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RESEARCH ARTICLE

Deciphering heat stress tolerance indices for identifying terminal heat-tolerant bread wheat genotypes

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Abstract

High temperatures during the grain-filling period are a significant constraint in wheat production. Effective selection criteria help plant breeders utilize genetic variation more efficiently, improving stress tolerance in wheat. This study intends to investigate bread wheat genotypes' heat stress tolerance indices to identify and select heat stress-tolerant wheat genotypes. This study assessed 48 bread wheat genotypes during the wheat growing seasons 2021 and 2022 under both optimum and heat stress conditions. Twelve different stress indices were calculated, followed by correlation analysis, principal component analysis (PCA), cluster analysis and Multi-Trait Genotype-Ideotype Distance Index (MGIDI) analysis, all performed using the stress indices. Analysis of variance results showed that genotypes differed significantly for each stress index examined in the study. The significant drop in average grain yield across all genotypes under stress compared to optimal conditions indicates a considerable effect of heat stress on grain production. The results of the correlation, PCA and MGIDI analyses revealed that mean productivity (MP), geometric mean productivity (GMP), harmonic mean (HM) and mean relative performance (MRP) were key discriminating indices in explaining heat stress tolerance among 48 wheat genotypes. Principal component, cluster analysis and MGIDI results were used to draw the inference that GS/2019-20/6046, GS-2018-19/1007, HPYT-2019-20/416, SAWYT-2018-19/309 and GS/2019-20/5042 show high yielding indices and are suitable under heat stress environment. Thus, the mentioned genotypes hold promise for cultivation in high-temperature environments or as genetic reservoirs for integrating genetic variants into wheat genotypes, enhancing their resilience to heat stress.

Keywords: bread wheat; cluster analysis; correlation analysis; heat stress tolerance; MGIDI; principal component analysis

Abbreviations

ANOVA: Analysis of variance; GMP: Geometric mean; HM: Harmonic mean; MGIDI: Multi-trait genotype-ideotype distance index; MP: Mean productivity; MRP: Mean relative performance; NS: Non-significant; PC: Principal component; PYR: Percent yield reduction; RSI: Relative stress index; SSPI: Stress susceptibility percentage index; STI: Stress tolerance index; TOL: Tolerance index; YI: Yield index; Yp: Grain yield of genotypes under normal condition; Ys: Grain yield of genotypes under heat stress condition; YSI: Yield stability index.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops globally, occupying more agricultural area than any other type of food crop globally. Wheat is a staple food and an essential feed source for over 4.5 billion people worldwide (1). In India, wheat contributes approximately 35 % of total food grain production. This golden grain winter cereal is cultivated across diverse agro-climatic conditions in India, encompassing an area of 31.76 million hectares, which accounts for 14.17 % of the global area (2). In the 2022-2023 season, India recorded wheat production of 108.75 million tons with an average productivity of 3424 kg/ha. Currently, wheat production exceeds demand. However, climate change and population growth threaten food security and shift production dynamics (3). Climate change has led to more frequent extreme temperatures characterized by reduced rainfall, altered rainfall patterns, dispersal and shorter winter seasons. One of the most damaging abiotic stresses on crop plants affects their growth development potential. Continued changes in global climatic conditions are expected to exacerbate high-temperature stress, further constraining the productivity of crucial crops like wheat (5). According to climate projections, the average temperature may rise by 1-4 °C by the end of the 21st century, potentially resulting in a 4.1-6.4 % decrease in wheat output (6, 7). Elevated temperatures substantially hinder plant growth and development, affecting physiological processes, grain formation and yield (8, 9). Moreover, heat stress negatively impacts several metabolic activities, including the production of proteins, the physiological processes and the inactivation of enzymes within cells and the deterioration of cell membranes. Cell division is adversely affected by heat stress as well (3). Temperatures between 22 and 25 °C are ideal for wheat from the flowering to the grain maturity stage; deviations from this range may cause pollen sterility, shrivelled grains and reduced yield (10). High temperatures of 25-32 °C during the flowering and grain-filling stages might produce early crop maturation with late planting of wheat genotypes, which can significantly reduce grain production (11). The significance of heat stress in reducing yield was initially emphasized. Research indicates that wheat production in India is a gamble with temperature (12). This assertion remains relevant today. It is estimated that wheat output will need to increase by 60 % to meet the growing populations' demands, which is expected to reach 9 billion people by the year 2050 (13). As a result, it will be crucial to breed wheat types that can withstand high temperatures to boost productivity and meet the worlds' food needs (14). Hence, increasing yields by at least 1.6 % annually is imperative while enhancing tolerance to abiotic and biotic stresses (15). However, meeting this demand faces a significant hurdle in the form of high-temperature stress during crucial wheat growth stages, notably the grain-filling stage. Achieving this goal necessitates the development of high-yielding, climate-smart wheat varieties resilient to abiotic stressors through rigorous selection in real-world field conditions. Therefore, identifying genotypes resistant to heat is essential for breeders. Plant breeders can create stress-tolerant wheat varieties by using genetic variation among various crop genotypes under normal and stressed conditions (16, 17). Therefore, devising a practical selection approach remains a significant challenge for breeders in identifying heat-tolerant cultivars.

Although researchers have offered many stress tolerance indicators, only a few have shown to be very helpful in selecting heat-tolerant wheat genotypes. The difference between grain yield under stress and normal conditions was characterized as the tolerance index (TOL) (18). The average genotype yield under both stress (Ys) and normal (Yp) circumstances is known as the mean productivity index (MP) (18). The Stress Tolerance Index (STI) detects tolerant genotypes in standard and heat-stressed environments (19). The geometric mean production index serves as its foundation. The stress susceptibility percentage index (SSPI) has been proposed to assess trait stability and variation under stress and non-stress conditions (20). The relative stress index (RSI) was first introduced in the context of drought stress in wheat cultivars (21). Since harmonic mean (HM) is the ratio of a doubling product of genotypes yield and their total under both conditions, (22) employed it on wheat–rye disomic addition lines. Another measure of stress indices is the mean relative performance (MRP). The yield ratio under stress to the yield under normal circumstances was used to compute the yield stability index, or YSI (23). The yield index (YI) was first defined as the ratio of the genotypes' yield under normal circumstances to the average yield of all genotypes under stress (24). Percent Yield Reduction (PYR) was employed in a study on minimal phosphorus stress in rice (25). It is best to select stable and tolerant genotypes using high STI, MP, YSI, GMP, HM and YI values and low values for RSI, TOL, SSI, PYR and HSI (26).

This study assessed 48 wheat genotypes across varied environmental conditions, including normal sowing and late sowing. We noted that during late sowing, the yield decreased due to elevated temperatures, roughly 3-4 °C higher than those experienced during normal sowing, occurring during the stages of anthesis and grain filling of plant development. To identify and choose heat-tolerant genotypes, eleven stress indices were used: mean relative performance (MRP), stress tolerance index (STI), mean productivity (MP), harmonic mean (HM), geometric mean (GMP), yield index (YI), relative stress index (RSI), stress susceptibility percentage index (SSPI), yield stability index (YSI), percent yield reduction (PYR), tolerance index (TOL) and heat susceptible index (HSI). The current investigation aimed to evaluate the variations in different stress indices that contribute to yield between heat tolerant and heat sensitive genotypes under both optimum and heat stress conditions. Additionally, the study aimed to determine the essential stress indices that might be useful in breeding and selecting heat-tolerant wheat using correlation coefficients. Furthermore, heat tolerant wheat lines were identified for breeding future heat tolerant cultivars using diverse multivariate approaches, including principal component analysis, cluster analysis and the Multi-trait Genotype-Ideotype Distance Index (MGIDI).

Materials and Methods

Experimental materials

The current studys' experimental materials consisted of 42 wheat germplasm/genotypes, including exotic and indigenous lines with six check varieties *viz,* K 1317 GW 499, HD 2932, LOK 1, GW 173 and GW 11; listed in Table 1. The Wheat Research Station of Navsari Agricultural University, Bardoli, provided the pure seeds of these genotypes.

Location, experimental site and environments

The field trial was conducted at the College Farm of N M College of Agriculture, Navsari Agricultural University (20° 37'N, 72°54'E). The farm is located in the South Gujarat agro-climatic zone, which is characterized by high rainfall, at 11.98 m above mean sea level. Throughout the growing season, the weather remained normal and conducive to optimal crop growth. The genotypes were sown in November 2021 (specifically November 29, 2021) to represent normal sowing conditions and in January 2022 (specifically January 6, 2022) to simulate late sowing conditions during the *Rabi* season of 2021-22.

Table 1. List of wheat genotypes

Sr. No.	Genotype	Sr. No.	Genotype
1.	HTWYT/2019-20/2	**25.**	GS/2019-20/3060
2.	HTWYT/2019-20/8	**26.**	EHT-2018-19/401
3.	HTWYT/2019-20/11	**27.**	EHT-2018-19/403
4.	HTWYT/2019-20/17	**28.**	EHT-2018-19/406
5.	HTWYT/2019-20/30	**29.**	GS/2018-19/7042
6.	HTWYT/2019-20/34	**30.**	GS/2019-20/7004
7.	HTWYT/2019-20/40	**31.**	EHT-2019-20/732
8.	EHT-2018-19/407	**32.**	EHT-2019-20/735
9.	HPYT-2019-20/416	**33.**	GS/2018-19/6027
10.	HPYT-2019-20/418	**34.**	GS/2019-20/4003
11.	HPYT-2019-20/449	**35.**	HTWYT/2018- 19/36
12.	EHT-2018-19/443	**36.**	QST 1910
13.	CWYT 2018-19-630	**37.**	RWP 2019-31
14.	CWYT 2018-19-644	**38.**	DT RIL 110
15.	GS-2018-19/1007	**39.**	WYCYT 2018-20
16.	SAWYT-2018-19/309	**40.**	DT RIL 1
17.	RWP-2019-29	**41.**	GS/2018-19/4049
18.	GS/2019-20/5042	**42.**	WYCYT-2018-13
19.	DBW-166	**43.**	K 1317 ©
20.	GS/2019-20/6046	**44.**	GW 499 ©
21.	HI 1628	**45.**	HD 2932 ©
22.	HTWYT/2019- 20/39	**46.**	LOK 1 ©
23.	GS/2019-20/1003	**47.**	GW 173 ©
24.	GS/2019-20/3056	**48.**	GW 11 ©

© = Check variety

Experimental layout

The experiment was set up in three replications utilizing a Randomized Block Design. The genotypes were seeded in the field after there was enough moisture. There may be roughly 80 plants in three rows (per genotype) if the spacing between plants and rows was kept between 10 and 22.5 cm. To promote the growth of a healthy crop, normal cultural and agronomic procedures were followed for wheat production, along with regular irrigation at suitable intervals.

Environmental evaluation

During the crop growing season (November to May), weather features were collected from the Department of Agricultural Meteorology at NMCA NAU, Navsari (Fig. 1). Based on the meteorological data provided, delaying the sowing date by 38 days was expected to better simulate heat stress conditions compared to sowing at the usual time. Following crop maturity, the genotypes from each row were harvested independently and the amount of grain produced by each plant (measured in grams) was noted.

All stress indices in Table 2 were computed based on grain yield, with Yp and Ys representing genotypes under normal and late-sown conditions, respectively and all genotypes' mean yield (Xp and Xs). Temperatures exceeding 30 °C during March can adversely affect late-sown genotypes' flowering and post-flowering stages (29).

Statistical analysis

Stress indices were computed using Microsoft Excel. Variance and correlation coefficient calculation analysis were conducted using the R software Variability package, edition R 4.1.2. PAST Statistics version 4.06b was utilized to create PCA and identify tolerant and sensitive genotypes. Cluster analysis aimed at identifying genotypes prone to heat stress and those tolerant to it, was also performed using PAST Statistics version 4.06b. The Multi-trait Genotype -Ideotype Distance Index (MGIDI) (30), a novel technique, provides a powerful tool for evaluating the proximity of genotypes to an ideal ideotype across multiple traits. This approach simplifies the selection process for genotypes with desirable characteristics. To compute MGIDI, the 'metan' R package (31) was utilized, using the trait-specific relative values (RVs) for the analysis.

Results

ANOVA for the stress indices and grain yield

The substantial variance ($P < 0.001$) observed among wheat genotypes for grain yield under normal conditions, heat stress and stress indicators, as indicated in ANOVA (Table 3) highlights the genetic diversity of the genotypes.

Stress tolerance indices

This study determined a number of stress indices based on yield under both normal and heat stress conditions, including STI, SSPI, TOL, YI, YSI, RSI, MP, MRP, HM, GMP and

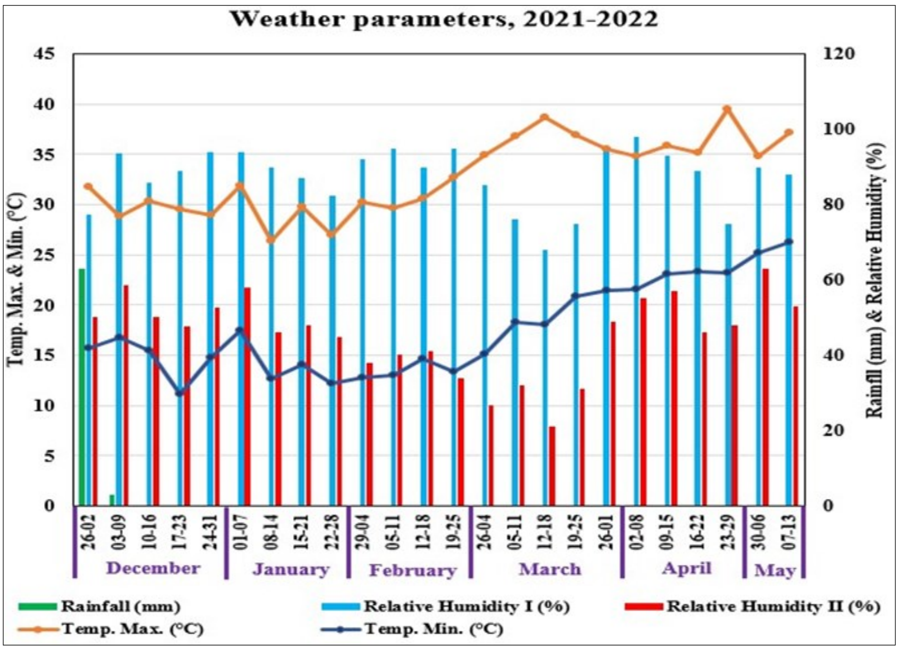

Fig. 1. Weather data on rainfall (mm), humidity and temperature during the 2021-22 crop season.

Table 2. Mathematical formulas for 12 heat tolerance and susceptibility indices

Sr.no	Index	Formula	Pattern of selection MGIDI	Reference
1	Tolerance index	$TOL = Yp - Ys$	Minimum	(18)
2	Stress susceptibility percentage index	$(SSPI) = Yp - Ys/2(Xp \times 100)$	Minimum	(20)
3	Percent yield reduction	$(PYR) = (Yp - Ys)/Yp \times 100$	Minimum	(22)
4	Heat susceptible index	$(HSI) = (1 - Xh/X)/(1 - Yh/Y)$	Minimum	(21)
5	Mean productivity	$(MP) = (Yp + Ys)/2$	Maximum	(18)
6	Geometric mean productivity	$(GMP) = \sqrt{(Ys \times Yp)}$	Maximum	(19)
7	Harmonic mean	$(HM) = 2\ (Yp \times Ys)/(Yp + Ys)$	Maximum	(27)
8	Stress tolerance index	$STI = (Yp \times Ys)/Xp^2$	Maximum	(19)
9	Yield index	$(YI) = Yp/Xs$	Maximum	(24)
10	Yield stability index	$(YSI) = Ys/Yp$	Maximum	(23)
11	Relative stress index	$(RSI) = \left(\frac{Yp}{Ys}\right)/\left(\frac{Xs}{Xp}\right)$	Maximum	(21)
12	Mean relative performance	$(MRP) = Ys/Xs + Yp/Xp$	Maximum	(28)

Yp and Ys denote the yield performance of the varieties, whereas the mean yield of all genotypes under normal and heat stress circumstances is represented by Xp and Xs, respectively; Xh = Average of individual genotype under stress situation; X = Average of individual genotype under

Table 3. Variance analysis for grain yield under stress (Ys) and normal (Yp) conditions for grain yield and heat tolerance indices for wheat genotypes

Source of variation	df	TOL	STI	SSPI	YI	YSI	RSI	MP	GMP	HM	MRP	PYR	HSI	YP	YS
Replication	2	0.23	0.03	11.7	0.00	0.01	0.02	0.34	0.37	0.39	0.00	101.44	0.07	0.13	0.67
Treatment	47	2.75*	0.23*	141.2*	0.13*	0.05*	0.30*	2.89*	3.05*	3.23*	0.31*	537.38*	1.27*	3.10*	4.06**
Error	94	0.61	0.02	31.4	0.01	0.01	0.04	0.19	0.19	0.20	0.02	111.93	0.24	0.37	0.33

For a description of the trait code, refer to Table 2

PYR (Table 4). The largest value for SSPI, RSI, YSI, TOL, PYR and HSI belonged to HTWYT/2019-20/39 and recognized as a heat-susceptible genotype because it is appropriate for a normal situation and has a high grain yield in non-stressful conditions and a low grain yield in stressful environment. The genotype HTWYT/2019-20/8, despite exhibiting characteristics indicative of heat stress tolerance such as minimal values for SSPI, TOL, YSI, HSI, RSI and PYR, was found to perform less effectively under both conditions. The decreased values of these indices are attributed to the modest yield distinction between the two environments. Indeed, minimal values of stress indices alone do not necessarily indicate high performance, as the genotypes' grain yield under both normal and stress conditions should be considered. The GS/2019-20/6046 genotype exhibited the largest values for MP, STI, HM, GMP and MRP, indicating it as the most reliable and productive genotype under both conditions. Conversely, the genotype GW 173 displayed the lowest values for the same stress indices. The genotype GS/2019-20/6046 exhibited the highest values for YI, indicating its superiority in yield stability. Conversely, the genotype EHT-2018-19/406 displayed the lowest value for YI. Furthermore, genotypes with the highest MP, STI, GMP, MRP and HM values were recognized as heat resistant. In contrast, GW 173, with the minimal values for these indices, was categorized as heat susceptible. The genotype HTWYT/2019-20/8 exhibited the least percent yield reduction, followed by K1317©, HTWYT/2019-20/17, EHT-2018-19/401 and EHT-2018-19/443. Indeed, these genotypes exhibited minimal differences in yield under both normal and stress conditions.

Correlation between stress tolerance indices and grain yield

The best stress-tolerant criterion was estimated by calculating the correlation coefficient between heat tolerance indicators and grain yield under normal and late-sown conditions (Fig. 2). The findings indicate that Yp and Ys have a statistically significant positive correlation of 0.54, indicating their potential usability in detecting high-yielding genotypes in both conditions. Grain yield was negatively correlated with RSI (-0.70), PYR (-0.67), HSI (-0.67), SSPI (-0.56) and TOL (-0.56) under stress conditions, indicating that an increase in these indices was associated with lower yield. However, under normal conditions, grain yield exhibited positive correlations with RSI, HSI, PYR, SSPI and TOL (0.20, 0.24, 0.20, 0.39 and 0.39, respectively), implying that higher values of these stress indicators were associated with higher grain yields under normal conditions. Grain output will therefore probably

Table 4. Stress tolerance indices of various wheat genotypes, grain yield/plot (g) and Yp and Ys under normal and stressful conditions

Sr.No	TOL	STI	SSPI	YI	YSI	RSI	MP	GMP	HM	MRP	PYR	HSI	YP	YS
1	1.25	0.87	8.97	1.07	0.83	1.58	6.55	6.49	6.43	2.10	16.71	0.73	7.18	5.93
2	-0.01	0.79	-0.06	1.12	1.00	1.26	6.21	6.21	6.20	2.01	-0.31	-0.04	6.20	6.21
3	0.45	0.55	3.23	0.90	0.92	1.39	5.20	5.19	5.18	1.68	8.04	0.38	5.42	4.97
4	0.34	0.91	2.44	1.17	0.95	1.34	6.65	6.63	6.62	2.15	4.58	0.13	6.82	6.48
5	0.86	0.98	6.18	1.18	0.89	1.45	6.94	6.91	6.89	2.23	11.18	0.62	7.37	6.51
6	1.53	0.78	10.95	0.98	0.79	1.62	6.19	6.14	6.09	1.98	21.45	1.00	6.96	5.43
7	0.92	0.65	6.62	0.93	0.85	1.49	5.63	5.61	5.59	1.81	15.01	0.71	6.09	5.17
8	1.61	0.88	11.53	1.05	0.78	1.62	6.59	6.53	6.48	2.11	21.92	1.03	7.39	5.78
9	1.41	1.53	10.10	1.42	0.85	1.49	8.60	8.56	8.53	2.76	14.83	0.94	9.30	7.89
10	0.66	0.70	4.72	1.00	0.89	1.42	5.86	5.85	5.84	1.89	10.52	0.47	6.19	5.53
11	1.21	0.56	8.63	0.84	0.80	1.59	5.23	5.19	5.16	1.67	20.05	0.87	5.84	4.63
12	0.34	0.91	2.41	1.17	0.96	1.33	6.65	6.64	6.63	2.15	4.49	0.22	6.82	6.48
13	0.75	0.75	5.40	1.03	0.88	1.44	6.07	6.05	6.04	1.95	11.51	0.61	6.44	5.69
14	1.22	0.94	8.72	1.12	0.84	1.57	6.81	6.75	6.68	2.18	15.87	0.86	7.42	6.20
15	1.32	1.52	9.43	1.44	0.87	1.47	8.60	8.56	8.52	2.76	12.72	0.79	9.26	7.94
16	1.27	1.33	9.08	1.34	0.86	1.49	8.07	8.04	8.01	2.59	14.27	0.70	8.70	7.44
17	0.67	0.85	4.82	1.10	0.90	1.41	6.43	6.43	6.42	2.07	9.88	0.48	6.77	6.10
18	1.24	1.06	8.89	1.19	0.84	1.51	7.19	7.17	7.14	2.31	15.90	0.74	7.81	6.57
19	0.91	0.72	6.49	0.99	0.86	1.48	5.94	5.92	5.90	1.91	14.08	0.60	6.39	5.48
20	0.87	1.73	6.24	1.58	0.91	1.39	9.18	9.17	9.16	2.96	8.98	0.53	9.62	8.75
21	2.96	0.58	21.17	0.73	0.58	2.20	5.49	5.29	5.09	1.73	42.39	2.05	6.97	4.01
22	3.30	0.55	23.61	0.68	0.53	2.37	5.43	5.17	4.93	1.70	46.62	2.27	7.08	3.78
23	0.19	0.65	1.39	1.00	0.98	1.31	5.61	5.59	5.58	1.81	1.73	0.32	5.71	5.51
24	0.79	0.53	5.63	0.85	0.87	1.48	5.09	5.06	5.03	1.63	12.65	0.81	5.48	4.69
25	2.79	0.64	19.99	0.79	0.61	2.08	5.75	5.58	5.41	1.81	38.98	1.94	7.15	4.36
26	0.59	0.65	4.20	0.97	0.94	1.47	5.68	5.61	5.55	1.83	6.28	0.13	5.97	5.38
27	3.13	0.61	22.42	0.74	0.57	2.24	5.65	5.43	5.21	1.77	43.39	2.05	7.22	4.09
28	3.05	0.43	21.82	0.60	0.52	2.44	4.83	4.58	4.34	1.51	47.79	2.30	6.35	3.30
29	0.27	0.88	1.91	1.15	0.95	1.35	6.50	6.49	6.48	2.10	4.64	0.31	6.64	6.37
30	1.02	0.94	7.34	1.13	0.86	1.47	6.78	6.76	6.74	2.18	14.10	0.67	7.29	6.27
31	2.45	0.78	17.58	0.91	0.67	1.88	6.29	6.16	6.04	1.99	32.50	1.59	7.51	5.06
32	3.05	0.75	21.83	0.85	0.61	2.10	6.24	6.05	5.86	1.97	39.17	1.94	7.77	4.72
33	2.05	0.42	14.67	0.65	0.64	1.99	4.61	4.49	4.37	1.46	36.08	1.86	5.63	3.58
34	2.85	0.64	20.39	0.78	0.60	2.12	5.77	5.58	5.40	1.82	39.55	1.97	7.19	4.35
35	3.10	0.68	22.20	0.80	0.59	2.19	5.97	5.75	5.54	1.88	40.79	1.97	7.52	4.42
36	0.88	0.59	6.27	0.89	0.86	1.49	5.37	5.34	5.31	1.72	13.90	0.85	5.81	4.93
37	1.47	0.97	10.50	1.12	0.81	1.63	6.92	6.84	6.77	2.22	18.92	0.94	7.65	6.19
38	2.32	1.10	16.65	1.13	0.73	1.75	7.40	7.30	7.20	2.35	27.24	1.31	8.56	6.23
39	1.84	0.93	13.21	1.06	0.76	1.67	6.81	6.74	6.68	2.17	23.74	1.19	7.73	5.88
40	1.15	0.66	8.21	0.93	0.82	1.55	5.70	5.67	5.64	1.83	18.12	0.81	6.27	5.12
41	3.51	0.69	25.17	0.78	0.55	2.31	6.05	5.79	5.53	1.90	44.89	2.15	7.81	4.29
42	1.43	0.55	10.27	0.81	0.76	1.67	5.22	5.16	5.11	1.66	23.78	1.08	5.93	4.50
43	-0.06	0.80	-0.42	1.13	1.01	1.25	6.23	6.23	6.23	2.02	-0.94	-0.17	6.20	6.26
44	1.28	0.98	9.18	1.14	0.83	1.52	6.94	6.91	6.88	2.22	16.75	0.74	7.58	6.30
45	1.09	0.72	7.80	0.98	0.84	1.53	5.95	5.91	5.87	1.91	15.81	0.70	6.49	5.40
46	0.78	0.79	5.57	1.05	0.89	1.45	6.22	6.19	6.16	2.00	11.20	0.52	6.60	5.83
47	1.52	0.41	10.86	0.68	0.71	1.80	4.51	4.44	4.37	1.43	28.80	1.37	5.27	3.75
48	1.61	0.91	11.56	1.06	0.79	1.62	6.69	6.64	6.59	2.14	21.48	1.02	7.50	5.88

For a description of the trait code, refer to Table 2

increase under non-stressed condition but may decline under stressful condition if selection is based on these criteria. The variable YSI showed a negative association (-0.25) with Yp, but a significant positive association (0.67) with Ys. This is a more useful measure for differentiating heat tolerant and sensitive genotypes. There was a strong positive significant association between the grain yield (Yp and Ys) and the MP, STI, GMP, YI, MRP and HM. These indicators were chosen as the superior ones to pinpoint genotypes yielding well in both situations. The genotypes GS/2019-20/6046, GS-2018-19/1007, HPYT-2019-20/416 and SAWYT-2018-19/309 were shown to be high yielding under both conditions based on these indices. It was shown that the stable and heat-tolerant genotypes may be distinguished using the positive significant association of YI and YSI with Ys and the negative correlation with TOL and SSPI. The genotypes treated as good yielder under heat stress conditions and classified as heat tolerant can be chosen using the lowest values of TOL and SSPI. The genotype with the lowest TOL and SSPI in this investigation was HTWYT/2019-20/8, followed by K 1317 and GS/2019-20/1003.

Principal component analysis

Principal component analysis (PCA) was performed to find stress-tolerant genotypes utilizing heat stress tolerance

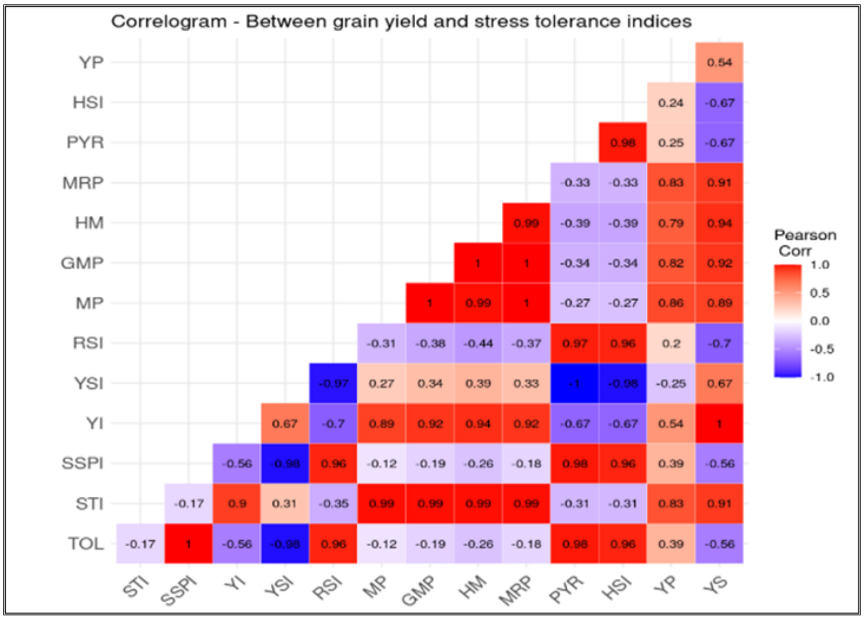

Fig. 2. Correlation coefficient between grain yield (Yp and Ys) of wheat genotypes and stress tolerance indices. For a description of the trait code, refer to Table 2.

indicators and grain yield (Yp and Ys). The first two principal components (PCs) having an eigenvalue >1.0 together accounted for 99.49 % of the variation among the fourteen PCs in Table 5. In particular, the contributions to the overall variation from the PC1 and PC2 components were 66.88 % and 32.61 % (Table 5). The strengths and orientations of correlation between attributes can be inferred from the angles and directions between attribute vectors (19). PC1 demonstrated a strong positive association with YI, YS, GMP, HM, STI, MRP and MP, with YI, YS, HM and GMP showing the highest variation among all stress indices. However, there was a noticeable positive association shown by PC2 with YP, SSPI, TOL, PYR, RSI and HSI. These findings align with correlation results, suggesting that HM, GMP, STI, MRP and MP could serve as reliable selection criteria for assessing terminal heat tolerance. To evaluate genotypes and the associations between heat tolerance indicators, a biplot was created using PC1 and PC2 (Fig. 3). Wheat genotypes GS/2019-20/6046, GS-2018-19/1007, HPYT-2019-20/416 and SAWYT-2018-19/309 were characterized as stable in both non stress and stress conditions, primarily because of their

Table 5. Principal component analysis (PCA) results are based on grain yield and stress tolerance indices. PC1 = First principal component; PC2 = Second principal component (For trait code descriptions, refer to Table 2)

Components	PC 1	PC 2
Eigen value	9.36	4.56
Variance %	66.88	32.61
Cumulative	66.88	99.49
YI	0.99	0.16
YS	0.99	0.16
HM	0.90	0.43
GMP	0.87	0.49
MRP	0.86	0.51
STI	0.85	0.51
MP	0.84	0.55
YSI	0.79	-0.60
YP	0.48	0.87
TOL	-0.68	0.73
SSPI	-0.68	0.73
HSI	-0.77	0.63
PYR	-0.79	0.60
RSI	-0.81	0.57

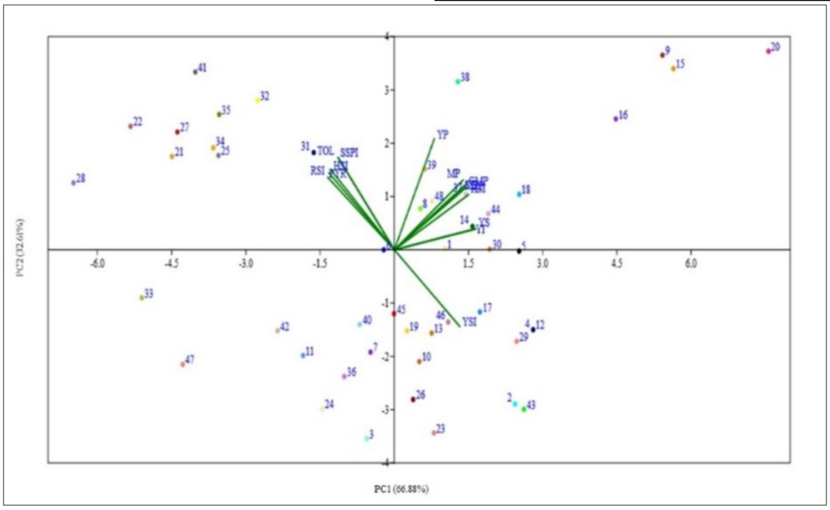

Fig. 3. PCA biplot showing the correlation between traits. PC1 = First principal component; PC2 = Second principal component (For trait code descriptions, refer to Table 2).

elevated PC1 values coupled with decrease PC2 values (Fig. 3). On the other hand, GS/2018-19/4049, HTWYT/2019-20/39, EHT-2018-19/406 and EHT-2018-19/403 genotypes were observed to be less effective or more susceptible to heat stress under challenging conditions. This was evident from their higher (positive) PC2 and lower (negative) PC1 values. The genotype GS/2019-20/6046 exhibited the highest PC1 value and was notably impacted by stress indices such as STI, HM, MP, MRP and GMP. As a result, this genotype was shown to be among the most heat-tolerant.

Cluster analysis

This study utilized correlation and PCA-selected traits for analysis, with the importance of these traits in explaining genotype variability validating this approach. Using heat-tolerance indices (HM, MP, STI, MRP and GMP), the genotypes were clustered into seven groups (Table 6 & Fig. 4). Eighteen genotypes comprised Cluster I, the most numerous clusters. In contrast, just one genotype was present in Cluster VII. The genotypes belonging to the same cluster showed more resemblances in their stress tolerance index values than those from different groups. The genotype in cluster VII showed the highest values of HM, MP, STI, MRP and GMP in this analysis. Cluster VI, III and IV genotypes were next in line.

On the other hand, genotypes from clusters I and II showed the lowest values, then cluster V genotype. Consequently, it was determined that the genotype GS/2019-20/6046 from cluster VII, which showed the maximum tolerance indices, was highly heat-tolerant. Conversely, the genotype GW 173 from cluster V, exhibiting lower values of these indices, was considered heat-susceptible.

Selection of heat-tolerant genotypes using MGIDI

The multi-trait genotype-ideotype distance index (MGIDI) was developed using various indices to identify heat-tolerant genotypes. The traits were categorized into two groups, with an average communality of 0.99, ranging from 0.98 for RSI and HIS to 1.00 for traits such as YI, YSI, GMP, MP, HM, MRP, PYR, YP and YS. The heritability (in a broad sense) values within this study exhibit considerable variability, with values exceeding 0.6 for all traits. Broad-sense heritability (h^2) ranged from 0.77 for TOL and SSPI to 0.93 for MP, GMP, HM and MRP, with an overall average of 0.866 (Table 7). These high heritability estimates indicate that these qualities are suitable for wheat development by selection. Among the heat -tolerance indices, the most significant gain observed was for STI, 66.70 %, followed by 33.4 % for YI, 31 % for HM, 29.9 for GMP, 29.5 for MRP, 28.8 for MP and YSI for 5.25 are enhancing overall yield potential. We also observed negative gains for the traits TOL, SSPI, RSI, PYR and HIS exhibited a marginal decline of -9.25, -9.25, -8.20, -21.0 and -16.2, respectively, indicative of relatively undesired gains performance in this

Table 6. Clustering wheat genotypes using average linkage (between groups) method based on heat stress indices

Clusters	No of genotypes	Cluster members
1	18	10, 19, 45, 35, 41, 7, 23, 26, 40, 25, 34, 13, 32, 6, 31, 46, 2, 43
2	8	21, 22, 24, 3, 11, 42, 27, 36
3	2	18, 38
4	13	8, 29, 1, 17, 5, 44, 37, 4, 12, 48, 14, 39, 30
5	3	28, 33, 47
6	3	9,15,16
7	1	20

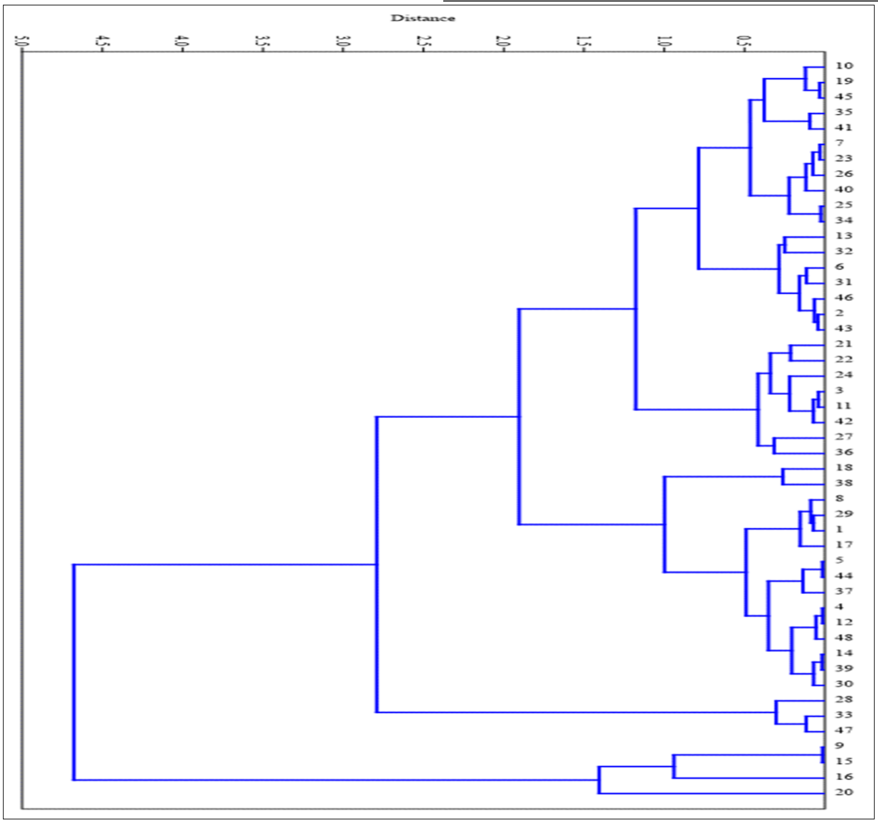

Fig. 4. Dendrogram indicating the clustering pattern of genotypes according to heat stress indices. MP, GMP and STI values are generally greater in genotypes performing better in normal and stressful conditions. Furthermore, it is anticipated that genotypes chosen using STI will have greater stress tolerance and grain yield.

Table 7. Estimated genetic gain for the PCA-selected seedling characteristics in the MGIDI analysis

Trait	FA1	FA2	Factor	h^2	SG (%)	Sense	Communality
TOL	-0.99	0.08	FA1	0.778	-0.133	decrease	0.99
SSPI	-0.99	0.08	FA1	0.778	-0.956	decrease	0.99
YSI	-0.97	0.24	FA1	0.792	0.042	increase	1
RSI	0.95	-0.28	FA1	0.861	-0.135	increase	0.77
PYR	-0.97	0.24	FA1	0.792	-4.20	decrease	1
HIS	-0.97	0.21	FA2	0.809	-0.159	decrease	0.98
STI	-0.13	0.99	FA2	0.929	0.539	increase	0.99
YI	-0.48	0.87	FA2	0.920	0.334	increase	1
MP	-0.09	1	FA3	0.932	1.80	increase	1
GMP	-0.16	0.99	FA3	0.935	1.85	increase	1
HM	-0.22	0.97	FA3	0.937	1.90	increase	1
MRP	-0.14	0.99	FA3	0.935	0.590	increase	1

Average communality = 0.99

trait within the context of FA2. The MGIDI discovered six heat-tolerant and stable genotypes as a result of this investigation: GS/2019-20/6046, GS-2018-19/1007, HPYT-2019-20/416, SAWYT-2018-19/309 and GS/2019-20/5042 (Fig. 5A). Interestingly, all of these selected genotypes came from Clusters VI and VII the highest performing group revealed earlier by cluster analysis. The figure illustrates the relative strengths and weaknesses of the evaluated genotypes based on the contribution of each component to the MGIDI score for each genotype. Genotypes SAWYT-2018-19/309 and GS/2019-20/5042 demonstrated strengths associated with FA1, particularly in characteristics such as TOL, SSPI, YSI, RSI PYR and HIS (Fig. 5B). Notably, these traits exhibited negative gains, indicating potential for improvement in these genotypes. FA2 demonstrated a significant impact on genotypes GS/2019-20/6046, GS-2018-19/1007 and HPYT-2019-20/416, particularly influencing traits related to YI, STI, MP, HM, GMP and MRP Fig. 5 (B). These genotypes exhibited notable responses to FA2, indicating their potential for enhancing overall yield and productivity traits in wheat cultivation. Through a thorough assessment of multiple indices, genotype ranking identified GS/2019-20/6046, GS-2018-19/1007, HPYT-2019-20/416, SAWYT-2018-19/309 and GS/2019-20/5042 as the highest performers among the selected genotypes.

Discussion

This study examined heat tolerance in wheat genotypes by screening stress indices based on grain yield. Additionally, highly heat-tolerant genotypes were identified through this analysis. High temperatures significantly reduced the grain yield of genotypes, consistent with similar findings observed in other studies (32). Heat stress was found to have a negative correlation with grain yield, which poses serious difficulties for plant breeders trying to maintain high yields. Different wheat-growing areas exhibit varying stress levels depending on their specific environmental conditions (33). Under late-sown conditions, heat stress particularly impacts wheat genotypes during the flowering and grain-filling stages, experiencing temperatures 3-5 °C higher than usually sown genotypes.

ANOVA results demonstrated significant effects of heat stress on wheat genotype growth and grain production. Significant differences in stress patterns were seen between several wheat-growing regions, suggesting a connection between genotype and environment. The diversity of genotypes with high-temperature tolerance is highlighted by the importance of the mean sum of squares for all stress parameters about grain production across all genotypes (34). Genotype variations across different environments underscore diversity within wheat genetic material for heat tolerance. Based on the variability across cultivars, plant breeders have used various techniques to

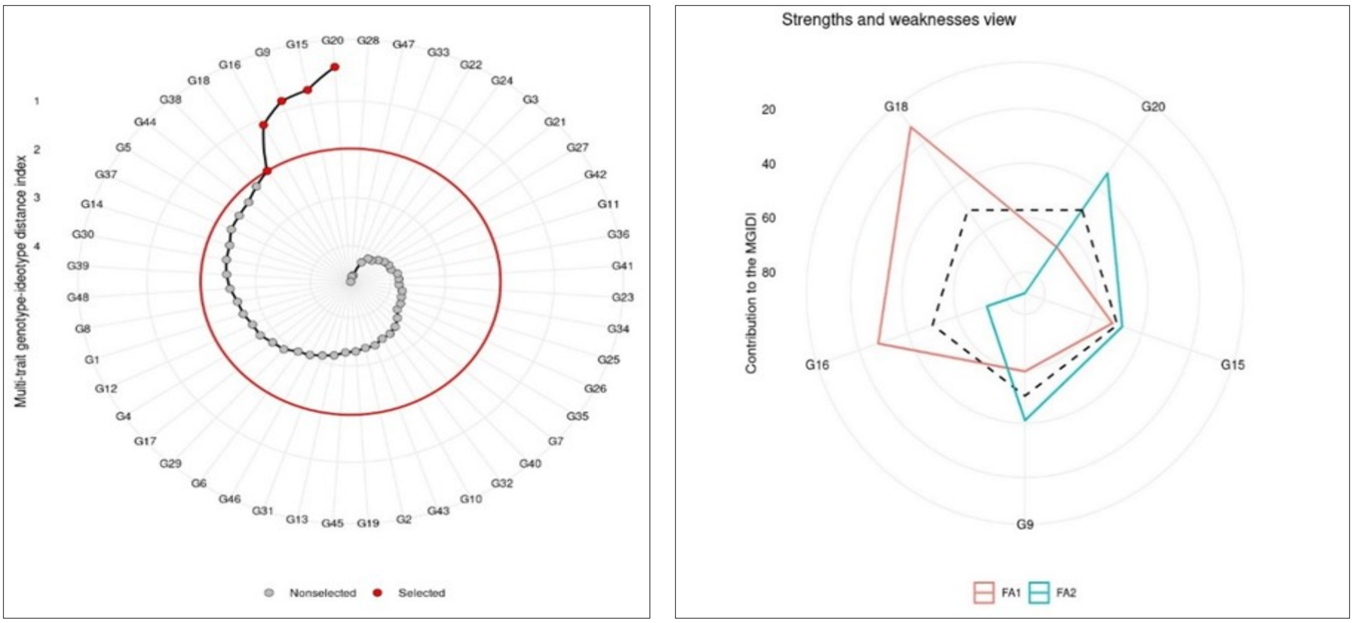

Fig. 5. MGIDI analysis of genotype ordering in ascending order. A) Top-ranked genotypes (red) are selected based on MGIDI values, with the red circle marking the selection cut-off. The percentage contribution of each factor indicates genotype strengths and weaknesses; B) Proximity of factor indices to the ideotype reflects lower explanatory fractions nearing the outer boundary. The dashed line represents the theoretical value from equal component contributions (For color interpretation, refer to the online version).

identify and choose high-yielding genotypes under stressful circumstances (25). One such research method is evaluating genotype performance using selection indices and ANOVA. Numerous indicators of stress tolerance are effectively utilized to pinpoint tolerant genotypes amidst challenging conditions. Specifically, stress tolerance indices in wheat have been applied in diverse studies to identify genotypes capable of enduring high temperatures. Preferably, smaller TOL values are favored when choosing genotypes that are tolerant since higher levels indicate a higher vulnerability to stress. As TOL and SSPI values decline, tolerance increases, but they are unable to distinguish between genotypes that yield more under either condition. Thus, preference is provided to genotypes with poorer yield performance under normal and higher yield under stressful conditions when genotypes are selected based on SSPI and TOL. The genotype HTWYT/2019-20/39 demonstrated the greatest values for RSI, TOL and SSPI. Its low yields under stress, in spite of yielding excellent grain yields under normal conditions, led to its designation as a genotype sensitive to heat. Conversely, the genotype HTWYT/2019-20/8 displayed the lowest PYR and TOL values, indicating minimal differences in yield between the two conditions. When selecting high-yielding genotypes, its crucial to consider both low TOL (stress tolerance index) and high grain yield. This ensures that the chosen genotypes exhibit resilience to stress conditions and deliver substantial yields under normal circumstances. Similar findings of wheat genotypes under various conditions were observed (4, 35). However, using the STI is more suitable for screening tolerant chickpea genotypes (36, 37).

However, choices based only on MP may fail to recognise the difference between high-yielding and stress-tolerant genotypes, even if they frequently improve genotypes' average performance under both stress and non -stress situations. MP often places a higher priority on yield potential at the expense of stress tolerance. Regarding MRP, GMP, HM and STI, genotype GS/2019-20/6046 showed the highest values in our study. Notably, GS/2019-20/6046 exhibited greater productivity under stress conditions than the other study genotypes. Research Indicates that greater values of MRP, GMP and STI might be used to identify genotypes that were higher yielding and heat-tolerant (17, 25). In line with these findings, the genotype GS/2018-19/6027 exhibited the least values for MRP, HM, GMP, MP and YI. The genotype K 1317 demonstrated the highest value for YSI, indicating its superior performance across varying conditions.

Additionally, K 1317 exhibited the least value for SSPI and the maximum value for YSI, suggesting its potential suitability as a heat-tolerant genotype. Similar outcomes were observed in rice, indicating that higher yielder genotypes under stress conditions may be efficiently identified using SSI and YSI, as opposed to standard conditions (25). The genotypes GS/2019-20/6046 and GS-2018-19/1007 exhibited the highest Yield Index (YI) values, indicating their potential as stress-tolerant genotypes. This aligns with the previous studies suggesting that the genotypes with maximum YI values are likely tolerant to stress conditions (38, 39). A single strategy based on the values of several stress indicators might not be adequate to choose between genotypes that are prone to heat stress or those that are heat tolerant. Therefore, correlation coefficients between grain yields (Yp and Ys) of both circumstances and several heat stress indices were investigated to determine the most appropriate stress indices for heat stress tolerance. Our study found a positive association between Yp and Ys, aligning with the results suggesting that genotypes performing well under one condition are likely to perform well under the other (17). Consequently, outcomes from normal conditions can indirectly aid in selecting genotypes suitable for heat-prone conditions. Under stress, there were negative associations between grain yield and RSI, TOL, SSPI, PYR and HSI; under normal circumstances, there were positive relationships.

On the other hand, YSI had a strong negative association with Yp but a positive correlation with Ys. These results were based on previous studies indicating minimal TOL and SSI values and maximum YSI values aid stress-tolerant genotype selection (14). Furthermore, a substantial positive significant association was found between YI, MP, STI, GMP, HM and MRP and grain yield (Yp and Ys). Similar correlations in wheat genotypes that are resistant to nitrogen shortage (40). Indicators might determine high-yielding genotypes under both environments (41).

Using heat stress tolerance indices and grain yield under both conditions, PCA was used to calculate the percentage contribution of key components and indices to total variance. While correlation coefficient analysis helps examine the association between two variables, PCA has been proposed by several authors as a better criterion for choosing the genotypes that will yield the highest yields under stress and normal circumstances (42, 43). Through PCA, relationships between all traits may be assessed concurrently and the number of characteristics contributing to the highest percentage of total variations can be decreased. In this study, components with eigenvalues greater than one were considered to have higher variation than Average and were thus selected as the foundation for choosing components. Yield was the primary variable examined in this study and the basis for the analysis. PC1 was positively associated with YI, YS, HM, GMP, MRP, STI, MP and YSI. Hence, PC1 can be referred to as a "yield potential and heat tolerance component" under both conditions.

In the same way, PC2 exhibited strong associations with SSPI, TOL, HSI, RSI and PYR, thus being labelled as a "stress susceptibility component." PC2 can effectively identify heat-susceptible genotypes. Using a similar methodology, the first two main components were identified by correlation analysis (17, 44). Under both conditions, genotypes that perform well usually have greater PC1 values and lower PC2 values. Genotypes with greater PC1 but lower PC2 levels are thought to be stable and vice versa (45). The cosine of the angle between their vectors in a biplot analysis shows relationships between the indices as described previously (46). As a result, an acute angle indicates a negative correlation, while an obtuse angle indicates a positive correlation between two indices. There is no correlation between two vectors when perpendicular to one another. The biplot showed that, as demonstrated by the obtuse and acute angles between their vectors, respectively, Yp and Ys had positive relationships with STI, HM, YI, GMP, MRP and MP, while Ys had negative correlations with RSI, TOL, PYR and SSPI (Fig. 2). RSI and GMP have no association because they are both at 90 °.

All genotypes of wheat that were evaluated were divided into seven clusters based on heat-tolerant indicators such HM, MP, GMP, MRP and STI. Cluster VII exhibited the highest HM, MP, GMP, MRP and STI values, followed by genotypes in clusters III and IV. The lowest values were observed in cluster V, followed by genotypes in clusters I and II. Similarly, stress tolerance indicators such as STI, MP and GMP were used to classify eight maize genotypes into three groups (47). They discovered that whereas genotypes with mean values were classified as semi-tolerant to stress, those with high values of these indices were thought to be stress-tolerant. All genotypes under study were divided into five clusters based on performance and stress tolerance level using measures of grain yield under both conditions and stress tolerance (48). The genotypes that performed best and exhibited the highest stress tolerance were those with high HM, MP, GMP, YSI and STI values. These indices can be effectively utilized in breeding programs to develop stress-tolerant genotypes by selecting parents with high values. A study grouped distinct clusters based on morphological features and multiple stress tolerance indices to identify stress-resistant chickpeas (46). They proposed using genotypes from different groups in breeding operations to generate genotypes of chickpeas that can withstand stress (40, 50). Additionally, their findings highlighted MP, GMP, SSI and YI as selection criteria for maintaining chickpea yield under stress conditions.

Cluster analysis has various drawbacks that can make it difficult to pick tolerant genotypes. These include subjective interpretation, difficulties managing missing data, inadequate dimensionality reduction, the inability to consider interaction effects and a lack of statistical rigour (51). To address these constraints, it is critical to incorporate additional analytical approaches. Cluster analysis can be integrated with quantitative indicators such as the multi-trait genotype-ideotype distance index (MGIDI) to help identify tolerant genotypes. The MGIDI is an ideal method for genotypic selection because it addresses multicollinearity and eliminates the need for assigning economic weights (31). This method enhances breeding efficiency by reducing the time and resources needed to develop stress-tolerant cultivars (52). This index relies on factor analysis, an efficient technique for modelling observed genotypes using unobserved latent components while maximizing the shared variance among related genotypes. The exploratory factor analysis grouped twelve traits into two categories. The high average communality of 0.99 indicates that the factors (FA) explain a substantial portion of the variance in each trait. Communality refers to common characteristics or traits amongst genotypes. High commonalities in FA1 and FA2 highlight the interconnectedness of the characteristics, emphasizing their shared dependence on underlying factors. Negative loadings in FA1 and FA2 indicate an inverse relationship between the respective FA traits, suggesting that underlying variables decrease as FA scores increase. These variables positively correlate with FA2, indicating that as FA2 scores increase, STI, YI, GMP, HM and MRP rise. These findings provide valuable insights into the complex architecture of plant traits, informing potential trait groupings and interrelationships.

The MGIDI predicts significant genetic gains across all traits, including STI, YI, HM, GMP and MRP. This discovery was further reinforced by the MGIDI analysis, which identified six highly heat-tolerant and stable genotypes: GS/2019-20/6046, GS-2018-19/1007, HPYT-2019-20/416, SAWYT-2018-19/309 and GS/2019-20/5042. In the current investigation, Clusters VI and VII genotypes had the greatest heat stress tolerance capability due to the beneficial contribution of most positive indicators. These findings highlight the potential of the selected genotypes, indicating their eligibility for prioritization in breeding efforts. Their exceptional performance under heat-stress conditions makes them ideal candidates for further research and development, as they are more likely to exhibit desirable performance in warmer environments. Increased predictability, stability, broad-sense heritability (h^2) and selection gain of traits can help breeders evaluate the effectiveness of selection methods (53, 54, 55). The MGIDI study found that characteristics such as MP, STI, GMP, HM and MRP had higher h^2 and selection gain, illustrating their potential as breeding targets.

Conclusion

The current study revealed that heat stress severely affects the performance of all genotypes, especially under late sowing conditions, resulting in decreased yields. Heat stress indices assessed 48 wheat genotypes produced under normal and stress conditions. A strong positive link was found between STI, MP, MRP, HM and GMP with Yp and Ys conditions based on the findings of the biplot analysis, PCA, correlation coefficient and MGIDI analyses. Considering the contributions of these diverse indices, genotypes such as GS/2019-20/6046, GS-2018-19/1007, HPYT-2019-20/416, SAWYT-2018-19/30942 and GS/2019-20/5042 have been found as tolerant and high-performing across both normal and stress conditions. These heat-tolerant genotypes show significant promise for establishing practical solutions in sustainable agriculture, such as improving yield stability and heat resilience. They exhibit characteristics that make them suitable candidates for breeding programs focused on developing heat-tolerant varieties, potentially minimizing the effects of climate change on wheat production. Collaboration among researchers, breeders and policymakers is essential for fully harnessing the future value of these heat-tolerant wheat genotypes.

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Authors' contributions

CP and SB contributed to the writing, including review and editing, as well as data curation investigation and were the primary drafters of the manuscript. BK and RD were responsible for designing the overall study and setting the methodology, as well as project administration and supervision. DS, MC and PM contributed to the data analysis. DS and HP were involved in the figure and table development. MC, DV and DS assisted in drafting the original manuscript and contributed to its review and editing. All authors reviewed and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None

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