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ANALYSIS OF SALINITY TOLERANCE POTENTIAL IN SYNTHETIC HEXAPLOID WHEAT

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Abstract

Salinity is an important environmental constraint for agricultural crops including wheat. This experiment was performed to examine salinity tolerance in synthetic hexaploid wheats as compared to three commercial wheat varieties (Zarghoon-79, Kharchia and Shorawaki). All genotypes were grown at various salinity levels (0 mM, 50 mM, 100 mM, 150 mM and 200 mM) and then screened based on seed germination rate, length of root and shoot and fresh and dry weight of root and shoot. Biochemical parameters including photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids), ionic content (Na^+, K^+, Ca^+), antioxidant enzymes; superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), total soluble protein (TSP) and total soluble sugar (TSS) were also conducted on these genotypes. The result of analysis of variance showed that for most traits, there were highly significant differences among salt concentrations, varieties and their interaction. The synthetic lines (genotypes 1 to 10) showed significant tolerance to different levels of salt (Sodium Chloride; NaCl) as compared to commercial wheat cultivars (Zarghoon-79, Kharchia and Shorawaki). Two synthetic lines; genotypes 2 and 4 performed the best under stress condition. It has also been observed that wheat seedlings performed better at 50 mM concentration of NaCl as compared to control (0 mM NaCl) and other levels of salt (100 mM, 150 mM, 200 mM). Correlation analysis revealed that the increase of germination percentage, shoot and root length, fresh weight, dry weight, chlorophyll a, chlorophyll b, carotenoids and potassium were significantly correlated with the decrease in germination rate, sodium, calcium, SOD, POD, CAT, TSP and TSS.

Key words: Salinity stress, Bread wheat, Synthetic hexaploids, Total soluble sugar, Antioxidant enzymes.

Introduction

The significance of wheat (*Triticum aestivum*) cannot be neglected for being a member of "Big-Three Cereal Crops" (Shewry, 2009). Apart from its daily global consumption, its nutritional value also contributes a lot to human needs. According to Food and Agriculture Organization (FAO), total wheat consumption for the year 2014/15 as per 2nd April 2015 was 710.9 million tons, whereas the total production was 728.2 million tons (Anon., 2015). It is established that wheat is consumed as staple food and one-fifth of the calories utilized globally by humans in a year is achieved from wheat (Waines & Ehdaie, 2007). A wheat grain contains high quantities of important nutrients like 60-70% starch (carbohydrates), and 8-15% proteins (such as glutenin and gliadin) (Shewry *et al.,* 1995; D'Ovidio & Masci, 2004; Slade *et al.,* 2012). The 65% of the total wheat grain produced worldwide is utilized as a part of daily diet by man while the remaining 35% is spread among; 8% as seed material, 21% as livestock feed and 6% as raw material to industries to be used as fermentation substrate for making vitamins and antibiotics, paper production and adhesives in various products (Shewry & Jones, 2005).

In the last 50 years, wheat yields have been increased to 41 kg per hectare due to technological advancement and research (Ewert *et al.,* 2005). With an increase in world's population at an exorbitant pace (Godfray *et al.,* 2010), the projected world's population will be 9-10 billion by the year 2025 (DeLong *et al.,* 2010), which, in turn, be challenging to fulfill the needs of food and energy resources (Ray *et al.,* 2013). Mathematically, the required rate of increment in wheat production should be doubled by 2050 (Tilman *et al.,* 2001). The determined yield of wheat in 2005 was 2.5 tons per hectare, while the expected yield is to get 4.0 tons/ha by 2020 (Rajaram, 2005). As a matter of fact, just 3 billion hectares of land out of 13.4 billion is accessible for cultivation purposes (Smith *et al.,* 2010). The answer to overcome world's nutritional demands is to make the uncultivable land arable (Gregory *et al.,* 2002). It has been evaluated that by using just 20% of aggregate uncultivated soil, we can proliferate crop yield up to 67% (Bruinsma, 2003, 2009). Unfortunately, per capita arable land is consistently diminishing due to housing, industrialization, deforestation and various other environmental issues (Gregory and George, 2011). The environmental issues account for 50-80% yield loss in crop plants (Lobell *et al.,* 2009). Many scientific groups are trying to reduce the gap between potential and actual yields (Jaggard *et al*., 2010) by addressing various biotic and abiotic stresses (like drought, salinity and heat) (Atkinson & Urwin, 2012). One of these abiotic stresses is higher concentration of salt in soil known as salt stress or salinity stress.

There is no direct method and/or parameter for calculation of tolerance potential of a plant living in stress condition; rather, comparative physiological, biochemical and molecular analysis can be performed. Each of these categories contains many cited protocols that are commonly utilized for evaluation of a plant's potential of stress tolerance. Some of these protocols in physiological observations include plant morphology like dry and fresh weights of shoots and roots, and lengths of roots and shoots (Khan *et al.,* 2017; Jamil *et al.,* 2012). The

biochemical protocols include determination of ionic contents (Awan & Salim, 1997), antioxidants (Nasim *et al.,* 2017; Zhou & Leul, 1999; Zhang *et al*., 2008; Aebi, 1984) and other cellular components like proteins (Bradford, 1976) and sugars (Qayyum *et al.,* 2011).

Salinity is one of the significant limitations to harvest high yield globally (Flowers & Yeo, 1995). Saline soils are prevalent in parched and semiarid districts particularly in the regions where overwhelming water system or over preparation is practiced (Reynolds *et al.,* 2005). It is assessed that 800-930 million hectares (7%) of world's aggregate arable land is impacted by salt stress (Shannon, 1997; Szabolcs, 1994) whereas 230 million hectares of flooded land is affected by salinity (Oldeman *et al.,* 1991). To fulfill nutritional requirement of mankind, it is vital to combat salinity stress. As the soil management practices are labor extensive, time-consuming and costly, biotechnological approaches such as development of salt resistant varieties provide the more productive way forward.

Synthetic hexaploid wheats provide a mean of bringing alien genes from wild relatives to help increase the genetic diversity of bread wheat cultivars (Yanga *et al.,* 2014). The identified tolerance in synthetic wheats can be introduced into local wheat cultivars by recombination breeding. This study is designed to assess the salt tolerance potential of synthetic hexaploid wheats.

Materials and Methods

The seeds of three commercial varieties (Zarghoon-79, Kharchia and Shorawaki) and ten synthetic wheat lines were obtained from Wheat Wide Crosses, National Agricultural Research Centre (NARC), Islamabad, Pakistan. The synthetic wheat lines are labeled as genotypes 1-10 and three commercial varieties are labeled as genotypes 11-13. The pedigree of all these genotypes is listed in Table 1.

Using method of Jamil *et al.,* 2012, healthy seeds were sorted manually and surface sterilized with 3.5% sodium hypochlorite followed by three rinses with distilled water. Five replicates, each of 10 seeds were then inoculated at four different NaCl salt concentrations in distilled water (50 mM, 100 mM, 150 mM and 200 mM) along with control on doubled folds of filter paper in petri-plates. The plates were kept in growth chamber in dark for two days at 25^{o}C followed by 16-hour photoperiod for five days. Morphological parameters like germination reading, total germination percentage and germination rate (T_{50}) were calculated followed by measuring root and shoot length as well as fresh and dry weight of root and shoot (Hussain *et al.,* 2013). The final number of seeds germinated in a petri plate (or a single treatment) after 7 days of seed inoculation divided by total number of seeds planted (10) followed by multiplication with 100 yielded the germination percentage.

For biochemical analysis, seeds were again grown on petri-plates with the same procedure as above except the application of salt concentrations. After a week of growth in petri-plates, the seedlings were transferred to hydroponic trays containing Hoagland's media (Hoagland & Arnon, 1950) without the application of salts. After growing in hydroponics for one week, wheat seedlings were treated with different concentrations of salt in Hoagland's media for one week. Then the leaves of seedlings were harvested in two forms; (1) fresh material taken from fresh leaves and ground in liquid nitrogen using pestle and mortar, and (2) dry material obtained after drying fresh leaves for two days at 80^{o}C. The fresh material was subjected to the analysis of photosynthetic pigments (Lichtentaler & Wellburn, 1985), ionic content (Awan & Salim, 1997), determination of antioxidants including peroxidase (POD) (Zhou & Leul, 1999), superoxide dismutase (SOD) (Zhang *et al*., 2008), catalase (CAT) (Aebi, 1984), total soluble protein (Bradford, 1976) and total soluble sugar (Qayyum *et al.,* 2011). The dry material was used to analyze ionic content like sodium, potassium and calcium ion.

The factorial experiment was conducted using a completely randomized design with five replications. All data is reported as mean ± SE. The data was analyzed using Proc GLM of Statistical Analysis Software Version 9.4 (SAS Institute Inc., Cary, North Carolina, USA).

Table 1. Pedigree information of synthetic wheat varieties selected for this study.

Genotype #	Varieties	Pedigree
Genotype-1	SH-Salt-01	68.111/RGB-U//WARD RESEL/3/STIL/4/Ae. tauschii (781)
Genotype-2	SH-Salt-02	68.111/RGB-U//WARD/3/FGO/4/RABI/5/Ae. tauschii (882)
Genotype-3	SH-Salt-03	68.111/WARD//AE.SQ (369)
Genotype-4	SH-Salt-04	ALTAR 84/AE.SQ (224)
Genotype-5	SH-Salt-06	ALTAR 84/AE.SQ (220)
Genotype-6	SH-Salt-07	ALTAR 84/AE.SQ (211)
Genotype-7	SH-Salt-08	ALTAR 84/AE.SQ (J BANGOR)
Genotype-8	SH-Salt-09	CETA/AE.SQ (1027)
Genotype-9	SH-Salt-12	D67.2/P66.270//AE.SQ(220)
Genotype-10	SH-Salt-13	D67.2/P66.270//AE.SQ(213)
Genotype-11	Zarghoon-79	(CC/INIA/3/TOB/CTFN//BB/4/7C)
Genotype-12	Kharchia	Land race from Rajasthan, INDIA
Genotype-13	Shorawaki	Land race from Baluchistan, PAKISTAN

Results

The result of analysis of variance (Table 2) reveals significant differences ($p<0.01$) in wheat varieties for all traits except sodium and soluble sugars. There are also highly significant differences ($p<0.01$) in different concentrations of salt for all investigated traits. In addition, interaction effects of variety × salinity (Table 3) are significant for investigated traits except root dry weight, sodium, potassium, calcium and soluble sugars which indicate different response of varieties under different concentrations of salt.

Germination percentage: Among all wheat varieties, synthetic lines showed 100% germination under various concentrations of salt (NaCl) whereas other varieties showed deviating results, as shown in Figure 1A. The increase in salt (NaCl) concentration showed no impact on germination of synthetic lines (genotypes 1-10), however, the germination pattern of three cultivars (genotypes 11-13) varied. The germination percentages of these three cultivars tend to decrease with the increase in salt concentration. The lowest germination percentages observed were 57.14%, 42.85% and 28.57% at highest salt concentration (200 mM NaCl) in genotypes 11, 12 and 13, respectively.

Germination rate (T_{50} days): Although most inoculated seeds germinated, the time to reach half percent germination (T_{50} days) varied from cultivar to cultivar as shown in Figure 1B. Our study showed that wheat seed took an average of two days (48 hours approximately) to germinate (radical reaches 2 mm in length) under controlled (0 mM NaCl) condition. Ten synthetic wheat lines (genotypes 1-10) showed minor difference in germination rate at different salt concentrations. The germination rates of these varieties lie within the range of 2.0 to 2.5 days at all concentrations of NaCl. Among the synthetic wheat lines, the minimum time taken for germination was 1.89 days by genotype 4 at 0 mM NaCl whereas the maximum time taken for germination was 2.69 days by genotype 5 at 200 mM NaCl concentration. The three check cultivars germinated in about two days under controlled conditions but took more time as compared to synthetic lines with the increase in NaCl concentration. At 200 mM NaCl concentration, genotypes 11, 12 and 13 took 3.35, 3.47 and 3.64 days to germinate, respectively.

Shoot and root lengths: All wheat seeds grown under five salinity treatments (0 mM, 50 mM, 100 mM, 150 mM and 200 mM) resulted in different seedling lengths. The 7-day old seedlings showing the impact of different levels of NaCl stress on root and shoot lengths are presented in Figure 2. The individual length of shoots and roots of 7-days old seedlings is presented in Figures 1C and D. A general pattern was observed with the increase in salinity levels i.e., the length of roots as well as shoots decreased. However, wide variation in shoot and root length existed among the tested genotypes. The maximum shoot and root length was observed in genotype 4 followed by genotype 2. Genotype 2 showed a lower deviation (22.88, 22.27, 21.91, 21.35 and 20.66 cm shoot) as compared to genotype 4 (23.90, 22.88, 21.58, 20.62 and 19.14 cm shoot) with the increase in salt levels (Control, 50 mM, 100 mM, 150 mM and 200 mM), respectively. Similarly, the remaining salt synthetic lines performed well in terms of shoot and root lengths. Overall, the synthetic lines showed less deviating results as compared to commercial cultivars (susceptible checks). The commercial cultivars (genotypes 11-13) showed comparable results under control (0 mM NaCl) condition. However, with the increase in salinity levels, shoot as well as root lengths of these cultivars decreased. The minimum lengths of roots (4.72cm) and shoots (4.38cm) are recorded in genotype 13 (Shorawaki) at 200 mM NaCl followed by genotypes 11 and 12.

Fresh and dry biomass: Both fresh and dry biomass decreased with the increase in NaCl concentration. This pattern was found in all varieties as shown in Figure 3A and B for fresh weight of shoots, fresh weight of roots and Figure 3C and D for dry weight of shoots and dry weight of roots, respectively. The fresh and dry weights of all wheat varieties decreased with the increase in salt stress but genotypes 2 and 4 performed comparatively better than other varieties. The best performance as shown by genotype 4 was 1.093 mg, 0.666 mg, 0.491 mg and 0.277 mg for fresh weight of shoot, fresh weight of root, dry weight of shoot and dry weight of root, respectively. On the other hand, genotype 13 (Shorawaki) underperformed at all concentrations.

Biochemical analysis: The photosynthetic pigment concentration of chlorophyll-a, chlorophyll-b and total carotenoids decreased under different salt concentrations in all genotypes (Fig. 4A, B and C). There was an increase in concentration of photosynthetic pigments in all tested genotypes, but the values were comparatively higher in genotypes 2 and 4. These genotypes were unique in a way because the concentration of photosynthetic pigments was higher at 50 mM as compared to control (0 mM NaCl). Although salt stress influenced all photosynthetic pigments including chlorophyll a, chlorophyll b and carotenoids but very small differences were observed at 0 mM and 50 mM of NaCl in all genotypes. Even in some cases, the concentration of chlorophyll a, chlorophyll b and carotenoids was higher at 50 mM than 0 mM of NaCl (genotypes 1, 2, 4 and 5). However, in check cultivars (genotypes 11 to 13; Zarghoon-79, Kharchia and Shorawaki), no such pattern was visible. In their case, the concentration of all photosynthetic pigments decreased with the increase in salt concentration.

In case of ionic content in shoot samples, sodium and calcium ion content increased with the increase in salt concentration (Fig. 4D and F). In contrast, potassium ion concentrations decreased with the increase in salt concentration in all genotypes (Fig. 4E). The highest concentration of sodium and calcium ions was found in genotypes 11 to 13 (check cultivars) whereas genotypes 2 and 4 (synthetic wheats) showed the lowest sodium ion content. In contrast, genotypes 11-13 showed the lowest potassium ion concentrations whereas genotypes 2 and 4 showed minimum variations at different levels of NaCl. The synthetic wheat lines performed well and maintained sodium, potassium and calcium ion concentration with a little variation whereas more variation was observed in commercial cultivars.

Table 2. Analysis of variance of the effect of salinity stress on investigated traits in commercial and synthetic wheat genotypes.

Source of variation	d.f	Mean square (MS)									
		Germination	T 50 days	Shoot length	Root length	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Chlorophyll a	Chlorophyll b
Genotype	12	72.9944**	1.3604**	455.6499**	191.4799**	1.2647**	0.5971**	0.3169**	0.0853**	0.3253**	0.2905**
Salinity	4	9.1000**	3.8575**	164.8657**	215.7496**	0.5939**	0.3683**	0.2361**	0.0560**	1.7747**	0.1000**
Genotype × Salinity	48	2.6450**	0.1772**	3.8278**	3.7616**	0.0130**	0.0095**	0.0048**	0.0008	0.0139**	0.0023**
Error	260	0.1400	0.0038	1.2291	1.3777	0.0024	0.0013	0.0011	0.0010	0.0029	0.0007
CV (%)	-	4.08	2.60	7.33	8.40	7.36	11.95	12.38	20.39	4.03	8.57

Source of variation	d.f	Mean square (MS)								
		Carotenoids	Sodium	Potassium	Calcium	SOD	Peroxidase	Catalase	Soluble proteins	Soluble sugars
Genotype	12	1.7662**	0.0525	0.2715**	0.0516**	45.9726**	113.8748**	180.9757**	140.0399**	0.7279
Salinity	4	2.3912**	0.1292**	0.1181**	0.1157**	1552.3034**	1562.5177**	6455.4422**	19777.7007**	37.0176**
Genotype×Salinity	48	0.0401**	0.0045	0.0021	0.0043	13.7713**	20.5071**	20.1495**	22.9500**	0.0764
Error	260	0.0033	0.0363	0.0096	0.0138	0.0218	0.4310	0.6644	0.2291	0.8801
CV (%)	-	5.82	23.78	17.36	5.28	1.01	1.14	1.31	0.84	6.44

Table 3. Correlation coefficient among investigated traits in saline conditions in commercial and synthetic wheat genotypes.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1.	Germination	1																		
2.	T50days	-0.845**	1																	
3.	Shoot length	0.784**	-0.855**	1																
4.	Root length	0.742**	-0.748**	0.886**	1															
5.	Shoot fresh weight	0.778**	-0.827**	0.989**	0.879**	1														
6.	Root fresh weight	0.617*	-0.787**	0.957**	0.872**	0.939**	1													
7.	Shoot dry weight	0.571*	-0.736**	0.924**	0.835**	0.906**	0.987**	1												
8.	Root dry weight	0.740**	-0.824**	0.950**	0.865**	0.910**	0.947**	0.943**	1											
9.	Chlorophyll a	0.564*	-0.711**	0.919**	0.834**	0.899**	0.977**	0.978**	0.934**	1										
10.	Chlorophyll b	0.779**	-0.854**	0.998**	0.897**	0.986**	0.961**	0.926**	0.950**	0.924**	1									
11.	Carotenoids	0.716**	-0.811**	0.844**	0.813**	0.777**	0.792**	0.735**	0.886**	0.749**	0.847**	1								
12.	Sodium	-0.512	0.591*	-0.843**	-0.800**	-0.823**	-0.870**	-0.881**	-0.897**	-0.867**	-0.845**	-0.795**	1							
13.	Potassium	0.754**	-0.859**	0.964**	0.876**	0.959**	0.950**	0.920**	0.914**	0.916**	0.976**	0.775**	-0.796**	1						
14.	Calcium	-0.512	0.587*	-0.845**	-0.795**	-0.829**	-0.874**	-0.886**	-0.897**	-0.872**	-0.848**	-0.782**	0.999**	-0.803**	1					
15.	SOD	-0.667*	0.712**	-0.733**	-0.760**	-0.700**	-0.699**	-0.700**	-0.747**	-0.702**	-0.743**	-0.681*	0.631*	-0.736**	0.617*	1				
16.	Peroxidase	-0.687**	0.738**	-0.805**	-0.769**	-0.765**	-0.799**	-0.798**	-0.823**	-0.814**	-0.827**	-0.685**	0.659*	-0.870**	0.657*	0.893**	1			
17.	Catalase	-0.627*	0.699**	-0.865**	-0.821**	-0.872**	-0.892**	-0.891**	-0.852**	-0.818**	-0.870**	-0.665*	0.815**	-0.873**	0.824**	0.506	0.644*	1		
18.	Soluble proteins	-0.729**	0.702**	-0.770**	-0.902**	-0.747**	-0.711**	-0.679**	-0.789**	-0.675*	-0.779**	-0.842**	0.778**	-0.726**	0.762**	0.843**	0.712**	0.638*	1	
19.	Soluble sugars	-0.716**	0.774**	-0.937**	-0.898**	-0.917**	-0.934**	-0.934**	-0.976**	-0.937**	-0.941**	-0.854**	0.929**	-0.914**	0.929**	0.758**	0.811**	0.848**	0.835**	1

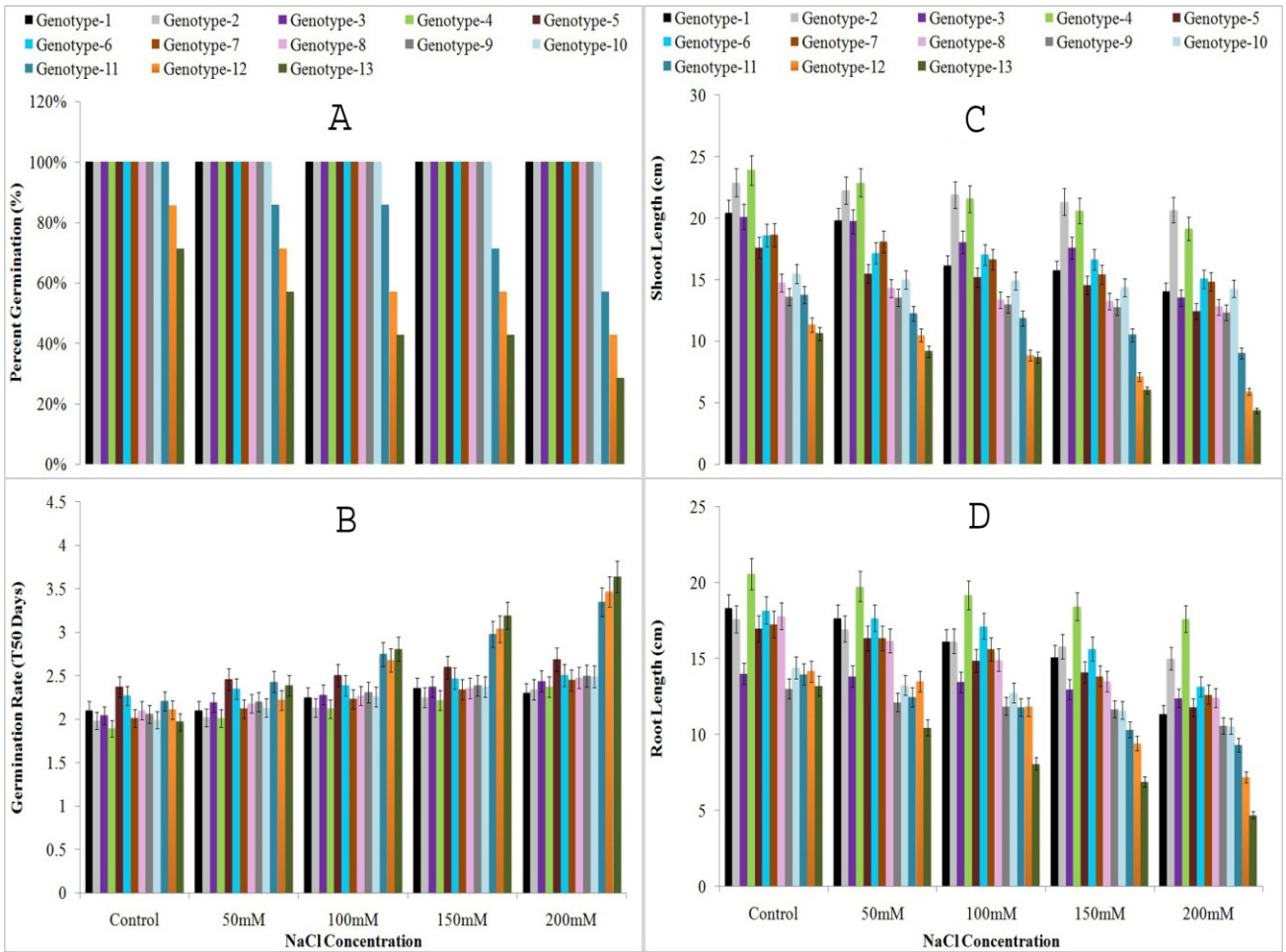

Fig. 1. (A) Percent germinated seeds, (B) germination rate (T_{50} Days), (C) Length of shoots and (D) roots of wheat varieties in response to varying NaCl concentration.

The activity of antioxidant enzymes plays a defensive role in plants and tends to increase when plants survive under stress conditions. The activity of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in wheat seedlings grown under different salt concentrations are shown in Figure 4G, H and I, respectively. At 0 mM NaCl concentration, minimum a of SOD, POD and CAT was observed, which remarkably increased with the increase in salinity levels. Comparatively, activity of all enzymes; SOD, POD and CAT was found higher in commercial cultivars as compared to synthetic wheat lines. The lowest activity of SOD, POD and CAT was recorded in genotypes 2 and genotype 4 whereas the highest activity of all these enzymes was found in genotypes 11, 12 and 13.

In response to increase in salt levels, the amount of total soluble proteins (TSP) increased as shown in Figure 5A. However, the accumulation of TSP was lower in synthetic wheat lines as compared to check cultivars (genotypes 11, 12 and 13). The lowest TSP accumulation was recorded in genotypes 2 and 4. The amount of total soluble sugar (TSS) also increased with the increase in salt stress as shown in Figure 5B. In comparison, levels of TSS were higher in salt sensitive varieties whereas lower TSS was recorded in salt resistant varieties. The lowest recorded TSS was found in genotypes 2 and 4. The highest rate of accumulation of TSS was found in all three commercial cultivars (genotypes 11-13).

Based on the result of correlation analysis, traits are separated into two groups. The first group includes germination percentage, shoot and root length, fresh weight, dry weight, chlorophyll a, chlorophyll b, carotenoids and potassium, which showed significant positive correlation with each other. The second group consists of germination rate, sodium, calcium, SOD, peroxidase, catalase, soluble proteins and soluble sugars, which also showed significantly positive correlation with each other but significantly negative correlation with the traits in the first group.

Discussion

Biotic and abiotic stresses are the major factors that cause severe damage to plant health leading to significant yield losses. Among abiotic stresses, salt stress or salinity is one of the serious concerns to plant growth and development. Initial results from our experiment revealed reduced germination of wheat seeds at higher concentration of NaCl. The average germination percentage decreased with the increase in salinity (NaCl) stress. The minimum germination was noted at the highest salinity treatment (i.e. 200 mM) as compared to 150, 100, 50 and 0 mM NaCl concentrations. Our findings are in full agreement with Hussain *et al.* (2013), who also reported delayed germination in wheat seeds under salinity stress.

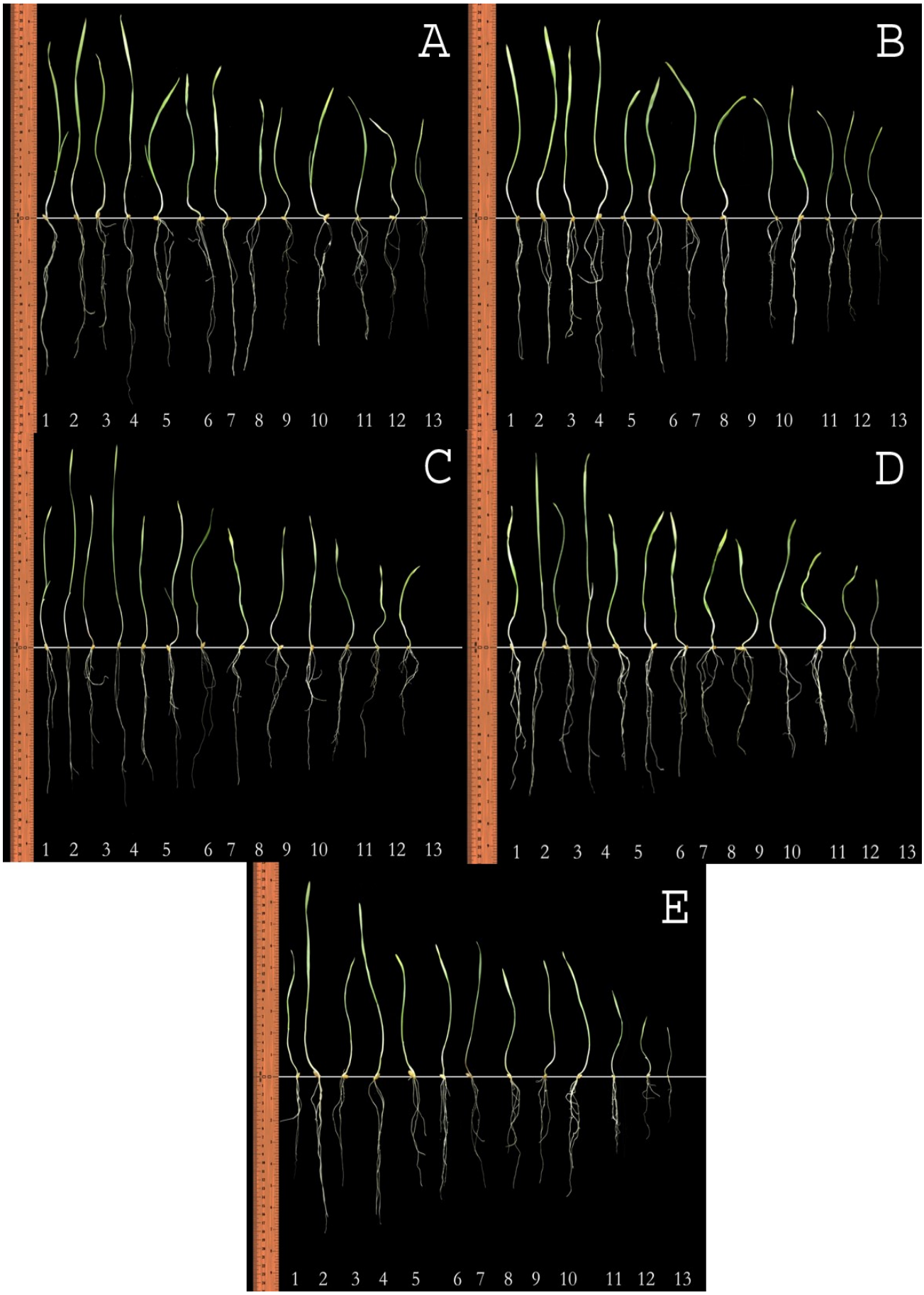

Fig. 2. Ten-days old wheat seedlings grown in (A) controlled condition (0 mM NaCl concentration), (B) 50 mM NaCl concentration, (C) 100 mM NaCl concentration, (D) 150 mM NaCl concentration, and (E) 200 mM NaCl concentration.

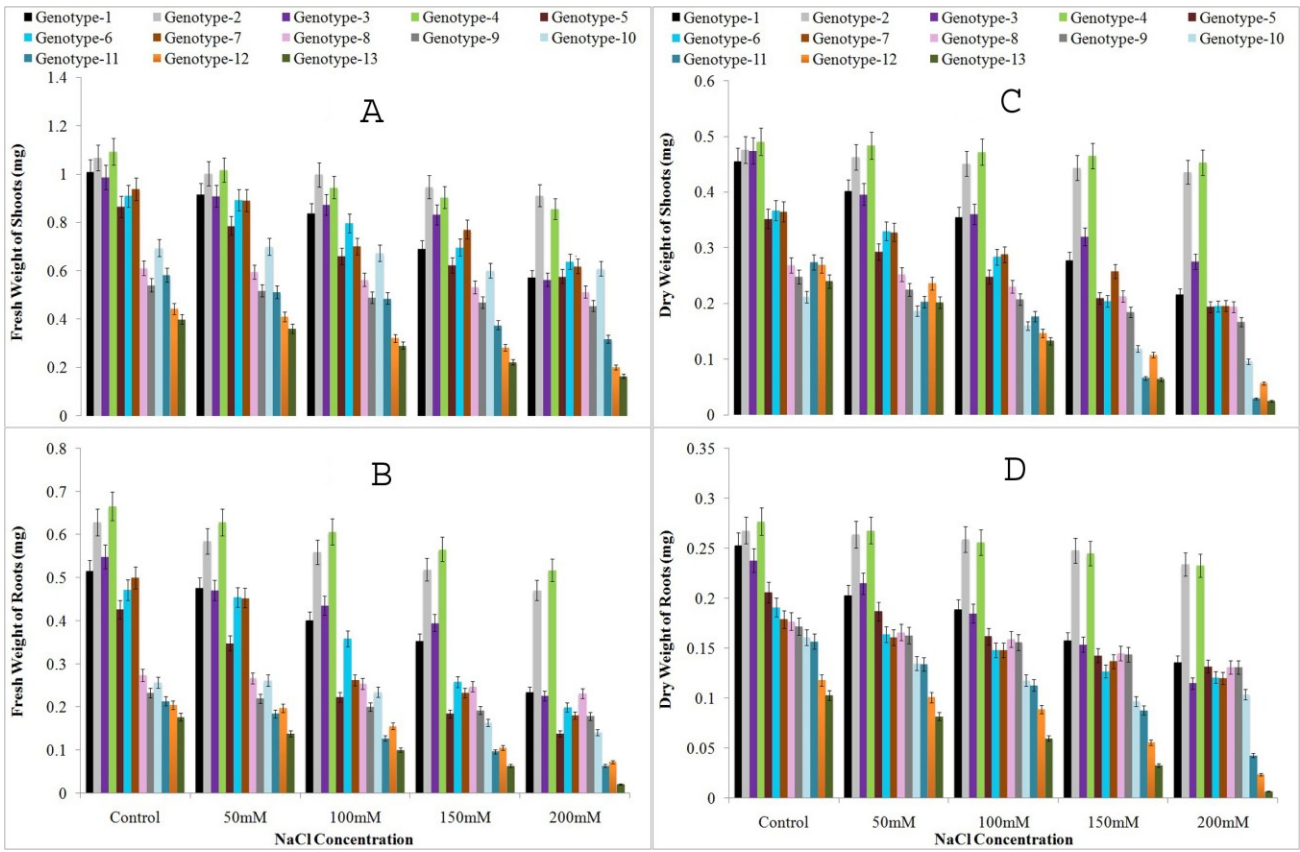

Fig. 3. (A) Fresh weight of shoots, (B) fresh weight of roots, (C) Dry weight of shoots, and (D) dry weight of roots of 10-days old wheat seedlings in response to varying NaCl concentration.

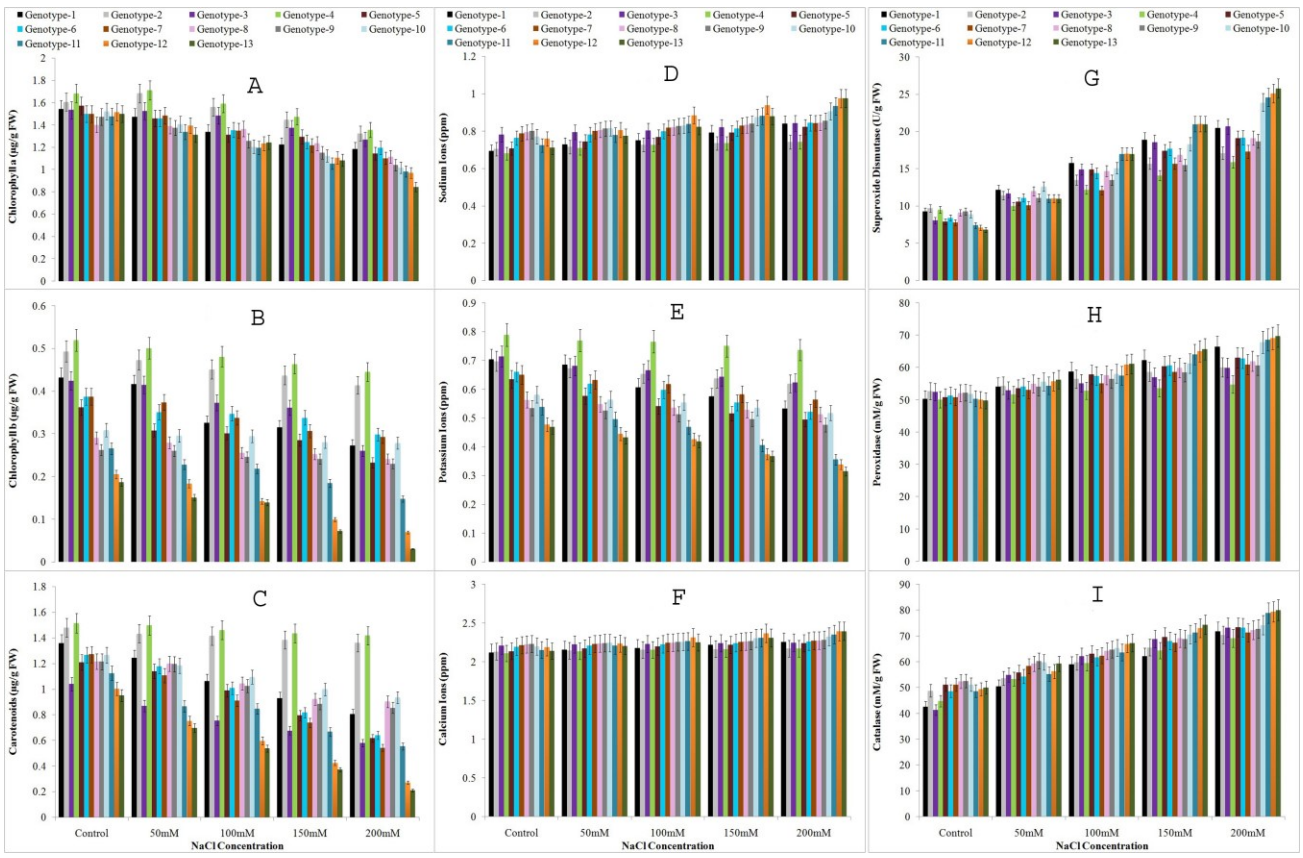

Fig. 4. (A) Chlorophyll-a, (B) chlorophyll-b, (C) carotenoids, (D) Sodium ions, (E) potassium ions, (F) calcium ions, (G) Superoxide dismutase (SOD), (H) peroxidase (POD), and (I) catalase (CAT) contents of 10-days old wheat seedlings in response to varying NaCl concentration.

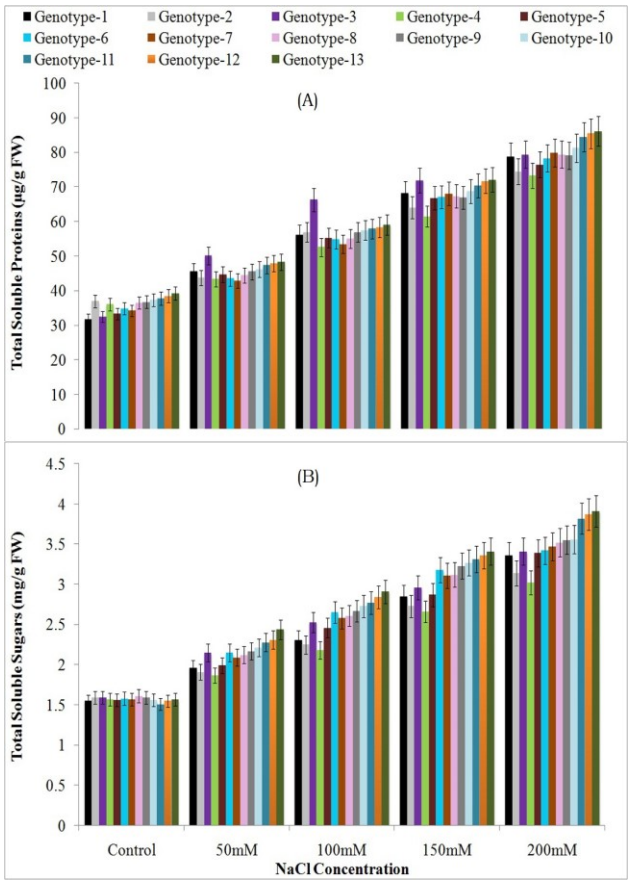

Fig. 5. (A) Total soluble proteins (TSPs) and (B) total soluble sugars (TSSs) content in 10-days old wheat seedlings in response to varying NaCl concentration

Radi *et al.* (2013) studied salt tolerance in wheat and bean varieties and established that non-tolerant varieties cultivated at higher salinity levels lose survival rate arbitrarily whereas tolerant varieties remained unaffected or showed only a little deviation. Our findings (Fig. 1A) corresponds to the reported results because of the germination percentage of genotypes 11-13 (Zarghoon-79, Kharchia and Shorawaki); our check cultivars, were affected when grown at higher salinity levels whereas the potential tolerant varieties; genotypes 1 to 10 stayed partially unaffected. The possible reason behind this issue may primarily lie in connection between the germination inducing mechanism inside the seed and the level of salt stress. The germination potential of seeds is usually achieved under optimum environmental conditions. However, under salt stress, the pathways and molecular players like transcription factors and enzymes do not work efficiently. This leads to minimal expression or inactivation of genes vital for seed germination, which ultimately affects germination percentage and germination rate negatively (Hu *et al.,* 2015). However, under the same level of salt stress, synthetic wheat lines (genotype 1-10) showed differnt results. As shown in Fig. 1A and B, no or little difference can be seen in the germination percentage and germination rates respectively under controlled and four different levels of salt stress. Our results are in agreement with Radi *et al*. (2013) who treated seeds of wheat as well as bean with 80 mM and 160 mM of NaCl and found that salt-tolerant cultivars not only were able to germinate under salinity stress but also showed an optimal germination percentage and germination rate. Their response against salinity levels makes them salt-tolerant varieties; however, a thorough investigation is mandatory to prove this statement. Likewise, salinity stress adversely affects morphological attributes of plants (Shabala, 2013) including length, fresh weight as well as dry weight of shoot and root. Afzal *et al.* (2007) observed reduction in length of roots and shoots under salt stress. Salt stress at germinating stage of wheat causes reduction in final germination rate and the length of radicle. In contrast, a salt-tolerant seedling remains either unaffected or show higher root and shoot length as compared to a non-tolerant variety.

In another study, Tammam *et al.* (2008) compared the weights of roots and shoots in wheat plants grown under different salinity levels. It was noted that the weight tended to decrease with the increase in salt level. In wheat seedlings grown at 320 mM, the root and shoot weights were less than those grown at lower salt concentrations (0 mM to 240 mM with intervals of 60 mM). Our results also indicated reduction in fresh and dry weight of root and shoot as salt concentration increased. There was variation in fresh and dry biomass of all varieties, both in roots and shoots. The decrease in fresh and dry weight under salinity stress could be due to disturbance in absorption of water and nutrients from roots under salinity stress leading to ionic imbalances ultimately leading to reduction in biomass.

When a plant is exposed to salinity stress, the rate of photosynthesis slows down due to reduction in chlorophyll a and chlorophyll b content. Din *et al*. (2008) also observed reduction in chlorophyll pigment under salt stress and the same results are also confirmed by our findings as shown in Figure 4A, B, C. It most likely happens due to higher oxidative stress in plants under salt stress. In this study, genotypes 2 and 4 exhibited less reduction in photosynthetic pigments thus greater tolerance to salinity stress than the control. The photosynthetic pigments present in the shoots of wheat seedlings are higher at 50 mM as compared to control (0 mM NaCl). It could be because a small amount of salt is required for germination, growth and photosynthesis in plants although higher concentration poses serious stress.

It is well-established that the mineral nutrition and ionic assimilation and acquisition in wheat are strappingly influenced under high salinity stress (Khan & Aziz, 2013). Our present study showed that the accumulation of ions under different salt concentrations differ from the control. The concentration of K^+ ions (Chao *et al.,* 2013) decreased as the concentration of salt increased and alternatively Na^+ ions (Maathuis, 2014) significantly increased as compared to the control. Under normal conditions, Na^+ ions help in maintaining membrane potential whereas K^+ ions keep osmotic balance and are also used by many enzymes as a co-factor (Din *et al.,* 2008). However, under stress conditions, the concentration of ions changes significantly, which causes diffusion of ions according to density gradients (Wu *et al.,* 2015). Calcium ions play a significant role as a messenger in various signaling pathways of plants under salt stress. Tammam *et al.* (2008) stated that concentration of Ca^{+2} ions increases by increasing salt available to the cytosol. The same pattern is also observed in our study. It can be indicated that the increase in Ca^{+2} signal might be responsible for salt stress signaling pathway resulting in diffusion of more ions across the membrane. Recent studies have shown that the existence of higher absorption of macronutrients i.e. (Na^+, K^+ and Ca^{+2}) in leaves tend to enhance the micronutrient level in wheat cultivars as compared to the control.

Salinity stress is responsible for the generation of reactive oxygen species (ROS) such as superoxide, hydroxyl-radicals and hydrogen peroxide in different parts of plant (Bhutta, 2011). Plants under stress produce various antioxidants to reduce deleterious effects of ROS. In our study, ROS accumulation increased with the increase in salinity stress, which indicates that the antioxidants (superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) enzymes) level in roots and shoots increased as a defense response to stress in wheat because this increase was not observed in control cultivars (susceptible checks). It can be implied that the elevated POD, SOD and CAT enzyme activities increase the ability of scavenging ROS thereby contributing to increased tolerance of plant to high salinity stress. We observed increase in SOD content in shoot at high salt levels as compared to control. Our results are similar to the findings of Bhutta, 2011, who also reported that progressive increment in SOD content in the leaf of wheat is indicative of better tolerance against salinity stress as compared to control. We observed significant increase in salt concentration of peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) in shoot with the increase in salt concentration. It may be because high salts act as strong inducers leading to localized oxidative damage in leaf cells resultantly disrupting metabolic function, causing loss of cellular integrity thus encouraging senescence. Our results also indicate that high salt concentration results in significant increase in catalase activity in shoot. It may be because catalase is one of the key enzymes involved in the removal of toxic peroxides and is universally present in plants as oxidoreductase to decompose ROS to water and molecular oxygen.

Total soluble protein (TSP) plays a protective role under salt stress in many plant species such as rice, wheat, maize and various dicots and the level of soluble proteins may increase or decrease depending on the response of salt tolerant or salt sensitive genotypes (Ashraf & O'leary, 1999). In our findings, it was noted that total soluble protein content increased in both shoot and root with the increase in salt concentration. Perveen *et al.* (2011) also found that soluble protein content increases in the shoots of wheat seedlings under salt stress. They also reported that salt tolerant wheat genotypes can generate more soluble proteins than salt sensitive genotypes. It can be argued that the increase in content of total soluble proteins has a protective role against salt stress and the soluble protein acts as a precursor in production of other metabolites in defense pathways in salinity stress response.

Total soluble sugar (TSS) content also varies in response to abiotic stresses. Production of osmo-protectants like soluble sugar is essential because they initiate positive responses in plants to tolerate various stresses including high salt stress (Khan *et al.,* 2010). Our results indicate increase in total soluble sugar in shoot and root with the increase in salt concentration, which is supported by Khan *et al.* (2010) who also reported increase in TSS like fructose, glucose, raffinose and sucrose in wheat leaves under salt stress. The increase in soluble sugar level protects plant cells from the damage exerted by salinity stress. They function as primary signal messengers in many transduction pathways and the accumulation of sugar might also play a vital role in plant defense mechanism of osmoregulation and energy preservation.

Conclusion

Among all 13 genotypes studied, 10 genotypes (1-10) have shown comparatively better results against different levels of salt (NaCl) as compared to genotypes 11-13. However, two genotypes; 2 and 4 have tolerated salt stress comparatively better than the rest. The check cultivars (genotypes 11-13; Zarghoon-79, Kharchia and Shorawaki) were more susceptible to higher concentration of salt. It is also observed that wheat seedlings performed better at 50 mM concentration of NaCl as compared to control (0 mM NaCl) and higher levels of salt (100 mM, 150 mM, 200 mM).

References

Aebi, H. 1984. Catalase *In vitro*. *Methods Enzymol.,* 105: 121-126.

Afzal, I., S.M.A. Basra, N. Ahmad and T.E. Lodhi. 2007. Counteraction of salinity stress on wheat plants by pre-sowing seed treatments. *Pak. J. Agri. Sci*., 44: 50-58.

Anonymous. 2015. FAO Cereal Supply and Demand Brief. http://www.fao.org/worldfoodsituation/csdb/en/ [Accessed: 8th April, 2015].

Ashraf, M. and J.W. O'leary. 1999. Changes in soluble proteins in spring wheat stressed with sodium chloride. *Biol. Plantarum*., 42: 113-117.

Atkinson, N.J. and P.E. Urwin. 2012. The interaction of plant biotic and abiotic stresses: from genes to the field. *J. Exp. Bot*., 63: 3523-3543.

Awan, J.A. and U.R. Salim. 1997. Food analysis manual. *Pak. Vet. J.,* 5: 2-7.

Bhutta, W.M. 2011. Antioxidant activity of enzymatic system of two different wheat (*Triticum aestivum* L.) cultivars growing under salt stress. *Plant Soil Environ*., 57: 101-107.

Bradford, N.M. 1976. Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding. *Anal. Biochem.,* 72: 248-254.

Bruinsma, J. 2003. World agriculture: towards 2015/2030, an FAO perspective. London: Earthscan Publications.

Bruinsma, J. 2009. The resource outlook to 2050: by how much do land, water, and crop yields need to increase by 2050? FAO Expert Meeting on 'How to feed the world in 2050'. 24-26 June 2009, Rome: FAO.

Chao, D-Y., B. Dilkes, H. Luo, A. Douglas, E. Yakubova, B. Lahner and D.E. Salt. 2013. Polyploids exhibit higher potassium uptake and salinity tolerance in Arabidopsis. *Science,* 341(6146): 658-659.

D'Ovidio, R. and S. Masci. 2004. The low-molecular-weight glutenin subunits of wheat gluten. *J. Cereal. Sci.,* 39(3): 321-339.

DeLong, J.P., O. Burger and M.J. Hamilton. 2010. Current demographics suggest future energy supplies will be inadequate to slow human population growth. *PLoS One.,* 5(10): e13206.

Din, J., S.U. Khan and I. Ali. 2008. Physiological response of wheat (*Triticum aestivum* L.) varieties as influenced by salinity stress. *The J.A.P.S.,* 18: 125-129.

Ewert, F., M.D.A. Rounsevell, I. Reginster, M.J. Metzger and R. Leemans. 2005. Future scenarios of European agricultural land use. I. Estimating changes in crop productivity. *Agr. Ecosyst. Environ.,* 107: 101-116.

Flowers, T.J. and A.R. Yeo. 1995. Breeding for salinity resistance in crop plants – where next? *Aust. J. Plant Physiol.,* 22: 875-884.

Godfray, H.C.J., J.R. Beddington, I.R. Crute, L. Haddad, D. Lawrence, J.F. Muir, J. Pretty, S. Robinson, S.M. Thomas and C. Toulmin. 2010. Food Security: The challenge of feeding 9 billion people. *Science,* 327: 812-818.

Gregory, P.J. and T.S. George. 2011. Feeding nine billion: the challenge to sustainable crop production. *J. Exp. Bot.,* DOI: 10.1093/jxb/err232.

Gregory, P.J., J.S.I. Ingram, R. Andersson, R.A. Betts, V. Brovkin, T.N. Chase, P.R. Grace, A.J. Gray, N. Hamilton, T.B. Hardy, S.M. Howden, A. Jenkins, M. Meybeck, M. Olsson, I. Ortiz-Monasterio, C.A. Palm, T.W. Payn, M. Rummukainen, R.E. Schulze, M. Thiem, C. Valentin and M.J. Wilkinson. 2002. Environmental consequences of alternative practices for intensifying crop production. *Agr. Ecosyst. Environ.,* 88: 279-290.

Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. *Calif. Agr. Exp. Sta. Circ.,* 347: 32.

Hu, W., Y. Yan, X. Hou, Y. He, Y. Wei, G. Yang, G. He and M. Peng. 2015. TaPP2C1, a Group F2 Protein Phosphatase 2C Gene, Confers Resistance to Salt Stress in Transgenic Tobacco. *PLoS ONE.,* 10(6). doi: e0129589. doi:10.1371/journal.pone.0129589.

Hussain, S., A. Khaliq, A. Matloob, M.A. Wahid and I. Afzal. 2013. Germination and growth response of three wheat cultivars to NaCl salinity. *Soil Environ.,* 32: 36-43.

Jaggard, K.W., A. Qi and E.S. Ober 2010. Possible changes to arable crop yields by 2050. *Philos. T. Roy. Soc. B.,* 365: 2835-2851.

Jamil, M., S. Bashir, S. Anwar, S. Bibi, A. Bangash, F. Ullah and E.S. Rha. 2012. Effect of salinity on physiological and biochemical characteristics of different varieties of rice. *Pak. J. Bot.,* 44: 7-13.

Khan, A. and M. Aziz. 2013. Influence of foliar application of potassium on wheat (*Triticum aestivum* L) under saline conditions. *Sci. Tech. Dev.*, 32: 285-289.

Khan, M.A., E. Islam, M.U. Shirazi, S. Mumtaz, S.M. Mujtaba, M.A. Khan, A. Shereen, M.Y. Ashraf and G.M. Kaleri. 2010. Physiological responses of various wheat genotypes to salinity. *Pak. J. Bot.,* 42: 3497-3505.

Khan, M.A., M.U. Shirazi, A. Shereen, S.M. Mujtaba, M.A. Khan, S. Mumtaz and W. Mahboob. 2017. Identification of some wheat (*Triticum aestivum* L.) lines for salt tolerance on the basis of growth and physiological characters. *Pak. J. Bot.,* 49(2): 397-403.

Lichtenthaler, H.K. and A.R. Wellburn. 1985. Determination of total carotenoids and chlorophyll a and b of leaf in different solvents. *Biochem. Soc. T.,* 11: 591-592.

Lobell, D.B., K.G. Cassman and C.B. Field. 2009. Crop yield gaps: their importance, magnitudes, and causes. *Annu. Rev. Environ. Res.,* 34: 179-204.

Maathuis, F.J.M. 2014. Sodium in plants: perception, signaling, and regulation of sodium fluxes. *J. Exp. Bot.,* 65: 849-858.

Nasim, S., G. Shabbir, M. Ilyas, N.M. Cheema and M.K.N. Shah. 2017. Contemplation of wheat genotypes for enhanced antioxidant enzyme activity. *Pak. J. Bot.,* 49(2): 647-653.

Oldeman, L.R., V.W.P. Van Englen and J.H.M. Pulles. 1991. The extent of human-induced soil degradation. In*: World map of the status of human-induced soil degradation: An explanatory note*. (Eds.): Oldeman, L.R., R.T.A. Hakkeling and W.G. Sombroek.International Soil Reference and Information Center (ISRIC). Wageningen. pp 27-33.

Perveen, S., M. Shahbaz and M. Ashraf. 2011. Modulation in activities of antioxidant enzymes in salt stressed and non-stressed wheat (*Triticum aestivum* L.) plants raised from seed treated with triacontanol. *Pak. J. Bot*., 43: 2463-2468.

Qayyum, A., A. Razzaq, M. Ahmad and M.A. Jenks. 2011. Water stress causes differential effects on germination indices, total soluble sugar and proline content in wheat (*Triticum aestivum* L.) genotypes. *Afr. J. Biotechnol.,* 10: 14038-14045.

Radi, A.A., F.A. Farghaly and A.M. Hamada. 2013. Physiological and biochemical responses of salt-tolerant and salt-sensitive wheat and bean cultivars to salinity. *J. Biol. Earth Sci.,* 3: B72-B88.

Rajaram, S. 2005. Role of conventional plant breeding and biotechnology in future wheat production. *Turk. J. Agric. For*., 29: 105-111.

Ray, D.K., N.D. Mueller, P.C. West and J.A. Foley. 2013. Yield trends are insufficient to double global crop production by 2050. *PLoS One*., 8(6): e66428.

Reynolds, M.P., A. Mujeeb-Kazi and M. Sawkins. 2005. Prospects of utilizing plant-adaptive mechanisms to improve wheat and other crops in drought- and salinity-prone environment. *Ann. Appl. Biol.,* 146: 239-259.

Shabala, S. 2013. Learning from halophytes: Physiological basis and strategies to improve abiotic stress tolerance in crops. *Ann. Bot.,* 112: 1209-1221.doi:10.1093/aob/mct205.

Shannon, M.C. 1997. Adaptation of plants to salinity. *Adv. Agron.,* 60: 75-119.

Shewry, P.R. 2009. Wheat. *J. Exp. Bot.,* 60(6): 1537-1553.

Shewry, P.R. and H.D. Jones. 2005. Transgenic wheat: where do we stand after the first 12 years? *Ann. Appl. Biol.,* 147: 1-14.

Shewry, P.R., A.S. Tatham, F. Barro, P. Barcelo and P. Lazzeri. 1995. Biotechnology of bread making: unraveling and manipulating the multi-protein gluten complex. *Nature Biotechnol.,* 13: 1185-1190.

Slade, A.J., C. McGuire, D. Loeffler, J. Mullenberg, W. Skinner, G. Fazio, A. Holm, K.M. Brandt, M.N. Steine, J.F. Goodstal and V.C. Knauf. 2012. Development of high amylose wheat through tilling. *BMC Plant Biol.,* 12: 69-85.

Smith, P., P.J. Gregory, D. van Vuuren, M. Obersteiner, P. Havlik, M. Rounsevell, J. Woods, E. Stehfest and J. Bellarby. 2010. Competition for land. *Philos. T. Roy. Soc. B.,* 365: 2941-2957.

Szabolcs, I. 1994. Soils and salinisation. In*: Handbook of plant and crop stress*. (Ed.): Pessarakli, M. Marcel Dekker. New York. pp 3-11.

Tammam, A.A., M.F.A. Alhamd and M.M. Hemeda. 2008. Study of salt tolerance in wheat (*Triticum aestivum* L.) cultivar Banysoif 1. *Aust. J. Crop Sci.,* 1:115-125.

Tilman, D., J. Fargione, B. Wolff, C. D'Antonio, A. Dobson, R. Howarth, D. Schindler, W.H. Schlesinger, D. Simberloff and D. Swackhamer. 2001. Forecasting agriculturally driven global environmental change. *Science,* 292(5515): 281-284.

Waines, J.G. and B. Ehdaie. 2007. Domestication and crop physiology: roots of gree-revolution wheat. *Ann. Bot.,* 100(5): 991-998.

Wu, H., L. Shabala, X. Liu, E. Azzarello, M. Zhou, C. Pandolfi, Z-H. Chen, J. Bose, S. Mancuso and S. Shabala. 2015. Linking salinity stress tolerance with tissue-specificNa^+ sequestration in wheat roots. *Front. Plant Sci.,* 6: 71.

Yanga, C., L. Zhaoa, H. Zhanga, Z. Yanga, H. Wangb, S. Wena, C. Zhanga, S. Rustgic, D. von Wettsteinc and B. Liua. 2014. Evolution of physiological responses to salt stress in hexaploid wheat. *PNAS.,* 111(32): 11882-11887.

Zhang, Z.F., F. Zhang, Raziuddin, H.J. Gong, Z.M. Yang, L. Lu, Q.F. Ye and W. Zhou. 2008. Effects of 5-aminolevulinic acid on oilseed rape seedling growth under herbicide toxicity stress. *J. Plant Growth Regul.,* 27: 159-169.

Zhou, W.J. and M. Leul. 1999. Uniconazole-induced tolerance of rape plants to heat stress in relation to changes in hormonal levels, enzyme activities and lipid peroxidation. *Plant Growth Regul.,* 27: 99-104.

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