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Research article

Overexpression of a chrysanthemum transcription factor gene, *DgWRKY3*, in tobacco enhances tolerance to salt stress

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ABSTRACT

WRKY transcription factor genes (TFs) play important roles in response to various abiotic stresses. However, the roles of the chrysanthemum WRKY genes in abiotic stress response remain obscure. In this study, we functionally characterized a novel WRKY gene, *DgWRKY3*, from chrysanthemum (*Dendranthema grandiflorum*). Its expression in the chrysanthemum was up-regulated by salinity or dehydration stress, but not by abscisic acid (ABA). The *DgWRKY3*-overexpression tobacco plants increase salt tolerance compared with wild-type (WT) tobacco plants. The increased levels of proline were observed in transgenic plants compared to WT plants under salt stress. In addition, the *DgWRKY3* transgenic plants reduced accumulation of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) compared with WT plants, accompanied by higher activities of antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) and the greater accumulation of antioxidants including ascorbate (AsA) and glutathione (GSH) under salt stress. Moreover, the *DgWRKY3* transgenic plants enhanced the expression of stress-related genes involved in osmotic adjustment and membrane protection (*NtP5CS*, *NtLEA5*, and *NtERD10D*) and oxidative stress response (*NtSOD*, *NtPOD*, *NtCAT*, and *NtAPX*) under salt stress. However, no significant difference in the expression of stress-related genes (*NtP5CS*, *NtLEA5*, *NtERD10D*, *NtSOD*, *NtPOD*, *NtCAT*, and *NtAPX*) was found between the *DgWRKY3*-overexpression and WT tobacco plants under normal conditions, despite the fact that the constitutive promoter was used to drive *DgWRKY3*. These findings suggest that *DgWRKY3* functions as a positive regulator to mediate tolerance of plants to salt stress.

1. Introduction

Plants are constantly challenged by various environmental stresses such as drought, high salinity, and low temperature, which can limit their growth and productivity. To cope with environmental stresses, plants have evolved complex physiological, molecular, and biochemical responses. Environmental stresses are perceived and transduced through a chain of signaling molecules that ultimately affect regulatory element of stress-inducible genes to initiate the synthesis of different classes of protein including transcription factors, enzymes, molecular chaperons, ion channels, and transporters or alter their activities [1]. Among the numerous stress-induced genes, many transcription factors (TFs) have been studied and identified. Numerous TFs, such as DREB, bZIP, NAC, and WRKY, can interact with *cis*-elements present in the promoter regions of various abiotic stress-related genes and thus regulate the expression of many genes resulting in imparting tolerance to abiotic stresses [2]. Among TFs, the WRKY genes received much attention in past decade.

The WRKY proteins, which contain one or two conserved WRKY domains consisting of 60 amino acid region with a conserved WRKYGQK motif in its N-terminus followed by a C2H2 or C2HC zinc finger motif, represent a large family of plant-specific TFs [3]. Many WRKY genes play regulatory roles in a variety of processes, such as pathogen defense, seed development and germination, leaf senescence, abiotic stress response [4–7]. To date, a series of stress-responsive WRKY genes have been identified and characterized from different plant species. Overexpression of soybean WRKY *GmWRKY54* in *Arabidopsis* elevated the expression of stress responsive genes of *DREB2A* and *STZ*, and enhanced *Arabidopsis* tolerance to salt and drought [8]. Similarly, *Arabidopsis* over-expressing a wheat

Abbreviations: ABA, abscisic acid; APX, ascorbate peroxidase; AsA, ascorbate; CaMV, the cauliflower mosaic virus; CAT, catalase; GSH, glutathione; H_2O_2, hydrogen peroxide; LEA, late embryogenesis abundant; MDA, malondialdehyde; POD, peroxidase; qRT-PCR, quantitative real-time polymerase chain reaction; RACE, rapid amplification of cDNA ends; ROS, reactive oxygen species; SOD, superoxide dismutase; WT, wild type.

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WRKY *TaWRKY19* elevated the expression of stress responsive genes of *DREB2A*, *RD29A*, *RD29B* and *Cor6.6*, thereby enhanced tolerance to salt, drought and freezing stresses [9]. In addition, *OsWRKY30* is activated by MAP kinases to confer drought tolerance in rice [10]. It inferred that WRKY gene is rather important for plant to respond to abiotic stresses.

Chrysanthemum is one of the most famous ornamental species in the world and its production is severely affected by high salinity conditions in the cutting-chrysanthemum industry [11]. In an objective of improving salt tolerance in chrysanthemum, we reported the isolation and characterization of a novel WRKY gene, *DgWRKY3*, and showed that it was induced by salt and drought stress. By stress assays, overexpression of *DgWRKY3* in tobacco plants improved tolerance to salt stress, via regulation of putative downstream genes and physiological changes.

2. Results

2.1. Isolation and characterization of DgWRKY3

DgWRKY3 (Genbank accession No. KC292215) contained a complete open reading frame of 945 bp, which encoded a protein of 314 amino acids with a calculated molecular mass of 35.5 kDa (Supplementary Fig. S1). Multiple alignment between DgWRKY3 and four other WRKY proteins by DNAMAN (Version 6.0) indicated that DgWRKY3 contains one WRKY domain comprising the highly conserved WRKYGQK peptide sequence and a C2HC zinc finger motif (Supplementary Fig. S2). The phylogenetic analysis based on the classification method [3] showed that DgWRKY3 belongs to group III and is more closely related to TcWRKY53 from *Thlaspi caerulescens* (Supplementary Fig. S3).

2.2. Expression analysis of DgWRKY3

The expression profiles of *DgWRKY3* were investigated in various tissues using qRT-PCR. As shown in Fig. 1A, *DgWRKY3* was detected in all organs. The transcription level in the stems and leaves was higher than that in roots and flowers. In addition, the expression patterns of *DgWRKY3* gene under different stress conditions was examined using qRT-PCR. Under salt stress, the transcripts of *DgWRKY3* increased gradually up to 12 h after NaCl treatment, thereafter decreased slightly (Fig. 1B). For drought stress, *DgWRKY3* was induced and peaked at 3 h after drought treatment, then declined gradually (Fig. 1C). However, ABA treatment did not affect *DgWRKY3* gene expression (Fig. 1D).

2.3. Salt tolerance analysis of DgWRKY3 transformed tobacco plants

To determine whether *DgWRKY3* overexpression enhances tolerance to salt stress, transgenic tobacco plants that overexpressed *DgWRKY3* was generated. With *NtUbiquitin* as the reference gene, the T_2 generation of the transgenic lines (OE-5, OE-11, OE-18, OE-21, and OE-26) showed higher expression levels of *DgWRKY3* while *DgWRKY3* expression was not detected in the WT control (Fig. 2A). Salt stress tolerance was compared between the OE-5, OE-21 transgenic tobacco plants and with WT. Under normal conditions, WT and two transgenic lines showed no significant difference in phenotypes during all life cycles. For salt tolerance assay, 3-week-old transgenic lines and WT were irrigated with 400 mM NaCl for 7 days. It was observed that the two transgenic lines were much better than WT (Fig. 2B). After 6 days of recovery from salt stress, the survival rate of two transgenic lines was significantly higher than that of WT (Fig. 2C). On 1/2 MS medium containing 150 mM NaCl, the percentage germination of two transgenic lines was significantly higher than that of WT (Fig. 3). On 10 μM ABA medium, no significant difference was observed between WT and the two transgenic lines (Fig. 3). These data indicate that *DgWRKY3* conferred tolerance of transgenic tobacco plants to salt stress during both seed germination and vegetative growth. In addition, *DgWRKY3* overexpression in tobacco did not affect ABA sensitivity in seed germination.

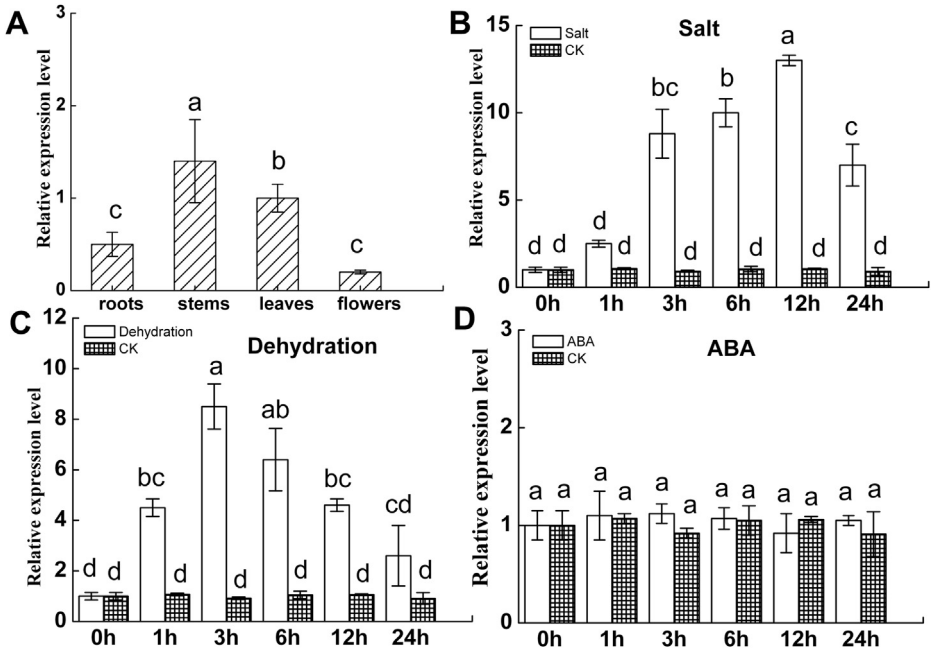

Fig. 1. Quantitative real-time PCR analysis of expression patterns of *DgWRKY3* in different organs and in response to various treatments. The relative expression of *DgWRKY3* in untreated leaves was used as CK. (A) Expression patterns of *DgWRKY3* in roots, stems, leaves, and flowers. (B) Dehydration. (C) Salt. (D) ABA. Data represent means and standard errors of three replicates. Different letters above columns indicate ($P < 0.05$) differences between treatments.