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Original Research Article

IMPACT OF WATER STRESS ON COTTON PHYSIOLOGY, ROS ACCUMULATION AND ANTIOXIDANT ACTIVITY IN UPLAND COTTON GENOTYPES

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Abstract *The current study was designed to compare the performance of ten cotton strains based on their key morphological, physiological, biochemical, antioxidant, and fiber quality-related traits under water stress conditions. The experiment was laid out under RCBD with three replicates and a 75 cm row-to-row distance was maintained. The data was recorded for CLCuV percentage, whitefly attack percentage, sympodia per plant, boll weight, ginning out turn, net photosynthetic rate, transpiration rate, stomatal conductance, cotton yield, total oxidative stress, sugars, ascorbic acid, total antioxidant capacity, glycine betaine, peroxidases, fiber length, fiber fineness, fiber strength, and fiber uniformity. The results revealed the occurrence of significant variations for agronomically important and fiber quality-related traits in cotton strains under water stress conditions except for boll weight. The correlation coefficient analysis revealed a strong positive correlation of cotton yield with transpiration rate, sugars, ginning out turn, stomatal conductance, peroxidases, net photosynthetic rate, and glycine betaine while a strong negative correlation with total oxidative stress, total oxidative stress, fiber fineness and fiber strength under water stress conditions. The PC-1/PC-2 based biplot analysis reconfirms the results obtained through the correlation analysis and further unveiled that most of the variations in the data were due to CLCuV percentage, fiber fineness, net photosynthetic, transpiration rate, ginning out turn, seed yield and total oxidative stress. The cluster analysis categorizes ten cotton strains into four clusters and found that cotton strains CRS-2202, CRS-2201, and CRS-2204 were the most productive.*

Keywords: *Drought stress; Antioxidants; ROS; Photosynthesis; Fiber Quality; Abiotic Stresses*

Introduction

Abiotic stresses, particularly drought stress, significantly impact crop production and sustainability, posing major challenges to global food security. Drought stress reduces crop yields by impairing plant physiological and biochemical processes, such as photosynthesis, nutrient uptake, and water use efficiency (Farooq et al., 2009). Crops under drought conditions exhibit stunted growth, reduced biomass, and lower reproductive success, which culminates in diminished harvests and economic losses for farmers (Raza et al., 2019). The severity of drought stress is exacerbated by climate change, which increases the frequency and intensity of drought events, thereby threatening the stability of agricultural systems worldwide (Lesk et al., 2016). Sustainable crop production under drought stress necessitates the development

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and adoption of drought-resistant crop varieties, improved irrigation practices, and soil management techniques that enhance water retention and reduce evapotranspiration (Kang et al., 2009). These strategies are essential for mitigating the adverse effects of drought stress, ensuring food security, and promoting agricultural sustainability in the face of increasingly erratic climate patterns.

Cotton is of paramount importance to Pakistan, serving as a backbone of the national economy and a critical component of its agricultural sector. It is often dubbed "white gold" due to its substantial contribution to Pakistan's GDP and its pivotal role in the livelihoods of millions of farmers and workers in the textile industry (Government of Pakistan, 2021). The cotton and textile sector is the largest industrial segment in Pakistan, accounting for approximately 60% of the country's exports, thus playing a crucial role in foreign exchange earnings (Pakistan Bureau of Statistics, 2020). Cotton is not only vital for textile manufacturing, which includes the production of yarn, fabric, garments, and home textiles, but its by-products, such as cottonseed oil and meal, are also significant for the food and feed industries. Moreover, the development of value-added cotton products has spurred industrial growth, created employment opportunities, and fostered technological advancements in agriculture and manufacturing (Soomro et al., 2019). Therefore, cotton remains a strategic crop for Pakistan's economic stability and industrial development, necessitating continued investment in sustainable cultivation practices and innovative technologies to enhance productivity and resilience.

Sustainable cotton production in Pakistan faces several significant challenges, leading to low yields and threatening the overall viability of the sector. Key issues include pest infestations, particularly from bollworms and whiteflies, which cause extensive damage to crops and necessitate heavy pesticide use, thus harming the environment and human health (Khan et al., 2018). Water scarcity and inefficient irrigation practices also impede cotton growth, especially in arid regions, exacerbated by climate change and erratic rainfall patterns (Abid et al., 2019). Additionally, soil degradation from continuous monoculture practices and insufficient use of organic fertilizers depletes soil fertility, reducing crop productivity. Limited access to high-quality seeds and inadequate agricultural extension services further contribute to suboptimal farming practices and poor yields (Ali et al., 2020). To address these challenges, adopting integrated pest management (IPM) strategies, improving water management through advanced irrigation techniques like drip and sprinkler systems, and promoting crop rotation and organic farming can enhance soil health and productivity. Investment in agricultural research and development to produce high-yield, pest-resistant cotton varieties, along with strengthening farmer education and support services, are crucial steps towards achieving sustainable cotton production in Pakistan.

Materials and Methods

The current research study was conducted during the cotton growing season, in 2023 at the research farm of Cotton Research Station, Bahawalpur. The experimental material was comprised of ten cotton strains including CRS-2201, CRS-2202, CRS-2203, CRS-2204, CRS-2205, CRS-2206, CRS-2207, CRS-2208, CRS-2209 and CRS-2210 developed by the Cotton Research Station, Bahawalpur. These cotton strains were sown in RCBD design under three replications. The plant-to-plant distance was maintained at 45 cm while row-to-row was kept at 75 cm. These soil and irrigation water properties are given in Table 1. The sowing was done under water stress conditions i.e., at flowering and early reproductive stage, three consecutive irrigations were halted to develop water stress. All other production technology was kept the same as standard.

Several, physiological, biochemical, and fiber quality-related parameters were recorded/measured at their desired time frame. The data for key morphological, insect/disease-related traits and physiological parameters including CLCuV percentage, whitefly attack percentage, sympodia per plant, boll weight, ginning out turn, net photosynthetic rate, transpiration rate, stomatal conductance, and cotton yield. Seeds of all cotton strains were subjected to chemical analysis through spectrometry to measure the accumulation of total oxidative stress, sugars, ascorbic acid, total antioxidant capacity, glycine betaine, and peroxidases. The fiber quality-related parameters i.e. fiber length, fiber fineness, fiber strength, and fiber uniformity were measured using High Volume Instrument (HVI).

Table 1: Properties of Irrigation water and Soil used for the Experiment

Properties of Irrigation water used in the study		
Sr#	**Parameter**	**Value**
1	Total Soluble Salts (TSS)	1145 ppm
2	Sodium Adsorption Ratio (SAR	6.9
3	Residual Sodium Bicarbonate (RSB)	1.44
4	Chloride	4.2 meL
Properties of Soil on which the experiment was carried out		

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1	Soil Texture	Clay Loam
2	pH	7.8
3	EC	4.7 dSms
4	Organic Matter	0.75
5	Available P	7.4 ppm
6	Available K	84 ppm
7	Zinc	1.35 ppm

Statistical Data Analysis

The obtained data were subjected to analysis of variance (ANOVA) and correlation coefficient analysis to observe the variations present in the data and to determine the relationship between different traits, respectively (Steel et al., 1997). Moreover, multivariate analysis approaches i.e., PC-1/PC-2 biplot and cluster analysis were also applied to categorize cotton strains based on their performance under water stress conditions as used by Yousaf et al. (2023) and Hussain et al. (2024). The Statistix 8.1, OriginPro, and XLSTAT statistical packages were used to analyze the data.

Results and Discussion

The results obtained from the analyzed data showed the occurrence of significant variations among the cotton strains under study (Table 2). The results revealed that variations among cotton strains were highly significant for all the traits under study i.e., CLCuV percentage (CLCuV), whitefly attack percentage (Whitefly), Sympodia per plant (S/P), Ginning out turn (GOT), Net photosynthetic rate (Pn), Transpiration rate (Tr), Stomatal conductance (Ci), Total oxidative stress (TOS), Sugars, Ascorbic acid (ASA), Total Antioxidant Capacity (TAC), Glycine betaine (GB), Peroxidases (POD), Fiber length (FL), Fiber fineness (FF), Fiber strength (FS), Fiber uniformity (FU) and Cotton yield (CY) except Boll weight (BW) for which the variations among cotton strains were non-significant. The variations present in the data/germplasm are fundamental to the development of new plant varieties, including cotton, as they provide the genetic diversity necessary for breeding programs to create improved cultivars. Several researchers also showed that variations among cotton strains for key cotton traits will provide a strong base for variability to run a breeding program to improve an existing genotype or to develop a new cotton variety (Yousaf et al., 2023; Aslam et al., 2022; Hussain et al., 2023a; Manan et al., 2022; Munir et al., 2020; Rahman et al., 2022).

Table 2: Mean Square (MS) values of studied cotton traits under water stress conditions

Traits/Source of Variance	**Replication**	**Genotypes**	**Error**
Degree of Freedom (df)	**2**	**9**	**17**
CLCuV Percentage	0.17	15.71*	8.68
Whitefly attack percentage	4.26	4.03**	0.17
Sympodia per plant	10.27	15.82**	3.83
Boll weight	0.01	0.07NS	0.08
Ginning out Turn	3.06	20.06**	4.50
Net photosynthetic Rate	25.84	24.43**	7.71
Transpiration Rate	0.00	0.01**	0.00
Stomatal Conductance	433	8398**	2423
Total Oxidative Stress	3908236	5936200**	1158948
Sugars	1.58	15.12**	3.61
Ascorbic Acid	1163	1764**	666
Total Antioxidant Capacity	0.48	2.42**	0.19
Glycine Betain	352	321**	33
Peroxidase	16145	7190**	1283
Fiber length	0.19	0.91*	0.46
Fiber fineness	0.00	0.07**	0.00
Fiber Strength	1.46	7.00**	0.85
Fibre Uniformity	7.06	12.55**	0.65
Seed Cotton Yield	150300	694361**	121342

Correlation coefficient analysis is one of the fundamental approaches to determine the strength and direction of association between cotton yield and other yield-associated parameters under water stress conditions. This approach helps plant scientists determine the interrelationship between different plant traits which could serve as

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the basis for parental selection in a breeding program. The current study revealed the presence of both strong positive and negative correlations between different traits in cotton strains under water stress conditions (Table 3 & Figure 1). The cotton yield was found to have a positive and significant correlation with transpiration rate (r = 0.823**), sugars (r = 0.782**), ginning out turn (r = 0.770**), stomatal conductance (r = 0.743**), peroxidases (r = 0.709**), net photosynthetic rate (r = 0.701**) and glycine betaine (r = 0.652**) while the strong negative correlation with total oxidative stress (r = -0.855**), total oxidative stress (r = -0.855**), fiber fineness (r = -0.799**) and fiber strength (r = -0.619**). The highest positive correlation was found between sugar and transpiration rate (r = 0.993**) followed by sugar and stomatal conductance (r = 0.991**). Similarly, the highest negative correlation was observed between total oxidative stress and transpiration rate (r = -0.959**) followed by Sugar and total oxidative stress (r = -0.956**) (Table 3 & Figure 1). Similar results were also reported by many researchers who showed that these parameters must be included in the selection criteria of selection of parental material for any cotton breeding program (Singh et al., 2007; Xu et al., 2020; Zafar et al., 2021; Thompson et al., 2022; Zafar et al., 2022; Yousaf et al., 2023; Zafar et al., 2023, Hussain et al., 2024).

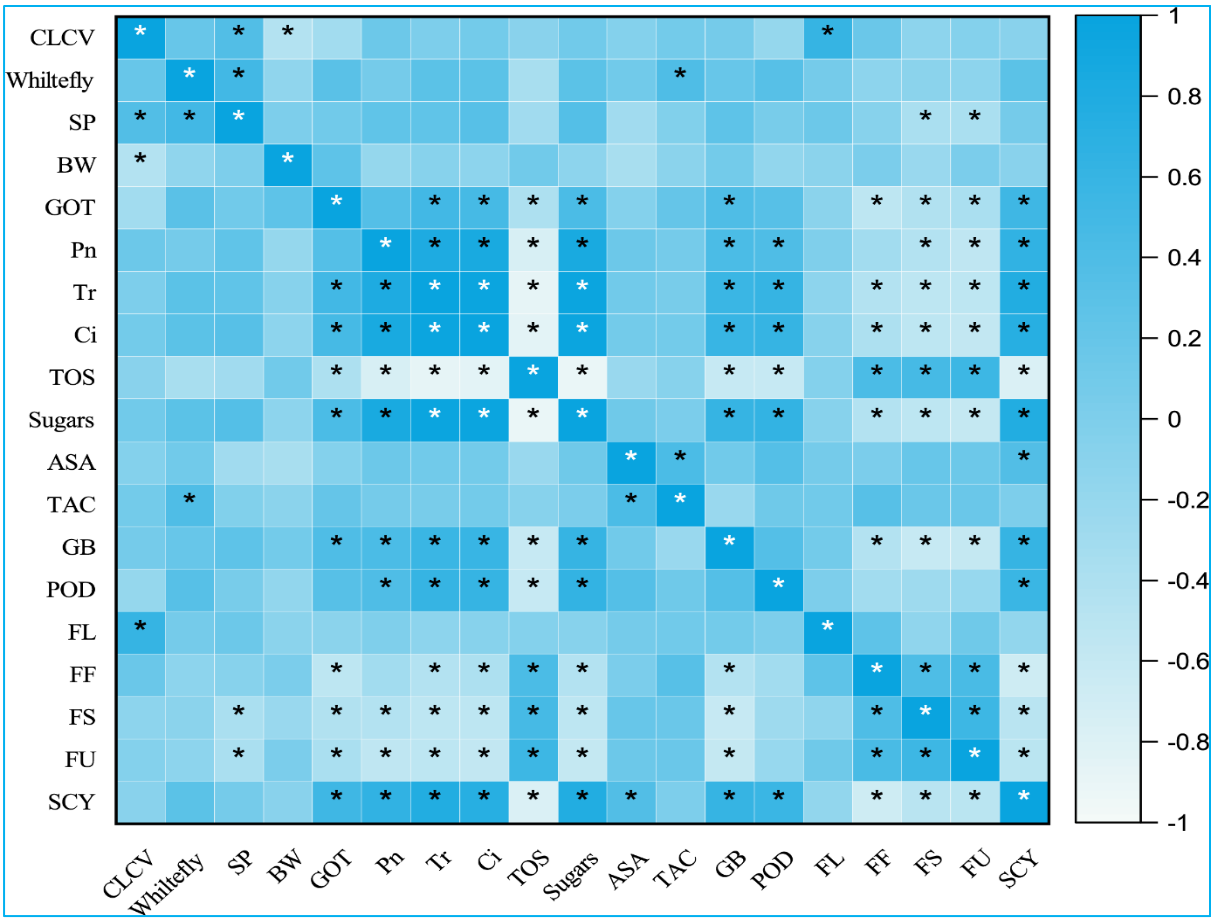

Figure 1: Correlation Matrix of studied plant traits in cotton strains under water stress conditions

The multivariate analysis approaches have become a fundamental method in characterizing, classifying, and categorizing crop varieties/strains/ecotypes under normal and stress conditions. In the current study, two multivariate approaches i.e., biplots and cluster analysis were used to classify the cotton strains based on their performance under heat stress conditions on clay loamy soil. The PC-1/PC-2-based biplot analysis showed a strong association of ginning out turn, peroxidases, glycine betaine, net photosynthetic rate, sugars, and transpiration rate with cotton yield as revealed by the angle of corresponding lines of these traits (Figure 2). Furthermore, most of the variations in the data were due to CLCuV percentage, fiber fineness, net photosynthetic, transpiration rate, ginning out turn, seed yield, and total oxidative stress as their corresponding lines

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were at maximum distance from the origin of the plot. However, ascorbic acid and total antioxidant activity showed the lowest variations in the data (Figure 2). Moreover, the cotton strains CRS-2202, CRS-2201, and CRS-2204 were the most productive strains under water stress conditions (Figure 2).

Table 3: Correlation coefficients between studied plant traits in American cotton strains under water stress conditions

Variables	CLCV	Whitefly	SP	BW	GOT	Pn	Tr	Ci	TOS	Sugars	ASA	TAC	GB	POD	FL	FF	FS	FU
CLCV	**1**	0.349	0.505	-0.446	-0.225	0.460	0.323	0.353	-0.389	0.351	-0.151	0.129	-0.061	-0.146	**0.641**	0.372	-0.126	-0.088
Whitefly	0.349	**1**	**0.711**	-0.233	0.402	0.377	0.436	0.435	**-0.634**	0.487	0.267	0.431	0.339	0.417	0.139	-0.147	-0.064	-0.340
SP	0.505	**0.711**	**1**	0.109	0.319	0.499	0.497	0.559	-0.573	0.569	-0.393	-0.080	0.508	0.337	0.307	-0.047	-0.373	-0.493
BW	-0.446	-0.233	0.109	**1**	0.205	-0.094	-0.048	-0.022	0.133	-0.072	-0.182	-0.226	0.450	-0.008	-0.092	0.034	-0.381	-0.043
GOT	-0.225	0.402	0.319	0.205	**1**	0.541	**0.660**	**0.659**	**-0.681**	**0.649**	0.046	0.148	**0.720**	**0.922**	-0.004	**-0.660**	**-0.705**	-0.539
Pn	0.460	0.377	0.499	-0.094	0.541	**1**	**0.975**	**0.976**	**-0.916**	**0.974**	-0.109	-0.028	0.585	0.591	0.087	-0.456	**-0.720**	-0.585
Tr	0.323	0.436	0.497	-0.048	**0.660**	**0.975**	**1**	**0.987**	**-0.959**	**0.993**	-0.077	-0.045	**0.688**	**0.671**	-0.014	-0.590	**-0.761**	**-0.690**
Ci	0.353	0.435	0.559	-0.022	**0.659**	**0.976**	**0.987**	**1**	**-0.932**	**0.991**	-0.158	-0.020	**0.685**	**0.670**	0.017	-0.510	**-0.772**	**-0.715**
TOS	-0.389	**-0.634**	-0.573	0.133	**-0.681**	**-0.916**	**-0.959**	**-0.932**	**1**	**-0.956**	-0.064	-0.084	**-0.687**	**-0.686**	-0.106	0.559	**0.687**	**0.653**
Sugars	0.351	0.487	0.569	-0.072	**0.649**	**0.974**	**0.993**	**0.991**	**-0.956**	**1**	-0.117	-0.048	**0.680**	**0.685**	-0.005	-0.571	**-0.727**	**-0.700**
ASA	-0.151	0.267	-0.393	-0.182	0.046	-0.109	-0.077	-0.158	-0.064	-0.117	**1**	**0.674**	-0.083	0.038	-0.011	0.004	0.274	0.335
TAC	0.129	0.431	-0.080	-0.226	0.148	-0.028	-0.045	-0.020	-0.084	-0.048	**0.674**	**1**	-0.209	0.065	0.174	0.386	0.217	0.223
GB	-0.061	0.339	0.508	0.450	**0.720**	0.585	**0.688**	**0.685**	**-0.687**	**0.680**	-0.083	-0.209	**1**	0.621	0.073	-0.552	**-0.842**	**-0.752**
POD	-0.146	0.417	0.337	-0.008	**0.922**	0.591	**0.671**	**0.670**	**-0.686**	**0.685**	0.038	0.065	0.621	**1**	0.019	**-0.739**	-0.579	-0.445
FL	**0.641**	0.139	0.307	-0.092	-0.004	0.087	-0.014	0.017	-0.106	-0.005	-0.011	0.174	0.073	0.019	**1**	0.392	-0.229	0.173
FF	0.372	-0.147	-0.047	0.034	**-0.660**	-0.456	-0.590	-0.510	0.559	-0.571	0.004	0.386	-0.552	**-0.739**	0.392	**1**	0.447	0.481
FS	-0.126	-0.064	-0.373	-0.381	**-0.705**	**-0.720**	**-0.761**	**-0.772**	**0.687**	**-0.727**	0.274	0.217	**-0.842**	-0.579	-0.229	0.447	**1**	**0.671**
FU	-0.088	-0.340	-0.493	-0.043	-0.539	-0.585	**-0.690**	**-0.715**	**0.653**	**-0.700**	0.335	0.223	**-0.752**	-0.445	0.173	0.481	**0.671**	**1**
SCY	-0.060	0.445	0.216	-0.014	**0.770**	**0.701**	**0.823**	**0.743**	**-0.855**	**0.782**	0.222	0.022	**0.652**	**0.709**	-0.206	**-0.799**	**-0.619**	-0.563

Note: Values in Bold represent the significance of correlation coefficients
CLCuV: CLCuV percentage, **Whitefly**: Whitefly attack percentage, **BW**: Boll weight, **S/P**: Sympodia per plant, **GOT**: Ginning out turn, **Pn**: Net photosynthetic rate, **Tr**: Transpiration rate, **Ci**: Stomatal conductance, **TOS**: Total oxidative stress, **Sugars**, **ASA**: Ascorbic acid, **TAC**: Total Antioxidant Capacity, **GB**: Glycine betaine, **POD**: Peroxidases, **FL**: Fiber length, **FF**: Fiber fineness, **FS**: Fiber strength, **FU**: Fiber uniformity and **CY**: Cotton yield

The cluster analysis grouped twelve cotton strains into four clusters, where cluster-1 comprised only one strain (CRS-2201), cluster-2 comprised three strains (CRS-2207, CRS-2202, and CRS-2209), cluster-3 consisted of five strains (CRS-2203, CRS-2210, CRS-2206, CRS-2205 and CRS-2208) and cluster-4 again comprises of only one cotton strain (CRS-2204), respectively (Table 4 & Figure 3). The most productive cluster was cluster-4, which produced 2574.0 kg per acre followed by cluster-1 (1844.0 kg per acre) and cluster-2 (1307.1 kg per acre), respectively. Multivariate approaches were extensively used by plant scientists to classify crop germplasm based on their performance under the given conditions and found it very useful in characterizing the germplasm (Zafar et al., 2021; Aslam et al., 2022; Yousaf et al., 2023; Zafar et al., 2023; Yousaf et al., 2024 and Hussain et al., 2024)

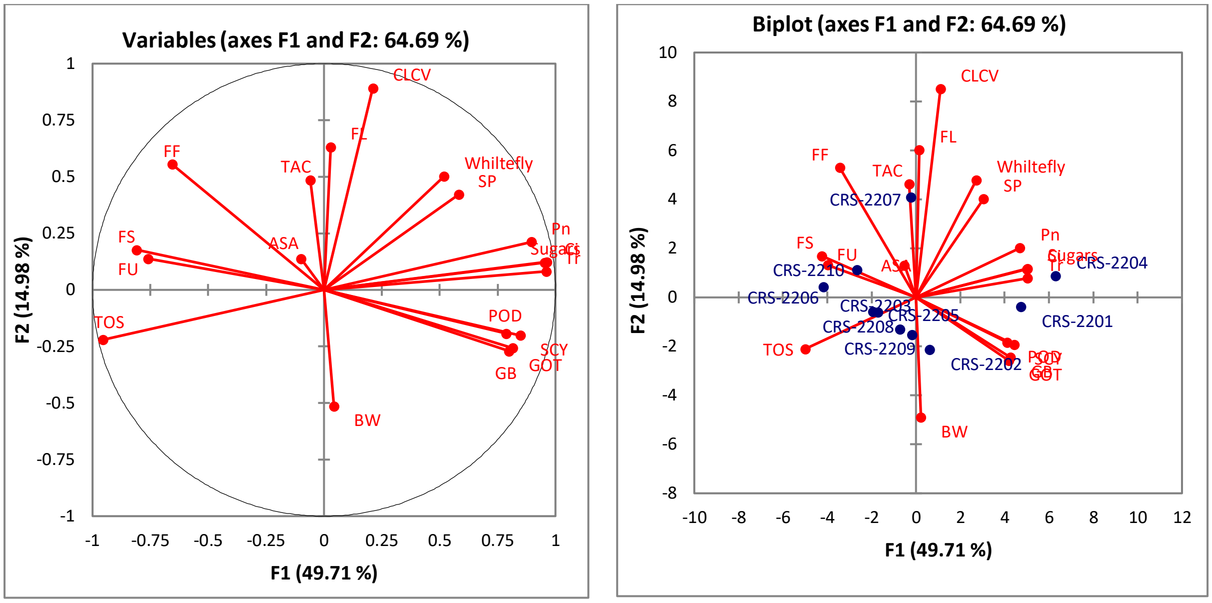

Figure 2: PC1/PC2 Cumulative Biplot between plant traits and cotton strains under water stress conditions

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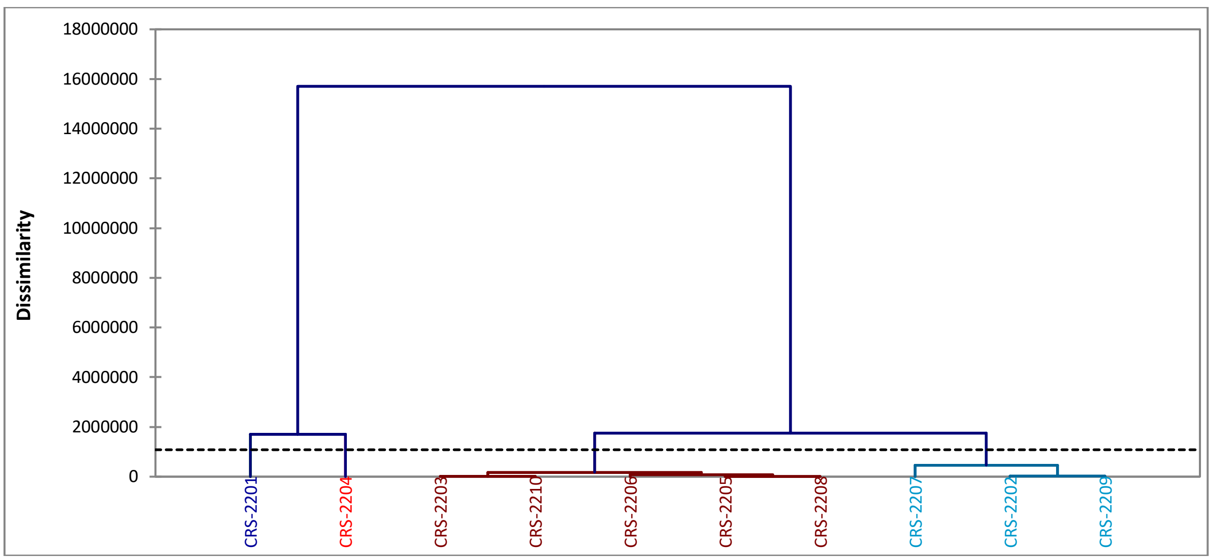

Figure 3: Cluster analysis-based Dendrogram of twelve cotton strains under water stress conditions

Conclusion

The current study revealed the occurrence of significant variations for agronomically important morphological, physiological, biochemical, and fiber quality-related traits in cotton strains under water stress conditions except for boll weight. The correlation coefficient analysis revealed a strong positive correlation of cotton yield with transpiration rate, sugars, ginning out turn, stomatal conductance, peroxidases, net photosynthetic rate, and glycine betaine while a strong negative correlation with total oxidative stress, total oxidative stress, fiber fineness and fiber strength under water stress conditions. The PC-1/PC-2 based biplot analysis reconfirms the results obtained through the correlation analysis and further unveiled that most of the variations in the data were due to CLCuV percentage, fiber fineness, net photosynthetic, transpiration rate, ginning out turn, seed yield and total oxidative stress. The cluster analysis categorizes ten cotton strains into four clusters and found that cotton strains CRS-2202, CRS-2201, and CRS-2204 were the most productive strains under water stress conditions. Therefore, these genotypes must be tested in multilocation trials and potential genotypes must be used for stress-prone areas.

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