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Research Article

Assessment of genetic architecture of cotton germplasm for drought tolerance: A focus on morpho-physiological and biochemical attributes

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Abstract

Cotton (*Gossypium hirsutum*) is a vital fiber and cash crop, but water scarcity significantly impacts its development and production. The drought tolerance of 15 genotypes was evaluated at the seedling stage under three water regimes: control, 40%, and 20% field capacity. Significant variations in sodium ions (Na^+) were observed across all morphological and physiological traits. Key traits like fresh root weight, shoot length, total chlorophyll, hydrogen peroxide (H_2O_2), K^+/Na^+, and potassium ions (K^+) showed strong interactions between drought stress and genotype (D × G). Excised leaf water loss (ELWL) was positively correlated with shoot length (SL) under both control and drought conditions, while negatively associated with fresh root weight. Shoot length had a positive correlation with all attributes except Na+. Fresh root weight was negatively correlated with H_2O_2 but positively with other traits. Potassium ions were positively associated with SL, fresh root weight, and chlorophyll content. Genotypic correlations showed positive relationships for all biochemical traits except H_2O_2. Traits like root length, shoot length, ELWL, relative water content, proline, peroxidase (POD), H_2O_2, and K^+/Na^+ can differentiate drought-tolerant genotypes. Genotypes RH-622, FH-144, CIM-608, and MNH-886 showed potential for developing drought-resistant cotton cultivars.

Keywords: allotetraploid; biplot analysis; climate change; drought tolerance; fiber crop; physiological characteristics

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Introduction

The specie *Gossypium* display greater morphological diversity from perennial herbaceous to tall tree with an approximate height of 15 m containing a wide range of reproductive and vegetative traits (Zahid *et al.*, 2021). cotton (*Gossypium hirsutum*) is a member of family Malvaceae which belongs to genus *Gossypium* (Ullah *et al.*, 2024). The genus *Gossypium* comprises of 50 species including 45 diploid species and 5 species are allotetraploid, there are 4 cultivated species and 46 are wild (Zafar *et al.*, 2023). Among these, *Gossypium arboreum* and *Gossypium herbaceum* are diploid (2n = 2x = 26) whereas, *Gossypium hirsutum* and *Gossypium barbadense* are allotetraploid (2n = 4x = 52) (Singh, 2004). In Pakistan, cotton cultivation has decreased from the last decade compared to *Saccharum officinarum*, *Zea mays*, *Solanum tuberosum*, and *Oryza sativa* have taken its place (Rafiq *et al.*, 2024). The area under cotton cultivation was recorded 2.4 million hectares for year 2023-24 whereas, year 2022-23 recorded 2.1 million hectares showed a growth of 13.1 %. Although, the area under cotton cultivation has declined over the years however, the production has been increased which is attributed to conducive weather, better input supply, improved crop cultural practices, and supporting price both at national and international markets (Pakistan economic survey, 2023-24). Pakistan is producing 10-12 million bales of cotton and importing 2 million bales to fill the supply gap. The both biotic, abiotic and unpredictable climate has caused a cumulative loss of 21% in cotton production from the last few year (Hussain *et al.*, 2023). According to an estimate, about 20% of the cotton land area in Pakistan predominantly affected by water scarcity (Hussain *et al.*, 2019).

Water shortage is becoming a serious threat to cotton crop (Singh, 2004). Plants have four different types of drought stress management mechanisms: avoidance, tolerance, recovery, and escape (Fang and Xiong, 2015). Among these, drought tolerance and avoidance are the major strategies used by plants to combat drought stress (Rasheed *et al.*, 2022). At the seedling stage, the tolerance variability is accessible. *Gossypium hirsutum* sensitivity and tolerance under drought stress could be evaluated through morphological attributes (Jaleel *et al.*, 2009). The water stress condition can be overcome via developing drought resilient cotton varieties with ability to provide sustainable crop production especially areas for areas under drought condition with no exception to Pakistan (Malik *et al.*, 2006). The global climate change taken place in recent times has gained attention around the globe (Trenberth *et al.*, 2015; Powell and Reinhard, 2016). Meanwhile, the delayed/reduced precipitation, its duration and spatial extent caused by extreme meteorological events influenced agricultural production and socio-economic development especially in agro-based economies (Wilhite *et al.*, 2014).

Cotton is being considered among the vital component of textile industry around the globe and a large source of natural fiber. Cotton production is influenced by both drought and salt stress once grown under arid or semi-arid conditions (Ameen *et al.*, 2024). Therefore, the high yield and quality has become the priority from limited available resources. Drought tolerance is considered an ability of plant to survive under water limited conditions. To cope with drought stress, plants have evolved multiple regulatory strategies, including reducing water loss, balancing water supply to important organs, and maintaining cell water content (Zhang *et al.*, 2024). Crop plants display three modes of actions known as drought tolerance, escape and avoidance (Singh, 2004). In literature, it has been documented that various physiological attribute i.e., relative leaf water contents, stomata size and frequency, osmotic potential, root morphology, leaf water content and stomatal conductance, etc., which could be the selection criteria for drought tolerance in plants (Başal and Ünay, 2006; Liu *et al.*, 2008). It is noteworthy, plants with higher water content are considered drought-tolerant (Brito *et al.*, 2011). Development of drought tolerant genotypes is a major challenge for the cotton breeders because of the complex inheritance pattern of quantitative drought response related traits and the difficulty in accurate measurement of the polygenic trait (Sharif *et al.*, 2024).

The present investigation was designed to determine the genetic origin and variation among drought tolerant and susceptible cotton cultivars at seedling stage. The selected genotypes and stress tolerance selection indices will be helpful for designing future plant breeding program based on climate smart agriculture.

The research study was conducted with the following objectives: A) To evaluate the drought tolerant and susceptible genotypes; B) To understand the genetics of drought tolerance.

Materials and Methods

Plant material and plant growth condition

The experiment was carried out in the Laboratory of Department of Botany, Postgraduate Agriculture Research Station (PARS), University of Agriculture, Faisalabad, Pakistan. Cotton germplasm consist of fifteen lines were collected from Cotton Research Station (CRS) Bahawalpur and Central Cotton Research Institute (CCRI) Multan. The polyfoam cups were filled with soil having normal pH. The cups were placed in laboratory. The seeds were thoroughly soaked in tap water for 5 hours. Three seeds were sown in each cup and irrigated under normal field capacity. Cotton seedlings were thinned after emergence and kept healthy seedlings. After 15 days, water stress was applied under various levels of field capacity. The experiment was carried out under completely randomized design (CRD) method. For this purpose, the experiment was conducted in triplicates under normal (100% moisture level), and two stress conditions that were medium stress (40% moisture capacity) and severe stress (20% moisture capacity). Afterwards, irrigation was done according to field capacity and stress level. The data was collected after 45 days related to morphological, physiological and biochemical attributes. The list of cotton varieties collected from various Institute are given in Table 1.

Table 1. Name of genotypes of upland cotton used in the experiment

Sr. No	Genotypes	Sr. No	Genotypes
1	CIM-598	9	CIM-608
2	FH-177	10	VH-329
3	FH-214	11	MNH-886
4	IUB-212	12	AA-703
5	RH-622	13	FH-144
6	KZ-189	14	VH-325
7	MNH-992	15	SAU-1
8	VH-295		

Data collection

To prevent the roots from breakage, seedlings were gently pulled out of the sand. The shoot was separated from root by cutting their joints. To remove sand, roots were thoroughly washed with water. The root length was measured by using measuring tape, mean value for each genotype was calculated and statistically analyzed.

Na^+ and K^+ determination

A homogenous mixture of 3 g of leaf tissues and 8 mL of distilled water was heated on a hot plate to 90 °C for 3 hours in order to determine the K^+ concentration. The K^+ content was then measured using an atomic absorption spectrophotometer (TA-S-986; Persee; China). Leaf tissues weighing about 2 g were crushed in 10 mL of distilled water by heating at 80 °C for three hours in order to determine the Na^+ content. Ion chromatography was utilised to determine the Na^+ concentration (DX-300; Sunnyvale, C.A., U.S.A.).

Chlorophyll content

Photosynthetic tissues of plant were collected from the middle part of the plant and then chlorophyll content was determined using chlorophyll meter (SPAD 502 Plus) as SPAD value.

Free proline content

By adhering to the procedure outlined by Bates *et al.* (1973) the proline contents were evaluated 5 mL of 3% sulfosalicylic acid were combined with 0.1 g of powdered leaf tissues. Centrifugation was performed for 10 minutes at 11,000 rpm to separate the supernatant. Then an equal amount of glacial acetic acid and 6 M ortho phosphoric acid was combined to create a 3% Ninhydrin solution. Then measure out 1 mL of each ingredient—glacial acetic acid, ninhydrin solution, and leaf extract supernatant—and pour them into cuvettes. Thereafter, incubation took place for 50 minutes at 100 °C. The mixture was then cooled using an ice bath, and an organic layer was produced by adding 0.5 mL of toluene and thoroughly mixed for 6 minutes while the aqueous layer was eliminated. After that, an organic layer was applied to an ELISA plate, and toluene was used as a blank to measure the absorbance at 520 nm for the standard curve. The organic layer was added to the ELISA plate, and toluene was used as the standard curve's blank to measure the absorbance at 520 nm.

Hydrogen peroxide

According to Bernt and Bergmeyer's (1974) approach, H_2O_2 content was measured. The crop's leaf tissues were maintained at -80 °C after harvest. With the aid of a SCILOGEX D2012 speed micro-centrifuge, 0.1 g of leaf tissue was homogenised and combined with 5 mL of pre-chilled acetone. Centrifugation was then performed at 3200 rpm for 9 min at 4 °C. One millimetre of supernatant was then mixed with 0.1 mL of 95% (v/v) hydrochloric acid (HCL), 0.3 mL of ammonia, and 30% (v/v) titanium tetrachloride before centrifugation was carried out at 11,000 rpm for 9 min at 4 °C as part of the subsequent analysis. The sediments were then rinsed using commonly used cold acetone, centrifuged for 9 minutes at 12,000 rpm, and then dissolved in 2 mL of 1 M H_2SO_4. Then, using a standard curve based on known concentrations, the absorbance was measured at 410 nm using a Nano Drop Spectrophotometer (Model No. ND-8000 Thermo Scientific).

Peroxidase activity

The method of Fielding and Hall (1978) was used to estimate the peroxidase activity, 0.05 M sodium phosphate buffer was used to homogenize the preserved leaf tissues. Centrifugation was performed at 10,000 rpm for 20 minutes, and the supernatant was then transferred into an Eppendorf tube. After that, an equal volume of guaiacol and H_2O_2 were combined to create a 3 mL reaction mixture, and the enzyme extract was added. Then, using a Nano Drop Spectrophotometer, absorbance was measured at 470 nm (Model No. ND-8000 Thermo Scientific).

Relative water content

From both control and drought stress condition, three-leaf samples were taken from selected plants. Fresh weight of leaves was taken by using digital balance. To obtain turgid leaf weight, leaves were then immersed in tap water overnight. The leaves were kept at room temperature for an hour to dry after the estimated weight of the turgid leaves was determined. The leaf tissues were kept in an oven at 70 °C for 72 hours to determine the dry weight.

Finally, relative water content was determined by using Ali *et al.* (2011).

$$RWC = [(Fresh\ weight - Dry\ weight) / (Turgid\ weight - Dry\ weight)]\ x\ 100.$$

Excised leaf water loss

Fresh leaves were retrieved from polythene bags for the calculation of excised leaf water loss, and weight was recorded using a digital scale. Leaves were kept at room temperature for 24 hours in order to provoke wilting. Weight of the wilting leaf was measured. The leaf tissues were kept in an oven at 70 °C for 72 hours to determine the dry weight. Excised leaf water loss was calculated according to formula used by Ali *et al.* (2011).

$$ELWL = (\ Fresh\ weight - wilted\ weight\ )/\ Dry\ \ weight$$

Statistical analysis

Using CRD, the screening was performed. It was decided to use variance analysis for significance estimation ($p<0.05$). Additionally, using Microsoft Excel and XLSTAT version 2012.1.02, principal component analysis, and simple correlation coefficients were calculated.

Results

The investigation was carried out employing factorial under completely randomized design (CRD) method. For this purpose, the experiment was conducted in triplicates under normal (100% moisture level), and two stress conditions that were medium stress (40% moisture capacity) and severe stress (20% moisture capacity). Analysis of variance showed significant differences for all of the observed variables, i.e., leaf relative water content (%), excised leaf water loss, total chlorophyll content, shoot length (cm), root length (cm), fresh shoot weight (g), fresh root weight (g), dry shoot weight (g), dry root weight (g), peroxidase (POD), hydrogen peroxide (H_2O_2), proline, sodium ion (Na^+), potassium ion (K^+), sodium to potassium ratio (Na^+/K^+ ratio) given in Table 2.

Table 2. Mean sum of square of all recorded parameters of upland cotton under various drought conditions

Source of variation	DF	RL	SL	FRW	FSW	DRW	DSW	RWC	ELWL
Drought	2	10.56**	2.56***	0.06***	0.03*	0.02**	0.02**	48.33*	1.21**
Genotypes	14	19.35**	12.99***	0.14***	0.06***	0.08***	0.01***	40.53*	0.46**
Drought × Genotypes	28	19.28**	21.03***	0.16***	0.08***	0.08***	0.04***	37.27*	0.16*
Error	90	6.46	0.13	0.01	0.07	0.02	0.03	12.38	0.04
Total	134								

Source of variation	DF	K^+	Na^+	K^+/Na^+	TChl	Pro	POD	H_2O_2
Drought	2	171.94***	1.85ns	4.39***	43.09***	0.09*	22.95*	0.21***
Genotypes	14	420.86***	19.90***	21.26***	69.62***	0.21**	23.19*	0.14***
Drought × Genotypes	28	590.53***	26.20***	27.14***	48.93***	0.08*	20.27*	0.15***
Error	90	3.93	0.79	0.37	0.59	0.02	6.17	0.09
Total	134							

*, **, ***, significant at $P<0.05$, $P<0.01$, $P<0.001$ respectively and ns non-significant at $P>0.05$.

DF: Degree of freedom, 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), RWC: Relative water content (%), ELWL: Excised leaf water loss, K^+: Potassium ion , Na^+: Sodium ion, Na^+/K^+ ratio: Sodium to potassium ratio, TChl: Total chlorophyll content, Pro: Proline, POD: Peroxidase, H_2O_2: Hydrogen peroxide

Highest hydrogen peroxide concentration (0.33 μmolg^{-1} FW) was found for RH-622 while lowest (0.17 μmolg^{-1} FW) for MNH-992 under normal sowing. For this parameter, VH-325 and MNH-886 exhibited maximum and minimum values under drought stress, respectively. Highest peroxidase activity was recorded for MNH-886 under drought stress while lowest value for this antioxidant was calibrated for AA-703 under normal sowing. AA-703 and FH-144 showed maximum proline content under control and stress conditions, i.e., 0.49 μmolg^{-1} FW and 0.60 μmolg^{-1} FW, respectively. Lowest value for this attribute was measured for FH-Noor under drought stress. Excised leaf water loss and relative water content under normal sowing was

estimated for IUB-212 and RH-622, respectively while under drought stress FH-144 and RH-622 exhibited highest values for these traits (Table 3).

Table 3. Maximum and minimum values and representative genotypes for the parameters of cotton grown under normal and drought conditions

Traits	Normal condition			
	Genotype	Max. value	Genotype	Min. value
Root length (cm)	FH-144	15.56	CIM-598	7.04
Shoot length (cm)	FH-144	23.73	IUB-212	11.01
Fresh root weight (g)	FH-144	0.68	AA-703	0.06
Fresh shoot weight (g)	RH-622	1.78	CIM-608	1.30
Dry root weight (g)	RH-622	0.28	AA-703	0.06
Dry shoot weight (g)	MNH-992	0.45	VH-329	0.31
Excised leaf water loss	IUB-212	0.71	CIM-608	0.42
Relative water content (%)	RH-622	68.8	IUB-212	59.6
Hydrogen peroxide (µmol g^{-1} FW)	RH-622	0.33	MNH-992	0.17
Peroxidase activity (U mg^{-1} protein)	AA-703	16.3	SAU-1	9.5
Proline content (µmol g^{-1} FW)	AA-703	0.49	CIM-608	0.15
Potassium content (mg/L)	KZ-189	21.4	FH-144	8.6
Sodium content (mg/L)	CIM-608	187.3	FH-177	144.1
Potassium/Sodium ratio	CIM-608	16.1	CIM-598	7.4
Total chlorophyll	RH-622	45.2	VH-329	38.1

Traits	Drought condition			
	Genotype	Max. value	Genotype	Min. value
Root length (cm)	FH-144	21.36	SAU-1	9.43
Shoot length (cm)	FH-144	19.93	IUB-212	9.03
Fresh root weight (g)	RH-622	1.45	FH-214	0.4
Fresh shoot weight (g)	FH-144	1.61	CIM-598	0.88
Dry root weight (g)	CIM-608	0.25	MNH-88	0.1
Dry shoot weight (g)	CIM-608	0.41	VH-295	0.22
Excised leaf water loss	FH-144	1.56	KZ-189	0.67
Relative water content (%)	RH-622	63.5	VH-325	53.8
Hydrogen peroxide (µmol g^{-1} FW)	VH-325	0.39	MNH-886	0.13
Peroxidase activity (U mg^{-1} protein)	MNH-886	22.9	FH-214	9.3
Proline content (µmol g^{-1} FW)	FH-144	0.60	CIM-598	0.14
Potassium content (mg/L)	CIM-598	18.3	FH-144	7.3
Sodium content (mg/L)	FH-144	198.3	AA-703	145.2
Potassium/Sodium ratio	VH-295	17.3	RH-622	8.2
Total chlorophyll	FH-144	44.8	AA-703	25.2

Correlation analysis

Genotypic and phenotypic association coefficients among the recorded attributes under control and two levels of drought stress are provided in Table 4. Shoot length was found to be positively associated with all the parameters except Na^+. Fresh root weight was negatively associate with H_2O_2 and positively corelate with all other attributes. The genotypic coefficient of fresh shoot weight is almost equal to the phenotypic coefficient of fresh shoot weight. Dry root weight had negative association with shoot length under control and drought levels. Chlorophyll content was positively associate with all the trait under control and negatively associate with all the parameters under both drought level. Hydrogen peroxide was negatively associated with all the parameters under control and drought levels. The genotypic coefficient was almost more than phenotypic

coefficient for all the traits except proline, peroxidase, and root length. ELWL was positively associated with SL for both control and drought regimes while negatively associated with fresh root weight. Peroxidase activity was negatively correlated with dry root weight under both control and drought level. Higher genotypic to phenotypic correlation coefficients observed for this trait. Positive association was found between potassium ion with SL, FSW and chlorophyll content. Genotypic correlation coefficient values were positively associated for all the biochemical trait except H_2O_2.

Table 4. Phenotypic (lower diagonal) and genotypic correlation (upper diagonal) matrix for recorded parameters in control and drought levels

Traits	Stress conditions	RL	SL	FRW	FSW	DRW	DSW	RWC	ELWL	K+	Na+	K+/Na+	Chlr	Pro	POD	H_2O_2
RL	C		1.009	1.006	0.983	1.009	0.889	0.965	0.948	- 0.921	-0. 973	0.976	0.764	0.729	- 0.561	0.564
	20%		1.008	1.010	0.959	1.010	0.915	0.945	0.657	- 0.960	- 0.545	0.614	0.846	0.784	- 0.297	0.583
	40%		1.005	1.025	0.957	1.115	1.119	0.845	0.564	-1.016	-0.461	0.467	0.956	0.795	-0.197	- 0.994
SL	C	1.001		0.083	0.103	0.769	0.754	1.015	- 0.911	0.829	- 0.873	0.422	0.918	0.787	0-576	- 0.912
	20%	1.006		0.064	0.189	0.854	0.757	1.019	-0.867	0.963	- 0.452	0.514	0.934	0.894	- 0.485	- 0.977
	40%	1.004		0.039	0.199	0.949	0.858	1.019	-0.764	0.948	-0.293	0.619	0.962	0.917	- 0.394	- 0.971
FRW	C	0.091	1.003		0.310	0.976	0.012	- 0.829	0.896	0.856	- 0.619	0.018	0.952	0.225	- 0.284	- 0.954
	20%	0.072	1.006		0.395	0.979	0.017	- 0.956	0.766	0.992	- 0.810	0.025	0.367	0.298	-0.298	- 0.928
	40%	0.095	1.009		0.465	0.998	0.041	-0.998	0.615	0.963	-0.913	0.077	0.589	- 0.485	0.191	- 0.849
FSW	C	0.225	0.038	0.938		0.159	- 0.045	1.013	0.914	0.498	- 0.314	- 0.670	- 0.567	0.983	- 0.172	- 0.853
	20%	0.301	0.024	0.950		0.710	- 0.048	1.022	0.959	0.947	-0.523	0.213	- 0.483	- 0.260	0.221	- 0.958
	40%	0.287	0.019	0.975		0.769	-0.194	1.076	0.521	1.025	- 0.875	0.150	-0.398	-0.298	-0.297	- 0.989
DRW	C	0.786	-0.038	0.173	1.006		0.192	0.736	0.645	0.673	- 0.745	0.815	0.302	- 0.218	- 0.323	- 0.961
	20%	0.735	-0.046	0.185	1.009		0.199	0.726	0.796	0.794	- 0.589	0.252	0.712	0.671	0.285	- 0.944
	40%	0.669	-0.096	0.195	1.110		0.129	0.996	0.636	0.875	-0.391	0.257	0.987	0.941	0.197	- 0.337
DSW	C	0.844	0.057	0.300	0.764	0.886		0.942	0.324	- 0.790	0.931	0.978	0.986	0.955	- 0.224	- 0.930
	20%	0.834	0.448	0.365	0.845	0.905		0.912	0.157	- 0.679	0.613	0.984	0.978	0.562	0.121	-0.945
	40%	0.915	0.024	0.415	0.943	1.110		0.662	0.514	-0.543	0.404	0.931	0.956	0.452	0.108	-0.978
RWC	C	1.007	0.073	0.911	0.957	0.755	0.956		0.382	0.162	0.967	0.975	0.665	0.567	0.127	- 0.964
	20%	1.005	0.079	0.920	0.987	0.758	0.905		0.142	0.293	0.991	0.924	0.947	0.912	- 0.284	- 0.921
	40%	1.002	0.084	0.963	0.989	0.851	0.854		0.129	0.347	0.896	0.901	0.998	0.967	-0.328	- 0.962
ELWL	C	0.039	-0.152	-0.043	0.059	0.002	1.005	0.984		- 0. 928	0.919	0.878	0.521	0.651	- 0.811	- 0.942
	20%	0.076	-0.245	-0.056	0.695	0.007	1.009	0.675		- 0.960	0.963	0.929	0.944	0.924	0.182	- 0.919
	40%	0.062	-0.301	-0.075	0.761	0.014	1.010	0.546		-0.977	0.997	0.903	0.997	0.987	0.148	- 0.978
K+	C	- 0. 136	0.614	- 0.039	0.206	- 0.054	- 0.819	- 0.901	- 0.912		- 0.937	- 0.879	- 0.103	- 0.331	- 0.775	- 0.806
	20%	- 0.156	0.715	- 0.046	0.349	- 0. 084	- 0.965	-0.876	- 0.959		- 0.923	- 0.951	- 0.977	- 0.923	- 0.792	- 0.876
	40%	-0.234	0.801	-0.078	0.451	-0.149	-0.989	-0.746	-1.006		-0.871	-0.923	-0.995	-0.976	-0.810	- 0.016
Na+	C	0.015	-0.577	-0.113	-0.132	0.092	1.010	0.869	0.892	-0. 970		0.971	0.421	0.02	- 0.615	- 0.037
	20%	0.073	-0.597	0.245	-0.245	0.099	1.032	0.766	0.936	- 0.554		0.984	0.964	0.982	0.452	-0.067
	40%	0.091	-0.671	0.346	-0.341	0.117	1.067	0.651	0.984	-0.461		0.997	0.988	0.945	0.345	-0.091
K+/Na+	C	0.703	0.952	0.966	0.969	0.845	0.969	0.512	0.865	- 0.870	0.967		0.195	0.231	0.321	0.214
	20%	0.774	0.918	0.975	0.651	0.866	0.924	0.654	0.929	- 0.451	0.641		0.234	0.321	- 0.122	0.451
	40%	0.691	0.898	0.983	0.481	1.005	0.910	0.769	0.936	-0.239	0.476		0.460	0.412	-0.095	0.615
Chlr	C	-0.081	1.009	- 0.437	- 0.551	0.785	0.626	0.633	0.499	- 0.609	0.422	0.087		- 0.156	- 0.862	0.789
	20%	-0.073	1.051	- 0.345	- 0.752	0.653	0.947	0.342	0.944	- 0.801	0.541	0.764		- 0.238	0.321	0.772
	40%	-0.059	1.086	-0.249	-0.760	0.751	1.006	0.175	1.020	-0.915	0.691	0.851		-0.186	0.175	0.819
Pro	C	0.0325	0.052	0.093	0.171	1.009	0.551	0.541	0.637	- 0.313	0.019	0.225	- 0.155		- 0.021	-0.045
	20%	0.0421	0.061	0.105	0.195	1.116	0.665	0.328	0.790	-0.532	0.052	0.275	-0.319		-0.038	-0.161
	40%	0.0416	0.073	0.115	0.259	1.276	0.912	0.124	0.876	- 0.874	0.076	0.328	- 0.432		- 0.047	-0.254
POD	C	0.016	-0.020	1.076	-0.857	- 0.806	0.798	0.789	- 0.799	- 0.754	- 0.607	- 0.312	- 0.854	0.015		0.917
	20%	0.029	0.542	1.081	0.051	- 0.163	0.377	0.401	- 0.678	- 0.589	0.231	- 0.098	0.532	0.179		0.985
	40%	0.036	0.612	1.097	0.084	-0.245	0.246	0.241	-0.534	-0.391	0.105	-0.004	0.284	0.201		0.983
H_2O_2	C	-0.161	0.071	0.080	1.007	1.175	0.455	0.645	0.126	0.915	0.851	0.798	0.241	-0.054	0.971	
	20%	-0.189	0.085	0.095	1.010	1.215	0.595	0.716	0.239	0.513	0.225	0.727	0.415	-0.116	0.958	
	40%	-0.143	0.091	0.097	1.034	1.311	0.641	0.901	0.341	0.404	0.275	0.891	0.651	-0.245	0.938	

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), RWC: Relative water content (%), ELWL: Excised leaf water loss, K^+: Potassium ion , Na^+: Sodium ion, Na^+/K^+ ratio: Sodium to potassium ratio, TChl: Total chlorophyll content, Pro: Proline, POD: Peroxidase, H_2O_2: Hydrogen peroxide

Principal component analysis

In this study, three principal components (PCs) showed value more than one. These components used for further explanation. The overall variation among the genotypes evaluated for seedling characteristics was explained by the first three PCs at 58.586%. (Table 5). Biplot analysis displayed the relationship among various indices (Figures 3 and 4). The Eigen values for all the traits in both conditions, i.e., control and drought stress were more than one which guarantee the validity of results. Under normal sowing conditions, the first and second component explained 48.31 and 11.73% variability, respectively. The PCI contributed maximum towards the variability (41.457%) followed by PC2 (8.946%). While all other traits had positive factor loadings, the trait H_2O_2 indicated negative loadings on PC I. In 2nd PC three traits RL, H_2O_2, Chlorophyll, POD, Proline, Na^+ and K^+ exhibited maximum positive factor loading while other parameters SL, ELWL, RWC, FRW, FSW, DRW, DSW and Na^+/K^+ had negative loading on PC2.

Table 5. Principle component analysis of different morpho-physiological and Biochemical traits in cotton under normal and drought conditions

Components	Control condition				Drought -condition		
	PC1	PC2	PC3	PC4	PC1	PC2	PC3
Eigenvalue	5.587	1.647	1.407	1.236	6.219	1.342	1.227
Variability (%)	39.908	11.761	10.050	8.828	41.457	8.946	8.183
Cumulative %	39.908	51.669	61.719	70.547	41.457	50.403	58.586

The scree plot certainly illustrates that seven factors contribute to the total variation in this study under both normal and water-stressed settings. However, under both situations, seven of the four principal components PCs and three PCs revealed eigen values ≥ 1. Among the cotton genotypes assessed for drought-related features, four PCs under normal conditions and three PCs under water stress interpolated cumulative variance of 70.547 and 58.586%, respectively (Figures 1, 2 and Table 6). Table 6 indicates that the remaining components, under both normal and water-stressed situations, exhibited the total variation. Under normal and water-stressed conditions, respectively, the PC1 displayed the largest variability of 39.90 and 41.45%, followed by the PCs 2, 11, 76, and 8.94%, and 3, 10.05, and 8.18%.

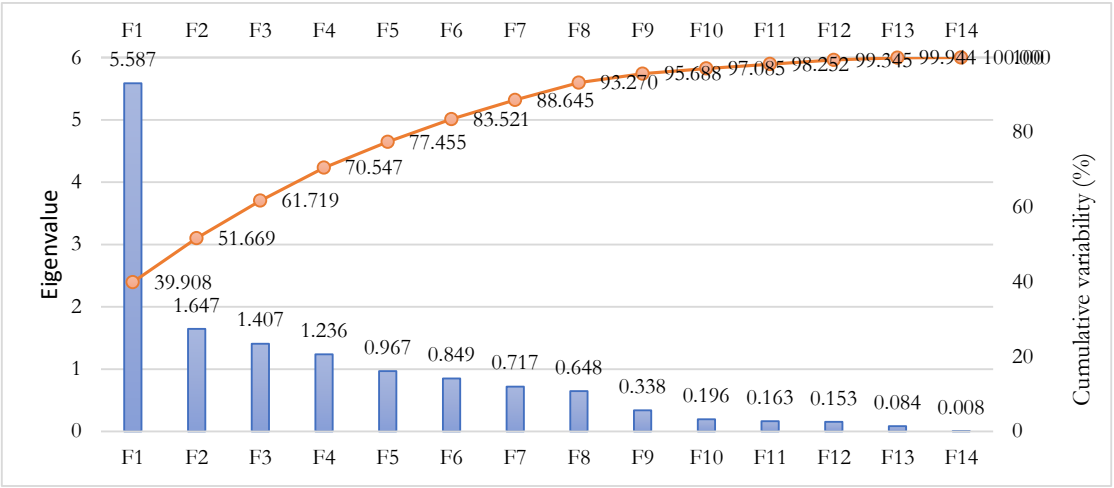

Figure 1. Scree plot between eigenvalues and factors under normal conditions

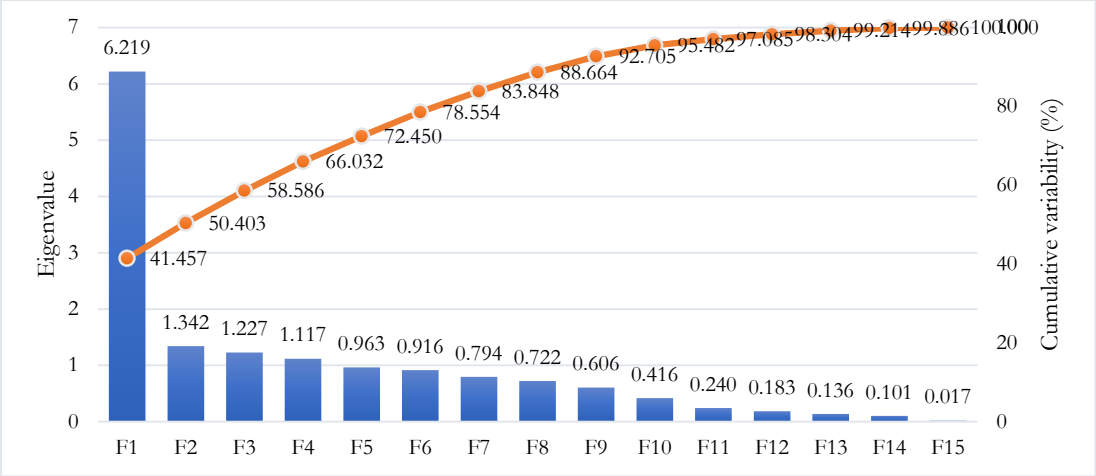

Figure 2. Scree plot between eigenvalues and factors under drought conditions

Table 6. Factor loadings by various seedling traits in cotton genotypes at 100% moisture level

Variables	PC1	PC2	PC3	PC4	PC5
RL	0.662	0.086	0.248	0.129	-0.048
SL	0.911	-0.054	-0.033	-0.038	-0.053
FRW	0.169	-0.004	-0.393	0.7	0.037
FSW	0.443	-0.134	-0.123	-0.362	0.572
DRW	0.891	-0.004	0.113	-0.107	-0.071
DSW	0.917	-0.036	0.004	-0.114	0.095
ELWL	0.013	-0.206	0.785	0.06	-0.293
RWC	0.036	-0.332	0.524	0.306	0.575
Chlr	0.693	0.18	0.095	0.207	-0.12
POD	0.004	0.405	0.062	0.517	0.185
H_2O_2	-0.166	0.661	0.19	-0.124	-0.151
Proline	0.099	0.721	0.172	-0.149	0.351
Na+	0.899	0.058	0.033	-0.07	0.038
K+	0.94	0.015	-0.105	-0.041	0.039
K+/Na+	0.938	-0.056	-0.044	0.04	-0.051

Variables and genotypes are superimposed on the plot as vectors, as seen by a PC biplot in Figure 1. The distance between factors and PC1, PC2, and PC3 revealed these variables' contribution in the variation of the various accessions under study. The biplot exhibited that the root length of genotype 2 (FH-177) contributed maximum towards variability in cotton genotypes because it had maximum distance from the origin (Figure 3).

The first and second components supported the presence of a 50.40% variance under drought stress circumstances. There were also many eigenvalues for each of the qualities. It was discovered that hydrogen peroxide was negatively correlated with other parameters. IUB-212, CIM-518, VH-325, and FH-177 were shown to have the most diversity for biochemically associated features, including H_2O_2, Proline, and POD on factor plane. For physiological features, such as excised leaf water loss and relative water content, AA-107 and FH-144 were deemed desirable.

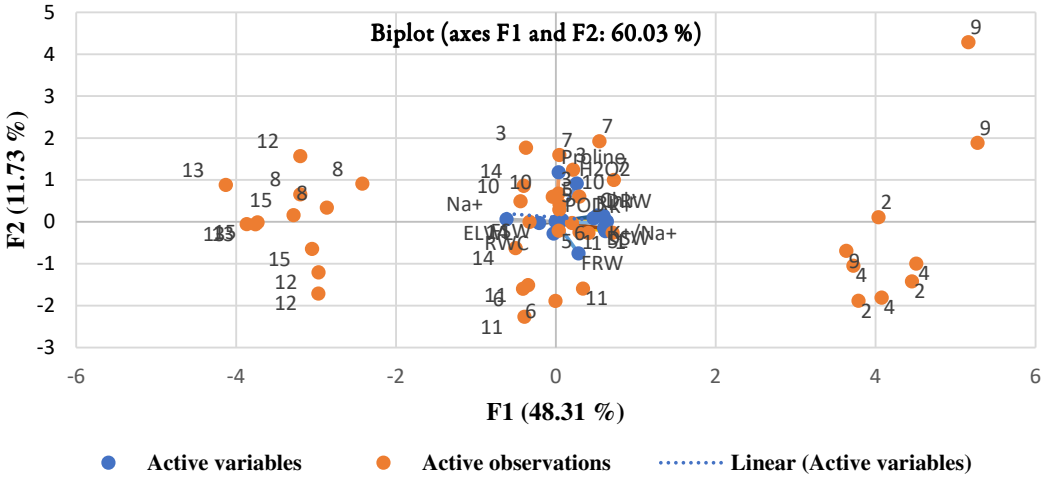

Figure 3. Biplot analysis of 15 cotton genotypes grown under control condition
RL: Root length (cm), SL: Shoot length (cm), FRW: Fresh root weight (g), FSW: Fresh shoot weight (g), DRW: Dry root weight (g), DSW: Dry shoot weight (g), RWC: Relative water content (%), ELWL: Excised leaf water loss, K^+: Potassium ion , Na^+: Sodium ion, Na^+/K^+ ratio: Sodium to potassium ratio, Chlr: Total chlorophyll content, Pro: Proline, POD: Peroxidase, H_2O_2: Hydrogen peroxide

For morphological features, RH-622 and CIM-608 performed best (Figure 4). Based on these analyses, it was observed that IUB-212, CIM-518 and VH-325 performed well under normal and drought stress conditions while FH-177, AA-107 and FH-144 showed good results under drought stress condition. Some genotypes did not perform under normal and drought stress conditions including SAU-1, VH-995 and KZ-189. Variables and genotypes are superimposed on the plot as vectors, as shown by a PC biplot in Figure 2. The variance of the variables in relation to PC1 and PC2 demonstrated the role that these variables involved in the variation of the various accessions under study. In the study of cotton genotypes, it was discovered that the total contribution of POD to variability was maximum (Figure 4).

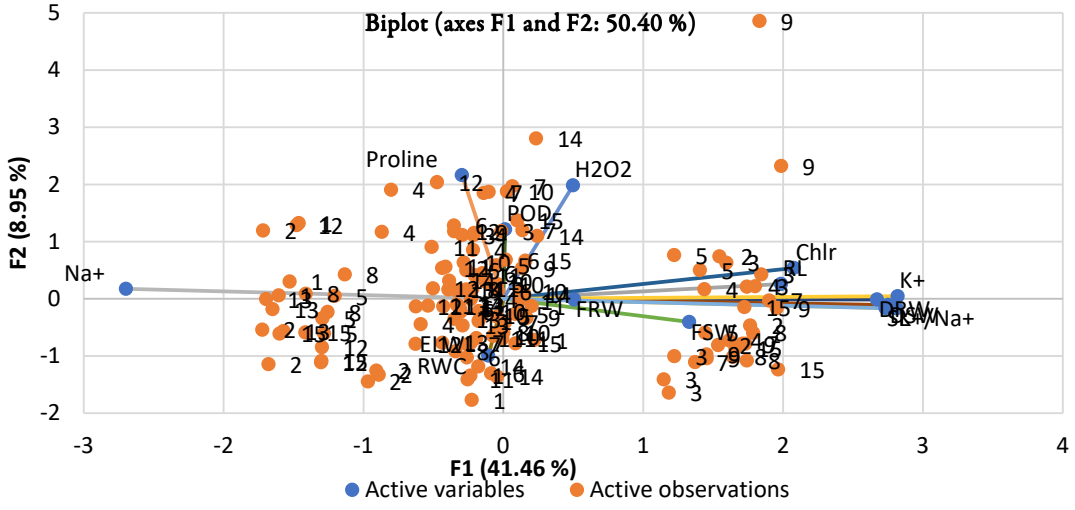

Figure 4. Biplot analysis of 15 cotton genotypes grown under drought stress condition
RL: Root length (cm), SL: Shoot length (cm), FRW: Fresh root weight (g), FSW: Fresh shoot weight (g), DRW: Dry root weight (g), DSW: Dry shoot weight (g), RWC: Relative water content (%), ELWL: Excised leaf water loss, K^+: Potassium ion , Na^+: Sodium ion, Na^+/K^+ ratio: Sodium to potassium ratio, Chlr : Total chlorophyll content, Pro: Proline, POD: Peroxidase , H_2O_2: Hydrogen peroxide

The factors' distance from PC1 and PC2 demonstrated their role in the variance of the various accessions under study. The biplot demonstrated that the total contribution of H_2O_2 and POD to cotton genotype variability was highest.

Discussion

Due to the rise in food demand for human consumption and the changing climate, food security is being compromised globally (Lesk *et al.*, 2016). These climatic variations cause heat stress and drought, which have an impact on agricultural output and ultimately threaten food security. Around the world, droughts frequently begin with low moisture content and irregular rainfall patterns (Lobell *et al.*, 2011). Two factors are necessary for cotton genotypes to be drought-tolerant: self-selection or natural selection. The drought negatively impacts plant growth, reproduction, and physiology, reducing crop plant yield (Yordanov *et al.*, 2000; Barnabas *et al.,* 2008). Therefore, it is the responsibility of the breeders to develop genotypes of crop plants that are tolerant to drought as there are more risks of water shortage as the temperature rises. Drought stress has an impact on the germination and growth of cotton seedlings. According to numerous research, drought has a detrimental impact on seed germination and seedling growth (Kaya *et al.*, 2006; Farooq *et al.*, 2009; Zafar and Azhar, 2015). To meet the demand of ever-increasing global population, cotton production must be sustainable because it is negatively impacted by a variety of biotic challenges, particularly water stress. Develop drought-tolerant cotton accessions that can withstand drought stress effectively so that there are fewer chances of yield losses in drought-stressed locations where water resources are insufficient. It has made incredible efforts to increase cotton production by implementing genomic metrics that consider physiological and yield-contributing features to balance cotton development programs suitable for our water-limited setting (Rasheed *et al.*, 2023).

To examine how plants behave and their variability during the seedling stage, fifteen genotypes were collected and planted in polyfoam cups under controlled conditions. The morphological and physiological traits RL, SL, FRW, FSW, DRW, DSW, RWC, and ELWL were examined under different water regimes. Biochemical characters were also analyzed, i.e., total chlorophyll, POD, Proline, H_2O_2, Na^+, K^+ and Na^+/K^+. In this study as the moisture level increases the root length of cotton plant also increases and then reduced gradually. As the water stress increases plants root length decreases significantly. Plants become unable to maintain their internal turgor pressure under water stress, so it results in cell division and cell elongation due to reduction in root length. These findings are consistent with those of (Khalid *et al.*, 2011) These results demonstrated that water stress at the seedling stage has an impact on most physiological and morphological features. Under moisture stress, it is crucial to ascertain how water stress affects a plant's root properties. The plant's root length at the seedling stage provides a useful indication of root growth at subsequent stages (Ali *et al.*, 2011). Pettigrew (2004) studied that due to drought stress there are variations in the performance of root length. Root parameters are considered as important criteria for screening of field crops because these are genetically controlled (Riaz *et al.*, 2013). The increase in root length might be the result of more photosynthates division for root elongation rather than shoot, as a result it takes plenty of water from the depth of soil.

Shoot length is a crucial factor to consider when analyzing how drought affects cotton and many other crops. In contrast to root growth, the principal effects of drought stress are on shoot growth. Water scarcity is responsible for the decline in performance of shoot characteristics (Pettigrew, 2004). The shoot length of cotton accessions was substantially reduced as the effects of drought stress increased. Less water loss from the shoot surface is possible if the shoot length is reduced. The findings of (Khalid *et al.*, 2011) are the outcomes for shoot length. Under drought stress, all nutrients flow to the roots to help plants absorb water from the soil's depth, which indirectly shortens shoots. Shoot length has been considered by many scientists as a selection criterion for evaluating the drought tolerance of cotton cultivars. When the osmotic pressure drops, it has a negative impact on shoot length, making it impossible for plants to survive under conditions of water stress. (Kaydan and Yagmur, 2008). Numerous efforts have been made to increase cotton's tolerance to drought.

Cotton has been the subject of extensive research into drought tolerance. The importance of the root in a plant's response to water stress and how it can also result in a slowdown in shoot growth, but how drought stress mostly impacts shoot growth as opposed to root growth. These findings were subsequently discovered by Iqbal *et al.* (2011) in *G. hirsutum* L. when it was negatively affected by water stress.

The diversification of drought-tolerant cultivars from the others was largely facilitated by morphological traits like ELWL and RWC. ELWL displays the cuticle thickness as water perspires through the epidermis after detaching from the plant (Saleem et al., 2016). Genotypes with the lowest values were suitable for excised leaf water loss because they lost the least amount of leaf water under stress. Indications of water status in plant leaves include relative water content, which is also a useful characteristic for identifying water stress (Sánchez-Blanco *et al.*, 2002). Physiological characteristics have an impact on RWC (Kramer and Boyer, 1995). Additionally, RWC declines as a result of dry conditions (Ullah *et al.*, 2012). This study found that under drought stress, cotton accessions produced more hydrogen peroxide (H_2O_2), a reactive oxygen species. Leaves affected by the increase of H_2O_2 concentration which leads to oxidative stress in plants and as a result lipid peroxidation occur (Zhang *et al.*, 2014). Moreover, at high water stress the protein present in membrane also denatured (He *et al.*, 2005). Therefore, the effects of drought stress change the permeability of cell membranes by degrading their lipids and proteins, which affects plasma membrane. As a result, the electrolyte leakage from the membrane increased, decreasing the cell membrane's capacity to withstand heat. Denatured proteins served as signalling molecules in the plasma membrane to start the genes responsible for producing enzymatic and non-enzymatic antioxidants (De Ronde *et al.*, 2000; Gulen and Eris, 2004). The concentration of peroxidase activity also increases in many high yielding genotypes. These molecules aggressively scavenged hydrogen peroxide to keep it at the ideal amount. The genotypes RH 622 and CIM 608 performed much better under all circumstances and might be regarded as drought-tolerant genotypes. A PC analysis determined the degree of character variation in the experimental material under observation, which may be used to choose the research programme to be built to increase drought resistance. Utilizing genetic resources involves breaking down total variation into its constituent parts.

Conclusions

Drought stress is currently the main factor limiting crop output and endangering the future of agriculture, it is imperative to create high-yielding and drought-tolerant cultivars. through means of morphological, physiological, and biochemical examination, i.e. RL, SL, FRW, FSW, DRW, DSW, RWC, ELWL, total chlorophyll, POD, Proline, H_2O_2, Na^+, K^+ and Na^+/K^+. We were able to pinpoint the types that would survive in arid conditions and would require less water to sprout and grow. By becoming used to the alterations brought on by water losses, these types were able to withstand the effects of drought. It is promising that these features have been discovered because they are simple to assess and may aid us in searching a gene pool for genes of particular interest All physiological, biochemical, and morphological parameters showed a wide range of variation, suggesting that we can choose between varieties that are resistant to and tolerant of drought. Due to their drought tolerance and significant genetic potential for improved performance under drought stress, out of 15 experimental varieties RH-622, FH-144, CIM-608 and MNH-886 could be exploited in breeding methods for the production of drought-tolerant varieties.

Authors' Contributions

Conceptualization, ZC, AM, and MTC; Data curation, ZC, MTC, and MMJ; Formal analysis, ZC, AM, and MTC; Funding acquisition SF, KAA, AHM and MJU; Investigation, ZC, MTC and AM; Methodology, BS; Resources, AM; Supervision, ZC, AM, MTC and MMJ Writing – original draft, revision,

proof-reading of the article. KAA, AHM, SF, MJU, ZC, AA, IH, SF and AM; Writing – review & editing, KAA, SF, AHM, MJU, ZC and AM.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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