



Full Length Article

Genetic Variability Studies for Salinity Tolerance in *Gossypium hirsutum*

Muhammad Awais Farooq¹, Amir Shakeel¹, Rana Muhammad Atif¹ and Muhammad Farrukh Saleem²

¹Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan 38040

²Department of Agronomy, University of Agriculture, Faisalabad, Pakistan 38040

*For correspondence: awaisfarooq724@gmail.com

Abstract

Fifty cotton genotypes were evaluated for NaCl tolerance in which plants were irrigated with nutrient solution with an electrical conductivity (EC) of 10 dS m⁻¹ and 15 dS m⁻¹ with the addition of NaCl from 10 d to 40 d seedlings. The chlorophyll contents, root and shoot lengths, root fresh and dry weights, shoot fresh and dry weights, Na⁺, K⁺ and K⁺/Na⁺ ratio was measured from the seedlings after three days of harvesting. There were significant differences among the genotypes at control and both salt stress levels. The maximum decline in growth and ionic traits was observed at 15 dS m⁻¹ salt stress among all cotton genotypes for chlorophyll contents, root and shoot length, root fresh and dry weights, shoot fresh and dry weights, Na⁺, K⁺ and K⁺/Na⁺ ratio, however, magnitude of reduction varied among all genotypes. High heritability and genetic advances were observed for the root length, fresh root weight, dry root weight, dry shoot weight and K⁺/Na⁺ ratio showing the presence of additive gene actions in the expression of these characters. Principal component analysis revealed NIAB-824, Mubarak, CIM-612, FH-114 and Kehkshan as salt tolerant genotypes. Existence of genetic variability in the cotton germplasm against salinity tolerance exhibited that it can be exploited for the genetic improvement in future cotton breeding program for salinity tolerance. © 2018 Friends Science Publishers

Keywords: Salinity; Salt tolerance; Upland cotton; Genetic variation; Seedlings traits

Introduction

Salinity is the rise of salt level in the soil which obstructs the healthy growth of the plant. Around the globe, the looming accumulation of salts in the soil is hampering the yield of various crops. All-important crops give only a fraction-average yield from 20 to 50%; of the expected yield mostly due to the high salinity (Shrivastava and Kumar, 2015). The levels of salts in the soil are rising due to the high evapotranspiration rate and irrigation by poor quality of water. The major issue of salinity persists in arid and semi-arid regions of the country. Soil is declared saline if its electrical conductivity is 4 dS m⁻¹ or of higher value. Germination and emergence of seedlings to take the good stand up are taken as primary criteria for salinity tolerance (Rozema and Schat, 2013). The plants with relatively high germination and seedlings growth in saline soils will be vigorous and highly tolerant to salt stress because, these growth stages are much crucial due to accumulation of salts at surface soil (Ashraf, 2002).

Cotton is moderately salt tolerant crop that can grow up to 7.7 dS m⁻¹ (Kamaran *et al.*, 2016), however, it shows decline in the germination and emergence percentage under the high salinity stress (Sevik and Cetin, 2015). At germination stage, cotton plant affected by salinity shows reduced germination of the seeds. At seedling stage, evaporation rate, photosynthesis and water efficiency are

reduced while respiration rate increases (Parihar *et al.*, 2015). Phenotypically, the plant height reduces, leaf expansion size is halted, stem thickness, shoot and root weight are also severely affected (Parida and Das, 2005).

The success of breeding program depends upon the presence of variability in the germplasm. Several physiological and morphological markers have been used to assess the variations under salinity stress. Morphological parameter includes longer tap root length, root to shoot ratio, reduced transpiration rate and chlorophyll contents can be exploited for the development of salt tolerant genotypes (Taibi *et al.*, 2016). Among physiological markers low Na⁺ uptake and enhanced K⁺/Na⁺ ratio are important (Liu *et al.*, 2017). Further, the knowledge of heritability and genetic variability can be employed to find a suitable breeding strategy and to estimate the amount of genetic advance to be expected from the selection (Soomro *et al.*, 2010).

For this purpose, it is deemed necessary to evaluate new cotton genotypes so that genetic resource for increasing salt tolerant genotypes can be sorted out.

In the present study, 50 cotton genotypes were examined for the variations in cotton plants under salinity stress at seedling stage. The results from present study may help breeders for the selection of potential germplasm for the salinity tolerance breeding mechanisms for salt affected areas.

Materials and Methods

The 50 phenotypically diverse genotypes of upland cotton were collected from different sources and subjected to salt stress at two levels under controlled conditions in the Department of Plant Breeding and Genetics in the University of Agriculture, Faisalabad (Table 1). The tested genotypes were sown in sand in the polystyrene cups (Height 5 inches, Diameter 3.5 inches), in three replications. Five cups for each genotype in one treatment per replication were maintained according to the Factorial Complete Randomized design. The Hoagland solution was provided to the plants for the first 10 days, then Hoagland solution of 10 dS m⁻¹ and 15 dS m⁻¹ was applied (Hoagland and Arnon, 1950). The salinity level was maintained by continuous testing of the EC of the sand material in the cups by pour through extraction method (Farhangi-Abriz and Torabian, 2017). One seedling was kept per cup. Chlorophyll contents in the leaves were measured at 1st true leaf stage by the use of chlorophyll meter (SPAD 502 PLUS Japan). When seedlings were 40 days old, they were uprooted and rinsed with deionized water and swapped with the paper towel. Then, seedlings were dissected into two portions *i.e.*, roots and shoots, and their length, fresh root and shoot weight were taken. Then, the roots and shoots were dried in an oven for 72 h at 70°C to take dry weight. For Na⁺ and K⁺ analysis, the dried leaves were grinded down with mortar and pestle and then digested with concentrated nitric acid and sulfuric acid in 2:1 ratio (molar ratio) on the hot plate. After digestion, material was cooled at room temperature by adding distilled water and readings were taken by flame photometer (410 Flame Photometer).

Analysis of variance was carried out (Steel *et al.*, 1997) to see whether the genotypic differences are significant. Heritability was estimated by the given formula (Frankham *et al.*, 2002) to find out the broad sense heritability. Genetic Advance was estimated following (Johnson *et al.*, 1955). Data of the traits showed significant genotype × environment interaction when subjected to PCA. PCA was performed on mean data using RStudio, Inc. (R Core Team 2014) following the method of (Husson *et al.*, 2017). To visualize and better understand the PCA-biplot, only genotypic numbering has been used as given in the plant material section.

Results

Analysis of variance revealed highly significant differences among the genotypes for the studied traits. There was also significant difference among the control and two levels of salinity stress ($P \leq 0.01$), and highly significant interaction (G×T), indicated differential response of genotypes to two salinity levels.

Chlorophyll Contents

The chlorophyll contents of 50 genotypes ranged from 46.46-26.93 (chlorophyll concentration index) under control conditions. Moreover, all the genotypes showed significantly reduced chlorophyll contents that ranged from 42.77–24.2 under 10 dS m⁻¹ salt stress and 39.4–23.1 at 15 dS m⁻¹. NIAB-824 indicated highest chlorophyll index under control (46.46) followed by 10 dS m⁻¹ (42.77) and 15 dS m⁻¹ (39.4) salt stress. SB-149 had lowest chlorophyll contents 26.93, 24.2, and 23.1 respectively under control, 10 dS m⁻¹ and 15 dS m⁻¹ salt stress. High genotypic and phenotypic variance than environmental variance was witnessed under control and salt stress condition (Table 3). However, high broad sense heritability and low genetic advance indicated that the characters were genetically controlled.

Root and Shoot Length

Analysis of variance showed that all the genotypes were significantly different for root and shoot length under control and both levels of salt stress. The root and shoot length decreased with the increase of the salt stress in all genotypes. The root length ranged from 18.7-6.13 cm under controlled conditions while it was 15.86-5.43 cm and 14.86-3.83 under 10 dS m⁻¹ and 15 dS m⁻¹ salt stress whereas, shoot length ranged from 22.03-11.3 cm under controlled conditions, 20.33-11.3 cm under 10 dS m⁻¹ and 18.66- 9.13 under 15 dS m⁻¹ salt stress. CIM-612 developed consistently longest root length while SB-149 showed shortest root length under control, 10 dS m⁻¹ and 15 dS m⁻¹. For shoot length AA-802 had the longest shoot length and BS-80 had the shortest shoot length under all treatments. The root and shoot length interaction with the salinity was significant showing a significant impact of salt stress on these traits. Genotypic variance and phenotypic variance for root length were almost equal while environmental variance was negligible to low under control and both salt levels (Table 2). While for shoot length genotypic and phenotypic variance was found gradually decreasing from control to the increasing salt stress. Root and shoot lengths indicated high heritability whereas genetic advance was high for root length and moderate for the shoot length under all treatments (Table 3 and 4).

Fresh and Dry Root Weight

All selected genotypes differed significantly from each other under control and salt stress conditions. The results showed that there was a decrease in the fresh and dry root weights of the plant with the increase of salt stress. The fresh root weight ranged from 1.18-0.21 mg under control, 0.98-0.16 mg under 10 dS m⁻¹ and 0.81-0.096 mg under 15 dS m⁻¹ salt stress conditions. Dry root weight ranged from 0.26-0.06 mg under controlled conditions while it was 0.19-0.06 mg under 10 dS m⁻¹ and 0.015-0.03 mg under 15 dS m⁻¹ salt

Table 1: Institutes and names of 50 genotypes of *Gossypium hirsutum* L. examined for salinity tolerance

Sr. No.	Genotypes	Institute Name	Sr. No.	Genotypes	Institute Name
1	FH-458	CRI, Faisalabad	26	KZ-189	Private Seed Company
2	BS-80	Private Seed Company	27	C-26	China
3	Mubarak	CRI, Faisalabad	28	SB-149	Private Seed Company
4	Debal	CRI, Faisalabad	29	IUB-222	IUB, Bahawalpur
5	Kehkshan	CRI, Faisalabad	30	MG-6	Private Seed Company
6	Lalazar	CRI, Faisalabad	31	IR-901	NIBGE, Fsd.
7	FH-312	CRI, Faisalabad	32	VH-333	CRS, Vehari
8	FH-444	CRI, Faisalabad	33	NIAB-824	NIAB
9	MNH-888	CRI, Multan	34	AS-01	Private Seed Company
10	FH-173	CRI, Faisalabad	35	AGC-2	Private Seed Company
11	FH-113	CRI, Faisalabad	36	RH-647	CRI, R.Y.Khan
12	FH-177	CRI, Faisalabad	37	CRS-456	CRS, Multan
13	FH-187	CRI, Faisalabad	38	AA-802	Private Seed Company
14	FH-171	CRI, Faisalabad	39	NS-131	Neelum Seeds
15	MNH-988	CRI, Multan	40	FH-154	CRI, Faisalabad
16	FH-170	CRI, Faisalabad	41	VH 259	CRS, Vehari
17	MNH-992	CRI, Multan	42	CIM 595	CCRI, Multan
18	FH-142	CRI, Faisalabad	43	VH 295	CRS, Vehari
19	FH-118	CRI, Faisalabad	44	VH 329	CRS, Vehari
20	FH-941	CRI, Faisalabad	45	CIM 602	CCRI, Multan
21	FH-4243	CRI, Faisalabad	46	AA 703	Ali Akbar Seeds
22	VH-325	CRS, Vehari	47	CIM 622	CCRI, Multan
23	FH-114	CRI, Faisalabad	48	FH 169	CRI, Faisalabad
24	CIM-612	CCRI, Multan	49	S 3	Private Seed Company
25	CRS-2007	CRS, Multan	50	IR 3701	NIBGE, Fsd

Table 2: Mean squares from analysis of variance for various seedling traits of 50 upland cotton accessions grown in control level and two NaCl concentrations (combine analysis at all levels)

Source of Variation	d.f	Chlorophyll contents	Root length	Shoot length	Fresh weight	root weight	Fresh shoot weight	Dry weight	Root weight	Dry shoot weight	Sodium (Na)	Potassium (K)	K/Na ratio
Accessions	49	142.74**	65.427**	44.67**	0.330**	0.429**	0.012**	0.026**	210**	1598.1**	11.85**		
Salinity	2	1193.96**	444.457**	349.99**	1.868**	4.930**	0.221**	1.071**	148190**	91534.7**	3597.46**		
Accessions×Salinity	98	2.08**	0.940**	0.33**	0.0061**	0.017**	0.0005**	0.0014**	28**	50.1**	5.51**		
Error	298	0.49	0.217	0.08	0.00009	0.00087	0.0001	0.0002	2	3.6	0.11		

*= Significant at 5%, **= Significant at 1%

stress. CIM-612 had highest fresh weight under control, while Kehkshan had the maximum at 10 dS m⁻¹ and 15 dS m⁻¹ salt stress. Maximum root dry weight was noted for Mubarak under controlled conditions while CIM-612 performed better at 10 dS m⁻¹ and 15 dS m⁻¹ salt stress conditions. The genotype FH-114 had lowest fresh weight while C-26 had lowest dry weight at control, 10 dS m⁻¹ and 15 dS m⁻¹ salt stress conditions. The interaction of root fresh and dry weights with application of salt stress was noted significant. The genotypic and phenotypic variance and the coefficient of their variances were found to be almost equal and higher than environmental variance for fresh and dry root weights. Heritability was high for the fresh root weight whereas decreasing trend was observed for the dry root weight with the increase of salt stress. Genetic advance remained high for fresh and dry root weights (Table 4 and 5).

Shoot Fresh and Dry Weight

All the genotypes differed significantly from each other

under control and salt stress conditions for shoot fresh and dry weights. There was significant impact of salt stress on the fresh and dry shoot weights causing the reduction of root and shoot weight of plants. CIM-612 had highest fresh weight under control, 10 dS m⁻¹ and 15 dS m⁻¹ salt stress. MG-6 had lowest weight under control, while BS-80 had the reduced weight under 10 dS m⁻¹ and 15 dS m⁻¹ salt stress conditions. For shoot dry weights MNH-988 had highest weight under control conditions, NIAB-824 performed well under 10 dS m⁻¹ and MNH-888 performed excellent under 15 dS m⁻¹ salt stress conditions. SB-149 under control and 10 d Sm⁻¹, while AGC-2 had lowest shoot dry weight under 15 dS m⁻¹ salt stress conditions. For fresh shoot and dry weights, the genotypic and phenotypic variance was higher than the environmental variance. Coefficient of genotypic and phenotypic variance was close to each other and higher than the environmental variance. It was also observed that environmental coefficient of variance increased with the increased of salt stress. Moreover, high broad sense

Table 3: Components of variance, heritability and genetic advance estimates of 50 cotton genotypes for chlorophyll contents (CCI) and root length (cm) under control level and salt stress

Sources of variations	Chlorophyll contents (CCI)			Root length (cm)		
	Control	10 dS m ⁻¹	15 dS m ⁻¹	Control	10 dS m ⁻¹	15 dS m ⁻¹
Genotypic Mean Square	53.33	50.34	43.229	26.810	20.45	20.046
Error Mean Square	0.55	0.302	0.466	0.311	0.180	0.162
Grand Mean of trait	36.50	33.881	30.865	10.712	8.729	7.284
Maximum	46.46	42.767	39.467	18.7	15.867	14.867
Minimum	26.93	24.2	23.1	6.133	5.433	3.833
Standard deviation	4.260	4.121	3.836	3.023	2.633	2.607
Environmental variance	0.557	0.302	0.466	0.311	0.180	0.164
Genotypic variance	17.591	16.681	14.254	8.832	6.756	6.629
Phenotypic variance	18.148	16.984	14.721	9.144	6.937	6.795
Environmental coefficient of variance	2.045	1.623	2.213	5.212	4.867	5.526
Genotypic coefficient of variance	11.489	12.054	12.232	27.742	29.777	35.34
Phenotypic coefficient of variance	11.670	12.163	12.430	28.228	30.172	35.778
Heritability Broad sense	96.927	98.218	96.828	96.590	97.397	97.611
Genetic advance i=1.76	7.226	7.083	6.501	5.111	4.489	4.451
Genetic advance %	19.795	20.906	21.063	47.715	51.427	61.110

Table 4: Components of variance, heritability and genetic advance estimates of 50 cotton genotypes for shoot length (cm) and fresh root weight (g) under control level and salt stress

Sources of variation	Shoot			Length (cm)			Fresh Root		Weight (g)	
	Control	10 dS m ⁻¹	15 dS m ⁻¹	Control	10 dS m ⁻¹	15 dS m ⁻¹	Control	10 dS m ⁻¹	15 dS m ⁻¹	
Genotypic Mean Square	15.99	14.537	14.820	0.144	0.102	0.095				
Error Mean Square	0.122	0.057	0.059	0.00019	6.44E ⁰⁵	8.51E ⁰⁵				
Grand Mean of trait	16.803	15.207	13.749	0.593	0.465	0.363				
Maximum	22.033	20.033	18.667	1.183	0.98	0.813				
Minimum	11.3	10.267	9.133	0.21	0.163	0.097				
standard deviation	2.323	2.209	2.231	0.219	0.185	0.176				
Environmental variance	0.122	0.057	0.059	0.00019	6.44E-05	8.51E-05				
Genotypic variance	5.299	4.826	4.920	0.048	0.034	0.039				
Phenotypic variance	5.412	4.885	4.979	0.048	0.038	0.036				
Environmental coefficient of variance	2.323	1.564	1.776	2.338	1.724	2.502				
Genotypic coefficient of variance	2.323	14.447	16.132	36.969	39.77	48.43				
Phenotypic coefficient of variance	2.323	14.528	16.230	37.043	39.813	48.50				
Heritability Broad sense	97.740	98.856	98.802	99.601	99.812	99.733				
Genetic advance i=1.76	3.948	3.826	3.858	0.382	0.323	0.312				
Genetic advance %	23.689	25.135	28.062	64.567	69.542	84.653				

heritability was observed under control conditions and values decreased with the increase of salt stress for shoot fresh and dry weights. Genetic advance was high for all treatments for shoot fresh weight, however for shoot dry weight genetic advance was moderate for control and 10 dS m⁻¹ while genetic advance was higher for 15 dS m⁻¹ (Table 5 and 6).

Sodium (Na⁺) and Potassium (K⁺) Concentration

Analysis of variance showed that there were significant differences among genotypes for Na⁺ and K⁺ concentration under control and both levels of salt stress. The concentration of Na⁺ was found maximum in FH-114 in control and 15 dS m⁻¹ while C-26 performed poorly at 10 dS m⁻¹ salt stress. AA-802 accumulated lowest amount of Na⁺ under control, Mubarak accumulated the lowest under 10 dS m⁻¹ while CIM-612 had highest accumulation under 15 dS m⁻¹ salt stress conditions. Maximum concentration of K⁺ was observed in NIAB-824 under control while FH-118 had

maximum accumulation under 10 dS m⁻¹, and 15 dS m⁻¹ salt stress. While SB-149 accumulated minimum K⁺ under control, FH-458 did poorly at 10 dS m⁻¹ and 15 dS m⁻¹ salt stress. The results dictated that there was a significant interaction of Na⁺ and K⁺ with the salinity. The environmental variance was lower than genotypic and phenotypic variance for Na⁺ and K⁺ concentration under all the treatments. Coefficient of genotypic and phenotypic variance was similar to each other whereas their values were higher than the coefficient of environmental variance. Further, broad sense heritability was higher for the all treatments. Genetic advance was found higher under control than the salt stress for Na⁺ concentration whereas genetic advance was noted low for K⁺ concentration for all treatments (Table 6 and 7).

Potassium-Sodium Ratio (K⁺/Na⁺)

All genotypes were significantly different from each other

Table 5: Components of variance, heritability and genetic advance estimates of 50 cotton genotypes for root dry weight (g) and shoot fresh weight (g) under control level and salt stress

Sources of variation	Root Dry Weight (g)			Shoot Fresh Weight (g)		
	Control	10 dS m ⁻¹	15 dS m ⁻¹	Control	10 dS m ⁻¹	15 dS m ⁻¹
Genotypic Mean Square	0.0076	0.0039	0.0025	0.207	0.133	0.123
Error Mean Square	3.38E05	0.000114	0.000125	0.00095	0.000604	0.00099
Grand Mean of trait	0.161	0.118	0.084	1.315	1.119	0.953
Maximum	0.267	0.19	0.15	1.867	1.627	1.42
Minimum	0.067	0.06	0.03	0.823	0.653	0.557
Standard deviation	0.050	0.037	0.03034	0.264	0.211	0.203
Environmental variance	3.38E-05	0.00011	0.000125	0.00095	0.000604	0.00099
Genotypic variance	0.0025	0.00128	0.000796	0.068	0.044	0.040
Phenotypic variance	0.0025	0.0014	0.000921	0.069	0.044	0.041
Environmental co-efficient of variance	3.608	8.999	13.26817	2.342	2.194	3.302
Genotypic co-efficient of variance	31.158	30.168	33.47145	19.928	18.779	21.19
Phenotypic coefficient of variance	31.366	31.479	36.00531	20.065	18.907	21.44
Heritability Broad sense	98.682	91.84	86.42034	98.637	98.652	97.629
Genetic advance i=1.76	0.083	0.061	0.045886	0.455	0.365	0.349
Genetic advance %	54.167	50.594	54.45284	34.63	32.642	36.647

Table 6: Components of variance, heritability and genetic advance estimates of 50 cotton genotypes for sodium concentration (ppm) and shoot dry weight (g) under control level and salt stress

Sources of variations	Sodium (ppm)			Shoot Dry Weight (g)		
	Control	10 dS m ⁻¹	15 dS m ⁻¹	Control	10 dS m ⁻¹	15 dS m ⁻¹
Genotypic Mean Square	21.875	98.21	145.6393	0.013693	0.00910	0.0065
Error Mean Square	0.552	1.832	2.298	0.000298	0.00025	0.000218
Grand Mean of trait	16.888	60.667	77.993	0.334	0.253	0.165
Maximum	21	69.667	90.333	0.457	0.353	0.28
Minimum	10.833	48.667	66	0.19	0.13	0.063
Standard deviation	2.768	5.827	7.075	0.069	0.0566	0.048
Environmental variance	0.556	1.832	2.298	0.00029	0.00025	0.000218
Genotypic variance	7.106	32.125	47.784	0.0044	0.00295	0.00212
Phenotypic variance	7.662	33.957	50.079	0.0043	0.0032	0.00234
Environmental co-efficient of variance	4.416	2.247	1.944	5.166	6.333	8.924
Genotypic co-efficient of variance	15.784	9.411	8.863	19.989	21.411	27.882
Phenotypic co-efficient of variance	16.39	9.675	9.073	20.62	22.321	29.276
Heritability Broad sense	92.738	94.603	95.409	93.742	91.958	90.706
Genetic advance i=1.76	4.492	9.647	11.815	0.112	0.0918	0.0768
Genetic advance %	26.609	16.018	15.149	33.861	35.936	46.472

Table 7: Components of variance, heritability and genetic advance estimates of 50 cotton genotypes for potassium concentration (ppm) and K/Na ratio under control level and salt stress

Sources of variation	Potassium (ppm)			K/Na ratio		
	Control	10 dS m ⁻¹	15 dS m ⁻¹	Control	10 dS m ⁻¹	15 dS m ⁻¹
Genotypic Mean Square	471.12	649.877	577.252	21.856	0.666	0.321
Error Mean Square	3.517	3.335	2.559	0.277	0.00361	0.0012
Grand Mean of trait	166.893	137.133	117.86	10.384	2.325	1.534
Maximum	188.333	168	147.333	17.327	3.419	2.221
Minimum	142.33	114.667	100	7.132	1.679	1.122
Standard deviation	12.624	14.793	13.932	2.733	0.479	0.328
Environmental variance	3.517	3.335	2.559	0.277	0.0036	0.0012
Genotypic variance	155.868	215.514	191.564	7.193	0.22	0.106
Phenotypic variance	159.385	218.849	194.123	7.470	0.224	0.108
Environmental co-efficient of variance	1.123	1.3317	1.357	5.071	2.585	2.336
Genotypic co-efficient of variance	7.480	10.705	11.743	25.827	20.202	21.298
Phenotypic co-efficient of variance	7.564	10.787	11.821	26.320	20.367	21.426
Heritability Broad sense	97.793	98.476	98.681	96.287	98.388	98.810
Genetic advance i=1.76	21.605	25.494	24.069	4.605	0.815	0.568
Genetic advance %	12.945	18.590	20.414	44.351	35.068	37.05

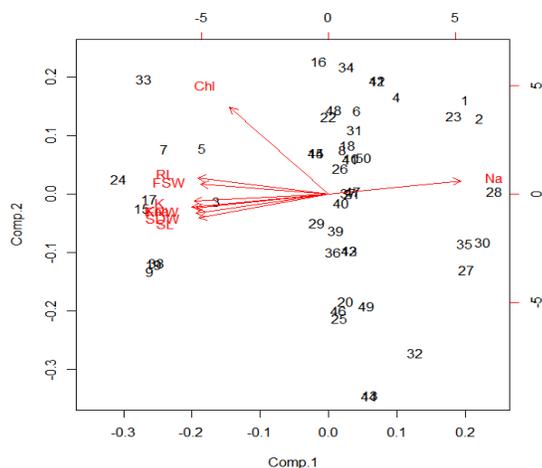


Fig. 1: Principal component biplot of genotype × Control × morpho-physiological traits interaction for all cotton genotypes. Where Chl (Chlorophyll Contents) RL (root length), SL (shoot length), FRW (fresh root weight), FSW (fresh shoot weight), RDW (root dry weight), SDW (shoot dry weight), K⁺ (potassium), Na⁺ (Sodium), K⁺/Na⁺ (Potassium to sodium ratio)

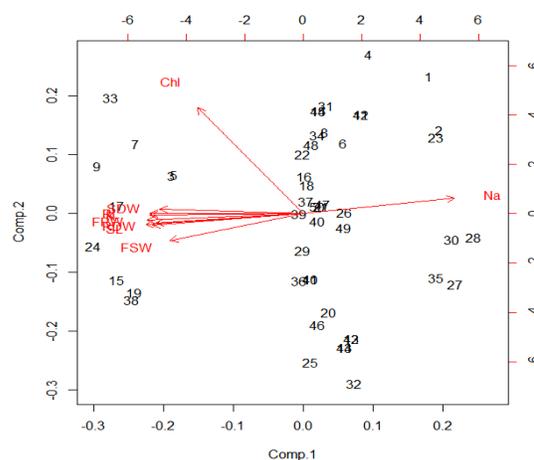


Fig. 3: Principal component biplot of genotype × salinity @ 15 dS m⁻¹ × morpho-physiological traits interaction for all cotton genotypes. Where Chl (Chlorophyll Contents) RL (root length), SL (shoot length), FRW (fresh root weight), FSW (fresh shoot weight), RDW (root dry weight), SDW (shoot dry weight), K⁺ (potassium), Na⁺ (Sodium), K⁺/Na⁺ (Potassium to sodium ratio)

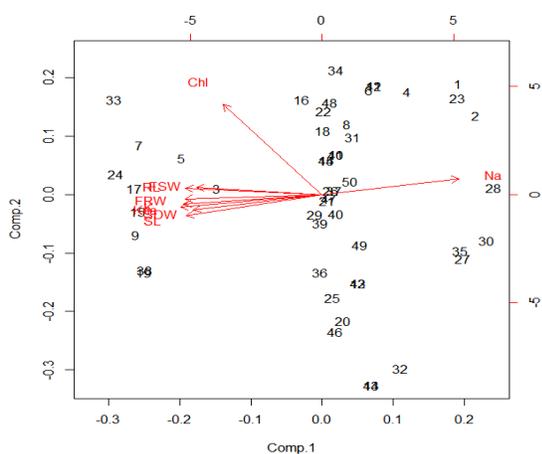


Fig. 2: Principal component biplot of genotype × Salinity @ 10 dS m⁻¹ × morpho-physiological traits interaction for all cotton genotypes. Where Chl (Chlorophyll Contents) RL (root length), SL (shoot length), FRW (fresh root weight), FSW (fresh shoot weight), RDW (root dry weight), SDW (shoot dry weight), K⁺ (potassium), Na⁺ (sodium), K⁺/Na⁺ (potassium to sodium ratio)

under control and both salt stress levels. The maximum K⁺/Na⁺ ratio was found for AA-802 and FH-118 under control and salt stress levels. AA-802 had showed highest value under control while FH-118 had maximum value at 10 dS m⁻¹ and 15 dS m⁻¹ salt stress. However, C-26 performed poorly under control, 10 dS m⁻¹ and 15 dS m⁻¹ salt stress levels. The genotypic and phenotypic variances were higher than environmental variance. The coefficient of genotypic and phenotypic variances

were negligibly different from each other while were higher than the environmental variance. There was high heritability estimated for the said character and high genetic advance was also reported for control and at both salt stress levels (Table 7).

Principal Component Analysis

In order to better understand the genotypic behavior at each salinity level for the studied morpho-physiological traits, PC-biplot was generated. Genotypes at control level showed peak values for morpho-physiological traits because scattered opposite to Na⁺ (Fig. 1). FH-312 (7), MNH-988 (15), MNH-992 (17), NIAB-824 (33) and CIM-612 (24) were found with trait vectors when a perpendicular is drawn for each morpho-physiological trait vector except Na⁺. When PCA was drawn for the higher levels of salt stress, there was a little depression of morpho-physiological factors noticed which further increased at the 15 dS m⁻¹. MUBARAK (3), FH-312 (7), NIAB-824 (33), MNH-992 (17), FH-118 (19), CIM-612 (24) and KEHKSHAN (5) looked more active while MG-6 (30), AGC-2 (35), SB-149 (28) and C-26 (27) were closely found with Na⁺ under 10 dS m⁻¹ and 15 dS m⁻¹ (Fig. 2 and 3).

Discussion

The presence of genetic variability in the genotypes provides breeder with necessary material for effective selection against biotic and abiotic stresses (Tyagi *et al.*, 2014). The studied traits showed significant differences under salinity. The interaction of genotypes × treatments was also significant indicating that genotypes behaved

differently under control and salt stress (Table 1). In the salt sensitive genotypes, chlorophyll contents are poorly degraded and reduced in quantity (Ismail and Horie, 2017). NIAB-824 and Kehkshan showed significantly high chlorophyll contents under successive high salt stress than other genotypes. High photosynthetic rate and high dry matter can be regarded as possible reason for the increase in chlorophyll contents and results in high crop yield even under salt stress (Ashraf, 2002). The decrease of chlorophyll contents was reported with the increase of salt stress (Khan *et al.*, 2009). However, the decrease in chlorophyll was significant in the salt sensitive genotypes of present study.

From breeding point of view, root and shoot related parameters carry much importance in the selection of genotypes against abiotic stresses. Performance of salt tolerant genotypes was remarkably less affected for root and shoot length under high salt stress (Bibi *et al.*, 2016). CIM-612, NIAB-824 and AA-802 had large root length under salt stress. Overall salt stress resulted in significant reduction of root and shoot length in all genotypes and root length was higher in salt tolerant genotypes in comparison to the salt sensitive genotypes and hence regarded as key parameter for the identification of salt tolerant genotypes (Hartmann *et al.*, 2015). Moreover, the fresh root and shoot weight was significantly different from each other under control and salt stress levels. Salt tolerant genotypes *i.e.*, CIM-612 and Kehkshan had high fresh root and shoot weights under salt stress. Salt sensitive genotypes displayed considerable reduction in fresh root and shoot weight of seedlings (Jiang *et al.*, 2016). It was due to the limited photosynthetic activity and the diversion of available photosynthates towards reducing the toxic effects of salts. Overall, genotypes indicated reduction of dry root and shoot weight under the salt stress (Deinlein *et al.*, 2014). Salt tolerant genotypes *i.e.*, Mubarak, CIM-612 and NIAB-824 exhibited high dry root and shoot weight which shows their capability to produce dry matter under salt stress. Among the 50 genotypes under studied, NIAB-824, Kehkshan, CIM-612, Mubarak, FH-312 had salt tolerant behavior for root and shoot related parameters. The trend in our results has been observed in various studies of strawberry, cotton and tomato (Garriga *et al.*, 2016). Root related parameters are also used in the screening and development of salt tolerant germplasm in grasses (Stavridou *et al.*, 2017). Even roots are more sensitive to salt and water stresses and reliable indicators for the selection and development of salt and drought tolerant germplasm.

Under salinity stress, accumulation of Na^+ in various parts of the plant is a primary response against salt stress. High Na^+ concentration inside the plant disrupts the different plant metabolic activities (Wang *et al.*, 2017). Salt tolerant genotypes differ from the salt sensitive in their capacity to retain Na^+ in roots under salt stress (Tsialtas *et al.*, 2017). In our experiment, genotypes were statistically significant from each other under control and both salt stress levels (Table 1). It was observed that NIAB-824, CIM-612 and Mubarak

accumulated least amount of Na^+ under control and increasing level of salt stress in their leaves and conceived as salt tolerant genotypes. The genotypes FH-114, C-26 and SB-149 had highest amount of Na^+ concentration in its leaves and were regarded as salt sensitive genotypes. The salt tolerant genotypes had less accumulation of Na^+ in their leaves in comparison to the salt sensitive genotypes (Zhang *et al.*, 2017). Salt tolerance is associated with Na^+ uptake, salt exclusion and its compartmentalization within cell or/and within plant. High Na^+ concentration inside the plant can be referred to high amount of salts in the growing material, lower efficiency of exclusion mechanisms or diffusion through damaged membranes (Wang *et al.*, 2017).

The results for heritability for the given traits under control and both levels salinity displayed that the most of the traits had high broad sense heritability means under genetically controlled (Azhar and Ahmad, 2000). Further, high genotypic and phenotypic variances and their coefficients than the environmental variance further confirm that these traits are less influenced by the environment. Among the given traits, fresh root weight had highest broad sense heritability. Besides cotton, these kind of studies have also been carried out in tomato (Saeed *et al.*, 2011), cotton (Dabbert *et al.*, 2017), and rice (Prasad *et al.*, 2017). Genetic advance was variable for each trait and maximum genetic advance was reported for the fresh root weight which shows that this trait had additive variation and selection for this character will be effective. Whereas, lowest genetic advance was estimated for K^+ concentration which shows that character was controlled by dominant genes and selection on the basis of this characters for breeding against salinity will be not useful (Sumanth *et al.*, 2017). Overall, salinity stress reduced the growth of all traits in the experiment and this reduction increased with the increase of salt stress (Negrão *et al.*, 2017). The reduction of growth can be referred to the ionic toxicity of Na^+ ions in the cell cytoplasm, water shortage in the plant due to the difference of osmotic pressure and ion imbalance or combination of all the above stated factors (Wang *et al.*, 2017). Principal component analysis illustrated generalized performances of all genotypes under studied across a single and multi-environments. PC-biplot categorized the best genotypes across the treatments and in particular revealed the best interaction of genotypes \times environment \times response. Among 50 genotypes of cotton the tolerant genotypes were found which had a longer vector with tolerant traits when a perpendicular was drawn from the genotype position (Yan, 2001). The genotypes in the positive quadrants were close to the desirable characters and selected as salt tolerant genotypes whereas the Na^+ was undesirable character which fell in the negative quadrant, so the genotypes close to the Na^+ vector and in opposite direction to the desirable character were considered salt sensitive. The results are in agreement with the (Raza *et al.*, 2017) where the genotypes present near the desirable traits were selected as salt tolerant at stress level of 10 and 15 dS m^{-1} .

Conclusion

The present study explored the genetic diversity in the cotton genotypes for salinity tolerance. Increase in salt stress had negative impact on the yield of the cotton crop and disturbs its metabolic pathways. Among fifty genotypes, NIAB-824, FH-118, Mubarak, Kehkshan and CIM-612 were identified as salt tolerant genotypes on the morpho-physiological basis. These identified genotypes can be used further in cotton breeding program under salt stress.

Acknowledgments

The authors are grateful to Higher Education Commission of Pakistan who funded for the experiment.

References

- Ashraf, M., 2002. Salt tolerance of cotton: some new advances. *Crit. Rev. Plant Sci.*, 21: 1–30
- Azhar, F. and R. Ahmad, 2000. Variation and heritability of salinity tolerance in upland cotton at early stage of plant development. *Pak. J. Biol. Sci.*, 3: 1991–1993
- Bibi, Z., N. Khan, A. Latif, M. Khan, G. Manyuan, Y. Niu, Q. Khan, I. Khan, S. Shaheen and G. Sadozai, 2016. Salt tolerance in upland cotton genotypes to NaCl salinity at early growth stages. *J. Anim. Plant Sci.*, 26: 766–775
- Dabbert, T.A., D. Pauli, R. Sheetz and M.A. Gore, 2017. Influences of the combination of high temperature and water deficit on the heritabilities and correlations of agronomic and fiber quality traits in upland cotton. *Euphytica*, 213: 6. Available at: <https://doi.org/10.1007/s10681-016-1798-8>. (Accessed: 26 April 2018)
- Deinlein, U., A.B. Stephan, T. Horie, W. Luo, G. Xu and J.I. Schroeder, 2014. Plant salt-tolerance mechanisms. *Trends Plant Sci.*, 19: 371–379
- Farhangi-Abri, S. and S. Torabian, 2017. Antioxidant enzyme and osmotic adjustment changes in bean seedlings as affected by biochar under salt stress. *Ecotoxicol. Environ. Saf.*, 137: 64–70
- Frankham, R., D.A. Briscoe and J.D. Ballou, 2002. Introduction to Conservation Genetics. *Mar. Turt. Newsl.*, 101:47-49
- Garriga, M., P.D.S. Caligari and J.B. Retamales, 2016. Salt stress induces differential responses in yield, fruit quality, vegetative growth and physiological parameters in commercial (*Fragaria × ananassa*) and Chilean strawberry (*F. chiloensis*) genotypes. In: *VIII Inter. Strawberry Symposium*, pp: 419-424. ISHS Acta Horticulturæ
- Hartmann, L., L. Pedrotti, C. Weiste, A. Fekete, J. Schierstaedt, J. Göttler, S. Kempa, M. Krischke, K. Dietrich and M.J. Mueller, 2015. Crosstalk between two bZIP signaling pathways orchestrates salt-induced metabolic reprogramming in Arabidopsis roots. *Plant Cell*, 27: 2244–2260
- Hoagland, D.R. and D.I. Arnon, 1950. *The Water Culture Method for Growing Plants Without Soil*, Vol. 347, p: 32. Circular California Agricultural Experiment Station, University of California, College of Agriculture, Berkeley, USA
- Husson, F., S. Le and J. Pages, 2017. *Explanatory Multivariate Analysis by Example Using R*. Chapman and Hall/CRC Press, Boca Raton, Florida, USA
- Ismail, A.M. and T. Horie, 2017. Genomics, physiology, and molecular breeding approaches for improving salt tolerance. *Ann. Rev. Plant Biol.*, 68: 405–434
- Jiang, K., J. Moe-Lange, L. Hennem and L.J. Feldman, 2016. Salt stress affects the redox status of Arabidopsis root meristems. *Front. Plant Sci.*, 7: 81
- Johnson, H.W., H. Robinson and R. Comstock, 1955. Estimates of Genetic and Environmental Variability in Soybeans I. *Agron. J.*, 47: 314–318
- Kamran, S., T.M. Khan, A. Shakeel, R. Ahmad and A. Zeb, 2016. Genetics of salt stress tolerance in upland cotton (*Gossypium hirsutum* L.). *Genetics*, 5: 31–37
- Khan, M., M. Shirazi, M.A. Khan, S. Mujtaba, E. Islam, S. Mumtaz, A. Shereen, R. Ansari and M.Y. Ashraf, 2009. Role of proline, K/Na ratio and chlorophyll content in salt tolerance of wheat (*Triticum aestivum* L.). *Pak. J. Bot.*, 41: 633–638
- Liu, X., S. Cai, G. Wang, F. Wang, F. Dong, M. Mak, P. Holford, J. Ji, A. Salih and M. Zhou, 2017. Halophytic NHXs confer salt tolerance by altering cytosolic and vacuolar K⁺ and Na⁺ in Arabidopsis root cell. *Plant Growth Regul.*, 82: 333–351
- Negrão, S., S. Schmöckel and M. Tester, 2017. Evaluating physiological responses of plants to salinity stress. *Ann. Bot.*, 119: 1–11
- Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicol. Environ. Saf.*, 60: 324–349
- Parihar, P., S. Singh, R. Singh, V.P. Singh and S.M. Prasad, 2015. Effect of salinity stress on plants and its tolerance strategies: a review. *Environ. Sci. Pollut. Res.*, 22: 4056–4075
- Prasad, K.R., K.R. Krishna, M. Bhawe and L.S. Rao, 2017. Genetic variability, Heritability and Genetic advance in Boro Rice (*Oryza sativa* L.) germplasm. *Int. J. Curr. Microbiol. Appl. Sci.*, 6: 1261–1266
- Raza, M.A., A. Saeed, H. Munir, K. Ziaf, A. Shakeel, N. Saeed, A. Munawar and F. Rehman, 2017. Screening of tomato genotypes for salinity tolerance based on early growth attributes and leaf inorganic osmolytes. *Arch. Agron. Soil Sci.*, 63: 501–512
- Rozema, J. and H. Schat, 2013. Salt tolerance of halophytes, research questions reviewed in the perspective of saline agriculture. *Environ. Exp. Bot.*, 92: 83–95
- Saeed, A., M. Saleem, M. Zakria, S. Anjum, A. Shakeel and N. Saeed, 2011. Genetic variability of NaCl tolerance in tomato. *Genet. Mol. Res.*, 10: 1371–1382
- Sevik, H. and M. Cetin, 2015. Effects of water stress on seed germination for select landscape plants. *Pol. J. Environ. Stud.*, 24: 689–693
- Shrivastava, P. and R. Kumar, 2015. Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi J. Biol. Sci.*, 22: 123–131
- Soomro, Z., M. Kumbhar, A. Larik, M. Imran and S. Brohi, 2010. Heritability and selection response in segregating generations of upland cotton. *Pak. J. Agric. Res.*, 23: 25–30
- Stavridou, E., A. Hastings, R.J. Webster and P.R. Robson, 2017. The impact of soil salinity on the yield, composition and physiology of the bioenergy grass *Miscanthus × giganteus*. *Glob. Change Biol. Bioener.*, 9: 92–104
- Steel, R.G., J.H. Torrie and D.A. Dickey, 1997. *Principles and Procedures of Statistics: A Biological Approach*, 3rd edition. McGraw Hill Book Co., New York, USA
- Sumanth, V., B. Suresh, B.J. Ram and G. Srujana, 2017. Estimation of genetic variability, heritability and genetic advance for grain yield components in rice (*Oryza sativa* L.). *J. Pharm. Phytochem.*, 6: 1437–1439
- Taïbi, K., F. Taïbi, L.A. Abderrahim, A. Ennajah, M. Belkhdja and J.M. Mulet, 2016. Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in *Phaseolus vulgaris* L. *South Afr. J. Bot.*, 105: 306–312
- Tsialtas, I.T., S. Shabala, D. Baxevanos and T. Matsi, 2017. Cation selectivity in cotton (*Gossypium hirsutum* L.) grown on calcareous soil as affected by potassium fertilization, cultivar and growth stage. *Plant Soil*, 415: 331–346
- Tyagi, P., M.A. Gore, D.T. Bowman, B.T. Campbell, J.A. Udall and V. Kuraparthi, 2014. Genetic diversity and population structure in the US upland cotton (*Gossypium hirsutum* L.). *Theor. Appl. Genet.*, 127: 283–295
- Wang, N., H. Qi, W. Qiao, J. Shi, Q. Xu, H. Zhou, G. Yan and Q. Huang, 2017. Cotton (*Gossypium hirsutum* L.) genotypes with contrasting K⁺/Na⁺ ion homeostasis: implications for salinity tolerance. *Acta Physiol. Plant.*, 39: 1–10
- Yan, W., 2001. GGEbiplot—a Windows application for graphical analysis of multienvironment trial data and other types of two-way data. *Agron. J.*, 93: 1111–1118
- Zhang, H., D. Li, Z. Zhou, R. Zahoor, B. Chen and Y. Meng, 2017. Soil water and salt affect cotton (*Gossypium hirsutum* L.) photosynthesis, yield and fiber quality in coastal saline soil. *Agric. Water Manage.*, 187: 112–121

(Received 08 March 2018; Accepted 17 August 2018)