AJAB

Assessing genetic potential and development of crosses for breeding cotton (*Gossypium hirsutum* L.) against drought

Muhmmad Kashif Shahzad Sarwar[1,2], Abdul Ghaffar[3], Saghir Ahmad[4], Shoaib Ur Rehman[1], Ummara Waheed[1*]
[1]Institute of Plant Breeding and Biotechnology, MNS University of Agriculture, Multan, Pakistan
[2]Cotton Research Station, Ayub Agricultural Research Institute, Faisalabad, Pakistan
[3]Departemnt of Agronomy, MNS University of Agriculture, Multan, Pakistan
[4]Cotton Research Institute, Multan, Pakistan

Abstract

Cotton diversity has long been studied using physiological and biochemical traits. This diversity has led to the development of various superior cotton cultivars over the year. At present and in face of climate change, development of high yielding and drought tolerant cotton varieties are necessary to fulfill the demand of ever-growing population of the world. In this study, *Gossypium hirsutum* L. germplasm (200) was evaluated under two irrigation regimes i.e., well-watered (W1) and limited water (W2) conditions. Various morphological and physiological traits were recorded under both irrigation regimes. A considerable reduction was recorded in W2 conditions in all the recorded traits except for glycine betaine, soluble sugars, and proline contents, highlighting the impact of drought on cotton germplasm. Cotton genotypes that maintained higher yield had positive correlation with biochemical traits. Out of 63 best performing genotype (superior parents based on the recorded data), FH-414, FH-415, FH-416, FH-326, FH-492, FH-Anmol, Gomal-105, Marvi, NIAB-878 and VH-327 were selected for hybridization to make crosses following Line x Tester fashion. F_1 hybrids (25 crosses) and 10 parents were again planted under W1 and W2 conditions. Out of 25 crosses, FH-326 × Marvi (CS5) and NIAB-878 × FH-414 (CS16) performed better under water deficit conditions. Quantitative real-time PCR was also performed using *GhHH3* and *GhIDD*. CS5 and CS16 had higher expression of drought tolerance causing *GhHH3* and *GhIDD* genes. The newly developed cotton crosses will pave the way for the development of high yielding drought tolerant cotton varieties in face of climate change.

Keywords: Biochemical attributes, Yield and yield components, *GhHH3*, *GhIDD*, Drought

How to cite this:

Sarwar MKS, Ghaffar A, Ahmad S, Rehman SU and Waheed U. Assessing genetic potential and development of crosses for breeding cotton (*Gossypium hirsutum* L.) against drought. Asian J. Agric. Biol. xxxx(x): 2024027. DOI: https://doi.org/10.35495/ajab.2024.027

Corresponding author email:
ummara.waheed@mnsuam.edu.pk

Introduction

Cotton (*Gossypium hirsutum* L.) is one of the main sources of natural textile fiber and holds vital position in agricultural economy (Gao et al., 2020). Inadequate cotton production in Pakistan can be attributed to the factors such as climate change, scarce quality seed and low genetic diversity (Qureshi et al., 2021). Drought is considered as serious threat that influences yield contributing features including boll number and boll weight (Sarwar et al., 2012). This scenario is predicted to worsen in the next 30 years, as over 50% of the world's regions are projected to experience water scarcity by 2050 (Gupta et al., 2020). Cotton's indeterminate growth pattern makes it more vulnerable to drought stress, which can result in a large drop in production. Compared to other crops, cotton, a glycophytic crop, has relatively higher resistance to stress. However, its production and growth are negatively impacted by extreme stress (Ullah et al., 2021). The growth and developmental processes of the cotton crop are significantly impacted by abrupt changes in climatic conditions (Rehman and Farooq, 2019). Any stage of growth can experience drought stress, and it causes huge yield losses in cotton (Mehmood et al., 2022). According to a careful estimate, drought stress can result in yield losses of up to 67%, which is more detrimental than other environmental pressures (Tokel et al., 2022). Therefore, it is very important to develop drought tolerant varieties for better cotton production. However, drought response being controlled by quantitative expression of genes hence breeding efforts alone have not met the needs till date (Loka et al., 2020).

Various morphological, physiological and biochemical features such as fiber related traits, relative water contents and proline contents have been identified as significant predictors of drought resistance in cotton (Sarwar et al., 2012; Zafar et al 2023). The importance of hybridization in biological invasions is becoming more widely acknowledged (Hovick and Whitney, 2014). As compared to their parents, hybrids can have intermediate traits or display transgressive segregation, in which some hybrid individuals deviate from parental phenotypic values in a positive or negative way (Vallejo-Marin and Hiscock, 2016). According to Birchler et al. (2006), hybrids may possess a higher mean fitness than their parents (a phenomenon referred to as heterosis or hybrid vigor).

In the recent years advanced genomic studies and genome sequencing have made it possible to analyze various genes controlling biotic and abiotic stress in various crops (Ali et al., 2019; Qanmber et al., 2019; Rauf et al., 2024; Zafer et al., 2023). In *Gossypium hirsutum*, 65 Indeterminate Domain (*IDD*) have been characterized. *GhIDD2, GhIDD7, GhIDD9, GhIDD11, GhIDD15, GhIDD21, GhIDD39* and *GhIDD42* take part in seed development and fiber elongation. *GhOIDD4* and *GhIDD32* expresses mostly in stem tissues whereas *GhIDD48* expressed in flowers indicating the importance of *GhIDD* for vegetative and seed development (Ali et al., 2019). Furthermore, *GhIDD4, GhIDD7, GhIDD11* and *GhIDD21* are also important for drought tolerance in cotton (Ali et al., 2019). In *Gossypium hirsutum*, thirty-four Histone H3 (HH3) genes have been characterized and potential role of *GhHH3-1, GhHH3-4* and *GhHH3-17* for breeding against drought been investigated (Qanmber et al., 2019).

Current study was aimed to analyze the drought resistance in cotton germplasm using physiological, biochemical and genetic techniques under well-watered and limited water conditions using split plot. The major objective of this study is to develop and identify cotton crosses that perform well under limited water conditions having higher expression of *GhHH3* and *GhIDD* genes during limited water conditions.

Material and Methods

Plant material, experimental design and agronomic practices

Two hundred cotton genotypes (Population 1) were collected from Agricultural Research Institutes of Pakistan (Cotton Research Institute (CRI), Multan; Stations: Faisalabad; Bahawalpur; Vehari; and Central Cotton Research Institute (CCRI), Multan and Nuclear Institute for Agriculture and Biology (NIAB) and screened based on morphological and physiological traits. Out of this large pool sixty-three genotypes of cotton Sub-Population 1 (SP1) were further selected to screen the most tolerant and susceptible accessions for the experiment. During cotton cropping season April 2020, SP1 was planted at the field experimental area of MNS University of Agriculture, Multan (30.08° latitude, 71.26° longitude and 189 m elevation from sea level) under two water regimes i.e., W1 (well-watered) =Total 12 irrigations for normal crop growth and W2 (Limited water) = Received normal

irrigations till flowering stage following RCBD split plot design with 4 repeats. Row-to-row distance of 75 cm and plant to plant distance of 30 cm was maintained along with standard agronomic practices. Nitrogen, phosphorus, potassium was applied at the rate of 100, 50, and 50 kg ha^{-1} respectively. Ten plants m^{-2} were established. Appropriate measures were practiced to control insect pests and weed control during both the growing seasons. Irrigation was stopped under the W2 conditions to impose drought stress.

Measurement of yield contributing attributes, fiber, and biochemical traits

Data was recorded for agronomic, fiber and biochemical traits from W1 and W2 regimes. Morphological traits including plant height (PH), monopodial branches (MB), sympodial branches (SB), boll weight (BW), boll number (BN), and seed cotton yield (SCY) were recorded manually. Fiber length, fiber strength, fiber fineness and uniformity were measured using USTER HVI 1000 (Uster Technologies).

A portable photosynthesis system (CIRAS-3, United Kingdom) was used to measure photosynthetic indicators. At the same time, the fully expanded leaves of the plants were used for measuring biochemical indicators including glycine betaine (Grieve and Grattan, 1983) proline (Bates et al., 1973) and total soluble sugars (Lowry et al., 1951) total chlorophyll (Chl) chlorophyll a, b, carotenoid (CAR) contents (Arnon, 1949) and leaf chlorophyll contents were measured using the SPAD-502 portable chlorophyll meter (Minolta Co. LTD, Osaka, Japan).

Calculation of reduction percentage and drought susceptibility index

Reduction/change in morpho-biochemical and yield attributes was calculated using the following formula:
Change in the performance of a trait (%) = 1-
Performance under limited water regime x 100
Performance under well water regime
Drought susceptibility index (DSI) was calculated by the following formula given by Fischer and Maurer (1978) for each genotype.
DSI= (1-Yd/Yp)/D
Where, Yd = Mean value of respective trait of a genotype under water-deficit condition.
Yp = Mean value of respective trait of a genotype under normal irrigation.
D = Mean of all genotypes for a corresponding trait under water-deficit condition/Mean of all genotypes for a corresponding trait under normal irrigation.
Variation for pigments and biochemical attributes has been revealed during both treatments.

Genetic material evaluation

Out of 63 genotypes, 10 suitable genotypes FH-326, FH-416, FH-492, NIAB-878, VH-327, FH-414, FH-415, FH-Anmol, Gomal-105, and Marvi were selected for hybridization. Among these 10 genotypes, five female parents (FH-326, FH-416, FH-492, NIAB-878, VH-327) tolerant, moderately tolerant and highly tolerant and 5 highly sensitive, sensitive and moderately sensitive to drought as testers/pollinators (FH-414, FH-415, FH-Anmol, Gomal-105, Marvi) grown in November 2020 to estimate inbred and parents through line x tester analysis. The genotypes were crossed in line x tester fashion when they reached the flowering stage. List of parents and cross combinations is given in (Table 1 (parents) and Supplementary Table 6).

Table-1: Performance of 10 diverse genotypes under both the regimes well water (W1) and limited water (W2)

Genotypes		FH-326	FH-414	FH-415	FH-416	FH-492	FH-Anmol	Gomal-105	Marvi	NIAB-878	VH-327
					Traits						
GB	(W$_1$)	1.25	1.31	0.59	0.98	1.02	1.22	1.50	0.99	1.40	0.67
	(W$_2$)	22.34	10.20	8.41	20.45	19.62	15.33	8.14	8.40	22.91	28.77
PRL	(W$_1$)	1.63	1.39	0.70	1.22	6.54	1.64	1.84	6.51	1.67	0.71
	(W$_2$)	28.42	11.86	11.88	27.70	25.65	12.98	11.57	11.59	29.97	29.92
TSS	(W$_1$)	4.57	2.75	2.74	5.49	5.06	2.96	2.20	2.68	6.32	2.73
	(W$_2$)	10.30	4.00	4.00	9.42	8.55	5.10	3.98	3.99	10.81	12.24
TSP	(W$_1$)	6.34	6.20	2.62	5.23	5.71	6.40	8.09	5.68	6.88	6.10
	(W$_2$)	4.41	2.41	2.44	5.21	4.65	3.00	2.02	2.42	6.07	5.12
Chl.a	(W$_1$)	2.29	1.81	1.31	2.22	2.29	1.68	1.24	1.30	2.22	2.08
	(W$_2$)	1.86	0.95	0.68	1.68	1.86	1.11	0.66	0.67	1.81	1.86
Chl.b	(W$_1$)	2.23	0.83	0.81	1.81	2.23	1.48	0.97	0.80	1.81	1.98
	(W$_2$)	1.29	0.37	0.28	1.25	1.29	0.65	0.26	0.27	1.20	1.29
CAR	(W$_1$)	1.59	1.01	0.78	1.34	1.55	1.12	0.78	0.74	1.36	1.43
	(W$_2$)	1.15	0.57	0.40	1.00	1.11	0.72	0.38	0.34	1.03	1.13
TCC	(W$_1$)	4.52	2.67	2.14	4.03	4.52	3.19	2.25	2.13	4.03	4.05
	(W$_2$)	3.14	1.36	0.96	2.92	3.14	1.77	0.96	0.96	3.00	3.14
CC	(W$_1$)	66.02	32.16	22.05	65.99	86.48	43.05	22.94	21.74	49.82	65.20
	(W$_2$)	77.53	20.28	15.67	57.87	75.75	33.53	11.63	10.83	62.90	66.67
FF	(W$_1$)	4.19	4.41	4.67	3.95	4.34	4.74	4.13	3.14	4.45	3.86
	(W$_2$)	4.55	4.96	4.33	4.14	4.55	4.74	4.75	4.16	4.76	4.04
FL	(W$_1$)	29.25	28.54	28.03	28.31	28.02	29.61	25.77	26.86	28.56	29.46
	(W$_2$)	28.08	25.51	25.33	27.98	27.91	25.72	24.06	25.11	28.01	28.11
GOT	(W$_1$)	36.20	38.50	38.50	30.41	40.92	38.50	31.79	33.47	37.83	34.94
	(W$_2$)	35.25	29.68	33.17	36.89	38.06	34.79	30.20	30.95	37.07	37.27
FS	(W$_1$)	30.14	27.08	28.34	25.72	29.37	23.27	27.08	22.55	29.63	32.65
	(W$_2$)	29.01	25.62	26.81	28.64	28.26	22.16	25.62	21.38	29.55	31.28
FU	(W$_1$)	82.14	80.68	87.21	79.52	80.90	85.05	78.93	90.53	81.36	83.01
	(W$_2$)	84.40	78.34	82.77	84.83	82.18	82.93	76.61	88.28	84.64	84.25
PH	(W$_1$)	146.23	108.14	112.38	144.42	139.70	126.84	95.97	97.91	155.94	145.38
	(W$_2$)	131.75	84.80	93.76	130.79	135.14	111.90	69.22	70.05	140.50	131.17
MB	(W$_1$)	2.64	1.25	1.40	1.37	1.38	1.32	2.60	2.04	1.38	1.38
	(W$_2$)	2.49	1.06	1.21	1.28	1.30	1.19	2.09	1.63	1.30	1.30
SB	(W$_1$)	29.89	19.09	28.20	28.13	29.89	29.36	17.21	17.43	33.30	34.20
	(W$_2$)	22.82	11.82	18.53	21.48	22.82	20.40	9.78	9.81	25.43	26.14
BN	(W$_1$)	44.38	24.59	24.67	32.67	37.27	38.48	19.53	24.91	35.60	36.38
	(W$_2$)	33.43	15.48	16.40	24.62	28.08	26.78	11.34	14.60	26.82	27.42
BW	(W$_1$)	3.39	3.66	4.14	3.51	3.01	3.88	3.75	3.36	4.28	4.16
	(W$_2$)	3.18	2.89	3.77	3.24	2.80	3.38	2.77	2.47	3.93	3.91
SCY	(W$_1$)	2851.92	2882.18	2866.78	2614.56	2705.88	2870.80	1878.25	2205.06	2870.35	2859.12
	(W$_2$)	2344.25	1166.75	1162.90	2130.00	2145.00	1367.57	611.07	1008.02	2288.00	2288.45

Twenty-five F_1 hybrids and 10 parents were planted in April 2021 under two moisture conditions, well water and limited water to study the genetics of water-deficit tolerance in cotton. During 2021, total irrigation applied to W1 and W2 regimes was 27.12-acre inches and 17.12-acre inches, respectively, and 4.58 inches more moisture was received in the form of precipitation. The crop was sown on April 13th, 2021, as a split-plot design with three replications, moisture levels as the main plot, and F_1 crosses and their parents as the subplot under a randomized complete block design. Each genotype had its own row in each replication. The separations between plants and rows were 75 and 30 cm, respectively. In contrast to the 90 cm separating each replication of a plot, the distance between the stress and non-stress plots was 100 cm. All suggested production methods and plant safety precautions were employed to explore the maximum combinations potential in both the regimes. Thus, a total of 25 genotypes were evaluated for morphological traits plant height (*PH*), monopodial branches (*MB*), sympodial branches (*SB*), boll numbers (*BN*), Boll weight (*BW*), seed cotton yield (*SCY*), fibre fineness (*FF*), fibre length (*FF*), fibre strength (*FS*), ginning out turn (*GOT*), fibre uniformity (*FU*), net photosynthesis rate (*A*), stomatal conductance (gs), transpiration rate (*E*), water use efficiency (*WUE*), chlorophyll content (*CC*), total chlorophyll contents (*TCC*), chlorophyll a (*Chl.a*), chlorophyll b (*Chl.b*), carotenoids (*CAR*), glycine betaine (*GB*), total soluble protein (*TSP*), total soluble proline (*PRL*) and total soluble sugars (*TSS*).

Gene expression analysis
Gene expression patterns of *GhHH3* and *GhIDD* genes under PEG 6000 @20 was investigated to decipher the functional implications of the *GhHH3*-4, *GhIDD*-11 genes (Ali et al., 2019; Qanmber et al., 2019). For gene expression studies, the samples were collected at 60 minutes, post PEG application at seedling stage. RNA was extracted using Total RNA extraction Kit (Beijing Solarbio Science & Technologies Co., Ltd.). *TrasnScript*® All-in-One first strand cDNA synthesis SuperMix (Transgen Biotech, Beijing, China) was used to synthesize cDNA using 1 µg total RNA. Ubiquitin was used as reference gene to normalize gene expression. qRT-PCR assay was performed using SYBR Green on BioRad (CFX Connect System Optic Module, USA).

Data analysis
ANOVA was performed to calculate differences between genotypes, irrigation regimes and their interactions were estimated in R using the package Agricola (de Mendiburu and de Mendiburu, 2019). Correlation using the R software's package was used to assess the relationship between morpho-biochemical characteristics and yield attributes (Wei et al., 2017). K-means clustering was used for cluster analysis and Ward's method was used to create a tree diagram based on elucidation distances. Using the R package Agricolae, the first two principal components were plotted against one another to determine the patterns of genotype variability and the associations between various clusters (de Mendiburu and de Mendiburu, 2019). The relative transcript level for each gene was calculated using the $2^{-\Delta\Delta CT}$ method (Schmittgen and Livak, 2008).

Results

Chlorophyll *a* (Chl. *a*)
For chlorophyll *a*, considerable reduction decrease percentage (RDI%) 30.78% and drought susceptibility index (DSI) 0.99 was found (Table 2). The mean values under W_1 and W_2 varied from 1.93 mg g^{-1} to 1.33 mg g^{-1} fresh Weight (Figure 2).

Table 2 Reduction decrease (RD) percentage and drought susceptibility Index (DSI) of attributes and mean reduction decrease

| Traits	RD%	DSI	Genotypes	RD%	Genotypes	SCY(RD%)	Genotypes	SCY(RD%)
GB	-1824.35	1.13	BH-167	41.05	FH-415	59.44	NIAB-135	36.88
PRL	-1721.91	1.56	BH-178	30.46	FH-416	18.53	NIAB-2008	40.02
TSS	-64.43	1.01	BH-180	28.77	FH-444	53.98	Reshmi	49.59
TSP	21.94	0.74	CKC	39.16	FH-490	50.16	RH-662	24.22
Chl.a	30.78	0.99	Chandi-95	43.78	FH-492	20.73	RH-667	27.89
Chl.b	46.60	1.02	CIM-343	38.45	FH-494	42.67	RH-668	23.91
CAR	34.60	1.03	CIM-602	46.66	FH-498	44.30	SAU-1	50.41
TCC	38.57	1.03	CIM-632	30.85	FH-Anmol	52.36	Sindh-1	47.59
CC	19.76	1.10	CIM-705	45.37	FH-Super cotton	55.22	Sitara-15	32.47
SCY	-7.76	1.01	CIM-717	31.81	GH-Mubarak	52.59	SLH-4	48.66
PH	6.55	1.04	CRIS-121	41.92	Gomal-105	67.47	SLH-8	31.95
SB	6.60	1.02	CRIS-34	43.01	IR-NIBGE-9	40.38	Sohni	51.13
BN	3.99	1.02	CRIS-342	51.79	IUB-264	49.86	Tarzan-1	47.14
BW	2.75	1.01	CRIS-607	34.45	Malmal	41.92	Thakar-808	39.93
MB	12.93	1.01	Cyto-177	59.85	Marvi	54.29	VH-189	36.01
FF	9.12	1.04	Cyto-608	40.05	MNH-1026	37.57	VH-259	30.01
FL	28.33	0.99	DNH-105	50.85	MNH-1035	33.20	VH-327	19.96
GOT	28.13	0.96	FH-142	39.86	MNH-990	29.11	VH-363	22.48
FS	10.76	0.98	FH-152	43.72	NIAB-878	20.29	VH-426	58.56
FU	41.02	0.98	FH-326	17.80	NIAB -Kiran	47.22	Weal-AG-Shahkar	46.73
GB	-1824.35	1.13	FH-414	59.52	NIAB-1048	39.16	Zakaria-1	49.32
PRL	-1721.91	1.56	BH-167	41.05	FH-415	59.44	NIAB-135	36.88
TSS	-64.43	1.01	BH-178	30.46	FH-416	18.53	NIAB-2008	40.02
TSP	21.94	0.74	BH-180	28.77	FH-444	53.98	Reshmi	49.59
Chl.a	30.78	0.99	CKC	39.16	FH-490	50.16	RH-662	24.22
Chl.b	46.60	1.02	Chandi-95	43.78	FH-492	20.73	RH-667	27.89
CAR	34.60	1.03	CIM-343	38.45	FH-494	42.67	RH-668	23.91
TCC	38.57	1.03	CIM-602	46.66	FH-498	44.30	SAU-1	50.41
CC	19.76	1.10	CIM-632	30.85	FH-Anmol	52.36	Sindh-1	47.59
SCY	-7.76	1.01	CIM-705	45.37	FH-Super cotton	55.22	Sitara-15	32.47
PH	6.55	1.04	CIM-717	31.81	GH-Mubarak	52.59	SLH-4	48.66
SB	6.60	1.02	CRIS-121	41.92	Gomal-105	67.47	SLH-8	31.95
BN	3.99	1.02	CRIS-34	43.01	IR-NIBGE-9	40.38	Sohni	51.13
BW	2.75	1.01	CRIS-342	51.79	IUB-264	49.86	Tarzan-1	47.14
MB	12.93	1.01	CRIS-607	34.45	Malmal	41.92	Thakar-808	39.93
FF	9.12	1.04	Cyto-177	59.85	Marvi	54.29	VH-189	36.01
FL	28.33	0.99	Cyto-608	40.05	MNH-1026	37.57	VH-259	30.01
GOT	28.13	0.96	DNH-105	50.85	MNH-1035	33.20	VH-327	19.96
FS	10.76	0.98	FH-142	39.86	MNH-990	29.11	VH-363	22.48
			FH-152	43.72	NIAB-878	20.29	VH-426	58.56
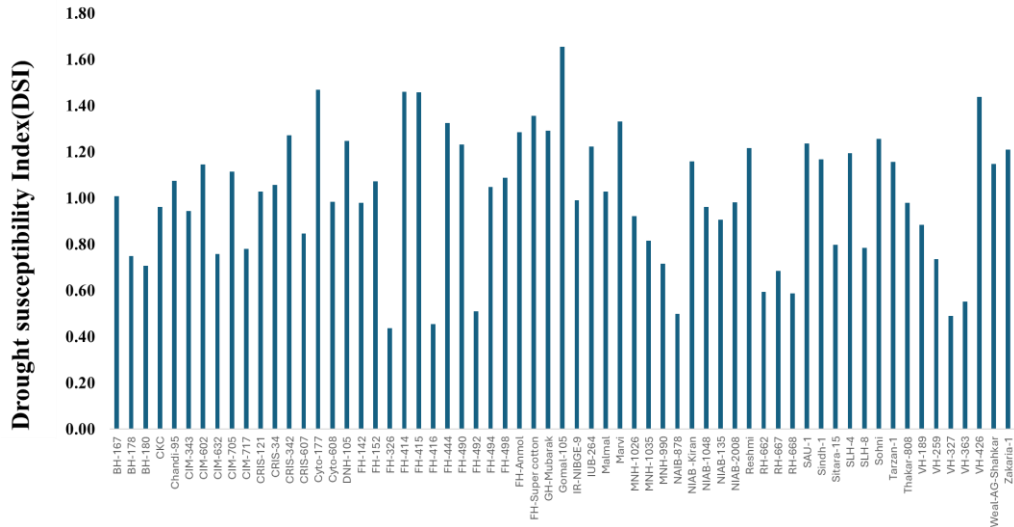
Figure-1. Mean drought susceptibilty Index based on SCY for cotton genotypes in W₁ and W₂ regime
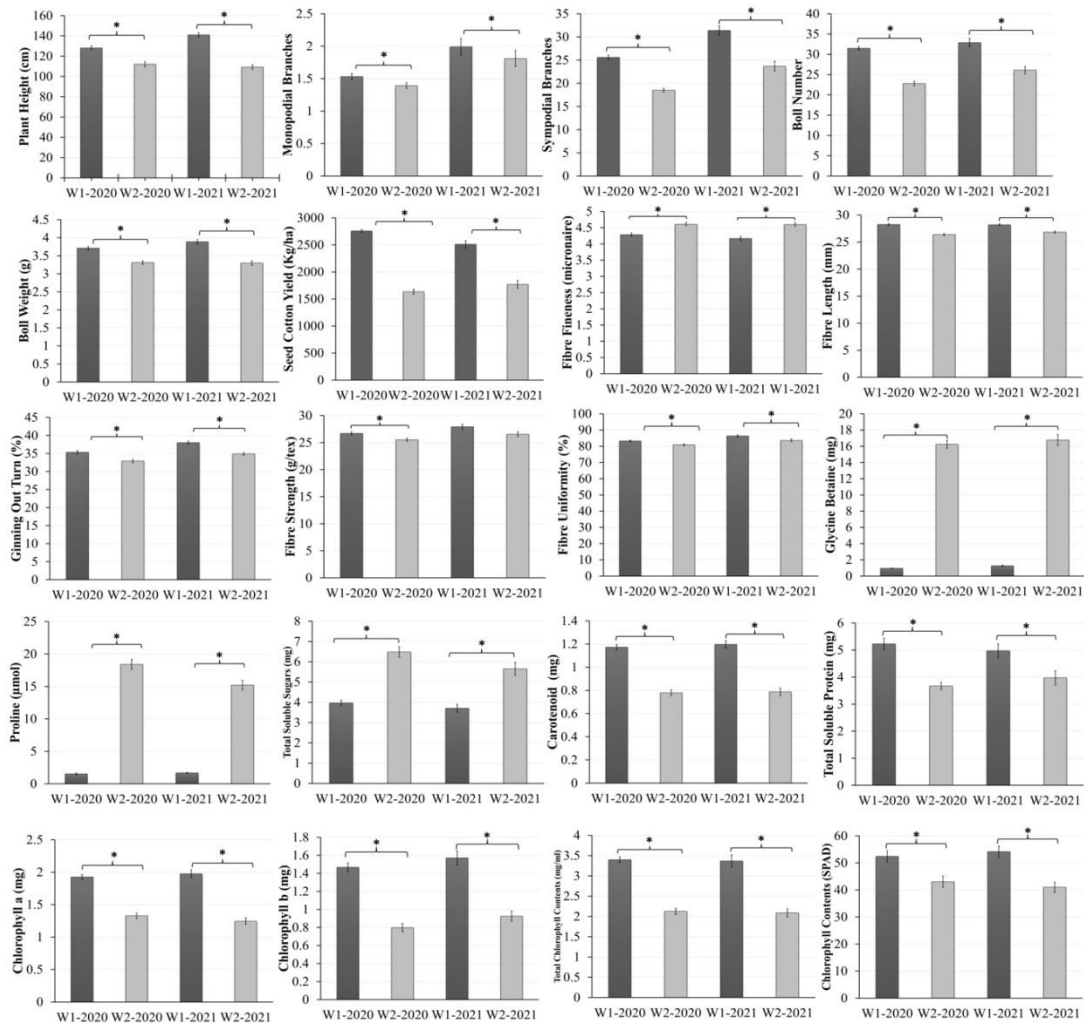

Figure-2. Mean statistics of various traits investigated in well water (W1) and limited water (W2) regimes during year 2020, 2021. Error bar denotes standard error. *= *P* value less than 0.05

Under well water conditions, highest concentrations of Chl. *a* was recorded in different genotypes including Gomal-105(1.24), Sindh-1(1.28), Marvi(1.30), CRIS-34(1.30), FH-415(1.31), FH-415(1.31), CRIS-607(1.54) and NIAB-2008(1.54) produced the lowest in folds of Chl.a, while IR-NIBGE(2.40), FH-498(2.40), NIAB-135(2.31), NIAB-Kiran (2.30), Sitara-15(2.30), and CKC-(2.30) demonstrated the highest concentration of Chl.a (Supplementary Table 1). Genotypes FH-152(1.71 mg g^{-1} fresh Wt), NIAB-1048(1.71 mg g^{-1} fresh Wt), RH-668(1.79 mg g^{-1} fresh Wt), NIAB-878(1.81 mg g^{-1} fresh Wt),VH-363(1.81 mg g^{-1} fresh Wt), FH-326(1.86 mg g^{-1} fresh Wt), FH-492(1.86 mg g^{-1} fresh Wt),VH-327(1.86 mg g^{-1} fresh Wt), FH-498(1.89 mg g^{-1} fresh Wt) and IR-NIBGE-9(1.89 mg g^{-1} fresh Wt) had the highest accumulation, while Gomal-105(0.66 mg g^{-1} fresh Wt), Marvi(0.67 mg g^{-1} fresh Wt), FH-415(0.68 mg g^{-1} fresh Wt), NIAB-2008(0.7 mg g^{-1} fresh Wt) and FH-142(0.71 mg g^{-1} fresh Wt) showed reduction in Chlorophyll *a* accumulation under the W_2 condition (Supplementary Table 1). Among all the cotton genotypes under study, CRIS-121 and IR-NIBGE-9 high value up to 2.40, the most pronounced increase in chlorophyll accumulation under W1, whereas Gomal-105 exhibited the lowest value of 1.24 (mg g^{-1} fresh Wt.) under W_1 conditions (Supplementary Table 1).

Chlorophyll *b* (Chl. *b*)

Genotypes experienced a 46.60% decrease and 1.02 DSI in Chl. b in all examined genotypes (Table 2). In all the tested cotton genotypes, chlorophyll *b* decreased under W_2 conditions. Significant variation among genotypes was depicted in both water regimes (Supplementary Table 1). The genotypes Sindh-1 (0.18), Cyto-177 (0.19), DNH-105 (0.19), Gomal-105 (0.26), Marvi (0.27), FH-415 (0.28), FH-414 (0.37) and CRIS-34 (0.40) produced the least amount of Chl. b under the well water regime (Supplementary Table 1). Genotypes FH-326 and FH-492 maintained the highest value i.e., (2.23 mg g^{-1}fresh weight) under W_1 regime (Supplementary Table 1). Genotypes highlighted maximum variation under W_2 condition that ranged from 0.18 in Sindh-1 to 1.64 mg g^{-1} in SLH-8. Least differences were observed for FH-494 under W_1 and W_2 conditions. Maximum variation was found in genotype Malmal under both water regimes. The entire mean value for the trait under examination was varied among genotypes, which revealed that it reduced from 1.47(W_1) to 0.8(W_2) in all genotypes during both the regime (Figure 2).

Total Chlorophyll contents (TCC)

All investigated genotypes showed genotypic variability and the trait RD% and DSI values for 63 genotypes were 1.03 and 38.57%, respectively (Table 2). It was demonstrated that TCC value in all the examined genotypes ranged from 3.40(W_1) to 2.13(W_2) during the experiment in both the regimes (Figure 2). Under W_1 regime FH-326 and FH-492 displayed a higher amount of TCC which was 4.52 mg g^{-1} fresh Wt. While lower level of TCC was found in Marvi (2.13 mg g^{-1} fresh Wt.) and FH-415(2.14 mg g^{-1} fresh Wt.) under W_1 regime (Supplementary Table 1). The maximum accumulation was recorded in FH-326, FH-492 and VH-327 (3.14 mg g^{-1} fresh Wt.) under W_2 regime, while it was the least in FH-415, Gomal-105 and Marvi (0.96 mg g^{-1} fresh Wt.) (Supplementary Table 1). Accession BH-178, MNH-990, RH-662, RH-667, FH-416, NIAB-878, VH-363, and RH-668 displayed the best performance during limited water regime., accumulated a higher concentration under W_2, while genotypes Cyto177, DNH-105, Sindh-1, CRIS-34, FH-414, Sohni, and FH-142 accumulated minimum amount for the attribute. However, the rest of the cotton genotypes in this experiment exhibited a moderate level of variable.

Carotenoids (CAR)

Variation in water availability also showed variation in the carotenoid concentrations with an average DSI (1.03) value for 63 genotypes and an RD% (34.60) (Table 2). Under W1 a range of 0.78 to 1.59 was found in Gomal-105 and FH-326 (Supplementary Table 1). Under W2 condition a range of 0.34 for Marvi to 1.15 for FH-326 was observed (Supplementary Table 1).

Chlorophyll contents (CC)

Chlorophyll contents showed high concentrations under the W_1 regime in FH-152 (88.54), FH-492 (86.48), FH490 (84.74), and FH-498 (82.34). In contrast, genotypes Marvi (21.74), FH-415 (22.05), Gomal-105 (22.04) and DNH-105 (27.27) had lower parameter levels (Supplementary Table 1). The highest value for CC was recorded by genotypes FH-326(77.53), which are followed during W_2 by FH-492(75.75), FH-490(74.24), FH-498(72.14), VH-327(66.67), Malmal (66.26), NIAB-1048(63.21), NIAB-878(62.90), and RH-667(62.87) (Supplementary Table 1).

Total soluble protein (TSP)

The present study highlighted the highest fold TSP accumulation in BH-167 (9.53 mg g^{-1}) followed by Chandi-95 (9.52 mg g^{-1} fresh Wt.) in well water regime (Supplementary Table 1). Minimum TSP concentration was determined in FH-415 (2.62 mg g^{-1} fresh Wt.) under W_1 and Gomal-105 (2.02 mg g^{-1} fresh Wt.) performed lower when exposed to W_2 (Supplementary Table 1). Highest concentration was revealed in RH-668 (7.49 mg g^{-1} fresh Wt.), NIAB-878 (6.07 mg g^{-1} fresh Wt.), RH-662 (5.48 mg g^{-1} fresh Wt), SLH-8(5.44 mg g^{-1} fresh Wt.) and MNH-1026 (5.44 mg g^{-1} fresh Wt.) while it reduced in Gomal-105(2.02 mg g^{-1} fresh Wt.) and DNH-105 (2.04 mg g^{-1} fresh Wt.) during W_2 (Supplementary Table 1).

Total soluble sugars (TSS)

In W_1 and W_2, the mean performance levels ranged from 6.48 to 3.96 (Figure 2). TSS level depicts values as 6.32 mg g^{-1} for RH-662 and NIAB-878 whereas it accumulated in minimum fold for Gomal-105 (2.2) followed by CRIS- 121 (2.44) in W_1 while under W_2 conditions VH-327 displayed (12.24 mg g^{-1} fresh Wt.) followed by RH-668 (11.3 mg g^{-1} fresh Wt.) the maximum concentration for the trait whereas it depicted lower folds in Gomal-105 (3.98), DNH-105 (3.99), CRIS-121 (3.99) and Marvi (3.99) (Supplementary Table 1).

Glycine betaine (GB)

Under limited water regime, the trait's percentage declined to -1824.35 was noted, and its concentration was revealed to an increase in W_2 (Table 2). The results of all genotypes showed a DSI score of 1.13. In W_1 and W_2, the mean performance levels ranged from 0.95 μmolg^{-1} to 16.24 μmolg^{-1} (Figure 2). The highest accumulation was recorded in Chandni (1.55 μmol g^{-1}) trailed by VH-363(1.53), FH-490(1.52), BH-167 (1.52 μmol g^{-1}) and Gomal-105(1.50) in well water regime, whereas it was lowest in Gomal-105 (8.14 μmol g^{-1}), DNH-105 (8.16 μmol g^{-1}), CRIS-121 (8.18 μmol g^{-1}), Marvi (8.4 μmol g^{-1}) and FH-415 (8.41 μmol g^{-1}) in W_2 (Supplementary Table 1). Considerable variation was observed for GB level, which varied from 8.14 to 28.77 μmol g^{-1} during limited water regime (Supplementary Table 1). Genotypes VH-327, NIAB-878, RH-668, RH-662, SLH-8, MNH-1026, FH-326, FH-416, RH-667, MNH-990, FH-492, FH-142, FH-498 and MNH-1035 had the higher concentration of GB under W_2 as compared to other genotypes (Supplementary Table 1).

Proline Contents (PRL)

It was observed that proline levels, such as glycine betaine, increased during W_2, which had an impact on the value of both the DSI (1.56) and the RD% (-1721.91). In W_1 and W_2 mean performance levels of proline fluctuated between 1.53 to 18.41 (Figure 2). All genotypes showed variations for proline accumulation in both regimes. In W_1 regime the higher accumulation in FH-492(6.54 μmol g^{-1}) and Marvi (6.51 μmol g^{-1}) was detected among accessions while the lowest was found in CRIS-34 (0.19 μmol g^{-1}) (Supplementary Table 1). During limited water regime lower concentration for proline were observed in Gomal-105 (11.57 μmol g^{-1}), Marvi (11.59 μmol g^{-1}), DNH-105(11.60 μmol g^{-1}), CRIS-121(11.61 μmol g^{-1}) and FH-414(11.86 μmol g^{-1}) (Supplementary Table 1). While higher contents were examined among RH-668 (30.10) followed by NIAB-878(29.97), VH-259(29.95), VH-327 (29.92), RH-662(28.91), SLH-8(28.80), FH-326(28.42) and FH-416(27.70) (Supplementary Table 1). A substantial decrease confirmed the effect of water limited regime on proline accumulation. Proline exhibits a diverse range of accumulation in studied genotypes from 1.53-fold to 18.41-fold in W_1 and W_2 regimes respectively (Figure 2).

Yield and fiber quality traits

When evaluating each genotype independently, it was observed that the drought stress had pronounced effect on plant height, boll number, boll weight, ginning out turn percentage (GOT %) and seed cotton yield across all genotypes (Figure 2). In well water regime maximum yield was produced in FH-498(3028.08 Kgha^{-1}) followed by FH-494(3011.61 Kgha^{-1}) VH-189(2999.10 Kgha^{-1}) and Cyto-608(2992.42Kgha^{-1}). All the genotypes produced above 2000 Kg ha^{-1} SCY except Gomal -105(1878.25 Kgha^{-1}) during W_1. In contrast, during W_2, FH-326(2344.25 Kgha^{-1}), VH-327(2288.45 Kgha^{-1}),NAB-878(2288 Kgha^{-1}), RH-668(2266.70 Kgha^{-1}), VH-363(2185.78 Kgha^{-1}), RH-662(2159.74 Kgha^{-1}), FH-492(2145 Kgha^{-1}), FH-416(2130 Kgha^{-1}), and RH-667(2048.12 Kgha^{-1}) demonstrated higher performance for yield in comparison to Gomal-105(611.07 Kgha^{-1}),Cyto-177(985.28 Kgha^{-1}), DNH-105(992.81 Kgha^{-1}), Marvi(1008.5 Kgha^{-1}), VH-426(1158 Kgha^{-1}), FH-415(1162 Kgha^{-1}), FH-414(1166.75) GH-Mubarik(1188.46 Kgha^{-1}), and

SAU-1(1277.17) (Supplementary Table 2). It was revealed that GOT% exhibits variation and exhibited the mean range from 35.35%(W_1) to 32.89%(W_2) (Figure 2). During limited water regime genotypes FH-492, MNH-990, Sitara-15, NIAB-878, SLH-8, FH-Anmol, VH-426, FH-498, FH-444 and NIAB-35 exhibited maximum ginning outturn (Supplementary Table 2). Performance of genotypes for yield components varied and it was revealed that mean value of genotypes influenced when examined during both the regimes for plant height (W_1: 128.11cm to W_2: 112.12cm), sympodial branches (W_1: 25.59 to W_2: 18.49), boll numbers (W_1: 31.50 to W_2: 22.80)and boll weight (W_1: 3.71g to W_2: 3.31g) (Figure 2). In limited water regime the maximum performance for yield components (SB, BN, BW) was revealed in RH-667, CKC, and NIAB 1048 (Supplementary Table 2).

The study demonstrated that both W_1 and W_2, such as drought, had significant impacts on fiber quality traits, including fiber fineness (FF), fiber length (FL) fiber strength (FS) and fiber uniformity (Figure 3a & b). Mean performance during W_1 to W_2 for fiber fineness (4.28 to 4.61 µg/inch), fiber length (28.26 to 26.4mm), fiber strength (26.7 to 25.53g/tex), and fiber uniformity (83.28 to 80.90%) was revealed that quality traits value decreased under limited water regime (Figure 2). However, the specific effects varied among different cotton genotypes. The study findings showed that CRIS-121, Cyto177, VH-327, FH-326, NIAB-878, SLH-8, FH-416, BH-180, FH-492, and CIM-602 exhibited the best performance under limited water regime for quality traits.

Drought susceptibility index (DSI)
The 63 genotypes that were evaluated on drought tolerance based for the reduction decrease (%) and the drought susceptibility value. Compared to other genotypes, genotypes FH-326, FH-416, FH-492, NIAB-878, RH-662, RH-668 and VH-327 yield the highest amount when water is limited (Figure 1 & Table 2). According to (Table 2), the lowest reduction was noted in FH-326 (17.8%), and the highest in Gomal-105 (67.47%) for end produce seed cotton yield (Table 2). Using the Fischer and Maurer (1978) procedure, the drought susceptibility index percentage (DSI%) was analyzed to determine the genotypes of cotton that are drought tolerant and sensitive. DSI values for genotypes FH-326, FH-416, VH-327, NIAB-878, FH-492, VH-363, RH-668, RH-662, RH-667, BH-180, MNH-990 , VH-259, BH-

178, CIM-632, CIM-717, SLH-8, Sitara-15, MNH-1035, CRIS-607, VH-189, NIAB-135, MNH-1026, CIM-343, NIAB-1048, CKC, FH-142, Thakar-808, NIAB-2008, Cyto-608,and IR-NIBGE-9 were <1, so they were classified as drought tolerant and for the BH-167, CRIS-121, Malmal, FH-494, CRIS-34, FH-152, Chandi-95, FH-498, CIM-705, CIM-602, Weal-AG-Shahkar, Tarzan-1, NIAB -Kiran, Sindh-1, SLH-4, Zakaria-1, Reshmi, IUB-264, FH-490, SAU-1, DNH-105, Sohni, CRIS-342, FH-Anmol, GH-Mubarak, FH-444 Marvi, FH-Super cotton, VH-426 ,FH-415 ,FH-414 ,Cyto-177 and Gomal-105 were classified as a drought-sensitive genotypes because their value was more than 1. It was found that the genotypes NIAB-878(0.50), FH-326(0.44), VH-327(0.49), FH-416(0.45) and FH-492(0.51) were superior genotypes that produced the highest yield and lower DSI value among all 63 genotypes. When their DSI values were examined, they also revealed promising outcomes. However, for SCY and maximal DSI and RD (%), genotypes FH-414, FH-415, FH-Anmol, Gomal-105, and Marvi were shown to be at the extreme in the W_2 regime (Figure 1 & Table 2). The fitness of five cultivated genotypes of cotton (NIAB-878, FH-326, VH-327, FH-416, and FH-492) to grow in regions impacted by drought stress episodes has been confirmed.

Correlation study between yield and biochemical traits
Yield contributing characters (SB, BN, BW) have shown significant distribution patterning the strong correlation with each other except the boll numbers which has no strong relationship with boll weight. Biochemical traits in W_1 have no significant association whereas chlorophyll contents exhibited significant correlation with BN, PH, SB, and SCY in W_1 (Figure 3c).

Correlation studies in W_2 describe the significant contribution of biochemical attributes and found to be best to enhance yield in cotton crop during drought phase. Highly significant correlation of chlorophyll a; with SCY, BN, BW, PH, SB and minimum association with MB was observed in limited water regime (Figure 3D). Correlation analysis showed a very significant relation of chlorophyll b; with SCY, BN, BW, PH, SB and positive significant association with monopodial branches was observed in W_2 (Figure 3D).

Stronger association was found for total soluble protein with SCY, SB, and BN while significant for

BW and no association with MB in W_2 (Figure 3D). Under W_2 conditions, significant correlation was detected for proline with SCY while with productive attributes BW, BN, PH, SB it has positive and no significant relationship was revealed with monopodia (Figure 3D) Total soluble sugar strongly linked with yield and yield contributing traits and it is revealed that its contribution was higher during limited water regime for SCY.

Strong positive correlation of seed cotton yield with glycine betaine that patented itself as the most contributing biochemical for drought tolerance in W_2. Glycine betaine has been recognized through its mapped association with SCY, BN, BW, PH, SB while non-significant association with monopodial branches was examined in W_2 (Figure 3D). Ginning out turn has been influenced through the accumulation of biochemical attributes. Biochemical

attributed showed positive association with GOT and their contribution maximizes its percentage. Correlation analysis showed a very significant relation of chlorophyll contents with SCY, BN, BW, PH, SB and positive significant association with monopodial branches was observed (Figure 3D).

Bolls per plant were found to be the primary determinant of SCY under well-watered and water-limited regimes. Bolls per plant demonstrated minimum association with boll weight. Boll numbers have strong correlation with all the biochemical attributes studied that enhanced SCY during limited water regime (Figure 3d). These findings evidenced that correlation coefficient analysis revealed about leaf chlorophyll content positive and significant association with the SCY and GOT, in addition to an increase in the leaf chlorophyll content may induce positive impacts on SCY under W_2.
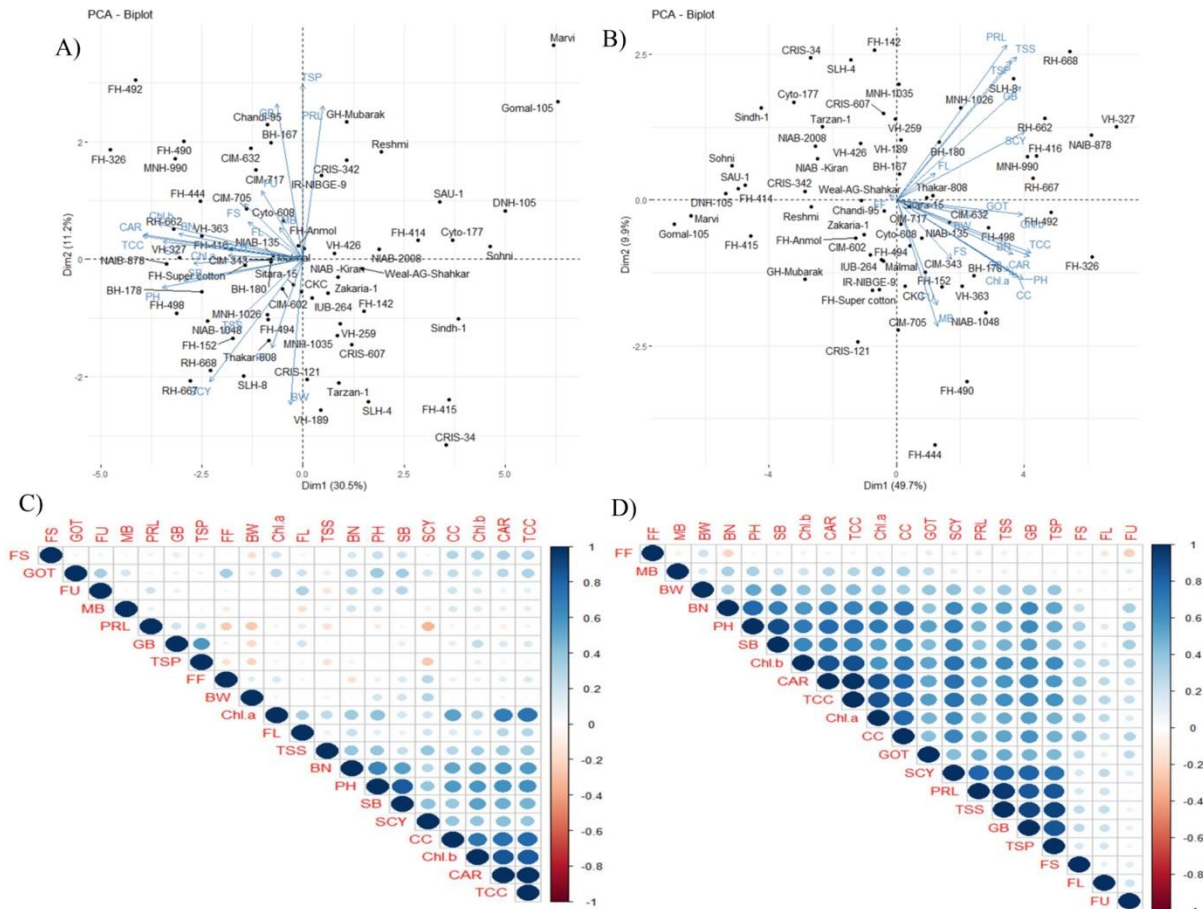

Figure-3. **Biplot between PC-1 and PC-2 highlighting the contribution of Morpho-physiological, biochemical and yield attributes.**

Well water regime (a) and limited water (b) regimes and correlation plot of yield parameters viz Plant height (PH), Monopodial branches (MB), sympodial branches(SB), Boll numbers (BN), Boll weight(BW), seed cotton yield(SCY)), Fibre parameters viz Fibre fineness (FF), Fibre length (FL), Ginning out turn(GOT), Fibre strength (FS) and Fibre uniformity (FU) biochemical viz Carotenoid (CAR), Total Chlorophyll (Chl.T), Chlorophyll a (Chl. a) Chlorophyll b (Chl. b), Glycine betaine (GB), Ginning out turn (GOT), Total Soluble protein (TSP), Proline (PRL), Chlorophyll contents (CC), and Total Soluble sugars (TSS) of 63 cotton cultivars/genotypes grown under well-watered (c) and limited regime (d) during 2020. Blue shade shows the positive correlation and pink shade shows negative correlation. Size of the circle shows how traits are associated with each other. More size means strong association. * Indicates significant (P≤0.05) and without sign (*) indicates non-significant (P ≥ 0.05)

Principal components, cluster and biplot analysis - well water and limited water regimes

Mean data of sixty-three cotton genotypes were analyzed and PCA was employed to sum up diversity from collected mean data. Out of 20, seven principal components (PCs) having an Eigen value greater than 1 were extracted in W_1 and five in W_2 regime. Contribution of seven PCs was 74.64% of the total diversity amongst the cotton genotypes assessed for biochemical, fibre and yield attributes during well water regime. In the limited water regime contribution of five PCs 77.42% of the total variability amongst the cotton genotypes assessed for physiology and yield attributes (Figure 3b). This shows that the first two PCs contain the most information on genetic variability among genotypes, which can be used in further selection.

Genotypes CIM-705, CRIS-342, FH-444, FH-Anmol, FH-Super Cotton, IR-NIBGE-9, IUB-264, Reshmi, SAU-1, Sindh-1, Sohni and Zakaria-1showed moderate susceptibility whereas Cyto-177, DNH-105, FH-414, FH-415, and GH-Mubarak are drought susceptible. Extremely low performance of genotypes Marvi and Gomal-105 were characterized, and these genotypes ranked as highly susceptible genotypes among 63 genotypes (Figure 3b).

The result of biplot analysis confirmed correlation analysis between studied criteria. Therefore, these lines were in the first and fourth regions of biplot identified as the good combiner for yield and biochemical (Figure 3b). Biplot for all the agronomic, yield, fibre and biochemical traits for 63 genotypes in well water regime during 2020 was mapped between PC I and PC II which influenced 41.7% of total interaction while it was 59.6% for PCI and PCII during W_2. The genotypes, including NIAB-878, RH-662, RH-668, VH-327, RH-668, VH-363, FH-416, FH-492, FH-494, FH-498 and FH-326 established strong positive interaction in W_1. Wide distributed diversity in cotton cultivars was observed through spread out graph named as polygon shaped in PCA (Figure 3A & B). Genotypes exhibiting their presence at vertex in biplot graph has longest distance from the origin, experienced tremendous variations for quantitative attributes and could be employed as breeding material in expanding the genetic base of cotton for breeding program. Genotypes clogged at the vertex and remotest from origin portrayed highest diversity while those adjacent to origin described lowest genetic diversity (Figure 3A & B).

Genotypes clogged at vertex of polygon in biplot graph have distance from the origin, carried extreme divisions for quantitative attributes and could be subjugated as parental lines in splaying the genetic base of cotton through breeding program. Genotypes labelled at the vertex of polygon and utmost from origin represented highest diversity while those nearest to origin received lowest genetic diversity (Figure 3A & B). The genotypes performed differently as shown by the biplot of the susceptibility and maximum yield (seed cotton) under both regimes W_1 and W_2. It was estimated that genotypes FH-326, RH-662, SLH-8, MNH-1026, FH-416, RH-667, MNH-990, FH-492, FH-142,FH-498, MNH-1035, VH-189, CIM-632, BH-180, SLH-4, VH-259 and CRIS-607 existed near to glycine betaine, proline, total soluble protein, soluble sugars and pigments trait at biplot destined that these genotypes will be the best when selection for high contents in water limited conditions. Selection for yield and yield components in limited water regimes, the genotypes that present at vectors of boll numbers per plant (CEMB-Klean Cotton, CIM-632, NIAB-135, FH-498, CIM-705, FH-490, NIAB-878, Cyto-608, FH-Anmol, BH-178, FH-494, FH-444,MNH-990, MNH-1026, BH-180, IUB-264, FH-416, VH-327,FH-326, FH-Super Cotton, Thakar-808, Weal-AG-Shahkar,NIAB-1048, MNH-1035, CIM-717, VH-259, RH-662, FH-492, FH-152, CIM-343, VH-363 and

Malmal), boll weight (RH-667, FH-490, BH-180, Thakar-808, FH-Super Cotton, FH-152, NIAB-135, Sitara-15, CIM-602, VH-189, RH-662, FH-498, MNH-1026, SLH-4, Cyto-608, BH-178, CIM-705, FH-Anmol, FH-444, Zakaria-1 and Tarzan-1) and seed cotton yield (CIM-632, VH-259, BH-180,MNH-1026, CRIS-607, Sitara-15, Thakar-808, Cyto-608, CIM-717, FH-142,FH-494, NIAB-1048,CRIS-121, BH-167, FH-498,NIAB-135,Chandi-95 and CRIS-34) will be the promising genotypes for these attributes. Analyses revealed that yield and yield components strongly associated with glycine betaine proline and protein. Boll numbers and boll weight closely linked with chlorophyll contents (Figure 3a & b).

Evaluation of breeding material using line x tester technique

Out of 63 genotypes, ten distinct genotypes were chosen based on their momentous results for the variables at both extremes (W_1, W_2) under study in the experiment and their aggregate performance (Table 1). Twenty-five crosses were made by employing the ten devise genotypes those selected from 63 for further evaluation following line x tester technique. Findings indicate the best performance of hybrids for the Physio-biochemical traits and seed cotton yield. Differential performance of parents and crosses was compared under both W_1 and W_2 (Supplementary Table 3, 4 & 5).

All the crosses confirmed variation for the traits that were examined during both the regimes (Supplementary Table 6). Among the crosses, CS21(VH-327 × FH-414), CS5(FH-326 × Marvi), CS7(FH-416 × FH-415), CS8(FH-416 × FH-Anmol), CS6 (FH-416 × FH-414), CS10(FH-416×Marvi),CS9(FH-416× Gomal-105), CS16(NIAB-878×FH-414) and CS24(VH-327×Gomal-105) had momentous performance for all the traits studied. Yield and yield contributing trait mean values for SB (CS7, CS6, CS9, CS24, CS4, CS22, CS11, CS1, CS13) BN (CS5. CS3, CS12, CS2, CS7, CS22, CS4, CS16, CS11, CS15) and BW (CS23, CS5, CS12, CS4, CS22, CS11, CS1. CS13, CS2, CS9, CS10) were higher under W_2 regime. while among the crosses, CS21, CS5, CS16, CS13, CS21, CS16, CS8 CS16, CS21and CS 16 had significantly high SB, BW, and BN under W_2 (Supplementary Table 3). In the physio- biochemical traits, including WUE, CC, TCC, CAR, TSS and TSP were higher in CS17, CS4, CS1, CS15, CS16 and CS5 under W_2 (Supplementary Table 4 & 5). Net

photosynthesis rate was also higher in crosses with a range of 29.76(CS5) to 17.39(CS20) during W_2 (Supplementary Table 5). Out of all combinations 15 hybrid combinations were observed the accumulation of Chl. b from 1 to 1.46 while out of 25, 22 hybrids exhibited the range of accumulation for Chl.a from 1.62 to 1.01 (Supplementary Table 4). Cross CS 16 accumulate the maximum concentration for Chl.a(1.62) while hybrid CS17(1.46) accumulates the maximum concentration for Chl.b during W_2. Maximum seed cotton yield was observed in CS24 (2967.28 Kgha^{-1}) during well water regime. Seed cotton yield ranged from 2967.28 Kgha^{-1}(CS24) to 2184.28 Kgha^{-1} (CS23) in W_1 (Supplementary Table 3).

Performance of genotypes for seed cotton yield exhibited that CS21 produce the maximum yield (2555 Kgha^{-1}) followed by CS5 (2344 Kgha^{-1}), CS7(2216.5 Kgha^{-1}), CS8(2168.75 Kgha^{-1}), CS6(2152.5 Kgha^{-1}), CS10(2148.25 Kgha^{-1}), CS9(2085.38 Kgha^{-1}), CS16(2055.38 Kgha^{-1}), CS24(2016.38 Kgha^{-1}), and CS4(1985 Kgha^{-1}) during limited water regimes. For the analyses of drought susceptibility index of seed cotton yield for these crosses highlighted that CS5, CS21, CS16 were more tolerant to drought followed by CS6, CS7, CS8 and CS10. As a result of the relationship between these findings and the fiber characteristics in cotton crops, it was revealed that under limited conditions, the value of fineness increases while the value of fiber length decreases. Fibre fineness mean value figured 4.17 µg/inch in well water regime whereas it was increased in limited water regime with a figure of 4.60µg/inch (Figure 2). Results for fibre length indicates that fibre length mean value in well water regime was higher than limited water regime and it was 28.22 mm and 26.81 in well water and limited water regimes respectively (Figure 2).

Significant differences among the crosses were estimated during both the water regimes W_1 and W_2 for seed cotton yield. Whereas some of the genotype's performance was momentous for yield during limited water regime. Out of 25 crosses the mean value for SCY for 25 crosses and drought susceptibility index highlighted that cross CS16(NIAB-878 × FH-414), CS5(FH-326 × Marvi) and CS21(VH-327 × FH-415) potential results for SCY. These 3 crosses showed minimum drought susceptibility index and that is ~0.5 which is the lowest among all the examined genotypes (Figure 4a & b). It was determined that the performance of these crosses for fiber fineness {CS5 (4.44 µg/inch) and

CS16 (4.55 µg/inch)}, fibre length{CS5 (28.07mm) and CS16 (28.05mm)}, seed cotton yield{CS5 (2344 Kgha^{-1}) and CS16 (2055 Kgha^{-1})} and drought susceptivity index {CS5 (0.12) and CS16 (0.15)} of these 2 crosses is momentous (Figure 4 A&D).
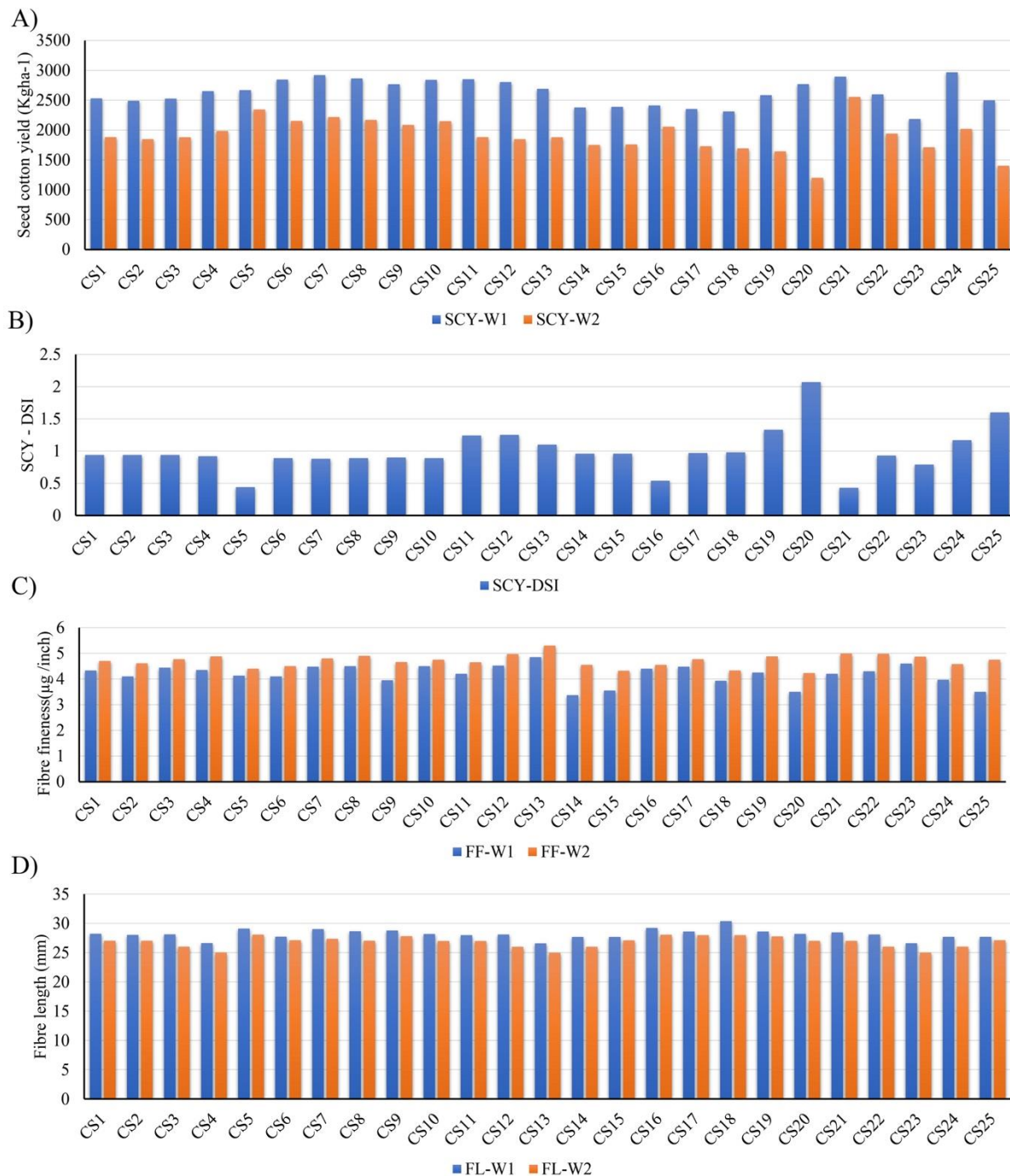
A)

B)

C)

D)

Figure-4. Seed cotton yield(a), drought susceptibility Index(b), fibre fineness(c) fibre length (d) of crosses examined in W1 and W2 regime. CS=Cross

Gene expression analysis of *GhHH3-4* and *GhIDD-11*

Normalized fold expression was studied in the selected crosses. We observed that *GhHH3-4* and *GhIDD-11* genes were up regulated in response to drought, suggesting that these genes may contribute towards drought tolerance. However, *GhIDD11* revealed higher expression comparatively. The cDNA was examined using the Ubiquitin housekeeping gene prior to qRT-PCR (Figure 5). Among the two selected crosses, CS16 showed higher transcript abundance as compared to CS5. Hence it can be deduced that CS16 has more potential to withstand and perform better in response to early drought conditions.
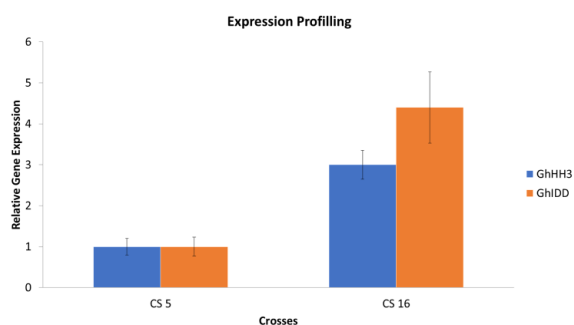
Figure-5. Relative gene expression *GhHH3* and *GhIDD* in the developed crosses

Discussion

A 3–5°C temperature increase in the following century indicated the occurrence of excessive drought, which has a direct impact on agriculture (Soong et al., 2020). The cotton is primary fibre producing crop in agriculture and drought has a significant impact on its yield (Loka et al., 2020; Ullah et al., 2022). The growing threat posed by climate-related events to global food security and agriculture, along with the world's population expansion, demands the timely development of crops that can withstand stress (Zafar et al., 2022). It is essential to consistently expose cotton plants to various challenges because of the continual fluctuations in the climate (Haroon et al., 2023). The most important factor in developing an initiative that will culminate in the development of tolerant genotypes is the selection of parents with desired qualities and survey of their genetic information (Khan et al., 2010; Li et al., 2019). Lower cotton yield in Pakistan appealed to address the causes that

are responsible. Limited water conditions are one of the major causes of lower yield. Considering plant drought resistance is a quantitative feature mediated by polygenes with micro effects, it makes more sense and is more scientific to assess plant drought resistance using a variety of multidimensional indicators (Sloane et al., 1990). Various studies indicated that the characteristics of cotton linked to drought resistance were found to be plant height, number of branches, boll weight, transpiration rate, glycine betaine, proline, soluble sugars and chlorophyll (Sarwar et al., 2012; Singh et al., 2021). Therefore, it is crucial for a breeding program to screen the cotton based on key indicators and to develop cotton that can produce appropriate yield under water stress (Celik, 2023). Finding genotypes that can be successfully used in upcoming breeding programs and are resilient to climate change requires the use of this strategy (Nawaz et al., 2023). The present study covers these prerequisites, and the findings associate with tolerant high yielding cotton genotypes. Study indicates the extensive amount of divergence of all the studied traits.

Several findings demonstrated that accumulation of adequate values for glycine betaine, proline, protein, soluble sugars, chlorophyll, yield, yield components and quality traits was observed in FH-326, FH-416, FH-492, NIAB-878, RH-662, RH-668, VH-327, and VH-363. Biplots and correlation studies using the yield-based drought susceptibility index demonstrated that the genotypes FH-326, FH-416, FH-492, NIAB-878, RH-662, RH-668, VH-327, and VH-363 were producing high yields during drought conditions. These genetic groups may serve as a foundation for the next generation of drought-tolerant, high-yield cotton genotypes.

Genotypes FH-Anmol, FH-414, FH-415, Gomal-105, and Marvi revealed themselves as susceptible genotypes with lower performance during limited water regime.

Pigments are important to plant mainly for harvesting light and production of reducing power such as ATP and NADPH. Both chlorophyll a and b are prone to soil drying damage. However, carotenoids have additional roles and partially help the plants to withstand adversaries of drought (Farooq et al., 2009). Carotenoids form a major part of the plant antioxidant protection system, but they are very sensitive to oxidative damage. β-carotene, found in the chloroplasts of all green plants is completely bound to the core complexes of PSI and PSII

(Havaux, 1998). Lack of water alters the internal structure of chloroplasts, which in turn impacts photosynthesis and chlorophyll (Huseynova et al., 2016).

In our research it was revealed that chlorophyll '*a*', '*b*', total chlorophyll and carotenoids showed significant differences under normal and limited water conditions in the genotypes under study. A decrease in chlorophyll content and carotenoids accumulation was observed in cotton cultivars in limited water regime however its concentration in tolerant genotypes was reasonable that may be related to the fact that under drought, tolerant genotypes adapt stomatal closure strategy and accumulates more relative water contents coupled with less excise water in their leaf tissue to maintain the photosynthetic activities as compared to susceptible genotypes (Chaves et al., 2009).

The decrement of chlorophyll content during drought stress could be related to photo-oxidation resulting from oxidative stress which reduces photosynthetic process by lowering the PSII quantum yield (Gill et al., 2016).) Our results agree with these researchers where in all genotypes, chlorophyll contents are decreasing (Aslam et al., 2023; Zafar et al., 2023; Goren and Tan, 2024).

Decrease in the synthesis soluble proteins may be due to breakdown of proteins by proteolytic process into amino acids that enhance reduction of NRA, NiRA and substrate under drought stresses (Parida and Das, 2004). Under stress conditions, plants produce some stress proteins and some of them are taken by phytohormones. When subjected to drought treatments, total soluble proteins significant drop in all genotypes as compare with control treatment. The results of the present study confirmed these findings and associated with the studies of and Zafar et al. (2023).

Limited water regime influenced total soluble sugars and a substantial increase was examined in investigated genotypes. Increase in total soluble sugars under various drought stress levels have been complemented by several investigations conducted on different crop plants (Akladious, 2012). The elevated sugar content might have resulted from starch degradation under drought stress due to amylase activity (Ghasempour et al., 1998). Total soluble sugar supports flowering and boll development during drought (Gao et al. 2020). Cotton plant accumulated high sugar levels during drought stress (Ullah et al., 2022). Sugar accumulation in drought stress conditions helps to maintain the stability of the membrane, prevent and protect membrane fusion and keep protein to remain functional. The current study validated these findings with an increase in total soluble sugars in drought stress to well-watered.

During drought stress glycine betaine accumulation increases that leads to protecting the photosynthesis and cell membrane integrity through osmotic adjustment in cotton plants (Marimuthu and Murali, 2018; Singh et al., 2021). This research revealed that under stress, the tolerant cultivars increased their levels of glycine betaine more than the sensitive cultivars. This was further supported by the finding that glycine betaine levels positively correlated with tolerance under drought stress. Genotypes with high levels of glycine betaine showed higher seed cotton yield, boll numbers plant and boll weight. It is since contents of glycine betaine contribute for better seed cotton yield and yield components. Our findings are in line with the previous findings where genotypes with higher levels of GB produced higher yields under water limited conditions (Singh et al., 2021).

Several plants accumulate high proline levels during drought that protect against this extreme condition (Moreno-Galvan et al., 2020). The production of proline in cotton under drought has been reported (Singh et al., 2021). Proline also scavenges free radicals, buffers cellular redox potential during stressful situations, and stabilizes subcellular level structures such as membranes and proteins. Drought-tolerant varieties of cotton have found to have higher proline contents (Singh et al., 2021). In this investigation, we found that the proline content of all our cultivars increased under drought stress. A similar trend of an increase in free proline content with a decrease in water content was described by Singh et al. (2021) in cotton. This may be because of proline's osmolyte function, which sustains the osmotic potential during stress relief. An association analysis showed that under low water conditions, cultivars with higher proline folds produced positive results for seed cotton yield and yield components.

Impact of drought on physio-biochemical attributes influence on peak flowering stage that leads to reduce seed cotton yield (Ullah et al., 2021). Agronomic and yield attributes mean value decreased during drought and genotypes showed more detrimental effects on traits, such as PH, SB, BN, BW, SCY, and GOT%. This may be due to the duration of time in limited water conditions that stimulate plants to reach earlier

maturity by shortened flowering-to-boll opening period (Xu et al., 2019). Similar trends were documented under limited water regime by Goren and Tan, 2024.

Water in adequate quantity required that keeps cell turgor and absorbs carbohydrates to develop quality fibre (Li et al., 2020). The quality of fiber traits deteriorated in limited water regime, and it was figured that FH-326, FH-416, FH-492, NIAB-878, VH-327, CS16 and CS5 retained it during water limited conditions with minimum variation. According to most of the previous studies, quality of fibre deteriorates during water stress (Avşar and Karademir, 2022; Ullah et al., 2021; Goren and Tan, 2024). The outcomes of our investigations are consistent with these findings on cotton.

Drought Susceptibility Index (DSI) provides an inside look at how crops react overall to water stress. Drought indices are therefore essential in the selection of genotypes with high yield potential. On the other hand, there was a trend showing low RD% and DSI scores to be associated with high yield under limited water conditions. The genotypes with high RD% and DSI values exhibited higher seed cotton yield under well water. It was found that FH-326 and VH-327 could withstand more water stress. Their genetic potential for exceptional performance in drought-stressed environments is high. A similar quintessence of trend was found by Ullah et al. (2019).

The findings suggest that parent selection contributes significantly and that, when there is a water deficiency stress, choosing the most suitable cross combinations may lead to successful outcomes. Most of the investigated variables, including physiological traits, proline, yield attributes, and glycine betaine, were found to be significantly controlled by genetics. These traits have a solid genetic foundation and hold great promise for enhancing cotton's ability to withstand drought. Furthermore, lines contributed more positive alleles for physio-biochemical (Net photosynthesis, glycine betaine, proline, total soluble protein) and yield qualities. This suggests that the features may be influenced by the maternal effects. Under conditions of water limited conditions, lines contributed much more to quality of fiber as well. Two combinations CS5 and CS16 revealed the better genotypes with their performance in physio-biochemical, yield and quality traits. It is recommended that the identified superior parents in this study may be used in future breeding programs to improve cotton growth and drought resistance.

The C_2H_2 transcription factor family, encoded by *IDD* genes, is one of the largest plant gene families and is essential for the growth and development of plants. Earlier research found the *IDD* gene family in rice, maize, apple, and Arabidopsis (Ali et al., 2019). However, genome-wide *IDD* and *HH3* gene identification and analysis have not yet been performed on cotton comprehensively. Identification of *IDD* genes in various plants have been observed during stress whereas in allotetraploid cotton (*Gossypium hirsutum* L.) *IDD* genes were studied to understand the role of the *IDD* gene family in cotton development (Ali et al., 2019). Considering the second intimate *HH3* gene in the study provided useful information for detecting stress phenomena in *Arabidopsis* and rice (Li et al., 2017) and these findings have now been verified in cotton (Qanmber et al., 2019). Analyses to qRT-PCR for both the genes in cotton genotypes CS16 and CS5 during stress revealed the expression of both the genes that provide indication that our findings are in support to the scientist's earlier discovery (Ali et al., 2019; Qanmber et al., 2019). According to expression analyses, *GhHH3* and *GhHH3* can be used in cotton breeding programs to identify genotypes that are the best in terms of yield and endurance during cotton development phases. We suggest that the newly developed crossed should be included in the cotton breeding program to develop drought resistance cotton varieties for future use.

Drought is a major constraint of low yield in cotton. At present it is important to develop high yielding and drought tolerant genotypes to revive the cotton production in Pakistan. The potential of the developed crosses CS5 and CS16 surely showcasing the opportunity to play its part in resolving the issue. The crosses can be utilized in cotton breeding programs for the development of improved germplasm. Such breeding material would be helpful for cotton breeders to proceed further.

Conclusion

Climate change significantly influence potential of already approved cotton genotypes. Despite several approaches, selection of cotton breeding material merely on the basis phenotype and physiology has limitation to attain desirable outcomes. Hence, coupling with gene expression studies will certainly aid in the selection of superior material.

Here 3 crosses CS16 (NIAB-878 × FH-414), CS5(FH-326 × Marvi) and CS21(VH-327 × FH-415) that produce valuable results for yield. Based on seed cotton yield and quality traits during limited water regime and gene expression studies, we recommend 2 crosses (CS5 and CS16) as the best and can be recommended to be included in the cotton breeding programs in Pakistan.

Acknowledgment

Contribution of Authors

Sarwar MKS: Experimentation, data analysis and drafted original manuscript
Ghaffar A & Ahmad S: Review of literature and manuscript editing
Rehman SU: Methodology, review and data analysis
Waheed U: Conceptualization, methodology and supervision

References

Akladious SA, 2012. Influence of different soaking times with selenium on growth, metabolic activities of wheat seedlings under low temperature stress. African J. Biotech. 11:14792-804. DOI: 10.5897/AJB12.2140

Ali F, Qanmber G, Li Y, Ma S, Lu L, Yang Z, Wang Z and Li F, 2019. Genome-wide identification of *Gossypium* indeterminate domain genes and their expression profiles in ovule development and abiotic stress responses. J. Cotton Res. 2:1-6. DOI:10.1186/s42397-019-0021-6

Arnon DI, 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24, 1-15. doi: 10.1104/pp.24.1.1.

Aslam S, Hussain, SB, Baber M, Shaheen S, Aslam S, Waheed R, Seo H and Azhar MT, 2023. Estimation of drought tolerance indices in upland cotton under water deficit conditions. Agron 13.p.984. https://doi.org/10.3390/agronomy13040984.

Avşar O and Karademir E, 2022. Evaluation of quality parameters in cotton production (*Gossypium hirsutum* L.) under water stress conditions. 55:45-61. DOI:10.46909/alse-551045

Bates LS, Waldren RA and Teare ID, 1973. Rapid determination of free proline for water-stress studies. Plant and soil. 39:205-7.

Birchler JA, Yao H and Chudalayandi S, 2006. Unraveling the genetic basis of hybrid vigor. Proc. Natl. Acad. Sci. U.S.A. 103:12957–12958. https://doi.org/10.1073/pnas.0605627103.

Celik S, 2023. Assessing drought tolerance in a large number of upland cotton plants (*Gossypium hirsutum* L.) under different irrigation regimes at the seedling stage. Life, 13: p.2067. https://doi.org/10.3390/life13102067

de Mendiburu F and de Mendiburu MF, 2019. 'agricolae'. R Package, version. 4. 1(3): 1143-1149.

Chaves MM, Flexas J and Pinheiro C, 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. 103:551-560. https://doi. org/ 10.1093/aob/mcn125.

Farooq M, Wahid A, Kobayashi N, Fujita D and Basra SMA, 2009. Plant drought stress: Effects, mechanisms and management. 153-88. In: Lichtfouse E, Navarrete M, Debaeke P, Veronique S, Alberola C, (eds) Sustainable Agriculture. Springer, Dordrecht.

Fischer RA and Maurer R, 1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. Aus. J. Agric. Res. 29:897-912. doi.org/10.1071/AR9780897

Gao M, Snider JL, Bai H, Hu W, Wang R, Meng Y, Wang Y, Chen B and Zhou Z, 2020. Drought effects on cotton (*Gossypium hirsutum* L.) fibre quality and fibre sucrose metabolism during the flowering and boll-formation period. J. Agron. Crop Sci. 206:309-21. DOI: 10.1111/jac.12389

Ghasempour HR, Gaff DF, Williams RPW and Gianello RD, 1998. Contents of sugars in leaves of

drying desiccation tolerant flowering plants, particularly grasses. Plant Growth Reg. 24:185-191.

Gill RA, Zhang N, Ali B, Farooq MA, Xu J, Gill MB, Mao B and Zhou W, 2016. Role of exogenous salicylic acid in regulating physio-morphic and molecular changes under chromium toxicity in black-and yellow-seeded *Brassica napus* L. Environ. Sci. Pollut. 23, 20483–20496. DOI: 10.1007/s11356-016-7167-2.

Goren HK and Tan U, 2024. Unraveling the complexities of drought stress in cotton: a multifaceted analysis of selection criteria and breeding approaches. PeerJ. 12, p.e17584. https://doi.org/10.7717/peerj.17584.

Grieve CM and Grattan SR, 1983. Rapid assay for determination of water-soluble quaternary ammonium compounds. Plant & Soil. 70:303-7.

Gupta A, Rico-Medina A and Cano-Delgado AI, 2020. The physiology of plant responses to drought. Sci. 368:266-9. DOI: 10.1126/science.aaz7614

Haroon M, Anas M, Naurin I, Afzal R, Irfa, U, Tariq H, Idrees F, Taj MH and Rukh M, 2023. Autoimmunity in plants; a powerful weapon in kingdom plantae to combat stresses. Int. J. Agric. Biosci. 12 :159–164. doi: 10.47278/journal.ijab/2023.059.

Havaux M, 1998. Carotenoids as membrane stabilizers in chloroplasts. Trends Plant Sci. 3, 147–151. doi.org/10.1016/S1360-1385(98)01200-X

Hovick SM and Whitney KD, 2014. Hybridisation is associated with increased fecundity and size in invasive taxa: meta-analytic support for the hybridisation-invasion hypothesis. Ecol. Lett. 17: 1464–1477. doi: 10.1111/ele.12355

Huseynova IM, Rustamova SM, Suleymanov SY, Aliyeva DR, Mammadov AC and Aliyev JA, 2016. Drought-induced changes in photosynthetic apparatus and antioxidant components of wheat (*Triticum durum* Desf.) varieties. Photosynth. Res. 130. pp.215-223.

Khan AI, Awan FS, Sadia B, Rana RM and Khan IA, 2010. Genetic diversity studies among coloured cotton genotypes by using RAPD markers. Pak. J. Bot. 1;42(1):71-7.

Li XR, Smith B Choat and Tissue DT, 2020. Drought resistance of cotton (*Gossypium hirsutum*) is promoted by early stomatal closure and leaf shedding. Funct. Plant Biol. 47:91-98. DOI. org/10.1071/FP19093

Li F, Li M, Wang P, Cox Jr KL, Duan L, Dever JK, Shan L, Li Z and He P, 2017. Regulation of cotton (*Gossypium hirsutum*) drought responses by mitogen-activated protein (MAP) kinase cascade-mediated phosphorylation of *GhWRKY59*. New Phytol. 215:1462-75. DOI:org/10.1111/nph.14680

Li X, Shahzad K, Guo L, Qi T, Zhang X, Wang H, Tang H, Qiao X, Zhang J, Wu J and Xing C, 2019. Using yield quantitative trait locus targeted SSR markers to study the relationship between genetic distance and yield heterosis in upland cotton (*Gossypium hirsutum*). Plant Breed. 138(1):105-13.

Loka DA, Oosterhuis DM, Baxevanos D, Noulas C and Hu W, 2020. Single and combined effects of heat and water stress and recovery on cotton (Gossypium hirsutum L.) leaf physiology and sucrose metabolism. Plant Physiol. Biochem. 148: 166-179. DOI: 10.1016/j.plaphy.2020.01.015.

Lowry OH, Rosebrough NJ, Farr AL and Randal RJ, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275.

Marimuthu G and Murali, PV, 2018. Effect of drought stress in *Paspalum scrobiculatum* L. biochemical and compatible solute accumulation. Int. J. Sci. Res. Sci. Technol. 4: 618-622.

Mehmood HZ, Abbas A, Hassan S and Ullah R, 2022. Socio-economic, farm, and information variables influencing farmer's decision to adopt a sustainable way of cotton production. Int. J. Agr. Ext. 10 :149-159. DOI: 10.33687/ijae.010.01.4010

Moreno-Galvan AE, Cortes-Patino S, Romero-Perdomo F, Uribe-Velez D, Bashan Y and Bonilla RR, 2020. Proline accumulation and glutathione reductase activity induced by drought-tolerant rhizobacteria as potential mechanisms to alleviate drought stress in Guinea grass. Appl. Soil Ecol. 147, 103367. DOI: org/10.1016/j.apsoil.2019.103367

Nawaz MS, Sami SA, Bano M, Khan MRQ, Anwar Z, Ijaz A and Ahmed T, 2023. Impact of salt stress on cotton. Int. J. Agric. Biosci. 12: 98–103. https://doi.org/10.47278/journal.ijab/2023.051.

Parida AK and Das AB, 2004. Effects of NaCl stress on nitrogen and phosphorus metabolism in a true mangrove Bruguieraparvi flora grown under hydroponic culture. J. Plant Physiol. 161: 921-928. https://doi.org/10.1016/j.jplph.2003.11.006.

Qanmber G, Ali F, Lu L, Mo H, Ma S, Wang Z and Yang Z, 2019. Identification of histone H3 (*HH3*) genes in *Gossypium hirsutum* revealed diverse expression during ovule development and stress responses. Genes. 9; 10:355. doi:10.3390/genes10050355

Qureshi H, Waheed U, Siddique AB, Ali Z, Ahmed R, Sidduqiue M, Shahzadi I, Naz A, Ejaz W and Raza N, 2021. Fruit drop in cotton: some causes. J. Agric. Food. 2:36-62. DOI:10.52587/JAF040202

Rauf A, Sher MA, Farooq U, Rasheed A, Sajjad M, Jing R, Khan Z, Attia KA, Mohammed AA, Fiaz S and Chen J, 2024. An SNP based genotyping assay for genes associated with drought tolerance in bread wheat. Mol. Biol. Rep. 51(1):527.

Rehman A and Farooq M, 2019. Morphology, physiology and ecology of cotton. In: Jabran, K., Chauhan, B.S. (Eds.), Cotton Production. John Wiley & Sons Ltd, pp. 23–46. https://doi.org/10.1002/9781119385523.ch2

Sarwar MK, Ashraf MY, Rahman M and Zafar Y, 2012. Genetic variability in different biochemical traits and their relationship with yield and yield parameters of cotton cultivars grown under water stress conditions. Pak. J. Bot. 44:515-20.

Schmittgen TD and Livak KJ, 2008. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc. 3(6): 1101-8. DOI:10.1038/nprot.2008.73

Sloane RJ, Patterson RP and Carter JrTE, 1990. Field drought tolerance of a soybean Plant Introduction. Crop Sci. 30:118-123. doi.org/10.2135/cropsci1990.0011183X003000010027x

Singh CK, Rajkumar BK and Kumar V, 2021. Differential responses of antioxidants and osmolytes in upland cotton (*Gossypium hirsutum*) cultivars contrasting in drought tolerance. Plant Stress. 1;2:100031.

Soong JL, Phillips CL, Ledn, C, Koven CD and Torn MS, 2020. CMIP5 models predict rapid and deep soil warming over the 21st century. J. Geophys. Res. Biogeosci. 125 :e2019JG005266. doi: 10.1029/2020JG005668.

Tokel D, Genc BN and Ozyigit II, 2022. Economic impacts of *Bt* (*Bacillus thuringiensis*) cotton. J. Nat. Fibers 19 :4622–4639. https://doi.org/10.1080/15440478.2020.1870613

Ullah S, Rehman A, Hussain M and Farooq M, 2021. Fiber yield and quality in cotton under drought: Effects and management. Agric. Water Manage. Pp255:106994. DOI:10.1016/j.agwat.2021.106994

Ullah A, Shakeel A, Malik TA and Saleem MF, 2019. Assessment of drought tolerance in some cotton genotypes based on drought tolerance indices. J. Animal Plant Sci. 29: 998-1009.

Ullah A, Shakeel A, Ahmed HG.D, Naeem M, Ali M, Shah AN, Wang L, Jaremko M, Abdelsalam, NR, Ghareeb RY and Hasan ME, 2022. Genetic basis and principal component analysis in cotton (Gossypium hirsutum L.) grown under water deficit condition. Front. Plant Sci. 13:981.369. https://doi.org/10.3389/fpls.2022.981369

Vallejo-Marin M and Hiscock SJ, 2016. Hybridization and hybrid speciation under global change. New Phytologist 211: 1170–1187. https://doi.org/10.1111/nph.14004.

Wei R, He Y, Liu J, He Y, Mi W, Yuen R and Wang J, 2017. Experimental study on the fire properties of nitrocellulose with different structures. Mater. 10:316. DOI:10.3390/ma10030316

Xu Y, Magwanga RO, Cai X, Zhou Z, Wang X, Wang Y, Zhang Z, Jin D, Guo X, Wei Y and Li Z, 2019. Deep transcriptome analysis reveals reactive oxygen species (ROS) network evolution, response to abiotic stress, and regulation of fiber development in cotton. Int J. Mol. Sci. 20 p.1863. DOI:10.3390/ijms20081863

Zafar MM, Razzaq A, Farooq MA, Rehman A, Firdous H, Shakeel A, Mo H, Ren M, Ashraf M and Youlu Y, 2022. Genetic variation studies of ionic and within boll yield components in cotton (*Gossypium hirsutum* L.) under salt stress. J. Nat Fibers 19 :3063–3082. doi: 10.1080/15440478.2020.1838996.

Zafar MM, Chattha WS, Khan AI, Zafar S, Anwar Z, Seleiman MF, Parvaiz A and Razzaq A, 2023. Drought and heat stress on cotton genotypes suggested agro-physiological and biochemical features for climate resilience. Front. Plant Sci. 14:1265700. doi:10.3389/fpls.2023.1265700

Zafer MZ, Tahir MH, Khan Z, Sajjad M, Gao X, Bakhtavar MA, Waheed U, Siddique M, Geng Z and Rehman SU, 2023. Genome-Wide characterization and sequence polymorphism analyses of *Glycine max* Fibrillin (FBN) revealed its role in response to drought condition. Genes. 14(6):1188.
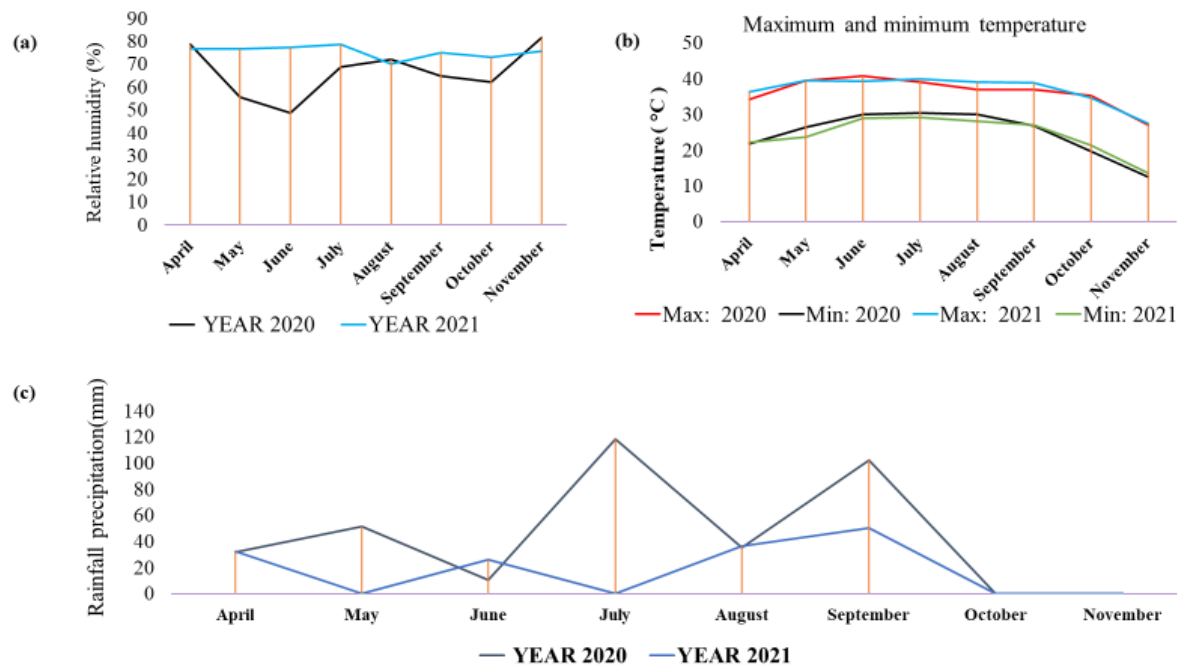
Supplementary Figure 1

Figure. Seasonal meteorological data during 2020 and 2021