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Full Length Article

Evaluation of Morpho-Physiological and Biochemical Attributes of Cotton under Salt Stress

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Abstract

The salinity level is increasing in soil due to multiple reasons like uneven rainfall areas and effects of climate change. Such increase in salinity forced the cotton breeder for the development of new germplasm suitable for saline soils. For this purpose, a set of one hundred accessions of *Gossypium hirsutum* was assessed for salt tolerance in hydroponic conditions at four levels of NaCl salt concentrations, *i.e.*, control, 100 m*M*, 150 m*M* and 200 m*M*. Significant differences were observed for morphological, physiological and biochemical parameters among the genotypes. At control, the genotypes showed enhanced growth, but some of genotypes exhibited similar performance at 100 m*M* as they were at normal. Whereas, response of some indices was significantly decreased at 150 m*M*, while at 200 m*M* highly significant response were noted among genotypes due to accumulation of Na+, increase reactive oxygen species levels and decreased level of K+ in leaves. K-means cluster and biplot analysis were used to identify the salt tolerance and susceptible genotypes. The accessions namely, NIAB-545, CIM-595, Coker-307, FH-113, FH-942 and DNH-40 were performed relatively better across all the treatments and Na^+/K^+, chlorophyll contents, free proline contents and peroxidase activity were found to be effective selection criterion for salt tolerance. The identified accessions (NIAB-545, CIM-595, Coker-307, FH-113, FH-942 and DNH-40) could be exploited in breeding programs as well as among farming community for cultivation on salt affected soils. © 2020 Friends Science Publishers

Keywords: Antioxidants enzymes; Biochemical assays; *Gossypium hirsutum* L.; Hydroponic; Salt tolerance

Introduction

Salinity is one of the abiotic stresses that affect crop productivity around the globe and resultantly reducing the yield in many field crops like cotton. Primarily *Gossypium hirsutum* plant is sensitive to salinity at germination and seedling stage. Approximately 30% of cultivated lands will be affected by salinity in next 25 years, which is one of the threats to food security around the globe (Manikandan *et al*. 2019). Due to these significant adverse effects of salinity about 800 million hectares of world's land that constitutes about 6% of the world's area has been shifted to barren soil (Ismail *et al*. 2007). Due to change in climate, level of salinity as well as area due to salinity is emergent issue in arid to semi-arid region of Pakistan and 6.82 million hectares are salt affected out of 22 million hectares of agriculture land in Pakistan (Hussain *et al*. 2019). The excess of salts has adversely affecting the productivity of crops due to the harmful effects including, retardation of growth and development in different crop plants (Munns and Tester 2008). Various mechanisms have been explored to dig out that how salinity exerts adverse effects on growth and developmental stages of crop plant (Munns 2002), like i) rhizosphere has low water potential due to the presence of salts that cause water shortfall in plant organs, ii) excess of Na^+ and Cl^- ions results in ion toxicity, iii) poor uptake of nutrients including micro and macro due to salt traces in root zone that lead to imbalance of ions (Zörb *et al*. 2019).

These effects prompted the plant researchers to develop salt tolerant genotypes of crops plants. To proceed this, in addition to ample genetic variability in available germplasm, the availability of amenable criteria is pre-requisite to have salt tolerant genotypes. The screening of large number of accessions of field crops for salt tolerance in laboratory and field conditions is a cumbersome work. For example, estimation of K^+/Na^+ ratio has been used as a dependable criterion for selection for salt tolerance in wheat (*Triticum aestivum* L.) (Wang *et al*. 2019), alfalfa (*Medicago sativa* L.) (Al-Farsi *et al*. 2020), sorghum [*Sorghum bicolor* (L.) Moench] (Forghani *et al*. 2018) and maize (*Zea mays* L.) (Farooq *et al*. 2015; Luo *et al*. 2019). In general, salt tolerant plants regulates the exclusion of Na^+ ions *via* roots, in contrary salt sensitive plants are unable to maintain Na^+ homeostasis. However, some studies

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reported that rather than Na^+ exclusion, maintenance of optimum K^+/Na^+ ions ratio determines the performance of plant under salt stress (Ding *et al*. 2010; Dai *et al*. 2014).

Salt stress exerts adverse effects on production of biomass *i.e*. decrease in leaf area, stem thickness, shoot and root weight and yield of seed cotton (Sharif *et al.* 2019). Stavridou *et al.* (2017) have reported that 50% of reduction in yield is recorded at 17 dS m^{-1}. Salinity has more harmful impact on cotton production at seedling stage, cotton plant grown under salt stress showed reduced rate of germination (Higbie *et al*. 2010), whereas at vegetative stage; rate of evaporation, photosynthesis and water use efficiency was also reduced, but respiration rate was increased. In later stages, plant height, expansion of leaves, stem diameter and root/shoot ratio of cotton was also affected significantly in salt stress conditions. In addition, increase in fruit shedding, delay in fruit initiation and poor fiber quality traits have also been witnessed due to the prolonged salt stress (Gupta and Huang 2014). Salt stress causes disturbance of cellular ions, osmotic stress and over production of reactive oxygen species (ROS). To cope with these effects, plants have efficient but complex enzymatic *i.e.,* catalase (CAT), peroxidase (POD) and non-enzymatic *i.e.,* free proline antioxidant defense systems to avoid the toxic effects of free radicals (Majeed *et al*. 2019). Salt stress leads to over-production of ROS such as superoxide anions, hydrogen peroxide (H_2O_2) and hydroxyl radicals (Sharif *et al*. 2019). Keeping in view the above-mentioned losses and effects of salinity, it is need of time to have such cotton accession which show enhanced level of tolerance to saline conditions. In this study, efforts are being made for the development of efficient and amenable selection criteria of salt tolerant cotton genotypes. The outcome from this study could be a contribution in various breeding program being executed in the country for the development of salt tolerant accessions of cotton.

Materials and Methods

Germplasm of cotton comprised of 100 genotypes/accessions collected from various cotton breeding institutes, centers and departments in the country *i.e.,* Cotton Research Station, Faisalabad, Nuclear Institute for Agriculture Biology, Faisalabad, Central Cotton Research Institute, Multan and Sakrand, Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad and Cotton Research Station, Vehari (Table 1). These germplasms were evaluated at four different salinity levels *i.e.,* (0 m*M* considered as control, 100 m*M*, 150 m*M* and 200 m*M*). The experiment was conducted following completely randomized design (CRD) under factorial arrangement. This experiment was carried out using glasshouse facility available at Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad (latitude 31.25' N, longitude 73.09' E and altitude 184.4 m).

Assessment of plant material

Three uniform and healthy seeds of each genotype were sown in a polythene bag measuring of 7.35 cm × 6.7 cm containing 20 g of sand. The electrical conductivity (EC) of 1.6 with pH of 8.1 was determined prior to sowing. All necessary plant production and protection measures were taken to have healthy seedlings. The seedlings were uprooted carefully from sand at first true leaf stage. The root system of seedlings was washed thoroughly with distilled water to remove sand and later on the seedlings of uniform size of each accession was selected for transplantation to hydroponic. One seedling per hole of thermopore sheet was transplanted which was placed in aerated half strength Hoagland nutrient solution in tin tub (dimension 1.52 m × 0.91 m) carrying 100 L of Hoagland solution (Hoagland and Arnon 1950). The tap water (EC= 0.22 dS/m) was treated as a control. Optimal aeration was maintained in each tin tub using air pumps. Three days after transplanting into aqueous media, four different levels of NaCl were gradually applied *i.e.,* control, 100 m*M*, 150 m*M* and 200 m*M*, respectively. The EC of each salinity level was maintained till the completion of experiment. After 30 days of salt stress, the data for each genotype was recorded for root length, shoot length, root/shoot ratio, fresh root weight, dry root weight, fresh shoot weight and dry shoot weight. The chlorophyll content was assessed using chlorophyll meter (SPAD 502 Plus) as SPAD value. At the same time, one fully opened fresh leaf tissue from each genotype was collected and immediately stored at -80°C freezer available at Centre for Advanced Studies (CAS), University of Agriculture, Faisalabad. These frozen leaf tissues were used for analyzing biochemical traits *i.e.*, K^+/ Na^+ ratio, free proline content, H_2O_2 concentration, peroxidase activity and catalase activity in Cotton Lab of the department.

For the determination of K^+ content, approximately 3 g leaf tissues were ground in 8 mL of distilled water by heating at 90°C using hot plate for 3 h to make homogenous mixture. Then K^+ content was measured using an atomic absorption spectrophotometer (TA-S-986; Persee; China). For the determination of Na^+ content, leaf tissues of approximately 2 g were ground in 10 mL of distilled water by heating at 80°C for 3 h. The Na^+ content was analyzed by ion chromatography (DX-300; Sunnyvale, C.A., U.S.A.).

Free proline content

Free proline contents were determined by following the protocol as proposed by Bates *et al*. (1973). 0.1 g ground leaf tissues were homogenized with 5 mL of 3% sulfosalicyclic acid. Then extract was centrifuged at 11,000 rpm for 10 min and separate the supernatant. Then 3% Ninhydrin solution containing equal volume of 6 M ortho phosphoric acid and glacial acetic acid was prepared. Then draw 1 mL from each component *i.e*., glacial acetic acid, ninhydrin solution and supernatant of leaf extract and

poured into cuvettes. Then incubation was done at 100°C for 50 min. Later, ice bath was used for cooling of mixture and then 0.5 mL toluene was added before vortex for 6 min to obtain organic layer while aqueous layer was discarded. Afterwards, organic layer was poured in ELISA plate and absorbance was recorded at 520 nm using toluene as blank for standard curve.

Hydrogen peroxide

H_2O_2 content was estimated according to the method of Bernt and Bergmeyer (1974). At the time of harvesting of crop, leaf tissues were stored at -80°C freezer for this analysis. For its estimation, 0.1 g leaf tissue was ground with 5 mL pre-chilled acetone and later centrifuged at 3200 × g for 9 min at 4°C using normal speed micro-centrifuge and SCILOGEX D2012. Then analysis further involved the mixing of one-millimeter supernatant with 0.1 mL of 95% (v/v) hydrochloric acid (HCL), 0.3 mL ammonia and 30% (v/v) titanium tetrachloride and centrifuged at 11,000 × g for 9 min at 4°C. Then cold acetone was repeatedly used to wash the sediments and centrifuged at 12,000 rpm for 9 min and finally dissolved in 2 mL of 1 *M* H_2SO_4. Nano Drop Spectrophotometer (Model No. ND-8000 Thermo Scientific) was used to determine absorbance at 410 nm and estimate H_2O_2 concentration by using a standard curve based on known concentration.

Peroxidase activity

The peroxidase activity was assessed following Fielding and Hall (1978). The leaf tissues were ground in pestle and mortar by using 0.05 M sodium phosphate buffer and centrifuged at 10,000 rpm for 20 min and poured supernatant in eppendorf tube. Afterward, 3 mL of reaction mixture was prepared by mixing equal amount of guaicol, H_2O_2 and finally poured in the enzyme extract. Then absorbance was measure at 470 nm by using Nano Drop Spectrophotometer (Model No. ND-8000 Thermo Scientific).

Catalase activity

Catalase activity was assayed according to Chance and Maehly (1955). Leaf tissues were ground with sodium phosphate buffer to prepared 0.1 mL enzyme extract. Then CAT reaction solution was made by using 40 m*M* phosphate buffer, 15 m*M* H_2O_2 and 0.1 mL enzyme extract. The absorbance was recorded at 240 nm by using Nano Drop Spectrophotometer (Model No. ND-8000 Thermo Scientific) after every 20 s.

Statistical analysis

Genetic variability among 100 accessions were assessed for these recorded attributes by using analysis of variance with factorial design (Steel *et al*. 1997), while K-means cluster analysis and biplot analysis were performed with the help of various statistical software tools, *i.e.*, SPSS v. 19 and STATISTICA v. 5.0 to determine the response of various upland cotton cultivars under control and various levels of salt concentrations.

Results

The data of morphological, physiological, and biochemical characters were analyzed through multivariate analysis that classify the germplasm into various clusters based on potential and performance of the traits. Likewise, this analysis was utilized for exploitation of data collected from this study. Significant genetic variability ($P < 0.01$) was found in the accessions for certain traits (Table 2). The presence of genetic variability allows the research worker to proceed for other biometrical analysis like biplot analysis used herein. One of feature and objective for using biplot analysis in this study is the characterization and identification of salt tolerant and susceptible lines. In addition, K-means cluster analysis was also exploited for grouping of 100 accessions based on mean values for certain variable *i.e.*, six clusters were found for certain variables.

In control conditions, the cluster No. 6 exhibited maximum mean values for the salinity related characters namely root length (14.44 cm), shoot length (20.87 cm), fresh root weight (0.93 g), fresh shoot weight (1.62 g), dry root weight (0.23 g), dry shoot weight (0.42 g), K^+/Na^+ (14.95) and chlorophyll content (40.81 chlorophyll concentration index) except for free proline content (0.25 µmol g^{-1} FW), POD (11.80 U mg^{-1} protein) and H_2O_2 (0.23 µmol g^{-1} FW) showed lower mean values. Accessions namely NS-121 (G13), FH-326 (G28), VH-326 (G49), NIA-86 (G56), VH-295 (G57) were found in cluster 6, likewise cluster no. 5 also had some of positively associated variable and certain number of accessions (Fig. 1). On contrary, cluster No. 3 had lower mean values for root length (7.13 cm), shoot length (13.55 cm), root fresh weight (0.29 g) and shoot fresh weight (0.96 g) (Table 3). Likewise, biplot analysis revealed that association among various parameters under control conditions. This analysis exhibited that high level of Na^+ and H_2O_2 were found to be negatively correlated with other traits. The genotypes namely, HG-HN-450 (G10), NS-121 (G13), FH-144 (G22), IUB-222 (G25), FH-326 (G28), CIM-595 (G36), VH-326 (G49) and VH-295 (G57) have maximum number of salt tolerance contributing traits. Besides, based on the importance of individual salt related physiological indices, VH-326 (G49) exhibited highest chlorophyll contents (37.78 CCI) while HG-HN-450 (G10), NS-121 (G13) and VH-295 (G57) revealed the presence of higher K^+ concentration and higher K^+/Na^+ ratio. Whereas, for morphological indices, dry weight of root and shoot were found to be high for FH-144 (G22), IUB-222 (G25) and FH-326 (G28). Higher H_2O_2 contents (0.56 μmol g^{-1} FW) were found in NIAB-878-B (G86) (Fig. 1).

Table 1: List of cotton genotypes used in the experiment

Code	Genotypes	Code	Genotypes	Code	Genotypes	Code	Genotypes
G1	Cemb 33	G26	VH-324	G51	IR-8	G76	IUB-212
G2	MNH-1016	G27	FH-942	G52	PB-896	G77	NIAB-112
G3	VH-259	G28	FH-326	G53	FH-458	G78	Cyto-179
G4	Sahara Buraq	G29	CIM-608	G54	FH-4243	G79	MNH-886
G5	RH-622	G30	AGC-2	G55	Rehmani	G80	DNH-40
G6	FH-214	G31	Coker-3113	G56	NIA-86	G81	MNH-988
G7	IR-3	G32	FH-113	G57	VH-295	G82	MNS-992
G8	VH-341	G33	CIM-602	G58	NS-131	G83	VH-329
G9	NIAB-824	G34	CIM-598	G59	CIM-612	G84	PB-900
G10	HG-HN-450	G35	CRS-1	G60	MNH-992	G85	FH-118
G11	NS-181	G36	CIM-595	G61	VH-283	G86	NIAB-878-B
G12	VH-228	G37	QM-IUB-65	G62	MS-71	G87	Saim-32
G13	NS-121	G38	BT-141	G63	Debal	G88	NIAB-545
G14	KZ-181	G39	RH-668	G64	AGC-99	G89	VH-148
G15	FH-115	G40	VH-330	G65	AA-307	G90	FH-113
G16	VH-171	G41	CRIS-9	G66	FH-634	G91	VH-363
G17	FH-158	G42	NIAB-414	G67	BS-15	G92	CIM-599
G18	SAU-1	G43	Sitara-008	G68	FH-175	G93	CIM-616
G19	VH-295	G44	KZ-189	G69	FH-Noor	G94	NIAB-BT-2
G20	FH-142	G45	FH-172	G70	AA-703	G95	Cyto-178
G21	NIAB-KIRN	G46	Coker-307	G71	VH-338	G96	FH-170
G22	FH-144	G47	MNH-992	G72	SLH-8	G97	Shahkar
G23	RH-647	G48	VH-325	G73	IR-901	G98	CIM-600
G24	MNH-786	G49	VH-326	G74	IUB-75	G99	SB-149
G25	IUB-222	G50	NIAB-1048	G75	FH-177	G100	MG-6

Where G = (Genotype)

Table 2: Mean squares for various quantitative traits of cotton under salt stress

Source of variation	DF	RL	SL	FRW	FSW	DRW	DSW	K^+	Na^+	K^+/Na^+	Chlr	Proln	POD	H_2O_2	CAT
Salinity	3	310.83**	237.96**	1.33**	3.18**	0.15**	0.69**	62308.7**	91906.4**	2209.99**	1024.66**	21.43**	2745.83**	8.58**	12375.5**
Genotypes	99	15.56**	11.202**	0.08**	0.11**	0.003**	0.007**	543.3**	524.5**	8.44**	38.87**	0.07**	24.22**	0.04**	140.2**
Salinity × Genotypes	297	12.23**	8.79**	0.05**	0.08**	0.002**	0.009**	542.8**	525.7**	8.22**	25.02**	0.07**	23.57**	0.05**	82.5**
Error	400	2.70	1.85	0.01	0.01	0.0005	0.001	77.1	11.9	0.79	5.17	0.006	3.44	0.006	11.3
Total	799														

*P < 0.05, **P < 0.01 and ***P < 0.001 Where, DF = Degree of freedom; RL = Root length; SL = Shoot length; FRW = Fresh root weight; FSW = Fresh shoot weight; DRW = Dry root weight; DSW = Dry shoot weight; K^+ = Potassium ion; Na^+ = Sodium ion; K^+/Na^+ = Potassium to Sodium ratio; Chlr = Chlorophyll content; Proln = Proline; POD = Peroxidase; H_2O_2 = Hydrogen peroxide; CAT = catalase

Table 3: K-means cluster analysis of 100 cotton genotypes grown under control conditions

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	F-value	*P*-value
RL	9.265	9.890	7.135	10.460	14.432	14.440	20.383**	0.0000
SL	15.374	16.200	13.550	16.826	18.950	20.870	26.486**	0.0000
FRW	0.503	0.549	0.293	0.581	0.812	0.932	23.455**	0.0000
FSW	1.141	1.269	0.966	1.304	1.622	1.628	23.270**	0.0000
DRW	0.127	0.149	0.092	0.160	0.215	0.235	30.180**	0.0000
DSW	0.262	0.316	0.232	0.336	0.415	0.427	36.679**	0.0000
K^+	142.180	162.975	147.300	167.707	181.500	184.900	52.832**	0.0000
Na^+	58.460	17.950	19.600	17.047	13.991	12.680	665.606**	0.0000
K^+/Na^+	2.485	9.395	7.565	10.062	13.523	14.950	288.709**	0.0000
Chlr	35.585	35.263	32.730	36.286	40.805	40.810	9.823**	0.0000
Proln	0.308	0.310	0.334	0.347	0.344	0.250	6.359*	0.0004
POD	11.920	11.675	12.700	12.431	13.409	11.800	5.758*	0.0006
H_2O_2	0.310	0.240	0.249	0.247	0.262	0.238	5.129*	0.0008
CAT	30.400	24.875	30.250	36.155	23.591	35.900	30.747**	0.0000

*P < 0.05, **P < 0.01 and ***P < 0.001 Where RL = Root length [cm] ; SL = Shoot length [cm]; FRW = Fresh root weight [g]; FSW = Fresh shoot weight [g]; DRW = Dry root weight [g]; DSW = Dry shoot weight [g]; K^+ = Potassium ion; Na^+ = Sodium ion; K^+/Na^+ = Potassium to Sodium ratio; Chlr = Chlorophyll content [CCI]; Proln = Proline content [μmol g^{-1} (FW)]; POD = Peroxidase [U mg^{-1} protein]; H_2O_2 = Hydrogen peroxide [μmol g^{-1} (FW)]; CAT = catalase activity [U mg^{-1} protein]

Under 100 m*M* salinity level, K-means cluster analysis had grouped these 100 accessions in six clusters. Genotypes in cluster no. 5 had higher mean values for root length (13.23 cm), shoot length (18.87 cm), K^+ and chlorophyll contents (37.78 CCI) (Table 4). It was found through biplot analysis that peroxidase activity and catalase were negatively associated with free proline content while high concentration Na^+ has negative association with K^+/Na^+ ratio.

Table 4: K-means cluster analysis of 100 cotton genotypes grown under 100 mM salinity level

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	F-value	*P*-value
RL	7.438	7.774	7.252	10.355	13.230	5.852	31.189**	0.0000
SL	14.104	15.055	13.337	15.966	18.870	12.468	27.711**	0.0000
RFW	0.417	0.418	0.371	0.551	0.748	0.264	19.417**	0.0000
SFW	1.085	1.052	0.942	1.183	1.433	0.868	20.864**	0.0000
RDW	0.101	0.113	0.083	0.119	0.174	0.067	28.289**	0.0000
SDW	0.213	0.248	0.175	0.253	0.308	0.137	39.563**	0.0000
K	124.958	130.500	120.100	142.273	161.100	107.800	235.784**	0.0000
Na	65.375	62.524	74.233	63.000	52.500	83.680	84.061**	0.0000
K/Na	1.932	2.126	1.636	2.279	3.090	1.296	165.380**	0.0000
Chlr	32.325	31.669	31.990	35.468	37.780	28.798	15.790**	0.0000
Proln	0.380	0.393	0.366	0.390	0.529	0.389	7.321*	0.0009
POD	15.250	17.595	14.733	15.318	13.000	17.880	4.536**	0.0010
H_2O_2	0.543	0.566	0.472	0.514	0.419	0.567	5.393*	0.0008
CAT	26.958	37.143	39.167	33.295	27.100	34.880	11.372**	0.0000

*P < 0.05, **P < 0.01 and ***P < 0.001 Where RL = Root length [cm] ; SL = Shoot length [cm]; FRW = Fresh root weight [g]; FSW = Fresh shoot weight [g]; DRW = Dry root weight [g]; DSW = Dry shoot weight [g]; K^+ = Potassium ion; Na^+ = Sodium ion; K^+/Na^+ = Potassium to Sodium ratio; Chlr = Chlorophyll content [CCI]; Proln = Proline content [μmol g^{-1} (FW)]; POD = Peroxidase [U mg^{-1} protein]; H_2O_2 = Hydrogen peroxide [μmol g^{-1} (FW)]; CAT = catalase activity [U mg^{-1} protein]

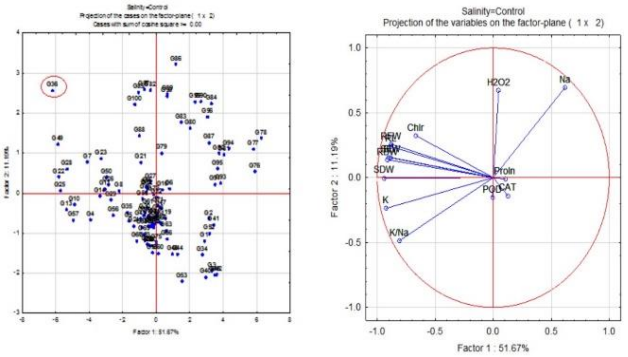

Fig. 1: Biplot analysis of 100 cotton genotypes for various seedlings traits grown under control salinity level**.** Where RL = Root length; SL = Shoot length; FRW = Fresh root weight; FSW = Fresh shoot weight; DRW = Dry root weight; K^+ = Potassium ion; Na^+ = Sodium ion; K^+/Na^+ = Potassium to Sodium ratio; Chlr = Chlorophyll content; Proln = Proline; POD = Peroxidase; H_2O_2 = Hydrogen peroxide; CAT = catalase activity

Note: Genotype G36 "CIM-595" (highlighted) at left top performed best among other genotypes under 200 m*M* salinity level

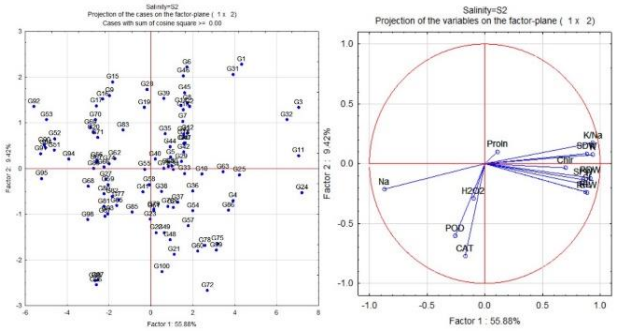

Fig. 2: Biplot analysis of 100 cotton genotypes for various seedlings traits grown under 100 m*M* salinity level**.** Where RL = Root length; SL = Shoot length; FRW = Fresh root weight; FSW = Fresh shoot weight; DRW = Dry root weight; K^+ = Potassium ion; Na^+ = Sodium ion; K^+/Na^+ = Potassium to Sodium ratio; Chlr = Chlorophyll content; Proln = Proline; POD = Peroxidase; H_2O_2 = Hydrogen peroxide; CAT = catalase activity

Higher mean values for root length (23.24 cm) and chlorophyll contents were noted for NS-181 (G11) and MNH-786 (G24) from cluster 5; while higher free proline contents (0.529 μmol g^{-1} FW) were found in Coker-307 (G46) (cluster 4). The genotypes namely CIM-600 (G98), MNH-988 (G81) and Cyto-178 (G95) showed poor response towards salinity stress due to the presence of high concentration of Na^+ (Fig. 2).

These 100 accessions were grouped in six clusters when exposed to 150 m*M* salinity stress. The genotypes in cluster No. 3 exhibited highest means for shoot length (17.07 cm), chlorophyll content (36.31 CCI), root length (11.67 cm) and K^+ concentration (Table 5). Likewise, biplot analysis revealed the positive association among same traits which were identified under 100 m*M* conditions. The genotype NIAB-545 (G88) had high chlorophyll contents (37.23 CCI) and K^+/Na^+ (2.5) ratio. The genotype namely, Coker-307 (G46) and FH-113 (G90) were also observed as tolerant lines based on highest mean values for biochemical characters. In contrary, higher concentration of Na^+ was found in Cyto-178 (G95) and VH-363 (G91) (Fig. 3). The same numbers of clusters *i.e.*, six were found for the accessions when tested at 200 m*M* salinity level in greenhouse conditions. Similarly, some of genotypes in cluster No.6 were proved to be good in biplot analysis *e.g.*, Coker (G46) and NIAB-545 (G88) for root length (10.65 cm), shoot length (16.25 cm), fresh root weight (0.56 g) and dry root weight (0.11 g) and K^+/Na^+ ratio (2.15). Moreover, DNH-40 (G80) exhibited highest chlorophyll contents (35.09 CCI) likewise FH-113 (G90) had highest concentration of catalase (51.18 U mg^{-1} protein), a required indices of salt tolerance. Highest free proline contents (1.02 µmol g^{-1} FW) were found in Coker-307 (G46). The genotypes AA-307 (G65) and AGC-99 (G64) had positive association with free proline and H_2O_2 while FH-214 (G6), NS-181 (G11) and KZ-181 (G14) had highest mean values for POD and chlorophyll content (Fig. 4 and Table 6).

Table 5: K-means cluster analysis of 100 cotton genotypes grown under 150 m*M* salinity level

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	F-value	*P*-value
RL	7.608	8.753	11.673	6.329	6.453	10.048	23.212**	0.0000
SL	14.677	15.217	17.077	13.243	12.791	16.273	15.553**	0.0000
RFW	0.427	0.489	0.610	0.301	0.311	0.511	14.312**	0.0000
SFW	1.122	1.046	1.282	0.912	0.904	1.256	18.262**	0.0000
RDW	0.114	0.102	0.140	0.078	0.078	0.146	17.827**	0.0000
SDW	0.235	0.210	0.254	0.160	0.150	0.320	40.750**	0.0000
K	127.654	129.033	148.308	116.044	110.500	161.775	111.476**	0.0000
Na	60.923	70.900	60.577	76.283	80.344	17.425	550.145**	0.0000
K/Na	2.108	1.842	2.501	1.528	1.367	1.458	54.844**	0.0000
Chlr	32.146	32.620	36.312	30.072	30.350	30.498	7.562**	0.0000
Proln	0.847	0.872	0.743	0.730	0.901	0.870	9.585*	0.0309
POD	17.923	19.500	16.731	19.022	19.250	17.050	5.198*	0.0309
H_2O_2	0.664	0.719	0.562	0.737	0.741	0.701	6.077*	0.0251
CAT	45.462	53.633	44.538	37.174	48.750	46.675	19.116**	0.0000

*P < 0.05, **P < 0.01 and ***P < 0.001 Where RL = Root length [cm] ; SL = Shoot length [cm]; FRW = Fresh root weight [g]; FSW =Fresh shoot weight [g]; DRW = Dry root weight [g]; DSW = Dry shoot weight [g]; K^+ = Potassium ion; Na^+ = Sodium ion; K^+/Na^+ = Potassium to Sodium ratio; Chlr = Chlorophyll content [CCI]; Proln = Proline content [μmol g^{-1} (FW)]; POD = Peroxidase [U mg^{-1} protein]; H_2O_2 = Hydrogen peroxide [μmol g^{-1} (FW)]; CAT = catalase activity [U mg^{-1} protein]

Table 6: K-means cluster analysis of 100 cotton genotypes grown under 200 mM salinity level

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	F-value	*P*-value
RL	8.306	6.396	6.052	5.257	7.606	10.656	29.080**	0.0000
SL	14.450	13.268	12.493	11.607	15.281	16.253	24.052**	0.0000
RFW	0.437	0.331	0.274	0.195	0.461	0.567	23.800**	0.0000
SFW	1.053	0.893	0.888	0.746	1.048	1.200	22.355**	0.0000
RDW	0.097	0.085	0.068	0.051	0.113	0.118	21.290**	0.0000
SDW	0.190	0.163	0.137	0.116	0.243	0.238	34.947**	0.0000
K^+	125.824	114.700	108.239	104.571	130.813	141.350	228.405**	0.0000
Na^+	72.235	77.860	83.391	86.357	63.938	65.750	72.925**	0.0000
K^+/Na^+	1.784	1.471	1.300	1.206	2.102	2.156	135.711**	0.0000
Chlr	32.774	30.314	29.093	29.043	32.419	35.090	12.065**	0.0000
Proln	0.959	1.025	1.045	0.983	0.919	0.968	3.518*	0.0007
POD	21.882	20.020	20.652	20.214	23.563	21.375	6.135**	0.0006
H_2O_2	0.701	0.711	0.714	0.627	0.674	0.703	5.253*	0.0003
CAT	51.588	41.920	51.522	31.857	36.813	48.850	31.894**	0.0000

*P < 0.05, **P < 0.01 and ***P < 0.001 Where RL = Root length [cm] ; SL = Shoot length [cm]; FRW = Fresh root weight [g]; FSW = Fresh shoot weight [g]; DRW = Dry root weight [g]; DSW = Dry shoot weight [g]; K^+ = Potassium ion; Na^+ = Sodium ion; K^+/Na^+ = Potassium to Sodium ratio; Chlr = Chlorophyll content [CCI]; Proln = Proline content [μmol g^{-1} (FW)]; POD = Peroxidase [U mg^{-1} protein]; H_2O_2 = Hydrogen peroxide [μmol g^{-1} (FW)]; CAT = catalase activity [U mg^{-1} protein]

Discussion

Various studies revealed that screening in hydroponic culture is very effective and it is comparable with soil conditions, and one can rely on selection of genotypes based on this method (Naher *et al.* 2014). Keeping in view the importance of this study on salinity, root and shoot related characters are important in the selection of salt tolerant genotypes of cotton, and because these characters has been used efficiently as selection criteria in several field crops namely, wheat (Tiwari *et al*. 2011), maize (Neto *et al.* 2006), tomato (*Solanum lycopersicon*) (Maggio *et al*. 2007), cowpea (*Vigna unguiculata* (L.) Walp.) (Farooq *et al*. 2020) and okra (*Abelmoschus esculentus* (L.) Moench) (Zhan *et al*. 2019). Root and shoot length of salt tolerant genotypes are less affected as compared to salt sensitive accessions *i.e.*, less root and shoot length whereas shoots are more sensitive than roots (Khataar *et al*. 2018; Razzaque *et al*. 2019).

The genotypes namely, NIAB-545 (G88), CIM-595 (G36) and Coker-307 (G46) has more root length under salt conditions. Whereas, reduced root and shoot length was noted in NS-121 (G13), CIM-602 (G33) and Rehmani (G55) that indicates the sensitivity to salt stress (Egamberdieva *et al.* 2015; Sharif *et al*. 2019). Fresh root and shoot weight of some of accessions were significantly varied under control and salt stress levels. For instance, salt tolerant genotypes *i.e.*, FH-942 (G27) and DNH-40 (G80) showed more fresh root and shoot weights under salt stress, in contrary to salt sensitive genotypes that exhibited considerable reduction for these sedling traits as reported by Deinlein *et al.* (2014) and Jiang *et al*. (2016). These traits were used efficiently as selection criteria for tolerant and susceptible accessions of strawberry (*Fragaria ananassa*) (Sun *et al.* 2015), cotton (Moussouraki *et al.* 2019) and tomato (Karlberg *et al.* 2006). Keeping in view the importance of these characters, NIAB-545 (G88), CIM-595 (G36), Coker-307 (G46) and FH-113 (G90) were identified as salt tolerant accessions. High Na^+ concentration in leaf sap is due to increase salinity in plant organelles is one of the primary plant responses to salinity stress (Meneguzzo *et al.* 2000) that disturbs the various metabolic activities of cells (Naik *et al*. 2019). The genotypes that can exclude Na^+ can survive in a better way under stress conditions (Akram *et al*. 2007; Dehnavi *et al.* 2019). It was also suggested that

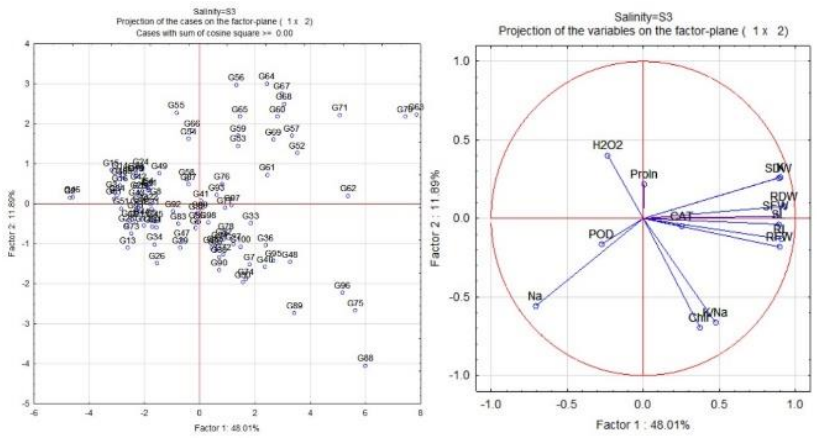

Fig. 3: Biplot analysis of 100 cotton genotypes for various seedlings traits grown under 150 m*M* salinity level. Where RL = Root length; SL = Shoot length; FRW = Fresh root weight; FSW = Fresh shoot weight; DRW = Dry root weight; K = Potassium ion; Na = Sodium ion; K^+/ Na^+ = Potassium to Sodium ratio; Chlr = Chlorophyll content; Proln = Proline; POD = Peroxidase; H_2O_2 = Hydrogen peroxide; CAT = catalase activity

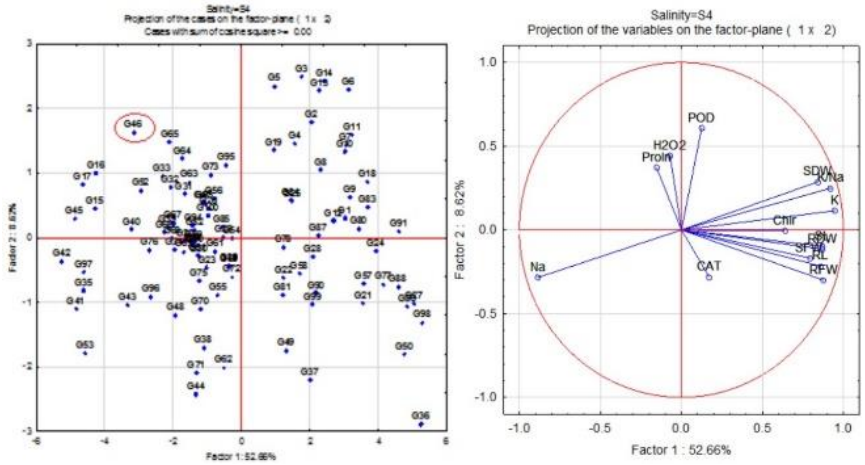

Fig. 4: Biplot analysis of 100 cotton genotypes for various seedlings traits grown under 200 m*M* salinity level**.** Where RL = Root length; SL = Shoot length; FRW = Fresh root weight; FSW = Fresh shoot weight; DRW = Dry root weight; K = Potassium ion; Na = Sodium ion; K^+/ Na^+ = Potassium to Sodium ratio; Chlr = Chlorophyll content; Proln = Proline; POD = Peroxidase; H_2O_2 = Hydrogen peroxide; CAT = catalase activity

Note: Genotype G46 "Coker-307" (highlighted) at left top performed best among other genotypes under 200 m*M* salinity level.

salt tolerance is associated with balancing of Na^+ ions (Santa-Maria and Epstein 2001) although it works in coordination with salt exclusion mechanism (Ashraf and Wu 1994; Colmer *et al*. 1995). The accessions in this study namely, NIAB-545, CIM-595, Coker-307, FH-113 and FH-942 were found with low Na^+ contents.

Several physiological characters like chlorophyll content have been used successfully as amenable selection criteria because these contents degrade due to salt stress that lead to the reduction in rate of photosynthesis and in plant growth. Because high concentration of chlorophyll is positively associated with rate of photosynthesis, dry matter production and yield (Harinasut *et al.* 2000; Ibrahim *et al.* 2019). The salt tolerant genotypes showed more chlorophyll contents as compared to salt susceptible lines (Iqbal *et al.* 2006; Nekir *et al.* 2019; Van *et al*. 2019). The accessions namely VH-338, IUB-75, FH-942, FH-177, DNH-40 showed higher chlorophyll contents while accessions IUB-222, FH-326 and Coker-3113 had lower chlorophyll contents. In addition to morphological and physiological mechanisms, the biochemical responses were also monitored in this study where positive association was found between antioxidants and ROS, because increased concentration of antioxidants in response to salinity leads to enhanced level of ROS generation (Kim *et al*. 2018). Salt stress leads to over-production of ROS such as superoxide anions, H_2O_2 and hydroxyl radicals. To mitigate this effect, several enzymatic and non-enzymatic antioxidants are

produced in plant that indicating the presence of positive association between ROS and antioxidants (Majeed *et al*. 2019). Some genotypes namely Coker-307, FH-113 and FH-942 have maintained higher level of antioxidant as compared to other accessions *i.e.,* VH-171, CRIS-9, and NIAB-414. The scavenging capability of antioxidants for ROS in accessions *i.e.,* CIM-595 (G36), Coker-307 (G46) and FH-113 (G90) was also high. This indicator was also exploited successfully for identification of salt tolerant lines from the germplasm of wheat (Yassin *et al*. 2019), cotton (Taghizadeh *et al*. 2018), maize (Chen *et al*. 2018) and rice (Vaidyanathan *et al*. 2003).

Conclusion

The potential of the identified salt tolerant genotypes namely, NIAB-545, CIM-595, Coker-307, FH-113, FH-942, DNH-40 for quantitative traits could be assessed after plantation on salt affected areas of the country. These genotypes could be used in breeding program for development of new salt tolerant germplasm. Such germplasm would be useful for cotton breeders and could provide the opportunity to increase the area of cultivation of cotton in the country.

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