The *Chrysanthemum lavandulifolium* *CINAC9* Gene Positively Regulates Saline, Alkaline, and Drought Stress in Transgenic *Chrysanthemum grandiflora*

Yu Liu¹ and Miao He¹

College of Landscape Architecture, Northeast Forestry University, Harbin 150040, China

Fengli Dong

College of Landscape Architecture, Northeast Forestry University, Harbin 150040, China; and Heilongjiang Ecological Engineering Training College, Harbin 150025, China

Yingjie Cai, Wenjie Gao, and Yunwei Zhou²

College of Landscape Architecture, Northeast Forestry University, Harbin 150040, China

He Huang and Silan Dai²

College of Landscape Architecture, Beijing Forestry University, Beijing 100083, China

ADDITIONAL INDEX WORDS. genetic transformation, overexpression, abiotic stress, physiology index, Northern blot

ABSTRACT. The NAC transcription factor is a peculiar kind of transcription factor in plants. Transcription factors are involved in the expression of plant genes under different conditions, and they play a crucial role in plant response to various biotic and abiotic stress. We transferred the *CINAC9* gene into *Chrysanthemum grandiflora* ‘niu9717’ by *Agrobacterium tumefaciens*-mediated transformation. The results of kanamycin-resistant screening, polymerase chain reaction (PCR) detection, and Northern blot analysis proved that the target gene had been integrated into the genome of the target plants. Wild-type (WT) plants and transgenic plants were treated with different concentrations of NaCl, NaHCO₃, and drought stress, and physiological indexes, such as antioxidant system activity (superoxide dismutase, peroxidase, catalase), malondialdehyde accumulation, and leaf relative water content, were measured. We also observed changes in plant morphology. The physiological indexes’ changing range and extreme values suggested that transgenic plants’ resistance to salinity, alkali, and drought stress was significantly higher than WT plants. Transgenic plant growth was less inhibited compared with WT plants, indicating that the *CINAC9* gene increased the resistance of transgenic plants under the stress of salinization, alkalization, and drought.

Drought, salinity, and other abiotic stresses have serious effects on plant photosynthesis, respiration, and the entire growth and development process. The ways and methods of plants dealing with various abiotic stresses in the environment are complex and orderly, and transcription factor regulation is an important method (Singh et al., 2002). Many transcriptomics studies have shown that regulating genes, especially those encoding transcription factors, such as *C2H2*, *WRKY*, *bZIP*, *MYB*, *SBP*, *HB*, *DREB*, *AP2/EREBP*, and *NAC*, are upregulated significantly after plants undergo adversity. The NAC transcription factor has multiple plant-specific biological functions and plays an important role in many aspects of plant growth and development as well as in biological and abiotic stress responses (Olsen et al., 2005). In 2003, NAC genes from wheat (*Triticum aestivum*), maize (*Zea mays*), *Arabidopsis thaliana*, and rice (*Oryza sativa*) were divided into two groups, Group I and Group II, by homologous evolution analysis (Ooka et al., 2003). In 2012, the NAC transcription factor was further divided into six functional groups by system evolution analysis: NAM/CUC3, SND, TIP, SNAC, ANAC034, and ONAC4

(Nakashima et al., 2012). In the genome-wide expression analysis of *A. thaliana* and rice, it was found that the 20% to 25% genes of NAC were induced by some kind of abiotic stress, such as salt, drought, cold, and abscisic acid (ABA), as well as plant hormones (Fang et al., 2008; Fujita et al., 2004; Nuruzzaman et al., 2012). By plant chip analysis, there were more than 45 NAC genes in rice induced by abiotic stress and 26 genes responding to biological stress. For example, the *CINAC* gene responds to drought, salt, low-temperature, high-temperature, and other abiotic stress (Huang et al., 2012), and the *ANAC092* transcription factor in *A. thaliana* responds to salt stress by promoting plant senescence (Balazadeh et al., 2010). Northern blot analysis showed that the expression of the *PeNAC1* gene was strongly induced by drought and salt stress. After the *PeNAC1* gene of *Populus euphratica* was overexpressed in *A. thaliana*, the ratio of Na⁺/K⁺ in roots and leaves was low, and the expression level of the *AtHKT1* gene was significantly inhibited, which enhanced the resistance of *A. thaliana* to salt stress (Wang and Dane, 2013). Overexpression of the *TaNAC2* homolog gene *TaNAC2a* in tobacco (*Nicotiana tabacum*) proved that *TaNAC2a* could enhance tolerance to drought in tobacco (Tang et al., 2012). The Rosa *RhNAC3* transcription factor is involved in response to drought stress in rose (*Rosa hybrida*) and participates in the stress response through an ABA-dependent regulatory pathway (Jiang et al., 2014). *BraNAC* responds to high-temperature and low-temperature stress (Ma et al., 2014), and *GhNAC8-GhNAC17* responds to

Received for publication 28 Mar. 2019. Accepted for publication 20 May 2019. This research was supported by the National Natural Science Foundation of China (31870687) and the Fundamental Research Funds for the Central Universities (2572017EA04).

¹These authors contributed equally.

²Corresponding authors. E-mail: dlzhyw@nefu.edu.cn or silandai@sina.com.

Fig. 1. Transgenes of *Chrysanthemum grandiflora*. (A) Resistant callus, (B, C) resistant shoots, (D) screening resistance of seedlings, (E, F) screening resistance of seedling root.

Fig. 2. Polymerase chain reaction analysis of *CINAC9* fragments in transgenic *Chrysanthemum grandiflora* plants: M = DL2000, 1 = positive control of pBI121-*CINAC9* plasmid, 2 = negative control of untransgenic line, A1–15 = different *CINAC9* transgenic lines. Bp = base pair.

abiotic stress, such as ABA, drought, salinity, and high or low temperature (Shah et al., 2013).

C. grandiflora has characteristics such as scrubby plants, long flowering period, rich color, abiotic and biotic resistance, and easy cultivation. It is an important groundcover plant material for landscape construction in north China and northeast China. To improve the adaptability of *C. grandiflora*, broadening the application area, especially for use in greening dry and barren areas, is a very meaningful breeding direction. In this study, the *CINAC9* gene was transferred into *C. grandiflora* 'niu9717' by the *A. tumefaciens*-mediated method, and corresponding resistance was studied by subjecting it to drought, saline, and alkali stress. This study provides a theoretical basis for expanding the scope of *C. grandiflora* in landscape architecture, thus enriching plant resources for landscape architecture in cities.

Materials and Methods

Materials

The *C. grandiflora* 'niu9717' was cultured in the plant cultivation room in the College of Landscape Architecture in Northeast Forestry University (Haerbin, China). The *C. grandiflora* 'niu9717' fully developed young leaves under the apical bud were used as explants and cut into 0.5×0.5 -cm squares for

genetic transformation studies. *A. tumefaciens* GV3101 was obtained from the Beijing Forestry University Landscape Architecture School (Beijing, China). The transgenic vector for overexpression of *CINAC9* *A. tumefaciens* culture medium luria-bertani (LB) (pH 7.0) contained $10 \text{ g}\cdot\text{L}^{-1}$ peptone, $5 \text{ g}\cdot\text{L}^{-1}$ yeast extract, and $10 \text{ g}\cdot\text{L}^{-1}$ NaCl.

Genetic transformation of *C. GRANDIFLORA*

The genetic transformation of *C. grandiflora* refers to the method of Liu (2015). After kanamycin sensitivity tests, selection for plant rooting pressure, precultivation, *A. tumefaciens* infection, cocultivation, and delayed cultivation, the resistant seedlings obtained were cultivated in medium containing kanamycin at $10 \text{ mg}\cdot\text{L}^{-1}$ Murashige and Skoog (Qingda Hope Bio-Technology Co., Qingdao, China) based on rooting screening integrant and PCR detection. Three positive strains (*CINAC9-5*, *CINAC9-6*, and *CINAC9-13*) were selected for further Northern blot detection (Li et al., 2007).

Salt and alkali tolerance of transgenic plants

When the root lengths of *CINAC9-5*, *CINAC9-6*, and *CINAC9-13* strains and WT plants were ≈ 1.5 cm long, they were transplanted to peat and vermiculite at a ratio of 1:1 in well-mixed cultures. The cultures were placed in an artificial climate chamber under a 16/16-h light/dark cycle, with light intensity of $120 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, temperature of $24/18^\circ\text{C}$ (day/night), and salt stress and alkali stress treatments were performed after 40 d. Concentrations of 100, 200, or $300 \text{ mmol}\cdot\text{L}^{-1}$ of NaCl salt solution and 50, 100, or $150 \text{ mmol}\cdot\text{L}^{-1}$ of NaHCO_3 base solution were used. Sterile water was used as the control, and plants were irrigated every 5 d. Salt stress was applied five times per day, and alkali stress was applied four times per day. Samples were taken after treatment, and relative water content (RWC), cell membrane permeability, malondialdehyde (MDA) content, superoxide dismutase (SOD) activity, peroxidase (POD) activity, catalase (CAT) activity, and other physiological indexes were detected in plants after salt stress. These physiological indexes were determined following the methods of Zhang et al. (2012). Proline content (Wang et al., 2016b), soluble protein content (Sami et al., 2015), MDA content, and antioxidant enzyme system (SOD, POD, CAT) activity were detected in plants after alkaline stress.

Detection of drought tolerance in transgenic plants

When the root lengths of *CINAC9-5*, *CINAC9-6*, and *CINAC9-13* strains and WT plants were ≈ 1.5 cm long, they were transplanted to peat soil and vermiculite at a ratio of 1:1 in

Fig. 3. Northern blot analysis of transgenic *Chrysanthemum grandiflora* plants: WT = wild type; A5, A6, A13 = *CINAC9* transgenic lines *CINAC9-5*, *CINAC9-6*, and *CINAC9-13*.

Fig. 4. Effects of different concentrations of salt stress on morphology of *Chrysanthemum grandiflora*: (A–D) Wild-type (WT) and transgenic plants grown under 0, 100, 200, and 300 mmol·L⁻¹ NaCl concentration.

well-mixed cultures. The cultures were placed in a culture room and drought stress treatments were performed after 40 d, applied by withholding watering of the plants in the culture room. The water stress treatment times of 5, 10, and 15 d were set, and the samples were taken at each time point. Treatment with no water stress (0 d) served as a control. After treatment, the RWC and chlorophyll content followed the method of Wang et al. (2015). MDA content, and antioxidant defense enzyme system (SOD, POD, CAT) activity indexes were detected.

Statistical methods

A one-way analysis of variance followed by Duncan's multiple range test ($P = 0.05$) was used to test if treatment

means differed statistically from one another. Excel (2007; Microsoft, Redmond, WA) and SPSS software (version 19.0J; IBM Corp., Armonk, NY) were used for all the statistical analyses. Three biological replicates were used for each analysis.

Results

Establishment of genetic transformation system for *C. grandiflora*

Through an *A. tumefaciens*-mediated method, an efficient genetic transformation system was established for 'niu9717'. Kanamycin at 10 mg·L⁻¹ is the best antibiotic screening concentration for leaves, and 8 mg·L⁻¹ is the best choice for rooting transformed plants; leaf preculture time is 2 to 3 d; if optical density at 600 nm (OD600) is ≈ 0.6 , the infection time is 15 min; if OD600 is ≈ 0.8 , the infection time is 10 min; and the coculture time is 2 d or a delayed cultivation of 3 d. Plants with kanamycin resistance were obtained (Fig. 1).

PCR identification and Northern blot analysis of transgenic plants

The resistant seedlings were further rooted and screened on the rooting medium containing kanamycin at 10 mg·L⁻¹. Finally, 15 transgenic *CINAC9* resistant seedlings were obtained and numbered A1 to 15. The genomic deoxyribonucleic acid (DNA) of the transgenic resistant seedlings was extracted, and the extracted genomic DNA was used as a template for PCR identification using *CINAC9* gene-specific primers. The results showed that a *CINAC9* gene-specific fragment whose length is the same as the positive control (≈ 650 base pairs) was amplified among the 15 detected strains of *CINAC9* transgenic resistant plants. There were no bands in the negative control plants without the transgene, so

that it can be inferred that the *CINAC9* gene has been integrated into the 'niu 9717' genome (Fig. 2). Northern blotting was performed using total ribonucleic acid (RNA) from *CINAC9-5*, *CINAC9-6*, *CINAC9-13*, and WT leaves. There were expression signals at the level of messenger RNA among transgenic strains, but no hybridization signal in the control WT group (Fig. 3). These results indicated that the *CINAC9* gene was expressed at the transcriptional level.

Salt tolerance of transgenic *C. grandiflora*

SALT STRESS PHENOTYPE. The effect of different concentrations of NaCl solution on the morphology of plants under 20 d of salt stress is shown in Fig. 4. Under mild salt stress (100

Fig. 5. Effects of different concentrations of salt stress on relative water content, relative electric conductivity, malondialdehyde (MDA) accumulation and superoxide dismutase (SOD), peroxidase (POD), and catalase activity (CAT) in leaves of *Chrysanthemum grandiflora*. Different letters indicate significant differences ($P < 0.05$). FW = fresh weight.

mmol·L⁻¹), WT plants showed symptoms of slight harm, and the top leaves of some plants were slightly yellowed. The morphology of transgenic plants was nearly unchanged. Under moderate stress (200 mmol·L⁻¹), all the plants showed different degrees of harm, and the WT plants were the most seriously affected. The shoot tip was withered, the leaves yellowed, and it was dry and drooping. It only had the ability to maintain vitality. The *CINAC9-5* strain was the lightest in the transgenic lines, and the *CINAC9-6* and *CINAC9-13* strains changed significantly. The *CINAC9-5* strain was the most susceptible strain of the transgenic lines, whereas *CINAC9-6* and *CINAC9-13* changed significantly. Leaves yellowed severely and stems withered, but to a lesser extent than WT plants; when severely

stressed (300 mmol·L⁻¹), the above-ground parts of the WT plants were all wilted, and individual chlorosis was observed in individual parts of the *CINAC9-6* and *CINAC9-13* plants. Although the *CINAC9-5* strain was not completely wilted, it was severely affected. The overall trend showed that the three transgenic lines were significantly more resistant to salt stress than WT plants, but when the salt stress reached a high concentration of stress (300 mmol·L⁻¹), all of the open pitted chrysanthemums were unable to tolerate the stress.

ANALYSIS OF PHYSIOLOGICAL INDEXES OF TRANSGENIC PLANTS UNDER SALT STRESS. As shown in Fig. 5, the effects of NaCl salt stress for 20 d on the RWC of transgenic and WT plants showed a decreasing trend with the increase in stress concentration.

WT CINAC9-5 CINAC9-6 CINAC9-13

WT CINAC9-5 CINAC9-6 CINAC9-13

Fig. 6. Effects of different concentrations of alkali stress on morphology of *Chrysanthemum grandiflora*: (A–D) wild-type (WT) and transgenic plants grown under 0, 50, 100, and 150 mmol·L⁻¹ NaHCO₃ concentration.

With the increase in stress, MDA and the relative conductivity of transgenic and WT plants showed an upward trend, but the rate of increase was reduced significantly in transgenic lines. The SOD activity of WT and transgenic lines was basically the same without stress, and the activity of POD and CAT in the transgenic lines was slightly higher than WT plants. With the increase in salt stress, the activity of SOD, POD, and CAT in three transgenic lines and WT plants all tended to increase and then decrease. Under mild salt stress, transgenic plants and WT plants showed a significant increase in CAT activity and reached the highest value. SOD and POD peaked at moderate stress, followed by a downward trend. Under severe stress, SOD, POD, and CAT did not resist the salt stress; although the transgenic lines also decreased, they were still higher than the WT plants.

Alkali tolerance of the transgenic *C. grandiflora*

ALKALI STRESS PHENOTYPE. The effect of different concentrations of alkali solution on the morphology of *C. grandiflora* at 15 d is shown in Fig. 6. Under mild stress (50 mmol·L⁻¹), WT plants and the three transgenic lines showed no obvious change, indicating that slight alkali stress did not affect the growth of *C. grandiflora*. Under moderate stress (100 mmol·L⁻¹), the WT plants showed severe leaf chlorosis and wilt sagging, while some of the leaves were withered at the edge of the leaves. The transgenic lines also had yellow leaves, and some dry sagging plant stems appeared in all the lines, but the extent of harm was significantly less than in WT plants. Under severe stress (150 mmol·L⁻¹), the WT plants could not withstand the stress. Most

of the plants wilted in the aerial region, whereas most of the plants showed wilting throughout the entire plant, but the leaves wilted seriously. Transgenic lines also showed severe stress, with leaves seriously chlorotic, suffering water loss and stem tip drooping but remaining intact.

ANALYSIS OF PHYSIOLOGICAL INDEXES OF TRANSGENIC PLANTS UNDER ALKALI STRESS. The effects on proline content, soluble protein content, MDA content, and antioxidant protective enzyme system of transgenic and WT *C. grandiflora* leaves after NaHCO₃ alkali stress for 15 d changed as the stress increased (Fig. 7). The proline content of transgenic lines and WT plants was almost the same without stress, and the content of soluble protein and MDA in transgenic lines was significantly higher than in WT plants. With the severity of stress, the proline content and MDA content in transgenic lines continued to increase, but the soluble protein content, SOD, POD, and CAT activity increased and then decreased, and they were higher than in WT plants. At the late stage of stress, the soluble protein content, SOD, POD,

and CAT activities of *CINAC9-13* transgenic lines were lower than those in *CINAC9-5* lines. The proline content and MDA content of WT plants tended to increase continuously, and the content of soluble protein and the activities of the antioxidant protective enzyme system increased at first and then decreased. Comprehensive analysis showed that transgenic plants increased alkali resistance.

Drought tolerance of transgenic *C. grandiflora*

DROUGHT STRESS PHENOTYPE. During drought stress, the morphological changes in *CINAC9-5*, *CINAC9-6*, and *CINAC9-13* transgenic lines and WT were observed and photographed. Figure 8 shows the morphological changes of *C. grandiflora* under drought stress at 0, 5, 10, and 15 d. With the prolongation of drought stress, the WT plants wilted faster than the transgenic plants under the conditions of water loss. WT plants began to lose water and to yellow after 10 d of stress, and the external morphology of the transgenic lines began to change slightly. Under stress at 15 d, the leaves of WT seedlings had severe dehydration, and the external appearance began to turn yellow, curly and drooping, whereas the transgenic lines began to dehydrate and wilt. The *CINAC9-13* line was more dehydrated but remained active. These observations indicate that the transgenic lines were significantly improved compared with the WT in terms of drought resistance; from the perspective of morphological changes, the transgenic lines *CINAC9-5* and *CINAC9-6* have strong drought resistance.

ANALYSIS OF PHYSIOLOGICAL INDEXES OF TRANSGENIC PLANTS UNDER DROUGHT STRESS. The changes in RWC, chlorophyll

Fig. 7. Effects of different concentrations of alkali stress on proline content, soluble protein content, malondialdehyde (MDA) accumulation and superoxide dismutase (SOD), peroxidase (POD), and catalase activity (CAT) in leaves of *Chrysanthemum grandiflora*. Different letters indicate significant differences ($P < 0.05$). FW = fresh weight.

content, MDA content, and antioxidant protective enzyme system of the leaves of transgenic and WT *C. grandiflora* under drought stress for 15 d are shown in Fig. 9. With the extension of drought conditions, the RWC and chlorophyll content of WT leaves continued to decrease and gradually lowered compared with the transgenic lines. The content of MDA increased steadily and was always higher than that of the transgenic lines. The activity of the antioxidant protective enzyme system was increased at first and then decreased, and it was lower than that of transgenic plants at the late stage of stress. The content of MDA in the transgenic lines kept increasing, while the chlorophyll content, SOD, POD, and CAT activity increased and then decreased. The transgenic

lines *CINAC9-5* and *CINAC9-6* showed similar changes, indicating strong drought tolerance. The results showed that transgenic *CINAC9* plants had stronger drought resistance than WT plants.

Discussion

Genetic transformation commonly uses antibiotics as a selection marker, with different varieties of antibiotic susceptibility screening. Selecting the correct antibiotics and determining the critical screening concentration of antibiotics is the key to successful genetic transformation. The toxicity of different antibiotics to *C. grandiflora* were chloramphenicol >

Fig. 8. Effects of different times of drought stress on morphology of *Chrysanthemum grandiflora*: (A–D) wild-type (WT) and transgenic plants grown under 0, 5, 10, and 15 d of drought stress.

rifampin > streptomycin > minomycin > ampicillin > antimicrobial. This research adopts the plant binary expression vector pBI121 containing the neomycin phosphotransferase gene, which produces kanamycin resistance in transformed cells. The effects of different concentrations of kanamycin on the induction of adventitious buds in vitro were studied. The results showed that *C. grandiflora* 'niu 9717' was sensitive to kanamycin. On the differentiation medium supplemented with kanamycin, the explants of leaves gradually became yellow or withered and died due to the increase in the concentration without any differentiation, and the lethality was obvious. In this study, resistant plants were obtained when screened by rooting. Negative reaction plants were identified by PCR, indicating that pseudotransformants were present in the study. According to the causes of pseudotransformation (Li et al., 2012), appropriate preventive measures include increasing the contact area of the transformation receptor and screening the medium in the process of transformation. The selection pressure in time is added, and after obtaining resistant buds, some methods were used, such as gradually increasing the selective pressure to reduce the occurrence of false positive seedlings. In this study, transgenic plants were detected by PCR and Northern blot analysis. The results showed that the *CINAC9*

gene was introduced and integrated into the genome of *C. grandiflora*.

The NAC transcription factor has a great response to different stress conditions (Wang et al., 2013). Many members of the NAC gene family have differential expression characteristics in their response to abiotic stresses. In this experiment, salt, alkali, and drought stress were measured, and the physiological indexes were measured after the transformation of the *CINAC9* gene. The relative conductivity of WT plants increased more than that of transgenic plants, and the damage to the cell membrane system of WT plants was more severe, consistent with the experimental results of Zhou et al. (2013). Under salt, alkali, and drought stress, the water content of WT plants decreased much more than in the transgenic lines. The activities of SOD, POD, and CAT in *C. grandiflora* increased with the prolongation in stress time and concentration and then decreased, and the activity in transgenic lines was higher than in WT plants. However, the content of MDA in WT plants showed a more significant increasing trend, indicating that the membrane peroxidation persists and increases continuously under stress, causing damage to plants. The change trend in enzyme activity in the protective enzyme system was consistent with that of Wang et al. (2016a) and An et al. (2016). With the

Fig. 9. Effects of different times of drought stress on relative water content, chlorophyll content, malondialdehyde (MDA) accumulation and superoxide dismutase (SOD), peroxidase (POD), and catalase activity (CAT) in leaves of *Chrysanthemum grandiflora*. Different letters indicate significant differences ($P < 0.05$). FW = fresh weight.

increase in alkali stress concentration, the proline content of the transgenic lines was slightly higher than that of WT plants. The content of soluble protein showed an upward trend under mild stress, indicating that the synthesized rate of soluble proteins increases and then declines under short-term and low-concentration stress. However, the transgenic lines were still slightly higher than the WT plants, indicating that introducing the *CINAC9* gene into *C. grandiflora* enhances the ability to maintain protein synthesis and decomposition ability. The contents of proline and soluble protein in the three transgenic lines did not appear to be significantly higher than in WT plants. It is speculated that the transfer of the *CINAC9* gene does not increase the tolerance of *C. grandiflora* to alkali

stress by increasing the content of free proline and soluble protein, and the specific reason remains to be further studied.

In late drought stress, the content of chlorophyll in WT and transgenic lines was decreased, but the chlorophyll content in transgenic lines was higher than in the WT. In addition, the chlorophyll content of the WT was more sensitive to the loss of water, and the recovery ability was also significantly weaker than the transgenic lines. Studies by Hao et al. (2011) demonstrated that the soybean *GmNAC20* transcription factor can regulate stress tolerance by activating the DREB/CBF-COR pathway, and its expression in transgenic *A. thaliana* enhances tolerance to salt and cold stress. *GmNAC11* may enhance the salt tolerance of transgenic *A. thaliana* by

regulating *DREB1A* and other stress-related genes (Hao et al., 2011). The *ONAC045* transcription factor is an important regulator of drought resistance in rice. Transient expression in onion epidermal cells indicated that the *ONAC045* protein was localized in the nucleus and in transgenic rice overexpressing *ONAC045*. The results showed that two stress-related genes are expressed, enhancing the tolerance of plants to drought stress (Zhang et al., 2009). In addition, other studies have shown that overexpression of the *OsNAC10* gene can significantly enhance the tolerance of rice to drought, high salt, and low temperature at the vegetative stage; and in the reproductive growth stage, the yield of rice can be increased by 25% to 42% under drought conditions or 5% to 14% under normal conditions (Jeong et al., 2010). The resistance of three transgenic lines to salinity, alkali, and drought stress were also not completely consistent. The *CINAC9-5* strain showed the most superior performance and the strongest resistance, whereas the *CINAC9-6* and *CINAC9-13* strains had stronger salt, alkaline, and drought stress resistance than the WT but weaker than the *CINAC9-5* strains. The same gene was transferred into the same plant, but the phenotypes of different strains are not completely consistent. The expression levels of transferred genes in different strains need further study.

Literature Cited

- An, Y.M., L.L. Song, Y.R. Liu, Y.J. Shu, and C.H. Guo. 2016. De novo transcriptional analysis of Alfalfa in response to saline-alkaline stress. *Front. Plant Sci.* 7:931.
- Balazadeh, S., H. Siddiqui, A.D. Allu, L.P. Matallana-Ramirez, C. Caldana, M. Mehrnia, M.I. Zanor, B. Köhler, and B. Mueller-Roeber. 2010. A gene regulatory network controlled by the NAC transcription factor ANAC092/AtNAC2/ORE1 during salt-promoted senescence. *Plant J.* 62:250–264.
- Fang, Y., J. You, K. Xie, W. Xie, and L. Xiong. 2008. Systematic sequence analysis and identification of tissue-specific or stress-responsive genes of NAC transcription factor family in rice. *Mol. Genet. Genomics* 280:547–563.
- Fujita, M., Y. Fujita, K. Maruyama, M. Seki, K. Hiratsu, M. Ohme-Takagi, L.P. Tran, K. Yamaguchi-Shinozaki, and K. Shinozaki. 2004. A dehydration-induced NAC protein, RD26, is involved in a novel ABA-dependent stress-signaling pathway. *Plant J.* 39:863–876.
- Hao, Y.J., W. Wei, Q.X. Song, H.W. Chen, Y.Q. Zhang, F. Wang, H.F. Zhou, G. Lei, A.G. Tian, W.K. Zhang, B. Ma, J.S. Zhang, and S.Y. Chen. 2011. Soybean NAC transcription factors promote abiotic stress tolerance and lateral root formation in transgenic plants. *Plant J.* 68:302–313.
- Huang, H., Y. Wang, S.L. Wang, X. Wu, K. Yang, Y.J. Niu, and S.L. Dai. 2012. Transcriptome-wide survey and expression analysis of stress-responsive NAC genes in *Chrysanthemum lavandulifolium*. *Plant Sci.* 193/194:18–27.
- Jeong, J.S., Y.S. Kim, K.H. Baek, J. Jung, S.H. Ha, D.C. Yang, M. Kim, C. Reuzeau, and J.K. Kim. 2010. Root-specific expression of *OsNAC10* improves drought tolerance and grain yield in rice under field drought conditions. *Plant Physiol.* 153:185–197.
- Jiang, G., X. Jiang, P. Lü, J. Liu, J. Gao, and C. Zhang. 2014. The Rose (*Rosa hybrida*) NAC transcription factor 3 gene, *RhNAC3*, involved in ABA signaling pathway both in rose and arabidopsis. *PLoS One* 9:e109415.
- Li, J.R., X.G. Ye, B.Y. An, L.P. Du, and H.J. Xu. 2012. Genetic transformation of wheat: Current status and future prospects. *Plant Biotechnol. Rpt.* 6:183–193.
- Li, Y.H., J.H. Liu, Q.J. Xu, and Z.R. Xu. 2007. Modern molecular biology module experimental guide. Higher Educ. Press, Beijing, China.
- Liu, C.X. 2015. Transferring *CICBF4* into *Chrysanthemum grandiflora* 'ZiYan' mediated by *Agrobacterium tumefaciens*. Northeast Forestry Univ., Heilongjiang, China, MA Thesis.
- Ma, J., F. Wang, M.Y. Li, Q. Jiang, G.F. Tan, and A.S. Xiong. 2014. Genome wide analysis of the NAC transcription factor family in Chinese cabbage to elucidate responses to temperature stress. *Scientia Hort.* 165:82–90.
- Nakashima, K., H. Takasaki, J. Mizoi, K. Shinozaki, and K. Yamaguchi-Shinozaki. 2012. NAC transcription factors in plant abiotic stress responses. *Biochim. Biophys. Acta* 1819:97–103.
- Nuruzzaman, M., A.M. Sharoni, K. Satoh, A. Moumeni, R. Venuprasad, R. Serraj, A. Kumar, H. Leung, K. Attia, and S. Kikuchi. 2012. Comprehensive gene expression analysis of the NAC gene family under normal growth conditions, hormone treatment, and drought stress conditions in rice using near-isogenic lines (NILs) generated from crossing Aday Selection (drought tolerant) and IR64. *Mol. Gen. Genet.* 287:389–410.
- Olsen, A.N., H.A. Ernst, L.L. Leggio, and K. Skriver. 2005. NAC transcription factors: Structurally distinct, functionally diverse. *Trends Plant Sci.* 10:79–87.
- Ooka, H., K. Satoh, K. Doi, T. Nagata, Y. Otomo, K. Murakami, K. Matsubara, N. Osato, J. Kawai, P. Carninci, Y. Hayashizaki, K. Suzuki, K. Kojima, Y. Takahara, K. Yamamoto, and S. Kikuchi. 2003. Comprehensive analysis of NAC family genes in *Oryza sativa* and *Arabidopsis thaliana*. *DNA Res.* 10:239–247.
- Sami, U., S.U. Khan, J.U. Din, A. Qayyum, N.E. Jaan, and M.A. Jenks. 2015. Heat tolerance indicators in Pakistani wheat (*Triticum aestivum* L.) genotypes. *Acta Bot. Croat.* 74:109–121.
- Shah, S.T., C. Pang, S. Fan, M. Song, S. Arain, and S. Yu. 2013. Isolation and expression profiling of *GhNAC* transcription factor genes in cotton (*Gossypium hirsutum* L.) during leaf senescence and in response to stresses. *Gene* 531:220–234.
- Singh, K., R.C. Foley, and L. Onate-Sanchez. 2002. Transcription factors in plant defense and stress responses. *Curr. Opin. Plant Biol.* 5:430–436.
- Tang, Y., M. Liu, S. Gao, Z. Zhang, X. Zhao, C. Zhao, F. Zhang, and X. Chen. 2012. Molecular characterization of novel *TaNAC* genes in wheat and overexpression of *TaNAC2a* confers drought tolerance in tobacco. *Physiol. Plant.* 144:210–224.
- Wang, G., S. Zhang, X. Ma, Y. Wang, F. Kong, and Q. Meng. 2016a. A stress-associated NAC transcription factor (*SINAC35*) from tomato plays a positive role in biotic and abiotic stresses. *Physiol. Plant.* 158:45–64.
- Wang, J.Y., J.P. Wang, and H. Yuan. 2013. A *Populus euphratica* NAC protein regulating Na^+/K^+ homeostasis improves salt tolerance in *Arabidopsis thaliana*. *Gene* 521:265–273.
- Wang, L.L., X.J. Zhou, and W.Z. Liu. 2016b. Effects of enhanced ultraviolet-B radiation on physiological indices and camptothecin content in *Camptotheca acuminata* Decne. *Acta Botanica Boreali Occidentalia Sinica* 36:979–986.
- Wang, Y.C., W.R. Gu, L.F. Ye, Y. Sun, L.J. Li, and H. Zhang. 2015. Physiological mechanisms of delaying leaf senescence in maize treated with compound mixtures of DCPTA and CCC. *J. Northeast Agr. Univ. (English ed.)* 22:1–15.
- Wang, Z. and F. Dane. 2013. NAC (NAM/ATF/CUC) transcription factors in different stresses and their signaling pathway. *Acta Physiol. Plant.* 35:1397–1408.
- Zhang, X., B. Chen, G.J. Lu, and B. Han. 2009. Overexpression of a NAC transcription factor enhances rice drought and salt tolerance. *Biochem. Biophys. Res. Commun.* 379:985–989.
- Zhang, Y., W.J. Jiang, H.J. Yu, and X.Y. Yang. 2012. Exogenous abscisic acid alleviates low temperature-induced oxidative damage in seedlings of *Cucumis sativus* L. *Trans. Chinese Soc. Agr. Eng.* 28:221–228.
- Zhou, M., D. Li, Z. Li, Q. Hu, C. Yang, L. Zhu, and H. Luo. 2013. Constitutive expression of *amiR319* gene alters plant development and enhances salt and drought tolerance in transgenic creeping bentgrass. *Plant Physiol.* 161:1375–1391.