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Morphological, physiological and molecular assessment of cotton for drought tolerance under field conditions



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ABSTRACT

Climate change could be an existential threat to many crops. Drought and heat stress are becoming harder for cultivated crops. Cotton in Pakistan is grown under natural high temperature and low moisture, could be used as a source of heat and drought tolerance. Therefore, the study was conducted to morphological, physiological and molecular characterization of cotton genotypes under field conditions. A total of 25 cotton genotypes were selected from the gene pool of Pakistan based on tolerance to heat and drought stress. In field trial, the stress related traits like boll retention percentage, plant height, number of nodes and inter-nodal distance were recorded. In physiological assessment, traits such as photosynthesis rate, stomatal conductance, transpiration rate, leaf temperature, relative water content and excised leaf water loss were observed. At molecular level, a set of 19 important transcription factors, controlling drought/heat stress tolerance (HSPCB, GHSP26, HSFA2, HSP101, HSP3, DREB1A, DREB2A, TPS, GhNAC2, GbMYB5, GhWRKY41, GhMCK3, GhMPK17, GhMCK1, GhMPK2, APX1, HSC70, ANNAT8, and GhPP2A1) were analyzed from all genotypes. Data analyses depicted that boll retention percentage, photosynthesis, stomatal conductance, relative water content under the stress conditions were associated with the presence of important drought & heat TF/genes which depicts high genetic potential of Pakistani cotton varieties against abiotic stress. The variety MNH-886 appeared in medium plant height, high boll retention percentage, high relative water content, photosynthesis rate, stomatal conductance, transpiration rate and with maximum number transcription factors under study. The variety may be used as source material for heat and drought tolerant cotton breeding. The results of this study may be useful for the cotton breeders to develop genotype adoptable to environmental stresses under climate change scenario.

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1. Introduction

Search for heat and drought tolerant germplasm has been increased because of climate change. Tolerant germplasm exists in the areas where climatic conditions are naturally severe. Pakistani cotton crop tolerates highest temperature as compared to any other country's cotton (Saleem et al., 2021). The country is also dropped to a water stressed country as freshwater availability is declining. Cotton varieties breed and grow here are comparatively more tolerant to heat and drought stress. A comprehensive assessment of stress tolerance includes morphological, physiological and molecular analysis. At molecular level, a variety of transcription factors, transcription variants, signaling genes and functional genes have been identified which became active under drought/heat stress and help plant grow normally. Among those, Annexins plays role in plant development and plant protection. Among eight reported genes of this family (Yadav et al., 2016), AnnAt8 a member of Annexins regulates abiotic stress tolerance (He et al., 2020). Under abiotic stress, overexpression of this gene shows better seed germination, growth and higher chlorophyll content (Yadav et al., 2016). Ascorbate peroxidase (APX) which catalysis H_2O_2 , a signaling molecule for fibre development is an important gene in cotton (Tao et al., 2018; Nawaz et al., 2020). The cytosolic APX gene possessed heat shock element in its promoter region to produces quick heat response (Storozhenko et al., 1998), drought response (Smirnoff & Colom  , 1988), salt and ABA response (Shi et al., 2001), sulphur dioxide response (Fryer et al., 2003) as well as involved in detoxification (Asada, 1992; Davletova et al., 2005). The proteomic analysis revealed its increased accumulation in cotton fibre cell during elongation period (Qin et al., 2008). The transcription factors DREB (Dehydration responsive element binding proteins) are associated with drought (Konzen et al., 2019; Zhang et al., 2020; Chai, et al 2020) and could be used for genetic improvement in crop plants (Niu et al., 2020).

A signaling family MAPK (Mitogen-activated protein kinase) improves drought tolerance by reducing water loss (Long et al., 2014) by phosphorylation (Hou et al., 2018) and through regulating salicylic acid signaling transduction (Long et al., 2020), work as an antioxidant (Zong et al., 2009), improves salt tolerance (Zhang et al., 2011; Taghizadeh et al., 2018) and considered as a multi-defense family against abiotic stress (Zhang et al., 2011). MAPKs are drought responsive (Zhou et al., 2020) involved in phytohormone signaling (Xie et al., 2020) could be used for molecular breeding for drought tolerance in cotton (Zhou et al., 2020). NAC proteins are produced in response to abiotic stress, improve tolerance by morphological changes as well as hormones production in cotton (Gunapati et al., 2016). TPS (trehalose-6-phosphate-synthase) is produced in cotton in response to drought stress (Kosmas et al., 2006). MYB transcription factors involved in drought and salt stress response (Ullah et al., 2017). In cotton, an important regulator for ABA mediated signaling pathways is GhABF2, encoding for typical cotton bZIP transcription factor, enhance fibre yield under osmotic stress (Liang et al., 2016).

Similarly, in cotton, some other transcription factors are reported to be involved in drought tolerance such as GhWRKY17 (Chen et al., 2015), GhWRKY41 (Chu et al., 2015), MYB (Chen et al., 2015; Zhai et al., 2017), and GhABF2 (Liang et al., 2016). GhMCK1 also proved to be an important regulator against environmental stresses as well as works as an antioxidant, whereas GhMCK3 regulates stomatal responses and root growth under drought stress (Wang et al., 2016). HSPs (Heat Shock Protein Genes) help cotton plants to withstand heat stress (Saleem et al., 2021). A variety of HSPs has been studied in plants. Among these GHSP26 increased drought tolerance (Shamim et al., 2013), HSA1

(Yu et al., 2012), HSA2 (Wang et al., 2014), HSP101 9 (Hong et al., 2000) improve heat tolerance.

Presence of such important genes would be a key to heat and drought stress in cotton. But very little attention has been given to conduct molecular characterization along with field performance physiologically and morphologically. This study has been conducted to investigate transcription factors, signaling and functional genes and their association with physiological and morphological analysis under heat and drought stress.

2. Material and method

2.1. Plant material

A set of 25 genotypes with high drought/heat stress response were raised in the field conditions. The genotypes were from different genetic backgrounds and were collected from different breeding stations of Pakistan such as Central Cotton Research Institute Multan, Central Cotton Research Institute Sakrand, Cotton Research Station Faisalabad, Cotton Research Station Multan, Cotton Research Station Vehari, and Nuclear Institute of Agriculture and Biology Faisalabad. All the genotypes were treated with two irrigation levels i.e. Normal irrigation and drought stress. In every replication there were ten plants for each entry following triplicated randomized complete block design.

2.2. Experimental treatments

The genotypes were planted in the field on 15 April 2019. Plant to plant and row to row distance was kept 45 and 60 cm, respectively. All agronomic practices such as plant protection measures and fertilizer applications were given as per cotton crop requirements uniformly to all the treatments. For drought management, all the genotypes were treated with two irrigation levels i.e., normal irrigation and drought stress. Drought stress was imposed by increasing the interval of irrigation. For normal irrigation, the crop was irrigated fortnightly (each of 75 mm) by flood irrigation while for drought stress, the crop was irrigated (each of 75 mm) after every three weeks. The data was recorded when the severe symptoms of drought stress were appeared on plants

2.3. Data collection

Data for morphological traits i.e., plant height and boll retention percentage were collected at crop maturity. Plant height was measured from ground level to the upper tip of the plant and each data value presents an average of five plants. For boll retention, was calculated by following formula by Iqbal et al., (2017):

$$\text{Boll Retention \%age} = \frac{\text{Total number of bolls opened}}{\text{Total number of buds formed}} \times 100$$

2.4. Physiological analysis

For physiological analysis, Infra-Red Gas Analyzer (CID Bioscience CI-340) was used to record traits such as net photosynthesis ($\mu\text{mol}/\text{m}^2/\text{s}$), stomatal conductance ($\text{mmol}/\text{m}^2/\text{s}$), transpiration rate ($\text{mmol}/\text{m}^2/\text{s}$), leaf temperature ($^{\circ}\text{C}$) and air temperature ($^{\circ}\text{C}$) from selected cotton genotypes. Data for physiological parameters were collected when the crop growth was at peak i.e. 30 July 2019 in late morning at 11:00 'O' Clock. The leaf-air temperature difference was calculated from each plant sample under control and stress by using the following formula (-Zhang et al., 2019):

$$\Delta T = (T_{leaf} - T_{air})$$

T_{leaf} = Leaf Temperature, T_{air} = Air Temperature

Relative water content (Clarke et al., 1986) and excised leaf water loss (Clarke et al., 1982) were assessed by following formulas:

$$RWC = \frac{(Fresh\ weight - Dry\ weight)}{(Turgid\ weight - Dry\ weight)} \times 100$$

$$ELWL = \frac{(Fresh\ weight - Wilted\ Weight)}{Dry\ Weight}$$

2.5. Molecular analysis

DNA was extracted from selected cotton genotypes (Table 2) using a standard CTAB method (Doyle & Doyle, 1987). For molecular screening, a set of 19 important transcription factors, variants and a functional genes controlling drought/heat tolerance were selected (Saleem et al., 2020) namely HSPCB, GHSP26, HSFA2, HSP101, HSP3, DREB1A, DREB2A, TPS, GhNAC2, GbMYB5, GhWRKY41, GhMKK3, GhMPK17, GhMKK1, GhMPK2, APX1, HSC70, ANNAT8 and GhPP2A1 (Table 1). Selected genes were amplified through PCR analysis using specific primers for each of the above genes (Table 1). PCR reaction mixture was prepared with 50 ng of cotton genomic DNA, 0.2 µg of 3' and 5' end primers, 200 mM of each dNTP, 1 × PCR buffer containing 50 mM KCL, 10 mM Tris HCl (pH 8.9), 2.0 mM MgCl₂ and one unit of *Taq* polymerase in a total of 25 µL solution individually for all eight primer

pairs. PCR thermal cycler was programmed for 2 min at 94 °C, 1 min at 47 °C and 1 min at 72 °C, and a final cycle of 10 min at 72 °C by using 35 PCR cycles. The amplification product was separated on 3% of metaphor agarose gel in 1 × TBE buffer, followed by staining with ethidium bromide. A 50 bp DNA ladder (Life Technologies-GIBCO BRL) was used to estimate the size of each band. Scoring of the bands was done based on the desired gene presence or absence. The primers were designed following NCBI data base. PCR products were checked on Agarose Gel Apparatus following standard protocols.

3. Results

3.1. Morphological traits

Maximum plant height was appeared in the variety VH-260 whereas lowest height was attained by CRIS-342 under normal irrigation conditions (Table 2). Boll retention percentage was observed highest in MNH-886, followed by CIM-600 and Sitara-008. Lowest boll retention percentage was observed in CRIS-342 under normal conditions. The range of BR% was between 40.21 and 77.70%. Under stressed conditions the maximum height was observed in the variety VH-305. The range of the trait plant height under drought stress condition was 49.25–106.5 cm among the varieties under study. The trait boll retention percentage was recorded highest in the variety MNH-886 under drought stress conditions followed by MNH-1035 and CIM-446. Lowest BR% was observed in CRIS-342 under drought stress (Table 2).

Table 1

List of transcription factors, transcription variant, signaling genes and functional gene screened in this study.

Serial #	TF/Gene	Primer Sequences	Function	Reference
1	HSPCB	FP: GTGCGTCTCTAGTGTCTTT RP: CTCCTTGAATGTATTTACTGCC	Heat shock protein	Voloudakis et al., 2002
2	GHSP26	FP: TGGGGCTACTCTCAAAGGGTTG RP: TGAGAAATTGCTGAAGCCGAAA		Maqbool et al., 2007
3	HSP101	FP: GGAAGTGAATCTGCGATAGT RP: GATTTTGTCCACCACTCTTTG		Yoshid et al., 2011
4	HSP3	FP: AGAAAAGTTGACCCCTGACCGC RP: AACCTCTCTTCGAGACCAAAC		Zhang et al., 2016
5	HSC70	FP: TTGTTACCGTCCCTGCATACTT RP: GACATCAAAAGTACCGCCACC		Qi et al., 2011
6	HSFA2	FP: GAAGGAAGGGCTTAACGAGG RP: GCTGACGAATGAACTGGAGA	Heat stress transcription factor (HSF)	Nishizawa et al., 2006
7	DREB1A	FP: CAGAGAATTCGGATCCCAATGAACTATTTCTGCT RP: CCGCACTCGAGGTGCGACCTGCGATCACACATCTC	Dehydration responsive element binding proteins	Liu et al., 1998
8	DREB2A	FP: GATCCGAATTCATGGCAGTTTATGATCAGAGTGG RP: CAGCACTCGAGGTGCGACGATCCTCTGTTTTAC		Liu et al., 1998
9	TPS	FP: CTGCGAGCCCAATATGCCAC RP: TAAGCTGCGAGGGATGG	Trehalose-6- phosphate synthase	Kosmas et al., 2006
10	GhNAC2	FP: ATGTGCATCGCAGTCCATC RP: CTCCGTACAACGCCAAATCT	NAC protein	Gunapati et al., 2016
11	GbMYB5	FP: GACATCAATGTTCAAAAGACAGC RP: ATTGAAGAACAGAAAGTTGAATCCC	MYB protein	Chen et al., 2015
12	GhWRKY41	FP: CTTACAGTGAAGGAAAGAAGA RP: TGAATGAAAGGGAGATGTATTGT	WRKY protein	Chu et al., 2015
13	GhMKK1	FP: GAAGAAGAAGCAAAACCTCAGATG RP: GTCATCACTACAGCCGCTC	MMK protein	Lu et al., 2013
14	GhMKK3	FP: CTGCGTGGATTGGGAAG RP: GAACTACTAACCTCAAGCGG		Wang et al., 2016
15	GhMPK2	FP: GGATCCCAGGAAAATGGCAATCCAG RP: GAGCTCCAGTGGTAAGACAACATCGT	Mitogen activated protein kinase	Zhang et al., 2011
16	GhMPK17	FP: GTTGCAAGCATCCGTGGAACCAAGAT RP: TAAGACAGATTAAAGAACCTCAGAGG		Zhang et al., 2014
17	APX1	FP: GGCACCTCAGCTGGAACCTTTG RP: AGCGTAGGTGAGGTAGGGAA	Ascorbate peroxidase (APX) enzyme	Hong and vierling 2000
18	ANNAT8	FP: CAGGATCTTGAGTACAAGGAGC RP: GTAAGTGCATCCTCATCGTTTC	Annexin	Shi et al., 2001
19	GhPP2A1	FP: GATCCTGTGGAGGAGTGGGA RP: GCGAAACAGTTCGACGAGAT	Phytochrome	Cantero et al., 2006

Table 2

Mean data for the traits Plant height (PH), Net Photosynthesis (NP), Stomatal Conductance (SC), Transpiration Rate (TR), Leaf-Air Temperature (ΔT), Relative Water Content (RWC), Excised Leaf Water Loss (ELWL), Boll Retention Percentage (BR%), and Screened Genes (SG) under Control (C) and under Drought stress (S) in selected varieties of cotton.

Sr. No	Genotypes	T	PH	NP	SC	TR	ΔT	RWC	ELWL	BR %age	Screened genes
1	CRIS-342	C	85.2	13.22	198.12	7.51	1.01	56.12	0.55	40.21	11
		S	64.1	08.9	140.31	5.10	1.21	41.01	0.34	27.11	
2	VH-260	C	119.0	21.05	345.61	7.29	-2.54	71.21	1.10	58.11	16
		S	106.5	18.91	329.13	7.00	-1.01	65.19	0.74	51.70	
3	VH-259	C	118.0	31.25	253.9	6.03	-2.13	68.90	1.21	69.6	17
		S	101.5	19.17	270.14	5.90	-1.10	54.28	0.45	48.17	
4	VH-Gulzar	C	104.5	25.10	263.7	7.01	-0.8	73.71	0.91	66.50	17
		S	92.5	21.14	281.82	5.82	-0.2	60.36	0.46	61.41	
5	VH-189	C	112.5	32.96	304.74	7.18	0.25	70.14	0.88	54.80	17
		S	101.0	27.17	287.34	6.11	0.41	56.24	0.41	41.23	
6	VH-305	C	118.8	28.80	501.90	8.73	-1.0	67.31	0.94	68.00	18
		S	109.0	18.31	439.10	6.44	-0.61	52.75	0.40	45.39	
7	VH-383	C	102.0	23.4	335.20	6.02	1.31	70.14	1.03	71.40	17
		S	77.5	19.21	340.13	6.10	1.10	61.30	0.62	60.93	
8	CIM-446	C	92.5	19.03	361.30	8.04	-2.06	69.13	1.02	68.20	16
		S	57.5	19.11	350.44	5.24	-1.30	62.89	0.53	63.51	
9	CIM-506	C	102.5	20.27	250.60	6.85	0.65	72.11	0.89	73.30	17
		S	61.6	20.15	248.91	4.81	1.21	63.78	0.48	50.51	
10	MNH-1035	C	105.5	22.40	431.03	6.91	0.15	69.90	0.99	68.90	16
		S	59.0	21.19	470.32	4.01	-0.10	65.69	0.56	63.39	
11	MNH-886	C	95.5	35.69	384.25	7.37	-0.75	74.15	0.73	77.70	18
		S	77.1	35.17	410.22	4.56	-1.20	69.55	0.29	63.88	
12	CIM-616	C	88.5	31.30	497.54	8.84	-0.60	71.14	0.79	63.30	17
		S	65.3	30.11	401.21	4.88	-0.41	60.00	0.36	48.98	
13	CIM-599	C	89.5	8.65	94.12	6.91	1.80	69.91	1.33	68.60	17
		S	49.25	7.19	110.10	4.16	1.41	59.47	0.63	43.45	
14	CIM-600	C	115.3	46.58	806.05	8.75	-1.0	70.81	0.97	72.20	18
		S	91.25	44.29	810.22	6.77	-1.34	64.24	0.48	60.33	
15	Sitara-008	C	90.4	35.60	321.09	8.35	0.85	68.13	0.79	76.60	17
		S	92.1	32.10	310.13	8.10	1.01	55.50	0.42	58.93	
16	CIM-534	C	110.0	22.49	265.59	7.06	0.35	67.07	0.66	60.41	17
		S	77.5	20.31	251.81	6.43	0.76	51.01	0.48	55.30	
17	CRIS-134	C	111.6	19.80	269.03	5.99	0.23	64.91	1.30	51.24	17
		S	88.5	14.21	220.21	6.81	0.91	49.70	0.70	45.80	
18	CRSM-38	C	103.5	18.90	353.05	7.61	0.06	69.01	0.83	48.34	18
		S	84.9	11.21	300.17	6.99	-0.47	62.09	0.20	40.90	
19	CIM-482	C	111.3	32.03	578.28	8.73	-0.5	71.23	0.69	66.08	17
		S	98.1	24.16	479.35	7.34	0.9	63.93	0.37	40.12	
20	NIAB-111	C	106.1	36.36	682.46	9.16	-1.15	65.29	0.63	59.01	18
		S	93.8	30.19	588.90	7.88	-0.31	59.38	0.51	46.60	
21	CIM-496	C	114.8	26.98	376.54	7.84	0.10	68.71	0.47	57.45	15
		S	89.3	24.10	349.21	6.13	0.32	49.52	0.32	31.60	
22	MNH-552	C	105.5	26.60	221.04	5.94	-1.09	62.93	0.81	60.31	15
		S	97.2	20.18	209.23	5.79	-0.33	44.85	0.36	39.02	
23	CYTO-124	C	107.3	14.28	222.23	6.54	0.62	65.39	0.91	63.01	17
		S	79.6	15.22	200.81	6.34	0.20	58.82	0.51	48.76	
24	CYTO-177	C	104.5	33.59	764.39	9.06	-1.0	69.01	0.77	70.00	16
		S	90.1	30.91	672.21	7.01	-1.03	56.00	0.48	59.91	
25	CYTO-178	C	101.4	22.48	188.72	5.83	0.65	66.42	0.98	63.41	15
		S	95.2	23.41	170.61	4.33	-1.03	54.79	0.76	60.80	
Probability		C	**	**	*	**	*	**	**	*	
		S	**	**	**	*	**	**	*	**	

3.2. Physiological traits

Net photosynthesis under normal conditions was highest in CIM-600, MNH-886, CIM-616 and VH-189. Lowest photosynthesis was recorded in CIM-599 under normal water availability. The range of net photosynthesis among varieties was between 8.65 and 46.58 $\mu\text{mol}/\text{m}^2/\text{s}$. Stomatal conductance was highest in CIM-600 followed by Cyto-177, NIAB-111, and VH-305 under normal conditions. Under drought stress CIM-600 had highest photosynthesis followed by MNH-886 and Cyto-177. Lowest photosynthesis was observed in CIM-599. The range of net photosynthesis in varieties under drought stress conditions was between 7.19 and 44.29 $\mu\text{mol}/\text{m}^2/\text{s}$. Lowest stomatal conductance under normal condition was shown by Cyto-178. The range of the trait stomatal conductance under normal conditions was between 188.72 and 806.05 $\text{mmol}/\text{m}^2/\text{s}^{-1}$. Under stressed conditions stomatal conductance was

highest in the variety CIM-600 under water limited conditions, followed by Cyto-177 and NIAB-111. Lowest stomatal conductance was observed in CRIS-342. The range of the trait under water limited conditions was between 140.31 and 810.22 $\text{mmol}/\text{m}^2/\text{s}^{-1}$. Lowest transpiration rate under normal environmental conditions was observed in the variety in Cyto-178, MNH-552 and VH-383. Transpiration rate was highest in NIAB-111 followed by CIM-616 and CIM-600. The range of the transpiration rate was between 5.83 and 9.16 $\text{mmol}/\text{m}^2/\text{s}$ in the varieties under normal irrigated conditions. Whereas under stressed conditions, lowest transpiration rate was observed in the variety Cyto-178 followed by MNH-1035 and CIM-616. Transpiration rate was highest in the variety Sitara-008 under drought stress conditions. The range of transpiration rate was between 4.81 and 8.16 $\text{mmol}/\text{m}^2/\text{s}$ in the genotypes. Assessment of Leaf-Air Temperature under normal conditions depicted the variety VH-260 had the coolest leaf tem-

perature under normal irrigated conditions, followed by CIM-446, CIM-600 and MNH-886. Leaf-Air Temperature was recorded highest in the variety VH-383. The range of temperature difference was between 1.31 and -2.54°C . Whereas, ΔT under stressed conditions was observed lowest in the genotypes CIM-446 followed by CIM-600 and MNH-886, whereas the highest ΔT was observed in the variety CIM-506. The range of ΔT among varieties under drought stress was 1.21 to -1.34°C . Highest relative water content under normal irrigated conditions was observed in the variety MNH-886 followed by VH-Gulzar, VH-260 and CIM-600. The physiological traits were observed lowest in the variety CRIS-342 under normal conditions. The range of relative water content was between 56.12 and 74.15%. Under water limited conditions, highest relative water content was observed in the varieties MNH-886, Cyto-178 and MNH-1035, whereas lowest relative water content was observed in CRIS-342. The range of the trait was between 41.01 and 65.69% under drought stress conditions. Excised leaf water loss was lowest in the variety CIM-496 followed by NIAB-111 and CIM-534 under normal irrigated conditions. Highest loss was observed in the variety CIM-599. The range of the water loss was between 0.47 and 1.33 g/g under normal irrigation. Lowest excised leaf water loss under water limited conditions was observed in CIM-599, CRSM-38 and CIM-496, whereas highest loss was observed in Cyto-178. The range of the water loss under stressed conditions was 0.03–0.76 g/g.

3.3. Molecular studies

A set of 19 important transcription factors, variants, signaling genes and a functional gene related to drought and heat stress

tolerance were used for screening selected cotton varieties (Table 4). Band size of all genes were same as were reported (Fig. 1). The variety CRIS-342 was appeared with lowest number of TF/genes, lacking GHSP26, DREB1A, DREB2A, TPS, GbMYB5, GhMCK1, APX1 and GhPP2A1. Whereas, five varieties had higher number TF/genes under study. Among these, the variety VH-305 appeared to possess a total number 18 transcription factors (Table 4), except the heat shock protein gene HSP101. The varieties MHH-886 and CRSM-38 also had maximum number of 18 transcription factor under study (Table 4), except GhMCK3. CIM-600 had not the transcription factor GhMCK2 and had all the other 18 genes under study. Similarly, the variety, NIAB-111 has all 18 TF/genes except GhWRKY41. The varieties with 17 transcription factors were VH-259, VH-Gulzar, VH-189, CIM-506, CIM-599, Sitara-008, CIM-534 and CIM-482, while the two genes missing in these varieties were GhMCK3 and GhMCK2. Similarly, CIM-616 has 17 TF/genes, except GbMYB5 and GhMCK2. CRIS-134 also had 17 TF/genes under study the missing genes were GhMCK3 and APX1 (Table 4). VH-383 has 17 genes, except HSP101 and GhPR2A1. Genotype Cyto-124 was without the two-transcription factor, GhMCK2 and GhPR2A1, while possessing all other TF/genes under study. The variety VH-260 had all other genes, but DREB2A, GhMCK2 and HSFA2 were missing. The variety CIM-446 appeared not possessing the transcription factors HSP101, GhPR2A1, and APX1 while exhibited all other TF/genes. In MNH-1035 genotype, GbMYB5, GhMCK3, and GhMCK2 were absent, while remaining 16 TF/genes under study were observed during analysis. Similarly, the variety Cyto-177 had 16 TF/genes, except the factors DREB2A, GhMCK3, and GhMCK2. The varieties CIM-496, MNH-552, and Cyto-178 were having 15 TF/genes under study (Table 4).

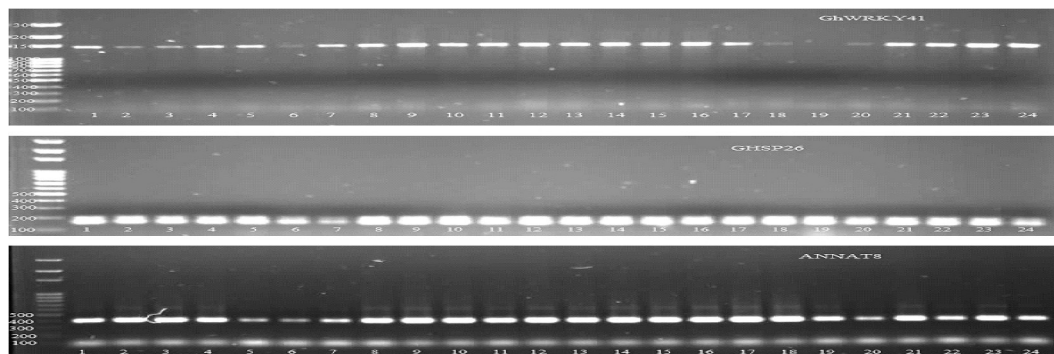


Fig. 1. Screening of transcription factors GbWRKY41, GHSP26, ANNAT8 in selected genotypes.

Table 3

Correlations among the traits Plant height (PH), Net Photosynthesis (NP), Stomatal Conductance (SC), Transpiration Rate (TR), Leaf-Air Temperature (ΔT), Relative Water Content (RWC), Excised Leaf Water Loss (ELWL), Boll Retention Percentage (BR%), and Screened Genes (SG) under Control (C) and under Drought stress (S) in selected varieties of cotton.

	T	PH	NP	SC	TR	ΔT	RWC	ELWL	BR
NP	C	0.22							
	S	0.31							
SC	C	0.02	0.51						
	S	0.01	-0.13						
TR	C	0.44*	0.39	0.39					
	S	0.29*	0.14	0.04					
ΔT	C	0.61*	0.04	0.51	0.40*				
	S	0.55*	0.09	-0.56^*	0.37*				
RWC	C	0.13	0.42*	0.33	0.38	-0.08			
	S	0.19	0.31*	0.18*	0.04	0.20			
ELWL	C	0.03	0.05	0.27	0.41*	0.08	-0.31^*		
	S	0.16	0.01	0.07	0.33*	0.04	-0.06		
BR	C	-0.32	0.21*	0.05	0.34	-0.20^*	0.21*	0.01	
	S	-0.11	0.09	0.10	0.19	-0.44^*	0.38*	0.005	
SG	C	0.01	0.31*	0.03	0.05	-0.39^*	0.08	0.10	0.31
	S	0.08	0.29*	0.09	0.17	-0.27^*	0.42*	0.27	0.28

Table 4
Molecular evaluation of selected varieties under drought stress.

Sr. No	Genotype	Genes																			Total
		HSPCB	GHSP26	DREB1A	DREB2A	TPS	GhNAC2	GbMYB5	GhWRKY41	GhMKK3	GhMPK17	GhMKK1	GhMPK2	HSFA2	HSP101	HSP3	APX1	HSC70	ANNAT8	GhPP2A1	
1	CRIS-342	✓	×	×	×	×	✓	×	✓	✓	×	✓	✓	✓	✓	×	✓	✓	×	11	
2	VH-260	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	×	×	✓	✓	✓	✓	✓	✓	16	
3	VH-259	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	17	
4	VH-GULZAR	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	17	
5	VH-189	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	17	
6	VH-305	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	18	
7	VH-383	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	×	17	
8	CIM-446	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	×	✓	✓	×	16	
9	CIM-506	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	17	
10	MNH-1035	✓	✓	✓	✓	✓	✓	×	✓	×	✓	×	✓	✓	✓	✓	✓	✓	✓	16	
11	MNH-886	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	18	
12	CIM-616	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	17	
13	CIM-599	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	17	
14	CIM-600	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	18	
15	SITARA-008	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	17	
16	CIM-534	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	17	
17	CRIS-134	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	17	
18	CRSM-38	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	✓	✓	18	
19	CIM-482	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	17	
20	NAIB-111	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	18	
21	CIM-496	✓	✓	✓	✓	✓	✓	✓	×	×	✓	×	✓	×	✓	✓	✓	✓	✓	15	
22	MNH-552	×	✓	✓	✓	✓	✓	✓	×	✓	✓	×	✓	✓	✓	✓	✓	✓	×	15	
23	CYTO-124	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	×	17	
24	CYTO-177	✓	✓	✓	×	✓	✓	✓	×	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	16	
25	CYTO-178	✓	✓	✓	✓	✓	✓	×	×	✓	✓	×	✓	✓	✓	✓	✓	✓	×	15	

3.4. Correlation analysis

The correlations analysis among morphological, physiological traits along with frequency of screened genes indicates that the higher number of genes under study were correlated with photosynthesis and leaf-air temperature under both conditions, whereas number of genes were correlated with relative water content under drought stress conditions (Table 3). The trait relative water content had positive correlations with boll retention percentage under both treatments. The trait excised leaf water loss had strong association with transpiration rate. Photosynthesis had positive correlation with relative water content under both control and drought conditions whereas the trait had positive correlation with boll retention percentage in stressed conditions. Stomatal conductance had negative correlation with leaf-air temperature and positive with relative water content under drought stress conditions. Transpiration rate was correlated with leaf-air temperature and excised leaf water loss under both environmental conditions. Leaf-air temperature had negative correlation with boll retention percentage under both treatments (Table 3).

4. Discussion

Water is the most important factor that regulates leaf temperature. During daytime, transpiration dissipates heat to the surroundings to lower leaf temperature, whereas condensation of water vapor as dew increases leaf temperature (Taiz and Zeiger, 2002). The trait leaf-air temperature ΔT , indicates potential of plant to keep leaf temperature lower under heat and drought stress was good in the varieties which had high boll retention percentage. Transpirational cooling in cotton improves yield (Taiz & Zeiger, 2002). The varieties showing good value of ΔT were also good in vegetative as well as reproductive growth. The trait ΔT may be used as criteria to select physiologically superior genotypes under high temperature/drought stress. In morphological analysis under drought stress, maximum plant height was observed from varieties of Cotton Research Station Vehari, as the varieties are known for high vegetative growth.

Stress related transcription factors/genes regulate the crop growth and reproductive development under stress conditions by regulating the physiological and biochemical processes of the plant. Among these transcription factors, GhMCK3 regulates stomatal responses (Wang et al., 2016), GbMYB5 helps recovering plant after drought stress (Chen et al., 2015), GhWRKY41 improves antioxidant enzyme activity (Chu et al., 2015) and GhMPK17 enhances roots under drought stress (Zhang et al., 2014). This set of transcription factors might have produced a major difference in tolerating drought stress in semi-arid sub-tropical conditions of Pakistan. Five varieties VH-305, MHH-886, CRSM-38, CIM-600, NIAB-111 had maximum number of transcription factors, showed higher drought stress tolerance than the varieties/genotypes having lower number of transcription factors. The presence of maximum transcription factors might have improved the relative water content, photosynthesis, low leaf-air temperature and high boll retention percentage under drought stress condition (Table 2). The variety MNH-886 has also been reported as heat tolerant by analysing the impact of HSPs (Saleem et al., 2021). The varieties breed here are naturally tolerant to heat stress as cotton withstand highest temperature here as compared to any other cotton growing area around the world. The transcription factors/gene such as MAPK, DREB, APX improves drought tolerance by improving physiological mechanism against drought stress (Zhang et al., 2020; Hou et al., 2018; Nawaz et al., 2020). It is well reported that drought stress reduces relative water content which ultimately reduces photosynthesis rate in cotton. In this study the varieties

with high relative water content were also superior in photosynthesis under drought stress. The results indicate these physiological traits could be improved simultaneously in cotton. Drought was applied during flowering period in this experiment and flower/bud shedding was increased during stress cycles. The varieties with higher relative water content were better in BR% and vice versa. Higher stomatal conductance was associated with higher relative water content and coolness of leaf temperature in this study as well as in literature. The plant with ability of keeping leaf cooler was associated with higher boll retention percentage which is associated with drought and heat stress tolerance (Iqbal et al., 2017; Saleem et al., 2021). The role of the presence of transcription factors/genes has been checked through the correlation analyses, where number of stress related transcription factors/genes showed a significant and positive correlation with stress related physiological traits and boll retention (Table 3). As a whole, the cotton varieties under study contained most of other TF/Gene except the two factors GhMCK3 and GhMPK2 for which the varieties showed variation (Table 3). These two are member of MAPK family. In cotton, GhMCK3 is a component of MAPK cascade induced by drought stress and involved in root hair development (Wang et al., 2016). The factor GhMPK2 regulates the ethylene synthesis in cotton, which help enhancement of ROS under drought stress conditions. The transcription factor triggers many antioxidant genes (Zhang et al., 2011) which scavenge the ROS and enhance stress tolerance. To improve local germplasm against drought tolerance, gene pyramiding may be undertaken to include these two transcription factors in molecular breeding for drought.

The overall results indicated that the genotypes having more number of stress related genes/transcription factors showed tolerance against drought stress at physiological and yield level. The variety MNH-886, CIM-600 and MNH-1035 had high relative water content, photosynthesis, stomatal conductance and low excised leaf water loss, showing overall better performance under drought stress. These varieties may be used as source material for breeding drought and heat tolerance in cotton under climate change scenario.

5. Conclusion

The genotypes with presence of transcription factors (DREB, MPK, MYB, NAC, HSPs) showed tolerance to drought stress under field conditions. Due to the strong association of above-mentioned stress related TF/genes with morphological and physiological traits, the genotypes with higher number of TF may be used in gene pyramiding for drought tolerance. The varieties MNH-886 and CIM-600 could be used a source of drought tolerance in cotton breeding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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