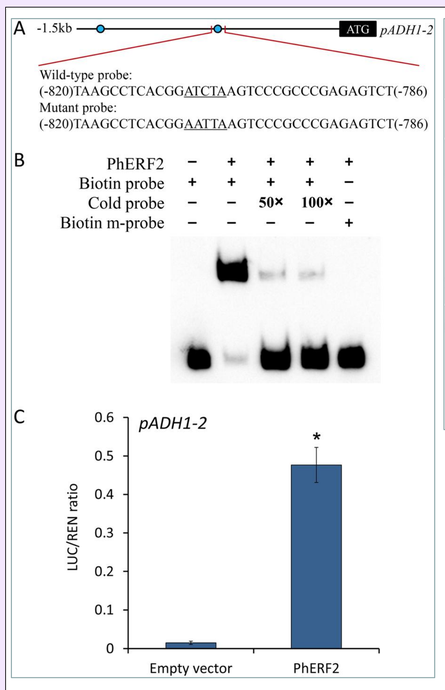

Fig. 5 Transactivation of the *ADH1-2* promoter by PhERF2. a Graphic representation of petunia *ADH1-2* promoter (*pADH1-2*) with a 1.5 kb region upstream of the coding sequence. Two putative PhERF2 binding sites (ATCTA) are marked by blue circles. The probe sequences used for electrophoretic mobility shift assay (EMSA) are indicated, with the wild-type cis-element and its nucleotide substitutions being underlined, respectively. b EMSA of PhERF2 binding to the biotinlabeled probe. Non-labeled probes (cold) at 50- and 100-fold concentrations were added for competition, and mutant probe (mprobe) for binding specificity test. c Dual luciferase transient expression assay of the *ADH1-2* promoter.

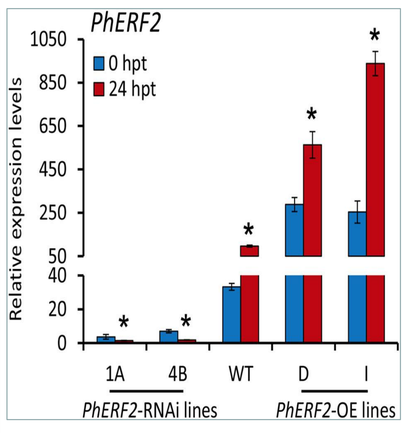

Fig.6 Expression of *PhERF2* in *PhERF2*-silenced and -overexpressing seedlings under waterlogging conditions. Quantitative real-time PCR analysis of *PhERF2* expression in the leaves of WT, *PhERF2*-RNAi lines (1A and 4B) and *PhERF2*-overexpressing (OE) lines (D and I) at 0 h and 24 h post treatment (hpt) with waterlogging. Data represent the means (±SD) of three biological replicates. Transcript abundances were standardized to 26S rRNA. Asterisks denote statistical significance using Student's t test at $P < 0.05$

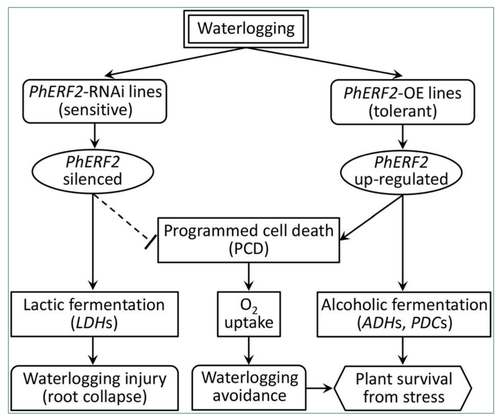

Fig.7 A proposed model for PhERF2's function in waterlogging responses. PhERF2 positively regulates the programmed cell death and genes associated with the adaptation to waterlogging stress.

Conclusions

Our results provide new evidence that PhERF2 transcriptionally regulates the PCD and genes of alcoholic fermentation system to protect plants from anaerobic respiration damage, and therefore plays an important role in defense responses against waterlogging stress. EMSA and dual luciferase assays confirmed the direct binding of PhERF2 to *ADH1-2* promoter in petunia. However, the mechanism on how PhERF2 and its target gene synergistically modulate plant defense against waterlogging is still unclear. To further dissect the biological function of *PhERF2* in response to waterlogging, future studies should include comprehensive transcriptomic or metabolomic analyses in *PhERF2* transgenic plants.

Acknowledgments

This work was partially supported by the National Science Foundation of China (grant numbers 31701963 and 31801895) and United States Department of Agriculture (USDA) CRIS project 5306-21000-019-00D.