

GENOTYPIC EVALUATION OF UPLAND COTTON FOR DROUGHT TOLERANCE USING PHYSIOLOGICAL TRAITS AND DROUGHT TOLERANCE INDICES

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ABSTRACT

Cotton production is negatively affected by abiotic stress mainly drought stress. It is challenging for breeders to develop tolerant genotypes which can withstand dry conditions without reducing yield. For this purpose 50 genotypes of upland cotton were evaluated under control and drought stress condition using drought tolerance indices and physiological traits. Significant variations were observed among tolerance indices and yield under normal and drought condition. Seed cotton yield under both conditions showed significantly positive association with mean productivity (MP), yield index (YI), geometric mean productivity (GMP), and stress tolerance index (STI), respectively. Relative water content (RWC) showed positively significant association with seed cotton yield under stress. In principal component analysis, PC1 and PC2 expressed 98.84% cumulative contribution in overall variability. Biplot analysis showed that Yp, MP, GMP were the highly significant indices for drought tolerance. Cluster analysis grouped genotypes into four major clusters based on yield, physiological traits and stress tolerance indices. From multivariate analysis, NIAB-111, MNH-1016, FH-142, MNH-886, NIAB-820, VH-295, CYTO-511 and CYTO-513 were recognized as the most stable genotypes under drought stress. Therefore, these genotypes can be used in future breeding programs for drought tolerance.

Keywords: Drought, Seed cotton yield, Drought tolerance indices, Multivariate analysis.

INTRODUCTION

Drought is a major abiotic stress limiting crop growth and development. Prolonged drought cause huge losses in crop productivity (Makamov *et al.*, 2022). Drought affects a number of morphological, physiological, and biochemical processes including stomatal closure, cellular dehydration, membrane lipid peroxidation, and reduced antioxidant capacity (Ahmed *et al.*, 2020; Huang *et al.*, 2019; Gao *et al.*, 2018).

From the last decade, cotton crop is drastically affected in Pakistan due to the climatic changes. 41% yield decline was recorded in 2022-23 than last year (Economic Survey of Pakistan 2023). Drought is a major hindrance to cotton, mainly at reproductive stage (Li *et al.*, 2022), leading up to 34% decreases in production (Ullah *et al.*, 2017). Different morphological traits, including bolls per plant, plant height, root length, and boll weight, vary significantly under water deficit conditions. Drought causes late flowering, delayed boll formation, and reduced fiber production in cotton. Cotton has developed different morpho-physiological and biochemical mechanisms to survive under drought stress (Shavkiev *et al.*, 2019; Mahmood *et al.*, 2021). Depending on the genotype, cotton plants respond differently to water deficit conditions. Plant response to water stress is indicated by plant height, root length, biomass, chlorophyll, proline content, photosynthesis rate, and drought response genes (Shavkiev *et al.*, 2020).

Plant morpho-physiological traits can be assessed using different indicators, including leaf water holding capacity, surface, permeability, size (Ashiralieva *et al.*, 2023). Plant physiological traits including relative water content (RWC), membrane stability index (MSI), electrolyte leakage (EL), and chlorophyll content are all affected by drought. An increase in RWC means plant performance is maintained under drought. RWC assesses the water levels in plant tissues and is the most significant indicator for selecting high-yielding genotypes under drought (Saidigani *et al.*, 2022; Rakhimov *et al.*, 2023). There is a strong correlation between relative water content and seed cotton yield under drought stress (Chorshanbiev *et al.*, 2023).

Plants have developed various morpho-physiological, biochemical, and molecular strategies to withstand drought (Mahmood *et al.*, 2021). Numerous morphological and physiological traits are genetically linked to drought

resistance. Crops mechanisms for resisting drought stress are quite complex. Any single indicator cannot completely assess the drought resistance of crops. Researchers have developed number of indices based on yield attributes, morphological, biomechanical, and chemical properties to assess drought tolerance (Uzilday *et al.*, 2012; Wang *et al.*, 2016). Yield potential can be improved using drought indices rather than drought tolerance mechanisms (Grzesiak *et al.*, 2019). Stress tolerance index, geometric mean of yield, and average yield are the indices being efficiently used in upland cotton (Singh *et al.*, 2016).

Assessing plants response to water shortage provides different management strategies (Sánchez-Reinoso *et al.*, 2020). Identifying drought tolerant genotypes is more difficult due to genotypes and environmental interactions. Therefore, many indices are used to evaluate the genetic differences in crop genotypes (Fernandez, 1992; Mardeh *et al.*, 2006). Fischer and Maurer, (1978) proposed Stress Susceptibility Index (SSI). Higher SSI i.e. SSI>1 shows drought-sensitive genotypes, whereas SSI<1 indicates drought-tolerant genotypes (Clarke *et al.*, 1992). Fernandez (1992) developed Stress Tolerance Index (STI) and GM indicators as statistical derivatives of yield. High YSI genotypes are more stable across environments (Rajamani, 1994). YSI is therefore an effective indicator of drought tolerance. The current study was aimed to study effects of different drought treatments on yield and physiological traits in upland cotton through studying drought indices.

MATERIAL AND METHODS

A set of 50 cotton genotypes (Table 1), obtained from Central Cotton Research Institute (CCRI), Multan, grown at Department of Plant Breeding and Genetics, BZU, Multan Pakistan in a triplicated trail under normal and drought stress condition. In control condition total eight irrigations on recommended time were given and four irrigations were used under stress treatment. First irrigation was applied at seeding, irrigation at 35 days after germination and two further irrigations spaced 30 days apart, up to 95 days following the first sowing.

Physiological traits measurements

For physiological assessment of cotton genotypes, RWC was measured by following Blum and Ebercon (1981)

$$\text{RWC (\%)} = (\text{Fresh leaf weight} - \text{Dry leaf weight} / \text{Turgid leaf weight} - \text{Dry leaf weight}) \times 100$$

Excise leaf water loss of leaf samples was measured by following Clark and Townley-Smith (1986).

$$\text{ELWL (\%)} = (\text{Fresh leaf sample weight} - \text{Wilted leaf sample weight}) / \text{Dry leaf sample weight}$$

Yield and drought tolerance indices

At maturity, five plants of each genotype were selected from each replication and seed cotton yield under normal (Y_p) and drought (Y_s) were recorded. Drought tolerance indices were calculated using standard formulae:

$$\text{Stress susceptibility index (SSI)} = \frac{1 - \left(\frac{Y_s}{Y_p}\right)}{1 - \left(\frac{\bar{Y}_s}{\bar{Y}_p}\right)} \quad (\text{Fischer and Maurer, 1978})$$

$$\text{Mean productivity (MP)} = \frac{Y_p + Y_s}{2} \quad (\text{Hossain } et al., 1990)$$

$$\text{Tolerance (TOL)} = Y_p - Y_s \quad (\text{Hossain } et al., 1990)$$

$$\text{Stress tolerance index (STI)} = \frac{Y_p \times Y_s}{Y_p^2} \quad (\text{Fernandez, 1992})$$

$$\text{Geometric mean productivity (GMP)} = \sqrt{Y_p \times Y_s} \quad (\text{Fernandez, 1992})$$

$$\text{Yield index (YI)} = \frac{Y_s}{\bar{Y}_s} \quad (\text{Gavuzzi } et al., 1997)$$

$$\text{Yield stability index (YSI)} = \frac{Y_s}{Y_p} \quad (\text{Bousslama and Schapaugh, 1984})$$

$$\text{Sensitivity drought index (SDI)} = \frac{Y_p - Y_s}{Y_p} \quad (\text{Khalili } et al., 2012)$$

$$\text{Drought resistance index (DI)} = Y_s \times \left(\frac{Y_s/Y_p}{\bar{Y}_s}\right) \quad (\text{Lan, 1998})$$

$$\text{Relative drought index (RDI)} = \frac{(Y_s/Y_p)}{(\bar{Y}_s/\bar{Y}_p)} \quad (\text{Fischer and Wood, 1979})$$

Whereas, Y_p and Y_s are seed cotton yield under control and drought stress, respectively. While, \bar{Y}_p and \bar{Y}_s are the overall mean of yield under control and drought stress, respectively.

Statistical analysis

Mean comparison of seed cotton yield, physiological traits and drought tolerance indices were performed. Multivariate analysis including correlation analysis, PCA and cluster analysis were performed using XLSTAT® to check genotypic performance.

RESULTS

Two physiological parameters viz., RWC and ELWL were used to assess drought tolerance in studied cotton genotypes (Supplementary Table S1). VH-295 expressed highest RWC (76.46) followed by NIAB-111 (75.55), CYTO-511 (74.83), MNH-886 (74.70), CYTO-513 (73.32), and MNH-1016 (72.98) under stress. Whereas, lowest RWC was recorded for CIM-446 (12.38) and CIM-240 (18.08). Lowest ELWL was recorded for MNH-1016 (0.60), VH-295 (0.82), NIAB-111 (0.85), MNH-886 (0.92), CYTO-511 (0.94) and NIAB-820 (1.02).

Yield and drought tolerance indices

Highest yield was recorded for CYTO-511 (136.22g) under normal and NIAB-111 (85.29g) under stress. CYTO-313 (16.45g) produced minimum seed cotton yield under stress condition. NIAB-111 showed highest value for MP (108.51), YI (2.16), GMP (106), STI (1.47) and DI (1.4). The highest TOL was recorded for CYTO-511 (95.11), whereas, VH-189 (16.44) expressed lower TOL. MNH-886 showed highest RDI (1.65) and YSI (0.74), however, it showed lowest SSI (0.46) and SDI (0.26).

Correlation analysis

The correlation between Yp and Ys was significantly positive (0.60). Yp and Ys showed positive association with MP (0.94, 0.84), YI (0.60, 1.0), GMP (0.86, 0.93), and STI (0.83, 0.92), respectively. DI (0.93), RDI (0.67) and YSI (0.67) showed significantly positive correlation with Ys (Table 2). A significant and positive association (0.36) was observed between Ys and RWC while, ELWL showed negative correlation (-0.36) with Ys. Negative association (-0.54) was observed between RWC and ELWL (Table 3).

Table 1. List of upland cotton genotypes used in current study.

Sr. No.	Genotypes	Sr. No.	Genotypes
1	MNH-786	26	VH-189
2	MNH-552	27	VH-295
3	MNH-147	28	CIM-240
4	VH-305	29	CIM-482
5	CIM-473	30	NIAB-111
6	CIM-600	31	CIM-707
7	CIM-620	32	NIAB-820
8	CIM-602	33	VH-148
9	AM-2017	34	IUB-222
10	AM-2012	35	CIM-446
11	VH-283	36	SB-149
12	MNH-1016	37	BH-118
13	Z-33	38	BH-160
14	FH-113	39	CYTO-124
15	FH-142	40	CYTO-177
16	MNH-886	41	CYTO-179
17	SS-32	42	CYTO-513
18	SS-102	43	CYTO-178
19	IS-29	44	CYTO-313
20	SIM-707	45	CYTO-511
21	FH-114	46	CYTO-515
22	FH-900	47	CIM-534
23	FH-901	48	CIM-663
24	FH-LALAZAR	49	CIM-554
25	MNH-789	50	CIM-506

Table 2. Correlation analysis among drought tolerance indices and seed cotton yield under normal and drought stress condition.

Variables	Yp	Ys	MP	TOL	YI	YSI	GMP	STI	SSI	SDI	RDI	DI
Yp	1											
Ys	0.60*	1										
MP	0.94**	0.84*	1									
TOL	0.78*	-0.021	0.53*	1								
YI	0.60*	1.00**	0.84*	-0.02	1							
YSI	-0.15	0.67*	0.18	-0.71*	0.67*	1						
GMP	0.86*	0.93**	0.98**	0.35	0.93*	0.36	1					
STI	0.83*	0.92*	0.96**	0.33	0.92*	0.34	0.99**	1				
SSI	0.15	-0.67*	-0.18	0.71*	-0.67*	-1.00**	-0.36	-0.34	1			
SDI	0.15	-0.67*	-0.18	0.71*	-0.67*	-1.00**	-0.36	-0.34	1.00**	1		
RDI	-0.15	0.67*	0.18	-0.71*	0.67*	1.00**	0.36	0.34	-1.00**	-1.00**	1	
DI	0.30	0.93**	0.59*	-0.35	0.93	0.86*	0.73*	0.74*	-0.86*	-0.86*	0.86*	1

* = significance level at 0.05, ** = significance level at 0.01. Where, Yp = seed cotton yield under normal condition; Ys = seed cotton yield under stress; MP = mean productivity; TOL = tolerance; YI = yield index; YSI; yield stability index; GMP = geometric mean productivity; STI = stress tolerance index; SSI = stress susceptibility index; SDI = sensitivity drought index; RDI = relative drought index; DI = drought resistance index.

Table 3. Correlation analysis among yield and physiological traits under drought stress.

Variables	Ys	RWC	ELWL
Ys	1		
RWC	0.55**	1	
ELWL	-0.36**	-0.54**	1

* = significance level at 0.05, ** = significance level at 0.01. Where, Ys = seed cotton yield under stress; RWC = relative water content; ELWL = excise leaf water loss.

Table 4. Cluster analysis for drought tolerance indices and seed cotton yield under normal and drought stress condition.

	Count	Yp	Ys	MP	TOL	YI	YSI	GMP	STI	SSI	SDI	RDI	DI
Cluster 1	27	86.91	41.21	64.06	45.70	1.05	0.48	59.41	0.47	0.95	0.52	1.06	0.53
Cluster 2	13	61.31	26.63	43.97	34.68	0.68	0.44	39.88	0.21	1.02	0.56	0.98	0.32
Cluster 3	04	124.96	71.12	98.04	53.84	1.80	0.57	94.18	1.17	0.79	0.43	1.26	1.03
Cluster 4	06	121.09	37.79	79.44	83.29	0.96	0.31	67.61	0.60	1.25	0.69	0.69	0.30

Where, Yp = seed cotton yield under normal condition; Ys = seed cotton yield under stress; MP = mean productivity; TOL = tolerance; YI = yield index; YSI; yield stability index; GMP = geometric mean productivity; STI = stress tolerance index; SSI = stress susceptibility index; SDI = sensitivity drought index; RDI = relative drought index; DI = drought resistance index.

Table 5. Cluster based performance of cotton for yield and physiological traits under drought stress condition.

	Count	Ys	RWC	ELWL
Cluster 1	16	37.83	55.90	1.89
Cluster 2	14	24.26	40.23	2.59
Cluster 3	14	44.41	42.18	2.08
Cluster 4	06	67.23	73.99	0.93

Where, Ys = seed cotton yield under stress; RWC = relative water content; ELWL = excise leaf water loss.

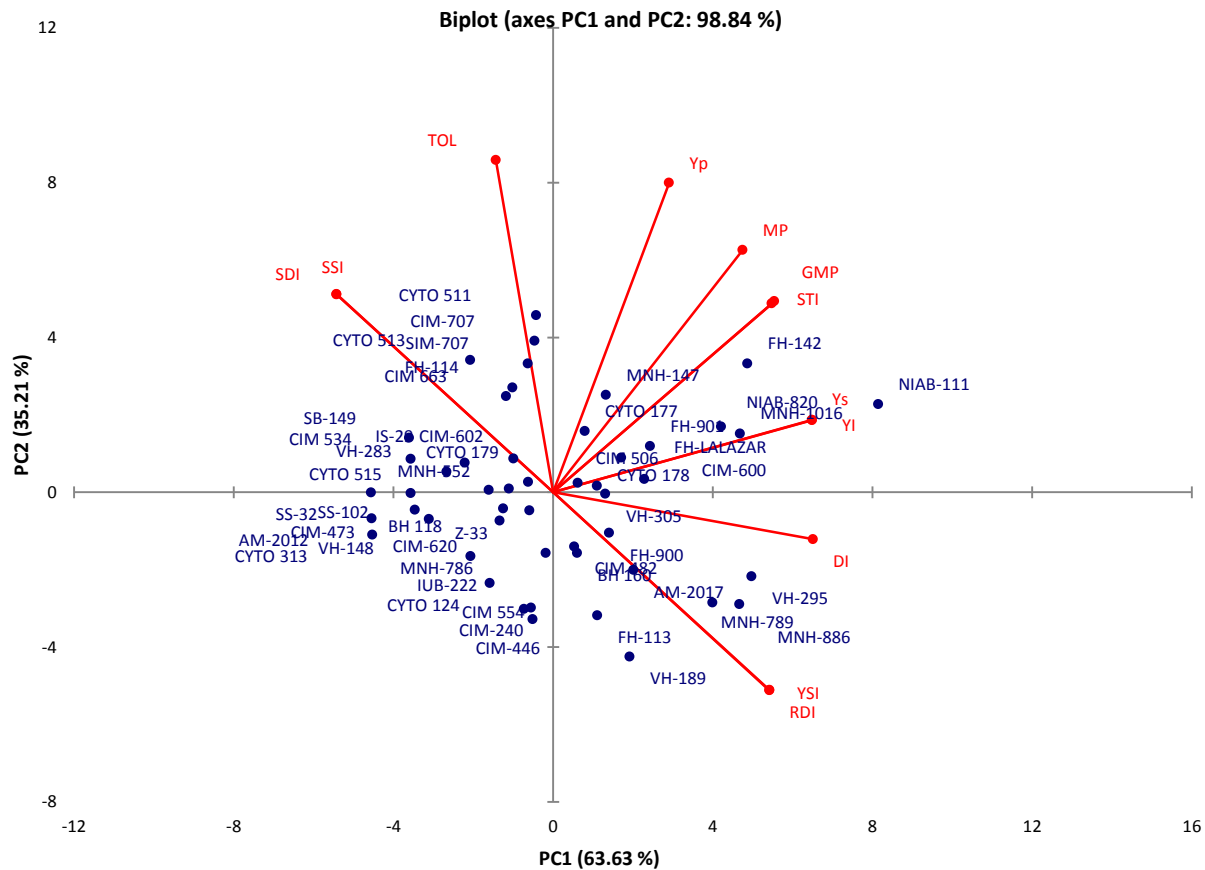


Fig. 1. PCA biplot of 50 cotton genotype based on drought tolerance indices and seed cotton yield under normal and drought stress.

Cluster Analysis

PCA biplot Analysis

In principal component analysis, first two PCs described 98.84 % cumulative contribution in total variability. PC1 expressed 63% and PC2 expressed 35.25% of overall variability. PC1 expressed highly positive coefficients for Ys, YI, DI, GMP, STI, YSI, RDI and MP. PC2 expressed significant coefficients for Yp, MP and TOL. Longer vector lengths indicate higher genetic variability and vice versa. So, Yp, MP, GMP and all other indices exhibited significant variability due to their large distance from the origin in the biplot (Fig. 1). In PCA biplot, FH-142, NIAB-111, VH-295, MNH-1016 and MNH-886 were observed to be highly efficient genotypes for drought tolerant indices.

50 cotton varieties were classified in to four major groups based on drought tolerance indices and seed cotton yield under normal and stressed conditions (Fig. 2). First cluster was largest with 27 genotypes followed by cluster 2 and cluster 4 having 13 and 6 genotypes, respectively. Cluster 3 depicted only 4 genotypes. The genotypes of cluster 1 were expressed moderate yield under both normal and stressed conditions. The genotypes in cluster 2 expressed low Yp, Ys, MP, TOL, YI and DI. However, genotypes in cluster 3 and cluster 4 showed high Yp, MP, YI and GMP. High STI, RDI, DI was observed in the genotypes of cluster 3 (Table 4).

Based on RWC, ELWE and seed cotton yield under drought stress condition cluster analysis divided cotton genotypes into 4 main clusters (Fig. 3). The first cluster was consisted of 16 genotypes showing average Ys, RWC and ELWL as 37.83%, 55.90 and 1.89g, respectively. Second and third clusters consisted of 14 genotypes with the average Ys (24.27g), RWC (24.27%) and ELWL (40.23%) for the 2nd cluster and Ys (44.41g), RWC (42.18%) and ELWL (2.08%) for 3rd cluster. Cluster 4 contained 6 potential genotypes with high Ys (67.23g), RWC (74.00%) and ELWL (0.93%) (Table 5).

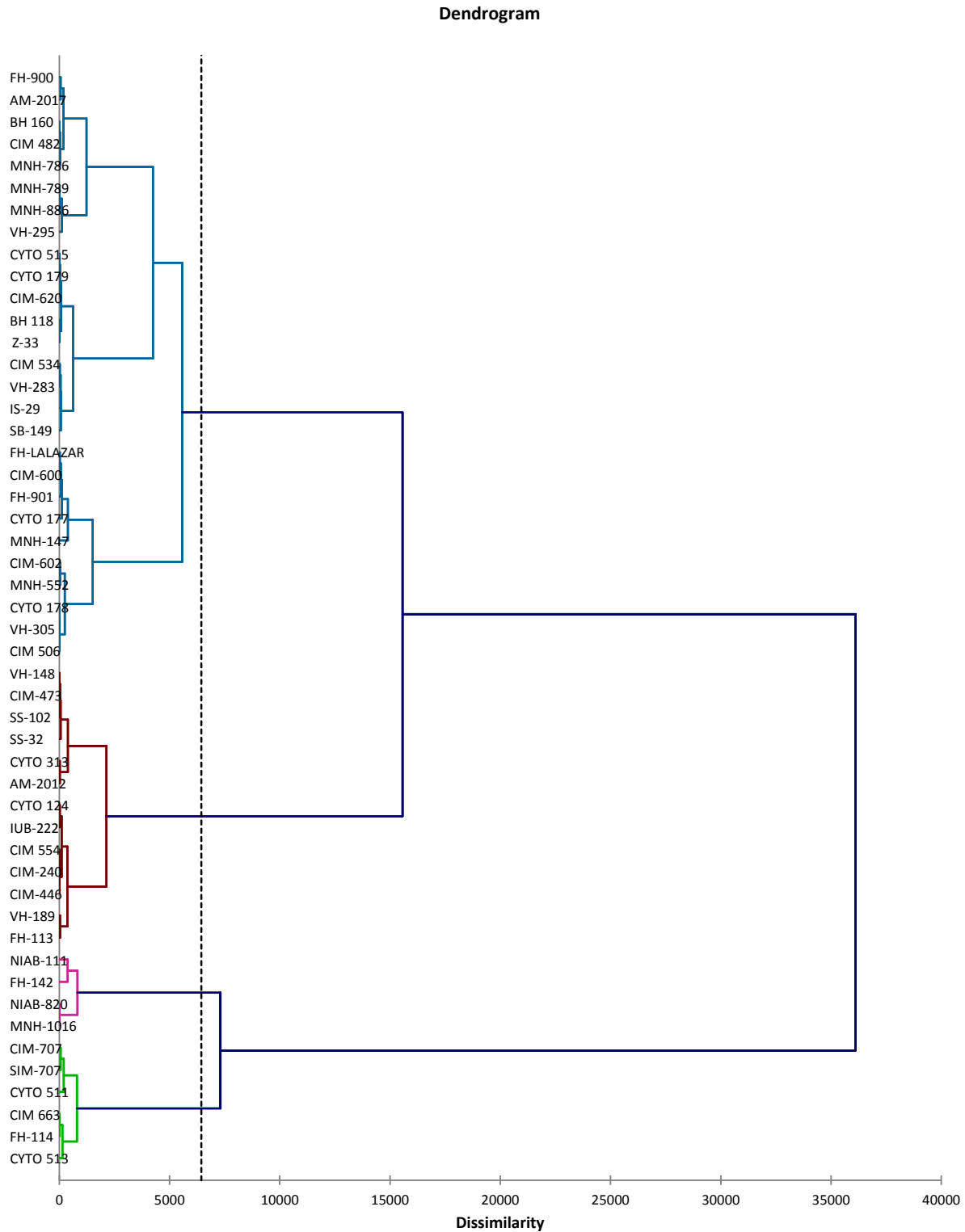


Fig. 2. Cluster analysis dendrogram for 50 cotton genotype based on drought tolerance indices and seed cotton yield under normal and drought stress.

Dendrogram

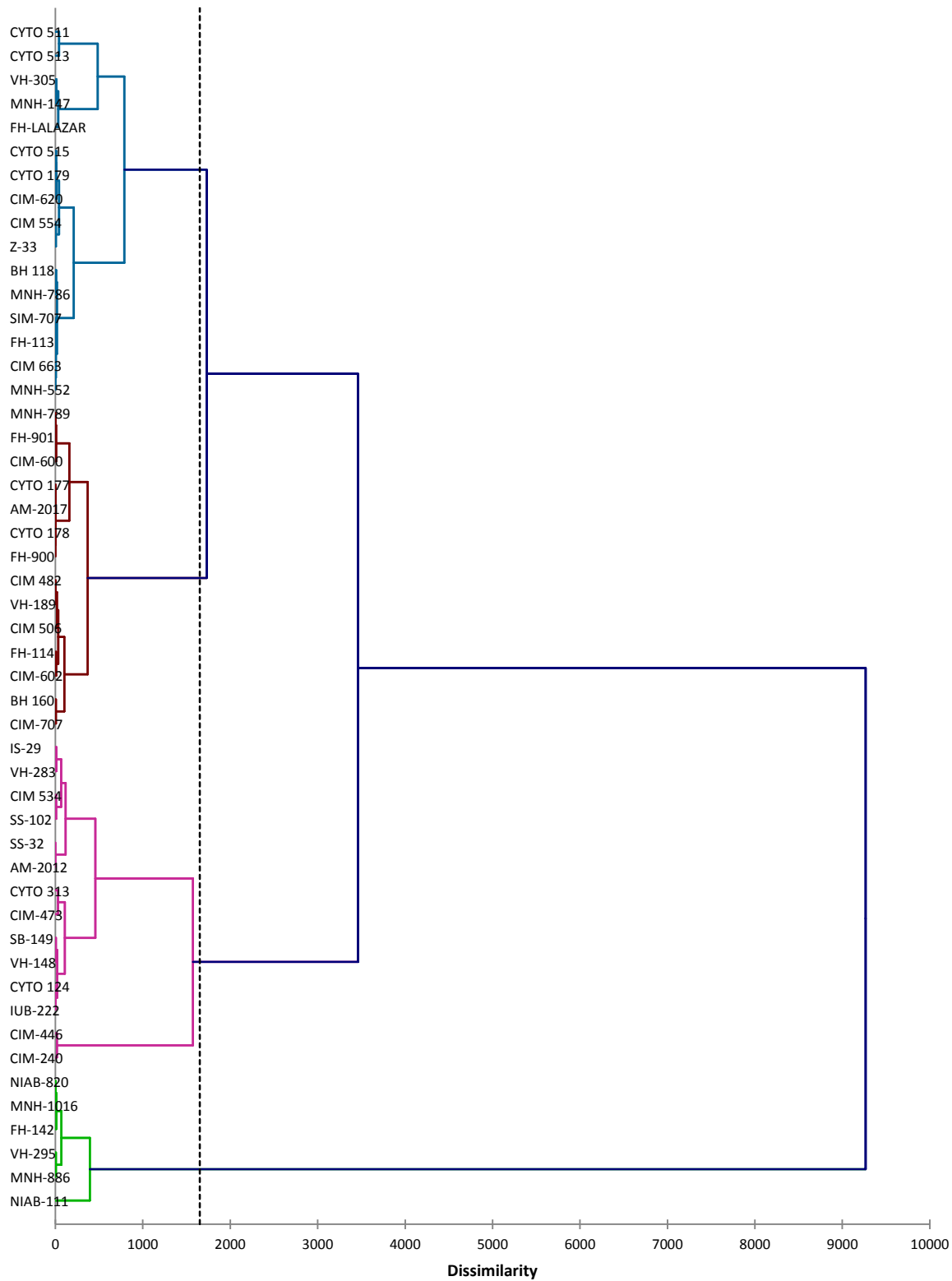


Fig. 3. Cluster analysis dendrogram for 50 cotton genotypes based on seed cotton yield and physiological traits under drought stress.

DISCUSSION

Drought stress has severe effects on plant growth and development ultimately resulting in yield reduction (Zafar *et al.*, 2023). It is necessary for breeders to assess and validate drought-tolerant cultivars. In present study, physiological traits (RWC and ELWL) and ten stress indicators namely MP, GMP, STI, SSI, TOL, YI, YSI, SDI, RDI and DI were evaluated under normal irrigation and water scarcity condition. Here, MP and GMP are indicators showing average cotton production under normal and water deficit condition (Zhao *et al.*, 2019). A comprehensive assessment and evaluation including this index needs to be completed. The genotype NIAB-111 expressed high estimate for MP and GMP followed by FH-142 and MNH-1016 for their high yield under normal and stress. The same genotypes showed high YI, STI and low SSI which indicates their stable yield and tolerance against drought stress. The corresponding results were obtained from a study on sweet potato by Gitore *et al.* (2021). MNH-886, VH-295, MNH-789 and VH-189 showed high values for RDI therefore, considered as drought tolerant genotypes. Ullah *et al.* (2019) also revealed similar results in their study. The current study can be validated if drought-tolerant genotypes are screened on physiological basis. The tolerant genotypes retain higher RWC and reduced ELWL through absorption of appropriate solutes under water stress conditions. Under drought stress, tolerant genotypes i.e. NIAB-111, MNH-886, MNH-1016, CYTO-511 and CYTO-513 depicted higher RWC whereas, lower RWC was recorded for susceptible genotypes i.e. CIM-446 and CIM-240. Aslam *et al.* (2023) found similar results in their study. The cotton genotypes with higher RWC are least affected by drought. Similarly, drought-tolerant genotypes showed less water loss from removed leaves than susceptible genotypes. Breeders can develop resistant cotton genotypes using ELWL as a screening tool along with other morpho-physiological traits without affecting seed cotton yield (Hasanuzzaman *et al.*, 2019).

Correlation analysis is very useful parameter in plant breeding as it helps to identify genetic relationships between various phenotypic traits. It is helpful for indirect selection for improving crop production (Baye *et al.*, 2020). In this study, results revealed that MP, YI, GMP and STI can be used for identification of high yielding and tolerant varieties. Similar results were obtained by Sun *et al.* (2023) and Zhao *et al.* (2019) in their studies. RWC was positively and ELWL was negatively associated with Ys, which shows that RWC and ELWL are useful parameters in selection for drought tolerance. Comparable results were found in a study by Arif *et al.* (2023). Multivariate approach is commonly used for sample classification. In this study, PCA identified drought tolerant genotypes. PC1 showed high contributions of drought indices including YI, DI, GMP and STI which is helpful in selecting tolerant cotton genotypes as higher values indicate better efficiency under drought stress. NIAB-111, MNH-1016, FH-142, MNH-886, NIAB-820 and VH-295 in PC1 and CYTO-511 and CYTO-513 in PC2 showed high yield and drought tolerance. Sun *et al.*, (2023); Abdel-Monaem *et al.* (2022) and Ullah *et al.* (2019) studied drought tolerance indices and reported similar finding for cotton selection. In cluster analysis, the distance among various groups of genotypes indicate the degree of variation between genotypes. Cluster dendrogram showed that diverse genotypes were arranged distantly whereas, comparable genotypes were grouped closer together (Ton, 2023). In this study, cluster dendrogram divided cotton genotypes in to four major clusters on the basis of yield, physiological performance and drought tolerance indices. Cluster 1 showed maximum number of genotypes showing moderate Yp and Ys. However the genotypes in cluster 3 and cluster 4 expressed high estimates for STI, MP, YI and GMP. Nandhini *et al.* (2022) used multivariate and association analysis with yield to study the stress tolerance indicators.

Conclusion

In current drought scenarios, it is imperative to develop tolerant cultivars that can withstand sudden variations in irrigation without reducing crop yield. Assessing the existing cotton germplasm to avoid drought stress is the first step towards the solution. In the present study, multivariate analysis indicated that drought tolerance indices MP, YI, GMP, YSI, STI, RWC and ELWL are suitable parameters for selecting tolerant and high yielding cotton genotypes. NIAB-111, MNH-1016, FH-142, MNH-886, NIAB-820, VH-295, CYTO-511 and CYTO-513 were identified as drought tolerant genotypes with stable seed cotton yield under drought stress and can be used in future breeding programs.

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