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Short Communication

Differential expression analysis of salt stress related genes *TaSRG* and *TaRUB1* in contrasting wheat genotypes

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Wheat is the second important food crop grown all over the world. However, several abiotic constraints such as drought, salinity, heat and cold severely affect wheat production and productivity. Among these stresses, salinity stress is major stress spreading worldwide and results from accumulation of soluble salts in the root zone (Ashraf and Foolad 2007; Bauder *et al.*, 2014). Therefore, developing wheat cultivars tolerant to salinity stresses is essentially required. Kharchia 65 (Kh 65) is well known wheat genotype tolerant to salinity stress (Munns *et al.*, 2006). However, very little is known about molecular basis of tolerance in Kh 65. Therefore, understanding molecular basis of salt tolerance in Kh 65 will lead to enhanced salinity tolerance in wheat cultivars. To adapt these changing environment conditions plants adopt various mechanisms (Shannon, 1997). At molecular level many signal transduction processes change gene expression resulting in various cellular changes (Hussain *et al.*, 2011). The expression of these genes is regulated by various transcription factors like ZIP, MYB, DREB, NAC etc. involved in abiotic stress (Lindemose *et al.*, 2013). Changes in expression of transcription factors genes usually lead to dramatic changes in plants (Liansen *et al.*, 1999).

Some specific transcription factors genes are expressed in response to salt stress (*Lindemose et al.,* 2013**).** One such transcription factor called salt response gene (SRG) was first identified in rice and arabidopsis and a homologous sequence to this transcription factor gene was identified in wheat (Xiaoliang *et al.*, 2011). Beside these transcription factors, many other proteins are involved in stress regulation. One such group of proteins are ubiquitin involved in post translational modification by regulating stability, activity, and trafficking of protein. Ubiquitin conjugation act as a major regulator of stress responsive transcription factors and other regulatory proteins (Lyzenga and Stone, 2011). Ubiquitin family proteins are divided into two classes; i. Ubiquitin like modifiers e.g. RUB and ii. Ubiquitin domain proteins e.g. RAD23 and DSK2. RUB is known as Nssd8 in fission yeast (Dreher *et al.*, 2007). Two closely linked RUB1 and RUB2 and one divergent RUB3 family member are found in Arabidopsis (Rao-Naik et al., 1998). RUB/NEED8 proteins help in DNA repair mechanism, DNA replication, cell cycle regulation, chromatin remodeling and chromatin organization (Zhang *et al.*, 2012). Earlier studies reported expression analysis of these transcription factors under various stress conditions (Zhang *et al.*, 2012, Xiaoliang *et al.*, 2011) but very few studies were related to differential expression. Therefore, present study was conducted on differential expression analysis of two transcription factors *TaSRG* and *TaRUB1* involved in stress response in salt tolerant Kharchia 65 (Kh 65) and sensitive HD 2009 under saline conditions.

Two wheat genotypes Kharchia65 (salt tolerant) and HD 2009 (salt sensitive) were grown under controlled conditions in growth chamber (Light intensity of 800 µmol/M^2S with 14h light at 20^{0}C (day) and 10h dark at 16^{0}C (night) at 70% relative humidity. Seeds were sown in pots with sandy soil (400 g) saturated with half strength of Hoagland solution. Salt treatment was initiated after 7 days of emergence of first leaf. The salt treatment was applied for three days with final concentration of 0.75 g of NaCl and 0.11 g of $CaCl_2$ (per pot) and the ECe was 12.0. Plants grown in pots in sand with half strength of Hoagland solution were used as control. Three replicates of each of each genotypes were analysed at 24, 48 and 72 hours of salt treatment.

For expression analysis of transcripts of two genes *TaSRG* and *TaRUB1*, total RNA was extracted from roots and leaf blade after an interval of 24, 48 and 72 hrs using RNAeasy Plant Mini kit (Qiagen). RNA quality and quantity was

checked using Nanodrop. DNA contamination from RNA was removed by treating RNA with RNase free DNase (Genei). The RNA extracts were then subjected to reverse transcription using RevertAid^{Tm}H Minus First strand cDNA synthesis kit (Thermo scientific).

The expression of transcript of *TaSRG* and *TaRUB1* of control and treated plant samples in triplicate was analyzed with 3 technical repeats by RT PCR (Bio Rad CFX96™ Real Time Detection System) using gene specific primers (sequence information given in Table 1). Wheat cyclophilin gene was used as an endogenous housekeeping gene to normalize expression with SYBR Green as fluorescent dye. PCR reaction conditions were 3 min at 95 °C followed by 39 cycles of 30 s at 95 °C, 45 s at 61 °C and 1min at 72 °C. Relative expression of the genes were carried out using the Pfaffl formula (ratio02$^{-\Delta\Delta Ct}$) (Pfaffl, 2001), where ΔΔCt (ΔCt sample−ΔCt control); ΔCt sample (ΔCt target−ΔCt ref) for all sampling times and NaCl concentrations; and ΔCt control (ΔCt target−ΔCt ref)

Table 1. Sequence of primers for expression analysis of *TaSRG* and *TaRUB1* genes

S.No.	Gene	Forward primer 5`-3`	Reverse primer 5`-3`	Reference
1	TaSRG	ATAGGATGCAGGG CGAAGTG	T T G T C C C T G A C C T C CATCTTC	Xiaoliang *et al.,* 2011
2	TaRUB1	GTTCAGTGCTCCA TCTTGTG	CGTCAATTACATG GCACTTC	Zhang *et al.,* 2012

Salinity stress is one of the major abiotic stresses spreading worldwide (Ashraf and Foolad 2007) and results from accumulation of soluble salts in the root zone (Bauder *et al.*, 2014). There are many genes involved in salinity stress tolerance and thus identification and functional study of these stress responsive gene is required for improving wheat under salinity stress (Radaie *et al.,* 2010). Present investigation was conducted to study the expression of two genes *TaSRG* (*Triticum aestivum* salt response gene) and *TaRUB* (*Triticum aestivum* related to ubiquitin) in response to salinity stress in wheat. The gene expression was measured as relative change in expression in at 24, 48 and 72 hrs of salt treatment. There was higher expression of *TaSRG* in roots of Kh 65 (16 fold) as compared to HD 2009 (2 fold) (Fig 1A). In shoot there was an increase in expression of *TaSRG* upto 48 hrs followed by decrease at 72 hrs in both the genotypes. There was 9 fold increases in expression of *TaSRG* in leaf of Kh 65 at 48 hrs and 4 fold in HD 2009. Earlier studies also indicated a higher expression of *TaSRG* in salt tolerant genotypes (Xiaoliang *et al.*, 2011).

Several ubiquitin related proteins are encoded by eukaryotic genome and among them the best characterized are SUMO/Smt3 and RUB/Nedd8. RUB family of proteins is involved in ubiquitin proteosome pathway (Hellmann *et al.*, 2002). Maximum expression (12 fold) of RUB1 was observed at 48 hrs in Kh 65 and no significant change in HD 2009. There was 7 fold increase in the expression of *TaRUB1* in roots of Kh 65 at 72 hrs of salt treatment while very little change in HD 2009 (Fig. 1B). Zhang *et al.*, 2012 identified a wheat RUB family gene *TaRUB1* and studied its expression on leaves infected with powdery mildew with RT-PCR. Therefore, the present investigation showed upregulation of both the genes under salt stress with more pronounced in Kh65 indicating their role in salinity tolerance.

The present study demonstrated the role of *TaSRG* and *TaRUB1* genes in imparting tolerance to salinity stress in wheat. The expression of the genes TaSRG and TaRUB1 was higher in tolerant genotype Kh 65 as compared to salt sensitive HD 2009 indicating the ability of Kh 65 in

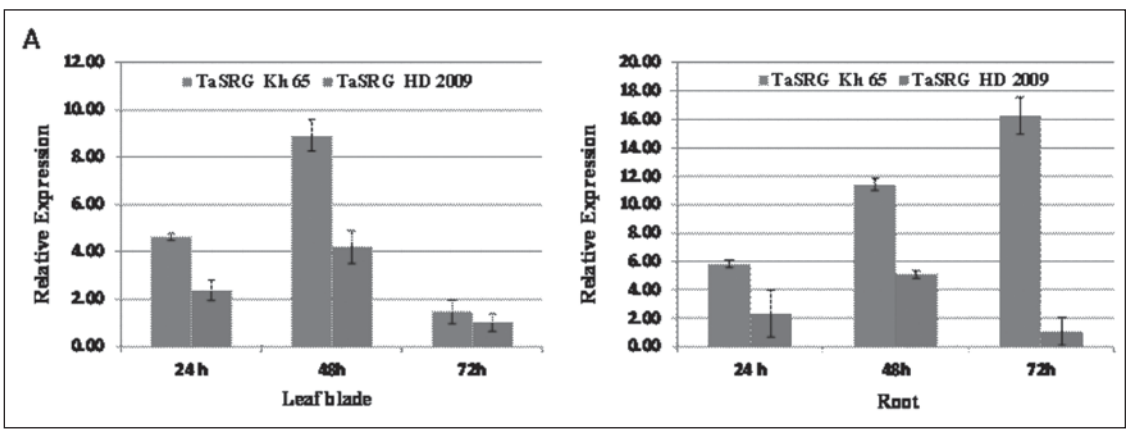

Fig. 1A

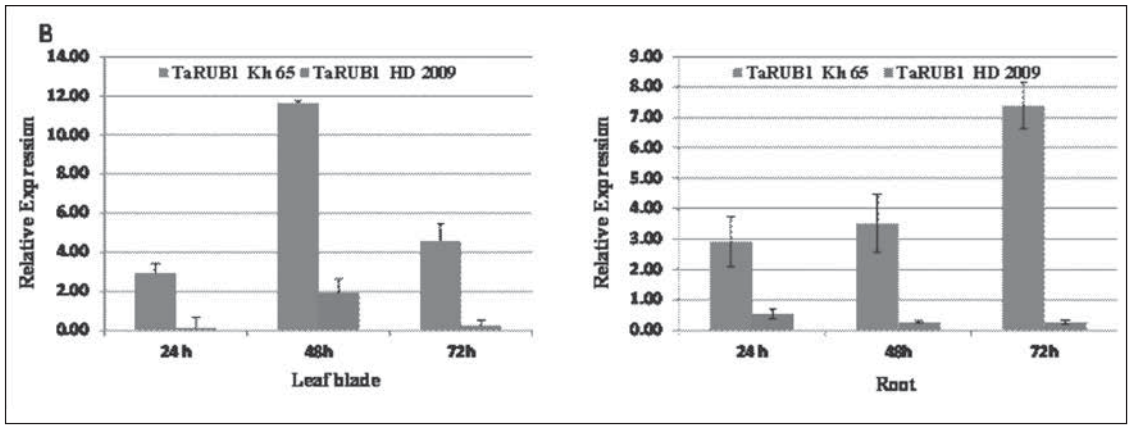

Fig. 1B

regulating expression of stress related genes allowing it to tolerate salt stress conditions. Since salinity tolerance is a complex phenomena and governed by so many genes, further studies are needed to identify additional genes related to salt tolerance. A better understanding of these genes and their regulation under salinity could be useful in breeding programmes to develop salinity tolerant genotypes.

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