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# Wheat NF-YA10 functions independently in salinity and drought stress

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**The transcription factor NUCLEAR FACTOR Y (NF-Y) plays an essential role in many developmental and stress-responsive processes in plants. NF-Y composed of 3 subunits, NF-YA, NF-YB, and NF-YC, targets the CCAAT box, a common *cis*-element in eukaryotic promoters. We recently identified a gene *TaNF-YA10–1* from the wheat salinity tolerant cultivar SR3 and found that recombinant TaNF-YA10–1 could successfully bind to the CCAAT motif *in vitro*. We also showed that the constitutive expression of *TaNF-YA10–1* in *Arabidopsis thaliana* significantly increased the plant's sensitivity to salinity. Here, we further demonstrated that *TaNF-YA10–1* -overexpressing plants conferred drought tolerance as judged from the relative root length and whole-plant growth under drought stress. These results suggest that *TaNF-YA10–1* functions independently in salinity and drought stress. Our findings are helpful in understanding the distinct roles of NF-YA in plant stress responses.**

Fine regulation of related genes at transcriptional level via different transcription factors is a vital part of plant defense responses in plant adaptive environmental stress response. [1-3] Currently, many kinds of transcription factors are known to be involved in plant life processes. Nuclear factor Y (NF-Y) is a type of typical multifunctional transcription factors, which is composed of 3 subunits: NF-YA, NF-YB and NF-YC. The three NF-Y subunits in the nucleus form an active heterotrimer that binds to the CCAAT box, a conserved motif in eukaryotic gene promoters, and thus regulate the transcription of downstream genes.[4-6] Each of the 3 NF-Y subunits in yeast and animals is encoded by a single gene, but in plants the NF-Y subunit proteins are present in the form of multigene families, which indicates a more complicated regulation of NF-Y proteins in plants than in other organisms.[7] For example, there are 36 NF-Y genes (10 *NF-YA*, 13 *NF-YB* and 13 *NF-YC*) in model plant *Arabidopsis thaliana*,[8] while in bread wheat (*Triticum aestivum*), the number of *NF-Y* genes is 80 (18 *NF-YA*, 34 *NF-YB* and 28 *NF-YC*).[9,10] By using the model plants (*Arabidopsis* and rice), it has been found that the members of NF-Y factors are involved in various processes of development and stress responses, including embryo development, flowering control, photosynthesis and the adaptive responses to drought stress, salt stress and nutrient deficiency.[9,11-14] Given the complex and large genome of wheat, the functions of NF-Ys in wheat have not been studied sufficiently. We recently isolated a wheat gene *TaNF-YA10–1* from the salinity tolerant cultivar SR3 and showed that the constitutive expression of *TaNF-YA10–1* in *Arabidopsis* significantly increased the plant's sensitivity to salinity.[15]

Moreover, NF-Ys have been identified as regulators of drought tolerance in different plant species. Transgenic *Arabidopsis* and maize (*Zea mays*) plants overexpressing *AtNF-YA5*, *AtNF-YB1* and the maize ortholog *ZmNF-YB2*, respectively, have improved performance and survival under drought conditions.[11,16] *TaNF-YB2*, was reported to confer drought resistance and increase crop productivity under drought field tests.[10] To assess whether *TaNF-YA10–1* also plays a role in drought stress response, we first examined the root elongation of the seedlings grown under

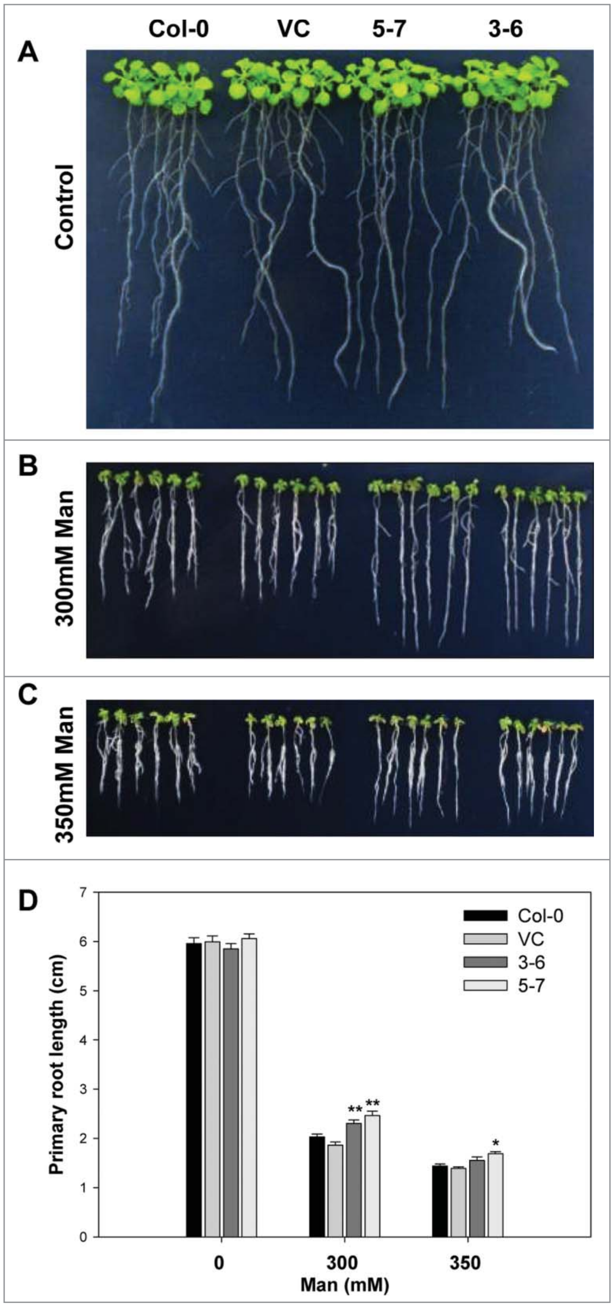

**Figure 1.** The constitutive expression of TaNF-YA10–1 in A. thaliana increased the tolerance of transgenic plants to osmotic stress. Comparison of root length between wild type (Col-0), the empty vector control (VC) and TaNF-YA10–1 overexpression lines (3–6 and 5–7) under normal conditions (**A**), 300 mM mannitol (**B**) and 350 mM mannitol (**C**). (**D**) Total root length of above seedlings. Man: mannitol. All data are given as mean ± SD from 3 independent experiments. The asterisks and double asterisks represent significant difference determined by the Student's t-test at $P < 0.05$ and $P < 0.01$ respectively.

medium containing a gradient concentration of mannitol. As shown in **Figure 1**, the root length of *TaNF-YA10–1* overexpression lines was longer than the wild type and vector control transgenic line when exposure to 300 or 350 mM mannitol. Assay of whole-plant drought tolerance confirmed that the seedlings of *TaNF-YA10–1* overexpression line were more tolerant of the drought stress (**Fig. 2**). These interesting observations suggest that *TaNF-YA10–1* is involved in different signaling pathways in response to different abiotic stresses.

In transgenic *A. thaliana* constitutively expressing *TaNF-YA10–1*, the ABA dependent pathway genes *AtRAB18*, *AtRD29B* and *AtABI5*, as well as the ABA-independent pathway genes *AtCBF1* and *AtCBF3*, were all down-regulated when plants were exposed to salinity stress.[15] The NF-Y transcription factors have mainly been considered as activators of transcription; however, recent evidence suggests their involvement in gene repression.[17,18] Thus, *TaNF-YA10–1* might function as repressor of transcription under salt stress conditions. It is suggested that NF-YAs could act as transcriptional activators of a subset of genes that contain the CCAAT-box and as repressors of genes whose promoters lack the CCAAT-box.[18] However, clear CCAAT motif enrichment was detected in the promoters of *AtCBF1*, *AtCBF3* and *AtRAB18*. Transcriptional activation or repression using protoplasts or yeast one-hybrid system will be required to provide direct evidences. Here, we further demonstrated that *TaNF-YA10–1*-overexpressing plants conferred drought tolerance, suggesting that *TaNF-YA10–1* functions independently in salinity and drought stress. Transcriptional behavior of stress responsive genes in *TaNF-YA10–1* overexpression lines under osmotic stress conditions remains to be elucidated.

The expansion of NF-Y families in plants, combined with their heterotrimeric nature, means that many possible NF-Y complexes can form. This leads to the formation of a flexible, combinatorial system of transcription factors that may allow subtle adjustments to many different environmental conditions.[19] These different functions of *TaNF-YA10*–1 might be explained by the diverse combination of NF-YA with other NF-YB/NF-YC factors, which thus affected different regulating pathways. We propose that there exists dual transcriptional control of *TaNF-YA10–1* through the interaction with different NF-YB/NF-YC heterodimers via its role in activating target drought-responsive genes or repressing target salt-responsive genes. Alternatively, other proteins associating with TaNF-YA10–1 subunit of NF-Y complexes may be different in

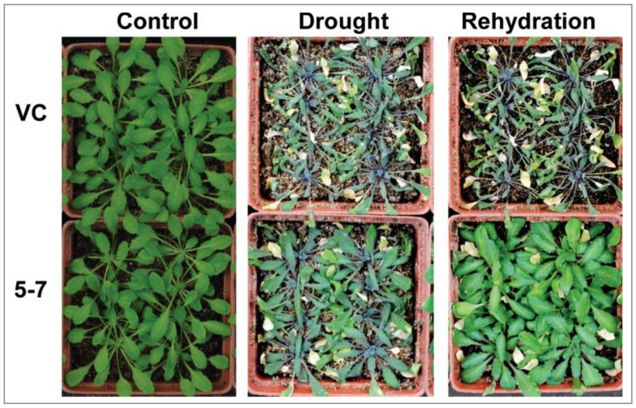

**Figure 2.** The phenotype of *TaNF-YA10–1* overexpression line under drought stress conditions. VC: the empty vector control; 5–7: *TaNF-YA10–1* overexpression line. For the drought stress treatment, watering was withheld from 3-week-old plants for 3 weeks before the photograph was taken. For the rehydration treatment, the photograph was taken 3 d after rewatering.

the presence of salinity and drought stresses. Further study on protein-protein interactions will be needed to elucidate the inner mechanisms.

## Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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