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Identification of physiological traits at seedling stage associated with salt tolerance in wheat variety KH 65 using RILs

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Abstract

Salinity is a major stress affecting production and productivity of wheat across the world. Understanding physiological traits associated with salt tolerance can help in breeding for improving wheat under salt stress. In the present investigation, physiological traits in 3rd leaf at seedling stage and grain yield at maturity were studied in a population of RILs derived from a cross between salt tolerant (Kharchia 65) and susceptible (HD 2009) cultivars under control (pH 8.2) and sodic condition (pH 9.2) in microplots. Though, HD 2009 had higher yield under control conditions, it exhibited higher reduction in yield (44.7%) under sodic stress as compared to KH 65 (9.8%). There was asignificantly lower accumulation of Na^+ content and higher accumulation of K^+, proline and chlorophyll content in 3rd leaf of KH 65 as compared to HD 2009 under sodic stress. Na^+ content exhibited significant negative correlation ($P<0.01$) while K^+/Na^+ ratio, proline content and chlorophyll content showed significant positive correlation ($P<0.01$) with grain yield (GY) under sodic condition. First and second principal component analysis (PCA) explained total variation of 66.43% (PCI 50.18 % and PCII 16.25%) among different traits under sodic conditions. Na^+ content made independent group with strong negative correlation with grain yield and K^+/Na^+ ratio while proline content and thousand grain weight (TGW) were grouped together along with GY. The study demonstrated that low Na^+ concentration and high K^+/Na^+ ratio, proline and chlorophyll content at seedling stage are important physiological traits contributing towards yield under sodic stress. The information is useful in breeding programme of wheat for salt tolerance.

Keywords: Sodicity, wheat (*Triticum aestivum* L.), RILs, salt tolerance, PCA.

1. Introduction

Wheat is one of the most important cereal crops contributing substantially in food and nutritional security. However, production and productivity of wheat is affected by several abiotic constraints including high and low temperature, drought and salt stress. Among these stresses, salt stress is the major stress spreading worldwide and results from accumulation of soluble salts in the root zone (Ashraf and Foolad, 2007) which causes annual losses of large area of arable land (Pressarakli and Szabolcs, 1999). Approximately, 900 million hectares of land in the world and 7 million hectares in India are salt affected areas (FAO, 2008). Among salt affected soils, sodic soilshave excess of Na^+ ion on exchange sites and associated with high pH (8.5-10.2) (Sharma *et al.*, 2004). Development of salt tolerant cultivars is required as soil remediation is difficult to apply in all situations by the farmers. However, breeding for salt tolerance is difficult because of unavailability of selection criteria and complexity of the trait. Therefore, understanding physiological basis of salt tolerance is needed to improve wheat varieties for salt stress (Munns and Tester, 2008).

Salt stress affects all stages of plant development including germination, seedling, vegetative growth and mature stage. It adversely affects seedling establishment at early growth stages and causes yield reduction (Bahrani *et al.*, 2012). Seedling stage can be used in selecting tolerant plants under salt stress (Aflaki *et al.*, 2017).The present investigation was conducted to know the effect of sodic stress on physiological traits including Na^+ content, K^+ content, proline content and chlorophyll content at seedling stage in a set of 114 RIL populations developed of a cross between KH 65 × HD 2009 cultivars. Kharchia 65 (KH 65) is a well-known wheat genotype tolerant to salt stress (Munns *et al.*, 2006, Sairam *et al.*, 2002; Rana *et al.*, 2015) and has been used extensively throughout the world in breeding programmes. However, very little is known about the physiological basis of tolerance in KH 65. Several physiological changes occur in response to salt stress in plant. Understanding physiological basis of salt tolerance in KH 65 can lead to enhanced efficiency in selection of desirable plants in breeding. This needs controlled conditions for experimentation as soil heterogeneity for salt concentration under natural conditions affect the true expression of genotype. Therefore, specially designed microplots were used in this investigation to identify physiological traits associated with salt stress in wheat.

2. Materials and methods

2.1. Experimental Design: A microplot experiment was conducted at ICAR-Indian Institute of Wheat & Barley Research (IIWBR), Karnal during 2015-16 crop season. A set of 114 recombinant inbred lines (RILs-F8) derived from a cross between KH 65 (salt tolerant) × HD 2009 (salt sensitive) were grown under control(pH~8.0) andsodic soil (pH 9.2) conditions in microplot (3m × 6 m) with row length of 75 cm and row to row space of 20 cm. Microplots were developed by adding the required amount of sodium bicarbonate ($NaHCO_3$) and sodium carbonate (Na_2CO_3) to the soil. Two replications of each sample were taken for estimating Na^+ content (mg^{-g} DW), K^+ content (mg^{-g} DW), proline content (μg^{-g} FW), chlorophyll content (mg^{-g} FW) in 3rd leaf at seedling stage and thousand grain weight (g) and grain yield (g) per row of 75cm after harvest.

2.2. Measurement of ions: 3rd leaf(10 days after leaf appearance) was used for the measurement of Na^+ and K^+ concentration at seedling stage. 100 mg leaf samples were dried for 48 hrs at 65°C and digested in 0.5 ml of 0.5N HNO_3 for 2hrs at 80°C as reported by Munns *et al.*, 2010. The extract was centrifuged at 10000 rpm for 5 minute followed by 100 times dilution of the supernatant. Concentrations of Na^+ and K^+ were measured by flame photometer using standards in the range of 0.25 ppm to 20 ppm and expressed on dry weight basis as milligram per gram dry weight (mg^{-g} DW).

2.3. Measurement of Proline: Proline content of 3rd leaf was extracted using ninhydrin reaction method (Bates *et al.*, 1973). 50 mg of fresh leaf sample was homogenized in 3% sulphosalicylic acid (5μl^{-mg} fresh weight), kept on ice for 5 minutes and centrifugedat 14,000 g for 10 min at room temperature. Reaction mixture contained 200µl glacial acetic acid, 200µl ninhydrin reagent and 100µl of supernatant. Incubatethe reaction mixture for 20 min at 90°C in water bath then, terminate the reaction on ice. 1ml toluene was added in reaction mixture and mixed by vortex. The upper toluene phase was used for spectrophotometric analysis at 520 nm. The concentration was measured using proline as the calibration standard. The proline content expressed in microgram per gram fresh weight (μg^{-g} FW).

2.4. Measurement of chlorophyll content: Chlorophyll content was estimated by extracting 50 mg fresh weight of the leaf material in 10 ml dimethylsulfoxide (DMSO) (Hiscox and Israelstam, 1979). Samples were heated in an incubator at 65 °C for 4 h and cooled to room temperature; the absorbance of the extracts was recorded at 663 nm. Chlorophyll content of the extract inµg/ml was calculated as Chl_{Total}: [20.2 x A_{645} + 8.02 x A_{663}]. Values of Chl_{Total}in mg^{-g} fresh weight were obtained by multiplying the above values with “V/W x 1000”, where V is volume of extract; W is fresh weight of sample as per Arnon (1949).

2.5. Statistical analysis: SAS software version 9.3 was used for statistical analysis for estimating correlation among different traits under control and sodicconditions. PCA was performed by Genstat software to identify the distribution of RILs vis a visdifferent traits and to identify traits contributing towards yield.

3. Results and Discussion

Tolerance to salt stress is a complex biological phenomenon governed by several physiological and genetic factors, and it is growth stage specific (Haq *et al.* 2010). Variations in physiological traits and their inter-relationships in seedling stage, and TGW and grain yield after harvest, under sodic stressare discussed below.

3.1. *Effects of salt stress on yield traits:* Though HD 2009 exhibited comparatively higher grain yield (GY) under control conditions, it showed higher reduction in yield (44.7%) under sodic conditions as compared to KH 65 (9.8%) (Table1). There was significantly higher GY in KH 65 (56.0g) as compared to HD 2009 (35.5g) under sodic condition. Similarly, RILs also showed wide range in yield and yield reduction under sodic condition. GY varied from 21 to 88g with the mean value of 50.53g in RILs under control condition and from 14.85 to 70.10 g with the mean value of 37.17 g under sodic condition. There are other reports also showing wide range of reduction in yield in tolerant and sensitive cultivars from control to sodic stress condition in wheat (Akbarpour *et al.*, 2015). There was also higher percentage of reduction in TGW in HD 2009 (23.0%) as compared to KH 65 (9.7 %). Thus, KH 65 exhibited tolerance to high pH with higher yield and TGW under sodic conditions. TGW varied from 24 to 49.6 g with the mean value of 39.19 g in RILs under control condition and from 20 to 46.05 g with the mean value of 36.02 g under sodic condition.

3.2. *Effects of salt stress on ion concentrations:* Though there was increase in Na^+ accumulation in 3rd leaf under salt conditions, it was significantly lower in KH 65 (6.01 mg^{-g} DW) as compared to HD 2009 (9.01 mg^{-g} DW) and varied from 4.47 to 16.75 mg^{-g} DW among RILs (Table 1). The important locationof Na^+ toxicity for most plants is the leaf blade, thus excluding Na^+ from the leaves blades is considered important for salt tolerance (Munns *et al.*, 2008). In general, more of sodium is accumulated under salt stress which results into ionic imbalance, and thus affect plant metabolism (Tavakkoli *et al.*, 2011). There was a significantly higher content of K^+ in KH 65 (21.6 mg^{-g} DW) as compared to HD 2009 (15.80 mg^{-g} DW) under sodic condition. In RILs K^+ content varied from 17.01 to 46.32 mg^{-g} DW with the mean value 31.25 mg^{-g} DW in control and 9.13 to 28.64 mg^{-g} DW with the mean value of 18.30 mg^{-g} DW in sodic condition (Table 1). Salt stress induced increase in sodium and depletion of potassium contents has also been reported earlier (Sairam *et al.*, 2002). Ratio of K^+/Na^+ was higher in KH 65 (3.18) as compared to HD 2009 (1.75) under sodic condition. High levels of Na^+ inhibit the K^+ uptake resulting into K^+ deficiency (Khan *et al.*, 2009). Thus regulation of K^+/Na^+ ratio has important role in imparting tolerance to salt stress in KH 65. Optimum K^+/Na^+ ratio can help in maintaining an ideal osmotic and membrane potential for cell volume regulation in plant under salt stress (El-Hendawy *et al.* 2009). The data showed that KH 65 had low leaf Na^+ accumulation and relatively high K^+ concentration and thus exhibited higher tolerance under sodic conditions.

Table1. Means (± standard error) and variation of physiological and yield traits for parental genotypes KH 65 (tolerant) and HD 2009 (susceptible), and RILs population under control (pH 8.0) and sodic condition (pH 9.2).

Traits	Parents				RILs			
	KH65		HD2009		Mean ± SD	Range	Mean ± SD	Range
	C	S	C	S	C	C	S	S
Na^+ (mg^{-g} DW)	0.98	6.01	0.88	9.01	1.21±0.34	0.47-2.05	9.32±1.21	4.47-16.75
K^+ (mg^{-g} DW)	36.67	21.6	34.66	15.8	31.25±1.19	17.01-46.32	18.30±1.37	9.13-28.64
K^+/Na^+	37.42	3.18	39.39	1.75	29.08±2.39	21.90-35.94	2.37±0.65	0.82-5.82
Proline (ug^{-g} FW)	0.76	2.15	0.61	0.91	0.57±0.27	0.22-0.99	3.12±3.81	0.60-2.50
CHL Content (mg^{-g} FW)	41.09	38.51	38.63	29.1	34.99±1.05	23.0-48.75	31.69±0.82	19.94-39.89
TGW (g)	39.9	36	39	30.2	39.19±0.78	24.0-49.6	36.02±1.13	20.0-46.05
GY (g)	62.1	56	64.2	35.5	50.53±2.27	21.0-88.0	37.17±2.62	14.85-70.10

C-Control; S-Sodic; Na^+-Sodium content; K^+-Potassium content; K^+/Na^+-Potassium sodium ratio; CHL- Chlorophyll content; TGW- Thousand grain weight; GY- Grain yield per row of 75 cm.

3.3. *Effects of salt stress on proline accumulation and chlorophyll content*: Though, there was significant increase in proline content under sodic condition in both the cultivars, it was significantly higher in KH 65 (2.15μg^{-g} FW) as compared to HD 2009 (0.91μg^{-g} FW).In RILs proline content varied from 0.22 to 0.99μg^{-g} FW with the mean value 0.57μg^{-g} FW in control and 0.60 to 2.50μg^{-g} FW with the mean value of 3.12μg^{-g} FW in sodic condition (Table 1). Accumulation of proline content under stress protects the cell by balancing the osmoticstrength of cytosol with that of vacuole and externalenvironment (Gadallah *et al.*, 1995). In addition, it has role in protecting enzymes activity under stress conditions. The data further support various earlier studies showing a positive role of proline in salt tolerance (Khan *et al.*, 2009; Ashraf and Foolad, 2007; Munns, 2005). Chlorophyll content was higher in KH 65 (38.51 mg^{-g}FW) as compared to HD 2009 (29.10 mg^{-g}FW) under sodic condition (Table 1). In RILs chlorophyll content varied from 23.0 to 48.75mg^{-g} FW with the mean value 34.99 in control and 19.94 to 39.89mg^{-g}FW with the mean value of 31.69mg^{-g}FW in sodic condition. Other reports indicated that the high Na+ lines lost chlorophyll more rapidly and died earlier than the low Na^+ lines (Munns *et al.* 2006; Oyiga *et al.*, 2016). Azadi *et al.* (2011) observed decrease in chlorophyll content at 150 mMNaCl treatment as compared to control in wheat. Similarly, Sai Ram *et al.*, 2002 also suggested the role of chlorophyll in imparting tolerance to salt stress in crop plants at seedling stage.

3.4. *Correlation between various traits:* A wide range of correlations wereobservedamong various traits under control and sodic conditions (Table 2). Significant positive correlations were found between GY and TGW under control (r = 0.339, P<0.01) and sodic (r = 0.569, P<0.001) conditions. Though Na+content showed no correlation with GY under control condition,it exhibited significantly negative correlation (r = -0.240, P<0.01) under sodic condition (Table 2). Other studies also showed negative correlation of Na^+ with GY under salt stress (Cuin *et al.*, 2008). Though K^+ content did not show any correlation with GY under sodiccondition, it exhibited significant positive correlation with K^+/Na^+ ratio, proline content, chlorophyll content and TGW. Furthermore, biochemical traits includes K^+/Na^+ ratio, proline content and chlorophyll content showed positive correlation with GY under sodic condition (Table 2). This is in agreement with some of the studies indicating role of K^+/Na^+ ratio, proline and chlorophyll in imparting salt tolerance (Goudarzi *et al.*, 2008; Thalji *et al.*, 2007). Significant positive correlation between proline and GY indicated the positive role of proline in combating salt stress by its increased accumulation under stressed condition. The data indicated that low Na^+ accumulation and high K^+/Na^+ ratio, proline content and chlorophyll content are important traits for tolerance in seedling stage. Screening for the tolerance at seedling stage may be helpful in finding tolerant genotypes with high yield potential under saltstress (Aflaki *et al.*, 2017).

Table 2. Correlation between different traits under control (lower diagonal) and sodic (above diagonal) conditions in RILs of the cross between KH 65 and HD 2009

Trait name	Na^+	K^+	K^+/Na^+	P	CHL	TGW	GY
Na^+	1	-0.303**	-0.703***	-0.491**	-0.356**	-0.431**	-0.240**
K^+	-0.244**	1	0.829***	0.449**	0.108	0.333**	0.176
K^+/Na^+	-0.847***	0.590***	1	0.558***	0.303**	0.483**	0.264**
Proline	-0.114	0.336**	0.200*	1	0.217*	0.544***	0.298**
CHL	0.052	0.357**	0.108	0.349**	1	0.252**	0.260**
TGW	-0.063	0.184*	0.165	0.003	0.045	1	0.569***
GY	-0.044	-0.047	0.035	0.063	0.059	0.339**	1

Na^+-Sodium content; K^+-Potassium content; K^+/Na^+-Potassium sodium ratio; CHL- Chlorophyll content; TGW-Thousand grain weight; GY- Grain yield.*P < 0.05; **P < 0.01; ***P <0.001

3.5. *Principal component analysis (PCA)*: Principal component analysis (PCA) is another way of understanding the interrelationships between traits and thus can identify screening criteria under sodic stress. Figure 1 shows the principal component analysis (PCA) of all seven traits under control conditions. Based on PCA, the first two components explained 54.02 % (PC1, 34.43%; PC2, 19.59%) of the total variation among different traits under control condition (Figure 1). However, under sodic condition the first and second PCA explained total variation of 66.43% (PCI 50.18 % and PCII 16.25 %) among different traits (Figure 2).Under control condition chlorophyll and proline content grouped together with GY while chlorophyll and TGW were grouped with GY under sodic conditions. The K^+ content and TGW were grouped together in control condition and showed correlation with GY (Figure1). While under sodic condition K^+ content, K^+/Na^+ ratio and proline content were grouped togetherand thus can be used interchangeable for selecting desirable genotypes under sodiccondition. Na^+ content showed independent group with strong negative correlation with grain yield and thus suggests that the exclusion of toxic ions may be an important component of salt tolerance in wheat as reported by EL-Hendawy *et al.*, 2017.

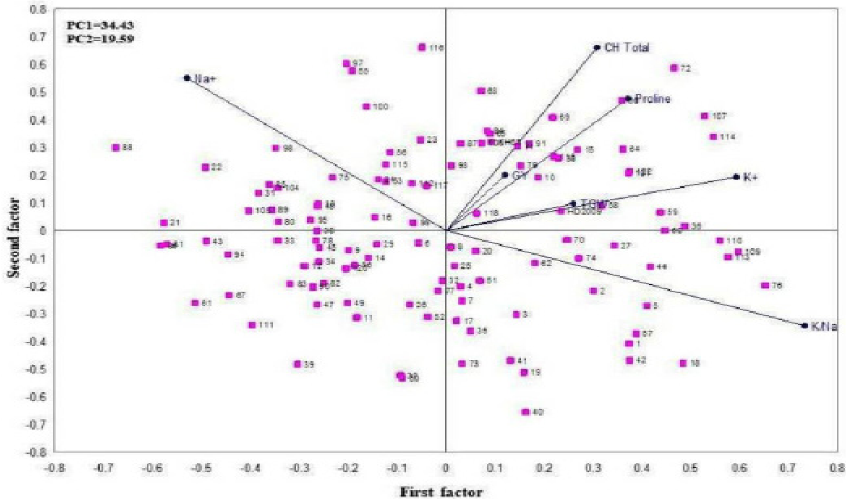

Figure 1. Vector view of the biplot showing interrelationships among traits under control conditions. Na^+- Sodium content; K^+- Potassium content; K^+/Na^+- Potassium sodium ratio; P- Proline content; CH Total- Chlorophyll content; TGW- Thousand grain weight; GY- Grain yield.

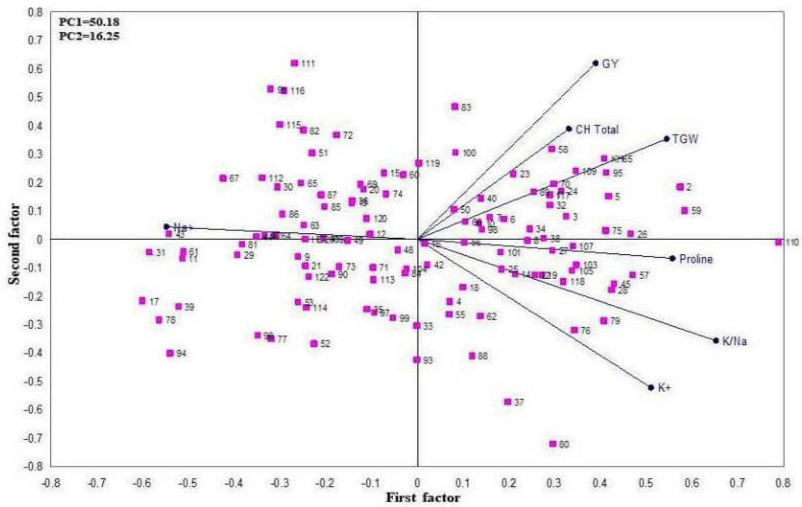

Figure 2. Vector view of the biplot showing interrelationships among traits under sodic stress conditions. Na^+- Sodium content; K^+-Potassium content; K^+/Na^+- Potassium sodium ratio; P- Proline content; CH Total- Chlorophyll content; TGW- Thousand grain weight; GY- Grain yield.

In conclusion, significant positive correlations were observed between grain yield and K^+/Na^+ ratio, proline content and chlorophyll content.While Na^+ content showed negative correlation with grain yield under sodic stress. This demonstrated that low Na^+ concentration and high proline and chlorophyll contentat seedling stage are important physiological traits contributing towards yield under salt stress and thus can be used as selection criteria in breeding programme.

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