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Physiological and molecular analysis of salt tolerance in wheat (*Triticum aestivum*) recombinant inbred lines population (HD2851 × Kharchia 65)

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ABSTRACT

Sodicity is a critical stress that significantly affects the yield and productivity of wheat. This stress can result in a range of physiological, biochemical, and molecular responses in plants, which can hinder their overall health and yield potential. Understanding these responses is key to developing salt-tolerant wheat (*Triticum aestivum* L.) varieties. The present study was carried out during 2022–23 and 2023–24 at ICAR-Central Soil Salinity Research Institute, Karnal, Haryana in which a population of 195 recombinant inbred lines (RILs) of wheat (HD2851 × KH65) was evaluated under control and sodicity conditions. Genotype HD2851 showed a more significant yield reduction (65.51%) under sodicity conditions compared to KH65 (45.08%). Following exposure to salt stress, the leaf tissues of KH65 exhibited 1.9-fold increase in Na^+ content, while HD2851 showed 3.1-fold increase. Significant positive correlations (P<0.01) were found between grain yield and several traits: chlorophyll content, K^+/Na^+ ratio, plant height, spike length, flag leaf area, and 1000-grain weight. Conversely, Na^+ content exhibited a significant negative correlation (P<0.01) with grain yield. The first two principal components accounted for 38.39% of the overall trait variation (PC1, 21.14%; PC2, 17.25%). In this study, the expression of *TaNHX1*, *TaSOS1* and *TaHKT2* genes was evaluated in the leaf tissues of salt-tolerant (KH65, RIL8 and RIL130) and salt-sensitive (HD2851, RIL61 and RIL154) wheat genotypes under salt treatment. The expression levels of *TaNHX1*, *TaSOS1* and *TaHKT2* genes were significantly higher in KH65, RIL8 and RIL130 genotypes following salt stress, suggesting enhanced capabilities for Na^+ exclusion at the plasma membrane and Na^+ sequestration in vacuoles. The information generated in the present study will be beneficial for improving salt tolerance in elite wheat genotypes.

Keywords: Gene expression, RIL, Salt tolerance, Sodium ion, *Triticum aestivum*

Wheat (*Triticum aestivum* L.) is an important cereal crop worldwide, contributing significantly to global food and nutritional security (Asseng *et al.* 2016). However, the challenge of fulfilling the growing worldwide demand for food is intensified by abiotic factors such as salinity and sodicity, which significantly affect the yield and productivity of wheat (Roy *et al.* 2011). Salt stress affects over 900 million hectares of agricultural land globally (Butcher *et al.* 2016). In India, approximately 6.74 million hectares of land are affected by salinity (Kumar and Sharma 2020). Sodic soils alter soil-water-plant interactions, reducing soil moisture content (Tiwari *et al.* 2021). Soil sodicity significantly risks crop productivity, primarily by increasing soil *p*H and sodium saturation (Yadav *et al.* 2024). Salt stress negatively impacts plants primarily through osmotic stress, ion toxicity, and disruption of mineral uptake (Hao *et al.* 2021).

Among cereals, wheat is considered a moderate salt stress tolerance, with significant variations observed among different cultivars (Munns *et al.* 2006). Several physiological and biochemical traits have been identified as contributors to salt tolerance in wheat, including the accumulation of compatible osmolytes, potassium selectivity, and the exclusion of sodium ions (Munns *et al.* 2012, Rana *et al.* 2015). Extensive research has been conducted to screen and evaluate wheat genotypes for salt tolerance, highlighting significant genetic variation and the potential for breeding programmes to improve this trait (Sabagh *et al.* 2021). The development and use of salt stress-responsive genes in breeding programme enhance wheat tolerance to salinity (Mehta *et al.* 2021). Therefore, a comprehensive understanding of wheat physiological, biochemical and molecular response to stress is crucial.

The present study aimed to investigate the impact of salt stress on various physiological, biochemical, and yield-

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related traits in a population of 195 recombinant inbred lines (RILs) developed from a cross between HD2851 and KH65 genotypes. The expression analysis of three salt-responsive genes (*TaNHX1*, *TaSOS1* and *TaHKT2*) was also performed in tolerant and sensitive wheat genotypes under stress conditions. These findings could provide valuable insights into the genetic basis of salt tolerance for breeding programmes focused on enhancing salt tolerance in wheat.

MATERIALS AND METHODS

Plant material and field experiment: The present study was carried out during 2022–23 and 2023–24 at ICAR-Central Soil Salinity Research Institute, Karnal, Haryana. In this investigation, a mapping population of 195 recombinant inbred lines (RILs) of wheat was developed from a cross between HD2851 (salt sensitive) and Kharchia 65 (salt tolerant). RILs and their parents were assessed under both control and sodic conditions in the field using an augmented block design with two replications. The field with sodic conditions (*p*H: 9.2) was developed by adding the required quantity of sodium bicarbonate to the soil. Each experimental unit was arranged in rows 2.5 m long and spaced 22.5 cm apart. Normal irrigation practices were employed, and fertilization followed standard recommendations (120 kg N, 70 kg P_2O_5 and 50 kg K_2O/ha) (Cao 2009).

Physiological measurements: Physiological and yield component parameters were measured in 10 randomly selected plants from each RIL and parent under control and salinity conditions. Parameters studied included days to heading (DTH) (the period until 50% of the plants in a plot reach the inflorescence phase), plant height (PH) (measured from the base to the top of the ear, excluding awns), spike length (SL), number of spikelets/spike (SPS), 1000-grain weight (TGW), grain yield (GY) (grain weight harvested/plot) and biological yield (BY) (weight of harvested grain and all non-grain plant parts/plot). Leaf area was determined using Quarrie and Jones formula, multiplying leaf length and width by 0.75 (Aldesuquy *et al.* 2014). Chlorophyll content in fully mature flag leaves was measured using a SPAD 502 Plus chlorophyll meter.

Measurement of leaf sodium and potassium content: The concentration of Na^+ and K^+ was determined in the wheat flag leaf. Leaves were collected and rinsed with distilled water to eliminate any surface contaminants. After being oven-dried for 48 h at 65°C, the leaves were grounded into a fine powder with a mortar and pestle. For the analysis, 0.5 g of this powder was taken and digested in 15 ml of a diacid mixture, a combination of nitric acid and perchloric acid, at 10:3 (v/v). This mixture was then left at room temperature for 2 h to digest (Sairam *et al.* 2002). The samples were then heated on a hot plate to 200°C until the reddish-orange liquid became clear, reducing its volume to 2–3 ml. The digested liquid was then diluted with 100 ml distilled water. Na^+ and K^+ content in the digested samples were measured using flame photometry. A Systronics FF128 model flame photometer was used for the analysis.

Statistical analysis: The data analysis was conducted using SAS (version 9.3). Tukey's Honestly Significant Difference (HSD) test was applied to identify significant differences among the means at a significance level of $P \leq 0.05$. Results were presented as mean ± standard deviation (SD). Pearson correlation coefficients were computed using SAS to analyze the relationship between traits under treatment and control conditions. Principal Component Analysis (PCA) was performed using OriginPro 2019b (version 9.6.5).

RNA extraction and qRT-PCR analysis: Total RNA was extracted from the leaf tissue of both control and treated plants after 15 days of salt stress using Trizol reagent (RNAiso Plus, TaKaRa®) following the manufacturer's guidelines. cDNA was synthesized using a PrimeScript™ first strand cDNA synthesis Kit (Takara Bio Inc) following the supplied protocol. Real-time PCR analysis for the genes *TaNHX1*, *TaSOS1* and *TaHKT2* was conducted using the GoTaq® qPCR Master Mix in line with the protocol. The specific primer sequences used are provided in Supplementary Table 1. Relative gene expression was measured using $2^{-\Delta\Delta CT}$ (Schmittgen and Livak 2008). The wheat ubiquitin gene was used as a housekeeping gene to normalize the expression levels. qRT-PCR data was analysed, and graphs were created using GraphPad Prism (version 9.1).

RESULTS AND DISCUSSION

High salt in soil, characterized by excess sodium ions at exchange sites, leads to several adverse effects on plant growth due to ionic imbalance and toxicity caused by the accumulated sodium ions (Balasubramaniam *et al.* 2023). Earlier studies reported a significant decline in the performance of wheat genotypes under conditions of high soil sodicity (Anzooman *et al.* 2023). In this study, salt tolerance of a wheat RIL population was assessed by examining physiological, biochemical, morphological, and yield-related traits.

Effects of salt stress on yield traits: Salinity stress adversely affects wheat growth, yield and physiological processes (Loudari *et al.* 2022). HD2851 displayed a relatively superior grain yield of 339.8 g under control conditions; however, it experienced a more substantial yield reduction of 65.51% under sodic conditions compared to KH65, which exhibited a yield reduction of 45.08%. Notably, KH65 showed a significantly higher grain yield of 134.77 g under sodic conditions compared to HD2851, 117.2 g. Furthermore, KH65 showed a lower reduction in 1000-grain weight (8.68%) compared to HD2851 (19.8%) (Table 1). Earlier studies have also reported higher grain yield in KH65 under salt stress (Devi *et al.* 2018). These indicate that KH65 is tolerant to salt stress. Similarly, the mean yield of RILs showed a reduction under sodic conditions compared to control conditions. GY ranged from 54.25–649.55 g with an average of 244.86 g in RILs under control conditions and varied from 33.34–294.48 g with an average of 115.8 g under stress conditions (Table 1). Previous studies have also documented a significant decrease in yield for both

Table 1 Mean values and variability of physiological and yield traits for the parent genotypes HD2851 (susceptible) and KH65 (tolerant), along with the RILs population, under control and sodicity conditions

Parents/ Trait	KH65		HD2851		RILs			
					Mean ± SD	Range	Mean ± SD	Range
	C	S	C	S	C	C	S	S
CHL	23.90±0.90	17.40±0.20	21.9±0.28	12.30±0.24	26.39±4.50	13.12-50.26	19.57±3.58	7.75-29.30
Na^+	2.68±0.05	5.12±0.22	2.69±0.01	8.34±0.26	1.46±0.58	0.10-4.12	3.65±2.10	0.14-11.91
K^+	118.2±2.09	21.48±0.91	102.7±0.28	11.50±0.22	45.43±19.52	1.96-119.94	34.63±18.03	0.87-86.17
K^+/Na^+	44.1±2.93	4.20±0.18	38.18±0.15	1.38±0.06	33.87±16.64	10.24-101.06	10.34±4.54	1.31-23.88
DTH	79.00±2.56	93.00±0.50	79.00±1.50	89.00±1.93	82.63±5.76	68.00-95.00	92.54±5.34	78.0-103.0
PH	111.0±3.28	76.33±3.06	84.33±2.52	63.00±5.0	98.09±15.76	57.0-148.0	72.73±13.44	18.0-105.0
SL	8.17±1.61	6.90±0.53	9.80±1.18	7.30±2.01	10.06±1.99	6.0 -22.1	7.84±1.23	4.0 -13.0
SPS	15.00±1.73	16.00±1.0	13.67±1.53	14.00±1.73	16.95±2.00	12.0-23.0	15.46±2.44	10.0-30.0
FLL	24.97±3.02	22.50±2.0	25.67±1.61	17.67±4.07	27.38±4.53	15.5 -42.5	18.27±4.05	9.0-35.0
FLA	33.18±5.55	24.14±1.40	39.71±3.58	20.58±6.35	36.09±8.91	14.03-73.31	18.79±6.36	3.0-39.9
TGW	44.93±1.57	41.03±4.60	41.40±0.47	33.20±0.18	30.19±5.30	13.65-51.73	34.61±4.27	21.39-48.29
GY	245.4±0.89	134.77±4.6	339.8±4.59	117.20±2.75	244.86±88.15	54.25-649.55	115.80±40.75	33.34-294.48
BY	1117±33.2	325.00±1.8	1146±26.9	338.00±8.53	1070.53±233	240.28-2074	329.03±107.76	111.73-954

C, Control; S, Sodicity; CHL, Chlorophyll content (mg^{g} FW); Na^+, Sodium content (mg^{g} DW); K^+, Potassium content (mg^{g} DW); K^+/Na^+, Potassium to sodium ratio; DTH, Days to heading; PH, Plant height (cm); SL, Spike length (cm); SPS, Spikelets/spike; FLL, Flag leaf length (cm); FLA, Flag leaf area (cm²); TGW, 1000-grain weight (g); GY, Grain yield (g); BY, Biological yield (g).

tolerant and sensitive wheat genotypes after exposure to sodicity (Sabagh *et al.* 2021).

Effects of salt stress on biochemical traits: The flag leaves exhibited more Na^+ accumulation when exposed to sodicity in wheat. The accumulation of Na^+ in the flag leaf under salt stress reduced growth and productivity in wheat genotypes (Hussein *et al.* 2023). Following exposure to sodicity, the leaf tissues of KH65 exhibited a 1.9-fold increase in Na^+ content, while HD2851 showed a 3.1-fold increase (Table 1). A significant variation in K^+ content in flag leaf was observed between these two genotypes under sodicity conditions. KH65 exhibited a 5.5-fold decrease in K^+ content compared to 8.9-fold decrease in HD2851 following exposure to salt (Table 1). Earlier studies have reported higher Na^+ accumulation and reduced K^+ levels in sensitive wheat genotypes under salt stress (Patwa *et al.* 2024).

A significant rise in sodium content within plants is accompanied by a general reduction in potassium content under salt stress condition (Lindberg and Premkumar 2023). The leaves of KH65 showed 10.5-fold reduction in K^+/Na^+ ratio compared to 27.7-fold reduction in HD2851 under sodicity conditions (Table 1). The tolerant genotypes (KH65) have a unique ability to maintain a high K^+ concentration while keeping Na^+ accumulation in the leaves low. High sodium concentrations can hinder potassium uptake, resulting in potassium deficiency (Ketehouli *et al.* 2019). Maintaining a favourable K^+/Na^+ ratio helps plants manage osmotic stress, promoting growth and survival in saline conditions (Lindberg and Kumar 2023). KH65 showed a reduction of 27.20% in chlorophyll content compared to 43.84% in HD2851 under salt stress conditions (Table 1). In recombinant inbred lines, the chlorophyll content ranged from 13.12–50.26 mg/g FW, averaging 26.39 mg/g FW under control conditions, and 7.75–29.30 mg/g FW, with an average of 19.57 mg/g FW under sodicity conditions (Table 1). Chlorophyll content in wheat genotypes showed a notable reduction following salt treatment (Irshad *et al.* 2022).

Correlation analysis among various traits: A diverse array of phenotypic associations was observed across various traits under control and sodicity conditions (Table 2). Under controled conditions, a significant positive correlation was observed between grain yield (GY) and 1000-grain weight (TGW) (r= 0.39, P<0.001). Similarly, under sodicity conditions, correlation analysis showed that chlorophyll content (CHL) (r= 0.09, P<0.05), K^+/Na^+ ratio (r= 0.15, P<0.001), plant height (PH) (r= 0.22, P<0.001), spike length (SL) (r= 0.11, P<0.01), flag leaf length (FLL) (r= 0.10, P<0.05), flag leaf area (FLA) (r= 0.17, P<0.001), and TGW (r= 0.51, P<0.001) had a positive and significant contributions to GY. However, under sodicity conditions, GY showed a significant negative correlation with Na^+ content (r= -0.09, P<0.05) (Table 2). Earlier studies have also shown a negative correlation between grain yield and sodium level under sodicity conditions (Tao *et al.* 2021).

Principal component analysis: Principal component analysis (PCA) identified the key traits contributing to salinity tolerance (Mubushar *et al.* 2022). PCA of all 13 parameters under both control and sodicity conditions was conducted in the present investigation (Fig. 1 and Fig. 2). Through PCA, the 13 variables were condensed into six

Table 2 Pearson's correlation coefficient (r) among various traits measured in parent and RILs population under control conditions (lower diagonal) and sodicity conditions (upper diagonal)

Trait	CHL	Na^+	K^+	K^+/Na^+	DTH	PH	SL	SPS	FLL	FLA	TGW	GY	BY
CHL	1	-0.24***	-0.15***	0.01	0.23***	-0.001	0.21***	0.09*	0.05	0.08	0.08	0.09*	0.12**
Na^+	-0.01	1	0.53***	-0.33***	-0.15***	0.02	-0.07	-0.13**	0.04	0.10*	0.02	-0.09*	-0.16***
K^+	-0.12**	0.41***	1	0.54***	-0.06	0.06	-0.09*	-0.1***	-0.02	-0.01	0.03	0.03	-0.01
K^+/Na^+	-0.09*	-0.43***	0.55***	1	0.10*	-0.001	-0.03	-0.01	-0.04	-0.09*	0.01	0.15***	0.17***
DTH	-0.08*	-0.06	-0.02	0.02	1	-0.4***	0.10*	0.27***	-0.2***	-0.20***	-0.2***	0.003	0.12**
PH	-0.03	0.11**	-0.02	-0.13**	0.15***	1	0.05	-0.1***	0.08	0.14***	0.27***	0.22***	0.20***
SL	0.08*	0.10*	0.03	-0.05	0.26***	0.14***	1	0.37***	0.01	0.09*	0.09*	0.11**	0.10*
SPS	0.09*	0.02	-0.07	-0.08	0.38***	0.18***	0.51***	1	0.09*	0.10*	-0.06	0.04	0.10*
FLL	0.16***	0.04	0.03	0.003	-0.08*	-0.08*	0.07	0.07	1	0.86***	0.03	0.10*	0.15***
FLA	0.13**	0.03	0.05	0.003	-0.01	-0.10*	0.11**	0.08	0.84***	1	0.17***	0.17***	0.18***
TGW	-0.09*	0.15***	0.25***	0.04	-0.17***	-0.05	-0.2***	-0.2***	0.03	0.11**	1	0.51***	0.36***
GY	0.08	0.15***	0.12**	-0.05	-0.41***	-0.2***	-0.2***	-0.2***	-0.0004	-0.02	0.39***	1	0.88***
BY	0.16***	0.12**	0.08	-0.04	0.12**	0.19***	0.02	0.05	0.03	0.05	0.07	0.47***	1

C, Control; S, Sodicity; CHL, Chlorophyll content (mg^g FW); Na^+, Sodium content (mg^g DW); K^+, Potassium content (mg^g DW); K^+/Na^+, Potassium to sodium ratio; DTH, Days to heading; PH, Plant height (cm); SL, Spike length (cm); SPS, Spikelets/spike; FLL, Flag leaf length (cm); FLA, Flag leaf area (cm²); TGW, 1000-grain weight (g); GY, Grain yield (g); BY, Biological yield (g). '*', '**' and '***' indicates significance at P<0.05, P<0.01, and P<0.001, respectively.

components, explaining 80.69% of the overall variance observed. The first two principal components accounted for 38.39% of the overall trait variation (PC1, 21.14%; PC2, 17.25%) (Table 3). The eigenvalues of PC1 and PC2 were 2.75 and 2.24, respectively (Table 3**)**. The most effective traits associated with the first and second components were grain yield and days to heading (Supplementary Table 2). K^+/Na^+ ratio and K^+ content was identified as the principal traits for the 3rd and 4th components, respectively.

Table 3 Eigen values of the correlation matrix

Principal component	Eigen value	Percentage of variance	Cumulative
1	2.75	21.14%	21.14%
2	2.24	17.25%	38.39%
3	1.86	14.29%	52.68%
4	1.44	11.10%	63.78%
5	1.16	8.94%	72.72%
6	1.04	7.97%	80.69%

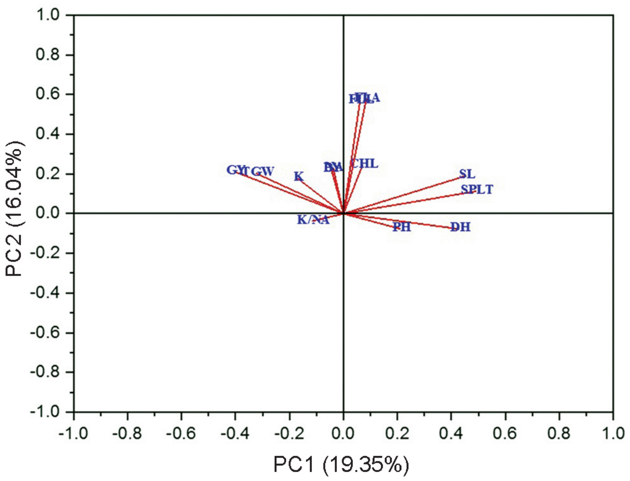

Fig. 1 Biplot representation showing the interconnections between traits under control condition. CHL, Chlorophyll content (mg^g FW); Na^+, Sodium content (mg^g DW); K^+, Potassium content (mg^g DW); K^+/Na^+, Potassium to sodium ratio; DTH, Days to heading; PH, Plant height (cm); SL, Spike length (cm); SPLT, Spikelets/spike; FLL, Flag leaf length (cm); FLA, Flag leaf area (cm²); TGW, 1000-grain weight (g); GY, Grain yield (g); BY, Biological yield (g).

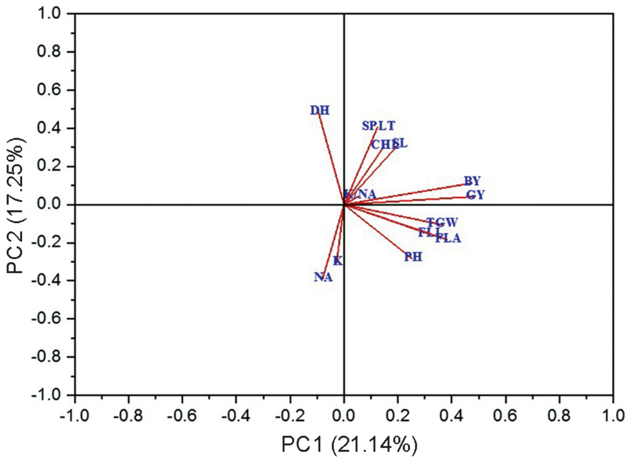

Fig. 2 Biplot representation using the first two principal components delineates various traits associated with sodicity tolerance. CHL, Chlorophyll content (mg^g FW); Na^+, Sodium content (mg^g DW); K^+, Potassium content (mg^g DW); K^+/Na^+, Potassium to sodium ratio; DTH, Days to heading; PH, Plant height (cm); SL, Spike length (cm); SPLT, Spikelets/spike; FLL, Flag leaf length (cm); FLA, Flag leaf area (cm²); TGW, 1000-grain weight (g); GY, Grain yield (g); BY, Biological yield (g).

Meanwhile, Na^+ content and spike length were the most influential traits for the fifth and sixth components, respectively (Supplementary Table 2**)**. Therefore, GY, DTH, K^+/Na^+ ratio, K^+ content and SL are crucial in enhancing salt tolerance in wheat genotypes. Earlier studies have also identified K^+ content and K^+/Na^+ ratio as the most effective traits contributing to salinity tolerance in wheat genotypes (Chaurasia *et al.* 2022).

The direction and magnitude of arrows within the biplot reflect the influence and contribution of specific traits to these first two principal components. Arrow length signifies the degree of contribution to the PCA components, with longer arrows representing greater influence and shorter arrows suggesting lesser influence by the traits.

Expression analysis of salt-responsive genes: In the present study, the expression levels of *TaNHX1*, *TaSOS1* and *TaHKT2* genes were evaluated in the leaf tissue of salt-tolerant (KH65, RIL8 and RIL130) and salt-sensitive (HD2851, RIL61 and RIL154) wheat genotypes following salt treatment. The expression levels of the *TaNHX1*, *TaSOS1* and *TaHKT2* genes varied among six wheat genotypes following salt treatment.

Sodium/hydrogen antiporter (*TaNHX1*) helps move Na^+ from the cytosol into the vacuole, thereby improving salt tolerance (Malakar and Chattopadhyay 2021). The upregulation of cation transporters is a crucial molecular mechanism that allows plants to endure salinity conditions (Karim *et al.* 2021). KH65 exhibited a significant upregulation of the *TaNHX1* gene (3.1-fold) compared to HD2851 (1.6-fold) following salt treatment (Fig. 3a**)**. Earlier studies have also reported a higher expression level of the *TaNHX1* gene in the leaves of KH65 under salt stress (Rana *et al.* 2016, Singh *et al.* 2019). RIL8 and RIL130 displayed significant upregulation of 5.5 and 5.1-fold, respectively. RIL61 and RIL154 displayed upregulation of 1.4-fold and 0.6-fold, respectively (Fig. 3a). Higher upregulation of the *TaNHX1* gene in the leaves of RIL8 and RIL130 indicates that these RILs are more efficient in sequestering excess sodium ions into vacuoles. However, lower expression of the *TaNHX1* gene in the leaf tissues of RIL61 and RIL154 suggests that these RILs are less effective at sequestering sodium into the vacuoles. Earlier studies reported that salt-tolerant wheat genotypes exhibited increased expression of *TaNHX* and more significant vacuolar Na^+ sequestration compared to sensitive genotypes (Wu *et al.* 2015).

The *TaSOS1* gene is responsible for the exclusion of sodium ions at the cell membrane under salt stress (Zheng *et al.* 2022). KH65 exhibited a 2-fold increase in *TaSOS1* gene expression, while HD2851 showed a 1.2-fold increase (Fig. 3b). Earlier studies have reported higher upregulation of *TaSOS1* genes in the root tissues of KH65 exposed to salt stress (Rana *et al.* 2016). Tao *et al.* (2021) observed an increase in the relative expression of *TaSOS1* gene in wheat under salt stress. RIL8 and RIL130 displayed significant upregulations with a 3.5-and 3.0-fold increase, respectively. RIL61 and RIL154 exhibited a lower upregulation of gene expression with 1.2- and 0.4-fold change, respectively (Fig. 3b). Wheat genotype with a high expression level of the *TaSOS1* gene showed enhanced tolerance to salinity stress (Jiang *et al.* 2021).

HKT2 transporters are essential for salinity tolerance by facilitating potassium uptake (Ali *et al.* 2019). The *TaHKT2* gene was upregulated 2.3-fold in KH65 and 1.1-fold in HD2851 following exposure to salt stress. RIL8 and RIL130 showed upregulation of 4-fold and 1.6-fold, respectively, while RIL61 and RIL154 exhibited upregulation of 0.9-fold and 0.5-fold, respectively (Fig. 3c). Earlier studies have reported that the expression of *TaHKT2* was upregulated in salt sensitive wheat genotype under salt stress (Irshad *et al.* 2022).

A least significant difference (LSD) test was performed to determine significant variations between means at a significance level of $P \leq 0.05$, presented as Mean ± Standard Error (SE).

In conclusion, there were significant positive correlations between grain yield and several traits, including

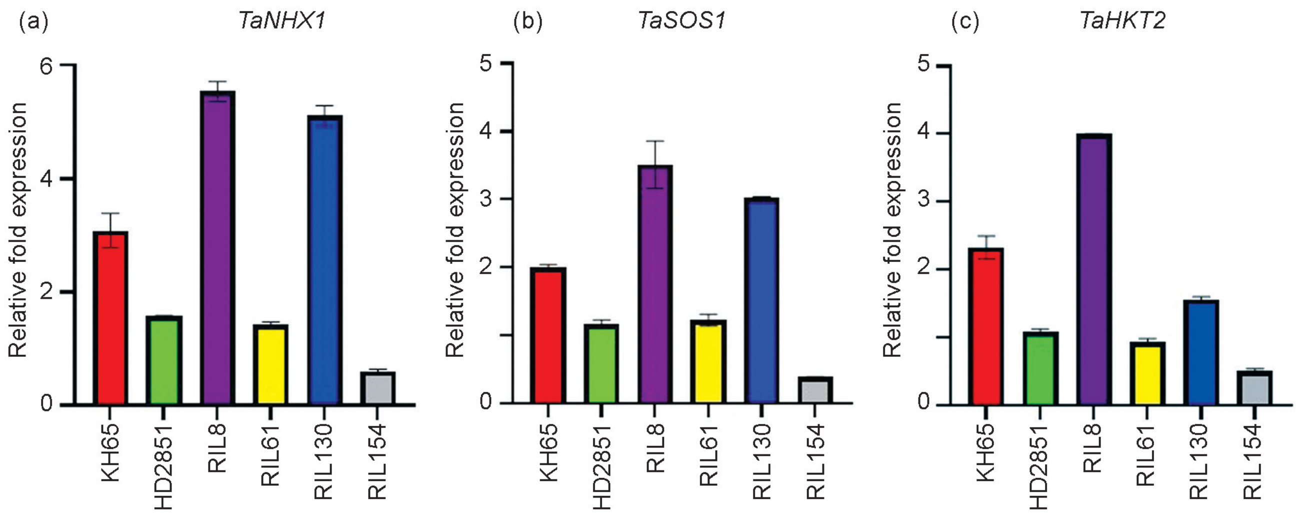

Fig. 3 Relative expression *of TaNHX1*, *TaSOS1* and *TaHKT2* genes across different genotypes following salt treatment.

chlorophyll content, K^+/Na^+ ratio, plant height, spike length, flag leaf area, and 1000-grain weight under sodicity conditions. Conversely, Na^+ content displayed a significant negative correlation with grain yield. The PCA identified grain yield as the most effective trait for assessing tolerance to salt stress. Expression analysis of *TaNHX1*, *TaSOS1* and *TaHKT2* genes has provided insights into the specific responses of salt-tolerant and salt-sensitive wheat varieties under salt stress conditions. The expression of *TaNHX1*, *TaSOS1* and *TaHKT2* genes were significantly upregulated in KH65, RIL8 and RIL130, suggesting that these varieties possess enhanced capabilities for Na^+ exclusion at the plasma membrane and Na^+ sequestration in vacuoles. A comprehensive understanding of these gene structural, functional, and regulatory mechanisms will enhance our ability to improve salt tolerance in wheat, especially in elite genotypes responding to salt stress.

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