

Screening for drought tolerance using physiological traits in upland cotton (*Gossypium hirsutum* L.)

Detección de tolerancia a la sequía utilizando rasgos fisiológicos en algodón americano (*Gossypium hirsutum* L.)

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ABSTRACT

Climate change patterns indicate a serious threat to freshwater availability for crops. A selection of drought-tolerant genotypes is essential for breeders. Three key physiological parameters of abiotic stress: relative water content, excised leaf water content, and cell membrane stability were assessed with 68 upland cotton genotypes in Pakistan. The most tolerant and susceptible genotypes were evaluated in a greenhouse under controlled conditions. From the selected genotypes, heat and drought stress-related transcription factors were screened and included the following: GhNAC2, DREB2A, GhABF2, HSC70, HSFA2, GbMPK3, GbMpK17, GhMKK1, APX1, GHSP26, TPS, ANNAT8, GhMPK2, GhMKK3, GhWRKY41, HSPCB, HSP101, HSP3, GhPP2A1, and GbMYB5. Cell membrane stability may be a screening criterion for drought tolerance in cotton under field and greenhouse conditions. Under these conditions, the physiological and molecular analyses revealed that the genotypes CRIS-134, BH-184, and FH-114 were the most tolerant, and the genotypes CIM-240, CIM-446, and FH-900 were susceptible. The selected tolerant varieties can be recommended for cultivation in drought-prone areas. They can be used in future breeding programs for drought tolerance in cotton.

Key words: transcriptional factors, physiological analysis, cell membrane stability, relative water content.

RESUMEN

Los patrones de cambio climático indican una amenaza seria a la disponibilidad de agua dulce para las plantas cultivadas. La selección de genotipos tolerantes a la sequía es esencial para los fitomejoradores. Se evaluaron tres parámetros fisiológicos clave del estrés abiótico: contenido relativo de agua, contenido de agua en hojas extirpadas y estabilidad de la membrana celular, en 68 genotipos de algodón americano "Upland" en Pakistán. Los genotipos más tolerantes y susceptibles se evaluaron en invernadero bajo condiciones controladas. Se evaluaron los factores de transcripción relacionados con el estrés por calor y sequía en los siguientes genotipos seleccionados: GhNAC2, DREB2A, GhABF2, HSC70, HSFA2, GbMPK3, GbMpK17, GhMKK1, APX1, GHSP26, TPS, ANNAT8, GhMPK2, GhMKK3, GhWRKY41, HSPCB, HSP101, HSP3, GhPP2A1 y GbMYB5. La estabilidad de la membrana celular puede utilizarse como criterio de selección para la tolerancia a la sequía en el algodón, tanto en condiciones de campo como de invernadero. En condiciones de campo y de invernadero, el análisis fisiológico y molecular reveló que los genotipos CRIS-134, BH-184 y FH-114 fueron los más tolerantes, y los genotipos CIM-240, CIM-446 y FH-900 fueron susceptibles. Las variedades resistentes seleccionadas pueden recomendarse para el cultivo en áreas propensas a la sequía y pueden utilizarse en futuros programas de mejoramiento para la tolerancia a la sequía en el algodón.

Palabras clave: factores transcripcionales, análisis fisiológico, estabilidad de la membrana celular, contenido relativo de agua.

Introduction

Crop production is affected by several environmental factors brought on by global climate change (Farooq *et al.*, 2022; Raza *et al.*, 2019). Crops and food security are negatively impacted by climate change and global warming (Jia *et al.*, 2022). Rising temperature trends and water scarcity are two significant problems. Drought stress affects the number of crops, mainly those grown in arid and semi-arid regions (Alamri *et al.*, 2020; Varshney *et al.*, 2021).

Reduced precipitation and altered rainfall patterns lead to drought stress worldwide (Cheng *et al.*, 2021). Drought means reduced water availability over an extended period (Abdelraheem *et al.*, 2015).

Drought stress affects yields and crop development in cotton. The built-in defense mechanisms in crops account for morphological changes such as small, thick leaves, thick cuticles, and waxy and hairy leaf surfaces that reduce water loss through transpiration (Waghmare, 2022). Multiple

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genes and environmental stimuli regulate drought tolerance in plants. Breeding for drought tolerance requires a genetic combination that enables plants to withstand drought stress. Pyramiding different traits into a single genotype requires source population and recurrent selection. Developing drought-tolerant cultivars needs specific genotypes with physiological traits related to drought tolerance. Water deficiency during the fiber growth phase negatively impacts cotton fiber quality, which shortens fiber length. Physiological processes, such as relative water content and excised leaf water loss, were disturbed by dry conditions of soil during the blooming stage (Makamov *et al.*, 2023).

Different transcription factors, signaling genes, and functional genes have been identified at the molecular level under abiotic stress in normal plant growth. The cytosolic APX gene has a heat shock element in its promoter region, which enables the production of quick heat responses (Storozhenko *et al.*, 1998), drought responses (Smirnov & Colombé, 1988), salt and ABA responses (Shi *et al.*, 2001). The transcription factors DREB (dehydration response element binding proteins) are linked to drought tolerance (Zhang *et al.*, 2020). They can be utilized for crop genetic improvement (Niu *et al.*, 2020).

The development, physiology, biochemistry, and reproduction of plants are affected by severe drought, which ultimately reduces agricultural output (Cui *et al.*, 2020; Rai *et al.*, 2021; Yang *et al.*, 2019). The effect, duration, and intensity of drought, plant genetics, and growth stage of plants all play a role in determining plant drought tolerance (Varshney *et al.*, 2021).

Cotton is susceptible to environmental changes (Yehia *et al.*, 2022). Reduced availability of irrigation water, erratic rainfall patterns, and heat stress are the main risks to cotton production (Ahmed *et al.*, 2022). The effects of drought stress on cotton include a reduction in shoot and root length, a blockage of vascular tissues, and a reduction in cell elongation (Mahmood *et al.*, 2022). As a result, plants grown under various stress conditions exhibit altered photosynthesis, chlorophyll content, fluorescence, and cell membrane thermostability (Azhar *et al.*, 2009). In particular, relative cell injury can be used to assess heat tolerance (Zhang *et al.*, 2014).

The water content of plants regulates various physiological and metabolic processes (Mubeen *et al.*, 2012). Reducing water content negatively impacts crop plant growth, *i.e.*, cotton growth from seedling to fiber maturity (Farooq *et al.*, 2009). Saleem *et al.* (2015) indicated that relative

water content, excised leaf water loss, and cell membrane stability are variables of quantitative nature in cotton. Cell membrane integrity is an important physiological trait for screening drought-tolerant plants in water limiting conditions (Levitt, 1980). Cellular dehydration negatively affects membranes, increasing their permeability. Various studies on genotypic variations in cell membrane stability have linked these variations to economic yield losses under water stress for many crops (Ashraf *et al.*, 1992; Saneoka *et al.*, 2004; Tripathy *et al.*, 2000). Stomatal closure and decreased activity of photosynthetic enzymes are two coordinated phenomena contributing to decreased photosynthetic activity under water stress (Aranjuelo *et al.*, 2011).

Water shortage builds up free solutes in cells, which lower osmotic potential; and due to water loss, the concentration of cellular solution can lower osmotic potential. When cellular water deficit rises above a particular threshold, osmotic adjustment occurs (Singh, 2015). The damage in cellular membranes causes leakage of numerous cellular solutes, including electrolytes. The electrical conductivity of the liquid in which the impacted leaf sample is placed detects electrolyte leakage. The method is applied to determine relative damage or stability of the cells by comparing leakage from stress-affected samples with leakage from control samples. Cell membrane stability is an important parameter to resist dehydration in leaves under stress (Singh, 2015).

Cotton is one of the most important fiber crops in the world, and it is severely impacted by drought stress compared to other crops (Yasmeen *et al.*, 2016). Drought stress decreases cellular growth (Turner *et al.*, 1986), root and stem growth (Hearn, 1994), number of fiber bolls per plant (Oosterhuis, 2000), relative water content, cell membrane integrity (Khan *et al.*, 2011), and cotton yield (Yagmur *et al.*, 2014). Both morphological and physiological characteristics, such as cell membrane integrity, excised leaf water loss, and relative water content, contribute to drought resistance. Drought resistance may be improved by plant mechanisms that keep the water content of leaves stable under drought stress (Xoconostle *et al.*, 2010). Relative water content measures a plant's ability to retain water, the most crucial defensive characteristic under drought stress (Tahara *et al.*, 1990). Similarly, preserving cell membrane integrity is another defensive strategy under drought stress (Bajji *et al.*, 2002). Breeding for drought tolerance may be improved by using genetic regulation of these properties.

Plants have acquired different mechanisms to develop tolerance against drought stress (Batool *et al.*, 2020; Mahmood *et al.*, 2021). They employ avoidance, escape, and tolerance

to resist drought stress (Galindo *et al.*, 2018). Phenotypic selection methods are ineffective against complex traits such as drought tolerance with narrow heritability (Abdelraheem *et al.*, 2015). It is possible to study the inheritance of drought tolerance through secondary techniques such as selection through secondary traits and correlation estimates (Saeed *et al.*, 2011). The third method compares genotypes under stressed and non-stressed environments, assesses germplasm drought resistance and susceptibility, and ranks genotypes (Abdelraheem *et al.*, 2015).

This study was aimed at: (i) screening cotton germplasm for drought tolerance, (ii) developing breeding material for cotton, and (iii) investigating the molecular and physiological traits contributing to drought tolerance.

Materials and methods

Experiment 1

We collected a set of 68 cotton genotypes from different cotton research stations in Pakistan, *i.e.*, CCRI Multan, CRS Faisalabad, CRS Multan, CRS Vehari, CRS Sakrand, and NIAB for use in the experiment in 2022-23. We grew the genotypes in a three-replicate trial in the field. We planted the experiment in sandy-loam soil, which is standard for cotton growth. The average temperature from germination to the flowering stage was recorded at 25-33°C and the average relative air humidity at 30-40%. We applied recommended local agronomic practices. We calculated physiological traits, including relative water content, excised leaf water loss, and cell membrane stability for all the genotypes under study as follows:

Relative water content (%) of the leaf samples was calculated using the following formula (Clark & Townley-Smith, 1986):

$$RWC = \left[\frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \right] \times 100 \quad (1)$$

Excised leaf water loss (%) was calculated using the following formula (Clarke & McCaig, 1982):

$$ELWL = \frac{\text{Fresh weight} - \text{Wilted weight}}{\text{Dry weight}} \times 100 \quad (2)$$

Cell membrane stability (%) of the leaf discs as the reciprocal of relative cell injury was calculated using the following formula (Blum & Ebercon, 1981):

$$CMS = \left[\frac{\left\{ 1 - \left(\frac{T_1}{T_2} \right) \right\}}{\left\{ 1 - \left(\frac{C_1}{C_2} \right) \right\}} \right] \times 100 \quad (3)$$

where

T_1 = Stressed sample conductance before autoclaving;

T_2 = Stressed sample conductance after autoclaving;

C_1 = Control sample conductance before autoclaving;

C_2 = Control sample conductance after autoclaving

Experiment 2

We selected a total of 15 tolerant and susceptible genotypes (based on cell membrane stability) grown in a greenhouse in three replicates under controlled environmental conditions. A total of nine plants of each genotype were grown per replicate. Optimum temperature and relative humidity were maintained for cotton's healthy growth under greenhouse conditions. Physiological traits such as RWC, ELWL, and CMS were evaluated as described in Experiment 1.

Molecular analysis

We extracted DNA from 15 selected genotypes using the standard cetyltrimethylammonium bromide (CTAB) method (Doyle, 1990). We used important drought/heat-related transcription factors, such as HSPCB, GHSP26, HSFA2, HSP101, HSP3, DREB1A, DREB2A, TPS, GhNAC2, GbMYB5, GhWRKY41, GhMKK3, GhMPK17, GhMKK1, GhMPK2, APX1, HSC70, ANNAT8, and GhPP2A1 for genotype screening (Saleem *et al.*, 2020). We performed PCR analysis using specific primers for all selected genotypes. We separated the amplification products on a 1% agarose gel in 1x TBE buffer stained with ethidium bromide on a 100 bp ladder (Life Technologies Gibco BRL).

Statistical analysis

We calculated an analysis of variance (ANOVA) of all traits (Steel *et al.*, 1997). Correlations for all characteristics were calculated to find an association between traits (Kwon & Torre, 1964). We used a t-test to compare RWC, ELWL, and CMS variations between the field and greenhouse data (Usman, 2016).

Results

Experiment 1

Relative water content: The genotypes NIAB-777 (89.19%), BH-184 (84.45%), and CIM-473 (83.79%) had the maximum relative water content in the field conditions. The genotypes CIM-240 (11.26%), CIM-446 (11.65%), and FH-900 (13.24%) showed minimum relative water content in the field (Tab. 1).

Excised leaf water loss: The genotypes CRIS-508 (0.24%), CRIS-9 (0.26%), and FH-114 (0.29%) showed minimum

excised leaf water loss under normal conditions. The genotypes CIM-446 (3.67%), CIM-240 (3.67%), and FH-900 (3.68%) had maximum excised leaf water loss in the field (Tab. 1).

Cell membrane stability: The genotypes BH-184 (80.61%), CRIS-134 (79.12%), and FH-114 (77.14%) had maximum cell membrane stability in the field. The genotypes CIM-446 (46.19%), CIM-240 (47.76%), and FH-900 (48.54%) showed minimum cell membrane stability in the field (Tab. 1).

Experiment 2

Out of 68 genotypes, twelve drought-tolerant and three drought-susceptible genotypes were evaluated in the greenhouse.

Relative water content: The genotypes CRIS-134 (76.16%), CRIS-9 (76.06%), and NIAB-777 (74.29%) showed maximum, whereas the genotypes CIM-240 (44.34%), CIM-446 (46.27%) and FH-900 (47.27%) showed minimum relative water content (Fig. 1A).

TABLE 1. Cotton genotypes selected based on cell membrane stability, relative water content, and excised leaf water loss.

Sr. No.	Genotypes	RWC	ELWL	CMS	Sr. No.	Genotypes	RWC	ELWL	CMS
1	CIM-63	54.58	1.23	56.43	35	FH-900	13.24	3.68	48.54
2	CIM-678	52.70	1.36	52.33	36	FH-118	46.47	1.89	67.50
3	CIM-785	44.33	1.06	51.42	37	CKC-2	63.04	1.75	65.11
4	CIM-343	53.00	1.34	54.13	38	CKC-3	36.82	1.44	64.93
5	CIM-600	57.49	1.67	61.95	39	MNH-552	77.53	0.58	70.35
6	CIM-240	11.26	3.67	47.76	40	MNH-554	58.97	1.17	66.66
7	CIM-109	41.25	1.76	64.83	41	MNH-147	60.28	1.23	67.50
8	CIM-499	70.67	0.31	70.25	42	MNH-1026	77.62	0.63	70.41
9	CIM-70	63.80	2.27	60.71	43	MNH-988	46.83	1.81	62.72
10	CIM-620	56.82	1.45	60.65	44	BH-184	84.45	1.92	80.61
11	CIM-443	65.80	1.27	61.81	45	BH-121	54.84	1.63	62.85
12	CIM-616	55.95	1.56	61.99	46	BH-118	45.69	1.45	66.66
13	CIM-506	59.95	1.52	62.54	47	G-93	61.87	1.36	57.14
14	CIM-446	11.65	3.67	46.19	48	G-105	82.72	2.09	60.00
15	CIM-632	46.26	1.52	56.25	49	AA-802	56.20	2.08	51.51
16	CIM-599	55.98	1.45	60.71	50	SLH-334	40.27	1.48	66.66
17	CIM-707	51.58	1.67	67.50	51	SLH-337	33.36	1.47	57.00
18	CIM-473	83.79	0.77	75.05	52	CRIS-134	75.99	0.96	79.12
19	CIM-482	58.31	1.13	56.25	53	CRIS-533	75.57	2.63	60.60
20	CIM-554	58.94	1.00	64.93	54	CRIS-510	57.40	2.18	53.96
21	CIM-534	57.62	2.45	67.50	55	CRIS-508	80.94	0.24	70.71
22	CIM-598	52.63	1.24	52.72	56	CRIS-121	75.15	0.34	69.81
23	CIM-573	53.54	1.36	60.00	57	CRIS-9	76.43	0.26	71.83
24	CYTO-515	52.50	1.45	59.37	58	CRIS-129	78.98	1.98	73.26
25	NIAB-111	58.50	1.28	62.74	59	SLH-317	60.42	2.24	67.50
26	NIAB-878	46.30	1.36	64.02	60	VH-Gulzar	68.53	2.61	65.00
27	NIAB-112	58.32	1.30	63.33	61	VH-189	46.94	2.37	58.69
28	NIAB-777	89.19	0.89	72.80	62	VH-418	45.27	1.99	54.54
29	BS-20	42.24	1.78	62.67	63	VH-383	44.92	1.65	58.82
30	FH Lalazar	53.77	1.87	67.50	64	VH-351	70.43	2.80	66.02
31	FH-114	70.83	0.29	77.14	65	VH-355	39.38	1.33	51.42
32	FH-444	44.98	1.38	51.81	66	VH-305	63.02	2.02	65.00
33	FH-490	38.36	1.64	60.00	67	VH-402	47.76	1.61	67.54
34	FH-113	62.34	1.24	70.00	68	Sitara-008	47.36	1.60	60.56

RWC – relative water content (%), ELWL – excised leaf water loss (%), and CMS – cell membrane stability (%).

Excised leaf water loss: The genotypes CRIS-129 (0.53%), CIM-499 (0.46%), and FH-114 (0.68%) showed minimum, whereas the genotypes CIM-446 (3.56%), CIM-240 (2.06%) and FH-900 (2.65%) showed maximum excised leaf water loss (Fig. 1B).

Cell membrane stability: The genotypes BH-18 (77.35%), FH-114 (77.21%), and CRIS-134 (76.82%) showed maximum, whereas the genotypes CIM-446 (48.83%), CIM-240 (46.53%) and FH-900 (51.55%) showed minimum cell membrane stability (Fig. 1C).

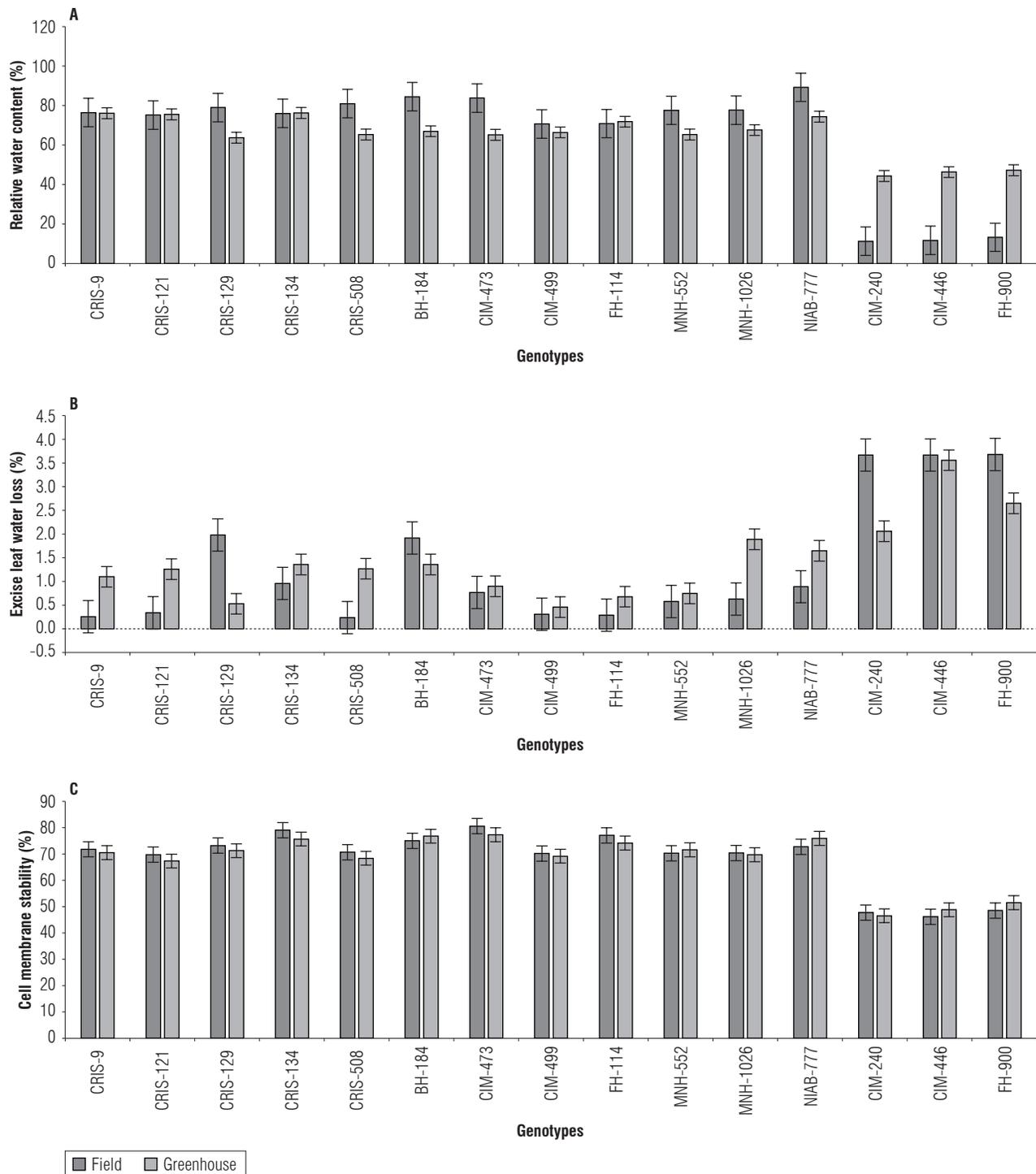


FIGURE 1. Comparative physiological traits in cotton genotypes: A) relative water content (RWC, %), B) excised leaf water loss and (ELWL, %), and C) cell membrane stability (CMS, %) under field and greenhouse. Error bars indicate standard error.

Molecular analysis

A set of 21 transcription factors was related to drought/heat tolerance for screening drought tolerance and susceptibility in selected cotton genotypes. The band sizes of

all genes were the same. Drought-tolerant varieties, *i.e.*, CRIS-9, CRIS-121, CRIS-129, CRIS-134, CRIS-508, CIM-473, CIM-499, FH-114, MNH-552 and MNH-1026 had all 21 TFs/gene (Figs. 2 and 3).

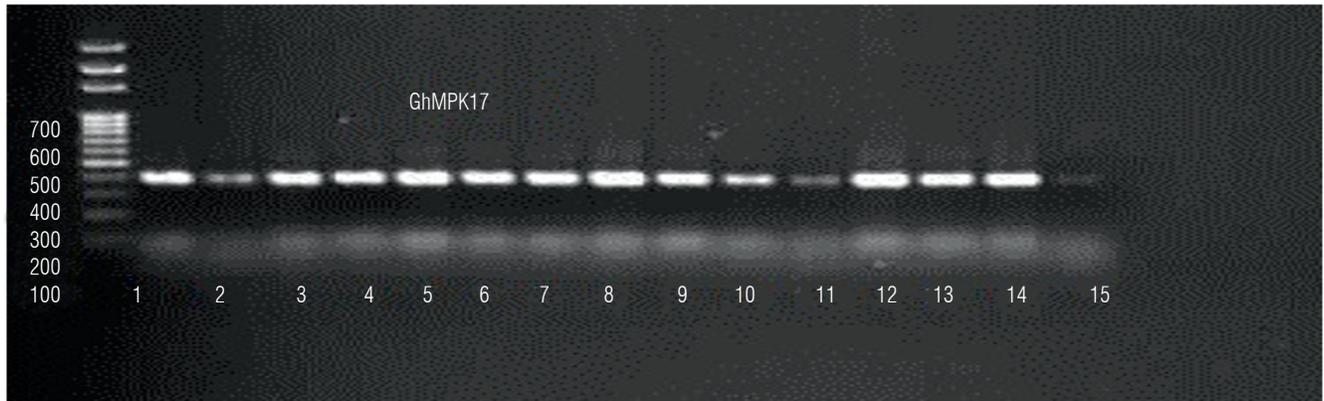


FIGURE 2. Molecular validation of the transcription factor GhMPK17 in selected cotton genotypes.

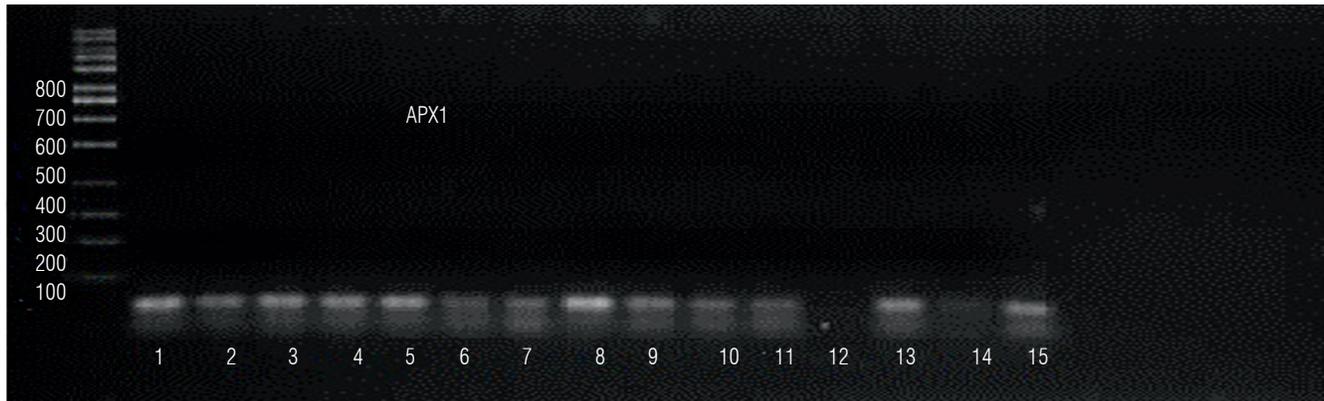


FIGURE 3. Molecular validation of the transcription factor APX1 in selected cotton genotypes.

TABLE 2. Molecular screening of 21 drought/heat-related transcription factors in selected cotton genotypes subjected to drought stress.

Genotype	GhNAC2	DREB2A	GhABF2	HSC70	HSFA2	GhMPK3	GhMpK17	GhMKK1	APX1	GHSP26	GHSP26	TPS	ANNAT8	GhMPK2	GhMKK3	GhWRKY41	HSPCB	HSP101	HSP3	GhPP2A1	GhMYB5		
CRIS-9	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
CRIS-121	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
CRIS-129	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	✓
CRIS-134	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
CRIS-508	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
BH-184	×	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
CIM-473	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
CIM-499	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
FH-114	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
MNH-552	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
MNH-1026	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
NIAB-777	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
CIM-240	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×
CIM-446	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
FH-900	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

The variance (ANOVA) analysis of all physiological traits, *i.e.*, RWC, ELWL, and CMS, was highly significant in both experiments. The correlation study showed that RWC positively correlated with CMS and negatively correlated with ELWL; CMS was negatively correlated with ELWL (Tab. 3).

TABLE 3. Correlations among physiological traits of cotton genotypes.

Variables	RWC	ELWL	CMS
RWC	1		
ELWL	-0.09**	1	
CMS	0.003*	-0.002*	1

RWC – relative water content (%), ELWL – excised leaf water loss (%), and CMS – cell membrane stability (%).

One sample t-test (significance level $\alpha=0.05$) of the arithmetic mean of all physiological traits (RWC, ELWL, and CMS) was used to evaluate the variations. We found highly significant differences between all the traits studied (Tab. 4).

TABLE 4. Comparative analysis (t-test) among physiological traits under both experiments. Null Hypothesis: $\mu = 0$; Alternative Hyp: $\mu < 0$. DF = 14.

Variable	Mean	SE	Lower value	Upper value	t-test
RWC	65.18	7.20	49.71	80.64	9.04**
	64.79	2.75	58.88	70.71	23.49**
ELWL	1.34	0.34	0.61	2.07	3.95**
	1.43	0.21	0.96	1.89	6.59**
CMS	68.25	2.90	62.02	74.48	23.50**
	67.64	2.62	62.00	73.28	25.73**

RWC – relative water content (%), ELWL – excised leaf water loss (%), and CMS – cell membrane stability (%).

Discussion

The varieties from the Cotton Research Institute Sakrand were tolerant to drought stress. These varieties have unique morphological traits, such as compact plants, early maturity, small leaves, and small fiber bolls. This study calculated the higher cell membrane stability and relative water content in these varieties. These varieties proved to be morphologically and physiologically drought-tolerant. Smaller bolls in these varieties are associated with more bolls, which keeps these varieties high-yielding genotypes. In this study, CIM-473 (Ullah *et al.*, 2008), CIM-499 (Khan *et al.*, 2009), CRIS-9, CRIS-121, CRIS-129, CRIS-134, CRIS-508 (Keerio *et al.*, 2022), FH-114 (Farooq *et al.*, 2009), BH-184 (Iqbal *et al.*, 2020), MNH-552 (Dahab *et al.*, 2012), MNH-1026 and NIAB-777 (Rehman *et al.*, 2021) were drought tolerant, whereas CIM-240 (Ahmad *et al.*, 2009), CIM-446 Iqbal *et al.*, 2010) and FH-900 (Nasimi *et al.*, 2016) were drought susceptible.

Cotton has moderate drought tolerance at the vegetative stage of growth. It is vulnerable to drought stress during the reproductive phase (Iqbal *et al.*, 2017; Niu *et al.*, 2018). Drought stress is commonly linked to oxidative and osmotic stress, leading to ion inequality, severe changes in cell membrane structure, and other cellular processes in plants (Bernardo *et al.*, 2019). Plants subjected to drought stress have lower relative water content and reduced cell membrane integrity (Hammad & Ali, 2014). Additionally, drought stress depletes lipid membranes, damaging membranes, which become more porous and increase electrolyte leakage (Petrov *et al.*, 2018).

Relative water content, leaf temperature, and osmotic potential are also impacted by drought stress (Fanaei *et al.*, 2012). Similarly, drought stress affects turgidity of cell growth and the development of plant tissues (Reddi & Reddi, 1995), leading to poor cell elongation (Nonami, 1998). The relative water content (RWC) is used to measure the water content in leaves as it reflects the ability of a genotype to survive under water deficit conditions (Silva *et al.*, 2007). A high RWC is preferred to maintain the water balance in a drought-stressed environment. Therefore, greater RWC is used as a base for developing drought-tolerant plants (Rahman *et al.*, 2000). A reduced transpiration rate and less water loss from excised leaves are crucial factors for selecting resistance against drought stress (Rahman *et al.*, 2000). Cell membrane thermostability (CMT) has been extensively used as an indicator of tolerance against water deficit stress, and Ur-Rahman *et al.*, (2004) used cell membrane stability to measure the heat tolerance of cotton.

The physiological and biochemical functions of stress-related transcription factors and genes control crop growth and reproductive development under drought conditions of different plants. GhMCK3 controls stomatal responses (Wang *et al.*, 2016), GbMYB5 helps in plant recovery during drought stress (Chen *et al.*, 2015), GhWRKY41 increases activity of antioxidant enzymes (Chu *et al.*, 2015), and GhMPK17 improves root strength under drought stress (Zhang *et al.*, 2014). This transcription factor set may have significantly impacted the ability of cotton to withstand Pakistan's semi-arid and sub-tropical climate. MAPK, DREB, and APX increase drought tolerance by strengthening physiological mechanisms against drought stress (Hou *et al.*, 2018; Nawaz *et al.*, 2020; Zhang *et al.*, 2020).

Saleem *et al.* (2015) reported a correlation between genes of relative water content and reduced excise leaf water loss in cotton. Relative water content and cell membrane stability sustain higher plant development and better performance

during drought by negatively correlating cell membrane stability with excised leaf water loss. Similar results were recorded in this study. Tolerant genotypes had higher RWC and CMS and lower ELWL values. The selected genotypes can be used as drought-tolerant materials in breeding.

Conclusions

Cell membrane stability was linked to relative water content and excise leaf water loss. This indicates that CMS is a rapid test for selecting cotton genotypes for drought tolerance. Field and greenhouse screening of cotton for drought tolerance showed similar results. The overall results indicate that the genotypes CRIS-134, BH-184, and FH-114 are drought tolerant and can be used for breeding programs.

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Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

NM and AM carried out the research. BMMA and NM prepared the manuscript. SMA supervised the research and reviewed the manuscript. All authors have read and approved the final version of the manuscript.

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