



Signaling Impact of ABA Responsive Pathway in the Regulation of Drought Stress in Plants

Ashutosh Singh¹, Sharwan Kumar Shukla¹, Anshuman Singh¹, Rakesh Kumar¹, Mitali Tiwari¹, Ritu Gaur¹, Chandu Singh², Sanjay Kumar Singh², Lalit Thakur³, Shivam Yadav^{1*}

¹R. L. B. Central Agricultural University, Jhansi 284003, U.P., India

²Division of Genetics, IARI, New Delhi 110012, India

³Dr. Y. S. P. University of Horticulture and Forestry, Solan 173230, H.P., India

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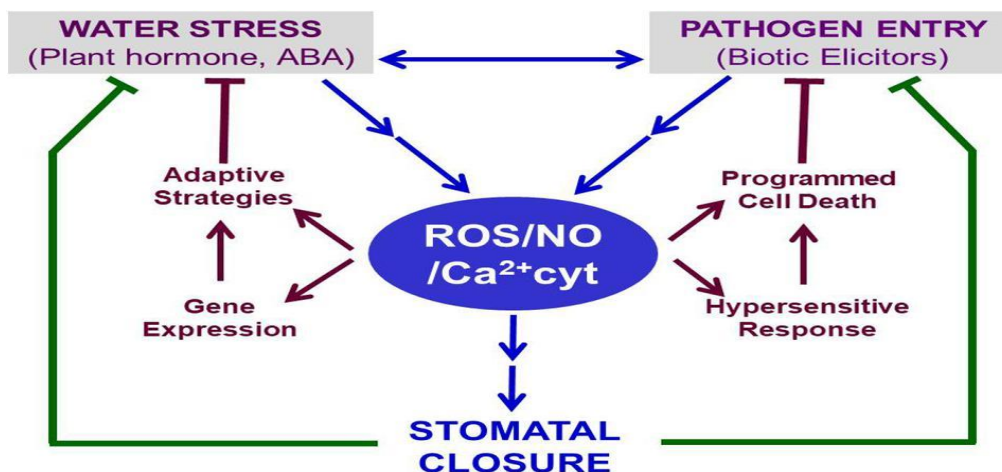
Overview

Abscisic acid (ABA) is one of the important Plant growth regulators plays major role in the regulation of drought stress in plants. Transcriptional factors associated with ABA – responsive regulation of genes optimized growth and development of plants under drought stress. In addition to drought regulation in plants, several drought responsive genes have been identified are associated with ABA-dependent pathway, whereas in ABA-independent pathways dehydration-responsive element binding protein (DREB), NAM, ATAF, and CUC regulons play an important role in the regulation of many droughts' responsive genes in the plants. In this literature, we focused on the various drought regulation strategies in plants by transcriptional factor (TF) families including abscisic acid-responsive elements and dehydration-responsive elements associated with ABA-pathway in plants.

1. Introduction

Drought or water deficit condition is the metrological factor limiting agricultural productivity across the world. Long period of water deficit condition due to uncertainty of rainfall and global warming leading to reduced available soil moisture to the plants (Gupta, et al., 2020a). In last few decades, drought stress has increased due to uncertainty of rainfall cause by global warming and environmental pollutants. Therefore, it is very essential to understand the mechanism of drought tolerance to improve crop plants for fulfil of the food demand of the increasing population of the world. In response to drought stress, stomatal closure is the primary response of the plants to reduce water loss (Raghavendra, et al., 2010). Secondary, rapid wax biosynthesis on the leaf surface reduces water loss, respiration rate and increase water uptake (Meng, et al., 2019). Several attempts have been made by the researchers and plant physiologist to understand the mechanism of drought tolerance. The plant response at cellular level, metabolic changers in plants under water deficit condition have been well studied and demonstrated. Furthermore, it has also been analyzed that drought stress affects expression of many genes in

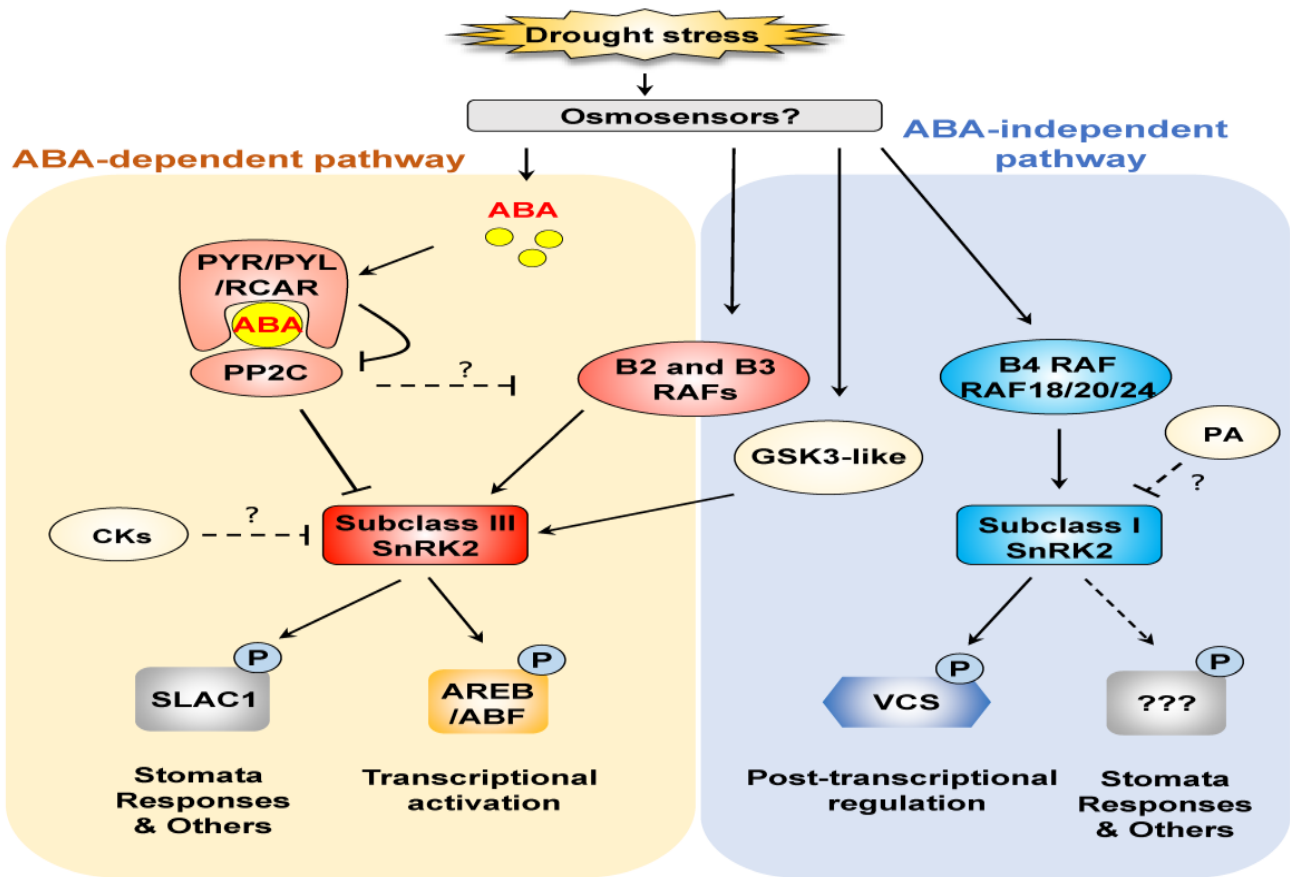
plant. The Genomic information of the model genetic plants “*Arabidopsis thaliana*” provided information about the gene and gene families associated with drought mechanism. In addition, advanced omic tools such as transcriptomic, proteomic and metabolomics studies provide strength about the mechanism of drought tolerance in plants. The changes in plants due to drought stress, many genes play an important role in the stress response the development of tolerance for the survival of plant. Many droughts responsive genes have been identified and characterized in response to drought tolerance. The responsive gene categories like LEA proteins, chaperons/HSPs, and reactive oxygen species sugar and proline biosynthesis enzymes are also essential for the development of drought tolerance in plants. Moreover, the gene encoding proteins involved in signaling and expression of the desirable genes like kinase, enzymes for lipid signaling and components of ABA and transcriptional factors have been also involved in stressed condition.



Source: Raghavendra, et al., 2010

2. ABA dependent plant response under drought stress

When plant subjected to water deficit condition the plant hormone abscisic acid (ABA) play important role for adaptation of plant under acute condition. Under drought condition, ABA is accumulated in the plants due to inductions of ABA biosynthetic gene families. Several physiological and biochemical changes in the plants under drought condition are regulated by the expression of the ABA responsive genes. Hence, existence of ABA–dependent signal transduction pathway play pivotal role under drought stress (Todaka, et al., 2015). Microarray study revealed that several genes involved in the up and down regulation have been identified in response to drought stress. In addition to ABA dependent drought stress response of the plants, microarray studies of rice, wheat, maize, barley and other crops have been compared to identifying the gene expression (Shaar-Moshe, et al., 2015).

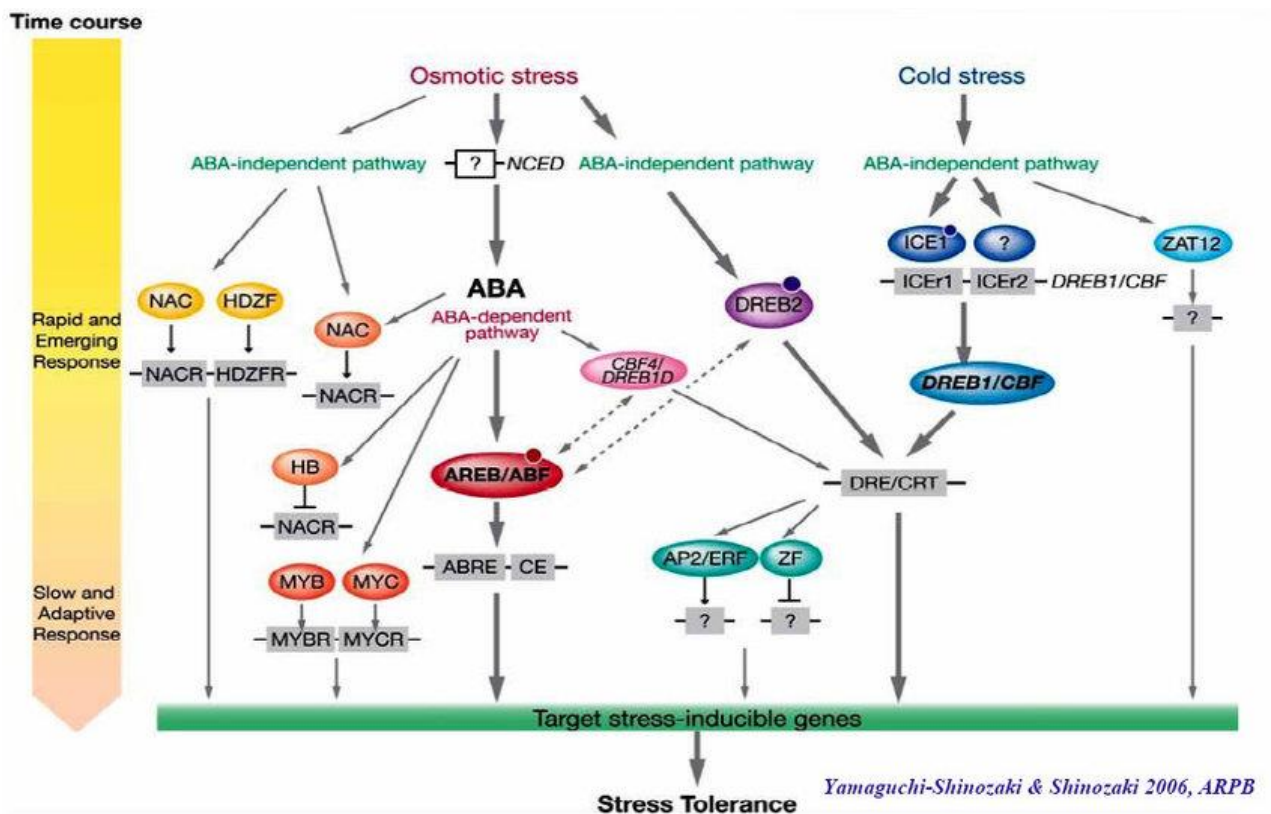


Source: D-Todaka, et al., 2015

3. ABA signaling pathway

ABA signaling plays an important role in response to drought stress by the regulation of transcriptional network. In water deficit condition, many genes are induced by exogenous application of ABA due to ABA signaling pathway regulated by transcriptional factors (Yamaguchi-Shinozaki and Shinozaki, 2006). ABA signaling pathway consists of receptor (RCAR/PYR/PYLs), protein phosphatase (PP2C) and kinase (SnRK2s). These receptors are bounded and activated to form trimeric complex with protein phosphatase (PP2C) and inhibited the activity of phosphatase (Park, et al., 2009). Just after the inhibition of the activity of phosphatase (PP2C), kinase SnRK2s (SnRK2.2, SnRK2.3, and SnRK2.6) released from associated PP2Cs. Released SnRK2s can be activated through autophosphorylation towards the downstream of Transcriptional Factors and ion channel proteins involved in the drought response (Ma, et al., 2009). Rather than transcriptional factors in the downstream in ABA signaling, some other transcriptional factors like WRKY, MYB, and NF-Ys, are also involved in drought response and tolerance mechanism in the plants. Hence, Transcriptional factors, ARE, DRE, WRKY, MYB,

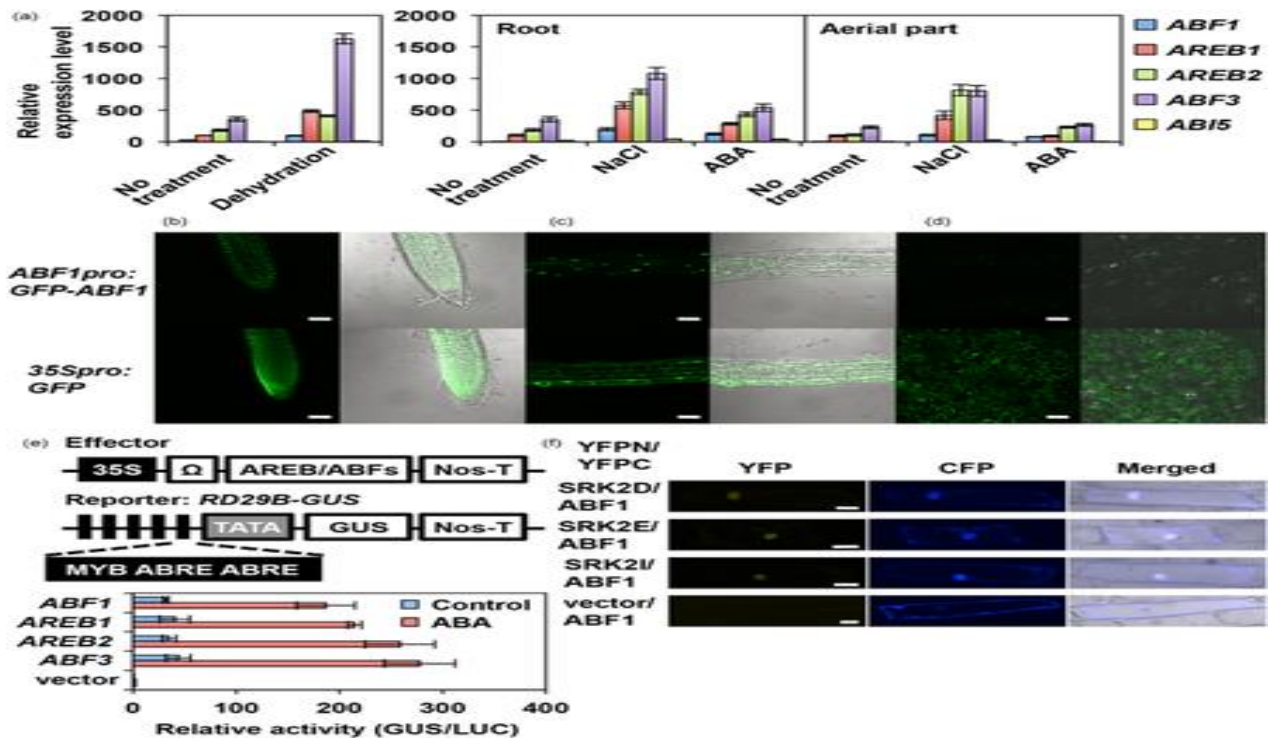
and NF-Ys, NACs plays very importance role when plants are under water deficit condition (Singh and Laxmi, 2015).



Source: Yamaguchi-Shinozaki and Shinozaki, 2006

4. Abscisic acid-responsive element (ARE)

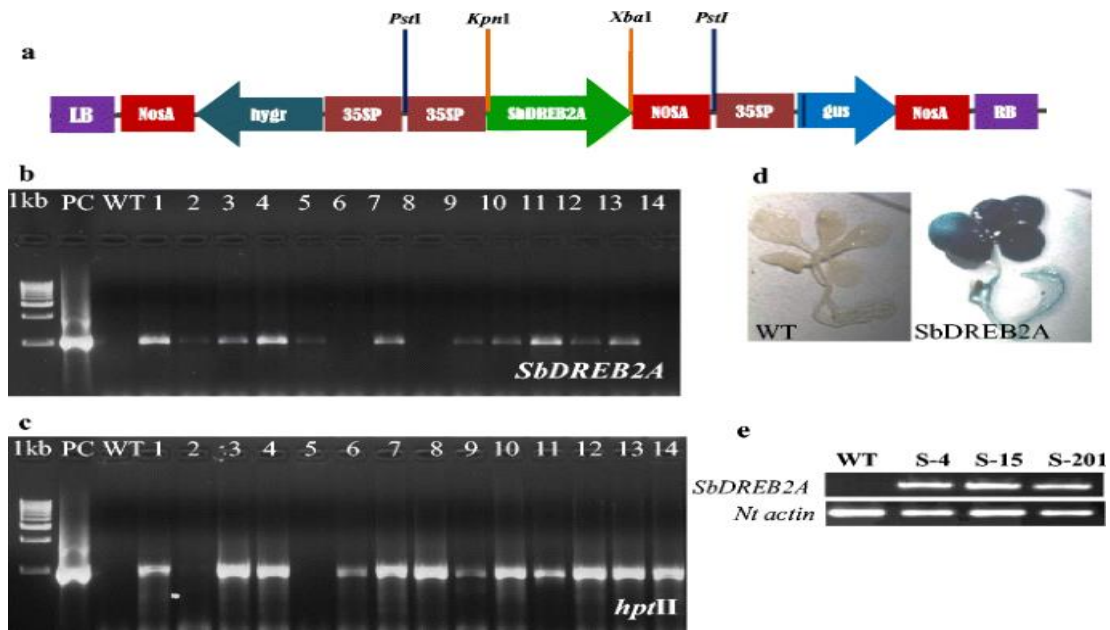
Abscisic acid-responsive element is ABRE factor, associated with abscisic acid-responsive element binding proteins (AREB). Abscisic acid-responsive element binding proteins are involved in the ABA-dependent regulation of gene expression when plants are under water deficit condition. ABA-responsive gene regulation is regulated in the promoter region of the gene (Yoshida, et al., 2015). ABRE is composed of conserved region of 8 bp long cis elements (PyACGTGG/TC) with one core sequence ACGT. In ABRE promoter region analysis it is found that single copy of ABRE is not sufficient in ABA-responsive gene expression under drought condition (Li, et al., 2019). The cis-acting elements of the ABRE needed close proximity of the other cis-acting elements of the other ABRE. Such type of proximity of the one cis-acting elements of the one ARBE to others is called as coupling elements (CE). These coupling elements (CEs) are GC rich region of the cis-elements (Yang, et al., 2020). Certain coupling elements such as coupling elements1 (CE1) and coupling elements3 (CE3) or c-repeats are involved in regulation of drought stress.



Source: Yoshida, et al., 2015

5. Dehydration-responsive elements (DRE)

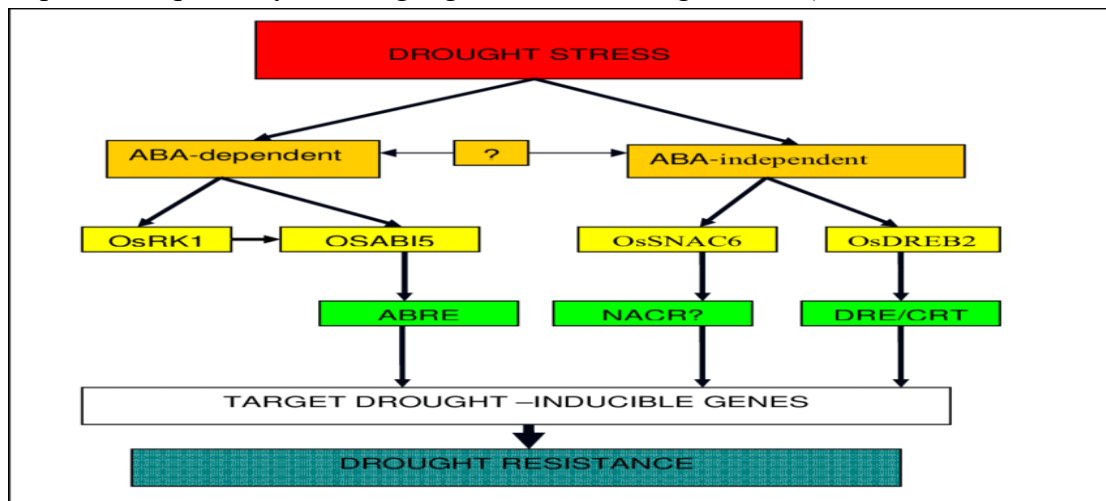
Dehydration-responsive elements (DREs) are A/GCCGAC rich sequences. These sequences have been identified in drought-responsive genes, which are recognized by DREB2 proteins. There are two types of DRE protein elements, namely DREB2A and DREB2B belongs to Apetala2 and ethylene-responsive factors of the TF family (Maruyama, et al., 2012). Dehydration-responsive elements (DRE) is associated with dehydration-responsive element binding proteins (DREB-2A regulon) are operated when plants are subjected to drought and heat stress. Overexpression of DREB-2A showed improved heat-tolerance by strategic expression of heat-sock related genes (Mizoi, et al., 2012). Hence, dehydration-responsive elements are not only involved in heat-tolerance, it is positively involved in drought stress tolerance (Lim, et al., 2007). Further study revealed that the stability of DREB2A is controlled by DRIP1 and DRIP2 through the ubiquitin/26S proteasome system under normal conditions. In *Arabidopsisthaliana*, tiny tiny2 tiny3 are the triple mutants of the TINY family showed hypersensitive phenotypes to drought stress whereas transgenic plants overexpressing TINY enhanced drought tolerance. Therefore, TINY family significantly regulate the genes associated with drought and promotes stomatal closure to reduce the dehydration rate by interacting BES1 in BR signaling (Xie, et al., 2019).



Source: Maruyama, et al., 2012

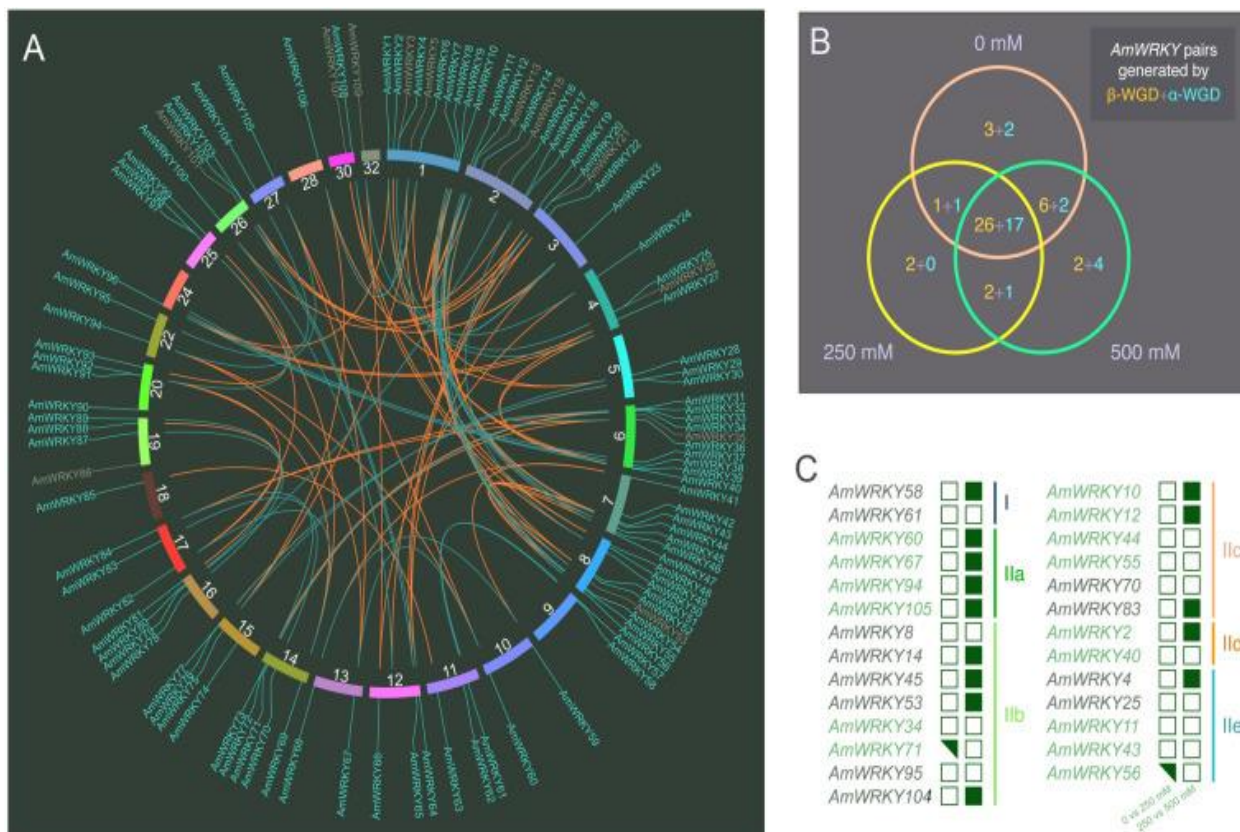
6. Transcriptional factors

Transcriptional factors play pivotal role in the modulation of drought-induced signal to several cellular responses. Single transcriptional factor have ability to modulate the expression of number of genes associated with respective stress (Takahashi, et al., 2018). Transcriptional factors are regulatory proteins that can regulate the drought stress in plants. Those regulatory proteins, which are involved in the modulation or regulation of genes under stressed condition, are collectively known as regulons. These regulatory proteins modulate the expression of respective genes by binding with promoter. Under acute drought condition, many regulons are activated to optimized plant growth (Yu, et al., 2017). In Arabidopsis thaliana, some regulons have been well studied and demonstrated in the regulation of drought stress (Nakashima, et al., 2009). In addition, ABA – independent and ABA – dependent pathways are actively involved in the regulation of transcriptional response by affecting regulons under drought stress (Nakashima, et al., 2009).



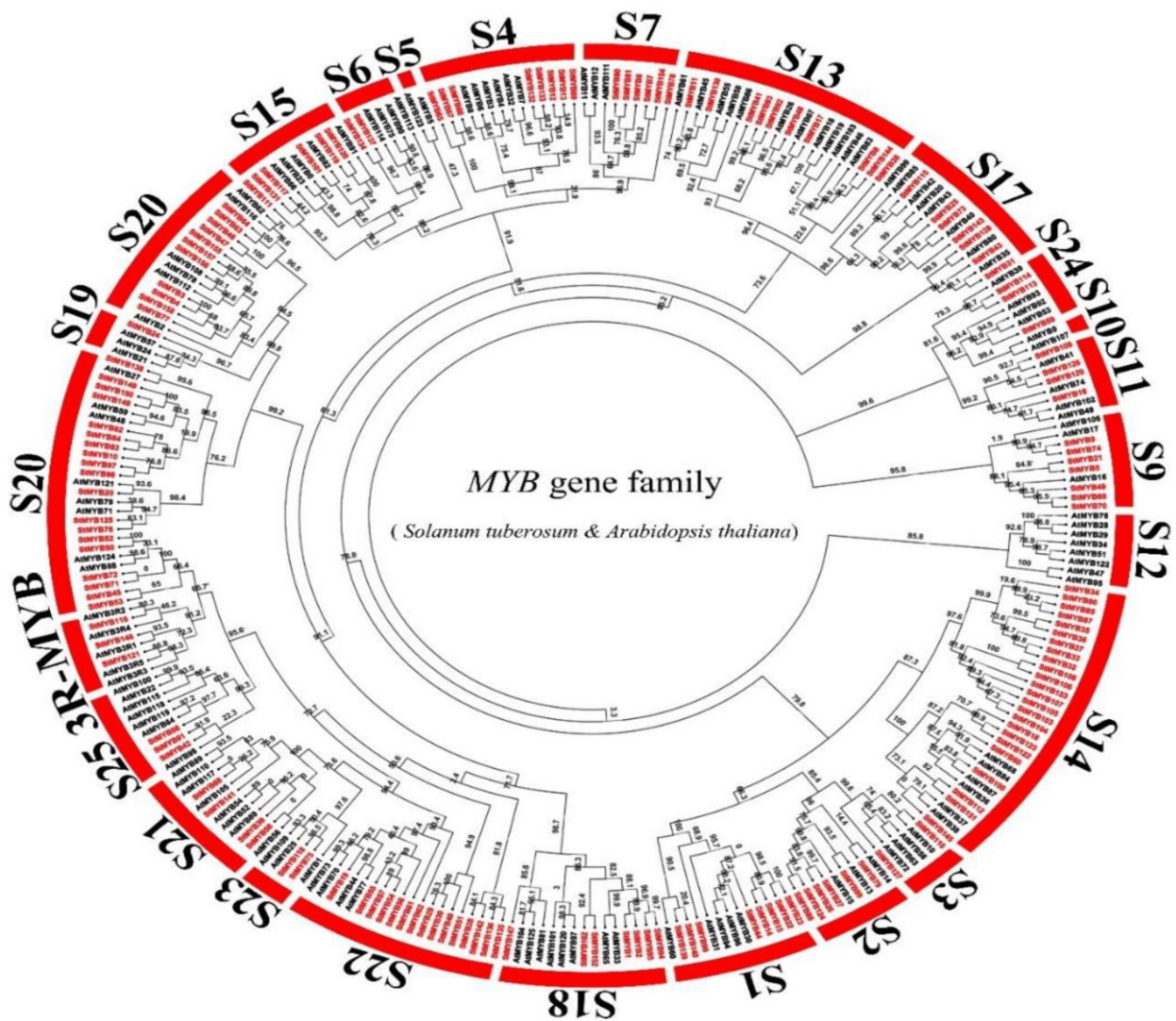
Source: Nakashima, et al., 2009

6.1. WRKY TF family: WRKY TF families do not recognize the ABRE motif but play important role in the ABA-dependent drought response. The member of this family have conserved region with DNA-binding potential (Singh and Laxmi, 2015). They have W-box of the TTGACC/T sequence involved in the acceleration of both biotic and abiotic stress responsive genes (Chen, et al., 2017). Some genes of this family viz. WRKY18, WRKY40, and WRKY60 are negatively regulating ABA-responsive genes through binding with their promoter region under normal condition (Chen, et al., 2019). Whereas, expression of WRKY18, WRKY40, and WRKY60 genes are accelerated under water deficit condition and ABA positively regulated the gene expression (Chen, et al., 2019). In addition to negatively regulating drought stress, WRKY40 and WRKY70 are known repressors in plant immunity and has been reported that drought stress-induced ABA biosynthesis inhibits salicylic acid (SA)-mediated plant immunity (Gupta, et al., 2020b).



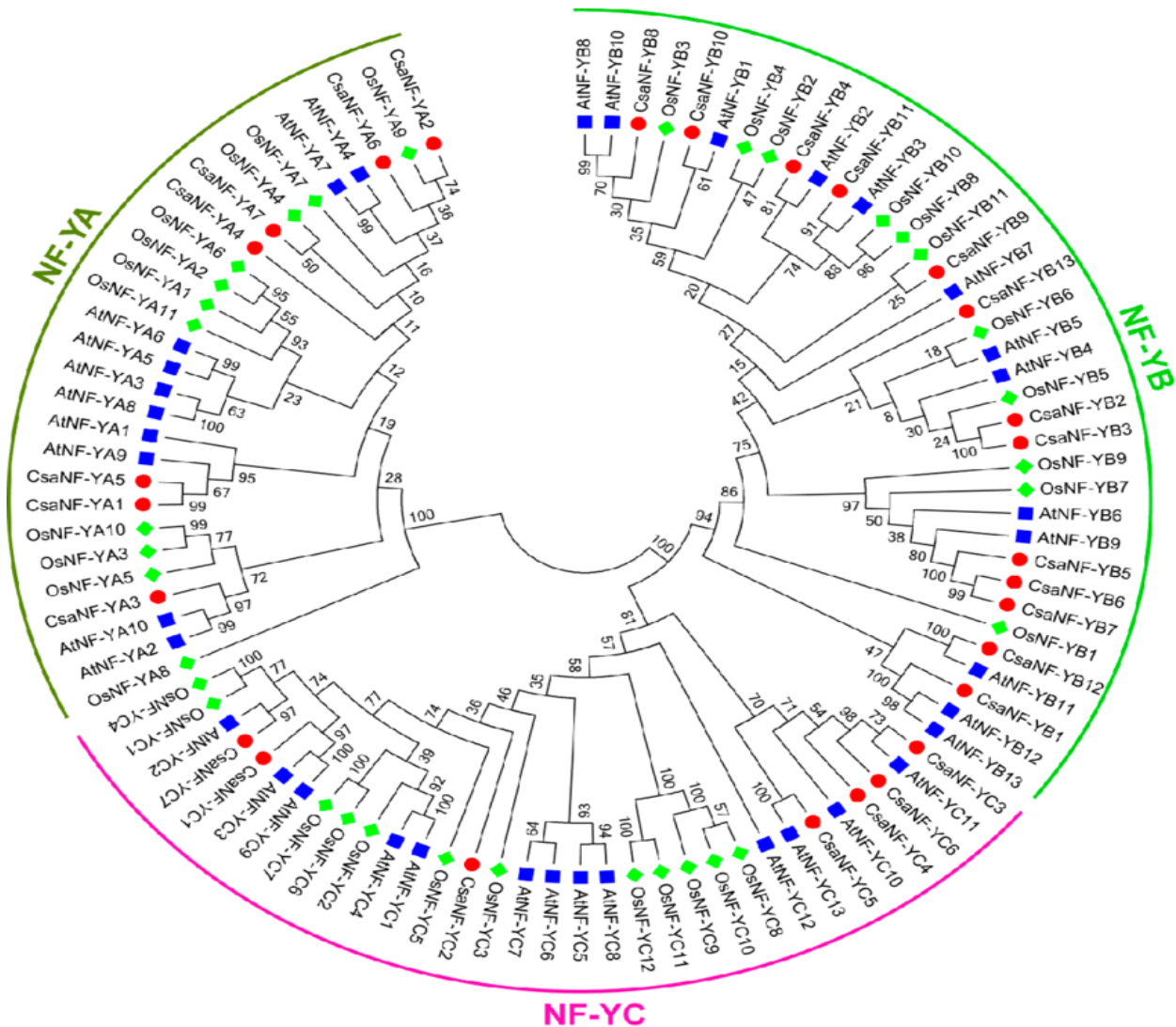
Source: Chen, et al., 2019

6.2. MYBs TF family: Expression of some cis-acting elements MYBRS (C/TAACNA/G) and MYCRS (CANNTG), in their promoter region governs drought responsive elements depend on ABA (Abe, et al., 1997). Further studies identified AtMYB2, AtAMYB96 and bHLH TF AtMYC2 member of the MYB TF family involved as drought responsive genes. In addition to the activation of target genes in response to ABA, AtMYB96 was reported to negatively involved in the regulation of ABA-repressible genes have been reported by Lee and Seo, 2019.



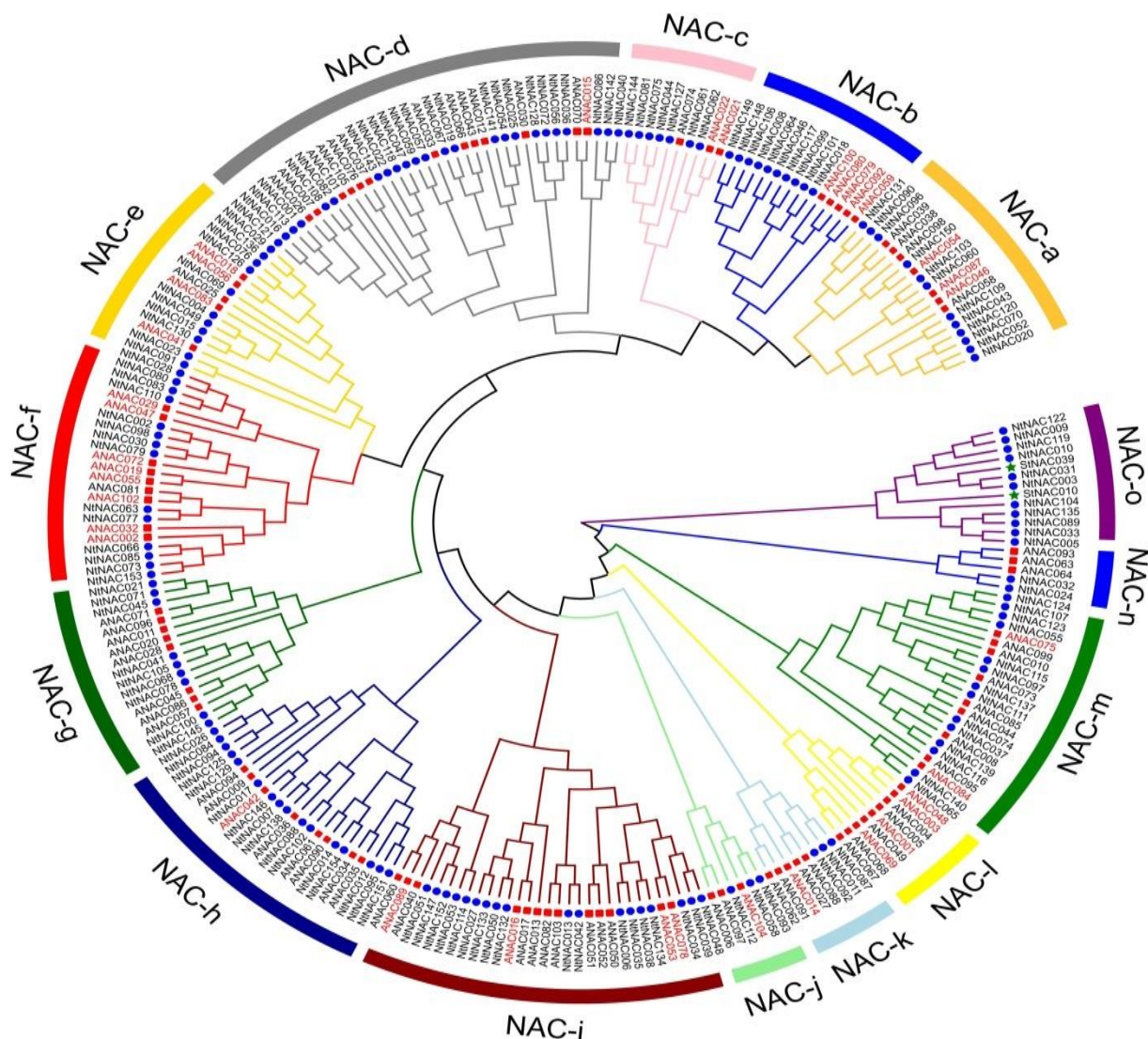
Source: Lee, et al., 2019

6.3. NF-Ys Unit: NF-Ys units are consists of three subunit viz. NF-YA, NF-YB, and NF-YC. These subunits recognize CCAAT box in the promoters of target genes. In *Arabidopsis thaliana*, AtNF-YA5 units of the NF-Ys family induced by drought stress in an ABA-dependent pathway and the mutant *nf-ya5* shows hypersensitivity to drought stress, whereas overexpression of NF-YA5 enhances drought tolerance. Other members like AtNF-YA3, AtNF-YA7, and AtNF-YA10 have been identified from NF-YA family significantly involved in the drought stress response (Leyva-Gonzalez, et al., 2012). In addition, one of the unique member of NF-Ys family i.e. AtNF-YB2 have been identified are involved in the regulation of drought stress in both ABA-dependent and ABA-independent pathways (Sato, et al., 2019). Recently in 2019, Hwang, et al., reported novel element AtNF-YC3/4/9 positively involved in the dehydration escape in most of the flowering plants while interact with ABF3/4 by activating SOC1 expression under drought condition (Hwang, et al., 2019).



Source: Leyva-Gonzalez, et al., 2012

6.4. NACs families: NACs is the largest transcriptional factor family includes NAM, ATAF, and CUC. Approximately hundred members in rice and Arabidopsis have been identified from this transcriptional families are involved in drought stress regulation. Most of the NAC transcriptional factors are encoded by NAC-gene family. NAC transcriptional family regulate drought stress responsive gene through ABA-dependent pathway, whereas the other member of NACs families do so through ABA-independent pathway (Singh and Laxmi, 2015). Further study revealed that the dehydration– responsive gene ERD1 expression induced by drought stress not by ABA. NAC transcriptional factors contain conserved N-terminal DNA-binding domain and variable C-terminal region (Nakashima, et al., 2012). The C-terminal region of NAC transcriptional factors play crucial role in the determination of drought responsive genes (Nuruzzaman, et al., 2013). These transcriptional factors are involved in the plant development process related to auxin signaling and development of meristematic tissues.



Source: Nuruzzaman, et al., 2013

7. Epigenetic regulation of drought stress

Epigenetic regulation significantly contributes to the drought and other abiotic stress response. Epigenetic regulation is associated with histone protein modification, chromatin remodeling, and DNA methylation (Chang, et al., 2019). Dehydration in plants due to acute drought, the antagonistic response of the histone 3lysine4 (H3K4) methyltransferase and histone demethylase maintain the gene expression for the development of stress tolerance. Whereas, histone demethylase control histone methylation homeostasis under drought stress. The histone demethylase JM17 of the KDM5/JARID1 family significantly response to dehydration stress in *Arabidopsis thaliana*. In addition to epigenetic regulation under dehydration stress, the *LIKE HETEROCHROMATIN PROTEIN* (lhp1) of the PRC1 complex also mediates transcriptional repression of drought-related TF gene like ANAC019 and ANAC055 (Huang, et al., 2019). Furthermore, the HDA9 of the histone deacetylase family significantly regulates drought response by maintaining ABA homeostasis in response to drought stress (Baek, et al., 2020).



References

- Abe, H., Yamaguchi-Shinozaki, K., Urao, T., Iwasaki, T., Hosokawa, D., and Shinozaki, K. (1997). Role of arabidopsis MYC and MYB homologs in drought- and abscisic acid-regulated gene expression. *Plant Cell* 9, 1859–1868. doi: 10.1105/tpc.9.10.1859
- Baek, D., Shin, G., Kim, M. C., Shen, M., Lee, S. Y., and Yun, D.-J. (2020). Histone deacetylase HDA9 with ABI4 contributes to abscisic acid homeostasis in drought stress response. *Front. Plant Sci.* 11:143. doi: 10.3389/fpls.2020.00143
- Chang, Y. N., Zhu, C., Jiang, J., Zhang, H., Zhu, J. K., and Duan, C. G. (2019). Epigenetic regulation in plant abiotic stress responses. *J. Integr. Plant Biol.* 62, 563–580. doi: 10.1111/jipb.12901
- Chen, J., Nolan, T. M., Ye, H., Zhang, M., Tong, H., Xin, P., et al. (2017). Arabidopsis WRKY46, WRKY54, and WRKY70 transcription factors are involved in brassinosteroid-regulated plant growth and drought responses. *Plant Cell* 29, 1425–1439. doi: 10.1105/tpc.17.00364
- Chen, X., Li, C., Wang, H., and Guo, Z. (2019). WRKY transcription factors: evolution, binding, and action. *Phytopathol. Res.* 1:13. doi: 10.1186/s42483-019-0022-x
- Gupta, A., Rico-Medina, A., and Caño-Delgado, A. I. (2020a). The physiology of plant responses to drought. *Science* 368, 266–269. doi: 10.1126/science.aaz7614
- Gupta, A., Sinha, R., Fernandes, J. L., Abdelrahman, M., Burritt, D. J., and Tran, L. P. (2020b). Phytohormones regulate convergent and divergent responses between individual and combined drought and pathogen infection. *Crit. Rev. Biotechnol.* 40, 320–340. doi: 10.1080/07388551.2019.1710459
- Huang, S., Zhang, A., Jin, J. B., Zhao, B., Wang, T.-J., Wu, Y., et al. (2019). Arabidopsis histone H3K4 demethylase JMJ17 functions in dehydration stress response. *New Phytol.* 223, 1372–1387. doi: 10.1111/nph.15874
- Hwang, K., Susila, H., Nasim, Z., Jung, J. Y., and Ahn, J. H. (2019). Arabidopsis ABF3 and ABF4 transcription factors act with the NF-YC complex to regulate SOC1 expression and mediate drought-accelerated flowering. *Mol. Plant* 12, 489–505. doi: 10.1016/j.molp.2019.01.002
- Lee, H. G., and Seo, P. J. (2019). MYB96 recruits the HDA15 protein to suppress negative regulators of ABA signaling in Arabidopsis. *Nat. Commun.* 10:1713. doi: 10.1038/s41467-019-09417-9411
- Leyva-Gonzalez, M. A., Ibarra-Laclette, E., Cruz-Ramirez, A., and Herrera-Estrella, L. (2012). Functional and transcriptome analysis reveals an acclimatization strategy for abiotic stress tolerance mediated by Arabidopsis NF-YA family members. *PLoS One* 7:e48138. doi: 10.1371/journal.pone.0048138
- Li, S., Lin, Y.-C. J., Wang, P., Zhang, B., Li, M., Chen, S., et al. (2019). The AREB1 transcription factor influences histone acetylation to regulate drought responses and tolerance in *Populus trichocarpa*. *Plant Cell* 31, 663–686. doi: 10.1105/tpc.18.00437
- Lim, C. J., Hwang, J. E., Chen, H., Hong, J. K., Yang, K. A., Choi, M. S., et al. (2007). Over-expression of the Arabidopsis DRE/CRT-binding transcription factor DREB2C enhances thermotolerance. *Biochem. Biophys. Res. Commun.* 362, 431–436. doi: 10.1016/j.bbrc.2007.08.007
- Ma, Y., Szostkiewicz, I., Korte, A., Moes, D., Yang, Y., Christmann, A., et al. (2009). Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science* 324, 1064–1068. doi: 10.1126/science.1172408
- Maruyama, K., Todaka, D., Mizoi, J., Yoshida, T., Kidokoro, S., Matsukura, S., et al. (2012). Identification of cis-acting promoter elements in cold- and dehydration-induced transcriptional pathways in Arabidopsis, rice, and soybean. *DNA Res.* 19, 37–49. doi: 10.1093/dnares/dsr040



- Meng, S., Cao, Y., Li, H., Bian, Z., Wang, D., Lian, C., et al. (2019). PeSHN1 regulates water-use efficiency and drought tolerance by modulating wax biosynthesis in poplar. *Tree Physiol.* 39, 1371–1386. doi: 10.1093/treephys/tpz033
- Mizoi, J., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2012). AP2/ERF family transcription factors in plant abiotic stress responses. *Biochim. Biophys. Acta* 1819, 86–96. doi: 10.1016/j.bbagr.2011.08.004
- Nakashima, K., Ito, Y., and Yamaguchi-Shinozaki, K. (2009). Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. *Plant Physiol.* 149, 88–95. doi: 10.1104/pp.108.129791
- Nakashima, K., Takasaki, H., Mizoi, J., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2012). NAC transcription factors in plant abiotic stress responses. *Biochim. Biophys. Acta* 1819, 97–103. doi: 10.1016/j.bbagr.2011.10.005
- Nuruzzaman, M., Sharoni, A. M., and Kikuchi, S. (2013). Roles of NAC transcription factors in the regulation of biotic and abiotic stress responses in plants. *Front. Microbiol.* 4:248. doi: 10.3389/fmicb.2013.00248
- Park, S. Y., Fung, P., Nishimura, N., Jensen, D. R., Fujii, H., Zhao, Y., et al. (2009). Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science* 324, 1068–1071. doi: 10.1126/science.1173041
- Raghavendra, A. S., Gonugunta, V. K., Christmann, A., and Grill, E. (2010). ABA perception and signalling. *Trends Plant Sci.* 15, 395–401. doi: 10.1016/j.tplants.2010.04.006
- Sato, H., Suzuki, T., Takahashi, F., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2019). NF-YB2 and NF-YB3 have functionally diverged and differentially induce drought and heat stress-specific genes. *Plant Physiol.* 180, 1677–1690. doi: 10.1104/pp.19.00391
- Shaar-Moshe, L., Hübner, S., and Peleg, Z. (2015). Identification of conserved drought-adaptive genes using a cross-species meta-analysis approach. *BMC Plant Biol.* 15:111. doi: 10.1186/s12870-015-0493-6
- Singh, D. and Laxmi, A. (2015). Transcriptional regulation of drought response: a tortuous network of transcriptional factors. *Front. Plant Sci.* 6:895. doi: 10.3389/fpls.2015.00895
- Takahashi, F., Kuromori, T., Sato, H., and Shinozaki, K. (2018). “Regulatory gene networks in drought stress responses and resistance in plants,” in *Survival Strategies in Extreme Cold and Desiccation: Adaptation Mechanisms and Their Applications*, eds M. Iwaya-Inoue, M. Sakurai, and M. Uemura (Singapore: Springer), 189–214. doi: 10.1007/978-981-13-1244-1_11
- Todaka, D., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2015). Recent advances in the dissection of drought-stress regulatory networks and strategies for development of drought-tolerant transgenic rice plants. *Front. Plant Sci.* 18:684. doi: 10.3389/fpls.2015.00084
- Xie, Z., Nolan, T., Jiang, H., Tang, B., Zhang, M., Li, Z., et al. (2019). The AP2/ERF transcription factor TINY modulates brassinosteroid-regulated plant growth and drought responses in Arabidopsis. *Plant Cell* 31, 1788–1806. doi: 10.1105/tpc.18.00918
- Yamaguchi-Shinozaki, K. and Shinozaki, K. (2006). Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu. Rev. Plant Biol.* 57, 781–803. doi: 10.1146/annurev.arplant.57.032905.105444
- Yang, Y., Li, H.-G., Wang, J., Wang, H.-L., He, F., Su, Y., et al. (2020). ABF3 enhances drought tolerance via promoting ABA-induced stomatal closure by directly regulating ADF5 in *Populus euphratica*. *J. Exper. Bot.* eraa383. doi: 10.1093/jxb/eraa383
- Yoshida, T., Fujita, Y., Maruyama, K., Mogami, J., Todaka, D., Shinozaki, K., et al. (2015). Four Arabidopsis AREB/ABF transcription factors function predominantly in gene expression downstream of SnRK2 kinases in abscisic acid signaling in response to osmotic stress. *Plant Cell Environ.* 38, 35–49. doi: 10.1111/pce.12351



Yu, J., Ge, H., Wang, X., Tang, R., Wang, Y., Zhao, F., et al. (2017). Overexpression of pyrabactin resistance-like abscisic acid receptors enhances drought, osmotic, and cold tolerance in transgenic poplars. *Front. Plant Sci.* 8:1752. doi: 10.3389/fpls.2017.01752