DIFFERATATION OF WHEAT WRKY TRANSCRIPTION FACTOR TaWRKY10 GENE EXPRESSION IN ABIOTIC STRESS RESISTANCE

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ABSTRACT

Drought, cold and salinity are the primary factors limiting wheat production. It has been shown that a wheat stress-responsive WRKY transcription factor encoded by the wheat WRKY10 gene plays an important role in drought, cold and salinty stress tolerance. The aim of the current study was to WRKY10 gene expression in drought, cold and salinity up-regulated in six varieties of Mongolian local wheat (Triticum aestivum L.), to select the stress tolerant best variety for breeding program. Plants expressing TaWRKY10 was up-regulated 48 hours stress by treatment with polyethylene glycol (PEG6000), 200 mM NaCl and 4^0C cold. Results of RT-PCR indicate that the all varieties show the gene expression level. Furthermore, reverse transcription polymerase chain reactions revealed that the expressions of WRKY 10 genes involved in abiotic stress signaling of six wheat varieties. These results indicated highest expression level of the WRKY10 in Darkhan-131 and lowest expression in Darkhan-34. And our study provided a promising approach to improve the tolerances of wheat cultivars to drought and salinity varieties of selection materials.

KEYWORDS: WRKY transcription factor, wheat, drought, cold, salinity

INTRODUCTION

Wheat (*Triticum aestivum L.*) is a staple food of about one third of the world's population. It is one of the most important cereals which provide more calories in the form of starch and proteins; besides vitamins I and diet than any other food crop (Govt. of Pakistan, 2004). Water stress reduces crop yield regardless of the growth stage at which it occurs in wheat. Arid and semi-arid environment besides other factors may induce water stress during crop growth and development, resulting reduction in crop yield (Ashraf et al., 1995). It is vital for plant breeding programmes to have sufficient diversity available to allow for the production of new varieties that are aimed towards the improvement of crop productivity and able to withstand damage from biotic and abiotic factors. In this respect, efforts have also been made to predict the prospects of developing superior genotypes from a cross by the measurement of a genetic similarity (GS) or genetic distance (GD) between the parents, since the later can be used as an estimation of expected genetic variances in different sets of segregating progenies derived, from different crosses. The availability of genetic variability

in elite wheat material is pre requisite for any breeding programme aimed towards the improvement of wheat productivity. Wheat breeding through hybridization also requires the selection of diverse genotypes, irrespective of whether the end product is pure line or a hybrid variety. Wheat is used mainly for human consumption and supports nearly 35% of the world population. It is nutritious, easy to store and transport and can be processed into various types of food. The demand for wheat is expected to grow faster than any other major agricultural crop. To meet the needs of the growing world population, the forecast demand for the year 2020 varies between 840 (Rosegrant *et al.* 1995) and 1050 million tons (Kronstad, 1998).The *WRKY* genes encode a large group of transcription factors. There are over 70 WRKY genes in *Arabidopsis (Arabidopsis thaliana*; Eulgem et al., 2000; Dong et al., 2003) and rice (*Oryza sativa*; Goff et al., 2002; Zhang et al., 2004). The characteristic feature of the WRKY family is its highly conserved 60 amino acids, the WRKY domain, which is comprised of a highly conserved *WRKYGQK* motif at the N-terminus and certain zinc finger motifs at C-terminus. The *WRKYGQK* motif in various plant species show slight variations in the amino sequences (for example, *WRKYGKK* and *WRKYGEK*). Depending on their domain structures, the *WRKY* proteins can be divided into three different groups. Proteins with two WRKY domains belong to group I; proteins containing one *WRKY* domain belong to groups II or III, depending on the type of zinc finger motif. The *WRKY* factors present high binding affinity to a DNA *cis*-acting element named as the W box, (C/T) TGAC (T/C), which permits signal transduction to regulate the expressions of stress-related genes, resulting in plant stress tolerance finally. In the current study, *TaWRKY10* gene was identified and its expression was found to be up-regulated by drought, salinity and stress, and selection of six Mongolian local wheat (*Triticum aestivum L*) variety were selected abiotic stress tolerance variety for breeding program.

MATERIALS AND METHODS

Plant materials and stress treatments

The seeds of six Mongolian local wheat *(Triticum aestivum L.)* varieties, Darkhan-34, Darkhan-74, Darkhan-131; Darkhan-141, Darkhan-144 and Khalkhgol-1 were obtained from the Plant Science and Agricultural Training and Research Institute of Darkhan-Uul. The seeds were sterilized in 70% ethanol for 3 min, and then were rinsed with sterilized water. The sterilized wheat seeds were germinated in a growth chamber (25°C, 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 16h light/8 h dark cycle). The 10 days old seedlings were transferred to petri dishes containing 20% *PEG6000* or 200 mM *NaCl* solutions, and incubated under light for 24 h. For cold treatment, seedlings were transferred to cold-chamber at 4°C under light for 24 h. In all these treatments, wheat seedlings at similar growth states were used, and untreated wheat seedlings were taken as controls. Leaf samples were frozen in liquid nitrogen, and then stored at −70°C until RNA extraction.

RNA isolation and cDNA synthesis

Total RNA was isolated by using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The RNA preparations were subjected to DNase digestion in the presence of a recombinant ribonuclease inhibitor.

Specific primers were designed to amplify these cDNAs. PCR amplifications were performed at 42^0C for 15 min, followed by 30 cycles of 95^0C for 5 min with a final extension at 4^0C for 5 min.

RT-PCR analysis

cDNA was used as the template in a 20 µl RT-PCR with the following thermal cycling parameters: 95 ^{0}C for 30 sec, 50 ^{0}C for 30 sec and 72^0C for 1 min. Gene specific primers (Table 1) were used RT-PCR analysis. A 250 bp *TaActin* gene fragment was amplified as a positive control using the primer pair 5'-CTTGTATGCCAGCGGTCGAACA3' and 5'-CTCATAATCAAGGGCCACGTA -3'.

RESULTS

To obtain abiotic stress responsive WRKY genes in wheat, we referred to the highly similar orthologs in rice. Then, we performed TBLASTN analysis in the DFCI database (http://compbio.dfci.harvard.edu/tgi/) using abiotic stress induced rice WRKY cDNA sequences. The *TaWRKY10* cDNA is 791 bp in length (GenBank accession no. HQ700327), including a complete ORF of 672 bp that encoding a putative protein of 223 amino acids (predicted relative molecular mass of 24.5 kDa). To elucidate the potential function of the *WRKY10* transcription factors in response to various stimuli, the expression patterns were analyzed by RT-PCR under various abiotic stress conditions.

Table1

Gene specific primers used for RT-PCR analysis

Gene name	forward	reverse
WRKY10	5’- GCTGCCTTCTACACATTCCAGT-3’	5’-CACCTCCAGCTGCTTCTCTAAT-3’
TaActin	5'- CTTGTATGCCAGCGGTCGAACA -3'	5'- CTCATAATCAAGGGCCACGTA -3'

Reverse transcription polymerase chain reaction (RT-PCR)

RT-PCR analyses showed that WRKY10 genes were significantly regulated by abiotic stresses including drought, cold, and salinity in six Mongolian local wheat varieties (Fig. 2). To clarify the tissue expression patterns of *TaWRKY10*, mRNA isolated from wheat leaves were using as the templates for RT-PCR. *TaWRKY10* was detected varying degrees of expression in leaves of 10-day-old seedlings.

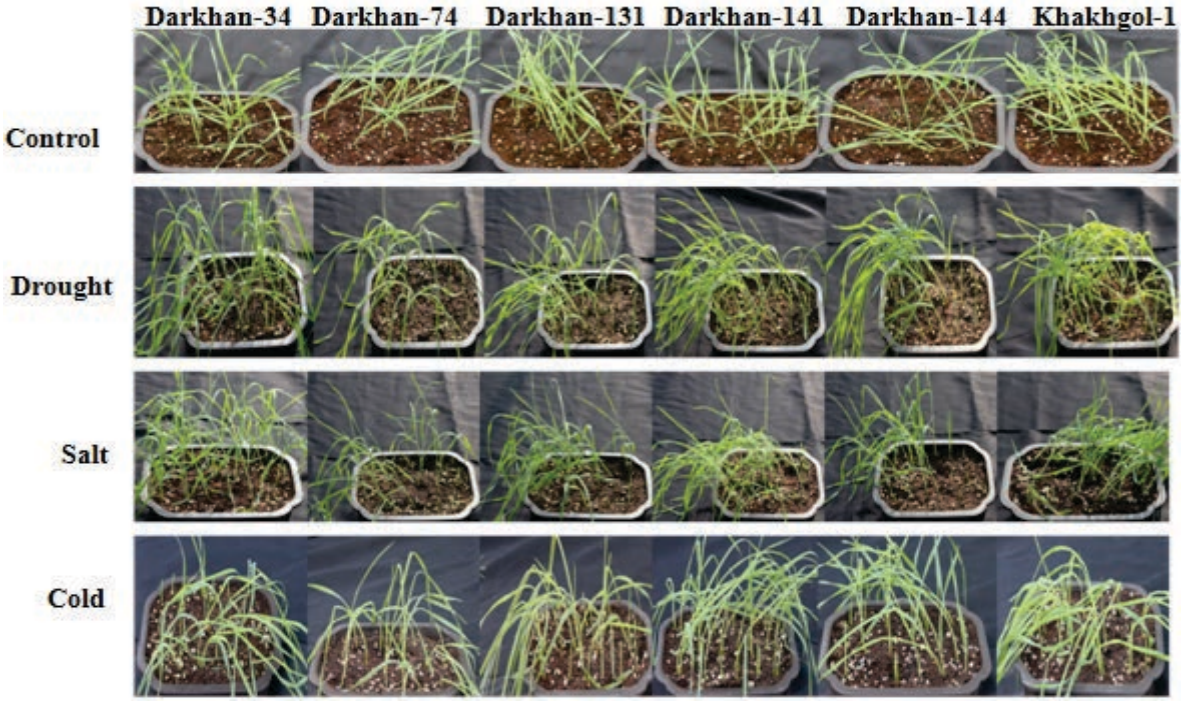

Figure 1. The phenotype of stress treated and control plants grown in the pots. Drought stress treated plants were irrigated by PEG6000 for 2 days, salt stress treated plants were irrigated by 200 mM NaCl for 2 days, and cold stress treated plants were stored at 4^0C for 2 days, respectively

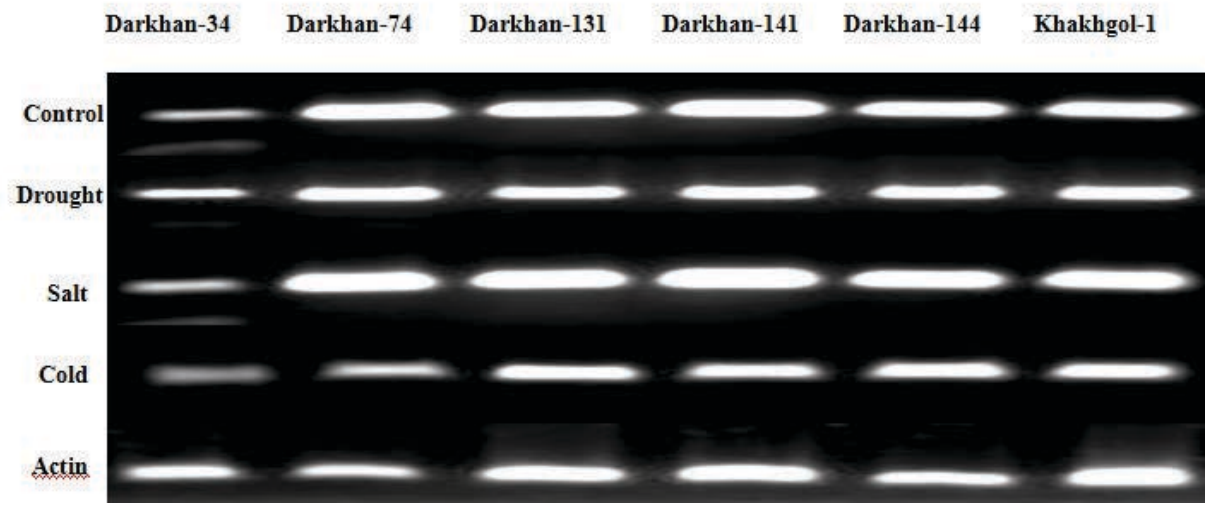

Figure 2. The *WRKY10* gene expression analysis of drought, salt and cold treatments and control (untreated plants). The 10 days old seedlings and control plants were used to extract RNA to detect *WRKY10* expression by RT-PCR. Actin was used as an internal control.

DISCUSSION

Wheat is the foremost staple food crop in the world which provides both calories and proteins to over 35% of the human population. The production of wheat is affected by multiple environmental stresses, including drought, salinity and extreme temperatures. However, only two wheat WRKYs have been characterized; *TaWRKY2* and *TaWRKY19* are involved in abiotic stresses responses. In our study, quantitative PCR demonstrated that *TaWRKY10* was induced by multiple stresses, including *PEG6000*, 200 mM *NaCl* and cold (4°C) (Fig. 1) Responses to abiotic stimuli of WRKY transcription factors are often extremely rapid and transient. The WRKY transcription factors mediate signals transduction via adaptive responses and regulation of downstream genes. During responses to multiple stresses, a single WRKY gene often participates in various signaling pathways, indicating its diverse regulatory mechanism. The expressions of certain stress-induced genes have been demonstrated to be associated with stress tolerance (Chen L, et al. 2012). In the present study, TaWRKY10 gene expression show the all 48 hours drought, salinity and cold stress treatment in six variety of Mongolian local wheat (*Triticum aestivum* L.*)*. The highest expression level was Darkhan-131 and Darkhan-141and lowest expression level Darkhan-34 respectively (Fig. 2).

CONCLUSION

In conclusion, our results clearly demonstrated that *TaWRKY10* is a stress-inducible wheat transcription factor, and that the show expression in six wheat variety of the Mongolian local variety *TaWRKY10* was up-regulated by *PEG, NaCl* and cold. Darkhan 131 was selected the most drought, cold and salinity resistant variety and Darkhan 34 was selected the most drought, cold and salinity sensitive variety depending upon TaWRKY10 gene expression level after 48 hours PEG6000, 200mM NaCl, and cold 4^0C stress treatment .

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