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Principal component analysis of Diverse Cotton Genotypes in Response to drought Stress at the Seedling Stage

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ABSTRACT In a completely randomized design, the study assessed 40 different cotton genotypes at the seedling stage under three different moisture levels, 100%, 75%, and 50% of field capacity, in triplicate under a split-plot. Principal component analysis was used to examine the data and choose genotypes that were sensitive to and tolerant of drought. Under three moisture levels, the first three principal components showed more than one eigenvalue. In 100%, 75%, and 50% of field capacity, respectively, the first two principal components (F1 and F2) contributed 49.65%, 56.73%, and 65.99% cumulative variability. The best selection markers under conditions of water deficit at the seedling stage were found to be RFW, SDW, RL, SFW, RWC, LRN, and ELWL. At 100% of field capacity, the genotypes IUB-212, IR-3701, NS-121, VH-295, FH-142, VH-144, AA-802, FH-113, NIAB-111, and IR-3 performed better and were shown to be drought tolerant; at 75% of field capacity, the genotypes IUB-212, VH-295, VH-144, NIAB-111, IR-3701, and NS-121 performed better and were proven to be drought tolerant. VH-295, IUB-212, and VH-144 were better performers while FH-1000 and CIM-443 were poor performers at all three moisture conditions.

Keywords: *Cotton; Water deficit; Field capacity; Principle component; Seedling*

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INTRODUCTION Global greenhouse effects enhance the likelihood of short-term high climate stress to agriculture, and drought is one of the primary abiotic stresses affecting crop development and output(Bahrami, Arzani, & Karimi, 2014; Zhang, Xu, Sun, Zhang, & Li, 2018). It was grown on 31.42 million hectares in 2020–21, producing an average of 773 kg ha 1 globally, or 111.48 million 480lb bales (USDA, 2022). Drought has been occurring more frequently in China's cotton zones since the 1980s, which has severely reduced the country's overall cotton crop. The drought has turn into the longest stress of the cotton growing season. As such, it is imperative to investigate the drought-resistant characteristics of cotton, analyze various genotypes' drought resistance fairly, then screen cotton for drought resistance indices, which act as a guide for figuring out how resistant cotton is to drought.

It is crucial to comprehend the mechanism and behavior of the plant during water scarcity conditions in order to develop resistance to it. Plants go through several developmental stages, including morphological, physiological, biochemical, anatomical, and molecular ones, to prepare themselves for the conditions of water scarcity. The process of drought tolerance in plants is intricate, both at the cellular and molecular levels and throughout the entire body. A number of factors, including the types of crops grown, the severity and duration of the stresses they face, and the phases at which the plants develop, add to the complexity of drought-tolerant systems (Bakht et al., 2020). Plants can withstand drought by adopting multiple tolerance strategies that work at once. A plant can become used to dealing with three primary mechanisms when there is a water deficit. (i) Resistance mechanism; (ii) avoidance or tolerance; and (iii) escape. The plant uses the first mechanism to finish its life cycles before there is a water deficit. In the second process, a plant closes its stomatal opening and reduces transpiration rates in response to a reduced water supply. In the third mechanism, plants create antioxidants at the cell level to sustain osmotic adjustments and at the tissue level to combat conditions of water deficit (Singh, Rajkumar, & Kumar, 2021).

In recent years, evaluation markers of cotton drought resilience have been presented in a number of research papers. As an example, (Feng et al., 2011) proved that indicators for drought resistance in cotton seedlings could be identified using, betaine, chlorophyll levels, soluble sugar, superoxide dismutase, proline (Pro), and peroxidase activity.(Chen et al., 2012) found that the primary and obvious indicators of colored cotton's tolerance to drought are the number of leaves, weight of bolls per plant, and number of bolls per plant (Shi et al., 2013).

Many unique morphological (leaf, stem, and root development parameters) and physiological (more than thirty features) traits have been suggested as critical selection factors in relation to drought tolerance in cotton (Loka, Oosterhuis, & Ritchie, 2011). However, there hasn't been any persistent positive correlation found between drought tolerance and any of the aforementioned physiological features (Loka et al., 2011). Considering the challenges in determining which physiological parameter serves as a valid predictor of yield in drought conditions, it has been proposed that yield performance in a variety of situations should serve as the main indicator of drought resistance (Voltas, Lopez-Corcoles, & Borras, 2005). There have been reports of certain morphological characteristics of seedlings that are significant for cotton's ability to withstand water stress (Riaz et al., 2013). These characteristics include lateral root density, root/shoot ratio, seedling vigor, taproot mass, and root/development speed. Based on the traits of the seedlings, cotton genotypes are chosen in an informal, affordable, and hassle-free manner. Similarly, the characteristics of the seedlings showed moderate to high variation, with additive genetic impacts on the surroundings (Rahman, Shaheen, Rahman, & Malik, 2000). Therefore, the current experiment was carried out to choose forty various cotton accessions for drought tolerance based on the performance of seedlings traits under three moisture levels, taking into account the current climate change condition. In order to meet the nation's needs for cotton production, this will provide a source of drought-tolerant soil for dry land agriculture in semiarid and rain-fed locations.

MATERIAL AND METHODS

Plant Material and Experimentation Site

A total of 40 cotton genotypes gathered from several research organizations were planted in polythene bags packed with pH 7.8 sandy loam soil having an EC of 1.7 dSm-1. Before seeding, all lines and varieties of seeds were soaked for a whole night. The next morning, holes were drilled at a depth of 2.5 cm in polythene bags. There were two seeds put in every hole. Throughout germination and growth, the glasshouse's temperature and humidity were kept at 35°C with the help of electric heaters and hot water circulating through pipes. The plants were exposed to sixteen hours of photoperiod, which consisted of both artificial and natural lighting. Following two weeks of sowing, one plant per bag was trimmed, and each bag received 0.25 g of urea (46 percent nitrogen) every fourteen days. For protection from pest attacks (chewing and sucking), plants were treated when necessary and received daily irrigations. Based on the estimated field capacity of soil in bags at the onset of the first true leaf, three distinct levels of moisture—100%, 75%, and 50% of the field capacity were used.

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Measuring the seedling traits

Six-week-old seedlings were used to measure the following characteristics: biomass, RFW, SFW, SL, RL, SDW, RDW, NLR, RWC and ELWL. Three plants of each genotype from each replication and treatment were taken out of the polythene bags to measure the seedling characteristics. The plants were gently washed to remove all the sand. The biomass of the uprooted plants was calculated in grams using an electronic balance. Each uprooted plant's shoot and root were divided by cutting at the point where the shoot and root met. Using an electronic balance, the fresh shoot and root weight were determined. Means were then computed for each genotype in each treatment. A centimeter measuring tape was used to determine the length of the shoot and roots, and the number of lateral roots was counted. After determining their fresh weight, the shoots and roots of each genotype were placed in separate craft paper bags. These shoots and roots were then oven-dried for 24 hours at 80°C to obtain dried shoots and roots. This process allowed for the measurement of the dry weight of the shoots and roots. Using an electronic balance, the weight of these dry shoots and roots of each genotype was determined, and the means for each treatment were calculated. The Relative water content (Aboughadareh, Naghavi, & Khalili, 2013) and Excised leaf water loss (R. T. Ahmad, Malik, Khan, & Jaskani, 2009) in this study

Statistical Analysis

Using Statistics 8.1, a basic ANOVA was performed to analyze the attributes of the seedling data that was gathered (Steel & Torrie, 1981). Principal component analysis was then used to further analyze the data (Sneath, 1973) using XLSTAT software. Biplot graph based on principle component analysis was used for the assessment of association among various traits at different moisture levels. The angle between vectors showed association between variables; acute angle, <90⁰ showed positive association, acute angle, <45⁰ showed strong positive association, right angle of 90⁰ no correlation, obtuse angle, >90⁰ showed negative association and obtuse angle of >135⁰ and <180⁰ showed strong negative association. In order to bestow the drought-tolerant cotton genotypes, favorable morpho-physiological associated seedling features and genotypes that are resistant to drought were chosen based on the results of the previously mentioned analysis

RESULTS AND DISCUSSION

Principal Component Analysis

Different methods are used for assessment of genotypes and among them the multivariate analysis is most suitable. Data mining is a highly helpful technique for selecting, evaluating, and modeling enormous datasets in order to identify fresh patterns and trends that will make the interpretation more elegant and compelling. Multivariate analysis is usually used for data mining. As a multivariate analysis, principle component analysis has numerous applications. This analysis's main goal is to achieve parsimony and minimize dimensionality by identifying the fewest components that can account for the majority of the original variation in the multivariate data. Therefore, some information is lost in the abbreviated data used in this procedure (Granato, Santos, Escher, Ferreira, & Maggio, 2018).

For every variable in this experiment, there were extremely significant differences between the genotypes and treatments. Except RDW, all traits showed a highly significant genotype × treatment interaction. For selection of principle components eigenvalue is very important criteria and the value 1 is used as cutoff for selection of principle components. A component that has an eigenvalue larger than one suggests that it accounts for more variance than one of the original variables (Abdi, Williams, & Valentin, 2013). Principle component analysis separated the data into nine separate principle factors (components); only the first three factors had an eigenvalue greater than 1 for all treatments. The total variability contributions of the first two components were 49.65%, 56.73%, and 65.99% in 50%, 75%, and 100% of field capacity, respectively (Fig. 4, 5, 6). Additionally, Zahid et al. (Zahid et al., 2021) and Zafar et al. (Zafar et al., 2022) noted that The total variability of cotton germplasm was largely influenced by the first fundamental components. For every treatment, eigenvalues and cumulative variability were also shown in scree plots (Figs. 1, 2, 3). Under various treatments, the contribution % of each characteristic to overall variability varied. Plant biomass contributed 6.46%, 16.92%, and 8.51% of the variability in the second factor for 50%, 75%, and 100% of the field capacity, respectively. In the first component, it contributed 23.15%, 16.26%, and 15.18% of the variability (Table 3). Shoot fresh weight gave relatively minimal percentage to F2, F3, and 100% of field capacity; however, this attribute contributed 20.15%, 14.07%, and 14.36% of variability to F1, 50%, 75%, and 100% of field capacity, respectively. Under 100%FC, the characteristics that contributed to the variability of F1 were shoot dry weight, root fresh weight, lateral root number, RL, and RWC (18.21%, 17.89%, 5.94%, 5.87%, and 4.81%) as in (Table 3).

Under 100%FC, the following factors have contributed to the F2 variability: RWC, ELWL, RDW, and lateral root number (32.50%, 25.48%, 21.62%, and 4.37%, respectively) (Table 4). Plant biomass, shoot fresh weight, root fresh weight, RL, and shoot dry weight were the variables that contributed more than 14% to the variability of F1 under 75%FC. Under 75% of field capacity, plant biomass, SL, RL, and ELWL all demonstrated contributions of more than 10% to the variability of F2. In the F1 variability under 50% of field capacity, biomass, shoot fresh weight, shot dry weight, SL, and RFW all contributed more than 10%. Table 3 demonstrates that while all traits had very low percent contributions to the variability of F2 under 50% of field capacity, the exceptions were RFW (21.61%), shoot dry weight (19.44%), and ELWL (44.86%). The traits that contributed more than 10% to the variability of F2 were SL, RDW, lateral root number, and ELWL contents. It is said that SL is an important consideration when assessing how drought affects crop plants (Ahmad et al., 2021). The nutrients moved within the root cells during the drought. These cells aid in the uptake of water and nutrients by the plants from the lower soil surface; nevertheless, poor plant development was caused by an excessive build-up of nutrients in these cells as a result of reduced growth of the shoot tissues (Mahmood et al., 2022).

Except for ELWL, which demonstrated a negative contribution under 100%FC and 75%FC, all features contributed positively to F1 of 100%, 75%, and 50% FC (Table 4). Within the F2 of 100%, 75%, and 50%FC, each feature had a positive effect on one treatment and a negative effect on another. SL, RL, shoot dry weight, RWC, and ELWL were the positive factors in F3 of 100%FC and 50%FC, while biomass, shoot fresh weight, shoot dry weight, and lateral root number were the negative factors. More RLWC is a reliable indicator of the water content of leaves and a plant's capacity to withstand drought. According to reports, during drought stress, accessions with higher RLWC are more productive (Anwar et al., 2022). In F3 of 75%FC, root fresh weight, RL, shoot dry weight, and lateral root number contributed negatively, but all other traits contributed positively (Table 4).

Biplot graph

For each water treatment, a separate set of biplot graphs representing F1 and F2 from the principal component analysis were created. The graphs' vector length and cosine of the angle were utilized to create various groups with various attributes. These groups showed comparable performance in genotype discrimination. Biplot categorized the characteristics into three main categories when the field capacity was 100%. Group 2 was created based on the relative water content, root dry weight, and quantity of lateral roots. Group 3 retained the water loss from the removed leaf. Traits showed extremely modest positive and negative associations between groups, whereas large positive associations existed within the group (Fig. 4). Group 2 had longer roots and shoots than Group 1 at 75% of the field capacity (Fig. 5). Groups were not distinguishable at 50% of field capacity because they were in 100% and 75% of field capacity, respectively (Fig. 6). The vector length showed how a trait may distinguish between different genotypes. These variables had poor discriminating power, and the current study should not use them to identify cotton genotypes. (Fig. 4, 5 & 6).

Association among seedling traits

In this study, PCA was used to describe the association among various seedling traits. According to (Maione & Barbosa, 2019), PCA is a commonly used multifactorial approach for sample classification. The degree of correlation between the attributes was indicated by the vector length and the cosine of the angle. Drought stress brought on by climate change are negatively influencing the quantity and quality of seed cotton produced (Ul-Allah, Rehman, Hussain, & Farooq, 2021). Under 50% field capacity (FC), relative water content exhibited a strong positive correlation with root fresh weight, fresh shoot weight, and overall biomass, including dry shoot weight. This correlation was less pronounced when moisture levels reached 100% FC or remained at 50% FC. Additionally, there was no significant relationship observed between shoot length at 100% FC and root dry weight at 75% FC when examining shorter vector lengths. A greater cosine of angle was found between excised leaf water loss and morphological features (shoot and root length, root dry weight, and lateral root number) indicating a negative correlation (Fig. 4, 5 & 6). These results are consistent with the research released by (Abdi et al., 2013).

Better and Worst Performer Genotypes

Genotypes that exhibited positive traits were more effective in differentiation, outperforming those associated with negative characteristics. The following genotypes—VH-144, AA-802, CRS-456, IR-3, FH-113, NS-121, and VH-295—excelled in terms of biomass, shoot fresh weight, root fresh weight, shoot dry weight, and root length. On the other hand, genotypes SB-149, CIM-707, CIM-240, VH-283, VH-282, and CRS-2007 underperformed in these areas. Looking at root dry weight, lateral root number, and excised leaf water loss, genotypes MG-6, CIM-443, and IUB-212 stood out with good performance, whereas (FH-1000), (FH-118), (AA-703), (S-12), and (IUB-222) lagged behind. Considering parameters such as 75% field capacity (75% FC), biomass, shoot fresh weight, shoot dry weight, and root fresh weight, genotypes (FH-113), (FH-142), (AA-802), and (VH-295) showed strong results. Conversely, (FH-175), (CIM-707), (CIM-443), (FH-1000), and AA-703 exhibited weaker performance. (FH-171), (SB-149), and (CRS-2007) demonstrated superior performance in terms of shoot length, relative water content, lateral root number, shoot dry weight, and root length compared to (CRS-456), (NIAB-820), (S-12), (IUB-222), and (KZ-181).

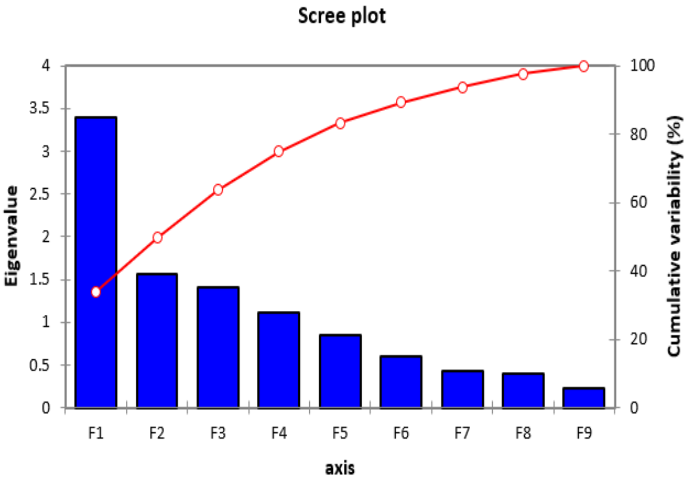

Fig. 1. Scree plot for 100% of field capacity

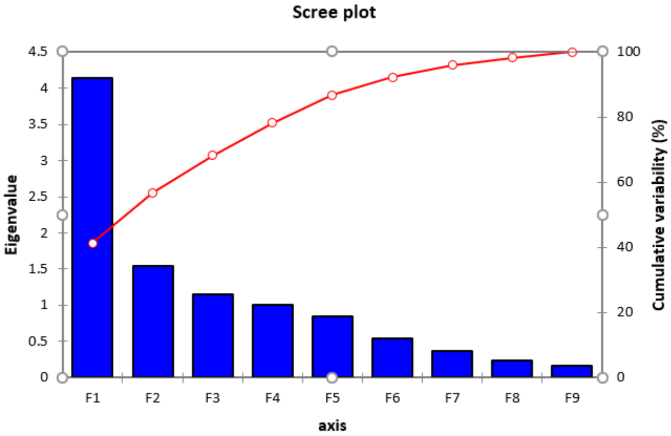

Fig. 2 Scree plot for 75% of field capacity

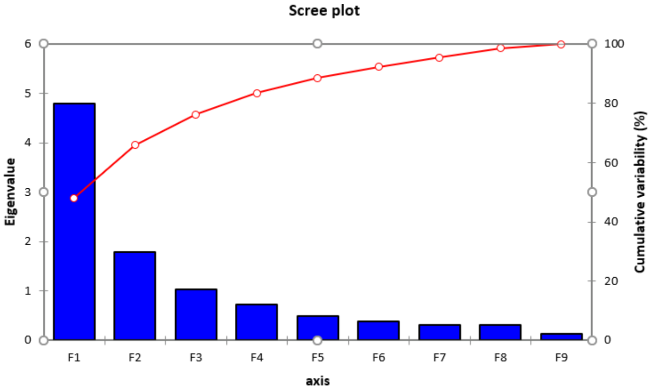

Fig. 3 Scree plot for 50% of field capacity

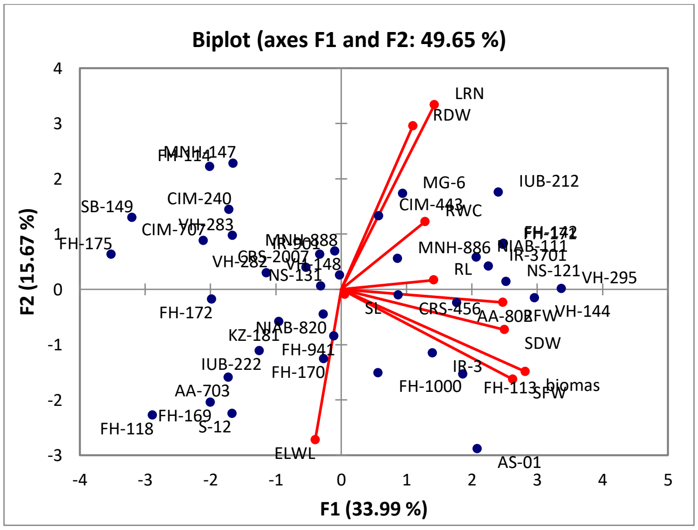

Fig. 4. Biplot graph for seedling traits in 40 cotton genotypes at 100%FC based on the first two components.

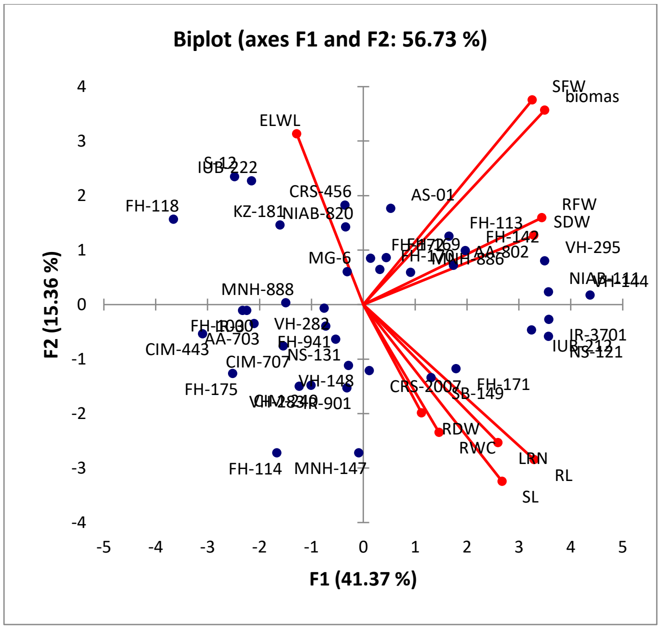

Fig. 5. Biplot graph for seedling traits in 40 cotton genotypes at 75%FC based on first two components.

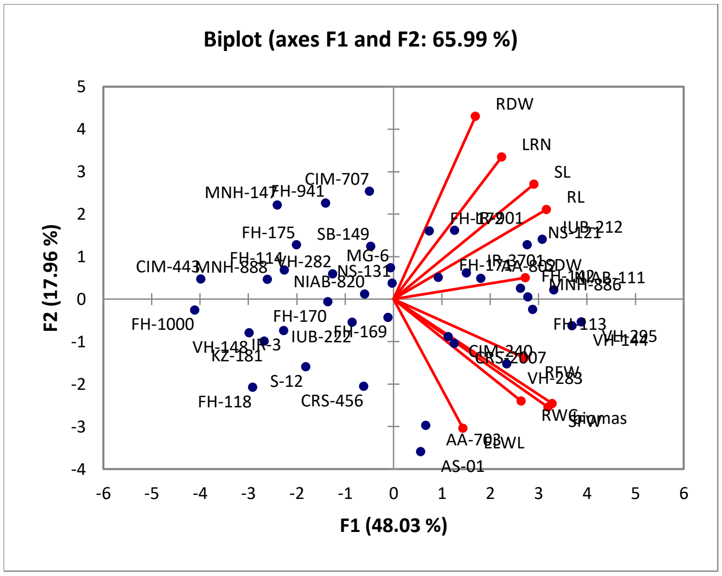

Fig. 6. Biplot graph for seedling traits in 40 cotton genotypes at 50%FC based on first two components.

Table 1: List of 40 cotton accessions used in the current study

Sr. No	Cultivar/Lines	Origin	Sr. No	Cultivar/Lines	Origin
1	IR-3701	NIBGE, Faisalabad	**21**	FH-142	CRI, Faisalabad
2	SB-149	Exotic	**22**	VH-144	CRS, Vehari
3	FH-172	CRI, Faisalabad	**23**	AS-01	Exotic
4	IUB-212	IUB, Bahawalpur	**24**	CRS-2007	CRS,
5	IR-901	NIBGE, Faisalabad	**25**	FH-1000	CRI, FSD
6	CIM-443	CCRI, Multan	**26**	IUB-222	IUB, Bahawalpur
7	KZ-181	Kanzo Seeds	**27**	S-12	Sitara Seeds
8	CIM-240	CCRI, Multan	**28**	FH-114	CRI, FSD
9	NS-121	Neelam Seeds	**29**	FH-118	CRI, FSD
10	FH-170	CRI, FSD	**30**	MNH-886	CRS, Multan
11	FH-941	CRI, FSD	**31**	MG-6	Exotic
12	CIM-707	CCRI, Multan	**32**	NS-131	Neelam Seeds
13	FH-171	CRI, FSD	**33**	FH-169	CRI, FSD
14	IR-3	NIBGE, FSD	**34**	VH-295	CRS, Vehari
15	AA-703	Ali Akbar Seeds	**35**	CRS-456	CRS, Multan
16	AA-802	Ali Akbar Seeds	**36**	FH-113	CRI, FSD
17	NIAB-111	NIAB, FSD	**37**	MNH-147	CRS, Multan
18	FH-175	CRI, FSD	**38**	VH-148	CRS, Vehari
19	MNH-888	CRS, Multan	**39**	VH-283	CRS, Vehari
20	NIAB-820	NIAB, FSD	**40**	VH-282	CRS, Vehari

Table 2: Using Principle Component Analysis, the eigenvalue, variability, and cumulative variability of several components under 100%FC, 75%FC, and 50%FC were calculated.

	Eigenvalue			**Variability (%)**			**Cumulative %**		
Treatment	100%FC	75%FC	50%FC	100%FC	75%FC	50%FC	100%FC	75%FC	50%FC
F1	3.4	4.14	4.8	33.99	41.37	48.03	33.99	41.37	48.03
F2	1.57	1.54	1.8	15.67	15.36	17.96	49.65	56.73	65.99
F3	1.41	1.16	1.03	14.1	11.56	10.3	63.75	68.29	76.29
F4	1.12	1	0.73	11.16	10.01	7.26	74.91	78.3	83.55
F5	0.84	0.85	0.5	8.43	8.49	5	83.35	86.79	88.55
F6	0.6	0.55	0.38	6.03	5.46	3.8	89.38	92.25	92.35
F7	0.44	0.37	0.32	4.37	3.68	3.19	93.75	95.93	95.54
F8	0.39	0.24	0.31	3.91	2.36	3.08	97.66	98.29	98.62
F9	0.23	0.17	0.14	2.34	1.71	1.38	100	100	100

Table 3: Proportion of the variables (%) under 100%FC, 75%FC, and 50%FC to the variation of various components with eigenvalues greater than 1.

		Biomass	**SFW**	**RFW**	**SL**	**RL**	**SDW**	**RDW**	**LRN**	**RWC**	**ELWL**	**Total**
100%FC	F1	23.15	20.15	17.89	0.01	5.87	18.21	3.5	5.94	4.81	0.46	100%
	F2	6.46	7.74	0.16	0.03	0.08	1.56	25.48	32.5	4.37	21.62	100%
	F3	4.71	7.39	1.4	37.59	33.65	0.8	8.36	0.05	5.96	0.1	100%
75%FC	F1	16.26	14.07	15.71	9.52	14.44	14.35	1.68	8.97	2.83	2.18	100%
	F2	16.92	18.7	3.37	14	10.77	2.13	5.27	8.53	7.31	13.01	100%
	F3	0.54	1.98	6.25	8.12	0.26	0.29	3.27	9.06	43.82	26.4	100%
50%FC	F1	15.18	14.36	10.3	11.87	14.05	10.47	4.04	7.02	9.81	2.91	100%
	F2	8.51	9.09	2.69	10.29	6.26	0.36	25.99	15.72	8.1	13.01	100%
	F3	7.43	4.38	21.61	0.65	0.26	19.44	0.01	0.02	1.33	44.86	100%

Table 4: Contribution of variables under 100%FC, 75%FC, and 50%FC for various cotton characteristics

		Biomass	**SFW**	**RFW**	**SL**	**RL**	**SDW**	**RDW**	**LRN**	**RWC**	**ELWL**
100%FC	F1	0.89	0.83	0.78	0.02	0.45	0.79	0.35	0.45	0.4	-0.13
	F2	-0.32	-0.35	-0.05	-0.02	0.04	-0.16	0.63	0.71	0.26	-0.58
	F3	-0.26	-0.32	0.14	0.73	0.69	0.11	-0.34	-0.03	0.29	0.04
75%FC	F1	0.82	0.76	0.81	0.63	0.77	0.77	0.26	0.61	0.34	-0.3
	F2	0.51	0.54	0.23	-0.46	-0.41	0.18	-0.28	-0.36	-0.34	0.45
	F3	0.08	0.15	-0.27	0.31	-0.05	-0.06	0.19	-0.32	0.71	0.55
50%FC	F1	0.85	0.83	0.7	0.75	0.82	0.71	0.44	0.58	0.69	0.37
	F2	-0.39	-0.4	-0.22	0.43	0.34	0.08	0.68	0.53	-0.38	-0.48

	F3	-0.28	-0.21	-0.47	0.08	0.05	0.45	-0.01	-0.01	0.12	0.68

CONCLUSION

This study led us to the conclusion that, at all moisture percentages of 75%, 50%, and 100% FC, MNH-886, NIAB-111, VH-144, FH-142, NS-121, AA-802, IR-3701, VH-295, FH-113, and IUB-212 did better and were chosen for their drought tolerant genotypes, whereas IR-3, CIM-443, S-12, VH-148, MNH-147, and FH-1000 performed poorly and were selected as drought-susceptible genotypes. The inheritance of physiological features and seed cotton production in a field trial demonstrated the material's complex genetic architecture and recommended delaying selection while dividing populations to increase drought tolerance in the plant material currently in use. Hence, these results and findings would be helpful for prospects and breeding programs to develop drought-tolerant varieties.

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