INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
16–831/2017/19–4–726–734
DOI: 10.17957/IJAB/15.0347
http://www.fspublishers.org

Full Length Article

Characterization of Salt Tolerant Wheat (*Triticum aestivum*) Genotypes on the Basis of Physiological Attributes

Wajid Mahboob*, Muhammad Athar Khan and Muhammad Ubaidullah Shirazi
Nuclear Institute of Agriculture, Tando Jam, Pakistan
*For correspondence: wajid_uaf@yahoo.com

Abstract

A glass house experiment was conducted to investigate the potential of different wheat genotypes for physiological traits, decisive for their salt tolerance. Ten wheat genotypes along with salt tolerant check (LU-26s) were evaluated under two salinity levels (control and 120 mM NaCl). Salt stress significantly affected growth and yield attributes, as well as physiological traits of wheat genotypes. However, LU-26s, CT-09117, NRL-1237, NRL-1235, Tatara and NIA-AS-14-2 had better growth in terms of plant height, productive tiller, plant biomass and grain yield per plant. These genotypes also demonstrated least degradation in chlorophyll contents with significant increase in endogenous level of proline, glycine betaine (GB), total phenols (TP), total soluble sugars (TSS) and maintained high potassium (K^+) content by restricting sodium (Na^+) uptake in response to salt stress. Genotypes NIA-AS-9 and 4 performed moderately but NIA-AS-14-8 and CT-09149 failed to adjust osmotically and resulted in poor growth and yield under saline stress. Wheat grain yield was positively correlated with K^+, proline and TSS; however, it showed negative relationship with Na^+ contents under salinity. Based on growth and yield parameters, physiological attributes and ion accumulation, wheat genotypes CT-09117, NRL-1237, NRL-1235, NIA-AS-14-2 and Tatara could be categorized as salt tolerant and may be further evaluated for mechanisms conferring salinity tolerance.

Keywords: Chlorophyll; Osmotic potential; Compatible solutes; Salinity; Wheat plants

Introduction

Soil salinity is one of the most devastating environmental stresses, causing reduction in cultivated land area and limiting factor for agricultural productivity and quality (Khan *et al.*, 2010; Turki *et al*., 2012; Maqbool *et al.*, 2016). Nearly 7% of total land area of the world is affected by salinity. In arid and semiarid areas of the world more than 20% of the irrigated arable land (~ about 45 million ha) is affected by salt stress, even so growing (Gupta and Huang, 2014). It has been estimated that more than 50% of the arable land will be salinized by the year 2050 (Jamil *et al*., 2011).

Salinity effects are the results of complex interactions among morphological, physiological and biochemical processes including seed germination, plant growth, water and nutrient uptake (Akbarimoghaddam *et al.*, 2011). The first response of plants to salinity is reduction in growth due to osmotic effect of salts and later on specific salt injury is caused by rising level of excess ions and consequently the feeding of photosynthates to the growing parts is reduced (Munns and Tester, 2008). Under saline conditions, high Na^+ concentration inhibits uptake of K^+ ions which is an essential element for growth and development that results into lower productivity and may even lead to death (Abbas *et al*., 2013). Plants failed to take up water under salinity which quickly leads toward the reduced growth rate, followed by an array of metabolic changes similar to those induced by drought conditions (Abbasdokht, 2011). Salt stress caused a significant reduction in leaf photosynthetic pigments as well as alterations in ionic balance which limits vegetative growth and economic productivity in wheat (Mahboob *et al*., 2016). However, several plant growth and development processes influenced by salinity results in low grain yield and poor quality of crops (Turki *et al*., 2012; Desoky and Merwad, 2015; Guo *et al.*, 2016).

The genetic differences exist among the plants like wheat in response to salt stress (Turki *et al*., 2012; Rahman *et al.*, 2014). This response may be in terms of significant increase in concentration of antioxidant phenols along with sugars, proline and glycine betaine, which act as osmoprotectants to secure the plants from salt injury (Khan *et al* 2014; Desoky and Merwad, 2015; Mahboob *et al*., 2016). It has been reported that high proline accumulation and chlorophyll contents, high K^+/Na^+ ratio and low Na^+, Cl^- accumulation have good correlation with salt tolerance in wheat (Hasan *et al.*, 2015).

As a huge genetic variability lies in wheat genotypes to tolerate salinity, thus, the use of salt tolerant wheat cultivars might be the most promising strategies for harvesting higher grain yield of best quality under saline

To cite this paper: Mahboob, W., M.A. Khan and M.U. Shirazi, 2017. Characterization of salt tolerant wheat (*Triticum aestivum*) genotypes on the basis of physiological attributes. *Int. J. Agric. Biol*., 19: 726–734

conditions (Turki *et al.,* 2012). Various approaches including screening of large germplasm collection has been used to improve the salt tolerance of wheat. According to the Ghars *et al.* (2008) screening technique based on salt tolerance mechanisms by the means of physiological traits to find out the germplasm with minimum Na^+ uptake or with high selectivity for K^+ over Na^+ has effectively contributed to the selection of genotypes for salt tolerance. Hence, the identification of salt tolerant wheat genotypes is relatively simple and useful way to improve crop yield and profitability of suboptimal soils.

The present study was conducted to examine the differences in salt tolerance of advanced wheat lines based on plant growth and physiological responses e.g. alteration in photosynthetic pigments, uptake of ions, osmotic potential and role of osmoprotectants (glycine betaine, proline, soluble sugars and phenolics content) in plant defense against salinity.

Materials and Methods

Planting Materials

Eleven wheat genotypes were used in this experiment. Seeds of six wheat genotypes (Lu 26s, NIA-AS-14-2, 4, 8, 9 and 10) collected from Plant Breeding Division, Nuclear Institute of Agriculture (NIA), Tandojam and five genotypes (CT-09117, CT-09149, NRL-1235, NRL-1235 and Tatara) obtained from Nuclear Institute of Food and Agriculture (NIFA), Peshawar, Pakistan were used.

Experimental Details

Before sowing, healthy seeds of uniform size and identical color from evaluating wheat genotypes were selected. The sorted seeds were washed in distilled water and surface sterilized with 1% (v/v) sodium hypochlorite for 3 min, rinsed thoroughly with distilled water and air-dried at room temperature (25°C) for 60 minutes. Seeds of different wheat genotypes were sown in cemented raised beds (30×12 ft^2) in gravel culture under glasshouse conditions with controlled environment (Temp: 25ºC to 35 ± 3ºC; RH: 60 ± 10%). Experiment was conducted in completely randomized design (CRD) with factorial arrangement using three replicates. After the completion of emergence, plants were thinned to maintain fifteen plants per replicate in a row with recommended plant to plant distance.

Imposition of Salinity

After uniform stand establishment, salt stress was imposed on two week old seedlings by irrigating them with nutrient solution ($1/4^{th}$ strength Hoagland solution) containing NaCl and applied with gradual increments until final concentration (12 dS m^{-1} NaCl) reached. Two salinity levels were maintained at 0 and 120 mM NaCl designated as control and saline stress.

Determination of Agronomic and Yield Related Traits

The growth and yield attributes including plant height, productive tillers, biomass and grain yield per plant were determined at time of maturity. A sample of five plants from each treatment was selected. Plant height was assessed with the help of measuring rod and productive tillers were computed manually. After that, biomass of selected plants was determined by weighing the plants with an electrical weighing balance (AND-3000; Japan). Wheat plants were threshed manually to measure the weight of grains and average was taken for final grain yield per plant.

Physiological and Biochemical Attributes

Leaf samples from each treatment were harvested for determination of physiological and biochemical parameters at booting stage (Khan *et al.,* 2010).

Determination of Chlorophyll Content

The contents of chlorophyll *a* and *b* were estimated by using the protocol described by Arnon (1949). For the extraction of chlorophyll contents, 1 g plant material was completely homogenized in 10 mL of 80% acetone. The extract was poured in cuvette and read at 663 and 645 OD's using spectrophotometer (Hitachi-150-20, Japan). The chlorophyll *a* and *b* contents were calculated by using the following formulae:

$$\text{Chlorophyll } a \text{ (mg/100 mL)} = 0.999\ A_{663} - 0.0989\ A_{645}$$
$$\text{Chlorophyll } b \text{ (mg/100 mL)} = -0.328\ A_{663} + 1.77\ A_{645}$$

Quantification of Compatible Solutes

The proline content was estimated using the acid ninhydrin method (Bates *et al.,* 1973). Wheat fresh leaf tissues (0.5 g) were ground in 10 mL of 3 % (w/v) sulfosalicylic acid solution and filtered. For analysis, 2 mL of the extract was taken to which 2 mL acid ninhydrin and 2 mL of glacial acetic acid were added. This reaction mixture was heated in a water bath at 100°C for 60 min and placed in ice bath to stop the reaction. Organic phase was extracted after adding 4 mL of toluene to the reaction mixture. The optical density of toluene soluble reddish chromophore was measured at 520 nm using spectrophotometer by keeping toluene as blank. Proline concentration was determined from a standard curve and calculated using following equation:

$$\mu moles\ proline/g\ fresh\ weight = \frac{\mu g\ \text{proline/mL} \times \text{mL toluene}}{[115.5\ \mu g/\text{umol}] \div [\text{g sample}/5]}$$

Following the method of Grieve and Gratan (1983), glycine betaine content was measured in wheat samples. Fresh leaf material (1.0 g) was homogenized with 10 mL of distilled water and after filtration 1 mL of extract was added to 1 mL of HCl (2N). From this acidified solution, 0.5 mL was taken in test tubes having 0.2 mL of potassium tri-iodide solution.

The final mixture was placed in ice bath for 90 min with random shaking and then 2 mL of ice cooled distilled water along with 20 mL of 1,2 dichloroethane (cooled at -10ºC) were added in the mixture. A continuous stream of air was passed for 1–2 min to mix the double layered solution. The upper aqueous layer was redundant and optical density of organic layer was read at 365 nm using double beam Spectrophotometer (Hitachi-150-20, Japan). The concentrations of the glycine betaine were calculated against the standard curve.

Total soluble sugars were quantified by anthrone method (Riazi *et al*., 1985). Wheat fresh tissues (0.5 g) were ground in 80% ethanol. Then, a volume of the supernatant 0.1 mL was reacted with anthrone reagent by exposing water bath at 100ºC for 10 min. The absorbance of reaction mixture was assessed at 630 nm and standard curve of glucose was used for calculation.

Total soluble phenols were determined according to method described by Waterhouse (2001). Leaf sample (1 g) was homogenized in 10 mL of 80% acetone and centrifuged it at 4000 rpm for 10 min. After centrifugation, 20 µL of extract was taken in a test tube along with 1.58 mL water, 100 µL of Folin-Ciocalteu reagent to which 300 µL of sodium carbonate solution was added and kept it at 40°C for 30 min. The absorbance of each sample was measured at 765 nm against the blank and phenolics level in the sample was determined with the calibration curve.

Osmotic Potential (Ψs)

Early in the morning at 6:30 a.m. the 3rd leaf was taken from each plant in test tubes containing few drops of chloroform to kill the tissues and kept for one week in a freezer at -20ºC. The frozen leaf material was brought to normal temperature and leaf sap was extracted manually by dispensable syringe, osmotic potential determined with an osmometer (OSMOMAT, Model 030; Germany).

Leaf Ionic Content (Na^+, K^+)

The contents of sodium and potassium were determined according to Ansari and Flowers (1986). Oven-dried (at 65ºC) leaf material was ground to make a powder. A sample (0.1 g) of tissues was extracted in 0.2 m*M* acetic acid (CH_3COOH) by placing it in pre-heated water bath for 60 min at 95ºC. The content of Na^+ and K^+ in extracted solution was measured by flame photometer (PFP-7, Jenway Ltd).

Statistical Analysis

The Fisher analysis of variance technique was employed to statistically analyze the collected data and significant treatments means were examine using least significance difference (LSD) test at 0.05 probability levels (Steel *et al*., 1997). The graphical presentation of data and computation of standard errors for comparison of treatments were done using Microsoft Excel (Microsoft Corporation, Los Angeles, CA, USA).

Results

Changes in Growth and Yield Attributes

Salinity significantly ($P \leq 0.05$) affected growth and yield contributing traits of wheat genotypes (Table 1). Under salinity, the average decrease in plant height, productive tillers, biomass and grain yield per plant was 9.2, 53.3, 49.4 and 57.6% respectively, as compared to control plants (Table 1). Plant height reduced considerably when subjected to salinity (120 mM NaCl) in all genotypes but at variable rate from 65.9 to 78.3 cm. Under control condition, wheat genotype CT-09117 showed superior plant height while minimum height was observed in genotype NIA-AS-14-2, while under salinity, wheat genotypes NIA-AS-14-10 and NIA-AS-14-8 produced maximum and minimum plant height respectively. Maximum percentage of relative reduction (25.1) in plant height was recorded in genotype NIA-AS-14-8 while plant height was least affected in genotype NIA-AS-14-2. Wheat genotypes LU-26s, NRL-1235, NRL-1237 and Tatara were least influenced by increasing salinity due to highest number of productive tillers. However, genotype Tatara exhibited maximum number of productive tillers with minimum relative decrease (36%). On other hand, NIA-AS-14-4, NIA-AS-14-8 and NIA-AS-14-9 produced better number of productive tillers under control conditions but failed to maintain it under salt stress (120 mM NaCl) and results in reduced number of productive tillers (Table 1). Similarly, significant ($P \leq 0.05$) reduction in plant biomass was noted in all wheat genotypes at 120 mM NaCl as compared to control (Table 1). Highest biomass per plant was produced by non-stressed plants of Tatara that were unable to maintain it at 120 mM NaCl stress and showed relatively high decrease (119.1%). Upon exposure to salinity, maximum biomass per plant was observed in genotype LU-26s that was statistically at par with NRL-1235 and NRL-1237 and showed less reduction by 39, 63.8 and 94.4% correspondingly, while minimum plant biomass was recorded in genotypes NIA-AS-14-4 under saline regime and behaved like CT-09149 and NIA-AS-14-10 which exhibited highest decrease of 142.7 and 138.1% in order. Moreover, salt stress comparatively resulted in reduced grain yield per plant in all evaluated wheat genotypes than non-saline control (Table 1). Under control, wheat genotype NIA-AS-14-9 produced best grain yield per plant followed by CT-09117 which reduced drastically (189.2%) at 120 mM NaCl. Among the wheat genotypes subjected to salinity, grain yield ranged from 1.43 to 2.02 g per plant however, maximum grain yield per plant was produced by LU-26s, NRL-1237, NRL-1235, Tatara and CT-09117 with comparatively less reduction at 120 mM NaCl stress. On other hand, wheat genotypes NIA-AS-14-8 showed minimum grain yield per plant with huge decline

Table 1: Impact of salt stress (120 mM NaCl) on plant height, productive tillers, plant biomass and grain yield per plant of different wheat genotypes

Genotypes	Plant height (cm)			Productive tiller			Biomass/Plant (g)			Grain yield/Plant (g)		
	Control	Saline	Relative Dec (%)	Control	Saline	Relative Dec (%)	Control	Saline	Relative Dec (%)	Control	Saline	Relative Dec (%)
LU-26s	84.3 a-c	77.0 d-i	9.5	2.8 b	1.8 cd	55.6	8.36 c	6.01 de	39.0	3.02 h	2.02 i	49.6
NRL-1235	79.4 b-g	70.4 jk	12.8	3.2 ab	1.75 c-e	82.9	8.78 bc	5.36 ef	63.8	3.82 f	1.81 jk	110.6
NRL-1237	84.7 ab	77.1 d-i	9.9	3.25 ab	1.75 c-e	85.7	10.40 a	5.34 f	94.6	4.27 ab	1.92 ij	122.0
CT-09117	85.8 a	75.8 e-j	13.2	3.15 ab	1.3 d-f	142.3	10.37 a	5.07 f	104.3	4.42 b	1.65 lm	168.4
CT-09149	81.0 a-e	73.1 h-j	10.8	3.25 ab	1.3 d-f	150.0	9.18 b	3.78 g	142.7	4.07 de	1.46 no	178.8
TATARA	80.2 a-f	70.5 jk	16.5	2.8 b	2.05 c	36.6	10.97 a	4.99 f	119.71	4.11 cd	1.70 kl	140.7
NIA-AS-14-2	71.8 i-k	70.7 jk	1.6	3.15 ab	1.2 ef	162.5	8.26 c	4.11 g	100.1	3.91 ef	1.60 l-n	153.8
NIA-AS-14-4	74.2 f-j	71.8 i-k	3.3	3.15 ab	1.1 f	186.4	6.67 d	3.59 g	85.7	3.10 h	1.52 m-o	189.2
NIA-AS-14-8	82.5 a-d	65.9 k	25.1	2.2 c	1.1 f	100.0	8.76 bc	4.14 g	112.2	3.63 g	1.43 o	127.4
NIA-AS-14-9	82.2 a-d	73.8 g-j	11.4	3.3 ab	1.1 f	200.0	8.90 bc	4.16 g	113.9	4.59 a	1.54 m-o	189.2
NIA-AS-14-10	79.9 a-g	78.3 c-h	2.0	3.5 a	1.3 d-f	169.2	9.13 b	3.83 g	138.1	4.15 cd	1.58 l-o	103.4
LSD value ($P \leq 0.05$)	*6.28*			*0.58*			*0.66*			*0.16*		

Table 2: Impact of salt stress (120 mM NaCl) on osmoprotectants and total phenolics content in different wheat genotypes

Genotypes	Proline (µmol/g FW)			Glycine Betaine (µmol/g FW)			Total soluble sugars (mg/g FW)			Phenolics contents (mg/g FW)		
	Control	Saline	Relative Increase (folds)	Control	Saline	Relative Increase (folds)	Control	Saline	Relative Increase (folds)	Control	Saline	Relative Increase (folds)
LU-26s	4.68 d-g	13.12 a	2.80	6.93 de	15.20 a	2.19	10.50 f	39.3 a	3.74	4.20 hi	6.64 e-g	1.58
NRL-1235	2.98 hij	12.98 a	4.35	7.28 e	13.70 b	1.88	9.15 hi	31.5 b	3.44	4.23 fg	5.07 ef	1.20
NRL-1237	2.86 hij	5.33 fg	1.87	13.30 b	7.510 e	0.56	6.75 k	15.8 d	2.35	4.21fg	7.00 d	1.66
CT-09117	3.37 hi	7.13 e	2.11	5.17 g	9.530 cd	1.84	6.35 k	13.7 e	2.17	3.20 h	5.04 efg	1.57
CT-09149	1.78 k	3.28 hi	1.82	3.86 h	3.765 h	0.98	6.10 k	17.5 c	2.88	5.36 e	9.26 bc	1.73
TATARA	2.65 ijk	7.54 de	2.84	8.900 d	9.425 cd	1.06	8.30 ij	11.2 fg	1.35	5.22 e	12.1 a	2.32
NIA-AS-14-2	3.60 h	9.27 bc	2.57	3.82 h	8.855 d	2.32	6.75 k	9.40 hi	1.39	5.10 e	9.08 bc	1.78
NIA-AS-14-4	1.78 k	5.60 f	3.14	7.13 e	7.745 e	1.09	9.05 hi	17.5 c	1.94	4.75 efg	6.74 d	1.42
NIA-AS-14-8	3.31 hi	9.40 b	2.84	6.94 ef	7.225 e	1.04	6.55 k	12.6 ef	2.62	4.66 efg	8.86 c	1.90
NIA-AS-14-9	2.73 hij	7.19 e	2.63	9.61 cd	6.070 fg	0.63	8.1 ij	14.0 e	0.77	4.52 efg	11.6 a	2.57
NIA-AS-14-10	2.32 jk	8.40 cd	3.62	15.85 a	10.01 c	0.63	2.35 l	7.50 jk	3.19	5.33 e	9.89 b	1.86
LSD value ($P \leq 0.05$)	*0.91*			*1.02*			*1.48*			*0.86*		

(189.2%) that was statistically at par with CT-09149, NIA-AS-14-4 and NIA-AS-14-9 whose grain yield was comparable (Table 1).

Osmoprotectant and Total Phenolics Content

Imposition of salt stress had a significant ($P \leq 0.05$) impact on production of osmoprotectants i.e. proline, glycine betaine, total soluble sugars and total soluble phenolics in all tested genotypes, but the effect of salinity differed substantially among the genotypes (Table 2). Plants exposed to salinity stress accumulated higher proline content as compared to their non-saline control. On average, leaf proline content increased approximately threefold from 2.91 in the absence of salt stress to 8.11 µmol/g fresh wt at 120 mM salinity. Wheat genotypes LU-26s, NRL-1235, NIA-AS-14-2, NIA-AS-14-8 and NIA-AS-14-10 had accumulated more proline under 120 mM NaCl increased by 2.80, 4.35, 3.14, 2.84 and 3.62 fold correspondingly. Minimum proline content was observed in salt-sensitive CT-09149 who failed to accumulate higher level of proline in response to salinity. Moreover, correlation analysis illustrated that free proline content showed a positive relationship ($r^2 = 0.59$) with grain yield per plant in wheat (Fig. 4a). Significant ($P \leq 0.05$) variation in content of glycine betaine (GB) was found in used wheat genotypes exposed to salinity (Table 2). Among the genotypes, glycine betaine content ranged from 3.76 to 15.20 (µmol/g FW) at 120 mM NaCl salinity. Maximum leaf GB content was given by stressed plants of LU-26s which had high relative increase (2.19 folds), followed by NRL-1235 and Tatara that also secured high GB content over their respective control. Surprisingly, the endogenous level of GB content was relatively higher in non-stressed plants of NRL-1237, NIA-AS-14-9 and CT-09149 than salt-stressed plants. Upon exposure to salinity, all the wheat genotypes showed a significant increase in production of total soluble sugars (TSS) as compared to control and ranged from 7.50 to 39.3 (mg/g FW) under saline regime (Table 2). In response to salinity, the average increase in leaf TSS over control was 136.6%. However, the maximum soluble sugars were found in genotype LU-26s followed by NRL-1235 and CT-09149, which also expressed highest relative increase in TSS by 3.74, 3.44 and 2.88 fold, respectively, whereas genotype

NIA-AS-14-10 accumulated least amount of soluble sugars under both control and saline environment as compared to other tested genotypes. Hence, a linear correlation (r^2= 0.49) was observed between total soluble sugars and grain yield per plant in evaluated genotypes (Fig. 4b).

Like osmoprotectants, total soluble phenols were also affected significantly ($P \leq 0.05$) by application of 120 mM NaCl (Table 2), however the degree of changes varied among the genotypes from 5.07 to 12.1 (mg/g FW). Under salinity, the average increase in leaf phenolics was observed by 78.1% with respect of non-saline control. At 120 mM salinity, significantly ($P \leq 0.05$) highest leaf phenolics content was produced by genotype Tatara followed by NIA-AS-14-9 and NIA-AS-14-10 with superior relative increase by 2.32, 2.57 and 1.86 fold respectively. Conversely, CT-09117, NRL-1235 and NIA-AS-14-4 produced minimum leaf phenolics under salinity and behaved alike.

Chlorophyll Pigments

A marked reduction in chlorophyll content in all wheat genotypes was observed due to 120 mM NaCl stress (Fig. 1a, b). The average decrease in chlorophyll *a* was comparatively less than chlorophyll *b* showing an overall reduction of 9.92% and 15.44%, respectively. In response to salinity, minimum chlorophyll *a* content was produced in NIA-AS-14-4 and CT-09149 with higher relative decrease by 19.02% and 17.24% correspondingly. The better stability in chlorophyll was observed in genotypes NRL-1237, LU-26s, NIA-AS-14-2, NIA-AS-8 and Tatara; however, NRL-1237 produced maximum chlorophyll *a* content by exhibiting less relative decrease (8.026%). Likewise, content of chlorophyll *b* was uneven among the tested wheat genotypes; however, NRL-1235 produced highest level of chlorophyll *b* at 120 mM NaCl stress. Imposition of salinity caused a greater reduction in chlorophyll *b* in NIA-AS-14-10, 8 and 4 by 30.1, 22.9 and 21.77% respectively and demonstrated lower values for chlorophyll *b* content.

Ionic Content and Osmotic Potential (Ψs)

Sodium is the characteristic ion of saline environment, which differed significantly ($P \leq 0.05$) from 0.86 to 2.13% under salinity in all wheat genotypes examined in this study (Fig. 2a). Overall accumulation of Na^+ content enhanced by 4.33 fold from 0.322% in optimal condition to 1.39% at 120 mM NaCl stress. Under control, maximum Na^+ content was accumulated by NIA-AS-14-2 but restricted Na^+ uptake of this genotypes results in minimum value of Na content at 120 mM NaCl. Furthermore, LU-26s, NRL-1235, CT-09117 and Tatara showed lower Na^+ content under control and saline conditions as well. In contrast, genotypes NIA-AS-14-10, NIA-AS-14-9, NRL-1237 and NIA-AS-14-8 had accumulated higher Na^+ content under salinity. Though, genotypes CT-09117 and NRL-1235 revealed least Na^+ accumulation but showed relatively higher increase by 12

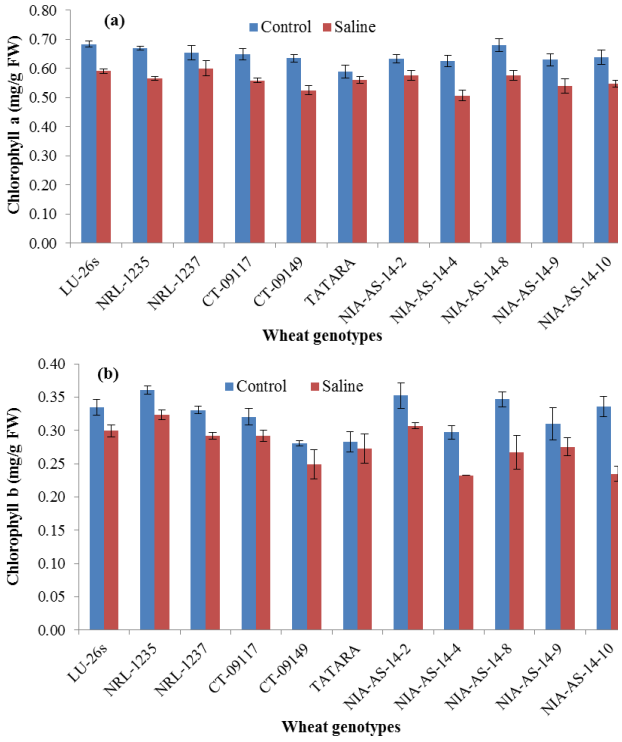

Fig. 1: Impact of salt stress (120 mM NaCl) on chlorophyll *a* content (a) and chlorophyll *b* content (b) ± S.E. of different wheat genotypes

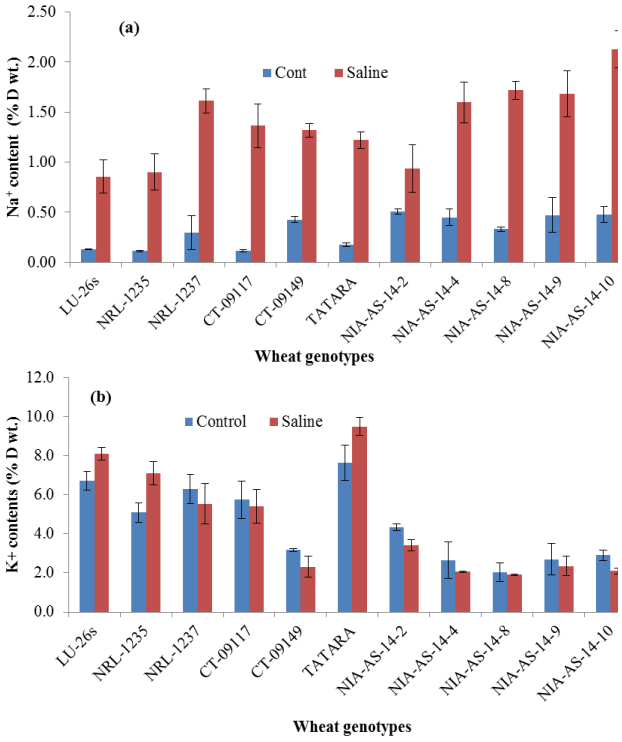

Fig. 2: Impact of salt stress (120 mM NaCl) on Na^+ (a) and K^+ contents (b) ± S.E. of different wheat genotypes

and 8 folds under salinity. Similar to Na^+ content, wheat genotypes also exhibited a variable response regarding leaf potassium (K) content under control and salinity stress (Fig. 2b). Salinity stress had caused an average decrease of 1.01 fold in leaf K content of studied genotypes over non-saline control. Wheat genotypes NIA-AS-14-10, 8, 4 and CT-09149 had accumulated lower content of K^+ under all experimental conditions. However, genotypes NRL-1237 and CT-091117 maintained significantly high K^+ content accompanied by restricted Na uptake. Hence, the accumulation of leaf K^+ content enhanced unexpectedly in Tatara, LU-26s and NRL1235 under saline environment. The relative increase in leaf Na^+ content caused a significant decline in grain yield and is further confirmed by correlation which showed a negative relationship between them (Fig. 4c). In contrast, leaf K^+ content had illustrated a positive correlation ($r^2 = 0.635$) with grain yield; resulting in a linear increase in grain yield with increasing K^+ content of wheat leaves (Fig. 4d).

The root zone salinization due to excessive salts in growing medium resulted in significant ($P \leq 0.05$) decrease (more negative) in leaf osmotic potential (OP) but uneven in all examined genotypes (Fig. 3). On average, leaf osmotic potential reduced from 0.63 Mpa in non-saline control to 0.82 MPa at 120 mM NaCl salinity. Osmotic potential varied subsequently from 3.76 to 15.20 (Mpa) among the genotypes exposed to salinity (120 mM NaCl). The genotypes NIA-AS-14-4, CT-09149 and Tatara illustrated a greater decline (53.05, 37.4 and 35.1%, respectively) in Ψs under saline conditions while less reduction was recorded in NIA-AS-14-8, CT-09117, LU-26s and NIA-AS-14-2 as compared to control by 8.16, 14.79, 16.77 and 24.3% individually.

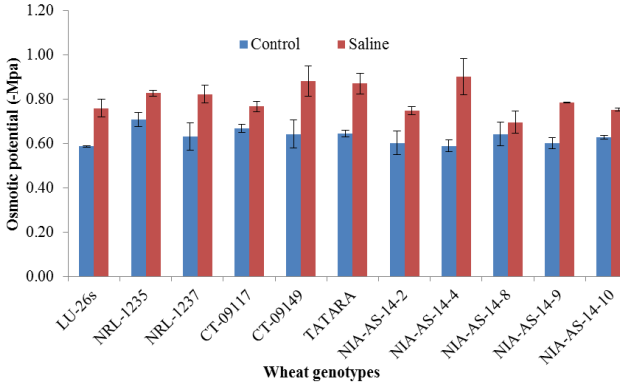

Fig. 3: Impact of salt stress (120 mM NaCl) on osmotic potential (MPa) ± S.E. in different wheat genotypes

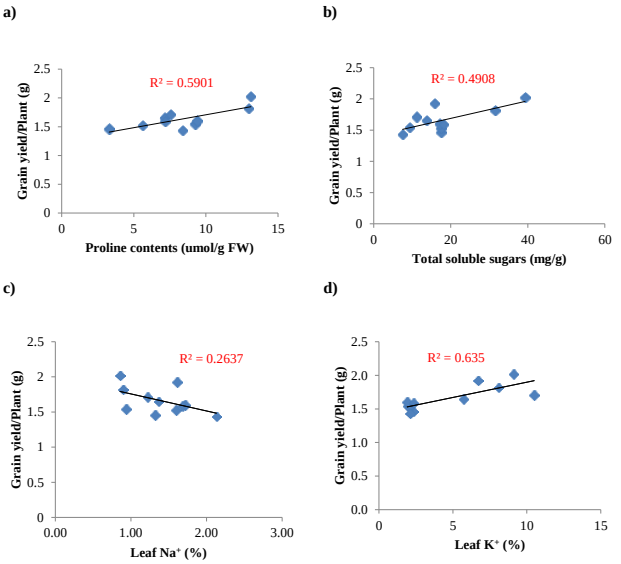

Fig. 4: Relationship of grain yield with (a) Leaf proline, (b) Total soluble sugars, (c) Na^+ and (d) K^+ content in different wheat genotypes

Discussion

Plant growth inhibition is a common response to salinity. The data illustrated that plant height, productive tillers, plant biomass and grain yield showed a considerable reduction on exposure to saline conditions but the effect varied in different wheat genotypes (Table 1). Likewise, hampered growth in salt-stressed plants of wheat was also reported by various investigators (Turki *et al*., 2012; Khan *et al*., 2014; Rahman *et al*., 2014). Reduction in plant growth by salinity might be due to the inhibitory effect of ions on cell division and expansion directly (Zhu, 2001). Moreover, retarded plant growth and development results from physiological water deficit under saline conditions that might be due to reduced solute potential which significantly disturbs uptake of water, thus leading water potential more negative (Munns, 2002; Cha-um *et al*., 2010). Excessive salts in growth medium caused a reduction in uptake of essential nutrients and available water, which result in restricted plant height (Desoky and Merwad, 2015) and decreased reproductive tillers (Khan *et al.*, 2014). Likewise, reduction in plant height and productive tillers was also noted among the wheat genotypes in present investigation (Table 1). The osmotic stress resulted from root zone salinity reduces the rate of tiller production (Munns, 2002), which caused more reduction in grain yield than later stages under salinity. It is also obvious from this study that plant biomass and grain yield adversely affected by NaCl salinity among wheat genotypes. Our results are parallel with the observations of Hasan *et al.* (2015) that salt-sensitive genotypes were affected more in their biomass production, succeeded by low final grain yield than salt-tolerant genotypes under saline environment. Upon exposure to salinity, the reduced grain yield could be result of poor tiller formation due to ionic toxicity and osmotic stress created by the excessive salts. The shortened duration of spikelet differentiation and grain filling caused further decrease in grain yield under salinity (Francoise *et al*., 1994).

Accumulation of compatible solutes is an important

tolerance mechanism exhibited by plants under stress conditions. The obtained data revealed that all wheat genotypes showed a significant ($P \leq 0.05$) increase in leaf proline content during salt stress (Table 2). Hence, salt-tolerant genotypes NRL-1235, Lu-26s and NIA-AS-14-2 showed maximum accumulation of proline which confirmed the previous reports that salt tolerant wheat cultivars generally exhibit higher proline content than the salt sensitive (Hasan *et al.*, 2015; Mahboob *et al*., 2016). It is obvious from present study that salt stress up-regulated the enzymes involved in biosynthesis and enhanced the level of proline which might be result of proline to stimulate the expression of salt stress respondent genes, which hold proline responsive elements in their promoters (Chinnusamy *et al*., 2005). Results of Khan *et al.* (2014) verified by this study as proline demonstrated a positive correlation with grain yield per plant (Fig. 4a), which indicates its potential to induce salt tolerance, might be due to its role in osmotic adjustment and stabilizing the structure of organelles and macromolecules (Sumithra *et al*., 2006). Like proline, glycine betaine is also considered to play a crucial role in salinity tolerance by protecting plant cells through osmotic adjustment (Raza *et al.*, 2006), stabilizes proteins to secure the photosynthetic apparatus (Cha-um *et al.*, 2010). In current study, most of the tested genotypes subjected to saline conditions (120 mM NaCl) expressed an increase in GB content (Table 2). Improved GB content in LU-26s, NRL-1235 and Tatara indicates their salt tolerance because GB mainly contributes to osmotic adjustment and is one of the important factors for improving photosynthetic capacity under salt stress (Raza *et al.*, 2006). Increased leaf GB content results from enhanced feeding of SAM and precursor glycine upon exposure to salt stress (Waditee *et al*., 2005). It was observed that non-stressed plants of CT-09149, NRL-1237 and NIA-AS-14-10 showed more GB than salt-stressed plants (Table 2). Thus, the elevated GB in non-stressed plants might be due to the distant transport of GB which may be phloem mobile driven by transpiration stream as non-stressed plants had relatively higher transpiration rate than salt stressed (Makela *et al*., 1996).

Accumulation of soluble sugars is commonly experienced and considered as one of the most notable consequences for osmotic adjustment under salt stress. Our data revealed a significant ($P \leq 0.05$) increase in endogenous level of TSS in all genotypes exposed to 120 mM NaCl stress (Table 2). Salt tolerant Lu-26s and NRL-1235 showed maximum values for TSS by producing 4.74 and 3.44 fold more sugars as compared to control plants. Accumulation of sugars under salt stress supported the well-established roles of sugars as an osmoprotectant that stabilizes cellular membrane, carbon storage and scavenging of reactive oxygen species (Gupta and Huang, 2014). Many researchers have already reported that leaf TSS significantly increased in both salt tolerant and sensitive wheat cultivars in response to NaCl stress (Radi *et al*., 2013; Khan *et al*., 2014; Mahboob *et al.*, 2016), which may encourage the salt tolerance either acting as an osmoticum or respiratory substrates. The correlation analysis showed that grain yield increases with increase of TSS in evaluated wheat genotypes which indicates a positive relationship (r^2= 0.64) between them (Fig. 4b) and these findings are strengthen by Khan *et al.* (2014).

The biosynthesis of phenolics usually changed in response of various biotic and abiotic stresses including salinity (Parida and Das, 2005), thus the accumulation of phenols could be a cellular adaptive mechanism for scavenging oxygen free radicals during stress (Mohamed and Aly, 2008). It is clear from present results that on average by 78.1% more phenolic compounds produced in wheat genotypes under salinity as compared to control (Table 2). The increase in phenolic compounds was recorded under abiotic stress would be credited to the activation of phenylalanine ammonia lyase (PAL) (Rivero *et al.*, 2001). Under salinity, a significant and gradual increase in level of phenolic content was observed in wheat (Desoky and Merwad, 2015; Mahboob *et al*., 2016), which illustrated the induction of secondary metabolism as one of the defense mechanisms adapted by the plants to face saline environment (Radi *et al*., 2013). The greater ability of phenolics to donate H^+ and to stabilize free radicals ranked them highly active than a range of other antioxidant metabolites (Rice-Evans, *et al*., 1997).

Chlorophyll played a vital role in photosynthesis being a photosynthetic pigment. Salinity stress induced accumulation of toxic ions and physiological water deficit in leaves delayed the chlorophyll biosynthesis and also accelerated the degradation of original chlorophyll (Zheng *et al.*, 2008). Similar outcomes were also found in present study, where a significant degradation of chlorophyll was noted in all the wheat genotypes at 120 mM NaCl stress (Fig. 1a, b). Our results are also in accord with those reported by many researchers in wheat (Cuin *et al*., 2008; Khan *et al*., 2010). The decreased chlorophyll content due to salinity is presumed as the stability of chlorophyll is associated with membrane strength, which under saline condition rarely remains intact (Ashraf and Foolad, 2005).

Salinity caused a significant ($P \leq 0.05$) increase in leaf Na^+ content but antagonistically lower K^+, which also differed substantially among the genotypes (Fig. 2a, b). Salt-sensitive genotypes NIA-AS-14-10, 8, 9 and 4 showed higher leaf Na^+ content accompanied by lower content of K^+ in response to salinity (Fig. 2a), which confirmed the previous findings (Abbas *et al*., 2013; Rahman *et al*., 2014; Hasan *et al*., 2015). In plants cells the net buildup of sodium (Na^+) might be because of equilibrium between influx via ion channels and efflux through a probable Na^+/H^+ antiporter (Tester and Davenport, 2003). It is cleared from data that in salt-sensitive genotypes K^+ level decreased by salinity, while tolerant genotypes, LU-26s, NRL1235 and Tatara increased their leaves K^+ content compared with their respective control (Fig. 2b). Cuin *et al.* (2008) also observed similar increase in leaf K^+ content in wheat plants exposed

to salinity and illustrated that the change in potassium levels at whole tissue level is primarily an indication of K^+ behavior within the vacuole, so hiding the degree of variations in activity of cytosolic potassium. Correlation analysis presented a negative relationship between wheat grain yield and leaf sodium content as escalating leaf sodium caused a linear decrease in grain yield (Fig. 4c). On the other hand, grain yield per plant showed a positive correlation with leaf K^+ content; resulting in increase in grain yield with an increase in K^+ contents (Fig. 4d). These results are in harmony with previous findings (Khan *et al*. 2014; Hasan *et al*., 2015).

Saline environment reduced the solute potential which significantly affects the ability of plant to take up water (Munns, 2002), thus manipulate the water potential to be more negative that caused reduction in plant growth (Cha-um *et al*., 2010). Our data showed that higher reduction (more negative) in osmotic potential was found in wheat genotypes when exposed to salinity for a long time (Fig. 3). Reduced Ψs under salinity was also reported by Rahman *et al*. (2014) in wheat. In our results, all the tested wheat genotypes under salinity had decreased their Ψs might be due to unrestricted flow of toxic ions like Na^+ inside the plant cells and/or accumulation of compatible solutes that is vital for osmoregulation; this outcome is in lined with Cha-um *et al*. (2010).

Conclusion

It is concluded from the results, that imposition of salt stress significantly affected the growth and yield attributes, as well as observed physiological traits. Salt-tolerant wheat genotypes LU-26s, CT-09117, NRL-1235, NRL-1237, NIA-AS-14-2 and Tatara were able to maintaining better growth and yield, photosynthetic pigments and also significantly enhanced level of osmoprotectants, antioxidant phenolics, K^+ content and low Na^+ concentration ultimately improved osmotic adjustment which is the characteristics of salt tolerant genotypes. Wheat genotype NIA-AS-17-4 and NIA-AS-17-9 exhibited sensitivity towards salinity while CT-09149 and NIA-AS-14-8 and 10 performed moderately under saline conditions. All physiological traits contributed to better growth and yield of salt tolerant genotypes under saline condition.

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(Received 09 December 2016; Accepted 04 April 2017)