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Full Length Article

Screening and Selection of Synthetic Hexaploid Wheat Germplasm for Salinity Tolerance Based on Physiological and Biochemical Characters

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Abstract

Salinity is one of the major abiotic stresses affecting plant growth and development as salinization of cultivated land is increasing globally. There is considerable variation in salinity tolerance of wheat genotypes and selection of salt tolerant genotypes is of great interest in salt affected regions. An experiment was conducted to evaluate the salt tolerance of 13 newly developed synthetic hexaploid wheats (2n=6x=42; AABBDD) along with two check varieties Kharchia-65 and Shorawaki. Thirteen-day-old seedlings, grown in a hydroponics system, were subjected to 0, 75 and 150 mM NaCl in Hoagland's nutrient solution for five days. Increasing salt stress generally affected all physiological aspects of the plants; however, various enzyme activities, proline content, soluble sugars and protein content increased with increased salt concentration. Exposure to salt stress affected plant dry biomass of all the genotypes; however, there was a difference in response of wheat genotypes to salinity stress. Among the tested genotypes, Kharchia-65, Shorawaki, N-7, N-9 and N-13 showed better performance in terms of plant biomass, K^+: Na^+ ratio, chlorophyll content, net assimilation rate (*A*), transpiration rate (*E*) and stomatal conductance (g_s). There was a strong correlation between K^+:Na^+ ratio, chlorophyll, proline, SOD, CAT and g_s against shoot dry biomass. Based on overall performance, the tested wheat genotypes were grouped as tolerant, moderately tolerant and sensitive. Wheat genotypes N-7, N-9 and N-13 were grouped as tolerant, N-33 and N-12 as moderately tolerant and the remaining genotypes were found sensitive to salt stress. In this regard K^+:Na^+ ratio, chlorophyll, proline, SOD, CAT and g_s may be used as potential biochemical and physiological selection criteria for screening of salt tolerance in wheat genotypes. © 2014 Friends Science Publishers

Keywords: Plant biomass; Physiological and biochemical attributes; Salt stress; Synthetic hexaploid wheat

Introduction

Wheat (*Triticum aestivum* L.) is an important cereal crop and a source of staple food for many countries. Wheat yield is reduced by abiotic stresses such as salinity, drought and heat in arid and semi arid regions of the world (Zhang *et al.*, 2010; Farooq *et al.*, 2011, 2013, 2014). Of these abiotic stresses, salinity is one of the major stresses, regarded as highly deleterious to the growth and productivity of wheat crop (Zhang *et al.*, 2010; Wakeel *et al.*, 2011; Jafar *et al.*, 2012). Salt effected soils can be brought under cultivation by producing salt tolerant germplasm. This involves identification of wheat gemplasm on the basis of physiological and biochemical traits that are tolerant to salinity or using new genetic resources to introduce new genes for salt tolerance into existing cultivars (Farshadfar *et al*., 2008). A method of introducing novel genes into hexaploid bread wheat is through synthetic hexaploid wheats via bridge crossing, which are produced from interspecific crosses between tetraploid *Triticum turgidum* L. (2n=4x=28; AABB) and diploid *Aegilops tauschii* (Coss) Schmal. (2n=2x=14; DD), as described by Mujeeb-Kazi *et al*., (1996). The salinity tolerance trait present in D-genome diploid ancestor of wheat (*Ae. tauschii*) is potentially transferable to synthetics when this donor is crossed with susceptible durum parents (Trethowan and Mujeeb-Kazi, 2008).

Salinity stress affects plant growth and development at all levels. In wheat, salinity decreases pant growth, due to osmotic effect and accumulation of excessive concentrations of Na^+ and Cl^-, and consequently declines in the availability of assimilates to growing tissues and organs (Munns, 2007). Elevated Na^+ level in plant tissue may harm membranes and organelles and ultimately affect growth (Hussain *et al*., 2013). Thus, less Na^+ uptake and higher K^+:Na^+ ratios in plant tissue has well correlated with salt tolerance (Flowers,

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2004). A reduction in photosynthetic pigments due to toxic level of Na^+ in plant tissue under saline conditions has been reported in various plant species (Ashraf and Harris, 2013). Excess cellular Na^+ and Cl^- impair the electron transport system and accelerate the production of reactive oxygen species (ROS) (Foyer *et al.,* 1994). Elevated ROS inside cells is highly injurious to membranes and other cellular components, including chloroplasts, proteins, nucleic acids and lipids. In order to combat excessive intercellular ROS, plants have developed antioxidant defense systems assisting them in managing ROS levels. Plants employ various mechanisms to protect themselves from the adverse effects of salinity. Plants respond to salinity stress by accumulating proline and sugar; these act as osmoprotectants against salt injury to plants (Din *et al*., 2008; Gurmani *et al*., 2013). Salinity also affects photosynthetic performance of plants such as net assimilation rate, transpiration rates, stomatal conductance and water use efficiency (Ashraf, 2004). Variation in gas exchange attributes not only prevail in different species, but within cultivars of the same species. Therefore gas exchange traits may be employed as screening parameters for salt tolerance; where, a positive correlation exist between growth and gas exchange attributes (Ashraf, 2004).

In order to assess the salinity tolerance of plants, physiological and biochemical parameters are used to evaluate genotypic variation for salinity tolerance in various crop species (Ahmadi and Ardekani, 2006; Munns, 2007). Researchers have emphasized inorganic ions, organic metabolites, water relations and photosynthesis as important variables for salinity tolerance in plants (Ashraf, 2004). Plants subjected to salinity stress produce several metabolites that differ widely among species and even between genotypes (Flowers, 2004; Munns, 2007). Screening for salinity tolerance can done under saline field conditions in an appropriate growing season (SamMons *et al*., 1978); however, greenhouse based studies to screen for salinity tolerant plant types can also be conducted. One of the limitations of field studies is the heterogeneity of the field conditions, since salinity varies in patches under field conditions. On the other hand, it is relatively easy to maintain and control various salinity levels under controlled environment conditions, so that greenhouse studies are generally preferred over field for screening of salinity tolerance in crop plants (Munns and James 2003).

There is considerable genetic diversity in crop plants for salinity tolerance and a number of studies have focused on this in various crops, such as rice (Hussain *et al.*, 2013), Canola (Ahmadi and Ardekani, 2006), cotton (Shaheen *et al.,* 2012) and wheat (Munns, 2007). Wheat is the most widely cultivated cereal crop in the world and a staple food for many countries of the world. Consequently, development of salt tolerant wheat will assist in feeding the increasing global population. The present study was conducted to evaluate the performance of newly developed D genome diverse wheat germplasms under saline conditions by measuring various physiological and biochemical traits. Evaluations were further made as to whether these biochemical and physiological traits could be utilized as selection criteria to screen wheat genotypes for salinity tolerance.

Material and Methods

Plant Material

Seeds of thirteen new synthetic hexaploid wheat genotypes (2n=6x=42; AABBDD) including two check varieties Kharchia-65 and Shorawaki were obtained from the Wheat Wide Crosses Program at National Agricultural Research Centre (NARC), Islamabad, Pakistan (Table 1).

Growth Conditions and Treatment

The experiment was conducted under hydroponic conditions in the growth chamber of Plant Physiology Program of the Crop Sciences Institute, National Agricultural Research Centre (NARC), Islamabad, Pakistan. Wheat seeds were sown in jiffy pots and were allowed to grow for ten days, after which the seedlings were transplanted into a hydroponic system containing 3.0 L of full strength Hoagland's solution (Hoagland and Arnon, 1950). The hydroponic system was artificially prepared and comprised of black polyethylene boxes (3.0 dm^3, 3.5 L capacity). The growing conditions were a 12 h photo period of a minimum of 400-500 µmol m^{-2} s^{-1} photosynthetically active radiation at 27°C. The temperature during the dark period was 25°C. The seedlings were allowed to grow in the hydroponics for 3 days after which the seedlings (13 days old) were subjected to 0, 75 and 150 mM NaCl levels, as described below. On day 4, NaCl was added to the Hoagland's solution (in two split doses 50% added on day 4 followed by 50% on day 5) with the quantity of NaCl was adjusted according the treatment dose. The plants were allowed to grow in the presence of the various treatments for 5 days under conditions described above. Twenty day old plants (after seeding) were harvested and data were collected immediately on shoot and root length. The plants were dried in an oven (65°C) for at least 72h after which shoot and root dry weights were collected.

Determination of Physiological and Biochemical Parameters

Gas exchange measurements were taken using a portable photosynthesis system (LI-6400, Li-cor Inc., Lincoln, NE) on the fully expanded 3rd leaf of each plant. Data on photosynthesis (*A*), transpiration rate (*E*) and stomatal conductance (g_s) were determined. Measurements

were taken between 14:00 and 16:00 h (Ben-Asher *et al.,* 2006).

At the end of the experiment, the 3rd fresh leaf of each plant was harvested and immediately immersed in 15 mL 80% ethanol in a Pyrex test tube, capped and incubated for 10 min in a water bath at 85°C. The tubes were subsequently cooled in a dark room. This crude extract was used for chlorophyll, Na^+ and K^+ determination.

For chlorophyll determination, samples were taken from the crude extract and readings were taken at A_{666} immediately, with care to minimize exposure to light, using a spectrophotometer (Unico-UV 2100 Japan). Chlorophyll content was calculated according to Arnon (1949).

For Na^+ and K^+ determination, acetic acid was added to the crude extract to a final concentration of 100 M m^{-3} and the tissue was re-extracted for 24 h at 4°C (Yeo and Flowers, 1983). Sodium and potassium contents in the extract were then determined by atomic absorption spectrophotometer (Perkin-Elmer Model 4100L). The remaining leaf material was dried, weighed and the concentration of chlorophyll, Na^+ and K^+ were presented on a leaf dry weight basis.

Sugar content of fresh leaves was measuredby the procedure of Dubois *et al*., (1956). Wheat fresh leaves were homogenized in 10 mL of distilled water using a clean pestle and mortar. The homogenate was centrifuged at 3,000 rpm for 5 min followed by the addition of 1 mL of 80% (v/v) phenol. The extract was incubated for 1 h at room temperature followed by the addition of 5 mL concentrated H_2SO_4 and vigorously mixed. Readings were taken at A_{420} using a spectrophotometer. A glucose standard curve was prepared and the concentration of samples was determined using this standard curve.

Proline content was analyzed by the method of Bates *et al.,* (1973). Fresh leaves were homogenized in 10 mL of 3% aqueous sulfosalicylic acid and filtered through Whatman 42 filter paper. The filtrate was mixed with 2 mL acid-ninhydrin and 2 mL of glacial acetic acid and placed in a water bath for 1 h at 100°C. It was then extracted with 4 mL toluene and readings were taken at A_{520} using a spectrophotometer.

The amount of total soluble proteins was estimated with a similar extract (phosphate buffer extraction) as used in SOD (K-Na-phosphate buffer; 60 mM, pH 7.8). The homogenate was centrifuged at 1000 rpm for 15 min; 0.5 mL of the supernatant and 0.5 mL distilled water were added to 3 mL 5 fold diluted Bradford reagent (Bio-Red protein assay dye reagent). Absorbance was measured at 595 nm. Protein content was calculated using a bovine serum albumin calibration curve and expressed on a fresh weight basis (Bradford, 1976).

The SOD activity was determined by measuring the ability of the enzyme to inhibit phytochemical reduction of tetrazolium blue, according to the method of Giannopolitis and Ries (1977). Fresh leaf tissue (0.25 g) was homogenized in an ice bath with 1 mL of 100 mM L^{-1} sodium phosphate buffer containing 1% polyvinyl pyrollidone (PVP). Then, it was centrifuged at 10,000g for 15 min at 4°C. For SOD, the reaction mixture (3 mL) contained: K-Na-phosphate buffer (60 mM, pH 7.8), methionine (13 mM L^{-1}), riboflavin (12 mM L^{-1}), P-tetrazoleum blue (80 µM), EDTA (0.1 mM) and 100 µL of enzyme extract. The reaction was run for 10 min under illumination with 15 W fluorescence light. Reaction was stopped by switching off the light. The absorbance of the mixture was measured at A_{560} nm. The reaction mixture, without the enzyme extract, was utilized as control and a dark control mixture acted as a blank. One unit of SOD activity was taken as the quantity of enzyme that is able to inhibit tetrazoleum blue reduction by 50%, as determined at A_{650} nm. SOD activity was expressed in arbitrary units per mg of fresh weight.

Table 1: Detail of genotypes studied comprising of two land races and thirteen synthetic hexaploid wheats

Genotypes	Pedigree/Germplasm detail
Kharchia-65	Land race from Rajasthan, INDIA
Shorawaki	Land race from Baluchistan, PAKISTAN
N-7	*Triticum turgidum/Aegilops tauschii* (N-7)
N-9	*Triticum turgidum/Aegilops tauschii* (N-9)
N-10	*Triticum turgidum/Aegilops t*auschii (N-10)
N-11	*Triticum turgidum/Aegilops tauschii* (N-11)
N-12	*Triticum turgidum/Aegilops tauschii* (N-12)
N-13	*Triticum turgidum/Aegilops tauschii* (N-13)
N-14	*Triticum turgidum/Aegilops tauschii* (N-14)
N-15	*Triticum turgidum/Aegilops tauschii* (N-15)
N-25	*Triticum turgidum/Aegilops tauschii* (N-25)
N-26	*Triticum turgidum/Aegilops tauschii* (N-26)
N-31	*Triticum turgidum/Aegilops tauschii* (N-31)
N-33	*Triticum turgidum/Aegilops tauschii* (N-33)
N-34	*Triticum turgidum/Aegilops tauschii* (N-34)

Peroxidase activity (POD) was assayed by the method of Pundir *et al.* (1999). Fresh leaf tissue (0.25 g) was homogenized in an ice bath with 1 mL of 100 mM L^{-1} sodium phosphate buffer containing 1% PVP. Then, it was centrifuged at 10,000 g for 20 min at 4°C. POD; CAT enzyme extract was determined with this supernatant. The assay mixture contained 1.8 mL 50 mM L^{-1} sodium phosphate buffer, 0.1 mL phenol, 0.1 mL 4-aminophenazone and 0.1 mL enzyme extract was incubated at 40°C for 5 min. Subsequently H_2O_2 (1 mM L^{-1}) was added after incubation and absorbance was recorded at 520 nm. The quantity of H_2O_2 consumed was calculated through a standard curve based on absorption A_{520} and H_2O_2. Enzyme activity (1 unit) was expressed as the quantity of H_2O_2 consumed per min per mg protein.

Catalase (CAT) activity was determined using the method of Aebi (1984). The supernatant was used for CAT determination. The reaction mixture included 50 mM L^{-1} sodium phosphate buffer, 50 mM L^{-1} H_2O_2 and 50 mL of enzyme extract. The activity was determined by observing the decline in absorbance at 240 nm as a result of H_2O_2

consumption and expressed as quantity of H_2O_2 utilized per minute per mg of protein.

Statistical Analysis

The experiment was organized as Completely Randomized Design. Data collected on variables were analyzed using Minitab software (Minitab 15.0, Minitab Inc., State College, PA, USA). One-way ANOVA (analysis of variance) was used and Duncan's Multiple Range (DMR) test was performed to test differences among treatment means. Only differences statistically significantly different at $P < 0.05$ are discussed in this report. In some cases, mean values are presented as mean value with standard errors (SE). Interactions between shoot dry weight and physiological or biochemical traits were analyzed by simple linear regression at 150 mM NaCl stress applying MS-Excel-2007 to determine the suitability of different physiological and biochemical traits as selection criteria for salt tolerance. In addition, salt tolerance indices were calculated by dividing each observation, at a given salinity, by the average of the control. Ranking numbers were allocated to groups on the basis of means and were applied to score the genotypes. Group rankings were acquired on the basis of Ward's minimum variance analysis of the averages of the salt tolerance indices for 4 plant biomass parameters (shoot length, root length, shoot dry weight and root dry weight) and 12 physiological and biochemical attributes (i.e. *A*, *E*, g_s, K^+:Na^+ ratio, chlorophyll, sugar, proline, soluble protein, SOD, POD and CAT). Group rankings were achieved from the average of means of the various parameters in each group. A sumMation was acquired by totaling the number of rankings at 150 mM NaCl in each genotype. Genotype rankings were finally determined on the basis of sumMations, most tolerant genotypes with the smallest sums (Zeng *et al*., 2002).

Results

Plant Biomass

Our data showed that shoot dry weight, root dry weight, shoot length and root length were decreased with increasing level of salinity (Tables 2, 3). Under low salt concentration (75 mM NaCl), the deleterious effects of salt stress were less pronounced than the higher salt concentration (150 mM NaCl). Reductions in shoot dry weight due to the application of 75 and 150 mM NaCl was 16 and 22%, respectively, for root dry weight was 15 and 20%, shoot length was 10 and 16% and root length was 17 and 30%, as compared to control plants (Table 2). The wheat genotypes Kharchia-65, Shorawaki, N-7, N-9 and N-13 were least affected by increasing salinity. For example, shoot dry weight at 150 mM NaCl were decreased by 12, 13, 16, 17 and 18% and root dry weight at 150 mM NaCl declined by 6, 7, 8, 9 and 10% for Kharchia-65, Shorawaki, N-7, N-9 and N-13, respectively, as compared to the control treatment (Table 2). Shoot dry weight for N-25, N-10, N-14 and N-26 were most sensitive, declining 32, 31, 30, and 29%, respectively, at 150 mM NaCl stress. Similarly, root dry weight showed maximum reduction in genotypes N-14, N-25, N-15 and N-10, decreasing 32, 28, 27, and 26%, respectively, at 150 mM NaCl. Of the 15 wheat genotypes, nine showed more than 10% reduction in their shoot length, whereas Kharchia-65, Shorawaki, N-7, N-9 and N-13 performed better at both 75 and 150 mM NaCl (Table 3). Maximum reduction in shoot length was observed for N-14, N-25 and N-15 (< 23%) and minimum for Kharchia-65, Shorawaki, N-7 and N-13 (< 8.0%). At 150 mM NaCl, 10 genotypes demonstrated more than 20% reduction in root

Table 2: Effect of different levels of NaCl on the shoot and root dry weight of wheat genotypes

	Shoot dry weight (mg plant^{-1})				Root dry weight (mg plant^{-1})			
Genotypes	0 m*M*	75 m*M*	150 m*M*	Mean	0 m*M*	75 m*M*	150 m*M*	Mean
Kharchia-65	37.6	36.0	33.0	36.0 a	25.1	24.1	21.1	23.2 a
Shorawaki	35.4	33.4	30.7	33.1ab	23.4	22.3	19.4	22.0 ab
N-7	36.2	34.1	30.3	34.0ab	22.1	21	20.3	21.1 abc
N-13	34.2	31.1	28.1	31.1a-d	20.3	18.7	18.2	19.0 a-d
N-9	35.2	32.2	28.8	32.1abc	21.3	19.7	19.2	20.0 abc
N-33	33.6	30.4	27.5	31.0a-e	20.1	18.2	17.4	19.0 a-d
N-12	25.0	21.3	18.6	21.6e-f	18.1	16.2	14.8	16.4 a-d
N-34	29.0	24.5	21.8	25.1b-f	16.2	14.1	13	15.0 a-d
N-26	27.0	22.0	19.0	23.0def	17.3	15	14.2	16.0 a-d
N-11	27.0	22.3	20.8	23.4c-f	16.5	14.5	13.2	15.0 a-d
N-31	22.0	18.0	16.5	19.0 f	16.2	13.6	12.5	14.1 bcd
N-10	26.0	20.0	18.0	21.3ef	15.6	12.8	11.6	13.3 bcd
N-15	27.0	21.0	20.0	23.0def	14.8	12.1	10.8	13.0 cd
N-25	25.0	19.0	17.0	20.3 f	13.1	10.7	9.4	11.0 d
N-14	23.0	18.0	16.0	19.0 f	12.5	10.1	8.5	10.4 d
Mean	32.0a	27.0b	25.0b		20.0a	17.0b	16.0b	

Table 3: Effect of different levels of NaCl on the shoot and root length of wheat genotypes

	Shoot length (cm)				Root length (cm)			
Genotypes	0 m*M*	75 m*M*	150 m*M*	Mean	0 m*M*	75 m*M*	150 m*M*	Mean
Kharchia-65	33.2	32.1	31.5	32.30a	14.5	13.5	13.0	13.8 ab
Shorawaki	32.5	31	30.5	31.3abc	13.7	12.5	11.8	12.7abc
N-7	32.5	30.9	30.2	31.2abc	11.2	10.1	11.4	10.2 bc
N-13	31.41	29.7	28.9	30.0abc	14	12.7	11.2	12.6abc
N-9	33.2	31.2	30.1	31.5abc	14	12.6	11.3	12.6abc
N-33	29.6	27.6	26.6	27.9a-e	11.3	9.8	8.6	9.9 c
N-12	31.3	28.4	26.8	28.8a-d	16.3	13.8	11.2	13.8 ab
N-34	30.7	27.6	24.3	28.2a-e	11.7	9.7	9.11	10.2 bc
N-26	29.4	26.4	24.8	26.9b-f	11.3	9.56	8.2	9.7 c
N-11	30.3	27.1	25.3	27.6a-e	14.3	12	10.2	12.2abc
N-31	25.3	21.6	19.5	22.1 f	12	10	8.7	10.2 bc
N-10	31.5	26.7	24.6	27.6a-e	16	13	9.5	12.8abc
N-15	29.6	26.1	22.8	26.2c-f	15	12.3	9.8	12.4abc
N-25	27.7	22.8	20.7	23.7ef	13	11.5	9.0	11.2bc
N-14	28.5	24.2	21.1	24.6def	16.3	14.6	12.3	14.4a
Mean	30.5a	27.3b	25.0 c		14.6a	12.0b	10.3 c	

Table 4: Effect of different levels of NaCl on K^+:Na^+ ratio, chlorophyll and soluble sugar content of wheat genotypes

Genotypes	K^+/Na^+ (ratio)			Chlorophyll content (mg g^{-1} D. wt.)			Soluble sugar content (mg g^{-1} D. wt.)		
	0 mM	75 mM	150 mM	0 mM	75 mM	150 mM	0 mM	75 mM	150 mM
Kharchia-65	5.8±0.8	4.2±0.32	1.9±0.19	22.8±1.5	19.5±1.0	13.8±0.60	21±1.6	30.0±2.4	41.5±4.0
Shorawaki	5.7±0.95	4.0±0.34	1.7±0.16	22.5±2.0	19.2±0.90	13.5±0.44	20.5±2.4	29.3±3.1	40.2±3.5
N-7	5.6±0.76	3.8±0.30	1.5±0.14	22.3±1.4	18.5±0.88	13.2±0.50	20.1±1.8	30.2±2.7	38.4±2.9
N-13	5.5±0.50	3.6±0.24	1.4±0.15	21.5±1.6	18.1±0.52	12.6±0.44	18.6±1.5	29.3±2.0	37.5±3.1
N-9	5.5±0.65	3.7±0.22	1.3±0.13	20.5±1.5	17.4±0.67	13±0.40	19.5±1.3	28.2±1.5	35.1±2.4
N-33	5.4±0.30	3.5±0.18	1.0±0.12	18.6±1.4	17±0.58	12.4±0.29	18.2±1.2	27.4±1.6	34.36±2.1
N-12	5.0±0.60	3.0±0.39	0.67±0.11	20.2±1.2	15.6±0.56	11.4±0.36	18.3±2.1	26.5±1.2	32.0±3.0
N-34	5.2±0.24	2.7±0.45	0.65±0.08	20.5±1.3	15.2±0.42	10.8±0.51	17.4±1.7	26.3±1.4	29.2±2.5
N-26	3.8±0.58	2.6±0.36	0.59±0.10	19.6±1.2	14.5±0.50	10.5±0.39	17.5±1.5	25.0±1.8	30.2±2.3
N-11	3.5±0.65	2.1±0.29	0.42±0.12	19.2±1.4	14.2±0.46	9.1±0.32	15.8±1.3	22.0±1.5	29.5±2.4
N-31	3.4±0.23	1.7±0.31	0.23±0.08	19±1.2	13.8±0.35	8.9±0.28	17.5±1.1	24.6±1.8	31.4±1.8
N-10	3.3±0.40	1.5±0.21	0.31±0.07	18.6±1.1	13.5±0.43	8.5±0.37	17.2±0.9	23.5±1.5	28.6±2.2
N-15	3.2±0.35	1.2±0.25	0.28±0.06	18.4±1.0	13±0.32	8.7±0.33	16.0±0.69	22.7±1.9	29.5±1.9
N-25	2.3±0.30	1.0±0.15	0.24±0.05	18±.93	12.6±0.38	8.6±0.26	15.4±0.43	23.5±1.2	27.8±1.8
N-14	2.0±0.21	0.9±0.14	0.26±0.04	18.2±.90	12.3±0.41	7.9±0.30	14.5±0.56	22.3±1.1	27.5±2.0
Mean	4.34 a	2.63 b	0.83 c	20.0 a	15.63 b	10.80 c	20.18 c	26.10 b	32.80 a

Table 5: Effect of different levels of NaCl on proline and soluble protein content of wheat genotypes

	Proline contet (µg g^{-1} fresh wt)			Soluble protein content (mg g^{-1})		
Genotypes	0 mM	75mM	150mM	0 mM	75 mM	150 mM
Kharchia-65	30±1.2	42±2.4	58±4.0	0.72±0.09	1.0±0.09	1.28±0.14
Shorawaki	29±1.5	40±3.1	55±3.5	0.67±0.04	0.94±0.1	1.25±0.16
N-7	30±1.4	38±2.7	56±2.9	0.68±0.06	0.89±0.08	1.2±0.12
N-13	27±1.2	36±2.0	52±3.1	0.64±0.05	0.76±0.07	0.95±0.10
N-9	27±1.1	40±2.6	50±2.4	0.58±0.3	0.88±0.08	0.9±0.09
N-33	28±1.0	35±3.0	47±2.1	0.49±0.42	0.7±0.06	0.82±0.06
N-12	20±1.6	30±0.8	45±3.0	0.52±0.06	0.66±0.04	0.89±0.08
N-34	18±1.3	28±1.3	42±2.5	0.62±0.05	0.82±0.03	0.9±0.06
N-26	16±1.2	26±1.8	40±2.3	0.43±0.02	0.62±0.04	0.86±0.07
N-11	12±.92	20±1.0	30±2.4	0.56±0.06	0.72±0.03	0.88±0.05
N-31	10±0.8	16±1.0	29±1.8	0.4±0.05	0.55±0.04	0.72±0.06
N-10	9±0.9	15±0.8	28±2.2	0.38±0.02	0.55±0.024	0.67±0.05
N-15	10±0.7	14±1.0	25±1.9	0.49±0.02	0.68±0.04	0.75±0.04
N-25	12±0.8	15±0.9	27±1.8	0.37±0.04	0.52±0.03	0.48±0.03
N-14	13±1.0	16±1.0	23±2.0	0.42±0.03	0.56±0.03	0.68±0.05
Mean	19.4c	27.40b	37.0a	0.53 c	0.73 b	0.87 a

length, while 5 genotypes showed less than 21%. The maximum decline in root length was observed for N-10, and the minimum for Kharchia-65 (Table 3). A positive correlation was found between shoot dry weight with root dry weigh (r^2: 0.82**) and shoot dry weight with shoot length (r^2: 0.60*). However, correlation between shoot dry weight with root length was not significant (r^2: 0.23 ns) (Table 8). Salt tolerance ranking based on plant biomass showed that wheat genotypes Kharchia-65, Shorawaki, N-7, N-9 and N-13 were ranked as salt tolerant, while genotypes N-12 and N-33 were grouped as moderately tolerant, and the remainder of genotypes were grouped as sensitive (Table 9).

Physiological and Biochemical Attributes

Our data on K^+:Na^+ ratio and chlorophyll content indicated that both were reduced with increasing levels of salinity (Table 4). At 75 and 150 mM NaCl, for example, K^+:Na^+ ratio was decreased by 39 and 80%, respectively, and chlorophyll content was decreased by21 and 45%, respectively, as compared to the control treatment (Table 4). The K^+:Na^+ ratio ranged from 0.9 to 4.2 at 75 mM NaCl and 0.26 to 1.9 at 150 mM NaCl; While, chlorophyll content ranged from 12.3 to 19.5 (mg g^{-1}) at 75 mM NaCl and 7.9 to13.8 (mg g^{-1}) at 150 mM NaCl. Minimum reduction in K^+:Na^+ ratio was recorded in genotypes Kharchia-65, Shorawaki, N-7, N-9 and N-13 (28, 29, 32, 34 and 32%), respectively, at 75 mM NaCl and 67, 68, 73, 74 and 76% at 150 mM NaCl. Conversely, maximum reduction in K^+:Na^+ ratio was obtained with genotypes N-10, N-15, N-25 and N-14, which were 54, 62, 56 and 55%, respectively, at 75 mM NaCl, and 90, 91, 89 and 87% at 150 mM NaCl. Maximum chlorophyll content was observed in the same genotypes (Kharchia-65, Shorawaki, N-7, N-13 and N-9) at both 75 and 150 mM NaCl levels. Whereas, minimum chlorophyll contents were recorded with N-10, N-15, N-25 and N-14 at 150 mM NaCl (Table 4).

Soluble sugar, proline and soluble protein contents were increased at 75 and 150 mM NaCl (Tables 4, 5). On average, soluble sugar content increased over the control by 29 and 62%, respectively, proline content by 41 and 90% and soluble protein content by 36 and 64% at 75 and 150 mM NaCl respectively. Among the evaluated genotypes, soluble sugar content ranged from 22 to 30 (mg g^{-1} dry wt.) at low salinity and from 27.5 to 40.5 (mg g^{-1} dry wt.) at high salinity. Proline content ranged from 14 to 42 (µg g^{-1} fresh wt.) at low salinity and 23 to 58 (µg g^{-1} fresh wt.) for high salinity. Soluble protein content ranged from 0.52 to 1.0 (µg g^{-1} fresh wt.) at low salinity and from 0.48 to 1.28 (mg g^{-1}) at high salinity. Maximum sugar contents (41.5, 40.2, 38.4, 37.5 and 35.1 mg g^{-1} dry wt.) were for genotypes Kharchia-65, Shorawaki, N-7, N-9 and N-13, respectively, at 150 mM NaCl; while, minimum sugar accumulations (17.2, 16.0, 15.4 and 14.5 mg g^{-1} dry wt.) were for N-10, N-15, N-25

Table 6: Effect of different levels of NaCl on superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities of wheat genotypes

	SOD (unit mg^{-1})			POD (unit mg^{-1})			CAT (unit mg^{-1})		
Genotypes	0 mM	75 mM	150 mM	0 mM	75 mM	150 mM	0 mM	75 mM	150 mM
Kharchia-65	15.0±1.5	20.4±1.6	26.3±2.0	128±3.6	132±3.6	145±4.6	40±2.5	48±2.6	54±2.8
Shorawaki	14.5±1.3	20.0±1.2	25.5±2.3	125±3.8	128±4.2	142±5.0	37±2.2	46±2.7	53±3.1
N-7	14.0±1.1	22.0±2.2	28.4±1.8	123±4.6	126±2.8	147±5.6	33±1.8	40±3.1	52±2.7
N-13	13.6±0.9	22.5±2.4	28.6±1.5	125±6.0	130±2.4	150±4.9	32±3.5	38±3.6	48±3.7
N-9	13.5±1.3	21.4±1.5	26.4±1.9	120±5.2	125±2.5	145±6.0	34±2.1	37±2.5	46±4.0
N-33	13.0±1.0	17.5±1.1	22.5±2.1	124±7.3	127±3.0	145±5.4	32±1.8	36±3.7	44±2.5
N-12	12.6±0.4	16.5±1.5	22.0±2.0	116±4.2	125±2.8	144±4.2	32±1.2	36±1.4	43±4.2
N-34	12.8±1.2	15.3±1.6	21.3±2.3	121±3.9	124±4.3	141±3.2	34±1.6	38±1.8	42±3.2
N-26	12.6±0.8	17.5±1.4	19.0±1.6	118±3.0	123±5.6	142±3.8	31±1.8	38±2.2	43±3.6
N-11	12.2±0.6	15.5±3.1	18.3±2.0	119±2.9	122±3.9	138±3.2	30±1.1	35±1.9	41±3.3
N-31	11.5±0.8	14.6±1.6	18.0±1.5	112±3.1	121±4.0	136±4.0	27±0.9	34±1.6	38±2.8
N-10	11.4±1.1	15.5±2.1	17.0±1.6	120±3.4	122±3.6	130±3.4	26±1.1	30±1.4	40±3.0
N-15	10.6±1.0	14.6±1.5	16.5±1.8	116±3.6	123±3.4	135±5.4	25±0.8	27±1.6	34±2.5
N-25	10.0±0.7	14.2±1.1	17.0±1.5	110±2.7	119±4.2	133±4.6	20±0.7	26±1.8	29±2.1
N-14	9.5±0.67	12.3±1.4	15.8±1.4	105±3.0	118±5.0	132±2.6	22±0.7	28±1.5	32±2.5
Mean	12.50c	17.30b	21.50a	118.50 c	124.3 bc	140.3 a	29.6 c	33.5 b	42.5 a

Table 7: Effect of different levels of NaCl on photosynthetic rate (*A*), transpiration rate (*E*) and stomatal conductance (g_s) of wheat genotypes

	A(u mol CO_2 m^{-2} S^{-1})			*E* (m mol H_2O m^{-2} S^{-1})			g_s (u mol m^{-2} S^{-1})		
Genotypes	0 mM	75 mM	150 mM	0 mM	75 mM	150 mM	0 mM	75 mM	150 mM
Kharchia-65	8.0±0.48	6.9±0.32	6.0±0.19	3.6±0.30	3.1±0.26	2.4±0.16	354±16	292±18	243±18
Shorawaki	7.8±0.56	6.8±0.34	5.80±0.16	3.5±0.20	3.0±0.12	2.3±0.15	342±20	286±20	240±16
N-7	7.9±0.50	6.2±0.30	5.80±0.14	3.2±0.14	2.3±0.18	1.55±0.14	350±23	285±17	236±15
N-13	7.5±0.42	6.2±0.26	5.40±0.15	3.1±0.24	2.2±0.14	1.45±0.21	346±21	283±20	238±12
N-9	7.6±0.47	6.1±0.22	5.50±0.13	3±0.16	2.3±0.20	1.39±0.29	335±24	280±17	230±14
N-33	7.5±0.30	6.3±0.19	5.00±0.12	2.8±0.18	2±0.14	1.4±0.25	326±22	276±14	225±16
N-12	7.0±0.20	5.4±0.39	4.80±0.11	2.5±0.14	2.1±0.24	1.2±0.20	318±15	262±15	220±18
N-34	6.8±0.24	5.6±0.45	4.60±0.09	2.4±0.20	2±0.16	1.2±0.19	316±18	256±16	206±20
N-26	6.5±0.52	5.0±0.36	4.20±0.10	2.5±0.26	1.8±0.18	1.1±0.24	311±17	245±17	200±21
N-11	6.3±0.62	5.3±0.29	3.90±0.12	2.3±0.31	1.7±0.14	0.96±0.17	305±23	250±14	195±15
N-31	6.4±0.23	4.6±0.31	3.70±0.08	2.4±0.16	1.8±0.20	0.92±0.10	302±19	251±16	194±12
N-10	6.0±0.40	4.8±0.21	3.60±0.07	2.4±0.18	1.6±0.14	0.85±0.11	295±16	236±21	190±16
N-15	5.8±0.35	4.4±0.25	3.10±0.06	2.6±0.14	1±0.19	0.52±0.15	293±20	245±18	187±19
N-25	5.6±0.30	4.6±0.15	3.40±0.05	2.3±0.17	1.3±0.13	0.81±0.11	280±26	235±16	180±14
N-14	5.5±0.21	4.5±0.14	3.20±0.04	2.2±0.10	1.5±0.23	0.84±0.12	286±16	240±15	184±10
Mean	7.00 a	5.50 ab	4.51 c	2.72 a	1.99 a	1.25 b	317.3 a	261.5 b	211.2 c

and N-14, respectively, in the absence of salt. Likewise, higher proline contents (58, 55, 56, 52 and 52 µg g^{-1} fresh wt.) were found for the aforementioned genotypes and minimum proline contents (28, 25, 27 and 23 µg g^{-1} fresh wt.) were for N-10, N-15, N-25 and N-14, respectively, in absence of salt (Table 5). Higher soluble protein contents were found in Kharchia-65, Shorawaki, N-7, N-9, N-34 and N-11 at 150 mM NaCl stress. Minimum soluble protein contents were recorded for N-10 and N-25.

Activities of the enzymes superoxide dismutase (SOD) and catalase (CAT) were increased with 75 and 150 mM NaCl. However, POD increased at 150 mM NaCl; there were no significant increases in POD due to treatment with 75 mM NaCl (Table 6). SOD activity ranged from 13.3 to 24.5 (units mg^{-1}) at low salinity and 15.8 to 28.6 (units mg^{-1}) at high salinity; while, POD activity ranged from 118 to 132 (units mg^{-1}) at low salinity and 130 to 150 (units mg^{-1}) at high salinity. Similarly, CAT activity ranged from 26 to 48 (units mg^{-1}) at low salinity and 29 to 54 (units mg^{-1}) at high salinity. Our results also indicated that SOD, POD and CAT activities increased 38, 4.5 and 13% with the application of 75 mM NaCl and 72, 19 and 43%, respectively, at 150 mM NaCl. At 150 mM NaCl, wheat genotypes Kharchia-65, Shorawaki, N-7, N-9 and N-13 had higher SOD activity levels (26.3, 25.5, 28.4 and 26.4 units mg^{-1}) and CAT (54, 53, 52, 48 and 46 units mg^{-1}); while maximum POD activities (150, 147, 145, 144 and 145 units mg^{-1}) were for genotypes N-13, N-7, Kharchia, N-9 and N-33 respectively at 150 mM NaCl.

Gas exchange attributes, such as leaf photosynthetic rate (*A*), transpiration rate (*E*) and stomatal conductance (g_s) were reduced with the application of 150 mM NaCl in all the wheat genotypes. At 150 mM NaCl, minimum reductions in photosynthetic rate were for Kharchia-65,

Table 8: Equation of linear regression between the values of shoot dry weight and physiological and biochemical attributes in wheat at 150 mM NaCl

Physiological/biochemical attributes	Regression equation	r^2
Root dry weight	y = 0.542X + 0.001	0.82**
Shoot length	y = 534.3 X + 14.25	0.60*
Root length	y = 116.7X + 7.593	0.23 ns
K^+/Na^+ ratio	y = 51.93X - 0.579	0.92***
Chlorophyll	y = 327.9 X + 3.32	0.87***
Soluble sugar	y = 849X + 18.19	0.78**
Proline	y = 2134X – 2.586	0.85***
Soluble protein	y = 34.19X + 0.075	0.74**
SOD	y = 985.0X + 0.337	0.90***
POD	y = 774.7X + 122.3	0.58*
CAT	y = 0.776X + 0.024	0.89***
A	y = 153.5X + 0.969	0.83**
E	y = 74.96X – 0.490	0.70**
g_s	y = 3522X + 129.9	0.85***

***, P < 0.001; **, P < 0.01; *, P < 0.05 and ns, not significant

Table 9: Ranking for relative salt tolerance of 15 wheat genotypes in terms of plant dry biomass at 150 mM NaCl

Genotypes	SDW	RDW	SL	RL	Sum	Ranking
Kharchia-65	1	1	1	1	4	Tolerant
Shorawaki	1	1	1	2	5	Tolerant
N-7	1	1	1	2	5	Tolerant
N-13	1	1	1	2	5	Tolerant
N-9	1	1	1	2	5	Tolerant
N-33	1	2	2	4	9	Moderate
N-12	3	2	2	2	9	Moderate
N-34	2	3	3	3	11	Sensitive
N-26	3	2	3	4	12	Sensitive
N-11	2	2	3	3	10	Sensitive
N-10	3	3	3	3	12	Sensitive
N-31	4	3	4	4	15	Sensitive
N-15	2	3	4	3	12	Sensitive
N-25	4	4	4	3	15	Sensitive
N-14	4	4	4	1	13	Sensitive

Shorawaki, N-7, N-13 and N-9, transpiration rate in Kharchia-65 and Shorawaki and stomatal conductance for genotypes Shorawaki, N-12, N-33, N-9 and Kharchia-65.

There were strong correlations between shoot dry biomass and K^+/Na^+ ratio, chlorophyll, proline, SOD, CAT and g_s (r^2: 0.92, 0.87, 0.85, 0.90, 0.89, 0.85***, respectively); hence, these could be used as selection criteria (Table 8). Different physiological and biochemical attributes for the suitability of selection criteria for salt tolerance in synthetic wheats was also evaluated by determining relationships between physiological attributes and shoot dry biomass using linear regression analysis (Table 9). Salt tolerance ranking on the basis of physiological and biochemical attributes indicated that, genotypes Kharchia-65, Shorawaki, N-7, N-9 and N-13 could be grouped as salt tolerant; genotypes N-33 and N-12 as moderately tolerant and genotypes N-10, N-15, N-25 and N-14 as sensitive (Table 10).

Discussion

Our results clearly indicate that increasing level of NaCl had adverse effects on wheat plant dry biomass production. However, there was variability in salt tolerance among the tested wheat genotypes. Salt sensitive genotypes had greater decrease in plant dry biomass than salt tolerant genotypes. The variation in response of the tested genotypes could be largely related to plant genetics. It has been reported that wheat genotypes having greater plant biomass at the seedling stage show better salt tolerance at maturity (Ahmadi and Ardekani, 2006). Establishment of more vigorous seedlings is an important step in the crop life cycle. Evaluation of genotypes for salinity tolerance at the seedling stage can save significant time. The decrease in plant dry biomass, plant height and root length under saline conditions was associated with higher K^+:Na^+ ratios and better photosynthesis. In the current study, wheat genotypes Kharchia-65, Shorawaki, N-7, N-9 and N13 maintained higher plant biomass and showed minimum growth reduction when exposed to 75 and 150 mM NaCl (Tables 2, 3). A positive correlation was observed between shoot dry weight and root dry weight at 150 mM NaCl. This is not surprising as plants with healthy and long roots are able to absorb more water and especially for plants under stress; this may result in higher photosynthetic levels and, consequently, increased shoot biomass. Under environmentally controlled conditions genotype salinity tolerance is most clearly indicated by dry plant biomass accumulation (Meneguzzo *et al*., 2000). In wheat, decreased shoot biomass was attributed to the reduction in water potential and growth associated with osmotic effects under salinity stress (Munns *et al*., 1995).

Salinity tolerance by plants includes the ability of plant to exclude Na^+ and the capacity to accumulate Na^+ in leaf tissue. Our data indicates that the K^+:Na^+ ratio declines with increasing salt concentration for all genotypes. In this regard, various wheat genotypes were differentially affected by salt stress due to their different genetic compositions. Salt tolerant genotypes, Kharchia-65, Shorawaki, N-7, N-9 and N-13, maintained higher K^+:Na^+ ratios at all the salinity levels (Table 4). Selection for physiological mechanisms of salt tolerance, such as selection for germplasm with low Na^+ uptake or with high selectivity for K^+ over Na^+, have successfully contributed in salt tolerance (Flowers, 2004). There was a strong correlation between shoot dry biomass and K^+:Na^+ ratio (Table 8). Salinity tolerance in glycophytes, such as wheat, is well known to be associated with Na^+ exclusion and wheat genotypes with a low capacity in this regard were categorized as salt sensitive genotypes (Din *et al*., 2008; Gurmani *et al*., 2013). The level of photosynthetic pigments, such as chlorophyll content, plays a vital role in photosynthesis. In the present investigation, increased concentrations of NaCl reduced chlorophyll contents in all the wheat genotypes. The current

Table 10: Ranking for relative salt tolerance of 15 wheat genotypes in terms of physiological and biochemical attributes at 150 m*M* NaCl

Genotypes	K/Na	Chlorophyll	Sugar	Proline	Protein	SOD	POD	CAT	*A*	*E*	g_s	Sum	Ranking
Kharchia-65	1	1	1	1	1	1	2	1	1	1	1	12	Tolerant
Shorawaki	1	1	1	1	1	1	2	1	1	1	1	12	Tolerant
N-7	1	1	1	1	1	1	2	1	1	2	1	13	Tolerant
N-13	1	1	1	1	2	1	1	1	1	2	1	13	Tolerant
N-9	1	1	1	1	1	1	2	1	1	2	1	13	Tolerant
N-33	2	2	2	2	3	2	2	2	2	2	2	23	Moderate
N-12	2	2	2	2	2	2	2	2	2	3	2	23	Moderate
N-34	2	2	2	2	2	3	3	2	3	3	3	27	Sensitive
N-26	3	3	3	3	3	3	3	2	3	3	3	32	Sensitive
N-11	3	3	4	4	3	3	3	2	3	4	4	36	Sensitive
N-31	4	3	4	4	4	4	4	3	4	4	4	42	Sensitive
N-10	4	4	4	4	4	4	4	3	4	4	4	43	Sensitive
N-15	4	4	4	4	4	4	4	3	4	4	4	43	Sensitive
N-25	4	4	4	4	4	4	4	4	4	4	4	44	Sensitive
N-14	4	4	4	4	4	4	4	4	4	4	4	44	Sensitive

work showed that tolerant genotypes, Kharchia-65, Shorawaki, N-7, N-9 and N-13, showed higher level of chlorophyll than moderate and sensitive genotypes at all the salinity levels; this is comparable with earlier findings in a range of crops e.g., cotton (Shaheen *et al.,* 2012), wheat (Gurmani *et al.,* 2013) and maize (Kaya *et al.,* 2013). A positive correlation (r^2 = 0.92) was observed between chlorophyll content and shoot dry biomass at 150 mM NaCl, suggesting that reductions in shoot dry biomass may have been partly due to decreases in chlorophyll content.

Another important tolerance mechanism exhibited by plants under stress conditions is the accumulation of compatible solutes such as sugar, carbohydrates and proline (Munns and James, 2003; Kaya *et al.,* 2013; Gurmani *et al.,* 2013). Genotypes having higher K^+:Na^+ ratios and chlorophyll contents under saline conditions were better able to make osmotic adjustments by increasing osmoprotectants such as proline, soluble sugar and soluble protein contents. Thus, the salt tolerance potential of tolerant genotypes (Kharchia-65, Shorawaki, N-7, N-9 and N-13) is associated with elevated level of osmolytes in leaf tissues. Increased production of compatible solutes under salt stress has already been reported in wheat (Din *et al*., 2008), maize (Kaya *et al.,* 2013), *Chenopodium quinoa* willd. (Prado *et al.,* 2000) and tomato (Amini and Ehsanpour, 2005). Afzal *et al.,* (2006), reported that increases in leaf soluble protein contents of wheat plants occur regardless of their sensitivity to salt stress; however, the level of increase or decrease in soluble protein under saline conditions is genotype dependent (Amini and Ehsanpour, 2005). In the present investigations, tolerant genotypes (Kharchia-65, Shorawaki, N-7, N-9, and N-13) accumulated higher levels of soluble protein under saline conditions (Table 4).

Salinity induced inhibition of plant growth is associated with damage caused by ROS. These ROS are free radicals in atoms or group of atoms containing minimum one unpaired electron (Polle, 2001). It is well known that salinity stress activate oxidative stress within the plant system generally known as oxidative burst (Foyer and Noctor, 2003). Superoxide dismutase (SOD) plays an important role in ROS detoxification by catalyzing the conversion of free O_2^- to O_2 and H_2O_2; SOD activity is correlated with stress conditions (Davies and Dow, 1997). A strong relationship between efficient antioxidant system and plant salinity tolerance has been reported by Kaya *et al.,* (2013), thus antioxidative enzyme levels could be a good indicator of plant performance under salt stress. In the present study, SOD activities in the evaluated wheat genotypes were increased with the increasing level of salinity stress and were greater in tolerant genotypes (Kharchia-65, Shorawaki, N-7, N-9, and N-13) than sensitive genotypes (Table 6). An increase in SOD activity in the leaves of salt stressed maize plants has been reported (Kaya *et al.,* 2013).

Peroxidases (POD) play a role in scavenging hydrogen peroxide generated in chloroplasts, as well as in plant growth processes and oxidation of toxic compounds (Dionisio-Sese and Tobita, 1998). Salinity stress enhances POD activity levels and higher POD activities have been reported in sensitive rice cultivars than tolerant ones (Mittal and Dubey, 1991). In the current investigations, accumulation of POD activity was uneven among the tested wheat genotypes; however, N-13 had the highest level of POD activity at 150 mM NaCl (Table 4).

Catalase (CAT) plays a vital role in plant defense against oxidative stress and can catalyse a redox reaction by dismutation of H_2O_2 to oxygen and water. Kaya *et al.,* (2013) reported that induction of salt stress is linked with enhanced CAT activity in leaves of maize plants. We also found an increase in CAT activity when NaCl levels increased, and higher CAT levels in tolerant genotypes (Kharchia-65, Shorawaki, N-7, N-9, and N-13) than sensitive genotypes (Table 6). Overall, a range of SOD, POD and CAT responses have been reported to occur under saline

conditions and across a range of crops, indicating that different crops and different levels of tolerance within the same crop could result in diverse kinds of antioxidant responses, related to scavenging of ROS, which could be due by genetic diversity.

Electron transport is a key part of photosynthesis and determination of photosynthetic levels is one of the basic criteria for the evaluation of stress tolerance in various crop species (Ashraf, 2004). Reductions in photosynthetic rate (*A*), transpiration rate (*E*) and stomatal conductance due to salinity stress have been reported in crops, such as cotton (Shaheen *et al.*, 2012), wheat (Gurmani *et al.*, 2013) and olive (Abusafieh *et al.*, 2011). The present work showed that photosynthetic rate (*A*), transpiration rate (*E*) and stomatal conductance (g_s) of all the genotypes diminished at higher salinity (150 mM NaCl). However, tolerant genotypes e.g. Kharchia-65, Shorawaki, N-7, N-13 and N-9, were least affected by salinity (Table 7). Salt induce inhibition variables related to leaf gas exchange by cotton has been reported by Shaheen *et al.*, (2012), who indicated that stomatal conductance and transpiration rate are the main determinants of growth and photosynthetic rate. In the present study, a positive correlation was observed between shoot dry biomass and *A*, *E* and g_s (r^2 = 0.83, 0.70 and 0.85, respectively) suggesting that salt induced reductions in shoot dry biomass are at least partly because of changes in photosystem activity. In the reported work, differences in salt induced reduction in plant biomass among wheat genotypes was potentially due to a disparity in genetic behavior with regard to gas exchange attributes.

Based on our physiological and biochemical data on 15 genotypes in growth chamber condition, we conclude that K^+:Na^+ ratio, photosynthetic capacity, proline level and SOD activity were at higher levels in salt tolerant genotypes than in moderately tolerant and sensitive genotypes. Based on these traits, we grouped the tested genotypes as tolerant, moderately tolerant and sensitive. Based on these traits, we grouped the tested genotypes as tolerant (Kharchia-65, Shorawaki, N-7, N-9 and N-13), moderately tolerant (N-33 and N-12) and sensitive (all the remaining genotypes) towards salinity. These promising findings argue for further work regarding the salinity tolerance of selected genotypes under saline field conditions.

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