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Original Research

EVALUATION OF GENETIC VARIABILITY FOR SALT TOLERANCE IN WHEAT

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Abstract: *Wheat is an important cereal crop which has been consumed as food crop throughout the globe. Present study discusses change in different morphological traits of six most common wheat varieties in Pakistan under the effect of salt stress. We have used two salt solutions; 10 dS/m NaCl and 15 dS/m NaCl concentrations were used in our research. Data collected during research indicates that all morphological traits decrease under salt treatments except that of two trait viz., root length and carotenoids level. It was noted that under the effect of both salt concentrations carotenoids content increased in significant amount in leaves and roots along with root length which was also increased. The outcomes from analysis of variance demonstrated that there was higher leaf careteniods for genotype 5 (Ujala-16) that was 998.32 mg/g of fresh leaf weight trailed by genotype 1 (Inqalab-91) 995.99 mg/g of fresh leaf weight) while lower carotenoids were found for genotype 2(Shafaq-06) that was 825.65 mg/g of fresh leaf weight. Highest root weight was found in Shafaq-06 under treatment of 15dS/m NaCl. While pooled all Pairwise comparison test revealed highest root length in genotype 4 (Galaxy-13). While linear regression suggests that carotenoids content contribute least in plant height. Genetic heritability was found highest for photosynthetic pigments i.e. 99.99% for chlorophyll b except that of carotenoids. Genetic advance was recorded higher for fresh stem weight (309.870%). Higher heritability and genetic advance revealed that from our study that the selection of salt stress wheat genotypes on the basis of root length may be help to develop salt stress tolerance wheat genotypes with higher grain yield.*

Keywords: salinity, wheat, NaCl, carotenoid content, genetic advance, broad sense heritability

1. Introduction

The king of cereal wheat belongs to poaceace family. It is a staple food of Pakistan. Total area of wheat cultivation in the world is about 13.4 billion hectors. Wheat is the most cultivated crop of the world and according to a report of 2014 it is grown on 220 million hectors worldwide. It is growing all over the globe and is second most growing crop after corn. Wheat was first ever cultivated by some 10,000 years ago during Neolithic revolution (Shewry, 2009). Enkiron and tetraploid was ever cultivated wheat (Hirzel *et al*., 2018). Wheat is most consumable food across the world as well. Gluten protein is present in wheat flour that helps to make roti. It has sticky powers. China is the world largest wheat producing country. In Pakistan 40% land area is consumed for wheat production. In Punjab, Pakistan wheat is grown on 6.97 million hector area that is 75% of the total wheat production. In Sindh Wheat is grown on 1.15 million hectors that is 12%. In KPK 0.73 million hectors i.e. 8% and Baluchistan with 0.38 million hectors (4% of total wheat production of Pakistan) is reserved for wheat production (Haider *et al*., 2019). According to an agricultural report Faisalabad 2008 and Sehar 2006 is most dominating wheat variety in Punjab almost 50% of the total area. Pakistan has also more than 30 wheat verities; each variety has its own requirement of water and nutrients (Abid *et al*., 2014; Mohsin *et al*., 2015; Raza *et al*., 2012). In my research Salinity condition in Pakistan is not different from the world as 6.30 million hectors out of 21.2 million hectors total cultivated area is affected by salinity. Out of this 1.89 million hectors are saline, 1.85 million hectors is permeable saline-sodic, 1.02 million hectors impermeable saline-sodic and 0.028 million hector is sodic. Deposition of salts in soil is salinity. Saline and saline sodic are the categories of salt containing soils with different amount of salts. Salts may be deposit by irrigation water. Different physiological stresses lay different sensitivity effects on plant growth (Gao *et al*., 2016; Zubair *et al*., 2016). According to a study hexaploid wheat is more salt tolerant than to its wild relative tetraploid but physiology is not clear that how this is possible (Yang *et al*., 2014). Due to less variation in genetic makeup plants show more salt tolerance (Oladosu *et al*., 2016). Salt reduces the photosynthetic ability of plants (Ali *et al*., 2015; Aly *et al*., 2018). As wheat is staple food of Pakistan. And salinity is increasing day by day so this problem will be severe. It's my estimation that in next 50 years salinity rate will be double and yield of wheat will be reduced that will lead to starvation. During my research I also found clear-cut morphological differences in both control and affected plants. Affected Leaves showed yellowing and brown margins. In developing countries it is very difficult to provide clean water to crops. Recently saffron can also be used in order to remove soil salinity (Sereshti *et al*., 2018).

2. Materials and method

The present study was conducted in institute of molecular biology and biotechnology at university of Lahore, Lahore (Pakistan). For this purpose seeds of six wheat verities Inqalab-91, Shafaq-06, Faisalabad-08, Galaxy-13, Ujala-16 and Anaj-17 with different genetic makeup and origin were collected from Ayub Agriculture Research Institute, Faisalabad.

2.1 Assessment of wheat germplasm at seedling stage:

Seeds of six varieties with different origin Inqalab-91, Shafaq-06, Faisalabad-08, Galaxy-13, Ujala-16 and Anaj-17 were grown in three different situations. Two salt solutions i.e. 10dS/m NaCl and 15dS/m were prepared for salt treatment.

Fig I: Salt solutions of two different concentration i.e. 1odS/m and 15dS/m used for treatment

First step was seed priming. For this seeds were dipped in tap water for two hours and then surface sterilized the seeds by adding 2% v/v commercial bleach for three minutes. After that seeds were rinsed with distilled water three times. Seeds were soaked on watman paper. Some other methods include treatment of seeds with 5% sodium hypochlorite for seed priming (Ghanbari *et al*., 2018). 18 neat and clean pots were chosen. All the pots were filled with soil best suitable for plant growth. Out of 18; three pots were reserved and labeled for each wheat variety. For each variety three pots were reserved i.e. one as control, 2^{nd} for treatment of solution of 15dS/m NaCl and 3^{rd} pot for treatment of 10dS/m NaCl. Pots were exposed to ideal condition required for germination. Proper care was taken in watering the pots. Aeration and light intensity was also kept in check and balanced. As seed priming was done so there were chances of even growth of seedling from every pot. After two weeks of germination of seeds; seedling germination data was collected. Healthy plant from each pot was removed very carefully along with its root and cleaned. For each of six variety root length (cm) and plant height (cm) was measured before and after treatment of 10d/S and 15 d/S NaCl. Each variety was morphologically distinct from each other (Kodikara *et al*., 2018).

Fig II: Measuring seedling parameters

2.2 Photometry

Fresh and healthy leaves of all six verities were selected from each pot including both controlled and treated by salt solutions to find out the carotenoid content. Fresh leaves were taken in falcons and dipped in 2.5 ml 95% pure ethanol according to their weight. I put the falcons in centrifuge machine and done their centrifugation. Centrifugation was done for 15 minutes at 4^oC and 10,000rpm. This process was repeated for each sample. Carotenoid content of both control and salt affected leaves of all varieties were also measured by using photospectrometry technique (Singh and Patidar, 2018).

Fig III: showing ethanol treatment and centrifugation of leaves of each variety

2.3 Statistical analysis of Morphological traits for salt tolerance

Ordinary analysis of variance followed by Tukey's range test was applied on all morphological traits to find out the genotypic differences between all accessions is significant or not (Zahra *et al*., 2018). General linear model of SPSS version 23 windows advance was used.

2.4 Correlation Analysis

By using SPSS version 23.1correlation was calculated between all morphological traits under salt treatment. All the morphological traits showed positive and negative significant correlation with one another.

2.5 Broad sense heritability (h^2b.s) for salt tolerance

Broad sense heritability was analyzed for all morphological traits of all accessions under salt stress. Variance within varieties calculated by a formula given by (Falconer and Mackay, 1996). Variance between accessions is due to environmental factors, as wheat is self pollinated crop.

3-Results and discussion

3.1 Carotenoids in leaves (mg/g fresh leaf weight)

It was convinced from results given in table 3.1 (given in supplementary data) that there were critical contrast among wheat genotypes, salt treatment and associations among genotypes and salt treatments. It was discovered that the normal caroteniods in average were 997.69±6.760mg/g of leaf weight in wheat seedlings under treatment of salt solutions. It was discovered that there was exceptionally low coefficient of variance (0.001%) for carotenoids in leaves showed that there was higher consistency for carotenoids in leaves. The outcomes from table 3.1a (given in supplementary data) demonstrated that there was higher leaf caroteniods for genotype 5 (998.32 mg/g new leaf weight) trailed by genotype 1 (995.99 mg/g fresh leaf weight) while lower carotenoids were found for genotype 3 (851.86 mg/g new leaf weight) and genotype 2 (825.65 mg/g new leaf weight). The higher leaf carotenoids showed that there were higher photosynthetic pigments in the leaves which might be useful for the improvement of natural pigments in the leaves and gives obstruction against different abiotic stresses. The higher leaf carotenoids in genotype 5 demonstrated that there was higher obstruction and survival capacity under salt treatments. The treatment of salt caused higher harming consequences for genotypes 2 and 3. The genotypes which demonstrated higher leaf carotenoids might be chosen as salt tolerant genotypes in wheat. It was influenced from figure 4.1(given in supplementary data) that there were little contrasts among the genotypes under treatments of salt solutions. The outcomes demonstrated that the majority of the genotypes indicated comparable sort of leaf carotenoids under salt treatments. Be that as it may, the collective effects of salt treatments for every genotype were distinctive as depicted by results in table 3.1 and 3.1a (given in supplementary data).

3.2 Root Length (cm)

Table 3.2(given in supplementary data) clearly indicates that there is difference between root lengths of wheat seedlings. Table shows there is significant difference between genotypes and salt treatments. It is clear from the table 3.2 that there is average of 8.9581±0.1218cm of Root length of wheat seedling. Table 3.2a (given in supplementary data) shows that higher leaf diameter was found in genotype 4 (12.993cm) followed by genotype 1 (8.800cm) of wheat seedling. While lowest root length was recorded in genotype 5 (7.890cm) and genotype 2 (7.081cm). In simple words we can say that genotype 1 and genotype 4 has showed more resistance to salt solutions. On the other hand genotype 5 and 2 are more affected under salt treatments. It is clear from figure 3.2 (given in supplementary data) that there are differences in root length among the genotypes under salt treatment. The results showed the differences in root length in all genotypes of wheat seedlings when exposed to 10dS/m NaCl and 15 dS/m NaCl. However, the collective effects of salt treatments for every genotype was distinctive are given by results in table 3.2 and 3.2a (given in supplementary data) in more detail. Salinity reduces photosynthetic pigments but increase only in carotenoids which cause increase in root length a little bit among all other morphological traits (Latef *et al*., 2017).

3.3 Plant Height (cm)

Table 3.3(given in supplementary data) clearly indicates that there is difference between plant heights of wheat seedlings. Table shows there is significant difference between genotypes and salt treatments. It is clear from the table 3.3 that there is average of 7.3729±0.0517cm of plant height of wheat seedling. Table 3.3 a(given in supplementary data) shows that higher leaf diameter was found in genotype 1 (9.18cm) followed by genotype 3 (8.48cm) of wheat seedling. While lowest plant height was recorded in genotype 5 (6.50cm) and genotype 6 (5.90cm). In simple words we can say that genotype 1 and genotype 3 has showed more resistance to salt solutions. On the other hand genotype 5 and 6 are more affected under salt treatments. It is clear from figure 3.3 (given in supplementary data) that there are differences in plant height among the genotypes under salt treatment. The results showed the differences in plant height in all genotypes of wheat seedlings when exposed to 10dS/m NaCl and 15 dS/m NaCl. However, the collective effects of salt treatments for every genotype was distinctive are given by results in table 3.3 and 3.3a (given in supplementary data) in more detail.

3.4 Correlation Analysis

Results from table 3.4 clearly shows that there is positive and significant correlation between wheat seedling carotenoids and fresh leaf weight(FLW), fresh root weight(FRW), fresh stem weight(FSW) and root length(RL). While negative but significant correlation was found with chlorophyll a, chlorophyll b, leaf diameter (LD), leaf length(LL), plant height(PH) and shoot diameter (SD). Positive correlation with root length shows that under stressed condition when there is deposition of carotenoids in root, stem and leaf plant try to survive and in this way they increase their root length. Off course with the increase in carotenoids there is positive correlation with stem, leaf and root weight under salt treatment. Previous study also indicates there is considerable increase in carotenoids and other phenolic compounds i.e. beta-carotenoids, lutin, β-solamargine and caffeic acid under 10dS/m Nacl solution. Infect increased content of carotenoid genes can be found significantly under salinity (Ben-Abdallah *et al*., 2018). According to table 3.4 chlorophyll.a has positive and significant correlation with chlorophyll b and leaf diameter (LD) while negative but significant correlation with caroteniods, fresh leaf weight (FLW), fresh root weight (FRW), fresh stem weight (FSW) and root length (RL). Negative correlation with carotenoids shows that if there is increase in carotenoids content which is obvious during salt stress then amount of chlorophyll start decreasing in plant seedling as given in past salinity research too (Ali *et al*., 2013; Piñero Zapata *et al*., 2019). According to table 3.4chlorophyll.b has positive and significant correlation with chlorophyll b, shoot diameter (SD) and leaf diameter (LD) while negative but significant correlation with caroteniods, fresh leaf weight (FLW), fresh root weight (FRW), fresh stem weight (FSW), and root length (RL). Negative correlation with carotenoids shows that if there is increase in carotenoids content which is obvious during salt stress then amount of chlorophyll start decreasing in plant seedling. Higher level of salinity caused degradation of chlorophyll b content in young seedling (Monteiro *et al*., 2018). Table 3.4 shows that plant height has positive, higher and significant correlation with chlorophyll a, b, stem weight, leaf diameter and leaf length. On the other hand significant negative correlation with carotenoids, leaf weight and root weight. A well know salt tolerant plant safflower also shows reduction in plant height because during salt stress a lot of secondary metabolites (Ali *et al*., 2014b; Ali *et al*., 2014c; Gengmao *et al*., 2015). Salinization cause increase in carotenoids in root, stem and leaves at dangerous level that cause reduction in plant height and stunt growth at seedling stage (Masood *et al*., 2014a; Serra *et al*., 2018). Shoot diameter shares significant correlation with root diameter is evidence that there is higher level of organic compounds aggregation in seedling under salt stress; salinity reduces the plant growth rates at seedling stage(Sallaku *et al*., 2019). When there is higher level of salt in plant seedling roots there is always negative relation between caroteniods and chlorophyll.a and chlorophyll.b (Ali *et al*., 2017; Vahtmäe *et al*., 2018). Table 3.4 shows that there is considerable, positive and significant correlation between root length and carotenoids and root weight. Whereas negative but significant correlation with chlorophyll a and b and plant height. As plant feels stress under salinity first and foremost response of plant seedling is to increase its root length. Carotenoids content is directly related to root length, simply we can say that increase in carotenoid increases root length. Salinity reduces photosynthetic pigments but increase only in carotenoids which cause increase in root length a little bit among all other morphological traits (Latef *et al*., 2017; Masood *et al*., 2014c; Naseem *et al*., 2015). Table 3.4 shows that plant height has positive, higher and significant correlation with chlorophyll a, b, stem weight, leaf diameter and leaf length. On the other hand significant negative correlation with carotenoids, leaf weight and root weight. A well know salt tolerant plant safflower also shows reduction in plant height because during salt stress a lot of secondary metabolites (Gengmao *et al*., 2015). Salinization cause increase in carotenoids in root, stem and leaves at dangerous level that cause reduction in plant height and stunt growth at seedling stage (Raza *et al*., 2015; Serra *et al*., 2018).

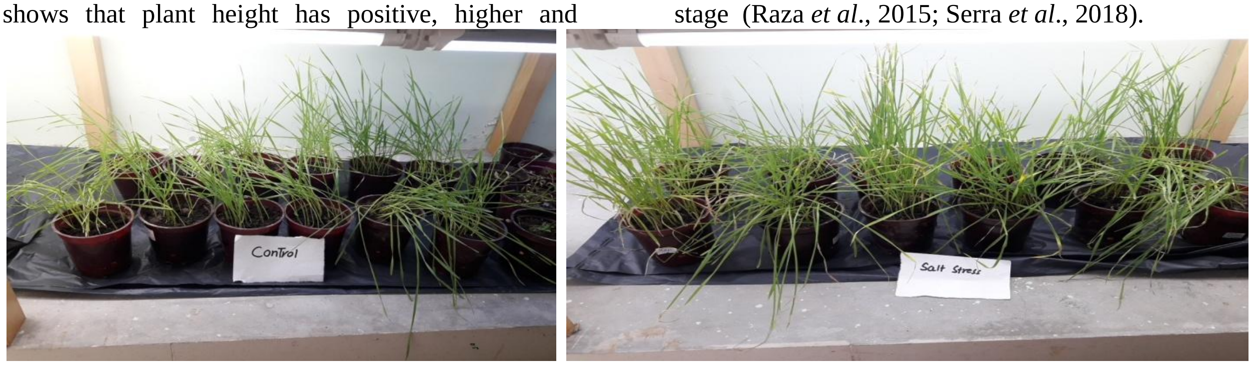

Fig IV: All six verities with and without salt treatment

Table 3.4: Pooled correlation among different morphological traits of wheat seedlings under salt treatments

Traits	Carotenoids	Chla.	Chlb.	FLW	FRW	FSW	LD	LL	PH	RD	RL
Chla	-0.997*										
Chlb	-0.9188*	0.9182*									
FLW	0.2954*	-0.2951*	-0.355*								
FRW	0.0219	-0.0208	-0.3181*	0.2879*							
FSW	0.3349*	-0.3351*	-0.2585*	0.8714*	0.0282						
LD	-0.1254	0.1254	0.1005	-0.3587*	-0.1777	-0.2562*					
LL	-0.1868	0.187	0.1092	0.0606	0.2073*	-0.0322	-0.4884*				
PH	-0.2308*	0.2305*	0.2941*	-0.1848	-0.1356	0.0586	0.158	0.0553			
RD	-0.4549*	0.4554*	0.305*	-0.1551	0.2567*	-0.1173	0.5569*	0.0698	0.2455*		
RL	0.4327*	-0.4328*	-0.3951*	-0.0673	0.105	-0.05	-0.2541*	0.0512	-0.4008*	-0.0808	
SD	-0.5579*	0.5574*	0.6201*	-0.6823*	-0.5423*	-0.5909*	0.3851*	-0.0802	-0.0722	0.1488	-0.0963

* = Significant at 5% probability level Ch.a=Chlorophyll.a, FLW=Fresh leaf weight, FRW=Fresh root weight, FSW=Fresh stem weight, LD=leaf diameter, LL=leaf length, RD=root diameter, RL=root length, SD=shoot diameter, Ch.b=chlorophyll b

3.5 Regression Analysis

Table 3.5 showing regression data was taken for twelve variables contributing to plant height. Regression analysis is showing that leaf carotenoids (1212.7) have higher and negative contribution for plant height under salt stress. This is because when there is salt stress there is increase in accessory photosynthetic pigments and increase in root length. On the same pace rapid decrease I necessary photosynthetic pigments cause decrease in plant height. While the other variables shows less contribution towards plant height. Previous studies on wheat regression was also conducted to find out different variables contribution towards wheat grain yield and plant height such as (Ali *et al*., 2014a; Leilah and Al-Khateeb, 2005; Mahmood *et al*., 2019) used this stepwise regression model to find out the weight of grain, harvest index, biological yield and spike length. Another study also shows the same as found in my results that carotenoids are significantly contributing towards phenotypic variations in plants under stress and specific genes are controlling this mechanism (Chander *et al*., 2008; Farooq *et al*., 2011). The data of table 3.5 represents accumulative medium coefficient of determination or R^2 for plant height and lower coefficient of determination or R^2 (0.07022%) that was found for leaf carotenoids. The regression equation was written as following:

Y= 1212710 -1212.7(carotenoids) +1213.21(Ch.a) + 0.12636(FLW)-0.79724(FRW)-2.10036(FSW) 2.5479(LD)+0.1882(LL)-3.43352(RD) -0.10799(RL) -4.20165(SD) +1.000(ch.b).

Table 3.5 : Pooled stepwise linear regression for plant height or seedling length under different salt treatments

Variable	Coefficient	Std Error	T	R^2
Carotenoids	-1212.7	649.896	-1.87	0.07022
Chl. A	1213.21	652.693	-1.86	0.07122
FLW	0.12636	1.22911	0.1	0.9187
FRW	-0.79724	0.69081	-1.15	0.2561
FSW	-2.10036	1.3668	-1.54	0.1331
LD	-2.5479	2.63942	-0.97	0.3408
LL	0.1882	0.03632	5.18	0
RD	-3.43352	1.47699	-2.32	0.0258
RL	-0.10799	0.0371	-2.91	0.0061
SD	-4.20165	1.16768	-3.6	0.001
Chl. B	1.000	-0.0973	-0.58	0.5667

Y = 1212710, R^2 = 0.9251, Adjusted R^2 =0.9022% , Standard Deviation = 0.45948, Ch.a=Chlorophyll.a, FLW=Fresh leaf weight, FRW=Fresh root weight, FSW=Fresh stem weight, LD=leaf diameter, LL=leaf length, RD=root diameter, RL=root length, SD=shoot diameter, Ch.b=chlorophyll b

3.6 Broad sense heritability for wheat seedling

Table 3.6 shows that there are considerable differences among all the morphological traits of wheat. Highest broad sense heritability was found for the Chlorophyll b (99.994%), Root length (99.747%), fresh leaf weight (99.961%), root diameter (99.426%) traits. Lowest broad sense heritability is found for leaf length (79.546%) and carotenoids (55.456%).

While genetic advance in carotenoids is (0.016%) contrary to this maximum found in chlorophyll b (101.049%).This is all due to change in environmental factors leads to changes in varieties. In current study broad sense heritabilities in wheat are quiet high as compared to all other species i.ie maize and four grass families studied by different researchers (Akbar *et al*., 2008; Masood *et al*., 2014b). Present values for genetic heritability shows variation for root length, root weight and photosynthetic pigments under treatment of two solutions 10dS/m and 15dS/m NaCl. These variations are due to variation in genetics of wheat germplasm. Environmental factors, accumulation of different ions and breeding plays a significant role in determing genetic heritability under salt treatment.

Table 3.6 Pooled Genetic components for various morphological traits of wheat seedling

Traits	M.S	G.M±S.E	GV	GCV %	PV	PCV %	EV	ECV %	h^2bs%	GA%
Carotenoids	0.057	999.690±6.760	0.015	0.388	0.027	0.521	0.01207	0.347	55.456	0.016
ch.a	0.066	0.300±6.747	0.022	27.033	0.022	27.144	1.82	2.449	99.186	86.265
ch.b	0.075	0.274±6.366	0.025	30.135	0.025	30.136	1.62E-06	0.242	99.994	101.049
FLW	0.383	0.203±3.505	0.128	79.400	0.128	79.416	0.00005	1.571	99.961	309.523
FRW	0.202	0.425±3.505	0.067	39.657	0.068	40.141	0.30241	6.213	97.604	105.496
FSW	0.180	0.138±9.986	0.060	65.810	0.060	66.030	0.0004	5.384	99.335	309.870
LD	0.015	0.073±4.931	0.005	24.958	0.006	27.488	9.726	11.519	82.439	146.892
RD	0.047	0.071±4.611	0.016	46.831	0.016	46.966	0.00009	3.558	99.426	307.346
RL	34.389	8.958±0.1218	11.443	113.023	11.502	113.314	0.059	8.116	99.487	66.103
PH	12.650	7.737±0.0517	4.213	75.593	4.224	75.689	0.0107	3.810	99.747	48.796
SD	0.127	0.469±0.0234	0.042	29.809	0.044	30.578	0.00218	6.815	95.033	74.437
LL	19.333	39.383±0.6177	5.936	38.822	7.462	43.528	1.526	19.686	79.546	9.683

*=Significant at 5% probability level, Mean Sum of Squares (M.S), Grand mean (G.M), Genotypic variance (GV), Genotypic coefficient of variance (GCV %), Phenotypic variance (PV), Phenotypic coefficient of variance (PCV %), Environmental Variance (EV), Environmental coefficient of variance (ECV %), Broad sense heritability (h^2bs %), Genetic advance (GA), Chlorophyll a(ch.a), Chlorophyll b(ch.b), Fresh leaf weight(FLW), Fresh root weight(FRW), Fresh stem weight(FSW), Leaf diameter(LD), Root diameter(RD), Root length(RL), Plant height(PH), Stem diameter(SD), Leaf length(LL).

4- Conclusions

For our study For this purpose seeds of six different wheat varieties with totally different genetic makeup and origin were selected. Main purpose of this investigation was to collect information to estimate genetic response of wheat genotypes in respect of salinity at seedling stage. For every morphological trait every genotype showed significant differences from each other, this means that they have different genetic makeup and so the genes response to salinity. On the basis of mean root length given in most stable wheat genotype was Faisalabad-08, which is also the variety that is grown on 50% in the Punjab agricultural land. Concluding my investigation; it was just analysis of different morphological traits of most used wheat varieties of Punjab, Pakistan under the effect of two salt solutions, how do they differ from each other because of their different genetic response to salt stress. So need of hour is we have to make our crops more tolerant and resistant against salinity. Further research is needed to analyze the genetic components, study on genes that may assist wheat plant to cope with salinity.

Conflict of interest

The authors declared the absence of any potential conflict of interest.

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