PLANT SIGNALING & BEHAVIOR
2020, VOL. 15, NO. 8, e1778920 (3 pages)
https://doi.org/10.1080/15592324.2020.1778920

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

An ERF-type transcription factor is involved in the regulation of the dehydrin *wzy1-2* gene in wheat

Ying Yang[a,b]*, Hao Liu[a]*, Aina Wang[b], and Linsheng Zhang[a]

[a]College of Life science/State Key Laboratory of Crop Stress Biology for Arid Areas, Northwest A&F University, Yangling, Shannxi, China; [b]College of Nursing, Weinan Vocational&Technical College, Weinan, Shannxi, China

ABSTRACT

As stress-inducible proteins, dehydrins exert functional protective role by alleviating the cell damage when plants are suffering from the stresses. However, the upstream regulatory mechanism of these proteins is not very clear. To unravel the regulatory mechanism of dehydrin, a screen of wheat cDNA library from cold and PEG-treated wheat seedlings was performed and a transcription factor TaERF4a (GenBank NO. AFP49822.1) interacting with wheat dehydrin *wzy1-2* gene (Gene ID: 100037544) promoter was identified by yeast one-hybrid assay. The regulator *TaERF4a* and the *wzy1-2* gene can respond to the abiotic stress, the induced transcripts of these two genes exhibit a similar expression trend under adverse environmental conditions. In planta, the dual luciferase transient assay analysis showed that TaERF4a can positively regulate the expression of WZY1-2 dehydrin. Therefore, the obtained results suggest that ERF transcription factor can regulate the expression level of the dehydrin gene in wheat.

ARTICLE HISTORY
Received 15 May 2020
Revised 1 June 2020
Accepted 2 June 2020

KEYWORDS
Dehydrin; transcription factor; abiotic stress; regulatory mechanism

Wheat, as one of the three major gramineous crops, is widely cultivated all over the world. In China, the main producing areas are the north China plain and the northeast plain.[1] In these areas, seasonal drought, low temperature, and other abiotic stress have seriously affected the growth and production of wheat. Dehydrins (DHNs), also known as LEA II (late embryogenesis abundant) proteins, are widely distributed in prokaryotic and eukaryotic organisms, including angiosperm, algae plants, and bacteria, fungi, evennematode.[2,3] Due to their unique amino acid compositions and structure character, dehydrins exhibit versatile abilities, including heat resistance, reduction of reactive oxygen species and enzyme cryoprotection, etc.[4,5] Therefore, they play an important role in protecting cell membrane and protein structure from dehydration and desiccation damage.[6] However, despite considerable progress made toward understanding the role of DHNs, the molecular mechanisms by which these proteins perform their functions inside the cells remain elusive.[7]

In a previous study, we have cloned the dehydrin gene *wzy1-2* (Gene ID: 100037544) and *wzy1-2* promoter in Zheng yin 1 cultivar of *Triticum aestivum* and showed its fundamental biological function in plant under adversity stresses.[8] Expression pattern analysis indicated that *wzy1-2* transcript and WZY1-2 protein accumulation can be induced by osmotic stress, cold, ABA, and MeJA treatments.[8] Histochemical analysis of GUS expression demonstrated that *wzy1-2* promoter activity could be upregulated by osmotic stress, ABA treatment.[8]

To elucidate the upstream regulation mechanism of *wzy1-2* dehydrin gene, cDNA library from cold and PEG-treated wheat seedlings was screened by using yeast one-hybrid (Y1H) assay with the *wzy1-2* promoter as bait. By sequencing the positive clones we isolated a transcription factor encoding 196 amino acids with a predicted molecular weight of 20.77 kDa, named TaERF4a (GenBank NO. AFP49822.1). To further verify the interaction between the TaERF4a and *wzy1-2* promoter, the plasmid containing the transcription factor *TaERF4a* gene was extracted and the cotransformation on selective media were performed. The bait yeast strains (Pwzy1-2 in pAbAi) containing the individual prey vector of the pGADT7-TaERF4a were able to grow on SD/-Leu (synthetic dropout nutrient medium) and SD/-Leu/AbA^{200} (Aureobasidin, 200 ng/ml) selective medium. However, Y1H Gold yeast cells containing pGADT7-TaERF4a could not grow on SD/-Leu/AbA^{200} selective medium (Figure 1a). Moreover, a typical AP2/ERF conserved domain was found in the gene sequence, indicating that TaERF4a was a member of the ERF protein family (Figure 1b). Ethylene-response factor (ERF) proteins are members of the second-largest transcription factor family involved in plant growth, development, and environmental stress responses.[9] ERF96, a positive regulator of ABA responses, can not only increase ABA response genes RD29A, ABI5, ABF3 expression, but it is also involved in the Se resistance and detoxification.[10]

Therefore, TaERF4a as the potential regulator of *wzy1-2* gene was selected for further research. Next, we aimed to determine the subcellular localization of TaERF4a using GFP translational fusions. The p35 S::GFP and p35 S::TaERF4a-GFP fusion constructs were used for transient expression analysis. The GFP fluorescent of the control was shown in the nucleus and the cytoplasm of the *Nicotiana benthamiana* protoplasts, but the TaERF4a-GFP signal was only detected in the nucleus (Figure 2a). In addition, tissue-specific analysis indicated that the

CONTACT Linsheng Zhang linszhang@nwsuaf.edu.cn College of Life science/State Key Laboratory of Crop Stress Biology for Arid Areas, Northwest A&F University, Yangling, Shannxi, China

*These authors contributed equally to this work.

Supplemental data for this article can be accessed on the publisher's website.

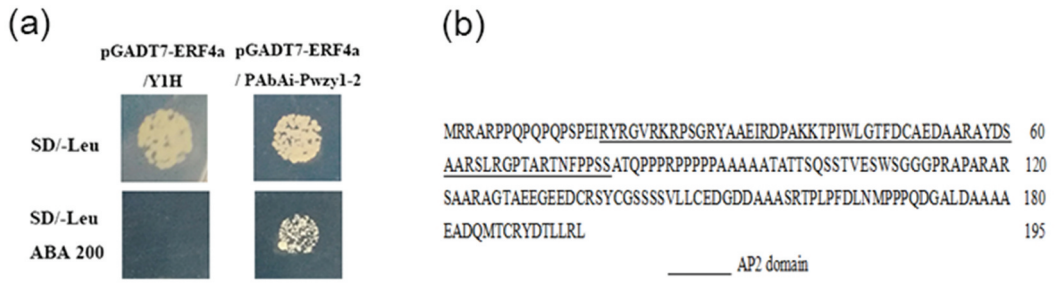

Figure 1. The interaction of TaERF4a with the promoter of *wzy1-2* gene in yeast one-hybrid assays (a) and the sequence feature of TaERF4a protein (b). (a) The transformation of pGADT7-TaERF4a with Y1H Gold yeast cells, bait yeast strains (Y1H Gold containing the PAbAi-Pwzy1-2) on selective media respectively to verify interactions. (b) The protein sequence of the TaERF4a.

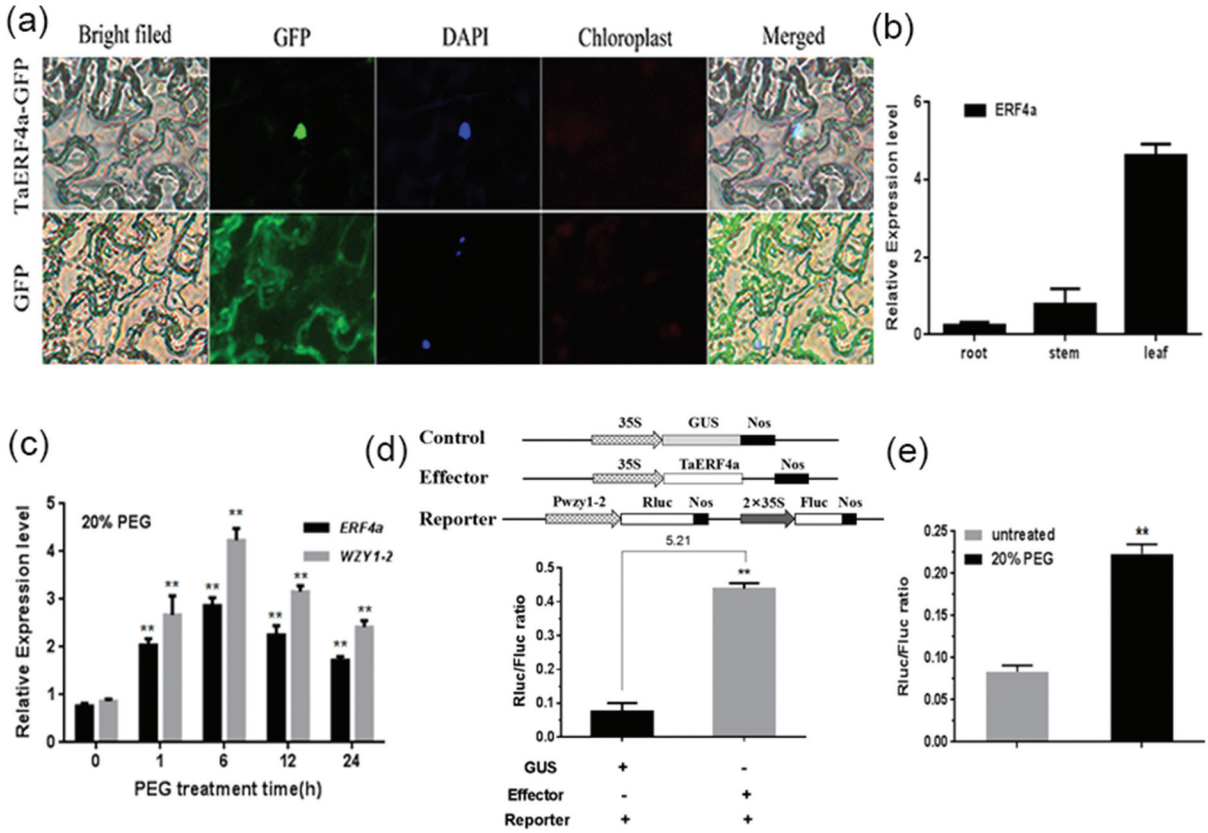

Figure 2. Identification of upstream transcription factor TaERF4a of dehydrin *wzy1-2* gene. (a). Subcellular localization of TaERF4a protein in *N. benthamiana* leaves performed by fluorescent microscope (Leica, DM5000 B, magnificant 20×). Tobacco leaf epidermal cells agro-infiltrated with p35 S::GFP and p35 S::TaERF4a-GFP vectors were used for transient expression of fusion proteins. The fluorescence images were visualized after 48 h infiltration. (b) Transcript level of *TaERF4a* in different tissues collected at 14 DAP seedlings. (c) The expression of *TaERF4a, wzy1-2* genes in response to PEG treatment at 14 DAP seedlings. Total RNA was extracted from leaves at the indicated times. Wheat actin gene (Accession No. AB181991) was used as an internal reference. (d) Transient luciferase activity assay of TaERF4a in *N. benthamiana* leaves. (e) The relative luciferase enzyme activity of TaERF4a after the PEG treatment. The experiments were performed with at least three biological repeats. Significance analysis was determined by Student's t-test (* = $P < .05$, ** = $P < .01$).

expression of the TaERF4a was highest in leaves during wheat seedling stage (Figure 2b). Moreover, we examined the expression patterns of the TaERF4a and WZY1-2 at different time points in response to abiotic stress. Under PEG treatment, the expression level of *TaERF4a* was gradually increased, reached a high level at 6 h and then declined. Interestingly, the expression of *wzy1-2* gene showed the similar tendency with this transcription factor (Figure 2c). To characterize the ability of TaERF4a to activate *wzy1-2* gene in plant, *A. tumefaciens* containing the p35 S::ERF4a (effector vector) together with the *wzy1-2* promoter::Rluc (reporter vector) were co-infiltrated into the tobacco leaves. The luciferase activity (Rluc/Fluc ratio) of the infected plants with TaERF4a protein was significantly increased (by 5.21 fold) (Figure 2d). In addition, the luciferase activity was also significantly increased after 24 h 20% PEG treatment compared with that of untreated (Figure 2e).

In conclusion, we identified an ERF transcription factor TaERF4a which presumably binds to the promoter of the dehydrin *wzy1-2* gene and regulate its expression. Some reports have shown that ERF can recognize and bind

specifically to the GCC box, dehydration responsive elements (DREs)/C-repeat elements (CRTs) in the promoter of stress-responsive genes.[11,12] The DRE as the *cis*-acting element also exist in the promoter of the dehydrin *wzy1-2* gene,[8] so we speculate that TaERF4a may bind to this motif to regulate the *wzy1-2* gene expression. Therefore, we further explore the binding site of the TaERF4a in the *wzy1-2* gene promoter and develop the transgenic plant to further confirm regulatory relationship between TaERF4a and *wzy1-2* gene and identify whether this regulator can improve the plant stress resistance.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 31671608) and Weinan basic research program in shaanxi province (2019ZDYF-JCYJ-133).

ORCID

Hao Liu http://orcid.org/0000-0002-2537-2551

References

1. Ren YJ, Gao C, Han HF, Li QQ. Response of water use efficiency and carbon emission to no-tillage and winter wheat genotypes in the North China Plain. Sci Total Environ. 2018;635:1102–1109. doi:10.1016/j.scitotenv.2018.04.204.
2. Gasulla F, de Nova PG, Esteban-Carrasco A, Zapata JM, Barreno E, Guera A. Dehydration rate and time of desiccation affect recovery of the lichen alga Trebouxia erici: alternative and classical protective mechanisms. Planta. 2009;231:195–208. doi:10.1007/s00425-009-1019-y.
3. Rorat T. Plant dehydrins–tissue location, structure and function. Cell Mol Biol Lett. 2006;11:536–556. doi:10.2478/s11658-006-0044-0.
4. Hara M, Fujinaga M, Kuboi T. Metal binding by citrus dehydrin with histidine-rich domains. J Exp Bot. 2005;56:2695–2703. doi:10.1093/jxb/eri262.
5. Hara M, Kondo M, Kato T. A KS-type dehydrin and its related domains reduce Cu-promoted radical generation and the histidine residues contribute to the radical-reducing activities. J Exp Bot. 2013;64:1615–1624. doi:10.1093/jxb/ert016.
6. Halder T, Agarwal T, Ray S. Isolation, cloning, and characterization of a novel Sorghum dehydrin (SbDhn2) protein. Protoplasma. 2016;253:1475–1488. doi:10.1007/s00709-015-0901-7.
7. Hanin M, Brini F, Ebel C, Toda Y, Takeda S, Masmoudi K. Plant dehydrins and stress tolerance: versatile proteins for complex mechanisms. Plant Signal Behav. 2011;6:1503–1509. doi:10.4161/psb.6.10.17088.
8. Zhu WN, Zhang DP, Lu XX, Zhang LS, Yu ZY, Lv H, Zhang H. Characterisation of an SKn-type dehydrin promoter from wheat and its responsiveness to various abiotic and biotic stresses. Plant Mol Biol Rep. 2014;32:664–678. doi:10.1007/s11105-013-0681-1.
9. Dong W, Ai X, Xu F, Quan T, Liu S, Xia G. Isolation and characterization of a bread wheat salinity responsive ERF transcription factor. Gene. 2012;511:38–45. doi:10.1016/j.gene.2012.09.039.
10. Jiang L, Yang J, Liu C, Chen Z, Yao Z, Cao S. Overexpression of ethylene response factor ERF96 gene enhances selenium tolerance in Arabidopsis. Plant Physiol Biochem. 2020;149:294–300. doi:10.1016/j.plaphy.2020.02.024.
11. Catinot J, Huang JB, Huang PY, Tseng MY, Chen YL, Gu SY, Lo W-S, Wang L-C, Chen Y-R, Zimmerli L, et al. ETHYLENE RESPONSE FACTOR 96 positively regulates A rabidopsis resistance to necrotrophic pathogens by direct binding to GCC elements of jasmonate - and ethylene-responsive defence genes. Plant Cell Environ. 2015;38:2721–2734. doi:10.1111/pce.12583.
12. Cheng MC, Liao PM, Kuo WW, Lin TP. The Arabidopsis ETHYLENE RESPONSE FACTOR1 regulates abiotic stress-responsive gene expression by binding to different cis-acting elements in response to different stress signals. Plant Physiol. 2013;162:1566–1582. doi:10.1104/pp.113.221911.