

## ROLE OF ANTIOXIDENTS ACCUMULATION AND PHOTOSYNTHETIC STABILITY FOR SUSTAINABLE COTTON PRODUCTION AND FIBRE QUALITY UNDER WATER STRESS CONDITIONS

# YOUSAF MI<sup>\*1</sup>, GHANI A<sup>2</sup>, ZUBAIR M<sup>3</sup>, TALIB I<sup>4</sup>, KAUSAR S<sup>5</sup>, AKHTAR I<sup>6</sup>, MURTAZA G<sup>5</sup>, REHMAN S<sup>7</sup>, BUKHARI MSJ<sup>3</sup>, NAZAR S<sup>8</sup>, QAMAR MJ<sup>9</sup>, AKRAM F<sup>10</sup>, ASHFAQ M<sup>11</sup>, SHAH SWH<sup>12</sup>, ALI B<sup>13</sup>, SHARIF S<sup>14</sup>, LUQMAN M<sup>15</sup>, ASIF M<sup>16</sup>, JAMIL S<sup>16</sup>, SATTAR A<sup>17</sup>, HAFEEZ Z<sup>18</sup>, KHALID M<sup>6</sup>, HUSSAIN S<sup>1</sup>

<sup>1</sup>Cotton Research Station (CRS), 63100, Bahawalpur, Pakistan <sup>2</sup>Maize and Millets Research Institute, Yusafwala-Sahiwal. <sup>3</sup>Agricultural Research Station, Bahawalpur, 63100, Pakistan <sup>4</sup>Institute of Industrial Biotechnology, Governmental College University, Lahore, Pakistan <sup>5</sup>Pesticide Quality Control Laboratory, 63100, Bahawalpur, Pakistan <sup>6</sup>Regional Agricultural Research Institute, Bahawalpur, 63100, Pakistan Soil and Water Testing Laboratory, Bahawalpur, 63100, Pakistan <sup>8</sup>Soil and Water Testing Laboratory, Gujranwala, Pakistan <sup>9</sup>Soil Fertility (Field), Bahawalpur, 63100, Pakistan <sup>10</sup>Soil and Water Testing Laboratory, Nankana Sahib, Pakistan <sup>11</sup>Central Cotton Research Institute, Multan, Pakistan <sup>12</sup>Entomological Research Sub-Station, Bahawalpur, 63100, Pakistan <sup>13</sup>Sorghum Research Sub-Station, Dera Ghazi Khan, 32200, Pakistan <sup>14</sup>Soil and Water Testing Laboratory, Okara, Pakistan <sup>15</sup>Govt Seed Farm, Dhakkar, Pakpattan, Pakistan <sup>16</sup>Soil and Water Testing Laboratory, Bahawalnagar, Pakistan <sup>17</sup>Soil and Water Testing Laboratory, Lodhran, Pakistan <sup>18</sup>Oilseeds Research Station, Bahawalpur, 63100, Pakistan \*Corresponding author email address: irfanpbg.uaf@gmail.com





(Received, 15<sup>th</sup> November 2023, Revised 10<sup>th</sup> January 2024, Published 1<sup>st</sup> March 2024)

**Abstract:** One of the significant steps in developing water stress-tolerant, climate-smart cotton genotypes is evaluating and screening available cultivated germplasm. The current study was designed to evaluate the elite cotton strains developed for release in drought-prone areas of Punjab and Pakistan. Twenty-four cotton strains, along with two check varieties BH-184 and FH-142 were sown under RCBD in triplicates. The plant-to-plant and row-to-row distances were maintained at 30 cm and 70 cm, respectively. The recommended dosage of fertilizers N-P-K (a) 100-36-37 kgs per acre was applied to all the treatments in all replications. The results revealed the presence of highly significant variations among cotton strains for studied plant parameters in twenty-four cotton strains. Correlation analysis unveiled the presence of highly significant and positive correlation of seed cotton yield with nodes per plant (r = 0.988\*\*), sympodia per plant (r = 0.987\*\*), transpiration rate (r = 0.968\*\*), flavonoids (r = 0.982\*\*), net photosynthetic rate (r = 0.979\*\*), peroxidases (r = 0.968\*\*), superoxide dismutase (r = 0.968\*\*), bolls per plant (r = 0.914\*\*), plant height (r = 0.885\*\*), monopodia per plant (r = 0.725\*\*) and ginning out turn (r = 0.430\*\*) while the significantly negative correlation with CLCuV occurrence percentage (r = -0.526\*\*). Cluster, principal component, and biplot analysis classify cotton strains into groups based on their performance under water-stress conditions. The results from PC1/PC2 biplot also verified the results obtained through correlations and further revealed that transpiration rate, net photosynthetic rate, peroxidases, superoxide dismutase, plant height, flavonoids, sympodia per plant, and superoxide dismutase were the most divergent traits and must be considered in developing a scheme to develop heat-tolerant cotton genotypes.

Keywords: Correlation, Cluster Analysis, Biplot, Photosynthesis, Transpiration rate, Antioxidants, CLCuV

#### Introduction

Climate change, characterized by shifts in temperature and precipitation patterns, poses significant challenges to sustainable crop production worldwide. One of the most pronounced manifestations of climate change is the increase in frequency and intensity of drought events, which exert substantial stress on agricultural systems (Kotir, 2011). Drought stress disrupts the delicate balance between water availability and crop water demand, leading to reduced yields, diminished crop quality, and increased vulnerability to pests and diseases (Farooq et al., 2009). Furthermore, prolonged drought conditions can result in soil degradation, depletion of water resources, and loss of biodiversity, exacerbating the resilience of agroecosystems. Sustainable

crop production practices, such as conservation agriculture, crop diversification, and improved irrigation techniques, are critical for mitigating the adverse effects of drought and enhancing resilience to climate variability. Implementing climate-smart agricultural strategies, including the use of drought-tolerant crop varieties and efficient water management practices, is essential for ensuring food security and promoting agricultural sustainability in the face of climate change-induced challenges (Arif et al., 2020).

Cotton, scientifically known as Gossypium, is a versatile and indispensable crop that has played a pivotal role in shaping human civilization for centuries. Its significance lies in its economic value and rich biological characteristics, historical importance, and global production impact. Cotton is a member of the Malvaceae family, a group of flowering plants known for their showy flowers and often fibrous fruits. Within the genus Gossypium, there are around 50 species, but only four are cultivated for their fibres: Gossypium hirsutum (Upland cotton), Gossypium barbadense (Pima cotton), Gossypium arboreum, and Gossypium herbaceum (Chaudhry & Guitchounts, 2003). These species vary in their characteristics, such as fiber length, strength, and fineness, making them suitable for different applications. These fibers' length, fineness, and strength determine their quality and utility in various industries, particularly textiles (Elmogahzy and Farag, 2018).

In 2022-23, cotton was grown on an area of 31.7 million hectares and 116.26 million 480 lb bales cotton was produced with an average of 798 kg ha<sup>-1</sup>. Cotton yield in Pakistan (472 kg ha<sup>-1</sup>) is for much less than top yielding countries, including China (2122 kg ha<sup>-1</sup>), Australia (1943), Turkey (1922), Mexico (1720), Brazil (1595 kg ha<sup>-1</sup>), United States (1068 kg ha<sup>-1</sup>) and Uzbekistan (684 kg ha<sup>-1</sup>) (USDA, 2024). Th major reasons for lower yield and production are heat and drought stresses, low inputs application, high input rates, insect-pest infestation especially aphid, Jassid, whitefly, pink bollworm and cotton leaf curl virus, non-availability of good quality seed (ESP, 2023).

In Pakistan, cotton is mainly grown for its usage in the textile and cooking oil industry. During 2022-23, the cotton crop was badly damaged in Pakistan due to the effects of climate change. The first damage was done by the raised temperature (7-10 °C) from the last few years in the month of March till May, combined with the limited water supply and heatwaves, which affected cotton germination and initial crop growth. In 2022-23, the cotton crop contributed 0.3 per cent of GDP and 1.4 per cent in value added in agriculture. The cotton crop was sown on an area of 2.144 million hectares compared to 1.937 million hectares (ESP, 2023). However, severe floods in Sindh and Balochistan swept the whole crops, resulting in a severe yield reduction (4.910 million bales) compared to last year's production (8.329 million bales). Moreover, in the Punjab Province, Rajanpur, DG Khan and Taunsa were also badly affected by the floods along with the insect pest infestation, especially pink bollworms, Whiteflies and Thrips (ESP, 2023).

Drought stress is one of the most alarming threats to the sustainable crop production in many parts of the world (Sinclair, 2005). Water stress not only effects the plant growth and development by alterations in plant morphology, physiology, anatomy but also degrade the quality of final out of crop (Saleem et al., 2016). Various plant development processes like germination, seedling growth, photosynthesis, transpiration, flower and fruit formation, fiber length and quality got badly effected by water stress. Recent research showed that cotton, being indeterminate in growth habit, is considered more susceptible to drought stress, leading to the significant loss of production. Studies also showed that flower and squares are the most sensitive parts of cotton plants affecting the source-sink ratio and severe reduction seed cotton yield (Ul-Allah et al., 2021; Cannon, 2023). Moreover, recent investigations also revealed that the reduction in seed cotton yield is in fact due to the reduction/hindrance in plant physiological processes under drought stress conditions (Seleiman et al., 2021; Zafar et al., 2023; Çelik e, 2023). Water stress was also observed to be link with reduction in cell and leaf expansion, stem longevity, alterations in root/shoot ration, stem reserve mobilization, hormonal imbalance and reduction in number of fruiting nodes in cotton

Screening and evaluation of existing cultivated germplasm is the first step in any plant breeding program aiming development of drought tolerance cotton genotypes. The one of the major causes of poor performance of many modern-day crop varieties under changing climatic conditions lies in their narrow genetic base (Ali et al., 2017). Hence, the initial stage in the development of heat-tolerant cotton cultivars involves assessing the currently cultivated germplasm under stressful conditions and choosing a range of parents for the hybridization program. The present investigation was carried out to evaluate the germplasm for the selection of suitable parents to be used in breeding programs.

# Methodology

The present investigation was carried out at the Cotton Research Station research farm, Bahawalpur, during the crop year 2023. The study was consisted of twenty-four cotton genotypes including two standard check varieties i.e., FH-142 and BH-184 and twenty-two elite cotton strains (Table 1). These strains were sown under RCB design in triplicates. These strains were sowed in the third week of April 2023 with the handheld seed drills @ 2 seeds per hill, which were thinned to 1 at the initial seedling stage to keep one healthy seedling to ensure optimum plant population. The water stress was given to the cotton strains before flowering for a period of four weeks, and then regular irrigation started. These strains were planted in four-rowed, 10-meter-long rows with a net plot size of 15cm2. Fertilizers, pesticides, hoeing, and other cultural/management practices were carried out to ensure plant health.

Table 1: List of cotton strains under study
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Sr. #	<b>Cotton Strains</b>	Sr. #	<b>Cotton Strains</b>	Sr. #	<b>Cotton Strains</b>
1	BRS-111	9	BH-184	17	BRS-125

2	FH-142	10	BRS-118	18	BRS-126
3	BRS-112	11	BRS-119	19	BRS-127
4	BRS-113	12	BRS-120	20	BRS-128
5	BRS-114	13	BRS-121	21	BRS-129
6	BRS-115	14	BRS-122	22	BRS-130
7	BRS-116	15	BRS-123	23	BRS-131
8	BRS-117	16	BRS-124	24	BRS-132

The water used for irrigation purposes was examined for its suitability and critical traits and the values are given as under;

 Table 2: Properties of Irrigation water used

Sr. #	Parameter	Value
1	Total Soluble Salts (TSS)	1153 ppm
2	Sodium Adsorption Ratio (SAR)	6.7
3	Residual Sodium Bicarbonate (RSB)	1.51
4	Chloride	4.2 meL <sup>s</sup>

Similarly, the soil was also tested for its properties so that optimum fertigation could be given to the soil to obtain good results.

Sr. #	Parameter	Value
1	Soil Texture	Sandy Loam
2	pH	7.8
3	EC	5.8 dSm <sup>s</sup>
4	Organic Matter	0.81%
5	Available P	7.1 ppm
6	Available K	83 ppm
7	Zinc	1.4 ppm

The data was obtained from ten fully guarded, randomly selected plants per replication per strain. The data for several plant traits, including plant populations per hectare (P. Pop), plant height (PH), nodes per plant (NP), monopods per plant (MP), sympods per plant (SP), bolls per plant (BP), CLCuV incidence percentage (CLCuV), net photosynthetic rate (Pn), transpiration rate (Tr), flavonoid (Flv), peroxidases (POD), superoxide dismutase (SOD), ginning out turn (GOT), staple length (SL), fiber strength (FS) and seed cotton yield (SCY). Moreover, the CLCuV incidence **Results and Discussion** 

#### ANOVA

The results obtained from ANOVA depicted the occurrence of significant variations among cotton strains for seed cotton yield and associated traits under drought stress conditions (Table 4).

The highest variations were observed for plant height (PH), nodes per plant (NP), monopodia per plant (MP), sympodia per plant (SP), bolls per plant (BP), ClCuV incidence percentage (CLCuV %), net photosynthetic rate (Pn), transpiration rate (Tr), Flavonoids (Flv), peroxidases (POD), superoxide dismutase (SOD), ginning out turn (GOT), staple length (SL), fibre strength (FS) and seed percentage (CLCuV) was determined according to the formula used by Aslam et al. (2022).

The obtained data were statistically subjected to analysis of variance and correlation analysis (Steel et al.,1997). Moreover, cluster analysis was performed to characterize cotton strains under heat stress conditions (Sneath & Sokal, 1973). To execute these analyses, Statistix 8.1 and XLSTAT 22.0. statistical tool was used. Moreover, Microsoft Excel was used to plot demonstration graphs to illustrate the mean performance of key traits.

cotton yield (SCY) except plant population per hectares (P. Pop) for which variations were non-significant (Table 4). Moreover, variations among cotton strains for these parameters were non-significant in replications except for plant height, sympodia per plant, CLCuV %, staple length and fibre strength which showed highly significant diversity among cotton strains. Similar findings were also reported by Soomro et al., 2011, Hafiz et al., 2015, Sezener et al., 2015; Saleem et al., 2016 and Ahmad et al., 2020 who revealed significant variations among cotton genotypes for similar/same traits under water stress conditions.

Table 4: Mean Square (MS	) of selected parameters	s of cotton strains under	water stress conditions

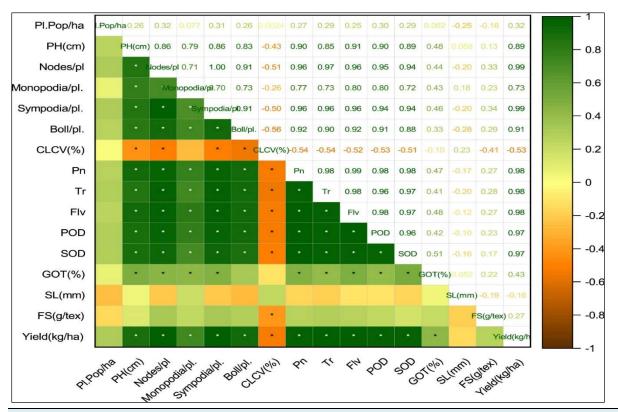
Source of Variation	Replications	Strains	Error	
Traits/Df	2	23	46	
Plant population per hectare (P.Pop)	2061828 <sup>NS</sup>	74900000**	1601158	

Plant Height (PH)	15.42**	284.06**	6.56
Nodes per Plant (NP)	3.39 <sup>NS</sup>	54.17**	2.01
Monopodia per plant (MP)	0.43 <sup>NS</sup>	1.30*	0.65
Sympodia per plant (SP)	4.67**	48.89**	1.52
Boll per plant (BP)	3.17 <sup>NS</sup>	78.52**	2.56
CLCuV incidence (%) (CLCuV%)	3.93**	9.04**	1.65
Net photosynthetic rate (Pn)	0.71 <sup>NS</sup>	45.72**	2.18
Transpiration rate (Tr)	0.00 <sup>NS</sup>	0.05**	0.003
Flavoniod (Flv)	835.0 <sup>NS</sup>	62223.8**	3267.3
Peroxidases (POD)	194.0 <sup>NS</sup>	8410.7**	513.7
Superoxide dismutase (SOD)	28.8 <sup>NS</sup>	559.6**	31.8
Ginning out turn (GOT)	0.08 <sup>NS</sup>	17.48**	0.04
Staple length (SL)	47.46**	2.01**	0.78
Fiber strength (FS)	33.84**	2.42**	0.62
Seed Cotton Yield (SCY)	5435 <sup>NS</sup>	116931**	4719

## **Correlation Analysis (CA)**

The correlation coefficient analysis was done to observe the direction and magnitude of correlation between different plant parameters under study in cotton strains under drought stress conditions (Figure 1). The results unveiled the presence of highly significant and positive correlation of seed cotton yield with nodes per plant ( $r = 0.988^{**}$ ), sympodia per plant ( $r = 0.982^{**}$ ), transpiration rate ( $r = 0.983^{**}$ ), flavonoids ( $r = 0.982^{**}$ ), net photosynthetic rate ( $r = 0.979^{**}$ ), peroxidases ( $r = 0.968^{**}$ ), superoxide dismutase

(r =  $0.968^{**}$ ), bolls per plant (r =  $0.914^{**}$ ), plant height (r =  $0.885^{**}$ ), monopodia per plant (r =  $0.725^{**}$ ) and ginning out turn (r =  $0.430^{**}$ ) while the significantly negative correlation with CLCuV occurrence percentage (r =  $-0.526^{**}$ ) (Figure 1). The strongest positive correlation was revealed between nodes per plant and symposia per plant (r =  $0.996^{**}$ ). In contrast, the strongest negative correlation was seen between bolls per plant and CLCuV occurrence percentage (r =  $-0.558^{**}$ ) (Table 4). Similar findings were reported by Rahman et al., 2008; Ullah et al., 2017; Khan et



al., 2018; Jie et al., 2020; Zahid et al., 2021 Babar et al., 2023 and Çelik et al., 2023 who showed a significantly stronger association of morphological, physiological, and biochemical traits with seed cotton yield and its quality

under heat stress conditions. Therefore, these characters must be considered while selecting the parental material and subsequent generations to develop drought-tolerant, climate-smart cotton genotypes.

## Figure 1: Correlation between different plant parameters in upland cotton strains under water stress conditions.

**Cluster Analysis (CA)** Cluster analysis is a multivariate analysis used to classify, categorize, or characterize crop varieties/genotypes based on their performance under different circumstances. The current study used cluster analysis to categorize upland cotton strains based on morpho-physiological, biochemical, and fiber parameters under water-limited conditions. The cluster analysis classified twenty-four cotton strains into three classes based on their performance under water stress conditions: Class I, II, and III (Table 5 & Figure 2).

Class-I was the smallest group comprised of six cotton strains, i.e., BRS-111, BRS-115, BRS-116, BRS-120, BRS-125, and BRS-129 (Table 5 & Figure 2). This group was regarded as the intermediately productive and droughttolerant group of cotton strains with an average seed cotton yield of 1151.6 kg per ha. The comparatively higher yield of this group was due to the higher mean values of positively associated traits, i.e., bolls per plant (31.6) and other parameters. The Class-II was the second largest with six cotton strains, i.e., BRS-114, BRS-119, BRS-121, BRS-126, BRS-131, BRS-132, and two standard check varieties, BH-184 and FH-142 (Figure 2). This group was characterized as the least productive and droughtsusceptible group of strains, with an average yield of 1021.9 kg. The lowest yield of the group was due to the lowest average of key contributing traits i.e., plant height (131.8), nodes per plant (25.1), monopodia per plant (1.25), sympodia per plant (20.6), bolls per plant (27.04), net photosynthetic rate (22.68), transpiration rate (0.375), flavonoids (653.9), peroxidases (815.29) and superoxide dismutase (150.79) (Table 5).

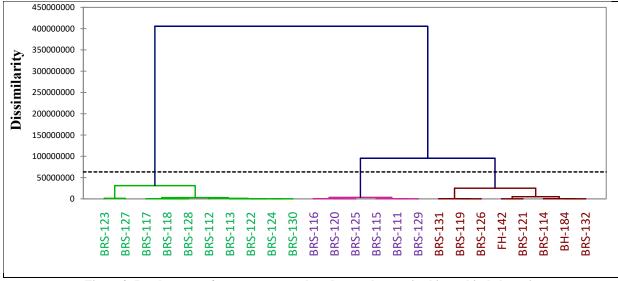


Figure 2: Dendrogram of cotton genotypes based on agglomerative hierarchical clustering

The third and last group, Class-III, was the group of most productive and drought-tolerant cotton strains comprised of ten cotton strains i.e., BRS-112, BRS-113, BRS-117, BRS-118, BRS-122, BRS-123, BRS-124, BRS-127, BRS-128 and BRS-130. The highest yield and drought tolerance revealed by the group genotypes was associated with the good average performance of its yield-related traits i.e., plant height (138.4), nodes per plant (28.38), monopodia per plant (1.433), sympodia per plant (23.6), net photosynthetic

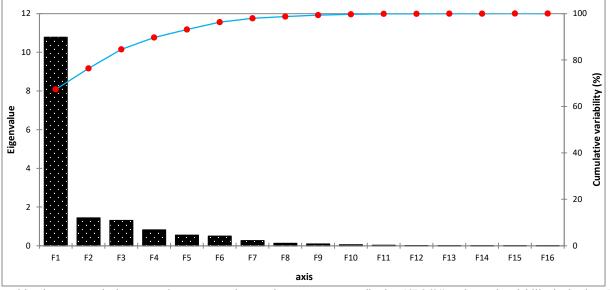
rate (25.48), transpiration rate (0.473), flavonoids (754.2), peroxidases (855.9) and superoxide dismutase (161.3) and ginning out turn (38.56) (Table 5). Several researchers used cluster analysis in categorizing cotton genotypes under different circumstances and found it very useful in classifying the genotypes based on their performance (Aslam et al., 2022; Zafar et al., 2022 and Manan et al., 2022; Hussain et al., 2023, Zafar et al., 2023, Yousaf et al., 2023).

Table 5: Class means of three clusters through	h agglomerative hierarchical clust	ering in cotton geno	otypes
Classes/Diant Traits	Class 1	Class 2	Clas

Classes/Plant Traits	Class-1	Class-2	Class-3	
Plant population per hectare (P.Pop)	28348.444	23071.869	33672.637	-
Plant Height (PH)	137.444	131.875	138.433	

Nodes per Plant (NP)	27.833	25.125	28.367
Monopodia per plant (MP)	1.389	1.250	1.433
Sympodia per plant (SP)	23.000	20.625	23.600
Boll per plant (BP)	31.667	27.042	30.067
CLCuV incidence (%) (CLCuV%)	6.834	7.177	6.914
Net photosynthetic rate (Pn)	24.861	22.679	25.483
Transpiration rate (Tr)	0.447	0.375	0.473
Flavoniod (Flv)	737.389	653.917	754.233
Peroxidases (POD)	841.944	815.292	855.900
Superoxide dismutase (SOD)	159.444	150.792	161.333
Ginning out turn (GOT)	38.211	38.088	38.560
Staple length (SL)	28.700	28.708	28.493
Fiber strength (FS)	30.733	30.979	30.680
Seed Cotton Yield (SCY)	1151.656	1021.984	1176.018

Principal Component-Based Biplot Analysis (PCBB) The principal component analysis (PCA) is another showed eigen more than 1 and contributed 84.6 % to the variability in the data. The PC1 showed the highest



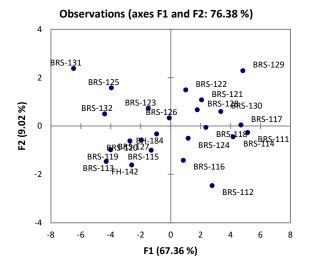
multivariate analysis used to characterize treatments/genotypes under given environmental conditions. The current study used PCA-based biplot analysis to classify the cotton strains under water stress conditions. The results obtained through PCA revealed that three principal components (PCs)/factors (Fs) out of sixteen contribution (67.36%) to the total variability in the data. The second PC gave an eigenvalue of 1.443 and contributed 9.017% variability in the data (Figure 3). Therefore, a PC1/PC2 biplot was drawn to consolidate the data and infer valuable findings.



The PC1/PC2-based biplot analysis also confirms the results obtained through cluster analysis and correlation analysis (Figure 4). The PC1/PC2 biplot showed that seed cotton yield has a significantly positive association with key yieldcontributing traits, including plant height, net photosynthetic rate, transpiration rate, superoxide dismutase, peroxidases, bolls per plant, sympodia per plant, nodes per plant and plant height as their corresponding lines lay near to the corresponding line of seed cotton yield while the highest negative association with CLCuV occurrence percentage as its corresponding line was in completely

<sup>[</sup>Citation: Yousaf, M.I., Ghani, A., Zubair, M., Talib, I., Kausar, S., Akhtar, I., Murtaza, G., Rehman, S., Bukhari, M.S.J., Nazar, S., Qamar, M.J., Akram, F., Ashfaq, M., Shah, S.W.H., Ali, B., Sharif, S., Luqman, M., Asif, M., Jamil, S., Sattar, A., Hafeez., Z, Khalid, M., Hussain, S. (2024). Role of antioxidents accumulation and photosynthetic stability for sustainable cotton production and fibre quality under water stress conditions. *Biol. Clin. Sci. Res. J.*, **2024**: 726. doi: https://doi.org/10.54112/bcsrj.v2024i1.726]

opposite direction (Figure 4). With respect to cotton strains, BRS-29, BRS-111, BRS-117, and BRS-130 were among the most productive and drought-tolerant cotton strains as they lay away from the base and are in line with the correspondence line of the seed cotton yield. the analysis is applied by several scientists and found helpful in categorize crop genotypes based on their divergence, performance, and



#### Conclusion

The genetic evaluation of the existing germplasm is a preliminary step in the development of drought-tolerant cotton genotypes. The current study observed highly significant differences among cotton strains for key yield-associated traits under water stress conditions. The correlation coefficient analysis unveiled the significantly positive correlation of seed cotton yield with net photosynthetic rate, peroxidases, superoxide dismutase, bolls per plant, nodes per plant, plant height, flavonoids, and sympodia per plant while strong negative correlation with ginning out turn. Cluster analysis classified cotton strains into three groups, with Group-III being the group of most productive and drought-tolerant cotton strains. The principal component-based biplot analysis revealed that transpiration rate, net photosynthetic rate, peroxidases, superoxide dismutase, plant height, flavonoids, sympodia per plant, and superoxide dismutase were the most divergent traits and must be considered in framing a strategy to develop drought-tolerant cotton genotypes.

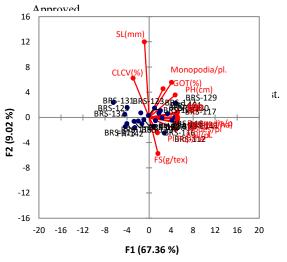
#### Declarations

### Data Availability statement

All data generated or analyzed during the study are included in the manuscript. **Ethics approval and consent to participate** Approved by the department Concerned.

**Consent for publication** 

performance under specified environments. Several researchers used principal component and PC1/PC2 biplot analysis in categorising cotton genotypes under different circumstances and found it very useful in classifying the genotypes based on their performance (Zafar et al., 2023, Yousaf et al., 2023, Aslam et al., 2022; Zafar et al., 2022, Manan et al., 2022 and Zafar et al., 2021).



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<sup>[</sup>Citation: Yousaf, M.I., Ghani, A., Zubair, M., Talib, I., Kausar, S., Akhtar, I., Murtaza, G., Rehman, S., Bukhari, M.S.J., Nazar, S., Qamar, M.J., Akram, F., Ashfaq, M., Shah, S.W.H., Ali, B., Sharif, S., Luqman, M., Asif, M., Jamil, S., Sattar, A., Hafeez., Z, Khalid, M., Hussain, S. (2024). Role of antioxidents accumulation and photosynthetic stability for sustainable cotton production and fibre quality under water stress conditions. *Biol. Clin. Sci. Res. J.*, **2024**: 726. doi: https://doi.org/10.54112/bcsrj.v2024i1.726]