Heterologous Expression of *ZmNF-YA12* Confers Tolerance to Drought and Salt Stress in *Arabidopsis*

Tongtong Zhang
Capital Normal University
Dengyu Zheng
Beijing Academy of Agriculture and Forestry Sciences
Chun Zhang
Beijing Academy of Agriculture and Forestry Sciences
Zhongyi Wu
Beijing Academy of Agriculture and Forestry Sciences
Rong Yu
Capital Normal University
Zhongbao Zhang (happyzzb@126.com)
Beijing Academy of Agriculture and Forestry Sciences https://orcid.org/0000-0001-5494-9762

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Abstract

Drought and salinity are serious environmental factors limiting the growth and productivity of plants worldwide. Therefore, it is necessary to develop ways to improve drought and salinity stress tolerance in plants. In this study, a drought-responsive nuclear factor Y subunit A gene, *ZmNF-YA12*, was cloned from maize. qPCR revealed *ZmNF-YA12* transcript in all vegetative and reproductive tissues, with higher levels in young roots. Expression analyses of maize revealed that *ZmNF-YA12* was induced by abscisic acid (ABA), jasmonic acid (JA), and abiotic stresses, including dehydration, high salinity, cold, and polyethylene glycol (PEG) treatment. The heterologous expression of *ZmNF-YA12* in *Arabidopsis* plants resulted in increased root length and better plant growth than in wild-type (WT) plants under conditions of mannitol, salt, and JA stress on 1/2 MS medium. Transgenic *Arabidopsis* showed improved tolerance to drought and salt stresses in soil, and higher proline content and lower malondialdehyde (MDA) content than WT controls. The transgenic plants also maintained higher peroxidase (POD) activities than WT plants under conditions of NaCl stress. A yeast two-hybrid experiment demonstrated that ZmNF-YA12 interacted with ZmNF-YC1 and ZmNF-YC15. Moreover, the transcript levels of stress-responsive genes (*RD29A*, *RD29B*, *RAB18*, and *RD22*) were markedly increased in transgenic lines under conditions of drought and salt stress. These observations suggested that the *ZmNF-YA12* gene confers drought and salt stress tolerance, and has potential applications in molecular breeding with maintenance of production under conditions of stress.

Introduction

The crop yield of maize (*Zea mays* L.), the most widely grown cereal crop in the world, is considerably limited by a range of abiotic stress factors (**Pechanova et al. 2013**). Drought and increased soil salinity are projected to lead to a 50% loss of arable land by the year 2050, which will severely affect crop yields. Therefore, it is becoming increasingly important to improve water use efficiency and salt tolerance for agricultural production in the ever-decreasing area of arable land (**Deinlein et al. 2014; Tiburcio et al. 2012**). Transcription factors play important roles in abiotic stress responses in plants, and represent promising targets for the genetic engineering of plants with elevated stress resistance (**Nowicka et al. 2018**). Many plant genes are regulated in response to abiotic stresses, and the products of these genes have functions related to the stress responses and tolerance (**Yamaguchi-Shinozaki and Shinozaki 2006**).

Nuclear factor Y (NF-Y), also called CBF and CP1, consists of three different subunits (NF-YA, NF-YB, and NF-YC), and is a unique DNA-binding protein that interacts with the CCAAT motif, a common element present in the promoters of a number of mammalian genes (**Maity and de Crombrugghe 1998**). The core domains of the NF-YC/NF-YB proteins interact through histone fold motifs. This histone-like pair is closely related to the H2A/H2B and NC2α/NC2β families, both of which have features common to this class of proteins and unique to NF-Y (**Romier et al. 2003**).

The plant NF-Y transcription factors have been reported to be key players in plant–microbe interactions, root development, and stress tolerance. Some members of the *NF-YA* and *NF-YB* gene families have been

shown to be involved in responses to water and nutrient scarcity in mono- and dicotyledonous plants (**Zanetti et al. 2017**). *AtNF-YB2* and *AtNF-YB3* are both essential for the normal flowering induced by long days in *Arabidopsis* (**Kumimoto et al. 2008**). Transcription of *OsNF-YA7* was shown to be induced by drought stress, and its overexpression in transgenic rice plants enhanced their drought tolerance (**Lee et al. 2015**). Overexpression of *TaNF-YA10-1* in wheat conferred drought tolerance, with longer root length and better whole-plant growth under conditions of drought (**Ma et al. 2015**). Transgenic tobacco plants overexpressing *CsNF-YA5* showed superior growth and photosynthetic rates under both normal conditions and drought stress (**Pereira et al. 2018**). *PdNF-YB21* overexpression promoted root growth with highly lignified and enlarged xylem vessels in poplar, resulting in increased drought resistance (**Zhou et al. 2020**). However, the biological roles of many members of the NF-Y family in maize are not clear.

Previously, we reported that *ZmNF-YA12* (GRMZM5G857944) could respond to abiotic stress (**Zhang et al. 2016**). To investigate the molecular biology function of *ZmNF-YA12*, its tissue-specific expression, gene expression patterns under different exogenous stresses, heterologous expression in *Arabidopsis*, and yeast two-hybrid experiment were performed. Transgenic *Arabidopsis* lines expressing *ZmNF-YA12* showed improved drought and NaCl tolerance.ZmNF-YA12 interacts with ZmNF-YC1 and ZmNF-YC15. Our data suggest that *ZmNF-YA12* may represent an important mechanism underlying the function of NF-Y under NaCl and drought stress.

Materials And Methods

Isolation of *ZmNF-YA12*

Total RNA was isolated from maize seedlings using TRIzol reagent (CW Biotech, Beijing, China) and reverse-transcribed to complementary DNA (cDNA) using HiScript II Q RT SuperMIX (Vazyme, Nanjing, China). The full *ZmNF-YA12*cDNA was amplified by PCR using the forward primer (FP) 5'-ATGCTTCTTCCCTCTTCGTCTT-3' and reserve primer (RP) 5'-TCATCTCATAACTGGAACCCT-3'.

Plant materials, growth conditions, and treatments

For tissue-specific analysis, leaves, stems, and roots were harvested from three-leaf seedlings grown in a greenhouse (28°C, 16/8 h day/night cycle). Mature leaves, roots, silks, tassels, and embryos were harvested at the grain-filling stage from plants grown in the field. All harvested materials were frozen immediately in liquid nitrogen and stored at −80°C.

To determine the *ZmNF-YA12* expression patterns under various stress conditions, maize seedlings were grown in 12-cm hydroponic barrels containing nutrient solution. Three-leaf maize seedlings were subjected to dehydration, NaCl, polyethylene glycol (PEG), cold, abscisic acid (ABA), and jasmonic acid (JA) treatments. For dehydration treatment, the whole seedling was removed, washed, and placed on an experimental table for natural dehydration at room temperature (25°C). For salt and PEG treatments, the roots of the seedlings were immersed in solutions containing 200 mM NaCl and 20% PEG, respectively. For cold treatment, seedlings were kept at 4°C. For each of the above four treatments, the shoots and

roots were collected at 0, 1, 2, 5, 10, and 24 hours after treatment. For the ABA and JA treatments, the leaves of the seedlings were sprayed with solutions containing 100 μM ABA or 100 μM JA and covered with plastic film. The leaves were then collected at 0, 1, 2, 5, and 10 hours after treatment. The samples were immediately frozen with liquid nitrogen for isolation of RNA.

Arabidopsis thaliana ecotype Columbia (Col-0) was used for transformation in this study. After vernalization treatment, seeds were surface-sterilized in a solution of 0.5% NaClO for 10 minutes, and washed five times with sterile distilled water. Following this treatment, the seeds were germinated and grown on half-strength Murashige and Skoog (1/2 MS) medium (pH 5.8–6.0). The plates were transferred to a growth chamber at 22°C for germination.

Gene expression analysis using qPCR

cDNA samples were obtained as described above. qPCR was performed in a CFX Connect Real-time PCR system (Bio-Rad, Hercules, CA, USA) using a Super Real PreMix (SYBR Green) kit (Tsingke Biotech, Beijing, China) according to the manufacturer's instructions. The primers used for qPCR were designed according to the *ZmNF-YA12* cDNA sequence (FP: 5'-AGCAACCTCCATTTGCGAGTCA-3' and RP: 5'-GGCTGCCCAAACATCTCCTGAT-3'). Each reaction was performed in triplicate, and the results are expressed relative to the expression levels of *Actin* (FP: 5'-GGTAACATTGTGCTCAGTGGTGG-3' and RP: 5'-AACGACCTTAATCTTCATGCTGC-3') and *GAPDH* (FP: 5'-CCCTTCATCACCACGGACTAC-3' and RP: 5'-AACCTTCTTGGCACCACCCT-3') in each sample using the $2^{-\Delta\Delta CT}$ method.

Vector construction and transformation of *Arabidopsis*

The *ZmNF-YA12* cDNA was cloned into the pCAMBIA-3301 vector driven by the cauliflower mosaic virus (CaMV) 35S promoter (**Supplementary Fig. 1**), as confirmed by sequencing. The resulting construct was transformed into *Arabidopsis* Col-0 by the floral dip method using *Agrobacterium tumefaciens* GV3101. The first generation (T_0) seeds of transgenic *Arabidopsis* were screened on 1/2 MS medium containing 50 mg/L kanamycin, and transgenic plants were confirmed by PCR and qPCR (FP: 5'-AACTCATCTGCGGCTTGG-3' and RP: 5'-GTATAATTGCGGGACTCTAATC-3'; and FP: 5'-AGCAACCTCCATTTGCGAGTCA -3' and RP: 5'-GGCTGCCCAAACATCTCCTGAT -3', respectively). Homozygous plants of the T_3 generation were used for further analysis.

Root growth assay

For root growth assay, transgenic and wild-type (WT) seeds were placed on 1/2 MS agar plates for germination. Seven days later, five germinated seedlings of the same size from each line were carefully transferred to 1/2 MS agar plates supplemented with 150 mM NaCl, 150 mM mannitol, 50 μM JA, or 10 μM ABA. Seedling root lengths were measured using ImageJ software (NIH, Bethesda, MD, USA) after 8 days of upright growth in treatment medium.

Drought and NaCl treatment of transgenic *Arabidopsis*

Drought and NaCl tolerance assays were performed on seedlings grown in pots in a greenhouse. Transgenic and WT seeds were germinated on 1/2 MS medium. One-week-old seedlings were planted in 7-cm pots containing mixed soil (vermiculite: humus = 1:1) of equal quality and well-watered for 3 weeks. For drought stress treatment, the seedlings were subsequently cultured without watering for 3 weeks and then re-watered for 2 days. For NaCl stress treatment, the plants were irrigated with a solution containing 450 mM NaCl for 1 week. Drought and NaCl tolerance experiments were performed in triplicate.Samples of *Arabidopsis* leaves were collected after the seedlings exhibited distinct phenotypes under drought and salt treatments. The peroxidase (POD) activity, proline, malondialdehyde (MDA), and chlorophyll contents were measured using a commercial assay kit (Solarbio, Beijing, China) according to the manufacturer's instructions.

Yeast two-hybrid assays

For yeast two-hybrid analysis, the *ZmNF-YA12* cDNA was cloned into the bait plasmid pGBKT7 (pGBKT7-*ZmNF-YA12*). The full-length cDNAs of *ZmNF-YB7* (GRMZM2G169884_T01), *ZmNF-YC1* (GRMZM2G089812_T01), *ZmNF-YC15* (GRMZM2G124421_T01), *ZmNF-YC17* (GRMZM2G311316_T01), *ZmCOI1*(GRMZM2G151536), and *ZmMYC2* (GRMZM2G049229) were separately cloned into the target pGADT7 plasmid. The bait and target plasmids to be tested for interactions were co-transformed into the yeast strain AH109 and plated on synthetic defined (SD) medium lacking leucine and tryptophan (SD/-LT) for screening transformants. The independent transformed colonies were then grown on SD medium lacking leucine, tryptophan, adenine, and histidine (SD/-AHLT). Survival of the yeast colonies on SD/-AHLT medium indicated that the target gene can interact with *ZmNF-YA12*.

Statistical analysis

The experiments were repeated three times and the data are presented as the mean ± SEM. The significance of the differences in the data was determined using SPSS statistical software (v. 25.0; SPSS Inc., Chicago, IL, USA). In all analyses, $p < 0.05$ was taken to indicate statistical significance.

Results

Isolation and characterization of *ZmNF-YA12*

The full-length *ZmNF-YA12* cDNA is 816 bp in length and encodes 271 amino acid residues with a predicted molecular mass of 29.3 kDa and isoelectric point (p*I*) of 10.96. Protein structure alignment showed that the ZmNF-YA12 sequence included an NF-Y transcription factor conserved domain (**Fig. 1A**). The results indicated that *ZmNF-YA12* is a member of the NF-YA transcription factor family. The deduced amino acid sequence of *ZmNF-YA12* was further compared to other NF-YA proteins from various organisms by phylogenetic analysis (**Fig. 1B**). The results indicated that ZmNF-YA12 is closely related to OsHAP2E. Analysis of the *ZmNF-YA12* promoter using the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) revealed a series of light-related and

hormone stress response elements, including CAT-box, G-box, and CGTCA motif (**Supplementary Fig. 2**). The results suggest that *ZmNF-YA12* may play important roles in responses to environmental stresses and regulation of plant growth and development.

Expression pattern of maize *ZmNF-YA12*

The expression levels of *ZmNF-YA12* in different tissues under various stresses were determined by qPCR. The results showed that *ZmNF-YA12* was expressed at higher levels in young roots than in other tissues (**Fig. 2**), and the expression levels of *ZmNF-YA12* in shoots and roots were upregulated by dehydration and PEG treatments (**Fig. 3A, B**). Under conditions of cold and NaCl treatment, the expression of *ZmNF-YA12* was markedly induced in roots but not in shoots (**Fig. 3C, D**). As shown in **Fig. 3E**, the *ZmNF-YA12* transcript level was downregulated at 1, 3, and 5 hours, and upregulated at 10 hours, with ABA treatment. With JA treatment, the expression of *ZmNF-YA12* first decreased and then increased, peaking at 3 hours (**Fig. 3F**).

Tolerance of transgenic *Arabidopsis* plants to salt, mannitol, JA, and ABA stress

To assess the effects of *ZmNF-YA12* in responses to abiotic stresses, *ZmNF-YA12* transgenic *Arabidopsis* plants (L-1, L-2, L-3) and WT seedlings were grown on 1/2 MS medium with different treatments. WT and transgenic plants showed similar root lengths under normal and 10 μM ABA conditions. However, the roots of transgenic lines were much longer than those of WT plants in the presence of 150 mM NaCl, 150 mM mannitol, or 50 μM JA (**Fig. 4; Supplementary Figs. 3–6**).

Heterologous expression of *ZmNF-YA12* confers enhanced drought and salt tolerance in *Arabidopsis*

Under control conditions, both WT and *ZmNF-YA12* transgenic plants exhibited a similarly normal growth phenotype. Drought and salt stress significantly inhibited the growth of WT plants, which exhibited more wilted and smaller leaves (**Fig. 5A and Fig. 6A**). However, *ZmNF-YA12* transgenic *Arabidopsis* plants showed less wilted leaves and more green and larger leaves (**Fig. 5B and Fig. 6B**). For drought treatment, after re-watering for 2 days, the *ZmNF-YA12* transgenic lines recovered more quickly, grew more green leaves and appeared to be healthier than WT plants (**Fig. 5C**).

To further characterize the function of *ZmNF-YA12*, we examined the MDA and proline contents under drought treatment, and POD and chlorophyll contents under salt stress in transgenic lines and WT plants. As shown in **Fig. 5D**, MDA content was lower in the transgenic plants than WT controls under drought stress. As expected, the proline content was much higher in the transgenic lines than in WT plants (**Fig. 5E**). Under NaCl treatment, the activities of POD in the transgenic lines were much higher than in WT plants (**Fig. 6C**). However, there were no obvious differences in chlorophyll contents between WT and transgenic plants (**Fig. 6D**). These results suggested that the heterologous expression of *ZmNF-YA12* improves drought and salt tolerance in *Arabidopsis*.

To determine the mechanism underlying the involvement of *ZmNF-YA12* in the stress response, the expression levels of stress-related genes were analyzed by qPCR in *ZmNF-YA12* transgenic and WT

plants grown under normal, NaCl, and drought conditions. As shown in **Fig. 7**, the expression levels of the stress-related genes *RD29A*, *RD29B*, *RAB18*, and *RD22* in the transgenic lines were much higher than in WT plants under NaCl and drought conditions.

ZmNF-YA12 interacts with ZmNF-YC1 and ZmNF-YC15

To investigate the mechanisms underlying the involvement of *ZmNF-YA12* in stress regulation, yeast two-hybrid assay was performed to identify proteins interacting with *ZmNF-YA12*. The results showed that *ZmNF-YA12* has no self-transcriptional activation activity. All of the transformed yeast cells grew well on SD/-LT medium, and the yeast cells transformed with both pGBKT7-*ZmNF-YA12* + pGADT7-*ZmNF-YC1* and pGBKT7-*ZmNF-YA12* + pGADT7-*ZmNF-YC15* grew well on SD/-AHLT medium, indicating that ZmNF-YA12interacts with ZmNF-YC1 and ZmNF-YC15(**Fig. 8**).

Discussion

The growth, development, and productivity of maize are seriously affected by abiotic stresses, such as drought, salinity, high and low temperatures, and by biotic stresses, such as fungi, viruses, and pests (**Gong et al. 2014**). The NF-Y transcription factors are important regulators of plant development and responses to environmental stress (**Petroni et al. 2012**). The maize genome includes 50 *ZmNF-Y* genes (14 *ZmNF-YA*, 18 *ZmNF-YB*, and 18 *ZmNF-YC*) (**Zhang et al. 2016**). In this study, we identified and characterized a gene, *ZmNF-YA12*, related to stress tolerance. *ZmNF-YA12* transcript levels were significantly induced by dehydration, PEG, cold, NaCl, ABA, and JA treatments (**Fig. 3**).

Several studies have indicated that *NF-Y* genes are involved in stress responses. *OsHAP2E*, a homolog of *ZmNF-YA12*, confers biotic and abiotic resistance, and increased photosynthesis and tiller numbers in rice (**Alam et al. 2015**). Overexpression of *SiNF-YA1* in transgenic tobacco lines enhanced drought and salt tolerance (**Feng et al. 2015**). Transgenic *Arabidopsis* plants overexpressing *AhNF-YC* showed increased seedling sensitivity to ABA, and influenced the expression of several genes associated with secondary metabolism, development, and ABA-related responses (**Palmeros-Suárez et al. 2015**). Our data showed that transgenic *Arabidopsis*seedlings expressing *ZmNF-YA12* had longer roots than WT plants when grown on 1/2 MS medium under mannitol, NaCl, and JA treatments (**Fig. 4**). Furthermore, seedlings of *ZmNF-YA12* transgenic plants grown in soil under drought and high NaCl conditions showed enhanced tolerance in comparison to WT plants (**Fig. 5A and Fig. 6A**). Several physiological and biochemical factors, such as MDA, proline, chlorophyll contents, and POD activity, play essential roles in plant tolerance to abiotic stresses. Proline plays a role as a compatible solute under conditions of environmental stress and contributes to the redox balance of the cell (**Lehmann et al. 2010**). MDA is the most frequently measured biomarker of oxidative stress, i.e., lipid peroxidation (**Tsikas 2017**). With drought treatment, transgenic *ZmNF-YA12* plants showed lower MDA content and higher proline content than WT controls (**Fig. 5D, 5E**). Therefore, we concluded that *ZmNF-YA12* transgenicplants have enhanced drought tolerance. Peroxiredoxins are thiol PODs with a variety of functions in the oxidation resistance and redox signaling networks of the cell (**Liebthal et al. 2018**). Leaf chlorophyll content

represents the photosynthetic capacity, and high oxidative stress inhibits its synthesis and accumulation (**Agathokleous et al. 2020**). The levels of POD activity in 35S:*ZmNF-YA12 Arabidopsis* were much higher than in WT plants under high NaCl conditions (**Fig. 6C**). However, there were no distinct differences in chlorophyll content between the transgenic and WT plants (**Fig. 6D**). These results indicated that *ZmNF-YA12* has a positive effect on salinity, osmotic, and drought stress responses in plants.

To investigate the mechanisms of action of *ZmNF-YA12* in stress responses, we examined the expression levels of stress-responsive genes. Previous studies showed that *RD29A* and *RD29B* can be induced by drought and salt stress, and responded to dehydration and ABA treatments (**Msanne et al. 2011; Nakashima et al. 2006**). In addition, *RD22* and *RAB18* are marker genes for ABA-induced gene expression and key nodes in ABA-responsive signaling networks (**Rushton et al. 2012; Yao et al. 2020**). In the present study, these four stress-related genes showed significantly elevated expression levels in the transgenic *ZmNF-YA12* compared to WT plants under drought and high NaCl treatments (**Fig. 7A, B, C, D**). These results indicated that *ZmNF-YA12* improves salt and drought tolerance by inducing the expression of stress-related genes in *Arabidopsis*.

Proteins interact with other proteins in complex network systems to perform their diverse and targeted functions (**Bhardwaj et al. 2016**). A previous study showed that the maize NF-Y family gene *NF-YA3* could interact with the JA activator MYC4 to improve drought and heat tolerance (**Su et al. 2018**). There have been no previous reports regarding the interactions of ZmNF-YA12 proteins in maize. In the present study, yeast two-hybrid experiments showed that ZmNF-YA12 interacted with ZmNF-YC1 and ZmNF-YC15. Our previous study showed that *ZmNF-YC15* was induced by drought stress (**Zhang et al. 2016**). Therefore, ZmNF-YA12 may respond to stress by interacting with ZmNF-YC15.

In conclusion, we cloned and characterized the NF-Y gene *ZmNF-YA12* from *Zea mays*. *ZmNF-YA12* was expressed at high levels in young roots and induced by abiotic stresses. Its heterologous expression conferred enhanced tolerance to drought and salt stress by regulating the expression of stress-related genes. The gene may also perform its diverse and targeted functions by interacting with ZmNF-YC1 and ZmNF-YC15. These results will be helpful to understand the roles of NF-Y in abiotic stress responses.

Declarations

Author contribution statement

ZZ, RY and ZW conceived and designed the experiments; TZ, DZ and CZ performed the experiments and analyzed the data; TZ wrote the manuscript and ZZ, RY and ZW revised and approved the publication.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Figures

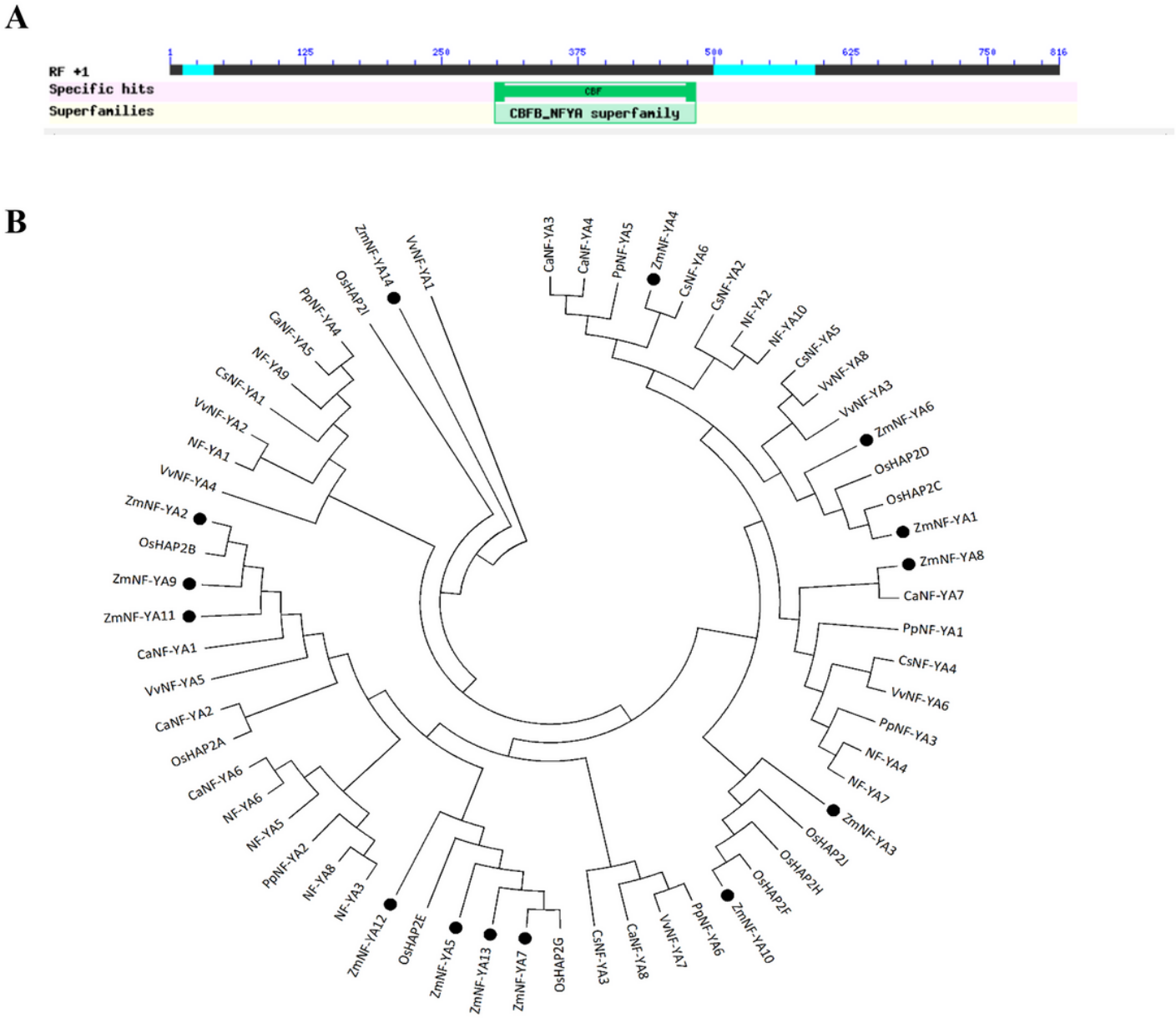

Figure 1

Bioinformatics analysis of the maize ZmNF-YA12 protein. A. Analysis of the ZmNF-YA12 conserved domain. B. Phylogenetic relationships of NF-YA family members in maize and other plants. All of the NF-YA family sequences were obtained from the NCBI database.

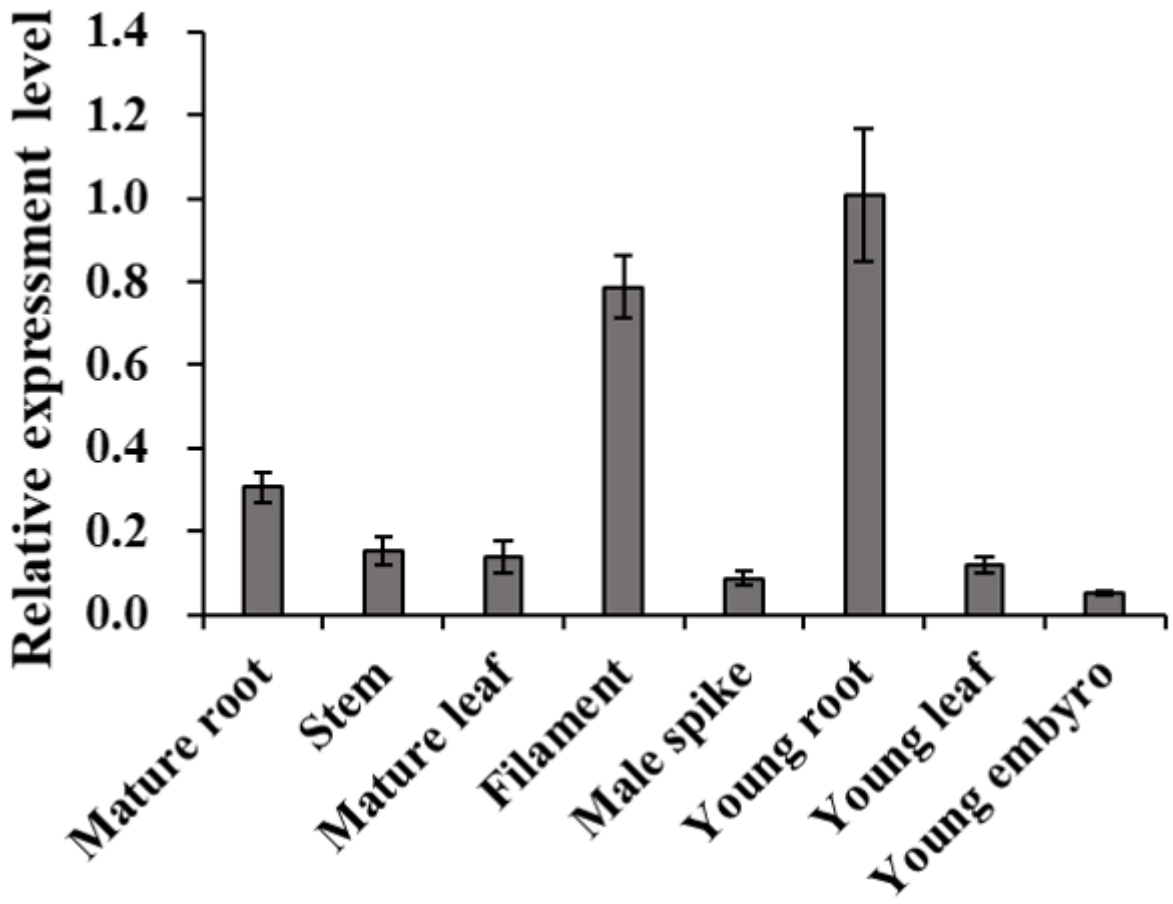

Figure 2

Tissue expression pattern of ZmNF-YA12 in maize. Total RNA was extracted from various tissues. GAPDH was used as an internal control. The vertical column shows the relative transcript level. Data are shown as the mean ± SEM (n = 3).

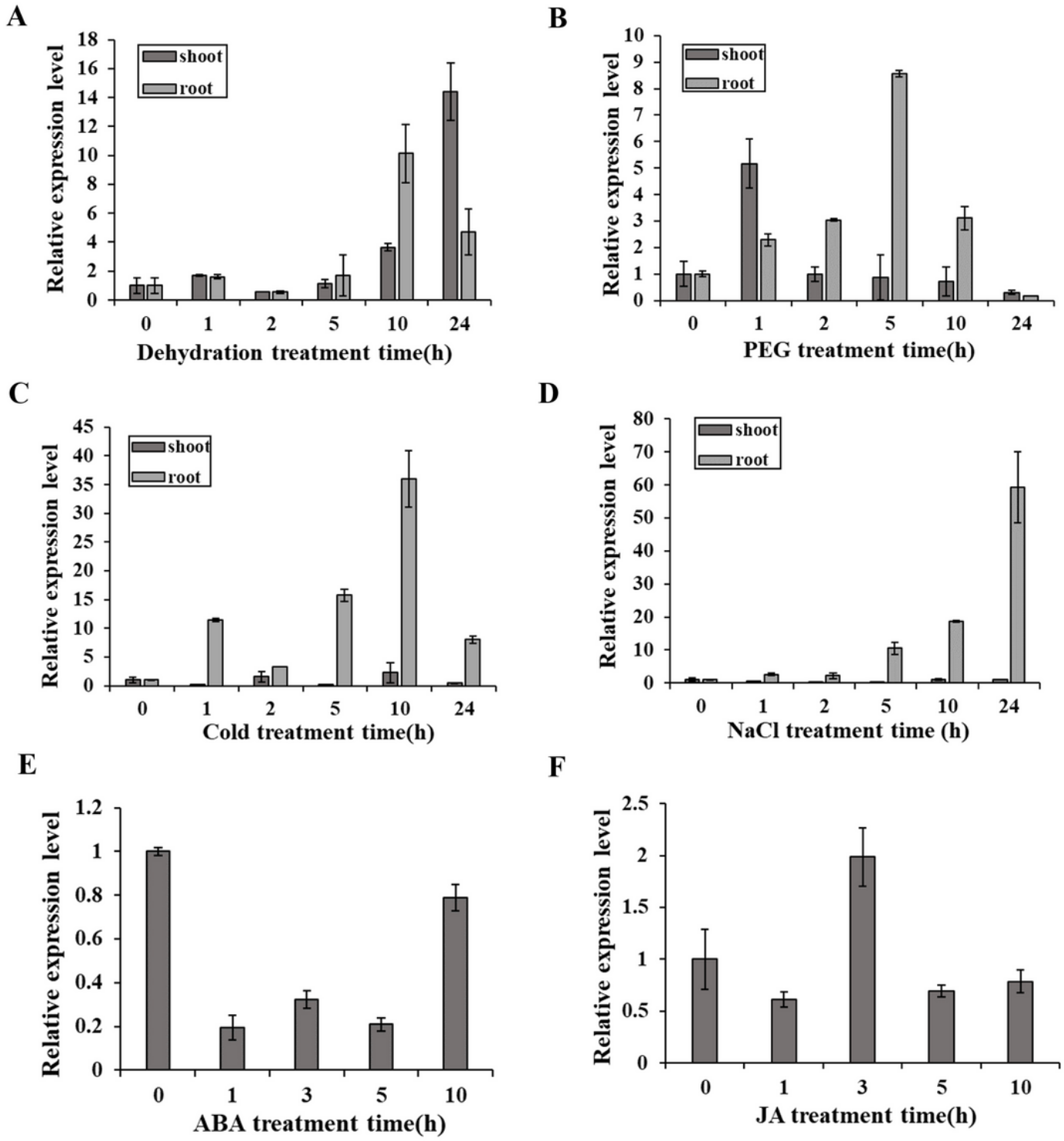

Figure 3

Expression of ZmNF-YA12 in response to abiotic and hormone stress in maize. A–F. ZmNF-YA12 expression under conditions of dehydration, PEG, cold, NaCl, ABA, and JA stress. GAPDH was used as an internal control. Data are shown as the mean ± SEM (n = 3).

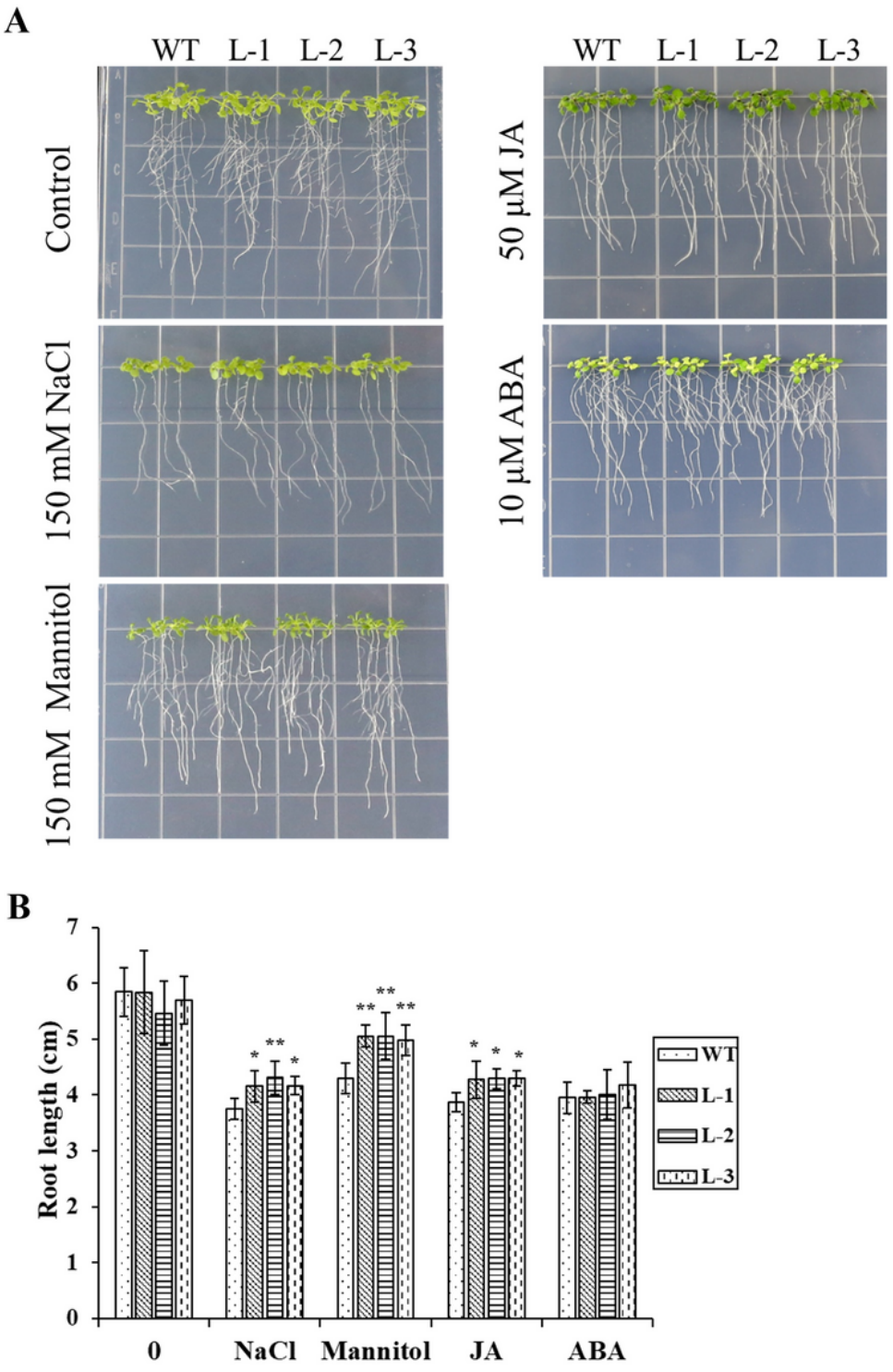

Figure 4

Phenotype analysis of WT and transgenic Arabidopsis lines under NaCl, mannitol, JA, and ABA treatments. A. Seedling development of transgenic and WT plants on 1/2 MS medium or 1/2 MS supplemented with 150 mM NaCl, 150 mM mannitol, 50 μM JA, or 10 μM ABA. B. Primary root length of plants grown on different media. Data represent the average root lengths. *p < 0.05; **p < 0.01 compared to the corresponding controls. Bar = 1.5 cm.

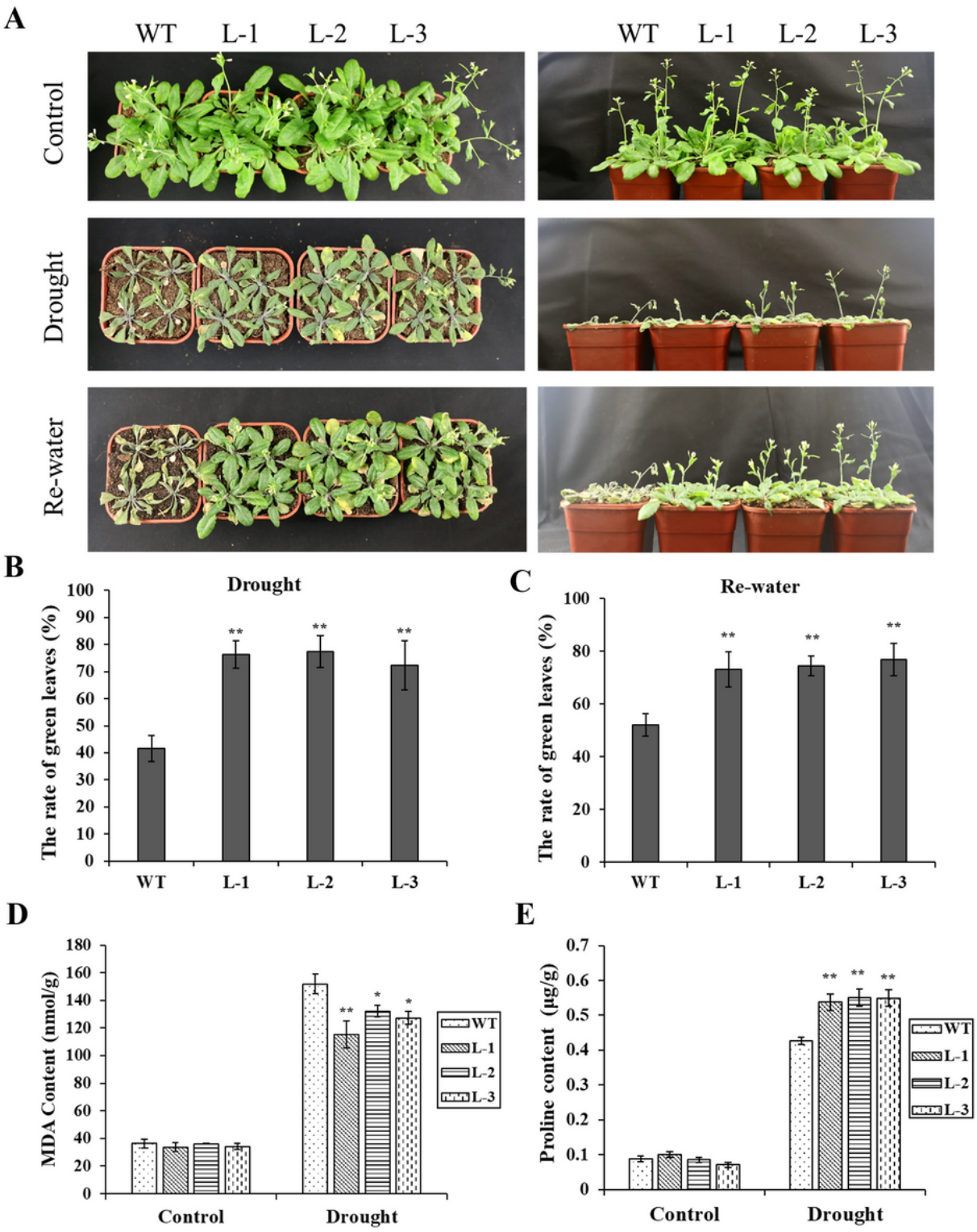

Figure 5

Improved drought tolerance in transgenic Arabidopsis plants expressing ZmNF-YA12. A. Assessment of drought tolerance in transgenic Arabidopsis plants. Healthy WT and 35S:ZmNF-YA12 plants were grown for 20 days with or without (control) water deficit, followed by re-watering for 2 days. B, C. The rates of green leaves in WT and transgenic lines under drought and re-watering treatments. D, E. Measurements of MDA and proline contents in transgenic lines and WT plants. *p < 0.05; **p < 0.01.

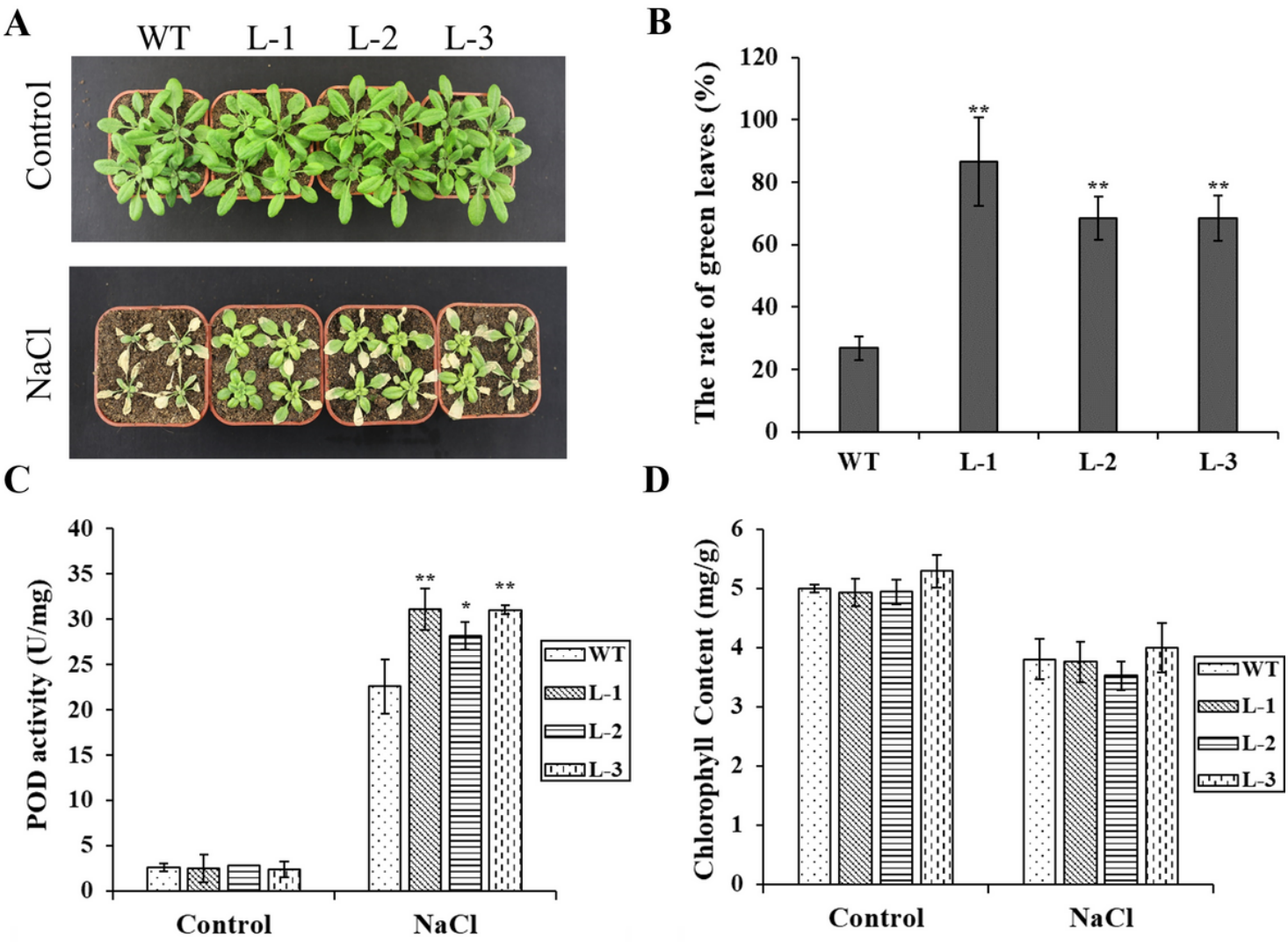

Figure 6

Enhanced salt tolerance of transgenic plants expressing ZmNF-YA12. A. Phenotypes of WT and transgenic seedlings in soil with or without (control) 450 mM NaCl treatment. Four-week-old transgenic and WT plants were irrigated with NaCl solution. Photographs were taken 7 days after treatment. B. Statistical analysis of green leaves of 35S:ZmNF-YA12 and WT plants. C, D. POD activity and chlorophyll content were measured in transgenic and WT plants. *$p < 0.05$; **$p < 0.01$ compared to the corresponding controls.

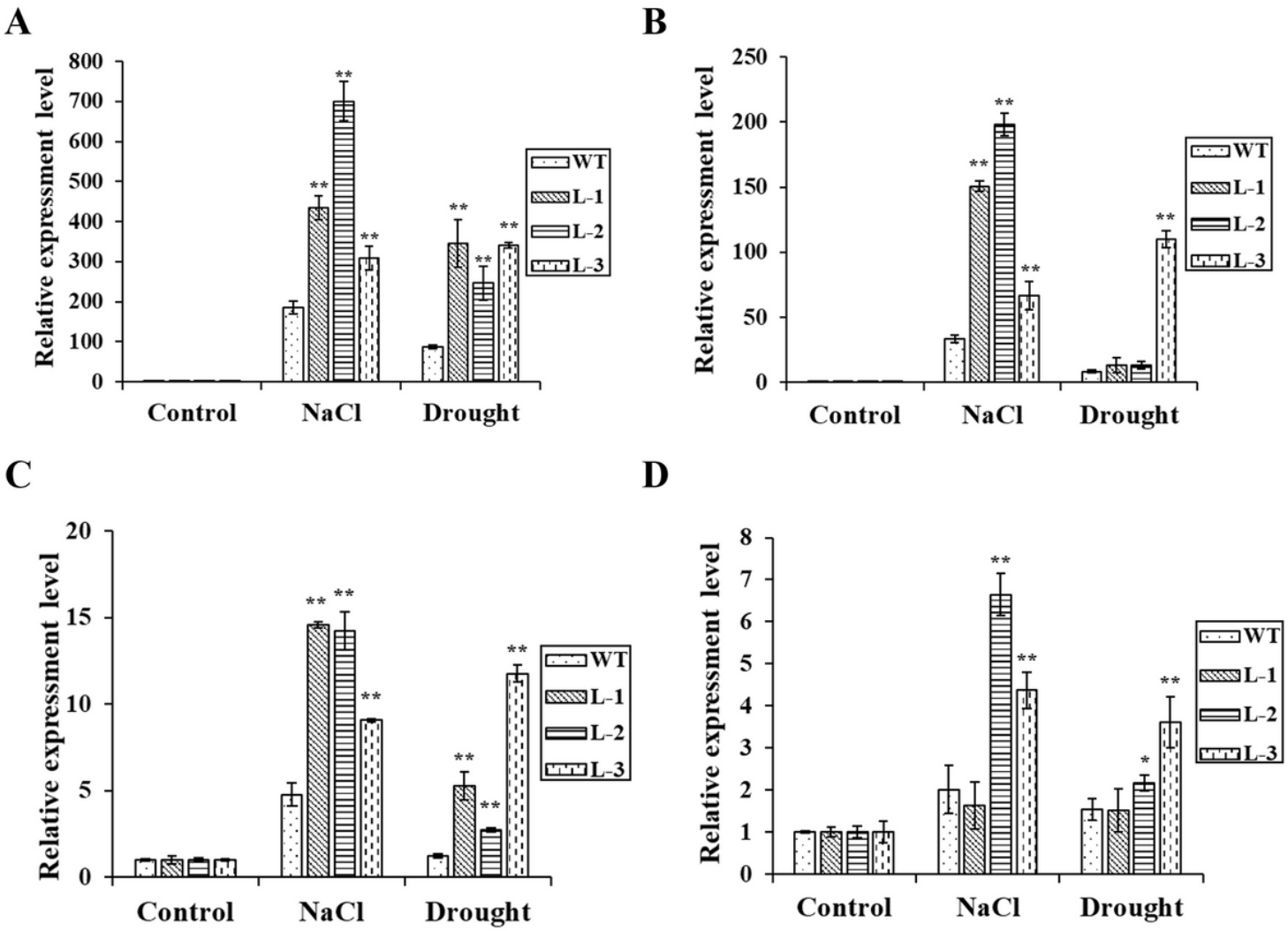

Figure 7

RD29A, RD29B, RAB18, and RD22 transcript levels in WT and ZmNF-YA12 transgenic plants under three growth conditions. Actin2 was used as an internal control. Data represent the mean ± SEM (n = 3). *p < 0.05; **p < 0.01.

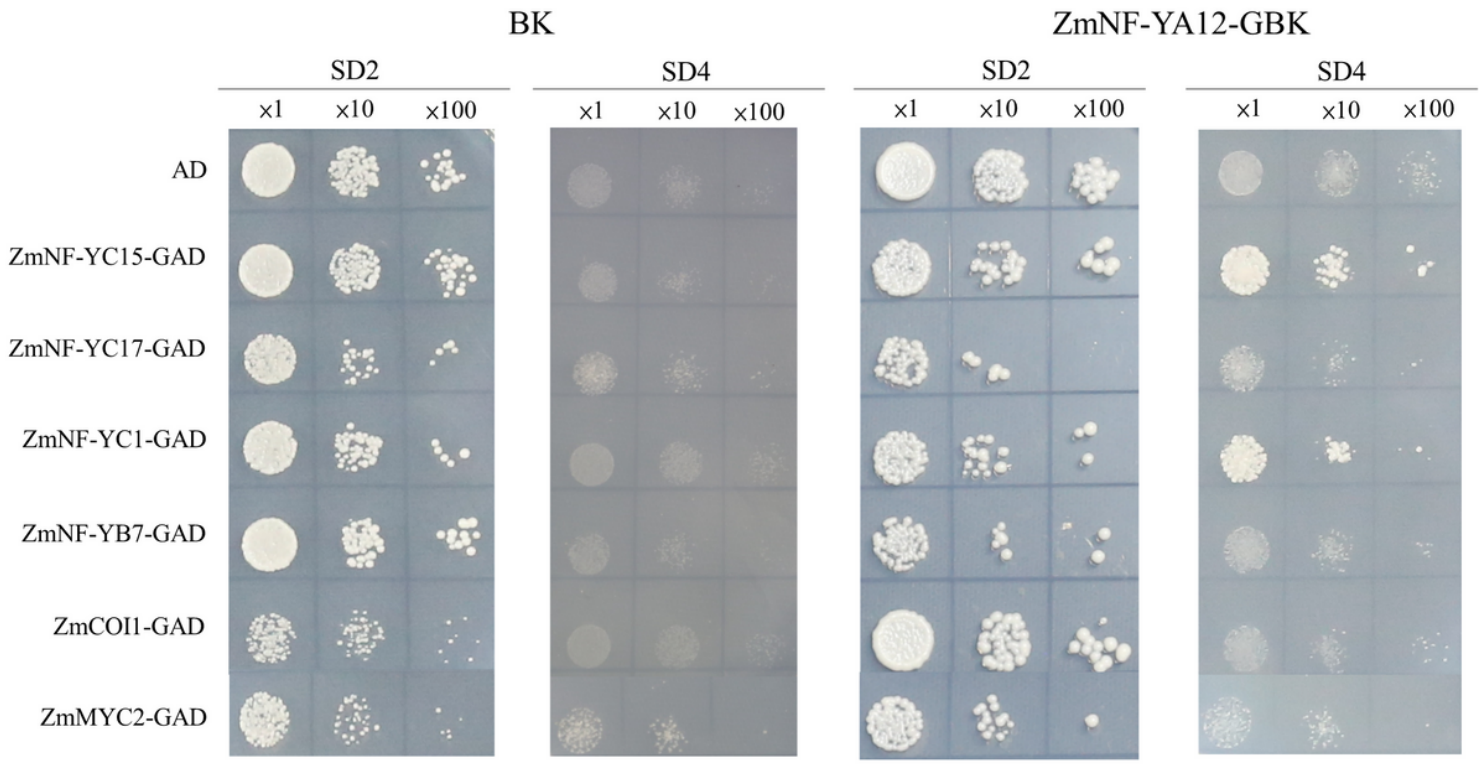

Figure 8

Protein interaction assays between ZmNF-YA12 and candidate genes.

Supplementary Files

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- SupplementaryFigures.docx