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MULTIVARIATE ANALYSIS OF DROUGHT TOLERANCE IN COTTON GENOTYPES BASED ON PHYSIOLOGICAL TRAITS AND YIELD PERFORMANCE

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ABSTRACT

Drought is a major constraint to cotton (*Gossypium hirsutum* L.) productivity, and its complex effects on plant water relations and yield formation require integrated approaches for genotype evaluation. This study evaluated 20 cotton genotypes under contrasting field irrigation regimes, comprising well-watered (optimal) and drought-stressed conditions, at the Regional Experimental Base of the Center of Genomics and Bioinformatics, Uzbekistan. Relative water content (RWC), excised leaf water loss (ELWL) after 1, 4, and 6 h, SPAD chlorophyll content, boll weight, productivity per plant, and seed cotton yield were assessed using a randomized complete block design with three replications. We examined genotypic responses using correlation analysis, principal component analysis (PCA), hierarchical cluster analysis, drought tolerance indices, and the Multi-Trait Genotype–Ideotype Distance Index (MGIDI). Drought reduced mean RWC from 70.72% under optimal irrigation to 61.51% under drought and reduced mean boll weight, productivity per plant, and seed cotton yield, whereas ELWL increased progressively with measurement time. Drought tolerance index (DTI) analysis identified Gulbahor_2 as the highest-ranked genotype, followed by Genafond_2, C_312, An_Boyovut_2, and Zakovat. MGIDI-based multi-trait selection identified Polvon, C_312, Shodlik_91, Zakovat, and Tafakkur as the genotypes closest to the predefined drought-response ideotype. C_312 and Zakovat were common to both selection approaches, indicating concordance between productivity-based drought indices and multi-trait physiological–agronomic selection. Multivariate analyses further showed that drought responses were characterized by coordinated variation in leaf water relations, ELWL, and yield-related traits rather than by a single indicator. The identified genotypes provide candidate materials for further validation and breeding studies aimed at improving cotton performance under water-limited conditions.

***Keywords*:** Cotton, Drought stress, Physiological traits, Drought tolerance indices, MGIDI, Multivariate analysis, Genotype selection.

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1. INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is the world's leading natural fiber crop and an important source of edible oil and livestock feed, underpinning both agricultural production and the textile industry. However, climate change increasingly challenges its sustainability, with drought among the most pervasive constraints on cotton productivity, particularly in arid and semi-arid regions (Zafar et al., 2022). Water deficit can reduce seed cotton yield and fiber quality while increasing production instability as cultivars differ in their capacity to maintain performance under fluctuating water availability (Ul-Allah et al., 2021; Rehman et al., 2021). Consequently, the development of drought-resilient cultivars has become a major priority in contemporary cotton breeding. This task remains challenging because drought tolerance is a quantitative and environmentally sensitive trait arising from interactions among multiple physiological, reproductive, and agronomic processes. Recent studies have demonstrated substantial phenotypic and agronomic variation in *Gossypium hirsutum* genotypes, including variation in morphological traits following gamma irradiation (Mohira et al., 2026) and differences in yield, fiber quality, and growth traits among cotton lines evaluated under field conditions in Uzbekistan (Bekmukhamedov et al., 2026). Such genotypic diversity provides an important basis for identifying cotton materials with improved adaptation to challenging environments. Nevertheless, phenotypic variation alone does not explain how genotypes maintain productivity when water

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availability becomes limiting (Zafar et al., 2020). Contemporary breeding therefore increasingly relies on integrative phenotyping, in which physiological attributes are interpreted together with yield-related characteristics to capture drought adaptation as a coordinated biological response (Shani et al., 2025a; 2025b). Water limitation initiates a cascade of physiological changes. Declining soil moisture restricts root water uptake and decreases leaf water potential, promoting stomatal closure, reducing CO_2 diffusion, and constraining photosynthetic carbon assimilation (Zafar et al., 2023; Patil et al., 2024). Prolonged stress may additionally disrupt chlorophyll stability, membrane integrity, and cellular metabolism, with subsequent consequences for boll retention, reproductive development, and seed cotton yield (Chaudhary et al., 2025). The intensity of these responses varies considerably among genotypes because cotton germplasm differs in its ability to preserve tissue hydration, regulate transpirational losses, and sustain photosynthetic activity during water shortage (Haghpanah et al., 2024; Safdar et al., 2024; Shani et al., 2025c). Drought adaptation is therefore unlikely to be adequately represented by a single physiological mechanism; instead, it reflects coordinated regulation of plant water relations, leaf water conservation, photosynthetic stability, and reproductive performance. Among readily measurable physiological attributes, relative water content (RWC), excised leaf water loss (ELWL), and chlorophyll content estimated by SPAD provide complementary information on plant responses to water deficit (Zahid et al., 2021; Mukhammadaliev et al., 2026). RWC reflects the capacity of leaves to retain cellular hydration and turgor under declining soil moisture and is consequently considered a reliable indicator of plant water status (Shani et al., 2025a; Chaudhary et al., 2025). ELWL, in contrast, characterizes the capacity of detached leaves to conserve water and is associated with stomatal regulation, cuticular properties, and leaf water-retention characteristics. Lower ELWL generally indicates greater water-conservation capacity under drought (Sajid-Ur-Rahman et al., 2000; Bi et al., 2017; Czyczyło-Mysza et al., 2018). SPAD measurements provide a rapid, non-destructive estimate of chlorophyll status and, indirectly, the stability of the photosynthetic apparatus during stress (Safdar et al., 2024; Shani et al., 2025a). Considered together, these variables represent distinct dimensions of drought response: tissue hydration, water dissipation, and photosynthetic integrity. Physiological maintenance, however, has limited breeding value unless it contributes to reproductive success and productivity. A genotype that preserves leaf hydration or chlorophyll may not necessarily maintain boll development, biomass accumulation, or seed cotton yield. Accordingly, physiological responses should be evaluated alongside agronomic attributes to determine whether stress-management mechanisms translate into economically relevant performance (Safdar et al., 2024; Chaudhary et al., 2025). This distinction is particularly important because physiological stress avoidance and productive adaptation are not necessarily equivalent. Simultaneous assessment of physiological and yield-related traits can therefore help distinguish materials that primarily withstand physiological perturbation from those that sustain agronomic performance under restricted water availability (Zafar et al., 2023; Patil et al., 2024). The interdependence of these characteristics further complicates drought phenotyping. Associations among water status, leaf water loss, chlorophyll content, boll traits, and yield may reveal coordinated mechanisms of adaptation that remain obscured when variables are examined independently. Multivariate techniques such as correlation analysis, principal component analysis (PCA), and hierarchical cluster analysis (HCA) are consequently valuable for summarizing correlated traits, identifying major axes of phenotypic variation, and differentiating genotypes according to their overall response patterns. Although these methods have been applied to cotton under water limitation, they have often been considered separately or using narrower combinations of physiological and agronomic variables (Zou et al., 2020; Sun et al., 2021). Their joint interpretation may therefore provide a more coherent representation of drought-response architecture. A related challenge concerns the transition from phenotypic description to breeding-oriented selection. Drought tolerance indices quantify relative productivity across favorable and stress conditions and can identify genotypes with stable performance; however, their emphasis on yield may provide limited insight into the physiological processes underlying adaptation (Nandhini et al., 2022; Çelik et al., 2023; Sharif et al., 2024). Conversely, physiological measurements offer mechanistic information but do not necessarily identify materials possessing the most desirable combination of traits. The Multi-trait Genotype–Ideotype Distance Index (MGIDI) addresses this limitation by integrating several traits according to a predefined breeding ideotype, thereby providing a complementary selection framework (Olivoto & Nardino, 2021). Despite substantial progress, the coordinated interpretation of physiological status, productivity, drought indices, multivariate structure, and ideotype-based selection remains comparatively limited in cotton exposed to contrasting irrigation regimes. Previous studies have frequently treated these analytical components as separate approaches, restricting the direct connection between physiological adaptation and breeding value (Guo et al., 2024). The novelty of the present work therefore does not lie in the individual use of RWC, ELWL, SPAD, drought tolerance indices, PCA, HCA, or MGIDI, all of which are established approaches. Rather, it derives from integrating these approaches within the same cotton genotype panel, enabling water status, water-retention capacity, photosynthetic condition, yield-related performance, stress-response indices, and multi-trait selection to be examined within a unified phenotypic framework. We hypothesized that drought adaptation cannot be sufficiently characterized by a single indicator and that combining physiological

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measures with agronomic performance and complementary analytical approaches would provide a more informative assessment of genotype responses. Specifically, integrating RWC, ELWL, and SPAD with boll weight, plant productivity, and seed cotton yield, together with correlation analysis, PCA, HCA, drought tolerance indices, and MGIDI, was expected to clarify the relationships between physiological maintenance and productive adaptation. To test this hypothesis, twenty cotton genotypes were evaluated under well-watered and drought-stressed conditions. The objectives were to: (i) quantify genotypic variation in physiological and agronomic traits under contrasting water regimes; (ii) determine associations among these characteristics using correlation analysis; (iii) identify major dimensions of phenotypic variation through PCA; (iv) classify genotypes according to integrated response patterns using HCA; and (v) identify materials combining favorable drought-response attributes and agronomic performance through drought tolerance indices and MGIDI. By integrating these complementary layers of evidence, this study seeks to provide a biologically interpretable and breeding-relevant framework for identifying cotton genotypes capable of maintaining productivity under water-limited conditions.

2. MATERIALS AND METHODS

2.1. Plant Material and Experimental Design

For the research, 20 varieties of upland cotton (*Gossypium hirsutum L.*) were used: Baraka_MAS, Tafakkur, Zakovat, Polvon, C_312, Bardosh, Gulbahor_2, Navbahor_2, Beshqahramon, Shodlik_91, Iqbol, Zamin, Mehnat, Ishonch, Genafond_2, An_Boyovut_2, L_478, L_480, L_485, S_1306. The field experiment was conducted in 2025 at the Regional Experimental Base of the Center of Genomics and Bioinformatics, Academy of Sciences of the Republic of Uzbekistan, located in the Kibray district of the Tashkent region, Uzbekistan (41.377858° N, 69.338751°E; 491 m a.s.l.). Wheat was grown as the preceding crop at the experimental site. The experimental fields were prepared in early April, and the cotton seeds were sown during the third week of April, according to a planting configuration of 0.76 × 0.15 m, with a sowing depth of 4–5 cm. Seed cotton was harvested during the third decade of September 2025. The experiment used a randomized complete block design (RCBD) with three replications. Within each replication, the 20 cotton genotypes were randomly assigned to individual plots to minimize positional effects and ensure an unbiased comparison among genotypes. Each experimental plot consisted of a single 10-m-long row, with 0.76 m spacing between adjacent rows and 0.15 m spacing between planting hills within the row. Seeds were sown in each hill, and the seedlings were subsequently thinned to one plant per hill. This corresponded to a planting configuration of 0.76 × 0.15 m, providing a gross plot area of 7.6 m² and approximately 67 plants per plot (genotype × replication × irrigation treatment). For physiological measurements, one plant was selected from each replication, resulting in three plants evaluated per genotype under each irrigation regime. The experimental plots were arranged according to a randomized design to minimize positional effects and ensure an unbiased comparison among genotypes and irrigation treatments. Two contrasting irrigation regimes were established to characterize genotype performance under optimal water availability and progressive water-deficit conditions. Under the optimal irrigation regime, plants were managed according to 1-2-1 irrigation schedule, comprising one irrigation during the seedling-development stage and two irrigations during flowering and boll development and one irrigation during maturation. The total irrigation water supplied under this regime was 4800 - 5000 m^3 ha^{-1}, while soil moisture during the flowering and boll-development period was maintained at approximately 65-70 of field capacity (FC).In contrast, drought stress was imposed through a substantially reduced irrigation frequency and water supply. Plants subjected to the drought treatment were maintained under a 0-1-0 irrigation schedule, with no irrigation during the seedling-development and maturation stages and a single irrigation during flowering and boll development. Total irrigation water under the drought regime was restricted to 1000–1200 m^3 ha^{-1}, and soil moisture during the flowering and boll-development period was maintained at approximately 40–45% of field capacity. Drought stress was initiated at the onset of flowering and boll development and maintained until the end of this developmental period. Soil moisture was monitored throughout the treatment period to maintain the target moisture ranges under both irrigation regimes.

2.2. Climatic Conditions

Climatic conditions during the 2025 cotton growing season were characterized by progressively increasing temperature and decreasing precipitation and relative humidity from spring to summer. From April to September, mean monthly air temperature ranged from 17.63°C in April to 29.48°C in July and subsequently decreased to 22.33 °C in September. Relative humidity ranged from 58.08% in April to 29.34% in July, with values of 31.11% in August and 35.77% in September. Total rainfall during April–September was 45.28 mm, with particularly low precipitation in July (0.37 mm), August (0.13 mm), and September (0.31 mm). Cumulative actual evapotranspiration/evaporation and potential evaporation during the same period were 314.39 and 1,694.64 mm, respectively. These climatic conditions provided a progressively warmer and drier environment during the crop-growth period and coincided with the period in which the contrasting irrigation treatments were imposed.

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2.3. Morphological, physiological and Biochemical Traits

Morphological, physiological, and agronomic characteristics were evaluated for all genotypes under two experimental conditions, namely well-watered (optimal) and drought stress. The measured traits included relative water content of leaves (RWC), water loss from excised leaves after 1, 4, and 6 h (ELWL_1h, ELWL_4h, and ELWL_6h), leaf chlorophyll content measured with a SPAD meter (SPAD), boll weight (BW), productivity per plant (Productivity), and seed cotton yield (Yield). All physiological measurements were conducted at the flowering and boll-development stages to ensure consistent sampling across the two irrigation regimes. For each analysis, three biological replicates were evaluated for each genotype. One plant was selected from each replicate, and one fully developed leaf was collected from the selected plant, resulting in three plants and three leaves per genotype for each measurement. Relative water content (RWC) was calculated according to the method described by Barrs and Weatherley (1962) using the following equation:

$$\text{RWC}\ (\%) = [\frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})]} \times\ 100]$$

Where FW is fresh weight (g), TW is turgid weight (g) after rehydration, and DW is dry weight (g) after oven drying. For RWC determination, leaves were collected during the flowering and boll-development stages under both optimal and drought conditions.

For RWC determination, three biological replicates were evaluated for each genotype. One leaf was collected from one selected plant in each replicate, resulting in three leaves per genotype. The samples were collected during the flowering and boll-development stages under both optimal and drought conditions.

2.4. Excised Leaf Water Loss (ELWL)

Excised leaf water loss was determined using fully expanded leaves collected from the upper canopy of cotton plants. For each genotype, three biological replicates were evaluated, with one fully expanded leaf collected from one selected plant in each replicate, resulting in three leaves per genotype for ELWL analysis. Immediately after excision, the fresh weight of each leaf was recorded. The leaves were maintained at room temperature under open-air conditions, and their weights were recorded after 1, 4, and 6 h to quantify water loss at each time point. After the final 6-h measurement, the leaves were oven-dried at 70°C for 72 h to determine dry weight. ELWL at each time point was calculated according to Matin et al. (1989) as:

$$\text{ELWL}\ (\%)\ =[\frac{(\text{FW} - \text{Wt})}{(\text{FW} - \text{DW})} \times\ 100]$$

Where FW is the initial fresh weight (g), Wt is the leaf weight (g) after t hours of desiccation (t = 1, 4, or 6 h), and DW is the oven-dry weight (g).

2.5. Leaf Chlorophyll Content (SPAD)

Leaf chlorophyll content was assessed using SPAD readings. For each genotype, three biological replicates were evaluated, and one fully expanded leaf from one selected plant in each replicate was used for a SPAD-502Plus chlorophyll meter (Konica Minolta, Inc., Tokyo, Japan), resulting in three leaves per genotype. Measurements were standardized to the third to fourth fully expanded leaf position, with readings taken from the middle portion of each leaf while avoiding the major vein. Several readings were obtained from each plant, and the average value was used for subsequent statistical analyses.SPAD values were expressed as SPAD units. Measurements were performed during the flowering and boll-development stages under both irrigation regimes. Higher SPAD values indicate greater chlorophyll content and reflect better maintenance of photosynthetic capacity under the experimental conditions.

2.6. Boll Weight (BW)

Boll weight (BW) was determined using fully mature, open bolls. Measurements were performed during the harvest period in the third decade of September 2025. For each genotype and replication, mature open bolls were randomly selected, and the weight of each sampled boll was recorded in grams using an analytical balance (Shimadzu AUW220D, Japan). The mean weight of the sampled bolls was calculated for each replication and expressed as grams per boll (g boll^{-1}). The mean boll weight was then calculated and used as an important agronomic trait for evaluating the yield potential of the genotypes. Boll weight was calculated using the following formula:

$$\text{BW} = \backslash\text{frac}\{\backslash\text{sum_}\{\text{i=1}\}\text{\^{}}\{\text{n}\}\ \text{W_i}\}\{\text{n}\}$$

Where (W_i) is the weight of the (i)-th sampled boll (g), and (n) is the number of sampled bolls.

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2.7. Productivity Per Plant

The productivity of a single plant was determined based on the total mass of seed cotton harvested from each plant. At final harvest in the third decade of September 2025, all open bolls from each selected plant were harvested and weighed individually using an Ohaus Pioneer PX4202 analytical balance. Productivity was expressed as g plant^{-1} calculated as:

$$P = \sum_{i=1}^{n} W_i$$

Where (W_i) is the seed cotton weight obtained from the (i)-th harvest of an individual plant (g), and (n) is the total number of harvests. One plant from each replication was evaluated, resulting in three biological observations per genotype under each irrigation regime.

2.8. Seed Cotton Yield

At physiological maturity, seed cotton yield (SCY) was determined from the total seed cotton harvested from each 10-m row plot (0.76 m × 10 m = 7.6 m²) across three replications and expressed as t ha^{-1}. SCY was calculated using the following formula: SCY (t ha^{-1}) = [plot yield (t) / plot area (m²)] × 10,000. Yield indicators were determined using the same method for all experimental variants and were used to assess the differences between optimal and drought conditions, as well as to determine the genotypes' degree of adaptation to drought.

2.9. Soil Conditions

The experiment was conducted on an old-irrigated typical sierozem with silt-loam texture at the Regional Experimental Base of the Center of Genomics and Bioinformatics, Academy of Sciences of the Republic of Uzbekistan. The soil was characterized by a pH of 6.4 and an organic matter content of 2%. Available N, P, and K concentrations were determined using standard soil analysis procedures. The main physicochemical characteristics of the experimental soil were recorded before planting to describe the initial soil conditions of the experimental site.

2.10. Statistical Analyses

All statistical analyses were performed using the R statistical programming environment (R Core Team, 2025). Analyses were conducted using R version 4.6.1, with the stats package for statistical calculations and additional packages including corrplot, FactoMineR, factoextra, dplyr, and ggplot2 for correlation visualization, multivariate analyses, data processing, and graphical presentation. Descriptive statistics of phenotypic data for each trait were evaluated by calculating the number of observations (N), mean (Mean), median (Median), standard deviation (SD), minimum (Min), and maximum (Max) values, as well as the coefficient of variation (CV, %). The coefficient of variation was determined using the following formula:CV (%) = (SD/Mean) × 100. Descriptive statistical indicators were calculated separately for optimal irrigation and drought conditions and were used to characterize the degree of phenotypic variability between genotypes. Pearson product-moment correlation analysis was performed to determine the linear relationship between phenotypic traits. Correlation analyses were conducted separately for the optimal irrigation and drought conditions using genotype-level trait means as observational units. Thus, each genotype contributed one observation per trait rather than individual plant- or replicate-level observations. Pearson correlation was applied to continuous quantitative variables after examination of approximate linearity and extreme observations; no transformation was applied. Correlation coefficients between pairs of traits were calculated using the Pearson method with the 'cor () ' function, and their statistical significance was evaluated based on the corresponding P-values ($P<0.05$ and $P<0.01$). The Pearson correlation coefficient (r) ranges from −1 to +1, where positive values indicate a direct linear relationship between the traits, and negative values indicate an inverse linear relationship (Yadav, 2018). To comprehensively assess drought resistance, 20 cotton genotypes were analyzed based on yield data obtained under optimal irrigation (ProductivityOpt) and drought (ProductivityDry) conditions. For each genotype, drought tolerance indices were calculated, including Tolerance Index (TOL), Mean Productivity (MP), Geometric Mean Productivity (GMP), Yield Stability Index (YSI), Stress Susceptibility Index (SSI), and Stress Tolerance Index (STI), as well as the Integrated Drought Tolerance Index (DTI) as a comprehensive assessment of these indicators. Using genotype-specific seed cotton yield under optimal irrigation (Yp) and drought conditions (Ys), TOL, MP, GMP, YSI, SSI, and STI were calculated as follows: TOL = Yp − Ys; MP = (Yp + Ys)/2; GMP = $\sqrt{(Yp \times Ys)}$; YSI = Ys/Yp; SSI = [1 − (Ys/Yp)]/[1 − ((\overline{Ys})/(\overline{Yp}))]; and STI = (Yp × Ys)/((\overline{Yp}))², where (\overline{Yp}) and (\overline{Ys}) represent the mean yields under optimal and drought conditions, respectively. Higher MP, GMP, YSI, and STI values and lower TOL and SSI values were considered favourable for drought tolerance. Because these indices differed in scale and directionality, they were normalized before integration. MP, GMP, YSI, and STI were normalized using min–max scaling, (I_{norm}=(I-I_{min})/(I_{max}-I_{min})), whereas TOL and SSI were

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reverse-normalized as (I_{norm}=(I_{max}-I)/(I_{max}-I_{min})). The DTI was calculated as the equal-weighted mean of the six normalized indices: (DTI=(TOL_{norm}+MP_{norm}+GMP_{norm}+YSI_{norm}+SSI_{norm}+STI_{norm})/6). Thus, higher DTI values represented a more favourable integrated drought-tolerance response. This comprehensive approach allows for the assessment of the ability to preserve yields under drought conditions based on several indices and is widely used in breeding programs to identify promising genotypes (Farshadfar et al., 2012). Genotypes were ranked according to their DTI values and assigned to four approximately equal-sized groups: highly drought tolerant, moderately tolerant, low tolerant, and drought susceptible. For the 20 genotypes evaluated, five genotypes were assigned to each category. The observed DTI ranges were 0.575–1.012, 0.482–0.563, 0.422–0.453, and 0.130–0.419 for the highly tolerant, moderately tolerant, low-tolerant, and sensitive groups, respectively. These category boundaries were therefore distribution-based and specific to the evaluated germplasm rather than universal biological thresholds. To assess multidimensional variability between phenotypic traits and the similarity of genotypes, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed.PCA was conducted using the genotype × trait matrix, with genotypes as observations and phenotypic traits as variables. All variables were standardized using Z-scores before PCA to remove differences in measurement scale, and components with eigenvalues >1 were retained for interpretation (Jolliffe & Cadima, 2016). To determine the phenotypic similarity between the genotypes, Euclidean distance and hierarchical clustering were first performed based on the Ward minimum variance algorithm. HCA was performed using the same standardized genotype × trait matrix, with Euclidean distance as the dissimilarity measure and Ward's minimum-variance method (hclust, method = "ward.D2") for agglomerative clustering.This method allows for the unification of phenotypically similar genotypes into a single group by minimizing intra-cluster dispersion and is widely used with high efficiency in the classification of biological data (Murtagh & Legendre, 2014). Based on the dendrogram, the formed clusters were analyzed for drought resistance characteristics and compared with PCA results to assess the reliability of phenotypic diversity and grouping between genotypes (Everitt et al., 2011).

2.11. Multi-Trait Genotype–Ideotype Distance Index (MGIDI)

Drought-tolerant genotypes were identified using the Multi-Trait Genotype–Ideotype Distance Index (MGIDI). The index simultaneously evaluates multiple agronomic and physiological traits by quantifying the distance between each genotype and an ideal ideotype (Olivoto & Nardino, 2020). MGIDI analysis was conducted using genotype-level mean values obtained under drought conditions, with the 20 cotton genotypes treated as observational units and the measured traits treated as variables in a genotype × trait data matrix.

Traits included: SPAD, ELWL_1h, ELWL_4h, ELWL_6h, RWC_Dry, BW, Productivity, and Yield. For the ideotype definition, higher values were considered desirable for SPAD, RWC_Dry, BW, Productivity, and Yield, whereas lower values were considered desirable for ELWL_1h, ELWL_4h, and ELWL_6h, reflecting the desired physiological and yield-related responses to drought. Factor analysis with varimax rotation was performed, and only factors with eigenvalues greater than 1 were retained. The factor analysis was performed on the standardized trait matrix, and the retained factors were used to calculate the factorial scores of both genotypes and the ideotype.

Euclidean distance:

$$MGIDI_i = \sqrt{\sum_{j=1}^{f}(F_{ij} - F_j)^2}$$

MGIDIi - value of the Multi-Trait Genotype-Ideotype Distance Index of the i-genotype; Fij is the factorial score of the i-genotype for the j-factor (factor score); Fy is the factorial score of the ideotype based on the j-factor; f is the number of factors preserved after factor analysis.The MGIDI value was calculated as the Euclidean distance between each genotype and the predefined ideotype in the retained factor space, with equal consideration of the retained factors. Genotypes with lower MGIDI values were considered closer to the ideotype and therefore more drought-tolerant. Analyses were performed in R using the metan package (Olivoto & Lúcio, 2020).

3. RESULTS

3.1. Descriptive Statistics of Phenotypic Traits

Descriptive statistics for the physiological and agronomic traits evaluated under drought (Dry) and optimum (Opt) conditions are presented in Table 1. Across both environments, all traits were assessed in 20 genotypes (N = 20), revealing substantial phenotypic variation among the studied materials. Under the Dry environment, SPAD exhibited a mean value of 48.21 ± 2.83 with the lowest coefficient of variation (CV = 5.87%), indicating high trait stability. Relative water content (RWC) averaged 61.51 ± 4.78% (CV = 7.78%), whereas boll weight (BW) showed a mean of 4.51 ± 0.39 (CV = 8.55%).

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Table 1: Descriptive statistics of the measured physiological and agronomic traits under drought (Dry) and optimum (Opt) environmental conditions. N = number of genotypes; SD = standard deviation; CV = coefficient of variation

Trait	Environment	N	Mean	SD	Min	Max	CV (%)
SPAD	Dry	20	48.21	2.83	43.53	52.90	5.87
SPAD	Opt	20	37.57	2.39	32.75	41.00	6.37
ELWL (1 h)	Dry	20	12.11	5.43	4.51	23.06	44.84
ELWL (1 h)	Opt	20	17.64	4.75	9.09	26.62	26.90
ELWL (4 h)	Dry	20	21.27	5.06	13.64	32.48	23.81
ELWL (4 h)	Opt	20	30.45	5.00	20.78	37.99	16.42
ELWL (6 h)	Dry	20	28.00	5.08	18.62	38.25	18.13
ELWL (6 h)	Opt	20	36.33	4.39	25.12	42.18	12.09
RWC	Dry	20	61.51	4.78	54.42	74.19	7.78
RWC	Opt	20	70.72	4.10	62.77	78.20	5.80
BW	Dry	20	4.51	0.39	3.84	5.28	8.55
BW	Opt	20	5.30	0.57	4.28	6.37	10.71
Productivity	Dry	20	30.03	6.21	15.03	42.07	20.67
Productivity	Opt	20	63.63	10.14	47.45	80.40	15.94
Seed cotton Yield	Dry	20	1.99	0.36	1.35	2.77	17.88
Seed cotton Yield	Opt	20	3.81	0.74	2.02	4.88	19.38

Excised leaf water loss (ELWL) increased progressively with incubation time, rising from 12.11 ± 5.43 after 1 h to 21.27 ± 5.06 after 4 h and 28.00 ± 5.08 after 6 h. The highest phenotypic variability was observed for ELWL after 1 h (CV = 44.84%), while ELWL after 4 h and 6 h displayed moderate variability with CV values of 23.81% and 18.13%, respectively. Agronomic traits showed moderate variation, with productivity averaging 30.03 ± 6.21 (CV = 20.67%) and seed cotton yield averaging 1.99 ± 0.36 (CV = 17.88%). Under the Opt environment, SPAD decreased to a mean of 37.57 ± 2.39 (CV = 6.37%), while RWC increased to 70.72 ± 4.10% (CV = 5.80%). Boll weight also increased, reaching 5.30 ± 0.57 (CV = 10.71%). Similar to the Dry environment, ELWL increased with incubation time, averaging 17.64 ± 4.75 after 1 h, 30.45 ± 5.00 after 4 h, and 36.33 ± 4.39 after 6 h. The corresponding coefficients of variation were 26.90%, 16.42%, and 12.09%, respectively. Productivity and seed cotton yield were substantially higher under optimum conditions, with mean values of 63.63 ± 10.14 (CV = 15.94%) and 3.81 ± 0.74 (CV = 19.38%), respectively. Comparison of the two environments revealed clear differences in both physiological and agronomic performance. Mean RWC increased by approximately 15% under optimum conditions (70.72% versus 61.51%), accompanied by increases in boll weight (5.30 versus 4.51), productivity (63.63 versus 30.03), and seed cotton yield (3.81 versus 1.99). In contrast, mean SPAD values were higher under drought conditions (48.21 versus 37.57). Across both environments, ELWL consistently increased with incubation time, while the highest relative variability was observed for ELWL measured after 1 h, particularly under drought stress (CV = 44.84%), indicating considerable phenotypic diversity among the evaluated genotypes.

3.2. Correlation Analysis Among Drought-Related Traits

Pearson correlation analysis was performed to evaluate the relationships among eight drought-related traits, including SPAD_Dry, ELWL measured at 1, 4, and 6 h (ELWL_1h, ELWL_4h, and ELWL_6h), RWC_Dry, BW_Dry, Productivity_Dry, and Seed cotton yield_Dry (Fig. 1a).The drought treatment revealed a distinct clustering of the ELWL variables, with consistently strong positive associations among measurements taken at 1, 4, and 6 h. The strongest relationships occurred between ELWL_1h and ELWL_4h ($r = 0.966$, $P<0.001$) and between ELWL_4h and ELWL_6h ($r = 0.964$, $P<0.001$), while ELWL_1h and ELWL_6h were likewise strongly correlated ($r = 0.896$, $P<0.001$; Fig. 1a). This coherence suggests that the three measurement intervals captured a common physiological component of excised-leaf water loss rather than independent responses. RWC_Dry was positively associated with the ELWL measurements, although these relationships were considerably weaker than the correlations within the ELWL group. The associations with ELWL_1h and ELWL_4h were statistically significant ($r = 0.445$ and 0.452, respectively; $P<0.05$), whereas the relationship with ELWL_6h was marginal ($r = 0.439$, $P=0.050$).

By contrast, SPAD_Dry showed no meaningful linear association with the other physiological or agronomic variables, indicating that chlorophyll-related variation was largely independent of the water-loss response under drought. BW_Dry was similarly weakly associated with the physiological traits and did not show statistically significant relationships with ELWL or RWC. Among productivity-related traits, the positive associations of BW_Dry and Productivity_Dry with Seed cotton yield_Dry were moderate in magnitude but statistically non-significant (Fig. 1a). Thus, the drought-induced correlation structure was dominated by the internal consistency of ELWL measurements, whereas relationships linking physiological indicators to yield-related traits were comparatively weak.

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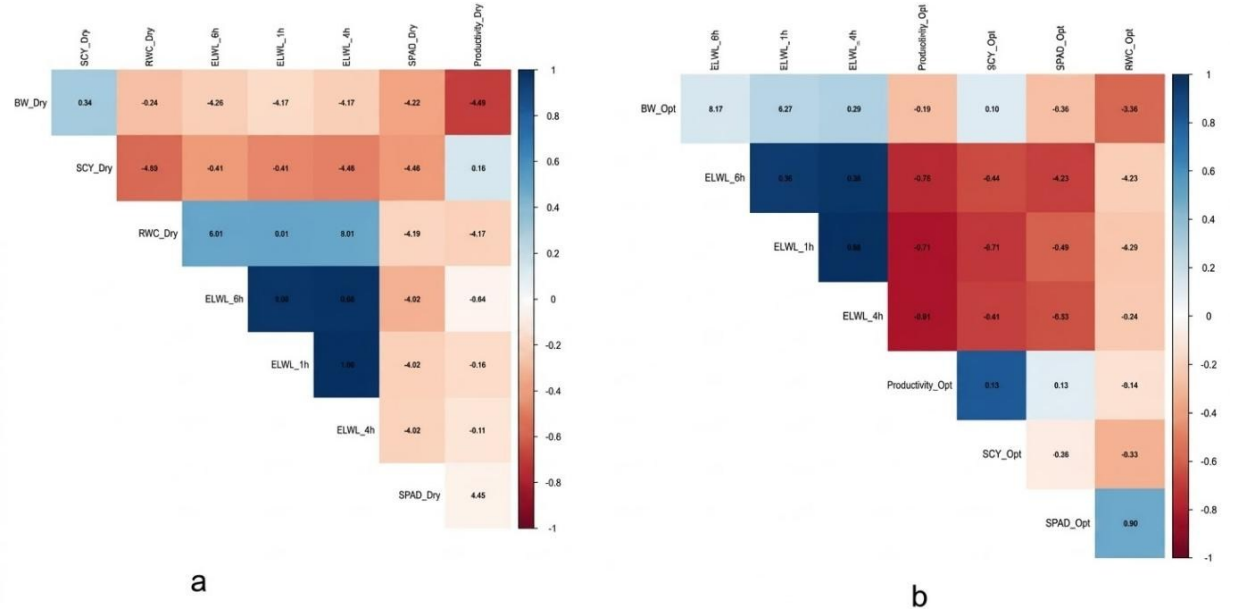


Fig. 1: Pearson correlation heatmaps of physiological and agronomic traits under drought (a) and optimal (b) growth conditions. Pairwise Pearson correlation coefficients (r) were calculated among boll weight (BW), Seed cotton yield (SCY), relative water content (RWC), excised leaf water loss measured after 1, 4, and 6 h (ELWL_1h, ELWL_4h, and ELWL_6h), SPAD chlorophyll content, and productivity. Panel **(a)** shows correlations under drought conditions (BW_Dry, Seed cotton Yield_Dry, RWC_Dry, ELWL_Dry, SPAD_Dry, and Productivity_Dry), whereas panel **(b)** presents the corresponding correlations under optimal conditions (BW_Opt, Seed cotton yield_Opt, ELWL_Opt, SPAD_Opt, and Productivity_Opt). Blue colors indicate positive correlations and red colors indicate negative correlations, with color intensity proportional to the magnitude of the correlation coefficient (r).

3.3. Pearson Correlation Analysis Under Optimal Growth Conditions

Pearson correlation analysis was performed to evaluate the relationships among eight physiological and agronomic traits measured under optimal growth conditions, including SPAD, excised leaf water loss (ELWL) at 1, 4, and 6 h, relative water content (RWC), boll weight (BW), productivity, and Seed cotton yield (Fig. 1b). Under optimal irrigation, the correlation structure was more differentiated. Under drought, the three ELWL measurements formed a strongly interconnected group, with the highest association between ELWL_1h and ELWL_4h (r = 0.909), followed by ELWL_4h and ELWL_6h (r = 0.872) and ELWL_1h and ELWL_6h (r = 0.757). This pattern confirms that ELWL measurements largely represented a shared physiological dimension across sampling intervals. In contrast, SPAD and RWC were only moderately related (r = 0.331), while their associations with the remaining traits were generally weak (Fig. 1b). A second biologically relevant pattern involved the relationship between water-loss dynamics and productivity. Productivity was negatively associated with all three ELWL measurements, with the strongest relationship observed for ELWL_4h (r = −0.331), suggesting that greater excised-leaf water loss tended to coincide with reduced productivity under non-stress conditions. Seed cotton yield was positively associated with productivity (r = 0.619), representing the strongest relationship among the non-ELWL trait combinations, while its association with BW was moderate (r = 0.318). The remaining relationships, including those involving SPAD, RWC, and yield-related traits, were comparatively weak (Fig. 1b). Overall, the correlation analysis identified two principal features of the trait network. First, ELWL measurements were highly redundant across measurement times, particularly under drought, indicating that they captured a common component of leaf water-loss behavior. Second, productivity was more closely aligned with seed cotton yield under optimal conditions than with the physiological traits, whereas the physiological indicators showed limited direct associations with yield components. These patterns indicate that drought-related physiological variation and agronomic performance were only partially coupled, highlighting the value of considering both physiological and productivity-related traits when characterizing genotype responses to contrasting water regimes.

3.4. Drought Tolerance Index (DTI)-Based Evaluation of Genotypes

The drought tolerance of the twenty evaluated genotypes was assessed using yield under optimal (Productivity_opt) and drought (Productivity_Dry) conditions together with several widely used drought tolerance indices, including tolerance index (TOL), mean productivity (MP), geometric mean productivity (GMP), yield stability index (YSI), stress susceptibility index (SSI), stress tolerance index (STI), and the integrated drought

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tolerance index (DTI). Based on the DTI values, all genotypes were ranked and classified into four drought tolerance categories: highly tolerant, moderately tolerant, low tolerant, and sensitive (Table 2).

Table 2: Drought tolerance indices and classification of 20 cotton genotypes under optimal and drought conditions

Rank	Genotype	Seed cotton yield (Optimal)	Seed cotton yield (Drought)	DTI	SSI	STI	DTI Category
1	Gulbahor_2	55.21	40.96	1.012	0.489	0.559	Highly tolerant
2	Genafond_2	77.63	42.07	0.759	0.867	0.806	Highly tolerant
3	C_312	59.75	35.60	0.707	0.765	0.525	Highly tolerant
4	An_Boyovut_2	66.67	33.99	0.577	0.928	0.560	Highly tolerant
5	Zakovat	56.96	31.35	0.575	0.851	0.441	Highly tolerant
6	L_485	64.44	33.00	0.563	0.924	0.525	Moderately tolerant
7	Zamin	47.88	27.82	0.538	0.793	0.329	Moderately tolerant
8	Bardosh	47.45	26.77	0.503	0.825	0.314	Moderately tolerant
9	L_478	60.81	30.18	0.499	0.954	0.453	Moderately tolerant
10	Shodlik_91	66.68	31.06	0.482	1.011	0.512	Moderately tolerant
11	S_1306	80.40	33.07	0.453	1.115	0.657	Low tolerant
12	Polvon	78.94	32.25	0.439	1.120	0.629	Low tolerant
13	L_480	66.81	29.66	0.439	1.053	0.489	Low tolerant
14	Tafakkur	59.30	27.73	0.432	1.008	0.406	Low tolerant
15	Beshqahramon	50.31	25.25	0.422	0.943	0.314	Low tolerant
16	Ishonch	80.37	31.79	0.419	1.144	0.631	Sensitive
17	Navbahor_2	68.12	27.93	0.381	1.117	0.470	Sensitive
18	Iqbol	67.71	24.06	0.285	1.220	0.402	Sensitive
19	Mehnat	59.38	20.94	0.246	1.226	0.307	Sensitive
20	Baraka_MAS	57.83	15.03	0.130	1.401	0.215	Sensitive

The integrated DTI analysis identified Gulbahor_2 as the highest-ranked genotype (Rank = 1), with the highest DTI value (1.012). This genotype maintained relatively high productivity under both optimal (55.21) and drought (40.96) conditions, resulting in one of the highest YSI values (0.742), a comparatively low SSI (0.489), and a relatively high STI (0.559) among the evaluated materials. These results indicate superior yield stability under water-limited conditions. The second-ranked genotype, Genafond_2, also demonstrated excellent drought performance with a DTI value of 0.759. Although this genotype exhibited a relatively high TOL value (35.56), it maintained the highest MP (59.85) and GMP (57.14) among all genotypes, reflecting consistently high productivity across contrasting environments. C_312, An_Boyovut_2, and Zakovat completed the top five DTI rankings, with DTI values ranging from 0.575 to 0.707.

These genotypes combined moderate-to-high productivity under drought conditions with relatively favorable values for MP, GMP, and STI, supporting their classification as highly drought-tolerant. Overall, the five highest-ranked genotypes maintained comparatively greater productivity under drought stress while exhibiting relatively favorable drought-response profiles across the reported indices. Five additional genotypes (L_485, Zamin, Bardosh, L_478, and Shodlik_91) were classified as moderately drought-tolerant. Their DTI values ranged from 0.482 to 0.563. These genotypes generally displayed intermediate productivity under drought conditions and moderate MP and GMP values. Although some exhibited relatively high TOL or SSI values, their overall performance remained sufficient to place them within the moderate tolerance category. The low drought tolerance group consisted of S_1306, Polvon, L_480, Tafakkur, and Beshqahramon, with DTI values between 0.422 and 0.453. Despite several of these genotypes exhibiting high yield potential under optimal irrigation (e.g., S_1306 and Polvon), substantial yield reductions under drought resulted in higher TOL values (25.06–47.33), lower YSI (0.411–0.502), and increased SSI (0.943–1.120), indicating greater sensitivity to water deficit. The lowest drought tolerance was observed in Ishonch, Navbahor_2, Iqbol, Mehnat, and Baraka_MAS, all of which were classified as sensitive. These genotypes recorded the lowest DTI values (0.130–0.419) and consistently showed poor performance under drought stress. Baraka_MAS, which ranked last, exhibited the lowest drought productivity (15.03), the lowest GMP (29.48), the lowest STI (0.215), and the highest SSI (1.401), indicating severe yield reduction under water deficit. Likewise, Mehnat and Iqbol displayed low YSI values (0.353 and 0.355, respectively) together with high SSI values (>1.22), reflecting poor yield stability during drought. Across all evaluated genotypes, Productivity_opt ranged from 47.45 to 80.40, whereas Productivity_Dry varied more substantially, from 15.03 to 42.07, demonstrating considerable genetic variation in drought response. TOL values ranged between 14.24 and 48.58, MP between 36.43 and 59.85, GMP between 29.48 and 57.14, YSI between 0.260 and 0.742, SSI between 0.489 and 1.401, STI between 0.215 and 0.806, and DTI between 0.130 and 1.012. The broad variation observed across all drought tolerance indices indicates substantial diversity among the tested germplasm for drought adaptation and provides a quantitative basis for discriminating genotypes according to their drought response. The DTI-based ranking clearly separated the evaluated germplasm into four distinct drought tolerance classes. Genotypes classified as highly drought-tolerant consistently maintained higher productivity under both environmental conditions and exhibited favorable values for MP, GMP, STI, YSI, and DTI, together with

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comparatively lower SSI values, whereas sensitive genotypes showed pronounced yield reductions under drought and correspondingly unfavorable drought tolerance indices (Table 2).

3.5. Ranking of Genotypes Based On The MGIDI Index

Analysis of the twenty cotton genotypes under drought conditions produced MGIDI values spanning from 1.71 in Polvon up to 5.19 in Baraka_MAS (Fig. 2a). Because a smaller MGIDI value signals closer proximity to the theoretical ideotype — meaning a more balanced performance across every trait measured — genotypes at the lower end of the distribution were treated as the strongest candidates for further breeding work. Applying a 25% selection intensity placed the cutoff at MGIDI ≤ 2.96, which captured five genotypes: Polvon (1.71), C_312 (2.19), Shodlik_91 (2.54), Zakovat (2.78), and Tafakkur (2.96). The remaining fifteen accessions, ranging from S_1306 (3.02) through Baraka_MAS (5.19), fell outside this threshold and were consequently excluded from the selected group, reflecting a greater overall deviation from the ideotype.

3.6. Trait Contributions Underlying Selection

To understand what drove each selected genotype's position relative to the ideotype, the contribution of the three trait-based factors (FA1, FA2, FA3) was examined (Fig. 2c). FA1 grouped the excised leaf water loss traits — ELWL measured at 1, 4, and 6 hours — along with relative water content, effectively capturing the water-retention component of drought response. FA2 combined boll weight and yield, representing the productivity dimension, while FA3 brought together SPAD readings and overall plant productivity. The pattern that emerged was not uniform across genotypes. In Polvon and Shodlik_91, FA1 accounted for the largest share of the MGIDI value, indicating that their selection was driven primarily by superior water-status maintenance rather than yield components alone. Zakovat showed the opposite trend, with FA2 dominating its contribution profile — its advantage stemmed mainly from boll weight and yield rather than water-related traits. C_312 displayed a more even split between FA1 and FA3, while Tafakkur stood out for having the smallest and most evenly distributed contributions across all three factors, suggesting this genotype approached the ideotype through balanced performance rather than excelling disproportionately in any single trait group.

3.7. Standardized Trait Performance of Selected Genotypes

The standardized heatmap (Fig. 2b) offered a trait-by-trait view of how each selected genotype deviated from the population mean. Polvon carried strongly positive z-scores for all three ELWL time points and for SPAD, consistent with its FA1-driven selection, but showed comparatively weaker values for relative water content and yield per hectare. C_312, despite a below-average SPAD score, registered positive z-scores for productivity and yield, pointing to more efficient partitioning of assimilates toward reproductive structures rather than vegetative traits. Shodlik_91 posted the highest z-score among all genotypes for ELWL at the one-hour mark and maintained above-average values across most remaining traits. Zakovat's boll weight stood out with the deepest red shading on the heatmap, visually confirming the FA2 dominance already noted in the radar plot. Tafakkur, in contrast, showed largely neutral (near-zero) coloring throughout, reinforcing the earlier observation of a balanced, non-extreme trait profile.

3.8. Selection Differentials

The selection differential (SD) values were positive for all eight traits evaluated, confirming that the five selected genotypes moved toward the ideotype in every measured direction rather than trading off one trait for another. The largest relative gains, expressed as percentage of the original trait mean (SDperc), were recorded for the excised leaf water loss traits: 46.20% for ELWL at 1 hour, 26.16% at 4 hours, and 18.21% at 6 hours. Seed cotton yield and boll weight followed with SDperc values of 12.57% and 8.07%, respectively, while productivity showed a 5.24% gain. The smallest relative shifts occurred in relative water content (2.27%) and SPAD (1.22%), suggesting these two traits were already close to their optimal expression across the broader genotype panel, leaving limited room for further improvement through selection. Taken together, these results indicate that MGIDI successfully identified a genotype subset — Polvon, C_312, Shodlik_91, Zakovat, and Tafakkur — that combines improved water-retention capacity with maintained or enhanced yield potential, offering distinct breeding value depending on whether water-status traits or productivity components are prioritized in subsequent selection cycles.

3.9. Principal Component Analysis of Physiological and Productivity Traits Under the Optimal Environment

Principal component analysis (PCA) was performed to summarize the variation among the cotton genotypes based on eight physiological and productivity-related traits measured under the optimal irrigation environment. The scree plot indicated that the first five principal components accounted for 93.8% of the total variation, while PC1 and PC2 together captured 58.7% (38.4 and 20.3%, respectively), providing an adequate representation of the major

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phenotypic structure among genotypes (Fig. 3a). Rather than reproducing individual loading values, the PCA biplot highlighted contrasting contributions of water-loss and productivity-related traits (Fig. 3b). PC1 was mainly associated with excised leaf water loss (ELWL after 1 h, 4 h, and 6 h), which exhibited long vectors directed toward the positive side of PC1, indicating that these variables contributed strongly to genotype discrimination. Boll weight (BW_Opt) also showed a positive association with PC1. Conversely, seed cotton yield (Yield_Opt) and productivity (Productivity_Opt) were positioned on the negative side of PC1 and the positive side of PC2, suggesting an opposite trend relative to excised leaf water loss variables. SPAD values were negatively associated with both PC1 and PC2, whereas relative water content (RWC_Opt) contributed primarily along the negative direction of PC2.

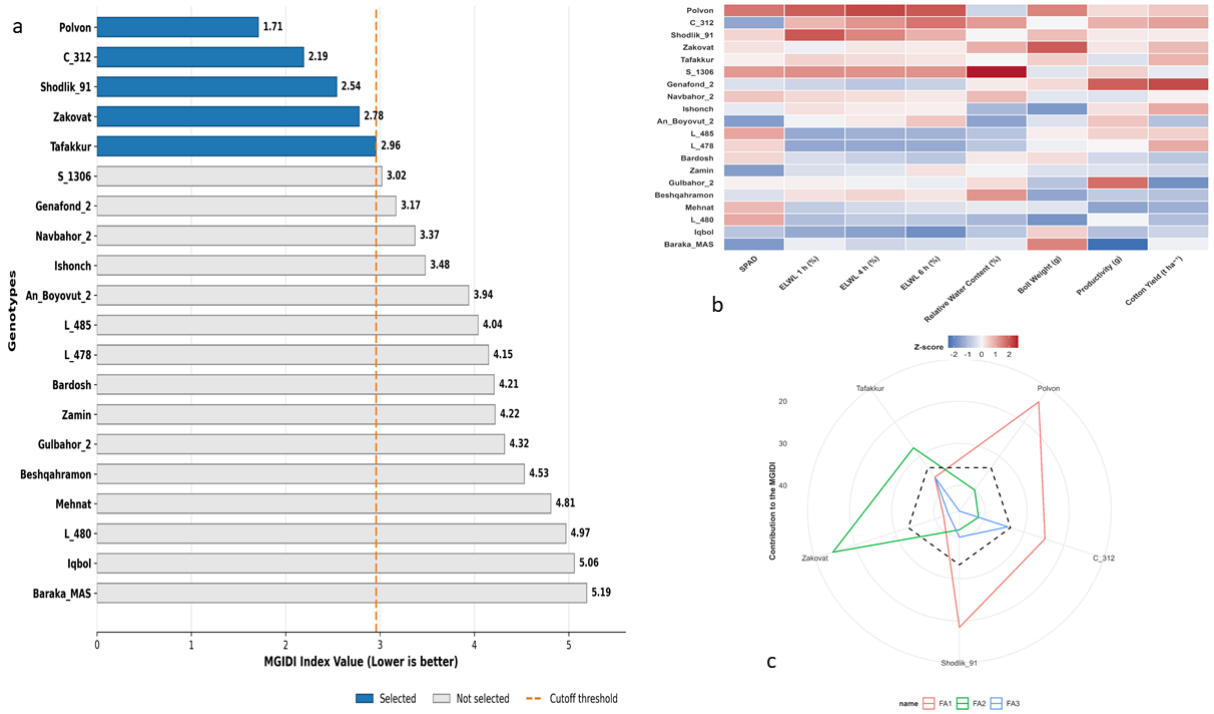


Fig. 2: Multi-Trait Genotype-Ideotype Distance Index (MGIDI) analysis of twenty cotton genotypes under drought conditions. **(a)** Genotype ranking by MGIDI value, from lowest (closest to the ideotype) to highest; the dashed line marks the selection threshold (MGIDI ≤ 2.96, 25% selection intensity), with blue bars indicating selected genotypes and grey bars indicating non-selected genotypes. **(b)** Standardized performance heatmap (z-scores) of the twenty genotypes across eight physiological and yield-related traits, with red indicating above-average and blue indicating below-average values. **(c)** Radar plot of the relative contribution of each trait-based factor (FA1: excised leaf water loss traits and relative water content; FA2: boll weight and yield; FA3: SPAD and productivity) to the MGIDI index of the five selected genotypes, where a smaller sector size reflects closer proximity to the ideotype; the dashed black polygon represents the ideotype reference.

The correlation circle further supported the major multivariate relationships among traits (Fig. 3c). The three excised leaf water loss measurements (ELWL_1h, ELWL_4h, and ELWL_6h) clustered closely together with nearly identical vector orientations, indicating a strong positive correlation among these variables. Similarly, Seed cotton yield_Opt and Productivity_Opt were closely aligned, reflecting their strong positive association. Their opposing orientation to the ELWL vectors suggests a broader contrast between water-loss behaviour and productivity-related variation among genotypes. In comparison, RWC_Opt was represented by a shorter vector, indicating a relatively limited contribution to the first two components. Complete trait loadings and correlations are presented in Fig. 3c.

3.10. Hierarchical Clustering of Cotton Genotypes

The optimal number of genotype clusters was determined using the silhouette method. The highest average silhouette coefficient was obtained at k = 5 (Fig. 3d). Accordingly, five clusters were retained for the final hierarchical-clustering interpretation, and the dendrogram classified the twenty cotton genotypes into five distinct clusters (Fig. 3f).Cluster 1 comprised Iqbol, Genafond_2, L_478, L_480, and L_485 (n = 5). This group represented a distinct genotype cluster characterized by relatively similar multivariate phenotypic profiles under the optimal environment. Cluster 2 included Bardosh, Gulbahor_2, Beshqahramon, and Zamin (n = 4), forming a separate group based on their overall similarity across the evaluated physiological and productivity-related traits. Cluster 3 consisted

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of Baraka_MAS, Polvon, and Shodlik_91 (n = 3), which were grouped together according to their multivariate phenotypic similarity. Cluster 4 contained C_312, Ishonch, and S_1306 (n = 3), representing another distinct group in the hierarchical dendrogram. Cluster 5 comprised Tafakkur, Zakovat, Navbahor_2, Mehnat, and An_Boyovut_2 (n = 5), forming the largest cluster together with Cluster 1. The five-cluster solution provided a more detailed representation of the phenotypic relationships among the genotypes than the previously considered four-cluster structure. The resulting groups reflected differences in the combined patterns of physiological and productivity-related traits rather than separation based on a single trait. Collectively, these results indicate that the evaluated genotypes could be differentiated into five phenotypically distinct groups under the optimal irrigation environment.

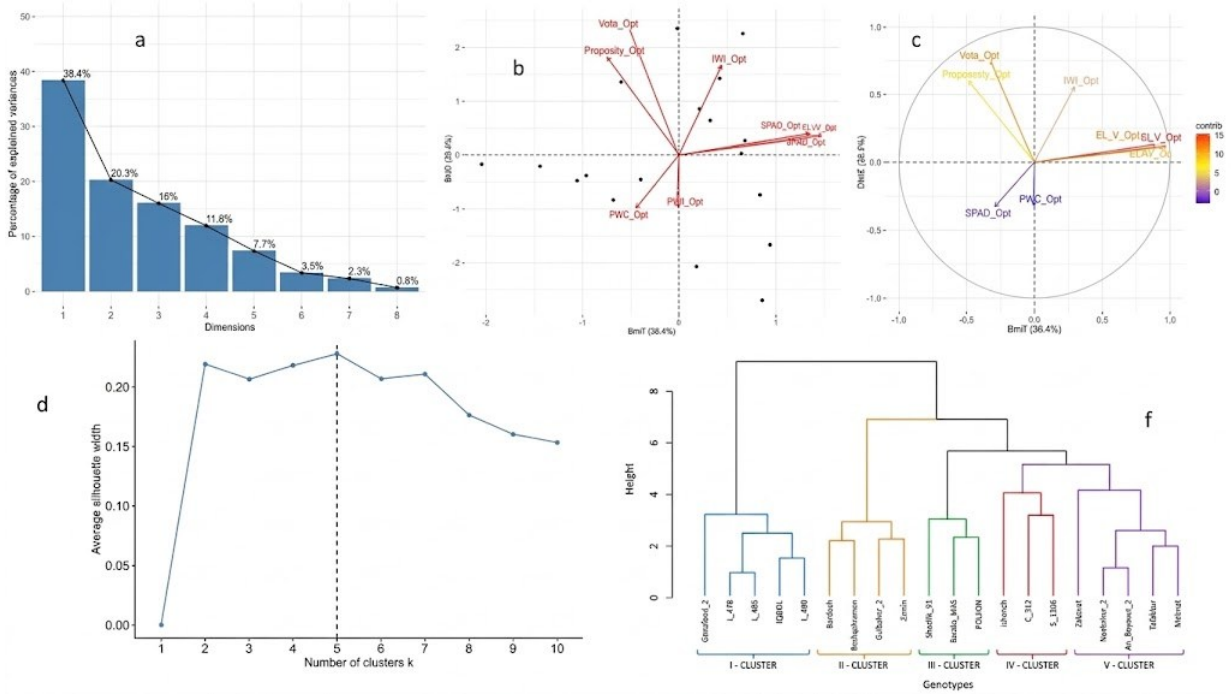


Fig. 3: Principal component analysis (PCA) and hierarchical clustering of cotton genotypes under the optimal (well-watered) environment. **(a)** Scree plot showing the percentage of variance explained by the principal components. **(b)** PCA biplot of 20 cotton genotypes and eight physiological and productivity-related traits. **(c)** Correlation circle showing trait contributions to the first two principal components. **(d)** Determination of the optimal number of clusters using the silhouette method. **(f)** Hierarchical clustering dendrogram of cotton genotypes generated using Ward's minimum variance (Ward.D2) method.

3.11. Cluster Characteristics Under The Optimal Environment

Distinct phenotypic profiles were evident among the five clusters. Cluster I was distinguished primarily by its productivity-related performance, whereas Cluster IV combined lower leaf water loss with comparatively high chlorophyll status and maintained productivity. Cluster II was characterized by stronger water-status and chlorophyll-related attributes, while Cluster III represented the lower-productivity group. Cluster V comprised Tafakkur, Zakovat, Navbahor_2, Mehnat, and An_Boyovut_2 and represented an additional phenotypic group identified by the five-cluster solution. Collectively, these patterns indicate that the five genotype groups differed not simply in yield potential but in the relative coordination of water relations, physiological status, and productivity under optimal irrigation.

3.12. Principal Component Analysis of Physiological Traits and Productivity Under Drought Stress

Principal component analysis (PCA) was performed using eight physiological and productivity-related traits measured under drought stress to investigate the multivariate variation among the twenty cotton genotypes. The first two components explained 58.02% of the total variation, with PC1 accounting for 41.69% and PC2 for 16.34%, while the first five components together captured 93.79% (Fig. 4a). Thus, the major structure of drought-associated phenotypic variation was concentrated in the first two axes. The PCA biplot revealed a clear separation of genotypes according to their physiological performance under drought stress (Fig. 4b). PC1 was primarily associated with leaf water-loss characteristics, whereas PC2 reflected variation in boll weight and yield-related attributes. Genotypes positioned toward the positive PC1 region tended to combine higher ELWL, RWC, productivity, boll weight, and

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seed cotton yield, whereas those on the opposite side generally exhibited lower values of these traits. The variable correlation circle further illustrated the relationships among the measured traits (Fig. 4c). ELWL measured after 1, 4, and 6 h formed a compact group with nearly parallel vectors, indicating a strong positive correlation among these variables. Relative water content was positively associated with ELWL variables, although its contribution was slightly lower. Likewise, boll weight and seed cotton yield exhibited similar vector orientations, suggesting a close positive relationship under drought stress. Productivity was positively associated with both yield and boll weight, whereas SPAD showed a comparatively short vector, indicating a relatively small contribution to the total variation explained by the first two principal components. Overall, the biplot and correlation circle indicate that drought-related differentiation was structured mainly around leaf water-loss behaviour and productivity, whereas SPAD contributed less strongly to genotype separation. Complete loading patterns are shown in Fig. 4b–c.

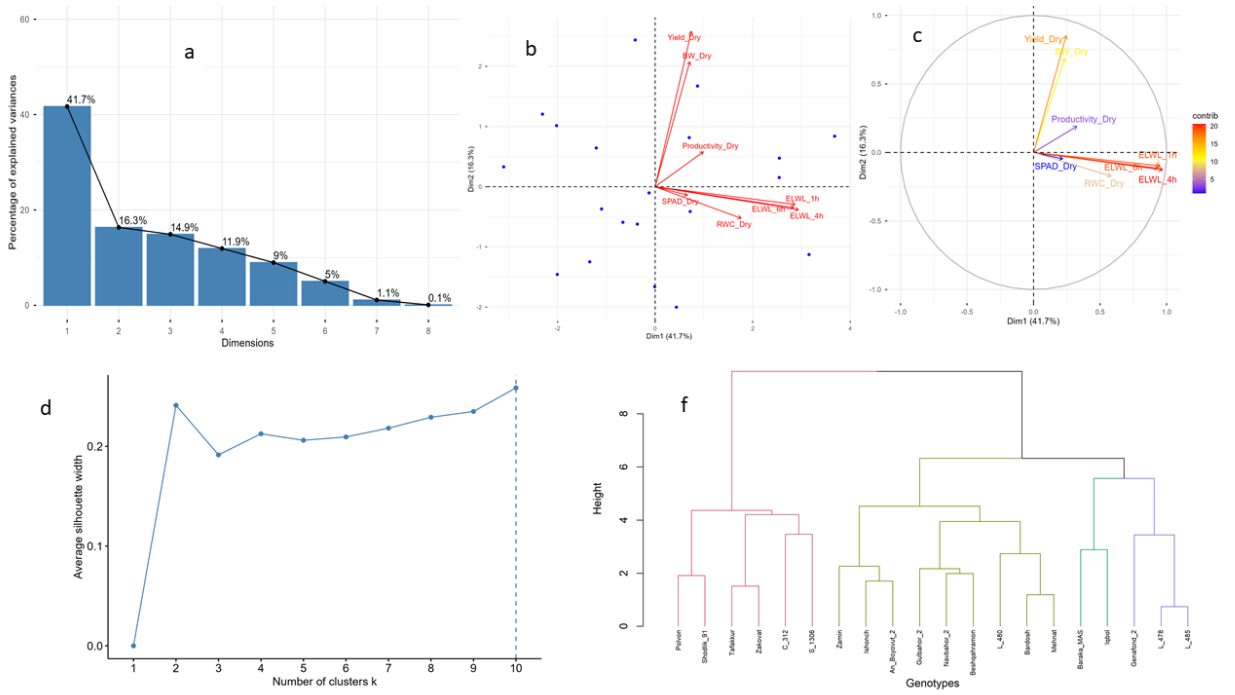


Fig. 4: (a) Scree plot showing the percentage of variance explained by each principal component for eight physiological and productivity-related traits under drought stress. (b) PCA biplot showing the distribution of 20 cotton genotypes and the relationships among the evaluated traits. (c) Hierarchical cluster analysis (HCA) dendrogram classifying the genotypes into four clusters. (d) Silhouette plot evaluating the quality of the identified clusters. (f) Integrated PCA–HCA plot showing the distribution of genotypes according to cluster membership under drought stress.

3.13. Hierarchical Cluster Analysis of Cotton Genotypes under Drought Stress

To further investigate the similarity among cotton genotypes under drought stress, hierarchical cluster analysis (HCA) was performed using Ward's minimum variance method based on Euclidean distances. Before dendrogram construction, the optimal number of clusters was estimated using the average silhouette width criterion (Fig. 4d). Although the average silhouette coefficient continued to increase toward higher values of k and did not show a distinct optimum at k = 4, four clusters were retained for the final interpretation because the Ward.D2 dendrogram provided a clear and phenotypically coherent partition of the genotypes at this level. The four-cluster solution also allowed biologically meaningful differences among genotype groups to be resolved without excessive fragmentation of the 20 genotypes into small clusters (Fig. 4d). The dendrogram was therefore cut at four groups, classifying the twenty cotton genotypes into four distinct clusters (Fig. 4f). Cluster I contained Baraka_MAS and Iqbol, representing genotypes with comparatively low productivity and seed cotton yield under drought conditions. Cluster II comprised Tafakkur, Zakovat, Polvon, C_312, Shodlik_91, and S_1306, which generally exhibited superior productivity and boll weight performance. Cluster III represented the largest group, including Bardosh, Gulbahor_2, Navbahor_2, Beshqahramon, Zamin, Mehnat, Ishonch, An_Boyovut_2, and L_480, indicating intermediate physiological responses under drought stress. Cluster IV consisted of Genafond_2, L_478, and L_485, which formed a distinct group characterized by comparatively high productivity and seed cotton yield despite differences in their physiological responses. Comparison of cluster centroids revealed a clear productivity gradient among the groups.

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Cluster IV showed the highest average productivity and seed cotton yield, whereas Cluster I represented the lowest-performing group. Cluster II was distinguished by relatively high boll weight and water status, while Cluster III occupied an intermediate position for most productivity-related traits. Importantly, the clustering pattern broadly corresponded with the PCA structure, reinforcing the interpretation that drought-associated genotype differentiation was shaped predominantly by coordinated variation in leaf water relations and productivity rather than by a single physiological trait.

4. DISCUSSION

This study was devoted to a comprehensive assessment of the drought adaptation of cotton genotypes using physiological traits, yield components, tolerance indices, and multivariate statistical approaches (correlation, PCA, cluster, and MGIDI analyses). The results showed that drought conditions altered not only the quantitative expression of individual traits but also the relationships among physiological and productivity-related characteristics, emphasizing the need to evaluate drought response as a combination of interacting traits rather than through a single indicator.

4.1. The Physiological Stress Response

The phenotypic variation observed among genotypes highlights substantial diversity in drought-related physiological responses and provides a useful basis for identifying drought-adapted material (Ullah et al., 2017; Zafar et al., 2023). One notable finding was the higher SPAD values under drought than under optimal irrigation. Although seemingly unexpected, this pattern may result from alterations in leaf structure or chlorophyll concentration per unit leaf area rather than increased photosynthetic capacity (Puangbut et al., 2017; Zafar et al., 2026). Moreover, the relatively limited contribution of SPAD to the principal components under both environments indicates that chlorophyll status was less discriminative than traits associated with leaf water balance (Wang et al., 2025). Accordingly, SPAD may be more informative when considered alongside other physiological traits than when used as a standalone measure of drought adaptation. RWC and ELWL showed clearer associations with the plants' water-status response. The decline in RWC under water limitation indicates reduced tissue hydration and supports its established value as an indicator of plant water balance (Zahid et al., 2021). ELWL increased with measurement duration in both environments, yet remained lower under drought. Since this assay is performed after leaf excision, such differences reflect post-excision water retention rather than whole-plant transpiration. Substantial variation among genotypes further points to differences in their ability to retain water following excision. The association between RWC and ELWL also depended on irrigation regime (Zafar et al., 2025).Their moderate positive relationship under drought, contrasted with a weaker association under optimal conditions, suggests that water-status characteristics may become more closely connected during water limitation. Nevertheless, this relationship should not be regarded as evidence of direct co-regulation, because the two measurements represent distinct physiological processes. Genotype, stress severity, developmental stage, and experimental conditions may all modify their relationship, as reported in cotton (Shani et al., 2025b; Abtahi et al., 2026).

4.2. Components of Productivity and their Relationship with Physiological Indicators

Water limitation reduced boll weight, productivity, and seed cotton yield, indicating that drought affected both vegetative performance and reproductive development. Restricted assimilate production and transport may limit biomass accumulation, while impaired reproductive retention and resource allocation further constrain final yield formation (Shani et al., 2025a). The weaker productivity–yield association under stress is particularly informative, as it implies that reproductive output becomes less dependent on productivity potential alone. Instead, carbon partitioning, osmotic adjustment, reproductive maintenance, and tissue hydration may collectively determine the amount of biomass ultimately converted into harvestable yield (Ullah et al., 2017; Zafar et al., 2023). An important implication is that ELWL should not be regarded as a direct surrogate for yield performance. Rather, post-excision water retention represents one component of a broader physiological strategy that may contribute to maintaining plant function when water availability declines. The relative importance of individual traits also appeared to shift between environments. Under adequate irrigation, differences in yield-related characteristics were more apparent, whereas drought placed greater emphasis on mechanisms associated with water conservation and maintenance of leaf hydration. This environmental dependence suggests that selection based exclusively on performance under favorable moisture conditions could overlook genotypes capable of sustaining production during water deficit.The contrasting responses of Genafond_2, L_478, and L_485 provide further evidence that comparable productivity under stress may be achieved through different combinations of physiological and agronomic attributes. Such variation supports a complementary selection framework in which yield is considered together with indicators of physiological stability. Integrating these trait classes may therefore provide a more informative basis for identifying

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cotton genotypes with reliable performance across contrasting moisture regimes (Baha et al., 2025; Greveniotis et al., 2026).

4.3. Distinguishing Genotypes by Tolerance Indices

The integrated DTI-based assessment provided a broader discrimination of drought-response profiles than individual traits because it incorporated performance across both favorable and water-limited environments. This supports the use of integrated indices such as DTI, MTSI, MGIDI, and STI for identifying genotypes that combine productivity with drought stability (Zuffo et al., 2020; Pour-Aboughadareh & Poczai, 2021). Cotton-specific studies likewise indicate that MP, GMP, STI, and related indices can effectively differentiate genotypes across contrasting water regimes (Nandhini et al., 2022; Zafar et al., 2024). The contrasting profiles of the identified genotypes highlight an important breeding principle: drought adaptation cannot be inferred from stress-environment yield alone. Genotypes such as Gulbahor_2 and Genafond_2 combined comparatively favorable productivity with greater stability across water regimes, suggesting that their performance was less dependent on favorable moisture conditions. In contrast, some genotypes with high yield potential under optimal irrigation experienced substantial performance reductions under drought. Thus, high yield potential and drought stability represent related but distinct breeding attributes, and their joint consideration can improve the identification of broadly adapted material (Ayed et al., 2021; Sabouri et al., 2022). The wide variation in DTI values further indicates substantial heterogeneity in drought-response strategies within the studied collection. The genotypes identified through the integrated indices, including Gulbahor_2, Genafond_2, C_312, An_Boyovut_2, and Zakovat, therefore represent promising material for subsequent multi-environment validation rather than definitive evidence of superior drought adaptation. Importantly, index-based classification reflects yield stability under the imposed drought treatment and should be interpreted together with physiological traits and environmental stability rather than as direct proof of a specific underlying adaptation mechanism (Varshney et al., 2021).

4.4. MGIDI-Based Multi-Trait Selection

Unlike single-trait indices, MGIDI integrated physiological and agronomic attributes into one measure, enabling genotypes to be assessed according to their proximity to the predefined multi-trait ideotype (Olivoto & Nardino, 2021). In the present dataset, the lowest values were observed for Polvon, C_312, Shodlik_91, Zakovat, and Tafakkur, indicating comparatively favorable combinations of measured characteristics (Zuffo et al., 2020). However, their underlying factor profiles differed considerably. Water-related attributes contributed more strongly to the profiles of Polvon and Shodlik_91, whereas productivity-associated characteristics were more prominent in Zakovat. Tafakkur, in contrast, exhibited a relatively balanced contribution across trait groups. Thus, similar MGIDI values can arise from distinct combinations of phenotypic attributes, emphasizing that the index represents a selection profile rather than a direct physiological mechanism (Martignago et al., 2020; Zhang et al., 2022; Bančič et al., 2023). The selection differentials further indicated simultaneous improvement across several measured characteristics, with notable responses among water-exchange traits. This finding supports the use of integrated selection strategies in which physiological attributes complement productivity rather than being considered separately (Mohammadi & Geravandi., 2024; Shani et al., 2025c). C_312 provides an additional example: its favorable multi-trait position despite relatively lower SPAD values demonstrates that chlorophyll status alone is insufficient to characterize drought performance. Taken together, the results point to a multidimensional basis of drought adaptation involving leaf hydration, post-excision water retention, chlorophyll status, and productivity. The relative contribution of these trait groups also varied with moisture availability. Combining MGIDI with DTI may therefore provide complementary information for identifying promising breeding material. Nevertheless, the selected genotypes should not be considered universally drought-tolerant; their breeding value requires confirmation across environments, seasons, and genetic backgrounds.

4.5. Several Limitations Should also be Considered when Interpreting these Findings

A major limitation of this study is that the evaluation involved only 20 genotypes and was conducted at a single field location during one growing season under the specific environmental and management conditions of the experiment. Therefore, the observed physiological and yield relationships may not fully represent genotype performance across contrasting locations, seasons, or drought intensities. Multi-environment and multi-year trials, together with additional physiological, biochemical, and molecular measurements, will be necessary to determine the stability of the identified trait combinations and to validate the breeding value of the highlighted genotypes.

5. CONCLUSION

This study demonstrated substantial phenotypic variation among cotton genotypes in physiological and

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agronomic responses to drought. DTI identified Gulbahor_2, Genafond_2, C_312, An_Boyovut_2, and Zakovat, whereas MGIDI identified Polvon, C_312, Shodlik_91, Zakovat, and Tafakkur as genotypes with favorable multi-trait profiles. The overlap of C_312 and Zakovat across both approaches provides particularly consistent evidence of favorable drought-response and productivity profiles. These genotypes may serve as candidate materials for breeding aimed at maintaining productivity under water-limited conditions. Multi-location and multi-season testing is required to validate the stability of these responses and their breeding value.

Declarations

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Conflicts of Interest: The authors declare that they have no conflicts of interest related to this study.

Data Availability: All the data generated and analyzed during this study are presented in the article.

Ethics Statement: This study involved only plant material (cotton genotypes) and did not involve human participants, human subjects, or animals. Therefore, ethical approval was not required.

Author's Contribution: A.X. Makamov conceived the study and provided overall scientific supervision. A.A. Yuldoshkhonov conducted the experiments, analyzed the data, and prepared the first draft of the manuscript. J.K. Norbekov, I.S. Normamatov, U.A. Boyqobilov, S.I. Mamanazarov, R.I. Mukhammadaliev, M. Kholmuradova, and G.X. Lutpillaev helped organize the field experiments, collect the data, and analyze the results. N.N. Khusenov and Z.T. Buriev contributed to interpreting and discussing the findings, critically revised the manuscript, and approved the final version. All authors read and approved the final manuscript.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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