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Expression of Genes Related to Na^+ Exclusion and Proline Accumulation in Tolerant and Susceptible Wheat Genotypes under Salt Stress

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In the present investigation, expression of genes related to Na^+ exclusion such as salt overly sensitive (TaSOS1) and Na^+/H^+ antiporter (TaNHX1) and proline accumulation such as pyrroline-5-carboxylate reductase (P5CR) and glutamate synthase (GOGAT) was studied in seedlings of Kharchia 65 (Kh 65, salt tolerant) and HD 2009 (sensitive) under salt stress (ECe, 12 dSm^{-1}) and controlled conditions. As compared to HD 2009, Kh 65 showed significantly lower accumulation of Na^+ ($p<0.01$) and higher accumulation of proline ($p<0.05$) in leaf blade under salt stress. The relative expression of TaSOS1 increased significantly ($p<0.001$) in roots of Kh 65 (4.31-fold) while it decreased in HD 2009. There was significantly higher ($p<0.01$) relative expression of TaNHX1 (27.57-fold) in leaf and 3.07-fold in roots of Kh 65 as compared to 3.65- and 0.87-fold increase in leaf and roots of HD 2009, respectively, under salt stress. There was significantly ($p<0.05$) higher accumulation of proline as compared to HD 2009 in leaf tissues. There was significantly higher ($p<0.01$) expression of P5CR (5.23-fold in leaf and 8.77-fold in the root) and glutamate synthase (6.0-fold in roots) in Kh 65 as compared to HD 2009. The study demonstrated that upregulation of genes for Na^+ exclusion in root and compartmentation in leaf and increased proline concentration are associated with tolerance to salinity stress in wheat. The information will be useful for improving wheat genotypes for salt tolerance.

Keywords: salinity tolerance, proline, Na^+/K^+, wheat, TaSOS, TaNHX

Abbreviations: P5CR – pyrroline-5-carboxylate reductase; SOS – salt overly sensitive; Kh 65 – Kharchia 65; PCR – polymerase chain reaction

Introduction

Soil salinity being a major abiotic stress constraint affects agriculture productivity worldwide. Almost 800 million ha land worldwide and 7 million ha in India is affected by soil salinity (Munns et al. 2006; Hollington 1998). Of the 240 million ha of irrigated land, 45 million ha are salt affected (FAO 2008). This problem is further aggravated by injudicious irrigation practices which cause water tables to rise leading to salt accumulation in root

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zone. Crop productivity in salt-affected soil can be improved either by soil reclamation methods such as applying gypsum or growing salt tolerant cultivars (Genc et al. 2013). Since soil remediation is difficult to apply in all situations by the farmers, development of salinity tolerant cultivars is viable alternative. However, breeding for salt tolerance by conventional means is difficult because of lack of selection criteria and complexity of the trait. Therefore, understanding molecular basis of salt tolerance can help breeding process to improve wheat varieties for salt stress.

High salt concentrations in soil inhibit plant growth in two ways I. Inducing water deficit in soil and II. Injury by excess salt inside the cell. Therefore, salt tolerance is associated with accumulation of compatible osmolyte such as proline, glycinebetaine etc. and exclusion of sodium while entering into the plant and its transport to leaves and also Na^+ tissue tolerance (Munns et al. 2006; Tester and Davenport 2003; Gouiaa et al. 2012; Roy et al. 2014). Several reports indicated the role of membrane transporters such as salt overly sensitive (TaSOS1) (Apse and Blumwald 2002; Cuin et al. 2011), Na^+/H^+ antiporter (NHX) family transporters such as TaNHX1 and TaNHX2 (Gaxiola et al. 1999; Brini et al. 2005; Xu et al. 2013) and high affinity potassium transporters (HKT) (Singh et al. 2015) in imparting tolerance to salinity stress. TaSOS1 is involved in sodium exclusion at plasma membrane level in root cells and TaNHX1 and TaNHX2 at vacuolar level. Other reports indicated the role of transcription factors (TaSRG and TaRUB) under stress conditions (He et al. 2011; Zhang et al. 2012) but very few studies were related to differential expression of these genes (Rana et al. 2015). Dubcovsky et al. (1996) reported Kna1 locus mapped on chromosome 4DL involved in Na^+ exclusion from leaves and discrimination of K^+ over Na^+ concentration in roots. However, the functional activity of many of these genes *in planta* remains to be demonstrated. In this investigation, we studied the functional activity of TaSOS1 and TaNHX1 in salt tolerant and sensitive cultivars.

In addition to Na^+ exclusion, functional analysis of genes related to proline synthesis was conducted. Proline, a multifunctional amino acid, plays a role in osmotic adjustment, in cell signalling, protein translation, maintaining a low NADPH: $NADP^+$ ratio and in scavenging free radical during stress (Szabados and Savouré 2009). Proline is synthesized mainly by glutamate pathway in which glutamate is first reduced to glutamate-semialdehyde (GSA) by the pyrroline-5-carboxylate synthetase (P5CS) enzyme, and spontaneously converted to pyrroline-5-carboxylate (P5C) (Szabados and Savouré 2009). P5C reductase (P5CR) further reduces P5C intermediate to proline. Glutamate is mainly synthesised from glutamine through GS-GOGAT pathway. GOGAT is also called glutamate synthase. In the present investigation, expression analysis of two major genes as P5C reductase (P5CR) and glutamate synthase (GOGAT) was conducted in salt tolerant (Kharchia 65; Kh 65) and sensitive (HD 2009) wheat cultivars under saline conditions. Kh 65 (an Indian land race of wheat) is highly tolerant to salinity (Munns et al. 2006; Díaz De León et al. 2010) and HD 2009 sensitive (Sairam et al. 2005). It is intriguing that so little is known about the genetics and physiology of the Kh 65, universally regarded as highly salt tolerant with low Na^+ uptake rates and successful osmotic adjustment and has high specific leaf area and early vigour.

Materials and Methods

Plant material and growth conditions

Two wheat varieties, namely Kharchia 65 (salt tolerant) and HD 2009 (salt sensitive) were grown under controlled conditions in growth chamber (800 µmol/M^2 S: 14 h light at 20 °C and 10 h dark at 16 °C) at 70% relative humidity. Seeds were sown in pots with 400 g of sandy soil saturated with half strength of Hoagland solution. The salt treatment was given with saline water containing 0.375 g of NaCl and 65 mg of $CaCl_2$ at 7^{th} and 9^{th} day of seedling emergence at one leaf stage as per method of Rana et al. (2015). Final content of NaCl and $CaCl_2$ was 0.75 g and 110 mg, respectively, in 400 g of sandy soil in each pot raising the electrical conductivity of extract (ECe) of the soil to 12 dSm^{-1}. Electrical conductivity of the soil extract (1 gram soil mixed in 2.0 ml distilled water) was measured using electrical conductivity meter (Delux Make 601). Three replicates of each of the leaf blade, leaf sheath and root were taken after 72 h of final salt treatment for the measurement of biomass and analysis of metabolites and at 24, 48 and 72 h for gene expression. Biomass of the shoot and root was taken after 72 h of final salt treatment. Shoot and root samples were thoroughly washed with running water followed by rinsing with doubled distilled water to remove soil particles. The biomass was measured as fresh weight in grams (gFW).

Measurement of ions

Na^+ and K^+ contents were measured using flame photometer. Around 100 mg of leaf, leaf sheath and root samples were taken and dried in oven and digested in 0.5 ml of 0.5N HNO_3 for 2 h at 80 °C as per the method of Munns et al. 2010). The digested samples were centrifuged and 100 µl of the supernatant was diluted 100 times by water. Concentration of Na^+/K^+ was measured in milligram per gram dry weight (mg/gDW) by using standards of NaCl and KCl in the range of 0.25 ppm to 20 ppm.

Proline concentration

Proline was extracted using ninhydrin reaction method (Cross et al. 2006). Fifty mg of fresh sample of each of leaf blade, lead sheath and root was kept overnight in 1.0 ml of 40:60 v/v ethanol and water mixture at 4 °C. The ethanolic extract was obtained by centrifuging for 10 min at 12000 rpm. A 200 µl of the extract was taken in 2 ml tubes containing 300 µl of ninhydrin reaction (1% ninhydrin in 20:60 v/v ethanol and acetic acid mix) mixture and incubated for 20 min at 90 °C in water bath. The mixture was centrifuged again for 2 min and the absorbance of chromophore was read at 520 nm. Proline concentration was expressed in microgram per gram fresh eight (µg/gFW).

RNA extraction and RT-PCR assay

Expression analysis of transcripts of four genes, namely salt overly sensitive (TaSOS1), Na^+/H^+ antiporter (TaNHX1), pyrroline-5-carboxylate reductase (P5CR) and glutamate synthase (GOGAT) were conducted in Ist leaf blade and roots of both the wheat genotypes (KH 65 and HD 2009) at 24, 48 and 72 h of short-term salt stress. Total RNA was extracted from roots and leaf blade at 24, 48 and 72 h after salt treatment using RNAeasy Plant Mini kit (Qiagen). RNA quality and quantity was checked using Nanodrop. The purified 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 all the genes was analysed with two biological and three technical repeats by RT PCR (Bio Rad CFX96^{TM}Real Time Detection System) using gene specific primers. The primer sequences for Tacyclophilin, TaSOS1 and TaNHX1 were used as described by Cuin et al. (2011) and for P5CR and GOGAT as per Kovács et al. (2011). All the primers were synthesized by Sigma Life Sciences. Wheat cyclophilin (Tacyclophilin) gene was used as an endogenous housekeeping gene to normalize expression. The forward and reverse primers used for amplification of Tacyclophilin were 5´CAAGCCGCTGCACTACAAGG3´ and 5´AGGGGACGGTGCAGATGAA3´; for TaSOS1 5´AGAAGCCGATCTGCAAAGAA3´ and 5´TGCTGCCATACATGCTGACT3´, for TaNHX1 5´GCCTGGTTCACCCATAGAGA3´ and 5´CACCGAAAGAATCCCAAGAG3´, for P5CR 5´TGGTCCGGCCTACATTTTCT3´, and 5´TTTACCCGTCTCGCTAACCA3´ and for GOGAT 5´ACCAAGGGACCTCAGTATTC3´ and 5´TCTTACCCTGGGCAGATATG3´. PCR reaction conditions were 3.0 min at 95 °C followed by 39 cycles of 30 s at 95 °C, 45 s at 61 °C and 1.0 min at 72 °C. Relative expression of the genes were carried out using the Pfaffl formula (ratio $2^{-\Delta\Delta Ct}$) (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).

Results

Biomass

Fresh weight of both root and shoot was measured under controlled conditions and after 72 h of salinity treatment. Significant difference ($p < 0.05$) was observed in shoot biomass of both Kh 65 and HD 2009 under salinity treatment. There was greater reduction in shoot biomass in the salt sensitive variety HD 2009 (53.3% reduction) as compared to tolerant Kh 65 (38.32% reduction). There was 22% reduction in root biomass of Kh 65 as compared to 32.95% reduction in HD 2009 under stress (Table 1). Higher reduction in root and shoot biomass of both the cultivars may be because of high salt concentrations (ECe 12.0) used in this investigation. Since fresh weight was measured, lower water absorption under high salinity stress would cause further reduction in fresh weight.

Na^+/K^+ concentration and gene expression analysis

Na^+ and K^+ contents were measured in individual leaf blade and leaf sheath (1st and 2nd) and root parts (Table 2). Significant differences in Na^+ concentration were observed be-

Table 1. Shoot and root biomass (gFW) of Kharchia 65 and HD 2009 under control conditions and salinity stress after 72 h of salt treatment.

Genotype	Shoot (gFW)			Root (gFW)		
	Control	Saline	% reduction	Control	Saline	% reduction
Kh 65	2.74±0.2	1.69±0.1	38.32	2.96±0.5	2.31±0.7	22.00
HD 2009	2.69±0.4	1.25±0.08	53.30	2.61±0.5	1.75±0.3	32.95

Values are mean + SD (n = 3)

tween Kh 65 and HD 2009 in first leaf blade ($p < 0.10$), sheath ($p < 0.10$), second leaf blade ($p < 0.05$) and sheath ($p < 0.05$) and root parts ($p < 0.05$) under salinity conditions. HD 2009 showed significantly higher accumulation of Na^+ and higher Na^+/K^+ ratio in leaf blade, sheath and root as compared to Kh 65. There was greater accumulation of Na^+ in 1st leaf (lower) as compared to IInd leaf (upper) in Kh 65. Significantly higher accumulation of Na^+ was observed in leaf sheath as compared to leaf blade ($p < 0.10$).

Since there was differential accumulation of Na^+ in tolerant and sensitive cultivars, expression analysis of genes involved in Na^+ exclusion (TaNHX1 and TaSOS1) was conducted under controlled conditions and at 24, 48 and 72 h after salt treatment in both the cultivars (Table 3). The relative expression of TaSOS1 increased 4.31-fold in roots of Kh 65 at 72 h of salt treatment. There was linear increase in expression of TaSOS1 in Kh 65 roots with the duration of salinity treatment, while no significant change in HD 2009 roots. The expression of TaSOS1 was significantly ($p < 0.001$) higher in root tissues of Kh 65 as compared to HD 2009. In leaf blade, there was no significant difference in the expression of TaSOS1 between HD 2009 and Kh 65. Relative expression of TaNHX1 increased 27.57-fold in leaf and 3.07-fold in roots of Kh 65 while it was 3.65- and 0.87-fold increase in leaf and roots of HD 2009, respectively. There was significantly higher expression levels of TaNHX1 in leaf ($p < 0.01$) and root ($p < 0.05$) of Kh 65 as compared to HD 2009 under salt stress.

Proline accumulation and gene expression analysis

Accumulation of proline was measured under controlled conditions and at 72 h of salt treatment in leaf blade, sheath and roots. Significant differences were observed in proline accumulation in leaf blade of both Kh 65 and HD 2009 under salt stress as compared to controlled conditions (Table 2). There was 2.64-fold increase in proline content in Ist leaf, 4.75-fold in IInd leaf, 6.31-fold in Ist leaf sheath and 10.1-fold in IInd leaf sheath of Kh 65. The increase in proline content in HD 2009 was 2.26-fold in Ist leaf, 1.55-fold in IInd leaf, 5.95-fold in Ist leaf sheath and 5.81-fold in IInd leaf sheath under slat stress. There was significantly higher concentration of proline ($p < 0.05$) in the 1st leaf of Kh 65 as compared to HD 2009 under salt stress. There was higher accumulation of proline in leaf sheaths as compared to leaf blade and root. In root tissues, there was 5.48 times increase in proline concentration in Kh 65 and 4.20 times in HD 2009 under salt stress as compared to control conditions.

Table 2. Na^+ and K^+ concentration (mg/gDW) and proline concentration (µg/gFW) in leaf blade, leaf sheath and root of Kharchia 65 and HD 2009 under control and saline conditions (ECe = 12 dSm^{-1})

Trait	Plant tissue		Kh 65		HD 2009	
			Control	Treatment (12 ECe)	Control	Treatment (12 ECe)
Na^+	Leaf blade	Ist leaf	0.51±0.09	4.60±0.38	0.74±0.05	6.40±0.5
		IInd leaf	0.48±0.15	3.43±0.15	0.63±0.08	6.51±2.07
	Leaf sheath	Ist leaf	0.35±0.04	7.60±1.6	0.57±0.02	12.23±0.9
		IInd leaf	0.31±0.17	4.09±0.6	0.64±0.19	10.39±1.1
	Root		5.06±0.38	11.19±2.24	5.37±0.96	18.22±0.28
K^+	Leaf blade	Ist leaf	34.39±4.44	39.58±3.0	39.95±5.51	41.45±3.36
		IInd leaf	33.42±2.19	28.92±1.02	44.81±2.2	43.94±2.40
	Leaf sheath	Ist leaf	34.42±0.95	37.68±2.99	43.38±0.34	29.90±0.9
		IInd leaf	37.68±2.99	22.46±1.56	29.00±0.71	29.75±1.23
	Root		13.41±1.44	11.59±1.8	13.93±2.17	15.32±1.96
Proline	Leaf blade	Ist leaf	0.87±0.11	2.30±0.19	0.78±0.07	1.77±0.04
		IInd leaf	0.24±0.02	1.14±0.08	0.68±0.01	1.06±0.03
	Leaf sheath	Ist leaf	0.22±0.03	1.39±0.15	0.19±0.005	1.13±0.02
		IInd leaf	0.18±0.02	1.83±0.06	0.21±0.01	1.22±0.06
	Root		0.21±0.002	1.15±0.36	0.24±0.06	1.01±0.42

Notes: 72 h of salinity treatment. Values are mean ±SD (n = 3).

Table 3. Relative expression changes of salt overly sensitive (TaSOS1), Na^+/H^+ antiporter (TaNHX1), pyrroline-5-carboxylate reductase (P5CR) and glutamate synthase (GOGAT) genes in 1st leaf blade and roots of wheat genotypes (Kharchia 65 and HD 2009) at 24, 48 and 72 h of short-term salt stress

Gene	Genotype	1st leaf blade			Root		
		24 h	48 h	72 h	24 h	48 h	72 h
TaSOS1	Kh 65	0.34+0.10	1.17+0.12	2.16+0.17	0.69+0.18	2.20+0.28	4.23+0.24
	HD 2009	0.54+0.04	1.47+0.1	2.13+0.15	0.20+0.06	0.19+0.08	0.25+0.06
TaNHX1	Kh 65	9.88+0.23	12.17+1.58	27.57+0.28	1.89+0.08	4.10+0.33	3.07+0.41
	HD 2009	2.29+0.71	2.36+0.54	3.65+1.82	1.12+0.11	0.90+0.24	0.89+0.20
P5CR	Kh 65	3.22+0.25	4.19+0.36	5.23+0.42	3.91+0.04	4.34+0.22	8.77+0.98
	HD 2009	0.85+0.05	1.15+0.32	1.30+0.01	2.21+0.27	1.82+0.11	1.30+0.07
GOGAT	Kh 65	–	–	–	0.47+0.06	2.09+0.05	5.99+0.44
	HD 2009	–	–	–	1.80+0.18	1.52+0.18	1.45+0.14

Notes: The expression levels of each gene are expressed as relative to the TaCyclophilin as fold expression change. Average relative expression values of 2 biological and 3 technical repeats are given in the table.

There was upregulation of P5CR and GOGAT genes involved in proline biosynthesis. The relative expression of P5CR increased 5.23-fold in leaf and 8.77-fold in the root of Kh 65 while it was 1.3-fold in both leaf blade and root of HD 2009 under salt treatment (Table 3). There was significantly higher expression of P5CR in both leaf ($p < 0.01$) and root ($p < 0.001$) of Kh 65 as compared to leaf and root of HD 2009, respectively in salt conditions. The expression of glutamate synthase increased 5.99-fold in roots of Kh 65 and 1.45-fold in HD 2009 after 72 h of salinity treatment. The differences in the relative expression of GOGAT were significant at $p < 0.01$ level between Kh 65 and HD 2009. The expression of both P5CR and glutamate synthase gene transcript increased with time of salt treatment in roots of Kh 65, however, there was initial increase in the relative expression of the gene in HD 2009 under salt stress followed by decrease in the expression level.

Discussion

Biomass

Under salt stress a general reduction in biomass is observed in both the genotypes. Kh 65 showed a lesser reduction in biomass in both shoot and root as compared to HD 2009, showing its tolerance to salt stress. Díaz De León et al. (2010) also reported Kharchia as salt tolerant with lower reduction in biomass under salt stress. HD 2009 was released in India during 1975 for cultivation in North Western Plains Zones under irrigated timely sown conditions. Kh 65 was released in India during 1970 under salinity/alkalinity conditions. Sairam et al. (2005) reported HD 2009 salt sensitive as compared to Kh 65. Our earlier report also exhibited that HD 2009 is salt sensitive (Rana et al. 2015). Higher reduction in root and shoot biomass (fresh weight) of both the cultivars may be because of lower water absorption under high salinity stress. In seedling stage, the major component of biomass is water and hence greater reduction in biomass under salinity stress. There was lower accumulation of salts in leaf and root parts of Kh 65 as compared to HD 2009. Munns et al. (2006) showed that a salt tolerant plant had ability to prevent salt from reaching toxic levels in root and shoot parts as compared to salt-sensitive plant. In addition, lower decrease in biomass of tolerant genotype could also be associated with other components such as higher antioxidant activity, osmolyte concentration and potassium contents, and lower H_2O_2 (Sairam et al. 2005; Haouari et al. 2013; Singh et al. 2015).

Na^+ concentration and expression analysis of TaSOS1 and TaNHX1

There was higher accumulation of Na^+ in lower leaf and sheath of both genotypes as compared to upper (younger) leaf. Generally grasses have been reported to have higher concentration of Na^+ in older leaves or leaf sheath (Colmer et al. 2006) under salt stress. The greater accumulation of Na^+ in sheath is considered a mechanism adopted by plants to avoid accumulation of higher concentration of Na^+ in leaf blade. Higher accumulation of Na^+ in older leaves may protect metabolically more active younger leaf from toxic effect of Na^+. There was higher accumulation of Na^+ in HD 2009 as compared to Kh 65 showing limited ability of HD 2009 in excluding Na^+ and thus prone to Na^+ toxicity.

A lower Na^+/K^+ ratio in Kh 65 exhibited its K^+ selectivity needed to maintain a lower Na^+/K^+ ratio. Several other reports also showed association of salt tolerance in plants with exclusion of Na^+ while entering into the system and also transport in the leaves (Tester and Davenport 2003; Munns et al. 2006; Gouiaa et al. 2012).

The relative expression of TaSOS1 increased 4.31-fold in roots of Kh 65 and no significant change in HD 2009 roots after 72 h of salt treatment. The relative expression of TaSOS1 was significantly ($p < 0.001$) higher in root tissues of Kh 65 as compared to HD 2009 which may have contributed towards lower accumulation of Na^+ in Kh 65 roots. TaSOS1 gene has been reported to be associated with sodium exclusion at plasmalemma level in root tissues (Apse and Blumwald 2002). Other reports also indicated higher expression of TaSOS1 gene transcript in roots (Benderradji et al. 2011; Cuin et al. 2011; Yamaguchi et al. 2013) under salt stress. There was 27.57-fold increase in the expression of TaNHX1 gene in leaf and 3.07-fold in the roots Kh 65 while 3.67-fold in leaf and 0.87-fold in root of HD 2009 under salt stress. NHX family transporters such as TaNHX1 and TaNHX2 have been reported in wheat (Brini et al. 2005; Xu et al. 2013). These are Na^+/H^+ antiporter and act at vacuolar level in transporting Na^+ driven by electrochemical proton gradient (Gaxiola et al. 1999). Higher expression of endogenous vacuolar Na^+/H^+ antiporters in tolerant wheat genotypes have been reported by several workers under salinity (Saqib et al. 2005; Benderradji et al. 2011; Cuin et al. 2011). Zörb et al. (2005) also reported overexpression of NHX gene under saline conditions in maize. A lower accumulation of Na^+ and a higher expression of TaNHX1 gene in Kh 65 indicates compartmentalization of Na^+ occurs in vacuoles at both root and shoot level. Na^+ sequestration in vacuole has role imparting salinity tolerance in wheat (Cuin et al. 2011; Roy et al. 2014).

Proline accumulation and gene expression analysis

There was 2.64-fold increase in proline concentration in I^{st} leaf of Kh 65 under salt stress while it was 2.26-fold increase in HD 2009 and the difference was significant ($p < 0.05$). Osmolytes like proline help in maintaining osmotic balance in plants during stress conditions (Ashraf and Foolad 2007). There was higher increase in proline concentration in leaf as compared to root. Earlier reports by Huang et al. (2013) also showed higher accumulation of proline in leaves as compared to root samples in artichoke. They suggested that leaves accumulate more proline in order to maintain chlorophyll level and cell turgor to protect photosynthetic activity under salt stress. Proline besides balancing the osmotic strength of cytosol plays an important role in cell signalling, protein translation, maintaining a low NADPH: $NADP^+$ ratio during stress and in scavenging free radical (Szabados and Savouré 2009). A higher accumulation of proline in Kh 65 indicated its adaptability to salinity stress. The proline concentration increased with the duration of salt treatment showing its positive role in combating salinity stress by maintaining osmotic balance.

The transcript levels of P5CR and GOGAT genes involved in proline synthesis increased significantly under salinity stress. Though, several expression studies related to P5CS gene have been reported, a few are related to P5CR and GOGAT. Ma et al. (2008) reported an upregulation of TaP5CR gene in wheat under salt, ABA, PEG and heat stress.

Similarly increased expression pattern of P5CR and glutamate synthase genes in response to cold stress also was reported by Kovács et al. (2011). There was significantly higher relative expression ($p < 0.01$) of P5CR in leaf blades of Kh 65 and both P5CR and GOGAT genes in roots of Kh 65 as compared to HD 2009. GOGAT was studied only in roots of both the genotypes. A higher accumulation of proline in Kh 65 and correspondingly higher expression of P5CR and glutamate synthase indicated an important role of proline in salt tolerance.

In conclusion, the study demonstrated the role of Na^+ exclusion and compartmentation along with proline accumulation in imparting tolerance to salinity stress in wheat. The relative expression of genes for Na^+ exclusion at plasma membrane (TaSOS1) and tonoplast (TaNHX1) levels were significantly higher in Kh 65 as compared to HD 2009, indicating the ability of Kh 65 to exclude Na^+ at plasma membrane level and compartmentalize Na^+ in vacuoles to minimise toxic effect of Na^+. Higher accumulation of proline concomitant with increased expression of P5CR and glutamate synthase demonstrated its role in combating salt stress conditions. The information will be useful for improving wheat genotypes for salt tolerance.

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