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RESEARCH ARTICLE

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Advancing Heat Tolerance in Cotton through Integration of Multiple Stress Tolerance Indices and Multivariate Analyses

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ABSTRACT

In the context of current abrupt climate change scenarios, the yield under both stressful and normal conditions stands as a key indicator for identifying genotypes resilient to stress. Various studies have proposed different yield indices to discern genotypes tolerant to stress. To ascertain desirable genotypes across regions prone to heat stress, 23 cotton genotypes were assessed for their response to normal and heat stress conditions. Nine stress tolerance indices were employed to evaluate seed cotton yield under both conditions, aiming to identify the most effective overall index. Analysis, including correlation and principal component analysis, indicated that mean productivity (MP), geometric mean productivity (GMP), harmonic mean (HM), stress tolerance index (STI), and yield index (YI) exhibited positive associations with seed cotton yield under both conditions. These indices identified five genotypes as the most heat-tolerant and three as the most heat-sensitive. Hierarchical clustering and ranking based on stress indices highlighted genotypes G15 and G7 as the most heat-tolerant, given their superior mean rank and relatively low standard deviation of rank. Additionally, the strong correlation of GMP with physiological traits such as STI, YI, and AR further validated the ranking based on yield indices.

Keywords: Cotton, Abiotic stress tolerance, Multivariate Analysis

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INTRODUCTION

Cotton production is negatively affected by several abiotic factors, including temperature, which regulates the growth and production of cotton (Kamal et al., 2019; Zafar et al., 2021). The elevated ambient temperature-induced heat stress is a chief constraint to achieving cotton's optimal yield potential. In Pakistan, the average temperature within cotton zone (37/25°C) is higher than in other cotton-growing regions of the world (Manan et al., 2021; Zafar et al., 2022a). The temperature remains high at earlier growth periods (May-June) of cotton (40-50°C). The cotton crop is sensitive to heat stress throughout all developmental stages (Zahid et al., 2016) but particular sensitivity observed during the reproductive stage (Zafar et al., 2022b). High temperature causes the shedding of flowers at the flowering stage and reduces boll weight and yield (Xu et al., 2020).

Heat stress causes adverse physiological, biochemical and molecular alterations in plants, including damage to lipids and proteins, lipid peroxidation of the cell membrane, oxidative stress, and impairment of photosynthesis and respiration (Bahrami et al., 2019; Haider et al., 2021). The cotton plant upregulates antioxidant activities like POD, CAT, TSP, and carotenoids. These antioxidants help the cotton plants detoxify the ROS to prevent cellular damage (Farooq et al., 2021). Heat stress also causes damage to the thylakoids (Chovancek et al., 2021) and reduced chlorophyll contents which are negatively associated with yield (Saha et al., 2016; Zafar et al., 2022b). The ideal temperature for germination is (12°C), seedling development (28 to 30°C), biomass (18.1 to 25.1°C), boll development and retention is 29.5°C. Each 1°C increase in field temperature decreases 110 kg ha^{-1} seed cotton yield (Singh et al. 2007). The reduction in yield due to heat stress became a major concern for cotton crops cultivated in arid and semi-arid areas of the world. The most sustainable approach to managing heat stress is the development of plants that can grow well at an extreme temperature (Zafar et al., 2023).

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Due to climate change, the frequency and severity of heat stress have been increased. Due to the devastating impacts of these stressors on crop yield in the last decades, these stressors became important (Vaughan et al., 2018). Understanding the relationship of yield performance with various selection criteria under multiple environmental and precise evaluations of stress tolerance in breeding materials can increase the efficiency of breeding programs (Collard and Mackill, 2008). The yield performance serves as the main criterion for evaluating tolerance to various environmental stress factors (e.g., temperature extremes, water deficit, and salinity) as a primary indicator of stress tolerance. The cultivars exhibiting higher yield under both conditions are declared tolerant (Sabagh et al., 2020). Some researchers use mathematical models to compare yields under stressful and normal environments. The reduction in seed cotton yield is a major concern for cotton breeders, demonstrating the necessity of emphasizing yield performance in heat-prone environments. The factors regarding acclimatization may cause variation in yield rather than stress resistance. Another challenge lies in determining the selection of materials, whether based on individual indices or in combination with other indices, to address both optimal and heat stress conditions (Zafar et al., 2022a). The most effective approach to tackle the issue of selecting for resistance to abiotic stresses is to establish a balanced compromise among yield stability, yield under stress conditions, and yield loss due to stress (Bahrami et al., 2021). Multivariate analysis including PCA is a robust method used to reduce the dimensions of variable matrix and several researchers reported the effectiveness of PCA to select genotype under normal and stress conditions with the help of multiple indices (Bahrami et al., 2014; 2019; 2021).

Different scientists suggested several stress indices for tolerance index (TOL), mean productivity (MP), geometric mean productivity (GMP), stress tolerance index (STI), stress susceptibility index (SSI), relative stress index (RSI), harmonic mean (HM), yield stability index (YSI), and yield index (YI). The TOL was defined as the difference between yield under stress and non-stress conditions and MP is the average of Yp (yield under normal conditions) and Ys (yield under stress conditions) (Rosielle and Hamblin, 1981). Different breeders suggested GMP as a powerful index for comparing yield under normal and stressed conditions (Raman et al., 2012). The STI is the ratio of the products' yield performance under stressful and normal environments and squared mean yield performance under normal conditions, and GMP was the square root of the product of the genotype performance under stress and normal conditions (Fernandez, 1992). (Fischer and Maurer, 1978) suggested SSI index in which the genotype with the least value of SSI is more tolerant to heat stress. The STI was suggested by identifying cultivars with high yield and stress tolerance potentials (Fernandez, 1992). were employed to evaluate the stability of genotypes under both normal and stress conditions (Gavuzzi et al., 1997).

In the last few years, the coincidence of high temperature with reproductive phases caused a significant global reduction in cotton yield. The objective of this study was to assess the performance of 23 cotton genotypes (including 8 parents and their 15F1 hybrids) under heat stress in terms of seed cotton yield by using different stress indices to identify heat-tolerant genotypes that can perform well under normal and heat stress conditions.

MATERIALS & METHODS

Experimental Design

In November 2018, healthy seeds of eight cotton genotypes were sown in clay pots in the glasshouse of the Department of Plant Breeding and Genetics, UAF, Pakistan. These genotypes were crossed in line × tester fashion at the flowering phase. As a line, five heat-tolerant genotypes were crossed with three heat-susceptible genotypes as testers. In the next normal cotton growing season, the F_1 seeds of 23 cotton genotypes (15 crosses and 8 parents) were sown under field conditions in normal and heat stress environments under RCBD following a split-plot arrangement in three replications. The chosen genotypes' seeds were planted in June and harvested in October. To mitigate humidity variations, tiny perforations were created in the polythene sheets. Plots without heat stress (control) were maintained in natural environmental conditions. The breeding materials used in this study are given in (Table S1).

Imposition of High-temperature Stress

In September, at 50% flowering, heat stress was given for 12 days. The temperature was elevated in the daytime by constructing a polythene tunnel and uncovered at night. A digital thermometer and humidity probe recorded the temperature and humidity in the tunnel (Fig. S1) (Both et al., 2015). The relative humidity inside and outside the tunnel during heat stress is given in (Table S2). Data were collected regarding seed cotton yield (SCY) at maturity as an average of five plants from each genotype. Different heat tolerance indices were used in this study to identify heat-tolerant and susceptible genotypes (Table S3).

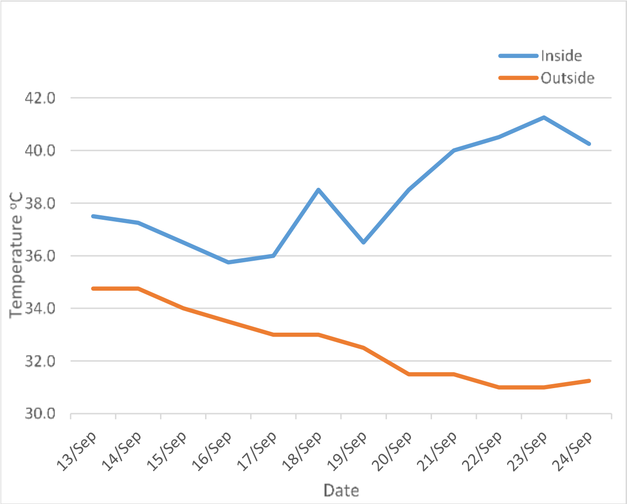

Fig. S1: Mean temperature ºC inside and outside tunnel during the heat stress implementation period.

Physiological Assay

After 12 days of heat stress, data on physiological traits from five selected plants were recorded under stressed and normal environments from each replication. The top young leaves of each genotype were used for physiological analysis. Catalase and peroxidase were determined by (Liu et al., 2009), total soluble proteins (Bradford, 1976) and chlorophyll contents and carotenoids (Arnon, 1949).

To identify genotypes demonstrating high seed cotton yield under both normal and heat stress conditions, 3D scatterplots were generated utilizing stress indices like TOL, MP, GMP, STI, SSI, RSI, HM, YSI, and YI. These plots were created using the iPASTIC software package developed by (Pour-Aboughadareh et al., 2019). The objective was to rank and identify genotypes with consistent and superior yields under both conditions, considering it as the representative trait (Ketata et al., 1989). In this approach, the average sum of rank (ASR) encompassing all variables/indices was utilized as an indicator for selecting the top-performing genotypes. According to this method, the genotype exhibiting the best performance for a specific variable receives the lowest rank. Therefore, genotypes with the lowest ASR values and lowest standard deviation values were considered the best performers. The extracted indices have then been subjected to multivariate analyses for further validation by using default analyses and standardization option with SAS-JMP Pro 16 (SAS Institute Inc., Cary, NC, USA, 1989–2021).

RESULTS

The seed cotton yield (SCY) for 23 cotton genotypes was recorded under heat stress and normal conditions. Compared to normal conditions, a considerable decline in SCY of all genotypes was noticed under a stressed environment. The genotypes G15 and G16 were the highest yielding cultivars in stressful and normal environments. The genotypes G21 and G23 revealed lower yields in both conditions, respectively (Table 1). Interestingly, the G21 showed the lowest reduction in SCY under a stress environment (Table 1). Different heat-tolerant indices were calculated based on SCY to investigate the heat-tolerant cotton genotypes for the heat-prone areas. a 3D scatterplot was created to classify 23 test genotypes of upland cotton, encompassing lines and their F1 hybrids, revealing four distinct groups (Fig. 1). Based on the framework proposed by Fernandez (1992), a 3D scatterplot was created to classify 23 test genotypes of upland cotton, encompassing lines and their F1 hybrids, revealing four distinct groups (Fig. 1). Group A consisted of genotypes demonstrating relatively consistent performance under both normal temperature and heat stress conditions. Group B comprised accessions exhibiting superior performance under normal conditions, while Group C consisted of genotypes displaying high performance under stress conditions. Conversely, Group D included genotypes with lower performance across both conditions (Fig. 1). The least TOL values were observed for G7 (1.95), followed by G21 (2.16) and G8 (6.20), whereas G14 and G2 revealed higher TOL values of 26.01 and 25.93, respectively. The genotypes G7, G21 and G8 exhibited the most negligible value for TOL and were considered heat-tolerant (Table 1, Fig. 1). The G21 genotypes are low yielding but revealed lower TOL values due to minimum yield difference under normal and heat stress conditions. Based on MP and GMP index, the genotypes G15, G16, G19 and G3 were identified as heat-tolerant, while the G21 and G23 were considered heat-susceptible genotypes (Table 1, Fig. 1).

The HM index revealed that the G15, G16, G19, G3 and G7 are heat tolerant, while the G23, G21 and G12 were recognized as heat-sensitive genotypes (Table 1 and Fig. 1). The G7, G21, G15 and G8 were the most heat tolerant genotypes, whereas the G12, G14, G2, G17 and G5 were the most heat susceptible genotypes. The G15 (1.71), G16 (1.24), G3 (1.00), G19 (1.00) and G7 (0.95) exhibited higher values for STI index and were declared as heat resistant and high yield genotypes (Table 1 and Fig. 1). The G21, G23 and G12 have been recognized as heat-sensitive genotypes with a low value of STI index. The YI index revealed that G15, G16, G7 and G3 are heat tolerant genotypes while G12, G22 and G23 are heat sensitive genotypes. The genotypes G8 and G21 were heat-tolerant due to higher values for the YSI index (Table 1 and Fig. 1). Based on the RSI index, the G7, G21, G15 and G8 are the most heat tolerant genotypes, whereas G12, G2 and G14 are heat susceptible genotypes (Table 1).

Table 1: Mean Seed cotton Yield (g) under non-stress and heat stress conditions and measures of different screening indices for 23 cotton genotypes

Genotype Code	Yp	Ys	RC	TOL	MP	GMP	HM	SSI	STI	YI	YSI	RSI
G1	45.500	34.295	24.626	11.205	39.898	39.502	39.111	0.857	0.652	0.984	0.754	1.058
G2	57.200	31.265	45.341	25.935	44.233	42.289	40.431	1.577	0.747	0.897	0.547	0.767
G3	59.150	40.620	31.327	18.530	49.885	49.017	48.164	1.090	1.004	1.165	0.687	0.964
G4	47.450	38.020	19.874	9.430	42.735	42.474	42.215	0.691	0.754	1.091	0.801	1.125
G5	44.350	28.700	35.287	15.650	36.525	35.677	34.849	1.227	0.532	0.823	0.647	0.908
G6	41.500	31.485	24.133	10.015	36.493	36.147	35.805	0.839	0.546	0.903	0.759	1.065
G7	48.800	46.845	4.006	1.955	47.823	47.813	47.803	0.139	0.955	1.344	0.960	1.347
G8	44.300	38.095	14.007	6.205	41.198	41.081	40.964	0.487	0.705	1.093	0.860	1.207
G9	45.550	36.320	20.263	9.230	40.935	40.674	40.415	0.705	0.691	1.042	0.797	1.119
G10	47.700	32.165	32.568	15.535	39.933	39.170	38.422	1.133	0.641	0.923	0.674	0.946
G11	48.600	30.175	37.912	18.425	39.388	38.295	37.233	1.319	0.613	0.866	0.621	0.871
G12	47.250	23.710	49.820	23.540	35.480	33.471	31.575	1.733	0.468	0.680	0.502	0.704
G13	43.500	31.600	27.356	11.900	37.550	37.076	36.607	0.951	0.574	0.907	0.726	1.020
G14	57.200	31.190	45.472	26.010	44.195	42.238	40.368	1.582	0.745	0.895	0.545	0.765
G15	67.650	60.725	10.237	6.925	64.188	64.094	64.001	0.356	1.716	1.742	0.898	1.260
G16	64.150	46.525	27.475	17.625	55.338	54.631	53.934	0.956	1.247	1.335	0.725	1.018
G17	52.800	32.790	37.898	20.010	42.795	41.609	40.456	1.318	0.723	0.941	0.621	0.872
G18	46.550	34.775	25.295	11.775	40.663	40.234	39.810	0.880	0.676	0.998	0.747	1.049
G19	55.950	42.910	23.307	13.040	49.430	48.998	48.570	0.811	1.003	1.231	0.767	1.076
G20	46.700	28.680	38.587	18.020	37.690	36.597	35.536	1.342	0.560	0.823	0.614	0.862
G21	31.900	29.735	6.787	2.165	30.818	30.798	30.779	0.236	0.396	0.853	0.932	1.308
G22	44.250	26.455	40.215	17.795	35.353	34.215	33.113	1.399	0.489	0.759	0.598	0.839
G23	37.200	24.600	33.871	12.600	30.900	30.251	29.616	1.178	0.382	0.706	0.661	0.928

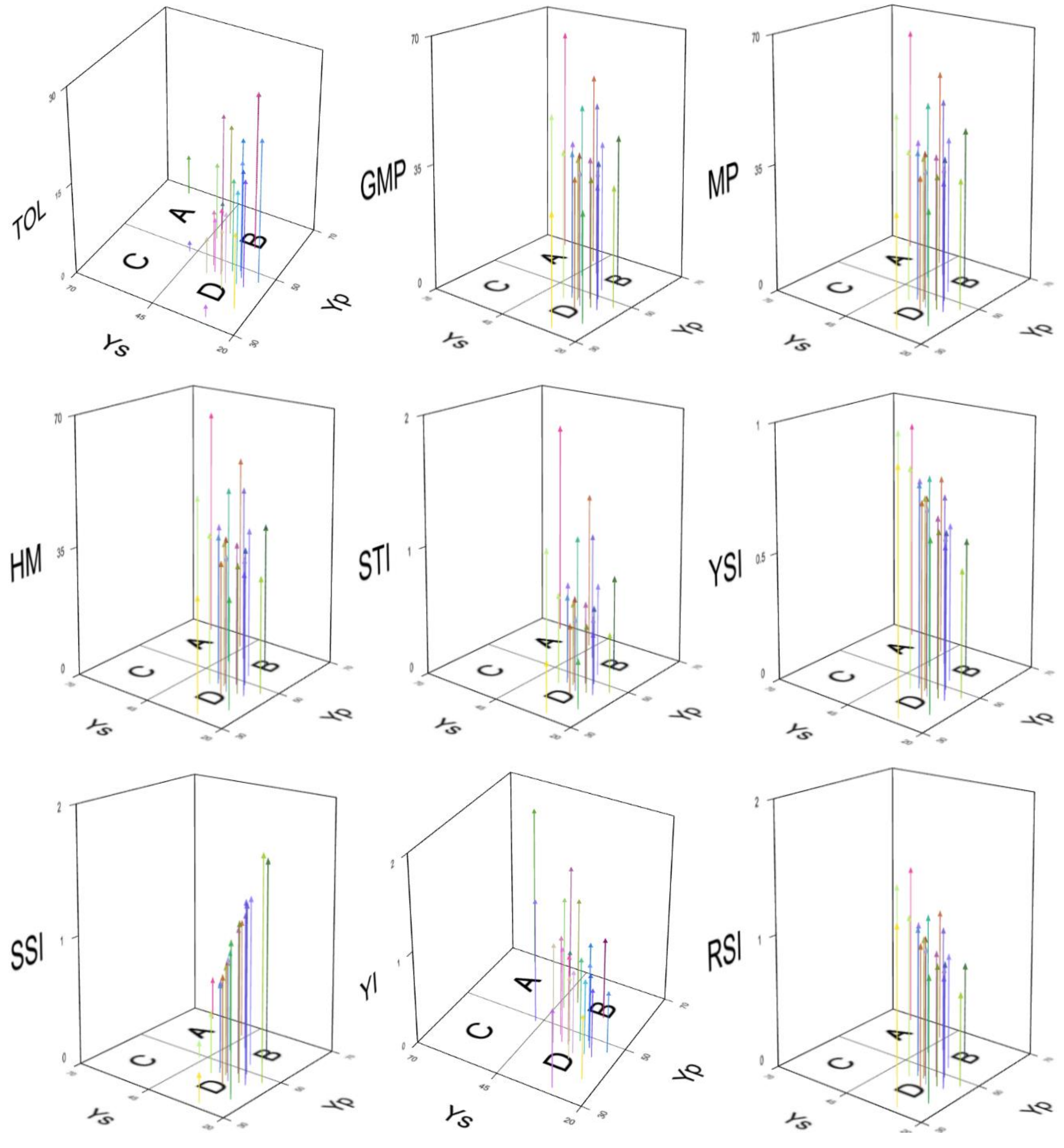

Fig. 1: Three-dimensional scatter plots were generated based on seed cotton yield under normal conditions (YP) and seed cotton yield under heat stress (YS), along with TOL, MP, GMP, STI, SSI, RSI, HM, YSI, and YI. The genotypes classified as Group A exhibited relatively consistent performance across both normal temperature and heat stress conditions. Group B consisted of accessions showing higher performance under normal conditions, while Group C comprised genotypes performing well under stress. In contrast, Group D comprised genotypes with lower performance across both conditions.

Hierarchical Clustering and Ranking based on Stress Indices

Based on stress indices, cluster analysis divided the 23 cotton genotypes into three groups with 9, 9 and 5 genotypes, respectively. The first group consists of G1, G18, G10, G6, G13, G4, G9, G8, and G21. The 2nd group contained G2, G14, G17, G5, G11, G20, G22, G23 and G12. The 3rd cluster consists of 5 genotypes, namely G3, G16, G19, G7 and G15 (Fig. 2). Based on various stress indices, the most heat tolerant genotypes are G15, followed by G7, G19, G16 and G3, respectively (Fig. 2).

The estimated indicators of heat tolerance revealed that selecting stress-tolerant genotypes by using single criterion is contradictory. Different indices introduced various genotypes as heat resistance. All indices' mean rank and standard deviation of all heat tolerance criteria were calculated to determine the most desirable heat-tolerant genotypes (Fig. 3 and Table 2). Based on ranked hierarchical clustering, the genotypes G15 and G7 were highly heat-tolerant as they revealed the best mean rank and almost low standard deviation of rank, while cultivars G12, G22, G23, G21 and G5 as the most sensitive genotypes (Fig. 3 and Table 2). Previous studies reported that our genotype's heat tolerance ranking agreed with the indices.

Table 2: Rank, rank mean (AR), the standard deviation of ranks (SD) and rank sum (SR) of stress resistance/tolerance indices

Genotype Code	Yp	Ys	TOL	MP	GMP	HM	SSI	STI	YI	YSI	RSI	SR	AR	SD
G01	16	10	8	14	13	13	9	13	10	9	9	124	11.273	2.611
G03	3	5	19	3	3	4	13	3	5	13	13	84	7.636	5.732
G04	11	7	6	9	6	6	5	6	7	5	5	73	6.636	1.859
G05	17	19	14	18	19	19	16	19	19	16	16	192	17.455	1.753
G06	21	14	7	19	18	17	8	18	14	8	8	152	13.818	5.212
G07	8	2	1	5	5	5	1	5	2	1	1	36	3.273	2.412
G08	18	6	3	10	10	7	4	10	6	4	4	82	7.455	4.367
G09	15	8	5	11	11	10	6	11	8	6	6	97	8.818	3.060
G10	10	12	13	13	14	14	14	14	12	14	14	144	13.091	1.300
G11	9	17	18	15	15	15	18	15	17	18	18	175	15.909	2.663
G12	12	23	21	20	21	21	23	21	23	23	23	231	21.000	3.194
G13	20	13	10	17	16	16	11	16	13	11	11	154	14.000	3.194
G14	4	16	23	7	8	11	22	8	16	22	22	159	14.455	7.133
G15	1	1	4	1	1	1	3	1	1	3	3	20	1.818	1.168
G16	2	3	15	2	2	2	12	2	3	12	12	67	6.091	5.356
G17	7	11	20	8	9	8	17	9	11	17	17	134	12.182	4.644
G18	14	9	9	12	12	12	10	12	9	10	10	119	10.818	1.662
G19	6	4	12	4	4	3	7	4	4	7	7	62	5.636	2.580
G2	4	15	22	6	7	9	21	7	15	21	21	148	13.455	7.048
G20	13	20	17	16	17	18	19	17	20	19	19	195	17.727	2.054
G21	23	18	2	23	22	22	2	22	18	2	2	156	14.182	9.806
G22	19	21	16	21	20	20	20	20	21	20	20	218	19.818	1.401
G23	22	22	11	22	23	23	15	23	22	15	15	213	19.364	4.411

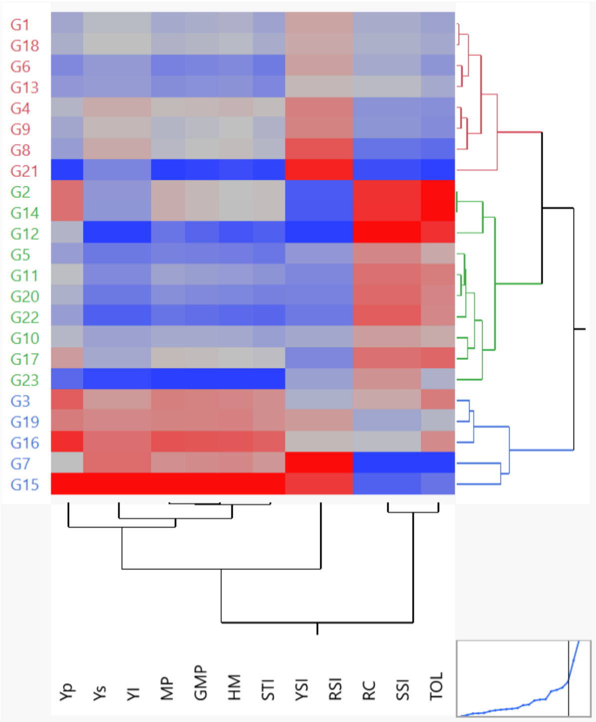

Fig. 2: There is hierarchical clustering of cotton genotypes for the seed cotton yield across YP and YS conditions; along with TOL, MP, GMP, STI, SSI, RSI, HM, YSI, and YI.

Biplot Analysis

Several studies suggested using a combination of stress indices to select stress-tolerant genotypes. Hence, PCA was conducted, and a biplot was generated as a more effective method compared to correlation coefficients to determine the optimal indices for selecting high-yielding genotypes under both normal and heat stress conditions. A PCA biplot for seed cotton yield (normal and heat stress conditions) and different stress indices (Fig. 4). The first two PCs revealed more than one eigenvalue and explained 99.60% of the total variation.

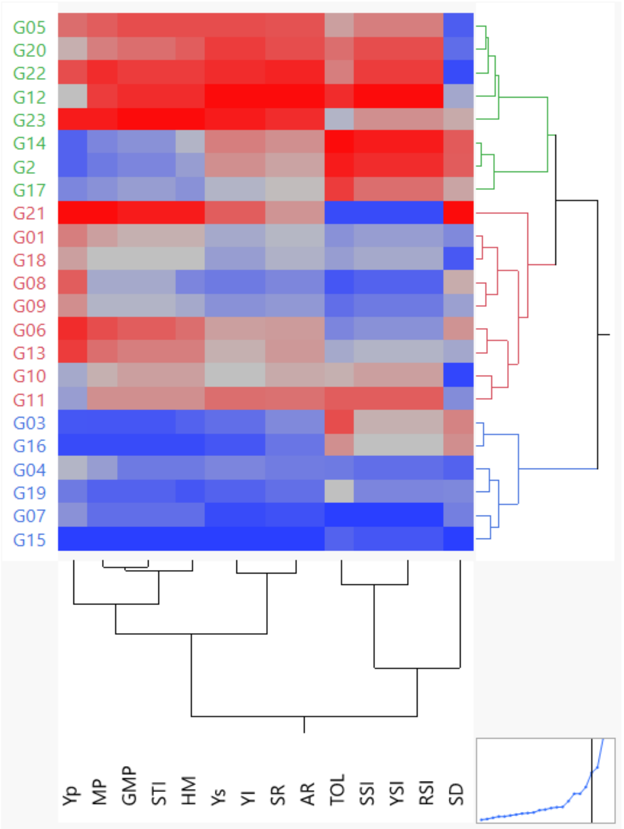

Fig. 3: Two-way clustering based on the ranking of cotton genotypes for YP, YS, TOL, MP, GMP, HM, STI, YSI, SSI, YI and RSI.

The first PC1 contributed 67.44% variation with positive loading factors; Ys (0.99), YI (0.99), HM (0.91), STI (0.89), GMP (0.88), MP (0.84), YSI (0.75), RSI (0.75) and Yp (0.55). The PC2 accounted for (32.16%) of the total variation with positive loading factors; Yp (0.83), TOL (0.82), SSI (0.66), RC (0.66), MP (0.53), GMP (0.48), STI (0.45), and HM (0.41) (Table S4 and S5). The biplot of PC1 and

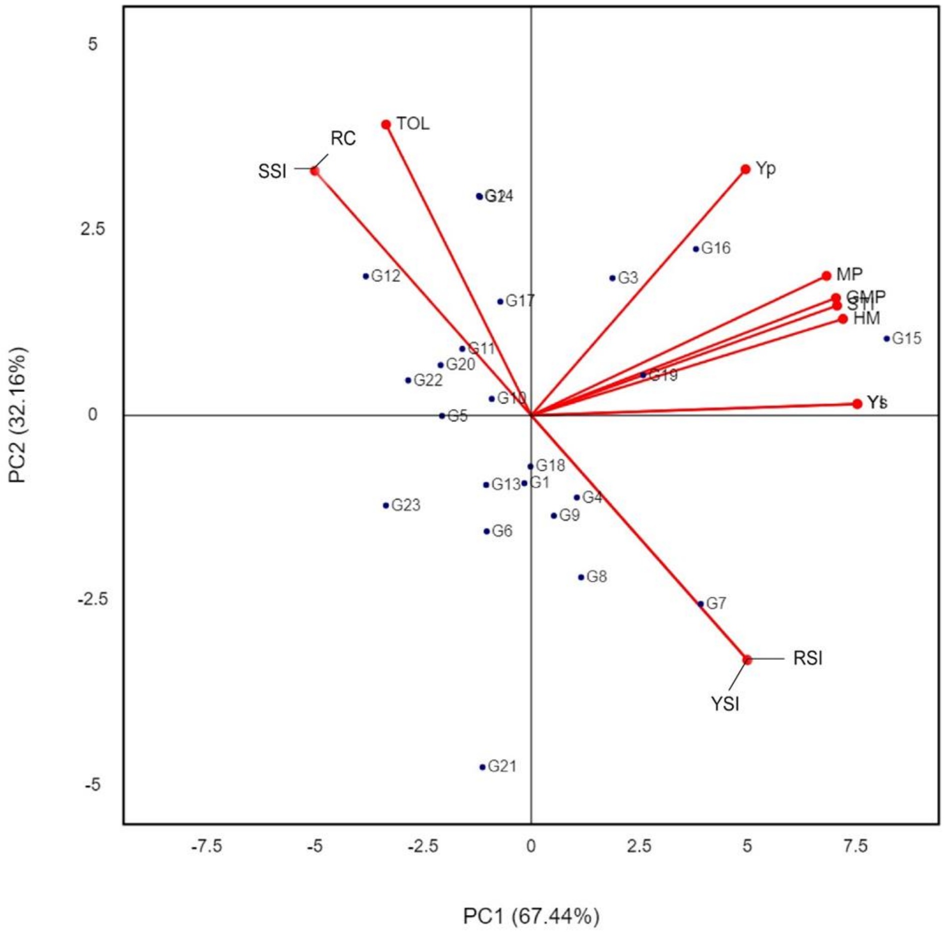

Fig. 4: Biplot between PC1 and PC2 displaying the distribution of 23 cotton genotypes for YP and YS, along with TOL, MP, GMP, HM, STI, YSI, SSI, YI and RSI.

PC2 explained (99.606%) of the total variation and the Ys, Yp, YI, HM, GMP, MP, and STI exhibited long vectors and lay closed to each towards the direction of PC1 and exhibited a strong positive correlation among themselves (Fig. 4). Selection based on these indices might help find out heat-tolerant cotton genotypes. The YSI and RSI also lay close to each other and are positively associated. The TOL, RC and SSI had long vectors lay close to each other towards the direction of PC2 and showed a strong positive association among themselves (Fig. 4). The PCA results revealed that the first two components (PC1 and PC2) contributed 67.44% and 32.16% variability to the total variation.

Based on correlation and biplot analysis, MP, GMP, HM STI and YI are the most suitable heat indices to identify high-yielding and heat tolerant cotton genotypes under normal and heat stress conditions. These stress indices revealed that the G15, G16, G19, G3 and G7 are the most heat tolerant genotypes, while the G23, G21 and G12 are heat sensitive genotypes.

Validation of Ranking with Physiological and Biochemical Indicators

The GMP of all physiological traits directly related to yield under heat stress conditions and the correlation of GMP of physiological traits with STI, YI, and AR were assessed to validate our ranking based on yield indices. The results revealed that the GMP of ROS, CAT, POD, TSP, Chl. a and b, and carotenoids were strongly related to STI, YI, and STI, whereas the AR showed a negative correlation with GMP of all physiological characters and other stress indices (Table 3). Interestingly all the physiological traits revealed positive associations among themselves. The negative correlation of AR with YI and STI confirmed our ranking based on the different yields mentioned above indices (Table 3).

DISCUSSION

Temperature is an important key factor in determining the growth and production of any crop (Yan et al., 2013). The cotton crop is more sensitive to heat stress, especially in the reproductive phase. The study investigated cotton genotypes and revealed significant differential responses at the morphological and physiological levels under stress conditions (Zafar et al., 2022b). Previous studies evaluated germplasm against heat stress based on high yield performance in normal and stress conditions (Clarke et al., 1992) and significant reductions were observed in SCY due to decreased boll retention and boll weight (Sharif et al., 2019). According to the genotype, the plants confront heat stress in various ways (Poudel et al., 2021).

This study calculated different heat-tolerant indices (TOL, MP, GMP, HM, SSI, STI, YI, RSI, and YSI) based on SCY to investigate the heat-tolerant cotton genotypes for the heat-prone areas. The heat-tolerant genotypes revealed minimum values for TOL and SSI, whereas higher values for MP, GMP, HM, STI, YI, RSI, and YSI under normal and stressed conditions. The low TOL observed in the current study might be related to higher resistance to stress and selection based on this index can be useful in selecting high-yielding cultivars under stress conditions (Rosielle and Hamblin, 1981). Interestingly, the G21 genotype is low-yielding but revealed lower TOL values due to minimum yield difference under normal and heat stress conditions. Therefore, a low TOL value doesn't mean high yielding; genotype yield should also be considered (Bahrami et al., 2021; Khodarahmpour et al., 2011). Limitations regarding the TOL index were also reported by previous studies on barley and wheat (Tahir et al., 2022).

Table 3: Relationship of various physiological indicators with stress tolerance indices and validation of average ranking

	POD	TPC	CAT	Chla	Chlb	Car	STI	YI	AR
POD	1.000**	0.919**	0.759**	0.885**	0.828**	0.856**	0.874**	0.787**	-0.698**
TSP	0.919**	1.000**	0.837**	0.879**	0.875**	0.867**	0.898**	0.825**	-0.721**
CAT	0.759**	0.837**	1.000**	0.758**	0.865**	0.828**	0.801**	0.666**	-0.566*
Chla	0.885**	0.879**	0.758**	1.000**	0.877**	0.855**	0.851**	0.820**	-0.799**
Chlb	0.828**	0.875**	0.865**	0.877**	1.000**	0.926**	0.865**	0.787**	-0.710**
Car	0.856**	0.867**	0.828**	0.855**	0.926**	1.000**	0.813**	0.698**	-0.580*
STI	0.874**	0.898**	0.801**	0.851**	0.865**	0.813**	1.000**	0.947**	-0.810**
YI	0.787**	0.825**	0.666**	0.820**	0.787**	0.698**	0.947**	1.000**	-0.928**
AR	-0.698**	-0.721**	-0.566*	-0.799**	-0.710**	-0.580*	-0.810**	-0.928**	1.000**

*= Significance (α=0.05), **= Highly Significant (α=0.01);

Table S1: List of genotypes used in this experiment

Genotype	Lines	Genotype	Tester	Genotype	Crosses	Genotype	Crosses	Genotype	Crosses
G1	NIAB-1048	G6	CRS-2	G9	NIAB-1048 × CRS-2	G14	NIAB-1048 × Shahkar	G19	NIAB-1048 × FH-313
G2	IUB-013	G7	Shahkar	G10	IUB-013 × CRS-2	G15	IUB-013 × Shahkar	G20	IUB-013× FH-313
G3	IUB-65	G8	FH-313	G11	IUB-65 × CRS-2	G16	IUB-65 × Shahkar	G21	IUB-65 × FH-313
G4	VH-329			G12	VH-329 × CRS-2	G17	VH-329× Shahkar	G22	VH-329 × FH-313
G5	FH-458			G13	FH-458 × CRS-2	G18	FH-458× Shahkar	G23	FH-458 × FH-313

Table S2: Average relative humidity outside and inside the tunnel

Days	Rain Fall (mm)	Avg. Relative Humidity %		Sun Shine (hours)
		Outside tunnel	Inside tunnel	
13-Sep	0.0	68	73	10.0
14-Sep	0.0	80	85	8.0
15-Sep	0.0	68	80	9.8
16-Sep	4.4	72	84	9.5
17-Sep	2.2	79	89	8.5
18-Sep	0.0	68	88	10.3
19-Sep	0.0	73	86	9.0
20-Sep	0.0	78	83	5.5
21-Sep	0.0	69	87	10.0
22-Sep	0.0	70	90	8.0
23-Sep	0.0	77	93	9.0
24-Sep	0.0	67	88	9.5

Table S3: Different Tolerance Indices used for evaluation of 23 Cotton genotypes for heat stress with formula and reference

Stress indices	Formula	Pattern of selection	Reference
TOL	$TOL = Y_P - Y_S$	Minimum value	(Rosielle and Hamblin 1981)
MP	$MP = \frac{Y_P + Y_S}{2}$	Maximum value	(Rosielle and Hamblin 1981)
GMP	$GMP = \sqrt{Y_S \times Y_P}$	Maximum value	(Fernandez 1992)
HM	$HM = \frac{2(Y_S \times Y_P)}{(Y_S + Y_P)}$	Maximum value	(Bidinger et al. 1987)
SSI	$SSI = \frac{1-(Y_S/Y_P)}{1-(\bar{Y}_S/\bar{Y}_P)}$	Maximum value	(Fischer and Maurer 1978)
STI	$STI = \frac{Y_S \times Y_P}{(\bar{Y}_P)^2}$	Minimum value	(Fernandez 1992)
YI	$YI = \frac{Y_S}{\bar{Y}_s}$	Maximum value	(Gavuzzi et al. 1997)
YSI	$YSI = \frac{Y_S}{Y_P}$	Maximum value	(Bouslama and Schapaugh Jr 1984)
RSI	$RSI = \frac{(Y_S/Y_P)}{(\bar{Y}_S/\bar{Y}_P)}$	Maximum value	(Fischer and Maurer 1978)

Table S4: Eigenvalue for yield and different stress indices for studied genotypes

Number	Eigenvalue	Percent	Cum Percent	Chi-Square	DF
1	7.9211	66.009	66.009	501.777	63.247
2	4.0316	33.596	99.606	328.605	65.459
3	0.0376	0.314	99.919	.	60.439
4	0.0084	0.07	99.989	.	49.224
5	0.0013	0.011	100	.	40.04
6	0	0	100	.	31.772

Table S5: Loading Matrix for yield and different stress indices for studied genotypes

Parameters	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6
Yp	0.5552	0.82893	0.06738	-0.00063	0.00942	0.00003
Ys	0.98577	0.16318	-0.0351	-0.01641	0.01122	0.00003
RC	-0.75306	0.65608	-0.04905	-0.00786	0.0007	0
TOL	-0.55397	0.82242	0.12782	0.0199	-0.00245	0.00001
MP	0.84248	0.53825	0.01716	-0.00936	0.01127	0.00003
GMP	0.8795	0.47541	0.00493	-0.01873	-0.00905	-0.00024
HM	0.90911	0.41478	-0.0063	-0.0269	-0.02671	0.00013
SSI	-0.75306	0.65608	-0.04905	-0.00786	0.0007	0
STI	0.88563	0.45307	-0.06572	0.07777	-0.00435	0
YI	0.98577	0.16318	-0.0351	-0.01641	0.01122	0.00003
YSI	0.75306	-0.65608	0.04905	0.00786	-0.0007	0
RSI	0.75306	-0.65608	0.04905	0.00786	-0.0007	0

Similar results were also reported in wheat and maize (Dorostkar et al., 2015; Kamrani et al., 2018; Khodarahmpour et al., 2011).

The SSI index showed that the G7, G21, G15 and G8 were the most heat tolerant genotypes, whereas the G12, G14, G2, G17 and G5 were the most heat susceptible genotypes. Different studies suggested that SSI is used to determine stress-tolerant cotton genotypes (Anwar et al., 2011; Yehia, 2020). The SSI recognizes only those cultivars having the least differences under normal and stress conditions (Fischer and Maurer, 1978). The value of SSI more than one suggests above-average susceptibility to heat stress (Saed-Moucheshi et al., 2022; Shojaei et al., 2022). Also, SSI is a powerful criterion for selecting maize genotypes under severe drought stress. Sánchez-Reinoso et al. (2020) also reported that the low value of SSI indices can develop water-deficit tolerant common bean cultivars.

Based on MP and GMP index, the genotypes G15, G16, G19 and G3 were identified as heat-tolerant, while the G21 and G23 were considered heat susceptible genotypes. (Kamrani et al., 2018) The selection based on MP and GMP would identify high-yielding heat-tolerant genotypes. (Etminan et al., 2019) The MP and GMP are efficient indices for screening drought-tolerant wheat genotypes. Our results showed contradiction (Devi et al., 2021), who reported that the combination of MP, GMP, and SSI is helpful for the selection of heat-tolerant wheat genotypes. The HM index revealed that the G15, G16, G19, G3 and G7 are heat tolerant, while the G23, G21 and G12 were identified as heat-sensitive genotypes. Our results were in agreement with the findings of (Yehia, 2020). The G15, G16, G3, G19 and G7 exhibited higher STI index values and were declared heat resistant and high yield genotypes. (Menezes et al., 2014) reported that a genotype's high value of STI is related to its tolerance against stress. Our results were in agreement with (Kumawat et al., 2017), who reported a similar rank for lentil genotypes selected based on MP, GMP and STI under salt stress. The genotypes G8 and G21 were heat-tolerant due to higher values for the YSI index. The higher value of YSI is helpful to identify heat-tolerant genotypes (Anwar et al., 2011; Poudel et al., 2021).

Based on the RSI index, the G7, G21, G15 and G8 are the most heat tolerant genotypes, whereas G12, G2 and G14 are heat susceptible genotypes. In our study, YSI and RSI revealed a similar ranking pattern in the characterization of heat-tolerant genotypes, which was harmonious with the findings of (Pour-Aboughadareh et al., 2019). The correlation between Yp and Ys with MP, GMP, HM, STI, and YI indicates that these heat indices have the potential to identify high-yielding cotton genotypes under both normal and heat stress conditions (Singh et al., 2016; Mau et al., 2019). El-Sabagh et al. (2018) also found a similar pattern correlation among grain yield of maize and the indices mentioned above under drought stress. Interestingly, Ys also revealed a strong positive association with YSI and RSI. The negative relationship among TOL and SSI also reported by Sánchez-Reinoso et al. (2020). The Yp was negatively related to YSI and RSI, whereas Ys showed a higher negative correlation with RC, TOL and SSI. The indices revealing a negative association with Ys and Yp are unsuitable for selecting resistant cultivars under stressed and normal conditions (Mitra, 2001; Saed-Moucheshi et al., 2022). According to all indices, the mean rank and standard deviation, the most desirable heat-tolerant cultivars were identified (Aliakbari et al., 2014).

Based on ranked hierarchical clustering, the genotypes G15 and G7 were highly heat-tolerant as they revealed the best mean rank and almost low standard deviation of rank. Previous studies reported that our genotype's heat tolerance ranking agreed with the indices (Ayed et al., 2021; Bakhshi and Shahmoradi, 2022). The ranking method is also used to screen other crops like wheat, maize and potato under stress conditions (Khalili et al., 2012; Abd El-Mohsen et al., 2015; Hossain et al., 2017).

Different studies purposed various physiological indicators like CAT, POD, carotenoids, total soluble proteins and chlorophyll (a and b) to select tolerant genotypes in stress breeding programs (Kumari et al., 2021; Manan et al., 2021). But at earlier stages of stress breeding programs, it is not easy to measure the physiological traits of the large population. Therefore, different breeders suggested yield as a good criterion for selecting tolerant genotypes. The physiological indicators' ranking confirmed our different yield-related stress indices. The higher value of GMP, STI and YI is negatively correlated with AR, revealing that the genotypes have high GMP values, STI and YI are heat tolerant. The up-regulation of antioxidants and defensive enzymes such as peroxidase POD and catalase CAT trigger the plants to respond to metabolize ROS. Different studies suggested that SCY is positively associated with CAT, POD, carotenoids, total soluble proteins and chlorophyll (a and b) under heat-stress conditions (Farooq et al., 2020).

Conclusions

In the current study, heat stress imposed at the flowering stage resulted in high indices reflecting that heat significantly affects productivity by affecting the crop. By combining multiple indices, the average sum of ranks and low standard deviation of rank were recorded for G15, G16, G19 and G3, indicating them as consistently heat-tolerant genotypes. Under heat stress, all stress tolerance indices have shown a significantly strong positive association except for RC, TOL and SSI with yield, reflecting that they are a better predictor for selecting a genotype with a higher yield potential under heat stress conditions. The ranking based on physiological indicators confirmed our ranking with different yield-related stress tolerance indices. This technique has been found to be a reliable screening method in the preliminary evaluation of genotypes with high yield performance under heat stress, efficiently assigning genetic variability in the genotypes with heat tolerance. The prime advantage of the technique can be its ability to screen genotypes rapidly at very early generations. Moreover, it can also efficiently help breeders narrow down the number of tolerant genotypes for further selection, saving a significant amount of energy, labor, resource, and time spent for massive selection from a large number of individuals in populations used for selection. Hence, the outcome of this study in terms of generated information and selected genotypes can be efficiently utilized by breeding programs aimed at developing varieties suitable for cultivation across high-temperature areas without the risk of losing productivity.

Ethics Approval and Consent to Participate

Consent for Publication

All of the authors declare their consent for publication in this journal.

Availability of Data and Materials

Data used to conclude the results is attached here to as supplementary files and the other is available from the corresponding authors on reasonable request.

Competing Interests

The authors declare no conflict of interest

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Authors' Contributions

Conceptualization, M.M.Z, and A.S.; methodology, M.M.Z., M.S.1 and Z.S.; software, M.S.I., and A.S..; validation, M.M.Z., A.I.K, and A.R.; formal analysis, M.M.Z., A.I.K and HK..; investigation, M.M.Z.; data curation, M.M.Z., and A.S..; writing—original draft preparation, M.M.Z. and A.R..; writing—review and editing, A.S., Z.S. and A.I.K; visualization, M.M.Z., and M.S.I; supervision, A.S., All authors have read and approved the final version for publication.

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