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Physio-chemical Distinctiveness and Metroglyph Analysis of Cotton Genotypes at Early Growth Stage under Saline Hydroponics

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Abstract

A hydroponic study was carried out to determine the response of 13 cotton (*Gossypium hirsutum* L.) genotypes at three salinity levels viz, 0.8, 8 and 16 dS m^{-1} for various physiological and biochemical traits. Data were subjected to metroglyph analyses for the estimation of genetic diversity under contrasting salinity regimes. The genotypes were grouped into five clusters with maximum index score of 97 per cluster. Minimum index score of 13 in case of cluster-V under non saline regime was observed. Three clusters were constructed with range of index scores from 119 to 23 at 8 dS m^{-1}, while all genotypes were grouped into five clusters with maximum and minimum index scores 114 and 23 in cluster-I and cluster-IV, respectively at 16 dS m^{-1}. Relative salinity tolerance was also observed among genotypes at two saline regimes. NIAB-111 and Russian (RL) were the most susceptible and SLH-41 and UCD-581 were found the most tolerant at 8 dS m^{-1}, whereas at 16 dS m^{-1} NIAB-111 and MS-39 were proved most susceptible and Groog-25, FH-982, TX-DOS-5-76C and Russian okra (RL) as most tolerant. We conclude that genotypes belonging to different clusters show diversity in salinity tolerance and can be used for further breeding programs with focus on development of salinity tolerant germplasm on the basis of easily detectable physiological and biochemical criteria.

Keywords: Physio-chemical; Metroglyph analysis; Cotton genotypes; Saline hydroponic

Introduction

Global food requirements are estimated to increase up to 70% by 2050, requiring gains in agricultural production with less land and resources. Rapid urbanization is forcing agriculture to saline, dried or more marginal lands. Among different abiotic stresses, salinity drastically suppresses the plant growth and productivity (Shazma *et al.*, 2011; Yousaf *et al.*, 2011).

Salinity is amongst the major abiotic ecological stresses and may be present either in topsoil or in subsoil (Grewal, 2010). High rate of evapo-transpiration with low rate of precipitation results in accumulation of excessive salts in soil. Soil salinity has become a severe threat to sustainable yield of field crops (Cha-um *et al.*, 2006) and occupies 20% of the total cultivated area and 50% of irrigated lands were under threat of soil salinity stress (Zhu, 2001). Salinity may deteriorate 30% of land within next 25 years and 50% up to 2050 (Wang *et al.*, 2003). Salt stress affects plant growth adversely by creating nutritional imbalance, specific ion effect and low osmotic potential of soil solution (Ashraf, 2002). The existence of toxic ions in soil brings negative effects on growth and developmental processes of plants due to salt induced drought stress, ion toxicity due to sodium and chloride ions and nutritional imbalance due to reduction in nutrient uptake and transportation of the nutrients to the aerial parts of the plants (Munns and Tester, 2008).

Genetic characterization of genotypes is a key step and major goal in evolutionary biology. This information is essential for accurate use of plant genetic resources for the development of new germplasm (Aladele, 2009). Estimation of genetic diversity within germplasm could help to reap information about the parental material to be used in hybridization. Hybridization between genetically diverse parental lines could generate high heterosis and genetically diverse trangressive segregants (Rauf *et al*., 2010). Salt tolerance involves many genes and different physiological and biochemical mechanisms, therefore categorized as complex trait (Cuartero *et al*., 2006). Therefore, evaluation of field crops for salt tolerance under field condition is laborious and time consuming and soil heterogeneity could mask the genetic variation. On the other hand, the use of solution culture medium for screening overcame the disadvantages of field evaluation (Akhtar *et al.*, 2010).

On the basis of these grounds the present study includes different physiological and biochemical standards for evaluation about the salt tolerance within cotton genotypes and genetic diversity estimation at variable salinity levels. The objectives of this study was to optimize

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the conditions for evaluation of cotton under saline hydroponic conditions, to set physiological and biochemical standards to screen against salinity, furthermore to identify salt tolerant and susceptible genomes to be used in future breeding program for the development of salt tolerant genotypes/cultivars.

Materials and Methods

Experiment was conducted in the screen-house of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan (latitude; 31° 25‘N, longitude 73°, 90‘E). Cotton (*Gossypium hirsutum* L.) genotypes viz. SLH-41, NIAB-111, BH-121, 4F, Grogg-25, MS-39, FH-982, TX-DOS-5-76C, Cedix-1176, Di-Xie-King, UCD-581, Russian Okra (RL) and Russian (RL) were collected from different research institutes.

Seeds of collected genotypes were delinted with the help of commercial sulphuric acid. Only healthy seeds were sorted out and sown in polythene bags filled with cleaned and washed sand by following triplicated completely randomized design. Sand was washed with distilled water, sun dried and debris were removed by using fine mesh sieve. Polythene bags were irrigated with tap water. Temperature and humidity was maintained by air cooler cum humidifier system. At three leaf stage, seedlings were transferred to hydroponic medium (Iron tubs (3′ × 2′) filled with tape water and thermopore sheet floating on the water surface, having equidistant pores in it) provided with proper aeration with the help of electronic air pump. Standard Hoagland solution was applied to provide nutrients to seedlings (Hoagland and Arnon, 1950). Three salinity levels (0.8, 8 and 16 dS m^{-1}) were applied in three different tubs each with capacity of 250 L. pH of the solution was adjusted by using NaOH and HCl and maintained on daily basis.

The tub of control treatment-1 (0.8 dS m^{-1}) was only provided with Hoagland solution and no salt was added. NaCl was added to attain the desired salinity levels (8 and 16 dS m^{-1}) in remaining tubs. EC was adjusted by using EC meter (TOA-CM-14P). Seedlings were harvested 45 days after the imposition of treatments. Root and shoot lengths of freshly harvested plants were measured with measuring tape and then root shoot ratio was calculated. Transpiration rate (E), photosynthetic rate (A) and water use efficiency were measured by using IRGA (L. MAN-LC1).

In metroglyph analysis (Anderson, 1957) two parameters having the highest variability or variation or coefficient of variability (CV) among all other parameters were selected as X- and Y-axis co-ordinates. After completion of all procedural steps of metroglyph analysis scattered diagram was obtained. In scattered diagram each genotype was represented by glyph and parameters of the genotypes were represented by rays on the relevant glyph. Genotypes were plotted on metroglyph graph by using the mean values of the parameters selected as X-axis and Y-axis co-ordinates. Each ray on the glyph shows a typical parameter obtained by classifying the range of values into three equal classes assigning the low, medium and high grades to each character. Length of the ray depends upon the index score of genotype for particular parameter i.e., 1 for low, 2 for medium and 3 for highest mean value. Total index score of genotype determined the performance of genotype. Total index score is the sum of index values related to all parameters.

Statistical Analysis

Data of ten randomly selected seedlings were recorded for traits under study by using standard recommended procedures and subjected to analysis of variances technique (Steel *et al*., 1997). Metroglyph Analysis proposed by Anderson (1957) was followed to study the morphological variation. Chlorophyll contents were estimated by following formulas designed by Nagata and Yamashita (1992).

Chlorophyll *a* (mg/100 mL) = $0.999A_{663}$-$0.0989A_{645}$
Chlorophyll *b* (mg/100 mL) = $0.328A_{663}$+$1.77A_{645}$
Beta carotenoids (mg/100 mL) = $0.216A_{663}$-$1.22A_{645}$-$0.304A_{505}$+$0.452A_{453}$.

Ascorbic acid contents in the plant samples were estimated by using Kampfenkel *et al*. (1995) method. These measurements were made by using spectrophotometer (UV-4000).

Results

Results of analysis of variance (Table 1) depicted significant differences ($P \leq 0.05$) among genotypes for all the parameters. Ranges of low, medium and high levels for different traits were calculated (Table 3A) and signs were allotted (Table 3B) to construct metroglyph. Clusters were numbered on the basis of index score of clusters in ascending order (Table 2). Scale for glyph’s diagram regarding 8 and 16 dS m^{-1} is presented in Table 5. Under controlled conditions (0.8 dS m^{-1}), 13 genotypes were divided into five clusters depending upon relative positioning of the genotype on glyph. Cluster-I possessed five genotypes and ranked highest index score of 97. Cluster-V consisted of only one genotype and had the lowest index score (13) among all other clusters (Fig.1).

Cluster analysis was used to group genotypes in to different clusters. Genotypes showing almost similar performances with non-significant differences ($P \geq 0.05$) tend to share similar cluster. On the other hand, significant differences among genotypes put them in different clusters. At 8 dS m^{-1}, 13 genotypes were divided into three clusters. Cluster-I consisted of seven genotypes and had the highest index score of 119. Cluster-II possessed five genotypes with total index score 97, while third cluster consisted of only one genotype and had index score of 23. In cluster-1 genotypes MS-39 and TX-DOS-5-76C had the highest index score (20), while genotype Russian (RL) showed the lowest index score (12). In cluster-II genotype, UCD-581

Table 1: Mean squares for the analysis of variance table for different traits in cotton

Source of variation	df	R/S	RD	Chl *a*	Chl *b*	BC	AA	PhR	TR	WUE	SC	SSCC
Genotype	12	0.21**	4.62**	0.82**	1.75**	0.17**	27.73*	16.1**	4.2**	4.21**	0.02*	23332.1**
Treatment	2	1.88**	500.8**	11.27**	26.74**	0.14**	75.81**	314**	54.10**	7.12**	0.18**	53443.**
Error	102	0.04	1.16	0.31	0.58	0.03	15.34	6.0	1.667	1.04	0.001	6476.2

** = Highly significant * = Significant df= degree of freedom, R/S = Root shoot ratio, RD = Root density,Chl *a* = Chlorophyll *a*, Chl *b* = Chlorophyll *b*, BC = Beta carotenoid, AA = Ascorbic acid, PhR = Photosynthetic rate, TR = Transpiration rate, WUE = Water use efficiency, SC = Stomatal conductance, SSCC = Substomatal CO_2 concentration

Table 2: Cluster number, index score and cotton genotypes included in each cluster following metroglyph technique under different salt conditions

Cluster number	Genotypes	Grand index score
At 0.8 dS m^{-1}		
I	Russian (RL), Russian okra (RL), UCD-581, BH-121, UCD-581	97
II	NIAB-111, Groog-25, Di-Xie-King	76
III	4F, Cedix-1176, FH-982	37
IV	MS-39	17
V	SLH-41	13
At 8 dS m^{-1}		
I	NIAB-111, BH-121, 4F, Groog-25, MS-39, TX-DOS-5-76C, Russian (RL).	119
II	FH-982, Cedix-1176, Di-xie-King, UCD-581, Russian okra (RL).	97
III	SLH-41	23
At 16 dS m^{-1}		
I	SLH-41, NIAB-111, BH-121, Groog-25, MS-39, FH-982.	114
II	4F, TX-DOS-5-76C.	43
III(a)	Cedix-1176, Di-xie-King.	37
III(b)	Russian (RL), UCD-581.	37
IV	Russian okra (RL).	23

Table 3A: Range of levels (low, medium, high) for different cotton traits under three salinity treatments for scoring of genotypes to construct Metroglyph plot

Trait	Low (1)			Medium (2)			High (3)		
	0.8 dS m^{-1}	8 dS m^{-1}	16 dS m^{-1}	0.8 dS m^{-1}	8 dS m^{-1}	16 dS m^{-1}	0.8 dS m^{-1}	8 dS m^{-1}	16 dS m^{-1}
Root Shoot Ratio	≤1.0417	≤1.354	≤1.666	≤0.4403	≤0.6005	0.7608	≤0.5851	≤0.866	≤1.147
Root Density (mL)	≤6.330	≤8.660	≤11.00	≤1.1667	≤1.8337	≤2.50	≤1.00	≤1.50	≤2.00
Chlorophyll *a* (mg/100 mL)	≤1.383	≤2.120	≤2.212	≤0.9484	≤1.5200	≤2.0917	≤0.546	≤0.7439	≤0.9432
Chlorophyll *b* (mg/100 mL)	≤2.185	≤3.613	≤5.043	≤1.1495	≤1.7392	≤2.3288	≤0.587	≤0.799	≤1.013
Beta carotenoids (mg/100 mL)	≤0.445	≤0.748	≤1.052	≤0.4149	≤0.5427	≤0.6739	≤0.358	≤0.578	≤0.798
Transpiration rat (mmol m^{-2} s^{-1})	≤2.163	≤3.706	≤5.250	≤2.183	≤3.7760	≤5.370	≤1.52	≤2.61	≤3.70
Photosynthetic rate (µmol m^{-2} $s^{-1)}$	≤6.106	≤11.473	≤16.84	≤2.56	≤4.36	≤6.16	≤0.933	≤1.8366	≤2.740
Substomatal C02 conc. (vmp)	≤122.660	≤229.334	≤336.00	≤145	≤219	≤293	≤133.33	≤253.66	≤374
Stomatal conductance (mol m^{-2} s^{-1})	≤0.060	≤0.110	≤0.160	≤0.06	≤0.11	≤0.16	≤0.03	≤0.05	≤0.07
Water use efficiency	≤2.250	≤4.023	≤5.795	≤1.4647	≤2.304	≤3.143	≤1.025	≤1.987	≤2.953
Ascorbic acid (µg mL^{-1})	≤1227.07	≤1227.68	≤1228.29	≤1220	≤1224	≤1228	≤1219.86	≤1223.733	≤1227.6

Table 3B: Signs of low (1) Medium (2) and high (3) scores for all the parameters in cotton under different salt treatments

Parameters	1	2	3
Root Shoot Ratio			
Root Density			
Chlorophyll *a*			
Chlorophyll *b*			
Beta carotenoids			
Transpiration rate (for control and treatment-2 only) and photosynthetic rate (for treatment-1and2)			
Sub-stomatal CO_2 concentration (for control and treatment-1)			
Stomatal conductance (only for control) and WUE (for Treatment-1and 2 only)			
Ascorbic acid			

had the highest index score (23), while genotype Cedix-1176 had the lowest index score in this cluster (Fig.2).

At 16 dS m^{-1}, metroglyph analysis grouped genotypes into five clusters (Fig. 3). Cluster-I had the highest index score (114) and consisted of six genotypes, while cluster-III (a and b) had the same index score and both consisted of two genotypes each. In cluster-I genotype FH-982 had the highest index score (23), while genotype MS-39 had the lowest index score (15) in this cluster. Cluster IV consisted of only one genotype i.e., Russian okra (RL), which had the lowest index score among all other clusters.

Total index score is the sum of index values related to all parameters. It reflects the performance of that particular genotype. Depending upon the results found after the application of 8 dS m^{-1}, genotypes with index score range 20.25 to 23 were grouped as tolerant, with index score 17.50 to 20.25 as moderately tolerant, with index score range 14.75 to 17.50 as moderately susceptible and with index score less than 14.75 as susceptible. These results were based on index score according to metroglyph scoring (Table 4). Among all genotypes SLH-41 and UCD-581 showed resistance at 8 dS m^{-1}, moderate tolerance was showed by seven genotypes i.e., 4F, Groog-25, MS-39, FH-982, TX-DOS-5-76C, Di-xie-King and Russian okra (RL). BH-121 and Cedix-1176 showed the moderately susceptible behavior. Whereas NIAB-111 and Russian (RL) showed the susceptible behavior having lowest index score.

At highest salinity level, genotypes with index score range 21.75 to 24 were grouped as tolerant, with index score range 19.50 to 21.75 as moderately tolerant, with index score range 17.25 to 19.50 as moderately susceptible and with index score less than 17.25 as susceptible. According to results exhibited in Table 4, four genotypes (Groog-25, TX-DOS-5-76C, Russian Okra (RL) and FH-982) exhibited the tolerant behavior. Moderately susceptible behavior was showed by seven genotypes (SLH-41, BH-121, 4F, Cedix-1176, Di-xie-King, Russian (RL) and UCD-581), whereas NIAB-111 and MS-39 were found as susceptible genotypes.

NIAB-111 showed susceptible behavior at salinity levels 8 and 16 dS m^{-1}. SLH-41 and UCD-581 showed moderately susceptible behavior at high salinity but found tolerant at medium. BH-121 and Cedix-1176 showed moderate susceptibility at 8 and 16 dS m^{-1}. Groog-25, 4F, MS-39, FH-982, TX-DOS-5-76C, Russian (RL) and Di-xie-King showed moderately tolerant behavior at medium salinity, while at high salinity no genotype is present in moderate tolerant group. Die-xie-King, 4F and Russian (RL) showed the moderately susceptible behavior at 16 dS m^{-1}. Groog-25, FH-39 and TX-DOS-5-76C showed tolerant behavior at 16 dS m^{-1}. Russian okra (RL) showed moderately tolerant behavior at 8 dS m^{-1} but tolerant behavior at 16 dS m^{-1}.

Table 4: Index score with grand total of thirteen genotypes under three treatments

Genotype	R/s			RD (ml)			Chl *a* (mg/100 mL)			Chl *b* (mg/100 mL)			BCr (mg/100 mL)			PR (A) (µmol m^{-2} $s^{-1)}$			TR (E) (mmol m^{-2} s^{-1})			SSCC (vmp)			SC (gs) mol m^{-2} s^{-1}			WUE			AC (µg mL^{-1})			Index score		
Treatments / Genotype	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
(Slh-41)	1	1	1	2	1	1	1	1	1	1	1	1	2	3	2	1	3	3	1	3	1	2	3	1	1	3	1	2	1	3	2	3	3	16	23	18
Niab-111	1	2	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	3	1	1	1	3	1	1	1	1	1	1	3	2	2	1	3	14	14	17
Bh-121	1	2	2	2	1	1	1	1	3	1	1	2	1	3	2	2	1	3	3	1	1	2	2	1	3	1	1	1	1	2	1	3	1	18	17	19
4f	2	3	2	3	1	1	1	1	1	1	1	1	2	3	2	2	1	2	1	1	1	1	2	2	1	1	1	3	1	3	1	3	3	18	18	19
Groog-25	1	3	3	2	1	2	3	1	3	3	1	2	3	3	2	1	1	2	3	1	1	3	2	1	2	1	1	1	1	2	1	3	3	24	18	22
Ms-39	1	2	1	3	3	1	1	2	1	1	2	1	1	2	1	3	1	2	3	1	1	2	1	1	3	1	1	2	2	2	2	3	3	22	20	15
Fh-982	1	2	3	2	2	1	1	1	3	1	1	3	2	3	3	2	2	2	1	1	1	1	2	1	1	1	1	2	2	2	1	3	3	15	20	23
Tx-dos-5-76c	1	2	2	3	1	3	3	2	3	2	2	2	2	3	3	2	1	2	3	1	1	3	1	2	3	1	1	1	3	2	2	3	3	25	20	24
Cedix-1176	1	1	1	2	1	1	2	1	2	2	1	3	2	2	1	2	1	2	1	1	1	1	3	1	1	1	1	3	1	2	1	3	3	18	16	18
Di-xie-king	2	1	1	2	3	1	3	1	2	3	1	2	1	3	2	1	2	2	3	1	1	3	1	1	3	1	1	1	3	3	2	3	3	24	20	19
Ucd-581	1	2	1	2	3	2	1	3	1	1	3	1	1	2	2	2	1	1	3	1	3	3	3	3	3	1	3	1	1	1	1	3	1	19	23	19
Russian okra (RL)	2	3	2	1	3	1	3	1	2	2	1	2	1	1	3	2	1	2	3	1	1	3	2	1	3	1	3	1	1	3	3	3	3	24	18	23
Russian (RL)	3	2	2	2	1	1	3	1	1	3	1	3	1	1	2	1	1	1	3	1	1	3	1	3	3	1	2	1	1	1	2	1	1	25	12	18

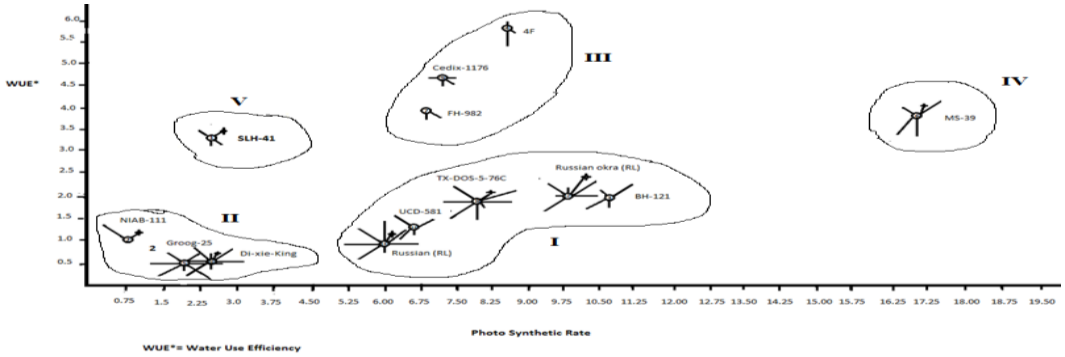

Fig. 1: Metroglyph diagram of 13 genotypes under control conditions (0 dS/m)

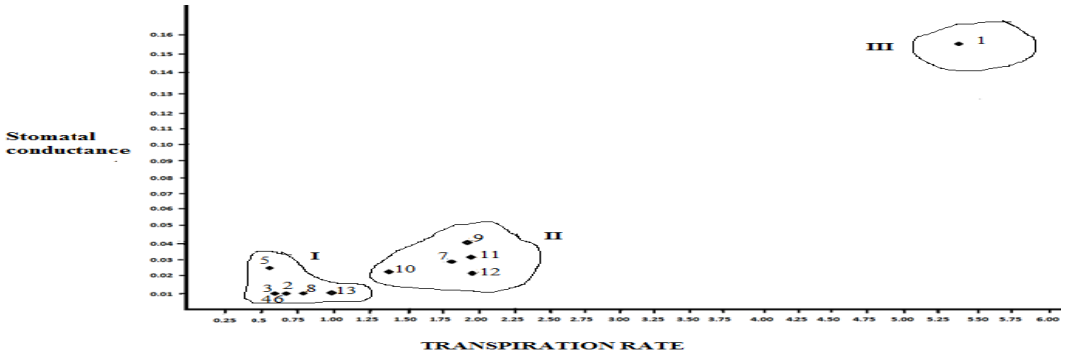

Fig. 2: Metroglyph diagram of thirteen (13) Genotypes under treatment 1(8ds/m)

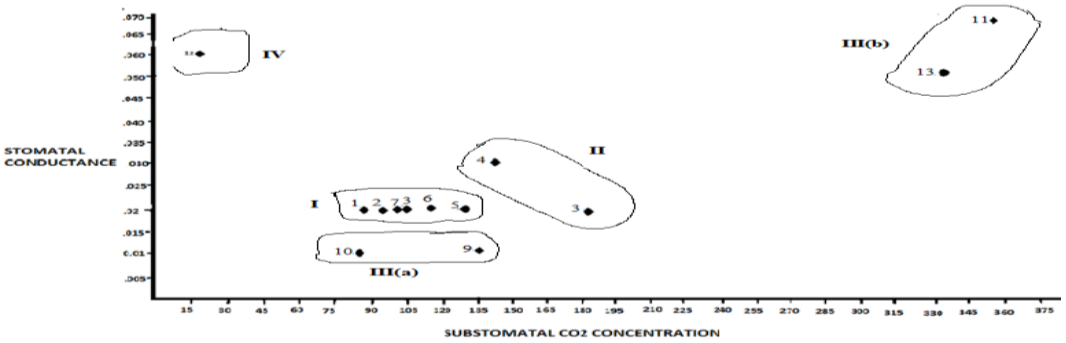

Fig. 3: Metroglyph diagram of thirteen (13) Genotypes under treatment 2(16dS/m)
Note: In case of Fig. 2 and Fig. 3, it was impossible to write genotype names and draw signs in figures therefore, elaborated in Table-5

Table 5: Scale for glyph's diagram plotted on metroglyph scatter diagram for treatment-2 (8 dS m^{-1}) and treatment-3 (16 dS m^{-1})

Serial No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Genotype	SLH-41	NIAB-111	BH-121	4F	Groog-25	MS-39	FH-982	TX-DOS-5-76C	Cedix-1176	Di-xie-King	UCD-581	Russian okra (RL)	Russian (RL)
Scale	1	2	3	4	5	6	7	8	9	10	11	12	13
Treatment # 2													
Treatment # 3													

Discussion

Development of salt tolerant cotton is cumbersome due to narrow genetic base of germplasm resources, lack of selection criteria, complex tolerance mechanism and variation in responses to salt at different developmental stages. Salt tolerance is a quantitatively controlled very complicated mechanism and involves multiple physiological and biochemical pathways. Plant breeders need well defined indicators as selection criteria for salt tolerance to screen the germplasm. It has been shown that seedling stage was more sensitive to salt stresses than adult stage and thus could provide more effective screening strategy (Lianes *et al.*, 2005). Moreover, it has been suggested that evaluation for salt tolerance could be more affective under controlled conditions using physiological traits (Flowers and Yeo, 1995) such as root and shoot growth reduction due to increase in salinity level (Jeannette *et al.*, 2002; Chachar *et al.*, 2008). Moreover, roots and shoots adjustment under saline conditions provides clue to

response of plants against salt stress (Jamil and Rha, 2004; Rauf *et al*., 2012).The accumulation of salts in root zone may be due to the ability of root system to check ion movement in shoot area which is necessary for plant survival (Hajibagheri *et al.,* 1989).

Osmotic adjustment is a key step in adaptation of plants to saline conditions by maintaining tissue metabolic activities. Excess deposition of ions in the cell modifies the metabolic activities due to decrease in early seedling growth and development (Yasar *et al*., 2006). Decreased photosynthetic area and reduced photosynthesis might be due to reduced development and differentiation of tissues, adverse effect on membranes, shrinkage and leakage of cell contents, unbalanced nutrient supply and lack of avoidance mechanism (Akram *et al.,* 2007). Stomatal closure due to salt induction is another vital factor, which retards photosynthetic activity under salt induced water stress (Saleem *et al.,* 2011). Due to salt stress susceptible genotypes show degradation of chlorophyll than tolerant ones (Khan *et al*., 2009), which ultimately reduced photosynthetic rate. Stomatal closure ultimately reduces CO_2 partial pressure in leaves (De Ridder and Salvucci, 2007). Findings of present study reveal that increasing salt stress significantly reduced the root/shoots growth, chlorophyll a and b pigments, photosynthetic and transpiration rate and stomatal conductance which ultimately reduce photosynthesis and plant growth. Similar results were found by Ahmad *et al.* (2012). Decrease in stomatal conductance might be due to less sap flow and guard cell turgidity in response to salt stress.

Ascorbic acid is involved in the regulation of many biological processes such as photoinhibition, cell elongation, and biosynthesis of ethylene (Smirnoff, 2000). Optimum concentration of ascorbic acid has beneficial effect on growth and yield of plants grown under salt conditions (Smirnoff, 2000; Bassuony *et al.,* 2008). Present study also proves that tolerant genotypes maintained high ascorbic acid concentration as compared to susceptible ones. Barth *et al*. (2006) reported that ascorbic acid restores hormone equilibrium disturbed in salt stress conditions and plays a protective role against reactive oxygen species formed from photosynthesis and respiratory processes (Athar *et al.,* 2008).

Present study supports the use of metroglyph analysis technique as used earlier in sorghum (Mehdi and Asghar, 1999), rice (Cheema *et al.,* 2004), sugarcane (Mujahid *et al.,* 2001) and brassica. Genotypes to be used as parents in different hybridization program to exploit polymorphism can be selected with variable response to prevailing conditions and of different origins. Thus, whole of the determined genetic variability between genotypes can be exploited to develop good combination between genotypes with variable response to prevailing conditions. Therefore, the ability of metroplyph to reduce the complexity of interrelationships among accessions is very simple. It explains the results in a simple pictorial scatter diagram, which is easier to comprehend (Akoroda, 1983; Khan *et al*., 2007).

Traditionally breeders do not use multidimensional response of plants indicators against salt stress as selection criterion to avoid complication but in present study cotton genotypes were evaluated on different physiological and biochemical standards and promising genotypes were selected.

Different genotypes like NIAB-111 and MS-39 were determined as most susceptible and Groog-25, FH-982, TX-DOS-5-76C and Russian okra (RL) as most tolerant genotypes, as they behaved differently and belong to different groups, so could be used as parental material for further breeding program to exploit genetic distance between these genotypes. The crossing between genetically distant parents may give good combination.

We conclude that screening for salinity tolerance under controlled conditions on the basis of different physiological standards using cluster analysis was efficient instead of using traditional time consuming screening methods. Root related indicators of plant growth, photosynthetic mechanism related indicators like chlorophyll contents and photosynthetic rate and stomatal behavior reflected significantly the differences on development at different levels of salt stress, therefore can be used as selection criteria.

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