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Research article

Salt tolerance function of the novel C2H2-type zinc finger protein TaZNF in wheat

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ABSTRACT

The expression profile chip of the wheat salt-tolerant mutant RH8706-49 was investigated under salt stress in our laboratory. Results revealed a novel gene induced by salt stress with unknown functions. The gene was named as *TaZNF* (*Triticum aestivum* predicted Dof zinc finger protein) because it contains the zf-Dof superfamily and was deposited in GenBank (accession no. KF307327). Further analysis showed that *TaZNF* significantly improved the salt-tolerance of transgenic *Arabidopsis*. Various physiological indices of the transgenic plant were improved compared with those of the control after salt stress. Non-invasive micro-test (NMT) detection showed that the root tip of transgenic *Arabidopsis* significantly expressed Na^+ excretion. TaZNF is mainly localized in the nucleus and exhibited transcriptional activity. Hence, this protein was considered a transcription factor. The *TaZNF* upstream promoter was then cloned and was found to contain three salts, one jasmonic acid methyl ester (MeJA), and several ABA-responsive elements. The GUS staining and quantitative results of different tissues in the full-length promoter in the transgenic plants showed that the promoter was not tissue specific. The promoter activity in the root, leaf, and flower was enhanced after induction by salt stress. Moreover, GUS staining and quantitative measurement of GUS activity showed that the promoter sequence contained the positive regulatory element of salt and MeJA after their respective elements were mutated in the full-length promoter. RNA-Seq result showed that 2727 genes were differentially expressed; most of these genes were involved in the metabolic pathway and biosynthesis of secondary metabolite pathway.

1. Introduction

Wheat is one of the most important food crops and is the main source of proteins for humans, Wheat contains high amounts of vitamin B, vitamin E, magnesium, phosphorus, cellulose, and other beneficial substances for humans (Vasil and Anderson, 1997). However, salt in soil is an important abiotic stress that limits wheat yield. Hence, varieties of salt-tolerant wheat must be cultivated to maximize the advantage of available cultivated land and improve wheat yield (Chinnusamy et al., 2005; Yamaguchi and Blumwald, 2005; Farooq and Azam, 2006).

Salinity stress and plant responses to high salinity have been investigated over the past two decades (Flowers et al., 1977; Hasegawa et al., 2000; Zhu, 2002). Numerous plant genes involved in responses to salt stress have been studied. Various transcription factors are also found to be involved in plant abiotic stress responses (Nakashima et al., 2014; Mao et al., 2012). The zinc finger protein, one of the common transcription factors widely exist in eukaryotes, is involved in cell differentiation, proliferation, apoptosis, and other important life processes. Study has indicated that some zinc finger proteins can regulate the stress resistance of plants (Ciftci-Yilmaz and Mittler, 2008). C2H2-type zinc finger proteins involved in plant development and stress resistance processes have been largely confirmed (Sakamoto et al., 2000; Kobayashi et al., 1998; Kim et al., 2001; Takatsuji et al., 1994; Yang et al., 2006).

Plant salt tolerance is a complex trait that involves many genes (Shi et al., 2002). Salt stress-resistant genes and their functions must be investigated to enrich wheat stress signal network and improve wheat tolerance to stress. The function of *TaZNF* and the

Abbreviations: ABA, abscisic acid; PEG, polyethylene glycol; NMT, non-invasive micro-test; GUS, beta-glucuronidase; EST, expressed sequence tag; MDA, malondialdehyde; MeJA, methyl jasmonate; MS, Murashige/Skoog.

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mechanism of salt tolerance were studied in this paper to provide a theoretical basis and application value for cultivation of salt-tolerant wheat varieties.

2. Materials and methods

2.1. Plant materials

The wheat salt-tolerant mutant RH8706-49 and salt-sensitive mutant H8706-34 (their genetic backgrounds were highly similar, but salt tolerance significantly differed), Columbia-type *Arabidopsis*, and tobacco were preserved in our laboratory.

2.2. TaZNF cloning

Through the gene chip partial SOM cluster analysis under the salt stress of wheat sal-tolerant mutant RH8706-49, the probe gb: BF199556, whose expression increased after salt stress for 12 h, was selected. Numerous wheat expressed sequence tags (ESTs) with high homology to the probe were obtained through BLAST search in EST library of the National Center for Biotechnology Information (NCBI). *TaZNF* was obtained by assembling the cloned sequences. The wheat salt-tolerant mutant RH8706-49 seeds were soaked in water until rooting and sprouting. The seeds were then cultivated in the light at 22 °C for 16 h and in the dark for 8 h. After growth to the two-leaf stage, the seedlings were treated by salt stress. Total RNA was extracted and reversely transcribed into cDNA (Ge et al., 2007). *TaZNF* was cloned using cDNA as the template and deposited in GenBank (accession no. KF307327). The primers used are shown in Table 1.

2.3. Expression pattern analysis of TaZNF

The wheat salt-tolerant mutant RH8706-49 and salt-sensitive mutant H8706-34 at the two-leaf stage were treated with 0.8% NaCl, 50 μmol/L abscisic acid (ABA), and 15% polyethylene glycol (PEG) for 0, 1, 6, 12, and 72 h. Total RNA was extracted from the leaves and roots and reverse transcribed into cDNA. Real Time-quantitative PCR (RT-qPCR) analysis was then conducted using Rotor-Gene 3000 quantitative PCR instrument (Gene Company Limited). The experimental results were analyzed using RG3000 6.0 software (Gene Company Limited). Quantitative analysis was performed using $2^{-\Delta\Delta C}{}_{T}$ method, with wheat β-actin gene as internal reference (GenBank accession no. AB181991) and SYBR Green as dye. Each sample was analyzed three times. The primers used are shown in Table 1.

2.4. Subcellular localization in TaZNF transgenic tobacco

TaZNF was subcloned into the pCAMBIA2300-35S-GFP-OCS vector (p2300-GFP). The p2300-*TaZNF*-GFP vector was transferred into *Agrobacterium tumefaciens* GV3101 through freeze–thaw method (Jyothishwaran et al., 2007). The transformed empty p2300-GFP *Agrobacterium* was used as control. Tobaccos growing under normal conditions for 6 weeks were selected, for transformation. *Agrobacterium* harboring experimental or control vector was injected into the tobacco leaves (Ma et al., 2015). The injected leaves were placed on the glass slides 45–48 h later and visualized through laser confocal microscopy. The exciting light wavelength was 488 nm, and the primers used are shown in Table 1.

2.5. Salt tolerance of TaZNF-overexpressing Arabidopsis

TaZNF was subcloned into the pCAMBIA1300 vector (P1300). The vector was transferred into *A. tumefaciens* GV3101 by using freeze–thaw method. We identified the *A. tumefaciens via* PCR. Then, the positive clone was selected to transform *Arabidopsis* (An et al., 2012; Clough and Bent, 1998). Transgenic *Arabidopsis* was selected using Murashige/Skoog (MS) medium containing hygromycin (25 mg/L) (Ishitani et al., 1997), and the transgenic nature was confirmed through RT-PCR. Homozygotes and wild-type seedlings were vertically cultured on the MS plate for 4 d and then they were moved to MS plate containing 150 and 170 mM NaCl. Root growth was observed after 3 d of culture. Homozygotes and control seedlings that germinated on the MS medium for 10 d were transplanted in pots filled with vermiculite/nutritive soil (1:1) and cultivated under normal light at 22 °C for 14 d. Plants in consistent growing status were irrigated using water and NaCl solution (200 mM NaCl) for 11 d. Phenotypic (plant height, leaf color and fructification) changes were observed.

2.6. Stomatal aperture of transgenic Arabidopsis leaves under salt stress

Stomatal aperture, that is, the ratio of stomatal width and the maximum length, is a widely used indicator to study stomatal movement. The data could reflect changes in stomatal area, particularly the opening and closing of stomata (Omasa and Onoe, 1984). Transgenic homozygous and control seeds were surface sterilized, sown on MS medium, and grown in a light incubator at 22 °C. Seedlings growing for 10 d were transplanted to pots filled with vermiculite and nutrient soil and grown under normal light at 22 °C for 14 d. The plants were irrigated with salt-free water and water containing 150, 180 and 200 mM NaCl for 11 d. Stomatal aperture was measured using an optical microscope (Carl Zeiss Microlmaging GmbH 37081 Gottingen, GERMANY).

2.7. Content of ions, chlorophyll, proline, malondialdehyde (MDA), and relative water content in TaZNF transgenic plants

TaZNF transgenic and the wild-type *Arabidopsis* plants were irrigated with salt-free water and water containing 200 mM NaCl

Table 1
List of oligo nucleotides used in the study.

Genes	Forward primers (5′ → 3′)	Reverse primers (5′ → 3′)
Arabidopsis overexpression	TCTAGACATATGGAGCTCGCCGG	GGATCCCGGAAGTTTCCGTATCACG
β-actin	TGCTATCCTTCGTTTGGACCTT	AGCGGTTGTTGTGAGGGAGT
TaZNF	GGTGGCAGGGAGCAAATG	GGTGGCAGGGAGCAAATG
Subcellular localization	GGTACCATGGAGCTCGCCGGAG	TCTAGACGTCGTCTCCTGGAAGGAC
Promoter	CTGCAGTGGGTGTGGCTTCTTTTCTG	TCTAGAATGGGGCAAGATCCGAC
Mutation promoter Y1	ACAAATGAACCAAAACAAAGAC	TCTTTGTTTTGGTTCATTTGTC
Mutation promoter Y2	GTTAATCATCCAAAAACGGAC	GTCCGTTTTTGGATGATTAAC
Mutation promoter Y3	TGAAATTCCAAAACAAAAATTTAC	ATTTTTGTTTTGGAATTTCAGAG
Mutation promoter M	GATTGATATTCCTTGACAAATG	TTGTCAAGGAATATCAATCGAC
Transcriptional activity	GAATTCATGGAGCTCGCCGGAG	GGATCCGCGTCGTCTCCTGGAAGG