Pak. J. Agri. Sci., Vol. 59(1), 63-74; 2022
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/22.531
http://www.pakjas.com.pk

Genetic variability in diverse cotton germplasm for drought tolerance

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Drought is one of the major abiotic stresses that significantly reduces seed cotton yield worldwide. Therefore, Drought tolerance is a complex phenomenon that comprises a combination of morphological and physiological parameters which results in the enhancement of drought tolerance in cotton. Therefore, in the present study 150 cotton genotypes were evaluated for drought tolerance by planting at two water regimes i.e., normal water and limited water conditions. Data were recorded for morphological and physiological parameters i.e. root fresh length (FRL), shoot fresh length (FSL), lateral root numbers (LRN), root fresh weight (FRW), shoot fresh weight (FSW), shoot dry weight (DSW), weight /length ratio(W), root dry weight (DRW), plant weight (PW), the difference in shoot fresh weight and shoot dry weight (SDWR), stomatal conductance (SC), canopy temperature (CT), water potential (WP), osmotic potential (OP) and relative water contents (RWC) at the seedling stage. Principal component analysis (PCA) of seedling at normal water conditions explained PCA1 35.21% and PCA2 15.25% of the total variance. The cluster analysis of the recorded data for the morpho-physiological parameters grouped 150 genotypes into six clusters. First Cluster included 16 cotton genotypes, 2nd cluster having 44, clusters 3, 4, 5, and 6 contains 22, 52, 10, and 6 genotypes respectively. In water stress conditions PCA1 containing 18 cotton genotypes, 2nd cluster having of 32 cotton genotypes, clusters 3, 4, 5, and 6 contain 14, 35, 30, and 21 genotypes, respectively. The use of morpho-physiological seedling traits associated with drought resistance can facilitate breeding strategies to evolve cotton genotypes having tolerance against drought stress in the changing climatic conditions. Screening of available cotton genotypes for drought tolerance in controlled greenhouse conditions can shorten the duration with improvement in efficiency for screening.

Keywords: Cluster analysis, cotton, drought tolerance, morpho-physiological traits, seedling traits, principal component analysis (PCA).

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is a significantly important fiber crop that provides about half of the world's fiber requirements (Pretorius, 2009). Despite synthetic fiber alternatives, cotton is still the primary source of fiber around the world in the textile industry (Sunilkumar *et al.*, 2006). Cottonseed is also economically very important (Pretorius, 2009) as it provides vegetable oil and seedcake which is used as a rich source of protein for ruminant livestock (FAO, 1994). Cottonseed is composed of 21% high-quality oil and 23% protein, (Rathore *et al.*, 2007). The world has witnessed frequently occurring extreme meteorological events, with rapidly changing climatic conditions that had drawn the attention of scientists around the world (Powell and Reinhard, 2016). Drought, being an extreme meteorological event had markedly affected agricultural production as well as social-economic development (Wilhite *et al.*, 2014). Therefore, to explore inadequate water reserves by maximizing efficiency, high yielding agriculture is the priority in climate-smart agriculture. Drought tolerance is a complicated trait in crop science having multi genetic controlling components that interrelate in different manners in crop plants (Cushman and Bohnert, 2000). High energy costs and depletion of groundwater resources affect cotton production badly in irrigated cotton areas. Therefore, breeding for drought stress conditions in cotton has been a major interest of plant breeders these days. Reduced water loss by stomata (rapid stomatal closure) is another important feature for water stress tolerance in plants (Franca *et al.*, 2000). Stomatal behavior in cotton plants and water losses in excised leaves was the possible indicator of water stress tolerance/resistance.

Asif, M., A. A. Khan, H. M. N. Cheema, S. H. Khan and Z. Iqbal. 2022. Genetic variability in diverse cotton germplasm for drought tolerance. Pakistan Journal of Agricultural Sciences. 59:63-74.
[Received 7 May 2021; Accepted 3 Mar 2022; Published 18 Mar 2022]

Plant breeders develop new cultivars by utilizing drought-tolerant genotypes as parents of new breeding segregating populations while sometimes new addition to the germplasm of breeding programs. Therefore, it is necessary to identify specific genes/alleles for drought tolerance in adapted elite germplasm. For effective evaluation and utilization of germplasm dissecting the available genetic diversity is much important for crop breeding (Zubair *et al.*, 2007). Therefore, the objective of the present study is (i) To find the morpho-physiological traits to screen the cotton genotypes at the seedling stage under drought stress (ii) To select the drought tolerant cotton genotypes for the future breeding program.

MATERIALS AND METHODS

The 150 cotton (*Gossypium hirsutum* L.) genotypes were evaluated in the glasshouse at the seedling stage during March-April, 2017. These cotton genotypes were phenotyped for morpho-physiological parameters under normal and water deficit conditions. 3-4 seeds of each genotype were grown after hydro-priming of seed for 8 hours at the depth of 2-3cm in polythene bags of 5×25 cm^2 size. A mixture of soil (peat: soil: peat, 1:1:1) was prepared to fill the polythene bags. Thinning of cotton seedlings was carried out after germination and only 1 plant per bag was kept. A factorial

Annexure-I. Coding of cotton genotypes

Code No.	Genotype	Code No.	Genotype	Code No.	Genotype	Code No.	Genotype
	AGC-777	41.	CH-019	81.	A-162	121.	COKER-310
	S-11/3	42.	CH-009	82.	BJAHL	122.	IR-NIAB-824
	SLH-74	43.	CH-003	83.	BLANCO-3363	123.	DP-148
	FH-312	44.	AR-25	84.	ALBACALA(70)19	124.	DP-165
	ABRI/5	45.	AR-22	85.	CIM-616	125.	IUB-2009
	IR-NIBGE-6	46.	AR-23	86.	CYTO-177	126.	SB-149
	FH-142	47.	AR-21	87.	CBS-1	127.	IR-NIBGE-3
	MNH-456	48.	108-F	88.	FH-118	128.	DP-15-26
	RH-627	49.	124-F	89.	VH-305	129.	FH-113
	SILKEE	50.	199-F	90.	IUB-13	130.	TARZEN-1
	BH-180	51.	208-HYBI	91.	CIM-599	131.	E-302
	PB-899	52.	268-F	92.	MNH-886	132.	EXOTIC
	FH-942	53.	281GL(443)	93.	BOSS-111	133.	F-281GL-44
	FH-4243	54.	407-26	94.	BROWN-BHW	134.	FE-4252
	FH-330	55.	448/4727C	95.	BS-1	135.	FH-1000
	RH-510	56.	4-F	96.	C2(37)1473	136.	FH-113
	AS-2	57.	AET-5	97.	C-24	137.	FH-1185
	AS-1	58.	ACALA-P3	98.	CAPTAIN-2833	138.	FH-2000
	CIM-600	59.	ACALA-7203-4-1	99.	CEDIX	139.	FH-2006
	CIM-598	60.	YU-MM2	100.	CIM-200	140.	FH-2925
	CEMB-55	61.	ACALA-157C	101.	CIM-240	141.	FH-900
	AS-3	62.	ACA-285	102.	CIM-243	142.	C-HIR-1628
	AGC-999	63.	AC-307	103.	CIM-443	143.	KZ-181
	MM-58	64.	61-F/89	104.	CIM-446	144.	FH-53
	FH-142S	65.	AMS-139	105.	CIM-473	145.	FH-901
	NS-161	66.	AMS-170	106.	CIM-482	146.	DPL-SL
	CIM-1100	67.	ASA\965)-650	107.	CIM-496	147.	KZ-191
	AR-9	68.	AU-59	108.	CIM-499	148.	SITARA-009
	AR-2	69.	AUBURH	109.	CIM-70	149.	N-131
	AR-3	70.	AUR-56	110.	CIM-83	150.	N-141
	AR-1	71.	B-403	111.	COKER		
	VH-300	72.	B-557	112.	SLH-2010-11		
	AR-14	73.	B-622	113.	CBS-2		
	AR-13	74.	BAR F/8	114.	DELCOTT-227		
	AR-17	75.	BH-580	115.	AONE		
	FH-324	76.	BH-118	116.	BH-175		
	FH-341	77.	BH-128	117.	SITARA-008		
	FH-314	78.	BARNT-205-4	118.	CRIS-468		
	FH-168	79.	BH-100	119.	CRIS-134		
	IUB-222	80.	BH-36	120.	CP-15		

arrangement under CRD (Completely Randomized Design) with three replicates was used under normal and water deficit conditions. List of the cotton genotypes used is with codes to be used in depicting the results are given in Annexure-I.

After the emergence of the first true leaves, seedlings were watered normally. After the development of the first true leaves, water deficit conditions were imposed on seedlings. Normal application of water was continued till the end of the experiment in normal irrigation treatment while stressed plants were irrigated to ½ of field capacity. Field capacities were maintained through the weight method (Colman, 1947). The experiment continued for fifty-five days till the full expansion of the third main stem leaf. Data were recorded for the following parameters:

***Phenotypic data measurements*:** Plant root, shoot, and physiological parameters were recorded for drought tolerance. Three seedlings were uprooted gently for each genotype and replicate. The length was measured by using a meter-rod. Plant fresh weight was measured with analytical balance in the Lab. The shoot length of three seedlings from each replicate was measured by using a meter-rod and the mean was recorded. The root length of three seedlings from each replicate was measured by using a meter-rod and the mean was recorded. Shoot fresh weight was recorded after measuring the fresh shoot length with analytical balance in the Lab and mean readings was observed. Root fresh weight was recorded after measuring the fresh root length with analytical balance in the Lab and means were recorded from each treatment. Root and shoots of all genotypes were oven (Memmert Schutzart DIN-40050) dried for 24 hours at 80ºC and weighed in grams by using weighing balance. The ratio of root-shoot was taken by using the formula:

$$\text{Root} - \text{shoot ratio} = \frac{\text{dry root weight}}{\text{dry shoot weight}}$$

W/L ratio was calculated by dividing weight by respective length.

From the middle of plant, canopy leaf samples were taken and covered with polythene bags. In the laboratory fresh weight (FW) was measured first and then the samples were kept for hydration in water for taking turgid leaf weight (TW). Leave were oven-dried at 70ºC overnight for the dry weight (DW). The RWC was taken by the following formula:

$$\text{RWC} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

A pressure chamber (Pressure Chamber Instrument Model 600, PMS International Company) was used to measure water potential by following the procedure elaborated by (Scholander *et al*., 1964). To avoid evaporation losses sampling was carried out from 6.00 to 9.00 a.m. Measurements were taken from fully expanded young leaf normally 16-18 days old leaves by placing the leaves in the pressure chamber quickly to avoid an error. Measurements were taken from both the normal and stressed plots separately. After measuring water potential cotton leaves were then frozen in a freezer (-20ºC) to measure osmotic potential. The frozen leaves were thawed in the first step. In the Eppendorf tubes, cell sap was collected by pressing the leaves with a glass rod. Calibration of osmometer was carried out first then osmotic potential was measured by pouring a drop of sap on cryoscopic osmometer (Cryoscopic osmometer printer, Genetec, Osmomat 030-D). An infrared thermometer (Model 510B; Everest Interscience Inc., Tucson, AZ, USA) was used to measure the canopy temperature (CT).

***Statistical Analysis*:** Analysis of variance was carried out using statistic 8.1statistical software (Steel *et al.*, 1997). Principal component, cluster, and correlation analyses were performed on observed data for normal and water stress conditions separately through Minitab 17. Cluster analysis and dendrogram were also carried out using Minitab 17 (Barbara *et al*., 1972).

RESULTS

Principal component analysis was carried out to identify the relationship between the different parameters (Fig.1). The first two PCs explained 35.21% and 15.25% variance of the total. In the biplot analysis, weights of different variables are explained with arrows that vary with distance from the origin. Biplot analysis for Fresh Root Length revealed that PB-899, MM-58, CIM-598, and CEMB-55 performed better as compared to other genotypes. The genotypes that are situated near the origin performed average for that character under investigation. If a perpendicular is drawn for FRL vector the genotypes that are lying on the opposite side of the vector performed below average for the parameter understudy. Performance of AMS-139, CAPRAIN-2833, AR-3, and DELCOTT-227 was below average concerning fresh root length. Biplot analysis for Fresh Shoot Length revealed that CIM-200, B-622, CP-15, and BH-580 performed better concerning Fresh Shoot Length while AR-9, CH-009, 407-26 NS, and sitara-008 performed below average. Biplot analysis for Fresh Root Length revealed that 448/4727C, 108-F, CEMB-55, FH-142 performed better for this character while N-141, AR-3, CIM-243, and AR-9 were low performers. Biplot analysis for Fresh Root weight revealed that FH-330, 268-F, VH-305, and SLH-2010-11 exhibited good performance while AGC-999, AR-9, CAPTAIN-2833, and BH-175 showed below-average performance. Biplot analysis for Fresh shoot weight revealed that FH-324, B-622, CIM-83, and KZ-191 displayed better performance while MNH-456, AS-1, FH-4243, and 407-26 showed below-average performance for FSW. Biplot analysis concerning DSW and W/L ratio revealed that FH-324, B-622, CIM-83, and FH-901 performed better conversely MNH-456, FH-4243, AS-1 and AR-22 displayed below-average performance. The vectors of both the parameters were laying almost close to each other so the behavior is almost the same. Biplot analysis for Dry root weight revealed that FH-341, BAR F/8, Cooker, and Sitara-

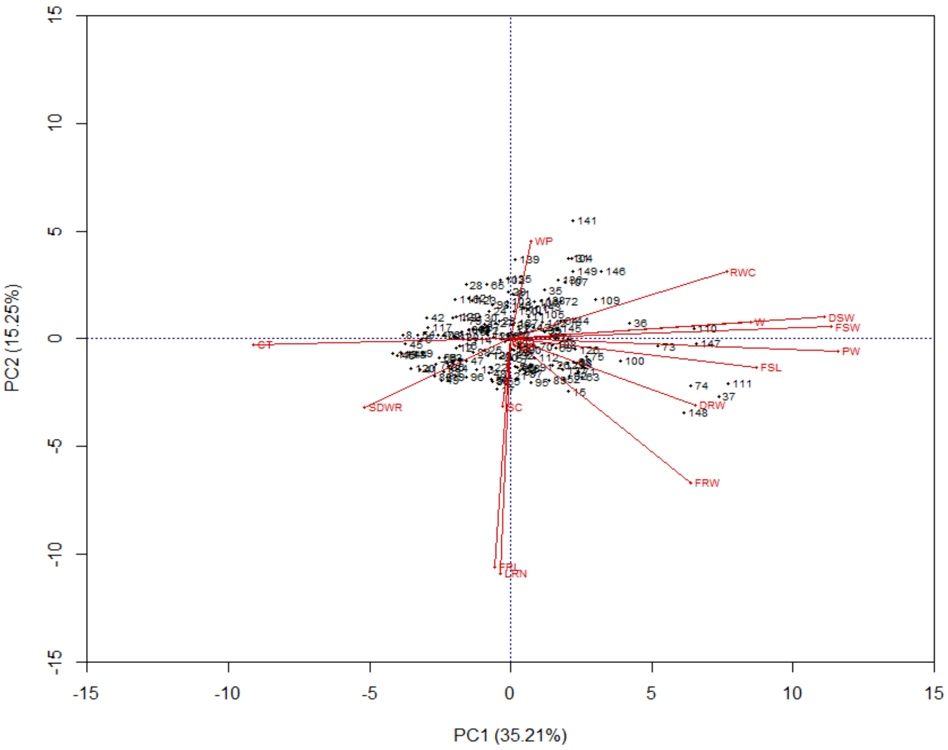

Figure 1. Biplot between PC-1 and PC-2 showing contribution of various traits under normal water condition.

009 performed better conversely FH-4243, AS-1, PB-899 and AR-22 displayed below-average performance. Biplot analysis for Plant weight revealed that B-622, KZ-191, AUR-56, and CP-15 performed better conversely 407-26, MNH-456, FH-4243, and Sitara-008 displayed below-average performance. Biplot analysis for Plant weight (root + shoot) revealed that PB-899, ALBACALA (70)19, AR-21, and CP-15 performed better conversely FH-312, FH-53, CIM-70 and AMS-139 displayed below-average performance. Biplot analysis for RWC reflected that FH-53, FH-312, AMS-139, and CIM-70 revealed that performed better conversely ALBACALA (70)19, PB-899, CP-15 and AR-21 displayed below-average performance. Biplot analysis for SC reflected that 448/4727C, AS-3, 108-F, and FH-142 performed better conversely AR-17, CIM-443, FH-2006 and BH-175 displayed below-average performance. Biplot analysis for water potential reflected that 42AR-17, CIM443, FH-2006, and BH-175 displayed better performance conversely 448/4727C, AS-3, 108-F, and FH-113 below-average performance.

Through cluster analysis, based on different morpho-physiological parameters 150 cotton genotypes were grouped into 6 clusters. Cluster analysis exhibited that cluster 1 consist

Table 1. Cluster membership of various genotypes in cotton under normal water condition.

Cluster 1	16	AGC-777, S-11/3, SLH-74, FH-312, ABRI/5, IR-NIBGE-6, GH-142, MNH-456, RH-627, CRIS-468, CRIS-134, CP-15, COKER-310, FE-4252, FH-1000, FH-2000
Cluster 2	44	SILKEE, BH-180, FH-942, FH-4243, FH-330, RH-510, AS-1, AS-2, AS-3,CIM-600, CIM-598, CEMB-55, FH-142S, 407-26, AU-59, CBS-1, FH-118, VH-305, MNH-886, BOSS-111, BROWN-BHW, BS-1, CIM-200, CIM-240, CIM-473, CIM-83, COKER, SLH-2010-11, CBS-2, BH-175, SITARA-008, IR-NIAB-824, IUB-2009, SB-149, IR-NIBGE-3, DP-15-26, FH-113, TARZEN-1, FH-2925, FVH-53, FH-901, KZ-191, SITARA-009, N-141
Cluster 3	22	PB-899, MM-58, CIM-1100, IUB-222, CH-019, CH-009, CH-003, AR-22, AR-23, AR-25, 108-F, C2(37)1473, CYTO-177, CIM-616, ALBACALA(70)19 ,BLANCO-3363, BJAHL, A-162, BH-36, BH-100, BARNT-205-4, 124-F
Cluster 4	52	AGC-999, NS-161, AR-1, AR-2, AR-3, AR-9, AR-13,AR-14, AR-17, AR-21,VH-300, FH-324, FH-341, FH-314, FH-168, 199-F, 208-HYBI, 268-F, 281GL(443),448/4727C, 4-F, AET-5, ACALA-P3, ACALA-7203-4-1, YU-MM2, ACALA-157C, ACA-285, AC-307, 61-F/89, AMS-139, AMS-170, ASA\965)-650, AUBURH, AUR-56, B-403, B-557, B-622, BAR F/8, BH-580, BH-118, BH-128, IUB-13, CIM-599, C-24, CAPTAIN-2833,CIM-243, CIM-443, CIM-446, CIM-482, CIM-496, CIM-499, CIM-70
Cluster 5	10	CEDIX, DELCOTT-227, AONE, DP-165, E-302, EXOTIC, F-281GL-44, FH-113, FH-1185, C-HIR-1628
Cluster 6	6	DP-148, FH-2006, FH-900, KZ-181, DPL-SL, N-131

Table 2. Cluster analysis of various traits in cotton under normal water conditions.

Variables	Culster-1	Culster-2	Culster-3	Culster-4	Culster-5	Culster-6
FRL	61.8954	65.0249	63.6403	60.5883	63.5860	52.6878
FSL	13.2500	18.9470	13.5182	18.3788	18.0633	16.1000
LRN	19.7081	21.5227	21.1364	19.8269	21.3000	14.5000
FRW	0.4385	0.5083	0.4248	0.4882	0.4920	0.3300
FSW	1.5531	2.2409	1.5564	2.2681	1.9320	2.7450
DSW	0.3625	0.5041	0.3514	0.5183	0.4190	0.6167
W	0.0069	0.0137	0.0058	0.0198	0.0219	0.0160
DRW	0.1756	0.2289	0.1909	0.2237	0.1940	0.2400
PW	1.9917	2.7492	1.9812	2.7563	2.4240	30.0750
SDWR	0.5727	0.4950	0.5994	0.4688	0.5020	0.4359
SC	45.2194	59.4091	78.0909	75.1923	35.4000	47.0000
CT	25.7162	22.2793	25.6382	21.8463	22.6260	21.6917
WP	2.6163	2.63452	2.5232	2.4479	2.6780	3.0883
OP	0.2783	0.2129	0.2000	0.2240	0.2161	0.2143
RWC	70.7945	84.4795	70.5055	89.3746	88.3380	91.6500

Table 3. Principle component analysis of different morpho-physiological traits in cotton under normal water condition.

	PC-I	PC-II	PC-III	PC-IV	PC-V	PC-VI
Eigen value	5.2810	2.2877	1.4684	1.2363	1.1224	1.0427
% of total variance	35.2	15.3	9.8	8.2	7.5	7.0
Cumulative variance	35.2	50.5	60.2	68.5	76.0	82.9

Table 4. Factor loadings of different morpho-physiological traits in cotton under normal water condition.

Variables	PC-I	PC-II	PC-III	PC-IV	PC-V	PC-VI
FRL	-0.020	-0.575	-0.284	0.129	0.043	0.063
FSL	0.310	-0.071	0.016	0.084	0.048	0.374
LRN	-0.013	-0.591	-0.207	0.128	-0.042	0.117
FRW	0.227	-0.362	0.033	-0.100	0.169	0.012
FSW	0.404	0.030	-0.096	-0.034	-0.003	-0.233
DSW	0.397	0.054	-0.124	-0.062	-0.022	-0.286
W	0.303	0.043	0.091	0.021	-0.107	-0.089
DRW	0.233	-0.167	0.513	0.180	0.238	-0.294
PW	0.413	-0.031	-0.084	-0.048	0.025	-0.245
SDWR	-0.185	-0.173	0.632	0.252	0.219	-0.023
SC	-0.010	-0.171	0.262	-0.203	-0.706	-0.104
CT	-0.325	-0.015	-0.093	-0.138	0.074	-0.459
WP	0.025	0.247	-0.249	0.593	0.227	-0.042
OP	0.005	-0.019	0.023	-0.660	0.532	0.135
RWC	0.272	0.168	0.183	-0.003	-0.101	0.541

of 16 genotypes, 2nd cluster of 44 and cluster 3, 4, 5 and 6 contains 22,52,10 and 6 cotton genotypes respectively (Table 1).

Cotton genotypes in 1st cluster exhibited a reasonable value for Osmotic Potential but for other parameters of drought, tolerance selection is not feasible in this cluster. The 2nd cluster contained the maximum number of cotton genotypes i.e., 44 which exhibited reasonable value for FRL, FSL, LRN, and FRW which is good for selection on the basis of seedling parameters (Table 2). The members in cluster 3 were categorized by the least values of DSW, W/L, PW, OP, and RWC. The cluster 4 and 5 is characterized by minimum value for WP and SC respectively. Cluster 6 had maximum values for FSW, DSW, PW, CT, WP, and RWC while it has minimum values for FRL, LRN, FRW, and root shoot ratio. The PCA of 150 cotton genotypes under normal water conditions revealed that among 150 cotton genotypes, 6 PCs had Eigen value >1. The contribution of these six PCs is82.9% of the total variability prevailing in cotton genotypes assessed for traits (Table 3). The remaining variability contribution is only 17.1%. The contribution of PC I is 35.2followed by 2nd PC (15.5%), 3rd PC (9.8%), 4th PC

(8.2%), 5th PC (7.5%) and 6th PC (7.0%). The traits like FSL, FRW, FSW, DSW, W/L, DRW, PW, WP, OP, and RWC showed significant positive factor loadings on 1st PC while CT had maximum negative loadings. The 2nd PC deals with diversity due to FRL, LRN, FRW and WP among cotton genotypes **(**Table 4**)**. No single cluster separation was exhibited. The tree diagram exhibited more or less identical results that comprised of two major groups which are further divided into sub clusters among different cotton genotypes (Fig. 2).

***Biplot analysis for water stress condition at seedling stage*:** Principal component analysis was carried out to identify the relationship between the different parameters (Fig. 3). The first two PCs explained 42.28% and 12.30% variance of the total. In the biplot analysis, weights of different variables are explained with arrows that vary with distance from the origin.

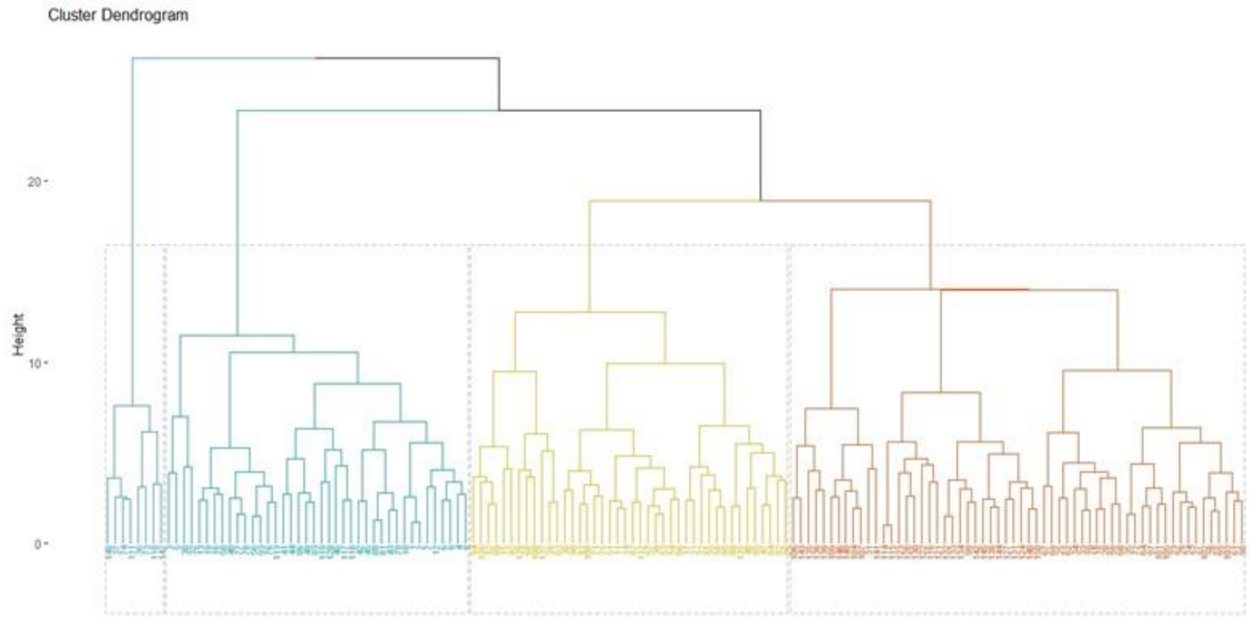

Figure 2. Dendrogram of 150 cotton genotypes under normal water condition

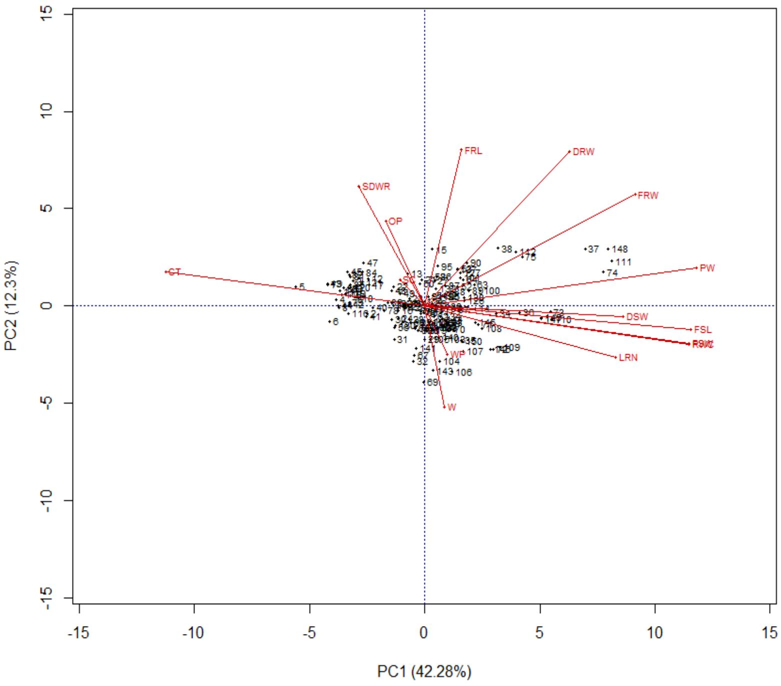

Figure 3. Biplot between PC-1 and PC-2 showing contribution of various traits under water stress condition

In the plot, the weights of the different variables are presented with arrows and are shown to vary with distance from the origin.

Biplot analysis regarding Fresh Root Length revealed that ABRI/5, FH-330, FH-324, and BS-1 performed better as compared to other genotypes. Performance of AR-1 FH-4243 VH-300 and ASA\965)-650 was below average concerning fresh root length. Biplot analysis concerning Fresh Shoot Length revealed that SILKEE, AR-3, B-557, and FH-900 performed better concerning Fresh Shoot LengthwhileABRI/5, RH-627, SILKEE and BH-580 performed below average. Biplot analysis concerning Fresh Root Length revealed that CIM-499, CIM-70, B-557, and FH-901 performed better concerning this character while PB-899, CH-019, AR-21, and ALBACALA (70)19 were low performers. Biplot analysis for Fresh Root weight revealed that 268-F, FH-314 BH-580, and C-HIR-1628 exhibited good performance while S-11/3, IR-NIBGE-6, BH-180 and BARNT-205-4 showed below-average performance. Biplot analysis for Fresh shoot weight revealed that B-557, SILKEE, AR-13 and AR-3, and KZ-191 displayed better performance while CIM-600, ABRI/5, FH-4243, and IUB-222 showed below-average performance for fresh shoot weight.

Biplot analysis for Dry shoot weight revealed that SILKEE, AR-3, AR-14, and B-557 and performed better conversely ABRI/5, CIM-600, CIM-598, and FH-312 displayed below-average performance. The vectors of both the parameters were lying almost close to each other so the behavior is almost the same. Biplot analysis for Dry root weight revealed that FH-341, FH-314, IUB-13, and IR-NIBGE-3 performed better conversely AR-3, AR-1, CH-009, and ACALA-P3 displayed below-average performance. Biplot analysis for Plant weight revealed that FH-341, BAR F/8, COKER, and SITARA-009 performed better conversely IR-NIBGE-6, IUB-222, BARNT-205-4 and BH-175 displayed below-average performance. Biplot analysis concerning RWC and SC revealed that SILKEE, AR-3, AR-13, and B-557 performed better conversely BH-180, CIM-600, BH-175, ALBACALA (70)19 and displayed below-average performance. Biplot analysis for SDWR and OP reflected that FH-942, AS-3, CH-

Table 5. Cluster analysis of various traits in cotton under water stress condition.

Variables	Culster-1	Culster-2	Culster-3	Culster-4	Culster-5	Culster-6
FRL	61.8954	65.0249	63.6403	60.5883	63.5860	52.6878
FSL	13.2500	18.9470	13.5182	18.3788	18.0633	16.1000
LRN	19.7081	21.5227	21.1364	19.8269	21.300	14.5000
FRW	0.4385	0.5083	0.4248	0.4882	0.4920	0.3300
FSW	1.5531	2.2409	1.5564	2.2681	1.9320	2.7450
DSW	0.3625	0.5041	0.3514	0.5183	0.4190	0.6167
W	0.0069	0.0137	0.0058	0.0198	0.0219	0.0160
DRW	0.1756	0.2289	0.1909	0.2237	0.1940	0.2400
PW	1.9917	2.7492	1.9812	2.7563	2.4240	30.0750
SDWR	0.5727	0.4950	0.5994	0.4688	0.5020	0.4359
SC	45.2194	59.4091	78.0909	75.1923	35.4000	47.0000
CT	25.7162	22.2793	25.6382	21.8463	22.6260	21.6917
WP	2.6163	2.63452	2.5232	2.4479	2.6780	3.0883
OP	0.2783	0.2129	0.2000	0.2240	0.2161	0.2143
RWC	70.7945	84.4795	70.5055	89.3746	88.3380	91.6500

Table 6. Cluster membership of various genotypes under water stress condition.

Cluster 1	18	AGC-777, S-11/3, SLH-74, FH-312, ABRI/5, IR-NIBGE-6, GH-142, MNH-456, RH-627, SILKEE, PB-899, BH-175, SITARA-008, CRIS-468, CRIS-134, CP-15, COKER-310, IR-NIAB-824
Cluster 2	32	CYTO-177, FH-118, VH-305, CIM-599, BOSS-111, BS-1, BH-180, FH-942, FH-4243, FH-330, RH-510, AS-2, AS-1, AS-3, CEMB-55, CH-019, AR-21, 108-F, 124-F, 199-F, 407-26, ACALA-P3, ACALA-7203-4-1, BH-128, BARNT-205-4, CBS-2, CIM-240, IUB-2009, SB-149, IR-NIBGE-3, DP-15-26, TARZEN-1,
Cluster 3	14	CIM-443, CIM-473, CIM-496, CAPTAIN-2833, CIM-243, CIM-600, DP-148, FH-1000, FH-113, CIM-446, FH-2006, FH-900, KZ-181, CIM-482
Cluster 4	35	YU-MM2, ACALA-157C, ACA-285, AMS-139, ASA\965)-650, AU-59, AUBURH, BH-100, BH-36, A-162, CIM-616, BROWN-BHW, CIM-598, AGC-999, MM-58, BJAHL, FH-142S, CIM-1100, AR-1, AR-2, AR-3, BLANCO-3363, AR-9, AR-9, AR-22, AR-23, AR-25, VH-300, FH-168, IUB-222, CH-009, CH-003, 448/4727C, 4-F, AET-5, ALBACALA(70)19
Cluster 5	30	C2(37)1473, C-24, NS-161, CBS-1, MNH-886, CEDIX, CIM-200, CIM-70, CIM-83, COKER, SLH-2010-11, DELCOTT-227, AONE, DP-165, FH-113, E-302, EXOTIC, F-281GL-44, FE-4252, FH-1185, FH-2000, FH-2925, C-HIR-1628, FVH-53, FH-901, DPL-SL, KZ-191, SITARA-009, N-131, N-141
Cluster 6	21	AR-14, AR-13, AR-17, FH-324, FH-341, FH-314, 208-HYBI, 268-F, 281GL(443), AC-307, 61-F/89, AMS-170, AUR-56, B-403, B-557, B-622, BAR F/8, BH-580, BH-118, IUB-13, CIM-499

Table 7. Principle component analysis of different morpho-physiological traits underwater stress condition in cotton.

	PC-I	PC-II	PC-III	PC-IV	PC-V
Eigen value	6.3412	1.8461	1.4273	1.2396	1.1064
% of total variance	42.3	12.3	9.5	8.2	7.4
Cumulative variance	42.3	54.6	64.1	72.3	79.7

009, and 124-F performed better conversely AUBURH, CIM-446,CIM-482, and KZ-181displayed below-average performance. Biplot analysis for W reflected that AUBURH, CIM-482, KZ-181 and CIM-446 performed better conversely FH-942, AGC-999, 124-F and YU-MM2 displayed below-average performance. Biplot analysis for CT reflected that FH-312, ABRI/5, CIM-600, and BH-580 performed better conversely B-557, AR-3, AR-13 SILKEE and displayed below-average performance (Fig. 3).

Cluster analysis of different 150 cotton genotypes was grouped into 6 different clusters based on different morpho-physiological parameters. Cluster analysis exhibited that cluster 1 consisted of 18 genotypes, cluster 2 of 32 while clusters 3, 4,5, and 6 contain 14,35,30, and 21 cotton genotypes respectively (Table 6). The genotypes in cluster 1 showed maximum value for SDWR and OP and minimum values FSL, LRN, FSW, FRW, DSW, W/L, PW, and RWC. The 2nd cluster displayed the maximum value for FRL. Cluster 3 has maximum value for W/L and WP while it displayed minimum value for FRL, DRW, and SDWR. The members of 4,5 and 6 have the minimum value for OP, SC, and WP respectively. Cluster 6 has the maximum value for FSL, LRN, FSW, FRW, PW, SC CT, and RWC. Cluster 6 seems to be a good one to select for desirable parents for drought tolerance (Table 5).

Five principal components (PCs) were extracted having Eigenvalue >1 out of a total of 15 PCs. The contribution of these 5 PCs is 79.7% of the total variability (Table 7).

The remaining components' contribution is only 20.3%. The PC I contributed maximum towards the variability i.e. 42.3 followed by PC II (12.3%), PC III (9.5%), PC IV (8.2%), and PC V (7.4%). PC I displayed maximum negative loadings for FRL, LRN, FSW, FRW, W/L, DSW, CT, and RWC while showed considerable maximum positive value for OP and SDWR. The correlation between a variable and a PC is called *loading.* PC-II was related to diversity prevailing in cotton genotypes due to FRL, LRN, FRW, and WP (Table 8).

Table 8. Factor loadings of different morpho-physiological traits in cotton under water stress condition

Variables	PC-I	PC-II	PC-III	PC-IV	PC-V
FRL	0.052	-0.483	-0.186	0.076	-0.039
FSL	0.375	0.073	0.107	0.011	0.126
LRN	0.270	0.159	0.151	0.036	-0.012
FRW	0.373	0.116	0.140	0.002	0.161
FSW	0.297	-0.344	-0.083	-0.011	-0.202
DSW	0.281	0.033	-0.442	-0.175	-0.217
W	0.027	0.314	-0.118	-0.250	-0.586
DRW	0.204	-0.479	-0.102	0.044	-0.318
PW	0.383	-0.119	0.037	-0.005	-0.015
SDWR	-0.093	-0.372	0.532	0.256	-0.068
SC	-0.035	-0.082	0.525	-0.414	-0.241
CT	-0.366	-0.106	-0.122	-0.010	-0.179
WP	0.032	0.149	-0.130	0.756	-0.124
OP	-0.055	-0.263	-0.279	-0.298	0.538
RWC	0.374	0.117	0.126	-0.005	0.157

No single cluster separation was exhibited. The tree diagram exhibited more or less identical results that comprised of two

Table 9. Correlation coefficients of different parameters of 150 cotton genotype under normal condition at seedling stage.

	FRL	FSL	LRN	FSW	FRW	DSW	W	DRW	PW	SDWR	SC	CT	WP	OP
FSL	0.060													
LRN	0.518**	-0.051												
FSW	0.474**	0.342**	0.364**											
FRW	0.486**	-0.088	0.980**	0.341**										
DSW	0.448**	-0.112	0.560**	0.435**	0.546**									
W	0.328**	0.062	0.508**	0.358**	0.469**	0.338**								
DRW	0.558**	0.008	0.988**	0.501**	0.967**	0.591**	0.530**							
PW	-0.250**	0.084	-0.480**	-0.056	-0.530**	-0.250**	0.411**	-0.460**						
SDWR	-0.038	0.122	-0.033	0.070	-0.026	-0.002	0.061	-0.020	0.085					
SC	-0.620**	-0.028	-0.570**	-0.270	-0.560**	-0.410**	-0.360**	-0.580**	0.214**	0.014				
CT	0.057	-0.193*	0.072	-0.127	0.073	-0.006	-0.050	0.046	-0.114	-0.290**	-0.060			
WP	0.006	-0.070	0.007	0.154	0.014	-0.115	0.009	0.031	-0.050	-0.117	0.054	-0.220**		
OP	0.478**	-0.187*	0.451**	0.142	0.424**	0.401**	0.238**	0.442**	-0.193*	0.001	-0.710**	0.026	0.036	
RWC	0.047	0.865**	-0.041	0.336**	-0.067	-0.155	0.027	0.017	0.045	0.051	0.028	-0.123	-0.028	-0.250**

major groups which are further divided into sub- clusters among different cotton genotypes (Fig. 4).

Seedlings correlation studies of different morpho-physiological parameters under normal and water stress condition: Correlation studies for seedlings parameters under normal reflected a positive significant correlation between important morphological and physiological parmeters under study where as negative significant correlation was also observed. Fresh root length was positively and significantly Fresh shoot length, lateral root number, fresh shoot weight, fresh root weight, dry shoot weight, W/L ratio, dry root wight, and osmotic potential consersely significantly negatively correlated with plant weight, SDWR and stomatal conductance. Correlation was also observed between other mopho-phyiological parameters (Table 9).

Similarly correlation studies in water stress conditions also reflected positive significant and negative singnificant correlations. Fresh shoot length was positively significantly correlation with lateral root number, frsh shoot weight, fresh root weight, W/L ratio, dry root wight whereas negetively and signigicatly correlated with water potential and stomatal conductance. Similarly significant correlation was also observed among other parameters (Table 10).

Table 10. Correlation coefficients of different parameters of 150 cotton genotype under water stress condition at seedling stage

	FRL	FSL	LRN	FSW	FRW	DSW	W	DRW	PW	SDWR	SC	CT	WP	OP
FSL	0.632**													
LRN	0.955**	0.623**												
FSW	0.597**	0.357**	0.541**											
FRW	0.551**	0.427**	0.515**	0.643**										
DSW	0.042	0.059	0.054	-0.039	0.188*									
W	0.360**	0.291**	0.296**	0.708**	0.438**	-0.019								
DRW	0.892**	0.564**	0.888**	0.867**	0.656**	0.011	0.562**							
PW	-0.195*	-0.104	-0.205*	0.038	-0.58**	-0.170*	0.189*	-0.101						
SDWR	-0.050	-0.055	-0.044	-0.011	-0.162*	-0.010	-0.041	-0.032	0.194*					
SC	-0.930**	-0.59**	-0.970**	-0.530**	-0.49**	-0.050	-0.280**	-0.860**	0.201*	0.081				
CT	0.080	0.050	0.064	0.028	0.050	-0.027	-0.004	0.053	-0.012	-0.270**	-0.051			
WP	-0.131	-0.195*	-0.153	0.017	-0.037	-0.170	0.058	-0.081	0.008	-0.130	0.143	-0.230**		
OP	0.951**	0.627**	0.993**	0.543**	0.523**	0.061	0.304**	0.880**	-0.220**	-0.051	-0.960**	0.061	-0.146	
RWC	0.062	-0.155	0.037	0.263**	0.110	-0.210*	0.399**	0.166*	0.058	-0.047	-0.075	-0.057	0.056	0.037

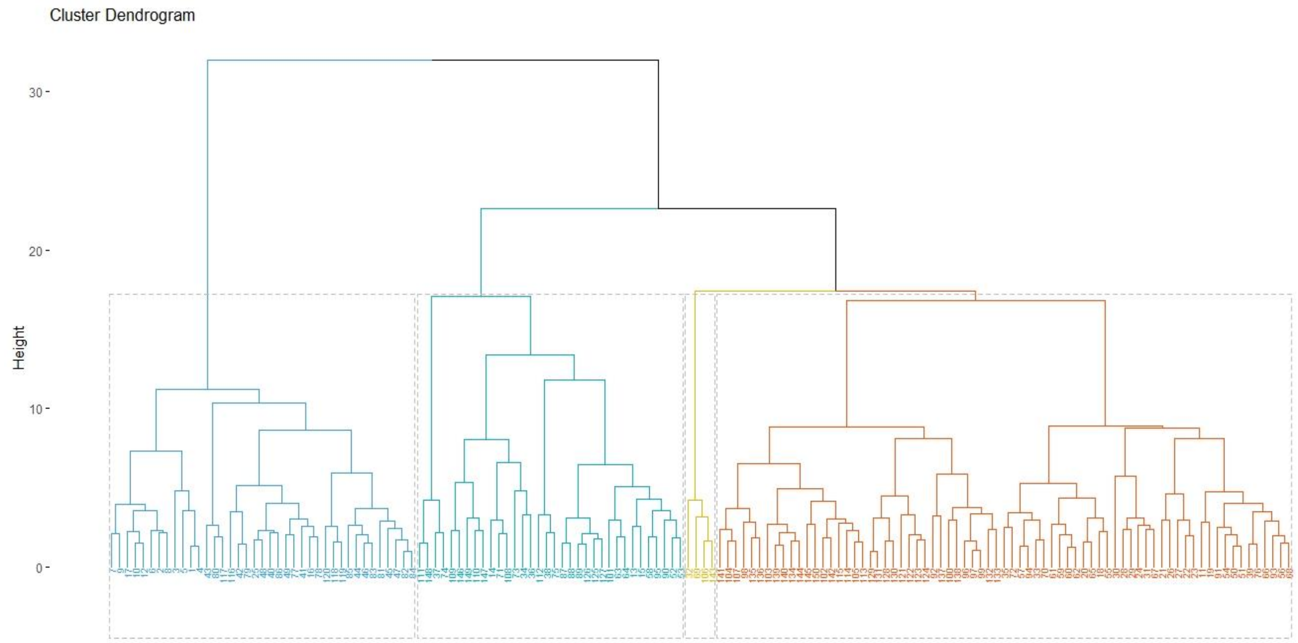

Figure 4. Dendrogram of 150 cotton genotypes under water stress condition

DISCUSSION

To start any crop breeding program the required information about the behaviour of different traits is an integral part of the program as it gives a prospect for the selection of desirable genotypes with traits of interest (Ali *et al.,* 2009; Wazeer *et al.*, 2020). This study was executed to work out the drought tolerance potential of available cotton germplasm by screening against the water deficit conditions. The objective was to make available the required data to improve the cotton breeding program regarding limited water conditions in the country. Drought considerably affects cotton growth by declining root shoot length, weight, lateral root number, relative water content, and leaf area, which collectively decreased the net photosynthetic rate and ultimately the total biomass of cotton seedlings. In almost all plants, the seedling growth stage is susceptible to environmental changes (Gutterman, 2002). Uniyal and Nautiyal (1998) anticipated assessing plant stress tolerance/resistance at the seedlings' growth stage. Different researchers have devised different morphological, physiological and biomechanical, traits to evaluate drought tolerance (Falkenberg *et al.*, 2007; Uzilday *et al.,* 2012; Wang *et al.,*. 2016). Root shoot parameters like root length, root fresh and dry weight, shoot length, shoot fresh dry weight remarkably declined under drought conditions. Cluster analysis is a method that aims to clarify and classify a sample of objects or subjects based on a set of deliberately chosen variables into several different groups keeping alike objects in a similar group. In water stress conditions strong reduction of root length was observed, therefore, genotypes in the water stress group may be referred to as susceptible for water stress conditions. Luo *et al*. (2016) also found the reduction of root length in water stress conditions. The parameters related to roots are directly linked with drought tolerance in cotton (Basal *et al.,* 2003; Iqbal, 2010). Shoot length has also been used as a selection parameter for drought tolerance (Chaturvedi *et al.,* 2012; Iqbal, 2010). Dwindling performance of different root and shoot parameters is due to the water stress conditions as being reported in many studies (Kumar *et al.,* 2001; Pace *et al.,* 1999). Under drought stress, the reduction of water loss through leaves is an important phenomenon in cotton. Excessive water loss results in wilting and rolling of leaves (Nayyar and Gupta, 2006). Relative Water Content was reduced remarkably in water deficit conditions. Reduced water contents under drought conditions have also been reported in many studies (Akbar and Hussain, 2019; Rashid *et al.,* 2015; Parida *et al.,* 2008). At the seedling stage, relative water content (RWC) is selection criterion for drought tolerance in cotton. Cotton is more responsive to low water potential for photosynthesis as compared with Stomatal conductance (Krieg, 1986). Reduction in cell expansion due to water limited conditions growth rate of stem & roots is adversely affected, which ultimately reduces cotton yield (Babar et al., 2009). Cultivar performance is very much dependent on root-shoot parameters on the basis of seedling vigor. Significant correlation of between root shoot traits is already known (Irum *et al.,* 2013). All genotypes exhibited raise in root–shoot ratio under limited water conditions. Different morpho-physiological traits play their role in drought tolerance in cotton. Those traits include root parameters like lateral root number, root length, root development rate, root shoot ratio (Pace et al., 1999); less transpiration rate (Quisenberry *et al.,* 1982); reduced stomatal conductance (Nepomuceno *et al.,* 1998); and leaf water content (Leidie*et al*., 1999). These parameters were recorded and the genotypes which exhibit better results regarding morpho-physiological traits are considered tolerant to drought. Negative effects on osmotic balance have been observed under water stress conditions, therefore, plants accumulate different organic and inorganic molecules to cope with osmotic potential (Efeoglu *et al*., 2009). The osmotic potential was also markedly reduced in water stress conditions. There was a significant and positive correlation between fresh shoot weight and fresh root weight, dry shoot weight, plant weight, and dry root weight. Similar results were also found in other studies (Basal *et al.*, 2005; Irum*et al.*, 2013). The results of the study would be useful to select the drought-tolerant genotypes from the PCA and Cluster analysis. The results from cluster analysis showed that the cotton genotypes i.e. FH-900, FH-901, FH-312, AS-1, AS-2, AS-3, RH-510, RH-627, AR-2, AR-9, BH-118, BH-175, SLH-74, CIM-1100, CIM-443, CIM-598, and MM-58 showed a minimum reduction in morpho-physiological parameters under water stress, therefore declared as drought-tolerant cotton genotypes for any breeding program. Principle component analysis resulted in six and five principal components (PCs) with Eigen value >1 in the normal and water stress environment. The cumulative score of the PC1 and PC2 is more than 50 % which is very effective. Correlated Variables with PC1 and PC2 are very effective in explaining the variability (Abdi and Williams, 2010). The scientists also described the higher contribution of the first two principal factors to the total variance. Ashokkumar and Ravikesavan (2011), and Malik *et al.* (2011) described enormous genetic diversity in cotton cultivars. Nazir *et al.* (2013) also concluded that the contribution between the first two PCs is very important in the total variation. PC1 contribution of normal and stress conditions was 35.2 and 42.3 respectively. Different studies reflected the vital contribution of the first two PCs in total variability concerning the investigation of different traits (Chozin, 2007; Mujaju and Chakuya, 2008). The genotypes that performed better concerning drought have to be selected from the PC, having maximum variability along with the highest Eigen value. Principle component analysis is a handful method for investigating extensive variation among different traits. The drought tolerance phenomenon in crops plants is very complex. Errors in evaluating the drought tolerance between materials by using a single parameter alone, no single parameter can fully and accurately evaluate the drought tolerance of crops. Meanwhile, there is a certain correlation among many evaluation indicators, such as multiple linear correlations, which leads to the overlapping of the information they provide on the response of crops to stress, so it is necessary to evaluate the drought resistance of plant's comprehensive character index by using multivariate analyses. PCA can reduce multiple variables to a few potential factors without little loss of information as much as possible (Wu and Bao, 2012). These factors can provide a high profile of the information in a large number of data.

***Conclusion*:** Narrow genetic variability is a serious issue among cotton cultivars in Pakistan. Under changing climatic conditions, assessment of genetic variability is of vital importance in starting any cotton breeding with respect to drought. Genetic variability of 150 cotton genotypes was assessed on the basis of morpho-physiological parameters using PCA, cluster analysis along with correlation studies. Different genotypes behaved differently and were categorized into different groups. FH-900, FH0901, CIM-1100, BH-180, AR-3, IUB-13, and FH-942 performed better under water limited conditions based on multivariate analysis. These genotypes can be used as potential candidates for the cotton drought breeding program.

***Conflict of Interest*:** The Authors declare that there is no conflict of interest

***Authors' Contribution Statements*:** MA executed the research, whereas AAK, HMNC, SH and ZI conceived the idea & supervised the work.

***Acknowledgement*:** Authors acknowledge the facilities provided by Ayub Agricultural Research Institute, Faisalabad.

REFERENCES

Abdi, H. and L. J. Williams. 2010. Principal Component Analysis. Wiley Interdisciplinary Reviews: Computational Statistics. 2:433-459.

Akbar, M. and S. B. Hussain. 2019. Assessment of drought tolerant cotton genotypes based on seedling & physiological attributes at different moisture levels. Pure and Applied Biology. 8: 93-107.

Ali, M. A., N. Shahid, A. Amjad, S. Waseem and J. Khawar. 2009. Genetic diversity and assessment of drought tolerant Sorghum landraces based on morpho-physiological traits at different growth stages. Plant Omics. 2:214-227.

Ashokkumar, K. and R. Ravikesavan. 2011. Morphological Diversity and per se Performance in Upland Cotton (*Gossypium hirsutum* L.). Journal of Agricultural Science. 3:107-113.

Babar, M., Y. Saranga, Z. Iqbal, M. Arif, Y. Zafar and E. Lubbers. 2009. Identification of QTLs and impact of selection from various environments (dry vs irrigated) on the genetic relationships among the selected cotton lines from f 6 population using a phylogenetic aroach. African Journal of Biotechnology. 8:4802-4810.

Barbara F. R., A. R. Thomas, Junior and B. L. Joiner. 1972. Minitab. Minitab Inc, State College, Pennsylvania.

Basal, H., C.W. Smith, P. S. Thaxton and J. K. Hemphill 2005.Seedling drought tolerance in upland cotton. Crop Science. 45:766-771.

Basal, H., P. Bebeli, C. Smith, and P. Thaxton. 2003. Root growth parameters of converted race stocks of upland cotton and two BCF2 populations. Crop Science. 43:1983-1988.

Chaturvedi, G. S., S. Anuradha and R. Bahadur. 2012. Screening techniques for evaluating crop germplasm for drought tolerance. Plant Archives. 12:11-18.

Chozin, M. 2007. Characterization of sorghum accessions and choice of parents for hybridization. Journal Akta. 2:227-232.

Colman, E. A., 1947. A laboratory procdure for determining the field capacity of soils. Soil Science. 63:277-284.

Cushman, J. C. and H. J. Bohnert. 2000. Genomic approaches to plant stress tolerance. Plant Biology. 3:117-124.

Efeoglu, B., Y. Ekmekci and N. Cicek. 2009. Physiological responses of three maize cultivars to drought stress and recovery. South African Journal of Botany. 75:34-42.

Falkenberg, N. R., G. Piccinni, J. T. Cothren, D. I. Leskovar and C. M. Rush. 2007. Remote sensing of biotic and abiotic stress for irrigation management of cotton. Agricultural Water Management. 87:23-31.

FAO (Food and Agriculture Organization) of the United Nations. 1994. Cotton pests and their control in the near east. Rome: Food and Agriculture Organization of the United Nations.

Franca, M. G., T. T. Pham, C. Pimentel, R. O. P. Rossiello, Y. Z. Fodil and D. Laffray. 2000. Differences in growth and water relation among Phaseolus vulgaris cultivars in response to induces water stress. Environmental and Experimental Botany 43:227337.

Gutterman, P. D. Y. 2002. Survival strategies of annual desert plants. Adaptations of Desert Organisms. 15:39-52.

Iqbal, K. 2010. The potential for breeding upland cotton under limited water conditions (PhD thesis). University of Agriculture, Faisalabad, Pakistan. Retrieved from http://www.prr.hec.gov.pk/Thesis/2295.pdf.

Irum. I., A. Tabasum and M. Z. Iqbal. 2013. Variability, correlation and path coefficient analysis of seedling traits and yield in cotton (*Gossypium hirsutum* L.). African Journal of Biotechnology. 10:18104-18110.

Krieg, D. R. 1986. Feedback control and stress effects on photosynthesis. In Proceedings of the Beltwide Cotton Conference, National Cotton Council of America, Memphis.pp. 227-243.

Kumar, A., C. Roach, I. S.Hirsh, S. Turley, S. de Walque, P. A. Michels and G. Hol. 2001. An unexpected extended conformation for the third TPR motif of the peroxin PEX5 from *Trypanosoma brucei*. Journal of Molecular Biology. 307:271-82.

Leidi, E. O., M. Lopez, J. Gorham, and J. C. Gutierrez. 1999. Variation in carbon isotope discrimination and other traits to drought tolerance in upland cotton cultivars under dryland conditions. Field Crops Research. 61:109-123.

Luo, H., Y. L. Zhang, and W. F. Zhang. 2016. Effects of water stress and re watering on photosynthesis, root activity, and yield of cotton with drip irrigation under mulch. Photosynthetica. 54 (1): 65-73.

Malik, W., M. Z. Iqbal, A. A. Khan, E. Noor, A. Qayyum and M. Hanif. 2011. Genetic basis of variation for seedling traits in *Gossypium hirsutum* L. African Journal of Biotechnology. 10: 1099-1105.

Mujaju, C. and E. Chakauya. 2008. Morphological Variation of Sorghum Landrace Accessions On-Farm in Semi-Arid Areas of Zimbabwe. . International Journal of Botany.4. 10.3923/ijb.2008.376.382.

Nayyar, H. and D. Gupta. 2006. Differential sensitivity of C3 and C4 plants to water deficit stress: Association with oxidative stress and antioxidants. Environmental and Experimental Botany. 58:106-113.

Nazir, M. S., A. W. Bambang, W. Yussof and A. M. Abdullah. 2013. Eco-Friendly Extraction and Characterization of Cellulose from Oil Palm Empty Fruit Bunches. Bioresources. 8:2161-2172.

Nepomuceno A. L., D. M. Oostrerhuis and J. M. Stewart. 1998. Physiological reponses of cotton leaves and roots to water deficit induced by Polythylene Glycol. Environmental and Experimental Botany. 40:29-41.

Pace P. F., H. T. Cralle, S. H. M. El-Halaway, J. T. Cothren and S. A. Senseman. 1999. Drought-induced changes in shoot and root growth of young cotton plants. Journalof Cotton Science. 3:183-187.

Parida, A. K., V. S. Dasgaonkar, M. S. Phalak and L. P. Aurangabadkar. 2008. Differential responses of the enzymes involved in proline biosynthesis and degradation in cotton genotypes during drought stress and recovery. Acta Physiologiae Plantarum. 30:619-627.

Powell, J. and S. Reinhard. 2016. Measuring the effects of extreme weather events on yields. Weather and Climate Extremes.12:69-79.

Pretorius, M. M. 2009. Evaluation of irrigated cotton cultivars in South Africa (MSc. Thesis). University of the Free State, South Africa.

Quisenberry, J. E., B. Roark and B. L. McMichael. 1982. Use of transpiration decline curves to identify drought-tolerant cotton germplasm. Crop Science. 22:918-922

Rashid, B., T. Husnain and S. Riazuddin. 2015. Genomic approaches and abiotic stress tolerance in plants. In: P. Ahmad and S. Rasool (Eds.), Emerging technologies and management of crop stress tolerance. Biological techniques, Elsevier, Academic press, USA. 1:1-38.

Rathore, K. S., G. Sunikumar, R. D. Stipanovic and T. C. Wedegaertner. 2007.RNAi-mediated, selective and substantial reduction in gossypol levels from cottonseed to enhance its food and feed value. In: Proceedings. World Cotton Conf., September 10–14, Lubbock, TX.

Scholander, P. F., E. D. Bradstreet, E. A. Hemmingsen and H. T. Hammel. 1964. Sap pressure in vascular plants. Science. 148:339-346.

Steel, R. G. D., J. H. Torrie and D. A. Dickey. 1997. Principles and procedures of statistics. In: A Biometrical Approach. 3rd Edition, McGraw Hill, Inc. Book Co., New York. 352-358.

Sunilkumar, G., L. M. Campbell and L. Puckhaber. 2006. Engineeing cotton seed for use in human nutrition by tissue specific reduction of toxin gossypol. Proceedings of the National Academy of Sciences. 103:18054-18059.

Uniyal, R. C. and A. R. Nautiyal. 1998. Seed germination and seedling extension growth in OugeiniadalbergioidesBenth under water and salinity stress. New Forests. 16:265-272.

Uzilday, B., I. Turkan, A.H. Sekmen, R.Ozgur, H.C. Karakaya. 2012. Comparison of ROS formation and antioxidant enzymes in Cleome gynandra (C4) and Cleome spinosa (C4) under drought stress. Plant Science. 182:59-70.

Wang, R., S. Ji, P. Zhang, Y. Meng, Y. Wang, B. Chen and Z. Zhou. 2016. Drought effects on cotton yield and fiber quality on different fruiting branches. Crop Science. 56:1265-1276.

Wazeer, S., M.W. Shahzad, A. Palwasha, Q. Ali, H.M. Ahmad, M. Faisal, S. Hussain, M.Z. Chudhry, A. Ali and W. Rehman. 2020. Evaluation of cotton varieties against whitefly and cotton leaf curl virus and its correlation with environmental conditions. Journal of Global Innovations in Agricultural and Social Sciences 8:80-97.

Wilhite, D. A., M. V. K. Sivakumar and R. S. Pulwarty. 2014. Managing drought risk in a changing climate: the role of national drought policy. Weather and Climate Extremes. 3:4-13.

Wu, X. L. and W. K. Bao. 2012. Statistical analysis of leaf water use efficiency and physiology traits of winter wheat under drought condition. Journal of Integrative Agriculture. 11:82-89.

Zubair, M., S. U. Ajmal, M. Anwar and M. Haqqani. 2007. Multivariate analysis for quantitative traits in mungbean [*Vignaradiata* (L.)] Wilczek. Pakistan Journal of Botany. 39: 103-113.