

EDITORIAL: REFLECTIONS ON *THE PLANT CELL CLASSICS*

DREB Duo Defines Distinct Drought and Cold Response Pathways^[OPEN]

Despite advances in agriculture over the millennia of human history, crop yields remain deeply dependent on climate and weather patterns. Abiotic stresses like drought, freezing, and flooding are dreaded for the devastation they can bring. Thus, understanding plant response to abiotic stress has long been a primary goal in plant biology research, and perhaps it is not surprising that some of the most highly cited articles in the history of the journal relate to transcriptional regulation during abiotic stress conditions. Two of the most highly cited articles in *The Plant Cell* (Yamaguchi-Shinozaki and Shinozaki, 1994 and Liu et al., 1998) come from the laboratories of Kazuko Yamaguchi-Shinozaki (now at the University of Tokyo; then working at Japan International Research Center for Agricultural Sciences, Tsukuba) and Kazuo Shinozaki (RIKEN Center for Sustainable Resource Science, Yokohama).

First, Yamaguchi-Shinozaki and Shinozaki (1994) described the *cis*-acting element DEHYDRATION RESPONSE ELEMENT (DRE), found in the promoter of *RD29A* and shown to be involved in the rapid response in the expression of this gene to dehydration or high-salt conditions. Liu et al. (1998) then extended this work by identifying two small families of transcription factors (TFs) in *Arabidopsis thaliana*—DREB1, which has three homologs, and DREB2, which has two homologs—that act in the regulation of two separate signal transduction pathways in response to low-temperature and dehydration conditions, respectively. One of the DREB1 proteins, DREB1B, and the DRE turned out to be the same as the CBF1 protein and a cold-regulated element (CRT), respectively, identified in independent studies performed by Michael Thomashow and colleagues at Michigan State University (Baker et al., 1994; Stockinger et al., 1997).

There are, of course, many publications that uncover aspects of transcriptional regulation under abiotic stress (far too many to cite here), and promoter deletion analysis had been in use in plants for some time prior to 1994. However, the Yamaguchi-Shinozaki and Shinozaki (1994) article certainly is one of the finest examples of early work in plants that directly connected a *cis*-element to induction of gene expression with a change in environmental conditions, in this case dehydration and high salt, and should be on the required reading list as a classic study for students and researchers in this field. The authors first show that two genes, *RD29A* and *RD29B*, exhibit differential responses in expression to dehydration, high salt, or low temperature, with *RD29A* expression responding rapidly to these treatments and *RD29B* responding more slowly (dehydration and high-salt treatments) or not at all (low temperature). Next, to pinpoint the region responsible for

the rapid induction of *RD29A*, they conducted a classic example of promoter deletion assessment with a chimeric gene construct consisting of a series of deletion fragments from the *RD29A* and *RD29B* promoter regions fused to the GUS reporter gene and expressed in transgenic *Arabidopsis* and tobacco (*Nicotiana tabacum*) plants. This allowed the identification of a *cis*-acting, dehydration-responsive element, known as DRE, involved in the rapid response of *RD29A* to conditions of dehydration or high salt. They further showed that DRE is involved in the induction by low temperature but not in a slower abscisic acid-responsive induction of *RD29A*.

Liu et al. (1998) extended this study by using yeast one-hybrid screening to identify two cDNA clones that encode the DRE binding proteins DREB1A and DREB2A, both of which bound specifically to the DRE sequence *in vitro* and activated the transcription of the GUS reporter gene driven by the DRE sequence in *Arabidopsis* leaf protoplasts. Interestingly, the two proteins showed no significant sequence similarity, except in their conserved DNA binding domains, and the expression of *DREB1A* and two homologous genes was induced by low temperature, whereas expression of *DREB2A* and its single homologous gene was induced by dehydration in *Arabidopsis*. Thus, they identified two independent families of DREB proteins, DREB1 and DREB2, that function as transcription factors in two different signal transduction pathways, regulating responses to different stresses—low temperature and dehydration—integrated through the same element.

It has since been shown that the DREB proteins belong to a large family of over 100 plant-specific DNA binding proteins containing a highly conserved ERF/AP2 DNA binding domain. The DREB subfamily in *Arabidopsis* includes 56 members divided into six subgroups (A1 to A6); the DREB1/CBF and DREB2 proteins are classified in the A1 and A2 subgroups, respectively (Sakuma et al., 2002). The DREB1/CBF proteins are now recognized as “master switches” constituting a “major hub” for gene regulation during cold acclimation (Thomashow, 2010; Kidokoro et al., 2017), whereas DREB2 proteins may function more specifically during drought and high-salt conditions (Sakuma et al., 2002).

A seminal finding with major implications for crop improvement reported by Liu et al. (1998) was that overexpression of one TF alone, DREB1A, resulted in enhanced tolerance to both dehydration and freezing stress. It was also shown by the Thomashow group that overexpression of CBF1 enhanced freezing tolerance (Jaglo-Ottosen et al., 1998). Although the transgenic plants were stunted relative to the wild type, this generated a lot of excitement, and early work on DREB/CBFs was cited as a promising avenue for engineering crop plants more highly tolerant of drought and other abiotic stresses (for example, Smirnov and Bryant, 1999).

Many subsequent studies have sought ways of altering DREB/CBF expression to enhance stress tolerance without negatively affecting growth (for example, Kasuga et al., 1999). In the last 10 years since publication of these two studies, citations have picked up, reflecting the importance of DREB/CBF pathways in plant stress tolerance and heightened efforts to achieve a breakthrough in overcoming the dichotomy between enhanced stress tolerance and growth retardation. The development of new methods and techniques, along with the characterization and deeper understanding of DREB/CBF pathways in different plant species (for example, Kudo et al., 2019; Li et al., 2019; Moon et al., 2019), gives renewed hope that such a breakthrough will not be far off.

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REFERENCES

- Baker, S.S., Wilhelm, K.S., and Thomashow, M.F.** (1994). The 5'-region of *Arabidopsis thaliana* cor15a has cis-acting elements that confer cold-, drought- and ABA-regulated gene expression. *Plant Mol. Biol.* **24**: 701–713.
- Jaglo-Ottosen, K.R., Gilmour, S.J., Zarka, D.G., Schabenberger, O., and Thomashow, M.F.** (1998). *Arabidopsis* CBF1 overexpression induces *COR* genes and enhances freezing tolerance. *Science* **280**: 104–106.
- Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K., and Shinozaki, K.** (1999). Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nat. Biotechnol.* **17**: 287–291.
- Kidokoro, S., Yoneda, K., Takasaki, H., Takahashi, F., Shinozaki, K., and Yamaguchi-Shinozaki, K.** (2017). Different cold-signaling pathways function in the responses to rapid and gradual decreases in temperature. *Plant Cell* **29**: 760–774.
- Kudo, M., Kidokoro, S., Yoshida, T., Mizoi, J., Kojima, M., Takebayashi, Y., Sakakibara, H., Fernie, A.R., Shinozaki, K., and Yamaguchi-Shinozaki, K.** (2019). A gene-stacking approach to overcome the trade-off between drought stress tolerance and growth in *Arabidopsis*. *Plant J.* **97**: 240–256.
- Li, X., Liang, Y., Gao, B., Mijiti, M., Bozorov, T.A., Yang, H., Zhang, D., and Wood, A.J.** (2019). *ScDREB10*, an A-5c type of *DREB* gene of the desert moss *Syntrichia caninervis*, confers osmotic and salt tolerances to *Arabidopsis*. *Genes (Basel)* **10**: 146.
- *Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K., and Shinozaki, K.** (1998). Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. *Plant Cell* **10**: 1391–1406.
- Moon, S.J., Min, M.K., Kim, J.A., Kim, D.Y., Yoon, I.S., Kwon, T.R., Byun, M.O., and Kim, B.G.** (2019). Ectopic expression of *OsDREB1G*, a member of the *OsDREB1* subfamily, confers cold stress tolerance in rice. *Front. Plant Sci.* **10**: 297.
- Sakuma, Y., Liu, Q., Dubouzet, J.G., Abe, H., Shinozaki, K., and Yamaguchi-Shinozaki, K.** (2002). DNA-binding specificity of the ERF/AP2 domain of *Arabidopsis* DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. *Biochem. Biophys. Res. Commun.* **290**: 998–1009.
- Smirnoff, N., and Bryant, J.A.** (1999). DREB takes the stress out of growing up. *Nat. Biotechnol.* **17**: 229–230.
- Stockinger, E.J., Gilmour, S.J., and Thomashow, M.F.** (1997). *Arabidopsis thaliana* CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. *Proc. Natl. Acad. Sci. USA* **94**: 1035–1040.
- Thomashow, M.F.** (2010). Molecular basis of plant cold acclimation: Insights gained from studying the CBF cold response pathway. *Plant Physiol.* **154**: 571–577.
- *Yamaguchi-Shinozaki, K., and Shinozaki, K.** (1994). A novel cis-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. *Plant Cell* **6**: 251–264.

*References highlighted for the 30th Anniversary of *The Plant Cell*.