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Heat tolerance indices as tools for characterizing resilient wheat (*Triticum aestivum*) RILs population under thermal stress

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ABSTRACT

The escalating impact of heat stress on agriculture due to climate change has necessitated the development of heat-tolerant crop varieties. To address this, a study was carried out at research farm of CCS Haryana Agricultural University, Hisar, Haryana during winter (*rabi*) seasons of 2018–19 and 2019–20 under two different environments (normal and late sown). Evaluation of multiple stress indices and their relationship with grain yield per plot was done using 200 recombinant inbred lines (RILs) of wheat (*Triticum aestivum* L.). Positive correlation was observed between grain yield and stress tolerance index, mean productivity, geometric mean productivity, harmonic mean and mean relative performance, while negative correlations existed with heat susceptibility index, tolerance, stress susceptibility index and reduction under stress conditions. Stepwise regression analysis revealed the importance of mean productivity, yield index, geometric mean productivity, stress tolerance index, and reduction in predicting grain yield. Principal Component Analysis highlighted the significance of tolerance and reduction in explaining the variance, with PC-1 labeled as the resilience and stress tolerance component and PC-2 as the yield stability and performance component. These findings were able to select 13 most heat tolerant RILs, performing better than national level check genotype WH730 and emphasized the role of stress indices especially HSI and TOL in characterizing genotypic responses to heat stress and guiding the selection of heat-tolerant genotypes for sustainable crop improvement. In the context of heat stress tolerance, understanding and harnessing transgressive segregants could lead to the development of crop varieties that not only tolerate, but thrive in challenging environments, ensuring sustainable food production under changing climatic conditions.

Keywords: Grain yield, Heat, Stress indices, Tolerance, Wheat

Wheat (*Triticum aestivum* L.) stands as one of the primary cereal crops on a global scale (Meena *et al.* 2023). The area harvested for wheat across India is about 31.87 million hectares in 2023/2024. One of the inevitable challenges is unpredictable fluctuations in rainfall patterns and temperature (majorly heat stress) (Chaubey *et al.* 2023). Worldwide, almost 40% of total irrigated area of wheat is severely affected by heat stress (Gurumurthy *et al.* 2023), with an estimated annual economic loss of around 7.7 billion dollars and it will rise to 18 billion dollars up to 2025 (Abay 2023). Heat stress can affect crops at different stages, starting from pre-emergence and continuing through the maturation process (Stone 2023). The impact of elevated temperatures on wheat growth, development and yield is multifaceted (Li *et al.* 2023). High temperatures can disrupt the crucial process of photosynthesis and impeding overall growth (Broberg *et al.* 2023). Moreover, the accelerated maturation caused by heat stress shortens the grain filling period (Djanaguiraman *et al.* 2020), limiting the time available for proper starch and nutrient accumulation, ultimately yielding smaller and lighter grains (Zhang *et al.* 2023). Additionally, the synergy between heat stress and water scarcity underlines the need for comprehensive strategies to ensure wheat resilience.

In this study we have evaluated different stress indices that play a pivotal role in selecting heat-tolerant genotypes when facing heat stress. Plant physiologists and researchers rely on these indices to study the effects of heat stress on plant metabolism, growth, and reproduction. Stress indices essentially serve as valuable tools to guide the selection of genotypes, thus contributing to the development of more resilient and adaptable crop varieties (Lamba *et al.* 2023,

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Jadon *et al.* 2022). By assessing the performance of different genotypes under stress conditions, scientists can identify the genetic basis of heat tolerance. Furthermore, stress indices provide data that can improve the accuracy of crop modeling, enabling researchers to predict how different varieties will perform under varying stress scenarios.

MATERIALS AND METHODS

Plant material: The present experiment was conducted at the research farm of CCS Haryana Agricultural University, Hisar, Haryana during winter (*rabi*) seasons of 2018–19 and 2019–20 under two different environments, viz. timely sown (1st week of November, 2018) and late sown (2nd week of December, 2018). The genetic material consisted of 200 recombinant inbred lines (RILs) of the cross WH 711/WH 1021.

Statistical analysis: Based on the data gathered, multiple stress indices i.e. HSI, Heat susceptibility index (Fischer and Maurer 1978); TOL, Tolerance (Rosielle and Hamblin 1981); STI, Stress tolerance index (Fernandez 1992); SSPI, Stress susceptibility percentage index (Moosavi *et al.* 2008); YI, Yield index (Gavuzzi *et al.* 1997); YSI, Yield susceptibility index (Bouslama and Schapaugh 1984); RSI, Relative stress index (Fischer and Wood 1979); MP, Mean productivity (Rosielle and Hamblin 1981); GMP, Geometric mean productivity (Fernandez 1992); HM, Harmonic mean (Bidinger *et al.* 1987); MRP, Mean relative performance (Ramirez and Kelly 1998); RED, Reduction (Farshadfar and Javadinia 2011) were calculated from the pooled data and the genotypes' performance was examined. These indices were further employed for correlation analysis. Additionally, principal component analysis was performed as an improved method over correlation coefficient to find the best performing genotypes under three stress conditions. Finding the relationships between all attributes at once is made easier by PCA. XLStat was used for the statistical analysis and generation of biplots.

RESULTS AND DISCUSSION

Variability for different heat stress indices: Several heat stress indices were calculated on the basis of pooled grain yield per plot obtained during 2018–19 and 2019–20 under timely and late sown conditions (Table 1). The heat susceptibility index varied from -0.26 to 2.07 (WH 711 = 1.10 and WH1021 = 0.64) with a mean of 0.97±0.46 indicating towards the presence of wide variations among RILs population. Out of 200 RILs, 47 RILs were found with lower HSI than heat tolerant parent (WH1021). The range for stress tolerance in RILs varied from -56 to 614 (WH711 = 390 and WH1021 = 195) with a mean of 291.07±9.14. Fourteen RILs had lower tolerance values than heat tolerant genotype WH730 (45). STI in RILs ranged from 0.26–1.19 (WH711 = 0.94 and WH1021 = 0.87) with an average of 0.66±0.21. Five RILs had higher STI than check variety HD3086 (1.00). Stress susceptibility percentage index in RILs ranged from -3.27 to 35.82 (WH711 = 22.80 and WH1021 = 11.40) with an overall mean of 17.02±2.21. Fourteen RILs showed lower SSPI value than WH711 (2.63). Yield index in RILs ranged from 0.44–1.58 (WH711 = 1.16 and WH1021 = 1.25) with an average of 0.99±0.19. Ten RILs had higher yield index value than check variety WH1124 (1.30). The range for yield stability index in RILs varied from 0.30–1.09 (WH711 = 0.63 and WH1021 = 0.78) with an overall mean of 0.67±0.19. Thirteen RILs had higher YSI value than WH730 (0.93). Relative stress index in RILs varied from 0.45-1.64 (WH711 = 0.95 and WH1021 = 1.19) with an overall mean of 1.02±0.23. Thirteen RILs showed higher relative stress index value than WH730 (1.41). Mean productivity in RILs ranged from 152.50–957.75 (WH711 = 855 and WH1021 = 807.50) with an average of 709.52±3.36. Only 4 RILs had higher mean productivity value than HD3086 (882.50). Geometric mean productivity ranged from 436.98–934.84 (WH711 = 832.47 and WH1021 = 801.59) with a mean of 690.02±3.42. Five RILs had higher geometric mean productivity than HD3086 (856.53). The harmonic mean in RILs varied from 387.47–912.45 (WH711 = 810.53 and WH1021 = 795.73) with an overall mean of 387.47±912.45. Nine RILs were found with greater harmonic mean than HD3086 (831.33). The mean relative performance in RILs ranged from 1.26 to 2.68 (WH711 = 2.39 and WH1021 = 2.31) with an average of 1.99±0.18. Six RILs had higher mean relative performance than HD3086 (2.46). The range of reduction in RILs varied from -8.75 to 70.18 (WH711 = 37.14 and WH1021 = 21.55) with an overall mean of 032.76±2.70.

The obtained results presented a comprehensive picture of the relationship between various heat stress indices and grain yield per plot under different conditions. Analyzing the stress tolerance indices, it was evident that variations existed among RILs in terms of their response to heat stress. The range of values for stress indices underscored the genetic diversity in their heat stress responses (Sareen *et al.* 2014). The RILs had a considerable amount of phenotypic variation for heat tolerance and exhibited transgressive segregants for most of the heat stress indices, even though they also performed better than heat tolerant checks WH1124 and WH730. These results were in accordance with the study of Ali and El- Sadek (2016). In the context of heat stress indices, these transgressive segregants might possess combinations of traits that confer exceptional heat tolerance, surpassing what is observed in the parental lines (Burnette and Eckhart 2021). This phenomenon opened new avenues for selecting superior genotypes that can excel under challenging conditions. The RILs possessing lower value for TOL, SSPI, RED and higher value for rest of the parameters were considered as tolerant RILs. A lower TOL signifies a genotype's ability to sustain its performance under heat stress conditions with minimal yield reduction compared to optimal conditions. Similarly, a lower SSPI value indicated less yield reduction when subjected to heat stress, while a lower RED value demonstrated better yield retention under stress (Lamba *et al.* 2023).

Correlation coefficient analysis for heat stress indices: Results (Fig 1) revealed a strong and positive association

Table 1 Mean and range of different heat stress indices in the parents, RILs of the cross WH711/WH1021 and checks

Stress indices	Parents		Checks			RILs	
	WH 711	WH1021	WH1124	HD3086	WH 730	Mean	Range
HIS	1.10	0.64	0.67	1.15	0.19	0.97±0.46	(-0.26-2.07)
Tol	390.00	195.00	215.00	425.00	45.00	291.07±9.14	-56-614
STI	0.94	0.87	0.95	1.00	0.61	0.66±0.21	0.26-1.19
SSPI	22.80	11.40	12.57	24.85	2.63	17.02±2.21	-3.27-35.82
YI	1.16	1.25	1.30	1.18	1.14	0.99±0.19	0.44-1.58
YSI	0.63	0.78	0.77	0.61	0.93	0.67±0.19	0.30-1.09
RSI	0.95	1.19	1.17	0.92	1.41	1.02±0.23	0.45-1.64
MP	855.00	807.50	842.50	882.50	667.50	709.52±3.36	152.50-957.75
GMP	832.47	801.59	835.61	856.53	667.12	690.02±3.42	436.98-934.84
HM	810.53	795.73	828.78	831.33	666.74	671.45±3.57	387.47-912.45
MRP	2.39	2.31	2.40	2.46	1.94	1.99±0.18	1.26-2.68
RED	37.14	21.55	22.63	38.81	6.52	32.76±2.70	-8.75-70.18

Refer to the methodology for Trait details.

of grain yield per plot under timely sown conditions (Yp) with MP (0.810**), SSPI (0.706**), TOL (0.704**), GMP (0.697**), MRP (0.696**), STI (0.696**), HM (0.575**), HSI (0.546**), RED (0.546**) and YI (0.150*). RSI showed a negative correlation (-0.546**) with grain yield per plot (timely sown). Furthermore, grain yield per plot under late sown conditions (Ys) showed significant negative correlation with HSI (-0.732**) and RED (-0.732**) TOL (-0.57**), SSI (-0.57**), whereas, positively correlated with STI (0.810**), YI (1.00**), RSI (0.732**), MP (0.703**), GMP (0.813**), HM (0.890**) and MRP (0.816**). The Pearson's correlation coefficient showed a positive and low association (0.152*) between Yp and Ys.

Correlation analysis is essential to identify influential factors, assess the strength and direction of relationships (Chawla *et al.* 2023) and predict stress outcomes. STI, MP, GMP, HM and MRP exhibited positive correlation with grain yield per plot under both environments, whereas HSI, TOL, SSI and RED showed significant negative correlation with grain yield per plot under stress conditions. Genotypes with stronger stress tolerance mechanisms, whether it's the ability to maintain yield levels, adapt to stress, or sustain productivity through alternative pathways, tend to exhibit better performance under heat stress. Genotypes with higher TOL values had a lower ability to maintain their performance under stress conditions, leading to a negative impact on grain yield. Similarly, as RED values increase, the corresponding decrease in grain yield leads to a negative correlation between RED and actual grain yield. Similar results were obtained by Farshadfar *et al.* (2013) for mean productivity and Rahmani *et al.* (2013) for tolerance under stress conditions. These findings were also in concordance with Anwaar *et al.* (2020).

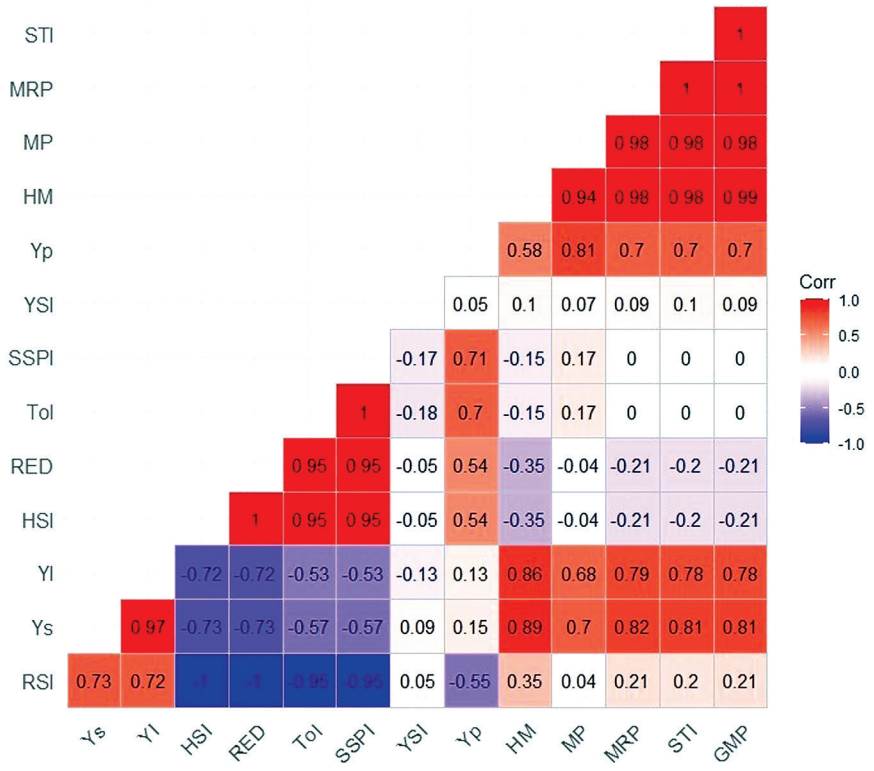

Fig 1 Correlation coefficients between grain yield per plot and heat stress tolerance/ susceptibility indices of RILs evaluated under normal and late sown conditions. The positive correlation is shown by red colour while the blue colour shows the negative correlation.

Regression analysis: The regression model for heat stress indices explained more than 99% of

the grain yield per plot variability. The significant and positive coefficient of regression was observed for TOL (0.5**), STI (2.885**), MP (1.0**), HM (2.048**) and RED (1.017**), whereas, a positive and non-significant coefficient of regression was observed for YI (1.365), YSI (566.91), RSI (377.14), GMP (0.959) and MRP (336.36).

Model for heat stress indices:

$$Y: -2.256 -172.53HSI + 0.5TOL + 2.885STI -6.612SSPI + 1.365YI + 566.91YSI + 377.14RSI + 1.0MP + 0.959GMP + 2.048HM + 336.36MRP + 1.017RED$$

The stepwise regression analysis retained five stress indices, viz. mean productivity (65.70%), yield index (34.27%), geometric mean productivity (0.01%), stress tolerance index (0.01%) and reduction (0.01%). The first two stress indices significantly attributed almost 100% of the variation in grain yield per plot.

Final model for heat stress indices:

$$Y= 8.861 + 2.0MP - 566.91YI – 4.801GMP – 1.185STI – 4.34RED$$

Regression analysis complements correlation analysis by not only quantifying relationships but also allowing for prediction, causality assessment, and control of confounding variables. It reinforced the importance of heat stress indices in predicting grain yield variability. The high percentage of explained variability (over 99%) demonstrated the strong relationship between these indices and grain yield. A higher MP value will contribute positively to grain yield prediction, while higher YI, GMP, STI and RED values will contribute negatively. The results are in accordance with the findings of Mansouri *et al.* (2018) and Sobhanian *et al.* (2019).

Principal component analysis for different heat stress indices: The results revealed that the first five components had more than one Eigen value and contributed 100% of the total variance (Table 2). First three principal components, PC 1 with Eigen value 25544.6, PC 2 with Eigen value 24621.3 and PC 3 with Eigen value 5618.9, contributed 99.8% of total variation (Fig 2). The study on loading factors revealed that PC 1 had high and positive loading for TOL (155.60) and RED (14.72), whereas, PC 2 showed high loading for GMP (90.14), HM (90.12) and MP (89.72). Yield susceptibility index was found to have high factor loading for PC 3. The RILs were plotted on the basis of PC 1 (45.75%) and PC 2 (44.09%) contributed 89.7% of the total variation (Fig 3). The vectors of ToL and MP showed better performance for the components with an acute angle, indicating presence of positive correlation between them. The vectors for GMP and HM performed better on the y-axis, i.e., contributed to only PC 2. These traits had an obtuse angle with tolerance index, indicating a negative correlation with tolerance.

Principal component analysis provided a comprehensive overview of the interrelation among different stress indices. The first component accounted 45.6% of total variation and exhibiting higher tolerance tend to maintain their performance even under stress conditions (Saoudi *et al.* 2023). Their relatively stable performance translated to a significant contribution to the variation captured by the first principal component. Reduction, being a direct measure of yield loss, is an essential indicator of how susceptible a genotype is to stress conditions. Genotypes with higher reduction values experienced more substantial yield reductions under stress (Darwish *et al.* 2023), making this trait a meaningful contributor to the first principal component. Therefore, PC 1 can be referred as resilience and stress tolerance component. Additionally, the PC 2 can be referred as yield stability and performance component as GMP, HM, and MP are all indices that provide information about the overall yield performance of genotypes.

In conclusion, the comprehensive analysis of various

Table 2 Principal components, eigen value and factors loading of different stress indices

Principal component	PC 1	PC 2	PC 3	PC 4	PC 5
Eigen values	25544.6	24621.3	5618.9	45.9	6.8
Variance %	45.6	44.1	10.1	0.1	0.1
Cumulative %	45.6	89.7	99.8	99.9	100
Trait	Factor loading				
HSI	0.43	-0.05	0.09	-0.04	0.07
Tol	155.6	16.73	7.61	-0.77	-0.31
STI	-0.02	0.17	0	0	0.01
SSPI	9.07	0.99	0.55	-0.05	-0.03
YI	-0.12	0.14	-0.03	0.01	-0.01
YSI	-18.41	6.5	74.32	-0.17	-0.09
RSI	-0.22	0.02	-0.05	0.02	-0.04
MP	6.01	89.72	0.08	4.84	0.29
GMP	-9.59	90.14	-1.85	-0.21	0.24
HM	-23.79	90.12	-4.95	-4.48	-0.41
MRP	-0.03	0.26	-0.01	0.01	0
RED	14.72	-1.56	2.97	-1.36	2.53

Refer to the methodology for Trait details.

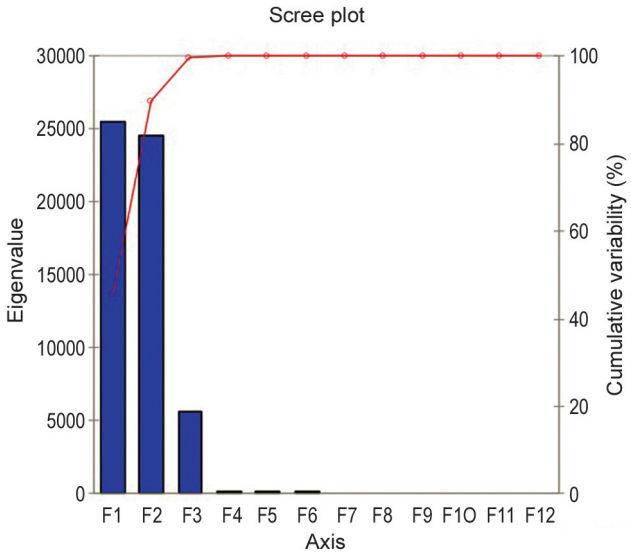

Fig 2 Scree plot depicting eigen values.

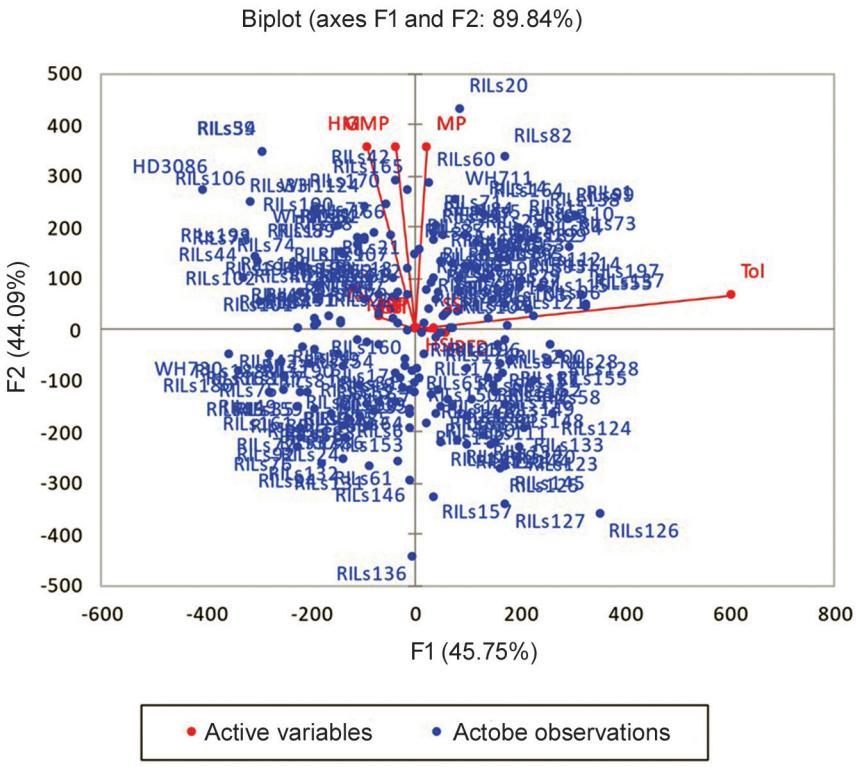

Fig 3 Biplot of heat stress indices based on PCA analysis.

heat stress indices and their relationship with grain yield provides valuable insights into the complex dynamics of genotypic responses to elevated temperatures. The presence of transgressive segregants among RILs, which exhibited superior performance compared to established heat-tolerant checks, signified the potential for selecting exceptional genotypes with superior heat tolerance attributes. This study could able to select 13 RILs as most heat tolerant as they recorded lower HSI than national level check genotype WH730.The positive correlations observed between stress tolerance indices (STI, MP, GMP, HM, MRP) and GYP under both conditions emphasized the importance of robust stress tolerance mechanisms in maintaining productivity under heat stress. These findings collectively emphasized the pivotal role of stress indices especially HSI and TOL in understanding and selecting genotypes for improved heat tolerance and productivity, contributing to the advancement of crop breeding for resilience in the face of challenging environmental conditions.

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