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Divergence Assessment of Advanced Bread Wheat Lines under Contrasting Heat Stress Regimes Utilizing Stress Tolerance Indices

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Heat stress during the post-anthesis phase significantly impacts wheat cultivation. To evaluate the tolerance of advanced wheat lines to heat stress, stress tolerance indices were used under both normal and post-anthesis heat stress conditions. The results showed a decrease in the average yield of advanced lines under both conditions, indicating the negative impact of heat stress on wheat cultivation. The analysis using PCs, biplot, and ranked clusters revealed that line V-21448 had below-average values for RHSI and %YDR, while V-21243 had values close to the average and all other lines had above-average values. Additionally, under normal conditions, V-19532 had the highest values for RHSI and HYT 100-47 had the lowest values for %YDR. In heat stress conditions, HYT 100-47 had the lowest values for both indices. HYT 100-74 and HYT 100-76 had the highest average values for MNP and YDI and were highly stable in the heat environment. Under normal settings, YDI, RHSI, and % YDR showed a positive correlation, while under heat stress conditions, the same traits showed a negative correlation. MNP had a high positive correlation with Yp and Ys, while YDSI had a negative correlation with Yp but a positive correlation with Ys. These results suggest that these traits are useful for identifying advanced lines with increased yield under both conditions.

Keywords: Wheat, divergence appraisal, normal and post anthesis, heat stress, stress tolerance index, advanced lines.

INTRODUCTION

Heat stress poses a significant challenge to wheat cultivation globally (Rehman *et al.,* 2009). In Pakistan, the main challenge for wheat cultivation is the concurrent harvesting of rice and cotton crops, which leads to late sowing of wheat. This exposes the wheat crop to high temperatures during the post-anthesis phase (Rehman *et al.,* 2009). It is widely acknowledged that heat stress can notably decrease both the yield and quality of wheat. In the irrigated zones of Punjab, Pakistan, 31.4% of wheat is sown during the normal period (mid-November), 56.1% is sown late (at the end of December), and 12.5% is sown very late (after December) (Mudasser *et al.,* 2001). Late sown wheat crop often experiences significant yield losses, sometimes up to 40-50% which is primarily characterized by loss in grain weight and size (Rehman *et al.*, 2009). Therefore, there's an urgent need to develop genotypes that can either withstand terminal heat stress or mature earlier without yield loss, thus avoiding the stress altogether.

Heat stress during the post-anthesis stage can cause both primary and secondary morphological and physiological damage to wheat, which is the main constraint on achieving high yield potential. The need of the hour for food security is the identification and development of heat-resistant wheat genotypes, as wheat is the major staple food of the country. Zulkiffal *et al.* (2022) developed heat-stress tolerant and susceptible wheat lines in diverse planting settings by using parametric stability models. However, several scientists have proposed various valuable stress tolerance indices for the selection of heat-tolerant lines under normal and stress environments (Rehman *et al.*, 2009).

We used five stress indices: yield index (YDI) (Gavuzzi *et al.*, 1997), relative heat stress index (RHSI) (Fischer and Wood, 1979), yield stability index (YDSI) (Bouslama and Schapaugh, 1984), mean productivity (MNP) (Rosielle and Hamblin, 1981), and percent yield reduction (%PYR) (Farshadfar and Javadinia, 2013) under normal and stress environments to study the ability of advanced wheat lines to combat heat stress. Several analyses including correlation,

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principal component analysis and cluster analysis were also done for the different indices under contrasting conditions of temperature. These analyses have already been used for contrasting treatments by the scientists (Jabran *et al.*, 2021)

The aim of the present study was to address heat stress during the post-anthesis phase by manipulating stress tolerance indices in advanced lines of bread wheat. This will help in battling heat and ensuring a robust state for national food security.

MATERIALS AND METHODS

***Evaluation material and treatments*:** The experiment consisted of two sets: the first set was conducted under normal field conditions, while the second set was conducted in a tunnel to expose the plants to heat stress during the post-anthesis stage according to Rehman et al. (2009). Sixteen advanced lines, along with two checks (Arooj-22 and Subhani-21), were evaluated at the Wheat Research Institute in Faisalabad, Pakistan (73º74 E, 30º31.5 N, 604 feet). The plots had dimensions of 5.0 m x 1.2 m rows and a randomized complete block design was used. During wheat cropping, the area experiences a mean maximum temperature of 27.73°C and a mean minimum temperature of 12.43°C. However, during the last fortnight of March and the first fortnight of April, the temperature can reach as high as 35.3°C and 21.5°C for maximum and minimum, respectively, on average. These days coincide with the anthesis stage of wheat, which is highly sensitive to elevated temperatures. The key criteria considered for hybridizing these lines were their potential resistance to heat stress. Therefore, the tunnel was covered with plastic sheeting from post-anthesis until maturity to reveal the effects of heat stress. The temperature inside the tunnel was maintained above 38.5°C by monitoring it daily, both inside and outside the tunnel.

***Assessment of divergence in advanced lines for heat tolerance indices*:** All other routine treatments were the same for both sets. Plants were harvested individually in the same plot dimensions. To determine stress tolerance indices, subsequent attempts were made to identify and select advanced lines that could withstand high temperatures given below.

Yield index (YDI) = Ys/Xs = Ratio of yield of advanced lines and mean yield of all advanced lines under normal and heat stress, respectively. (Gavuzzi *et al*., 1997).

Relative heat stress index (RHSI) = (Yp/Ys) / (Xs/Xp)=Ratio of average yield of advance line under normal and heat stress divided by the ratio yield performance of advance line under normal and under heat stress (Fischer and Wood, 1979)

Yield stability index (YDSI) = (Ys/Yp) = Ratio between the yield under heat stress and normal conditions (Bouslama and Schapaugh, 1984)

Mean productivity (MNP) = (Yp+Ys)/2= Average yield of advance line under normal and heat stress (Rosielle and Hamblin, 1981)

Percent yield reduction (%YDR) = (Yp-Ys)/ Yp*100= Percentage ratio between difference of yield of advance line under normal and heat stress and yield of advance line under heat stress (Farshadfar and Javadinia, 2013).

While, Ys & Yp =Yield performance of advance line under heat stress and normal, respectively, Xs and Xp=mean yield of all varieties under heat stress and normal, respectively.

***Statistical inspection*:** For future statistical analysis to determine principal components, clustering and character associations, R-Studio Package and STATISTICA version 5.0. These analyses were done following Arshad *et al.* (2021) and Sabar *et al.* (2021) who used these software for same purposes.

RESULTS AND DISCUSSION

***Exploration of variance for heat stress tolerance index*:** The pooled ANOVA showed significant differences in variances for the stress indices and yield among wheat advanced wheat lines under both heat stress and normal conditions (Table 1). This demonstrates that the elite germplasm under evaluation had significant diversity for these stress related indices which could be used to improve heat and drought tolerance in wheat through selection breeding (Ali *et al.*, 2015). This finding is consistent with a previous study (Ahmad *et al.*, 2022).

***Mean performance of stress tolerance indices*:** The mean performance on yield ground was evaluated in both stress settings, as shown in Figure 1 and 2. Under heat stress conditions, the lowest visible value for % YR was observed in HYT: 100-47 (9.59%), followed by HYT: 100-76 (10.15%) and HYT: 100-74 (11.47%). The highest value was observed in Subhani-21 (35.67%), followed by V: 20347 (31.83%) and V: 19532 (30.74%). The lines that displayed inferior value for %YR were able to withstand heat stress and are considered heat stress lenient, and vice versa. The at-parity %YR was

Table 1. Joint analysis of variance under two contrasting heat stress regimes

Source	DF	Means square						
		MNP	RHSI	YDSI	YDI	%YDR	Yp	Ys
Reps	01	66978	1.9066	0.0012	2E-11	11.76	14234.5	32.2
Lines	15	505759**	0.0419**	0.0116**	0.042**	116.44**	12110.2**	4346.4**
Error	15	14764	0.0080	0.0012	2E-04	11.53	12.3	19.3

* = P ≤ 0.05, **= P ≤ 0.01, MNP=mean productivity, RHSI=Relative heat stress index, YDSI=Yield stability index, YDI=yield index, %YDR=% yield reduction

established in lines V: 7016 S (21.8%) and V: 20337 (21.5%), indicating their identical influence under heat stress circumstances. This disclosure was also signposted in Figure 3, which showed the same effect. According to Dubey *et al.* (2020), the consequences revealed that terminal heat stress will decline wheat yield by 11.1% by 2050. Proper tactics can support in decreasing the effect of heat stress to 9%.

Among all lines, HYT-100-47 had the lowest RHSI value of 1.2 and was recognized as a heat-tolerant line due to its high Ys value of 5938.1 and MP value of 6252.8. The drop in MP values for all advanced lines along with Ys was due to a smaller rate and duration of grain filling at elevated temperatures, which eventually increased %YR. Similarly, Subhani 21 had the highest RHSI value of 1.73 and was classified as the most heat-sensitive line due to its low MP value of 4324.4 under stress conditions.

Among all the lines tested, HYT 100-47 showed the highest peak value for MP, making it the most stable and productive advance line under both situations. However, Subahani-21 had the lowest value for the same stress indices. The heat tolerant lines were identified as HYT 100-74, HYT 100-47, and HYT 100-76, which had the highest values for YDI and MP. On the other hand, the heat susceptible lines were Subhani-21, V-19532, and V-20347, which had the lowest values for these indices. Kamrani *et al.* (2017) reported parallel outcomes and suggested that heat-tolerant and high-yielding genotypes could be selected based on high values of MP and YDI. Basavaraj *et al.* (2021) recommended using genotypes with the highest values of yield stability index and yield index to identify higher yielding genotypes under stress conditions rather than normal conditions. The results are informative and suggest that the highest values for YDSI were found in the advanced line HYT 100-47 and HYT-100-76 (0.9), followed by HYT-100-74 (0.89), V-21448 (0.83), and Arooj-22 (0.83). The highest value for YDI was found in HYT-100-47 (1.28), followed by HYT-100-74 and HYT 100-76 (1.20), which had the same value.

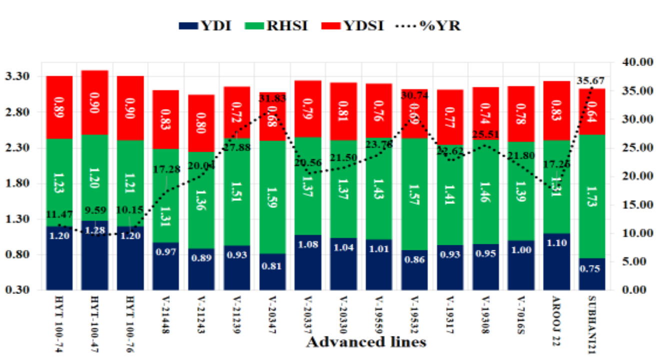

Figure 1. Mean performance of YDI, RHSI, YDSI and %YR over advanced wheat lines.

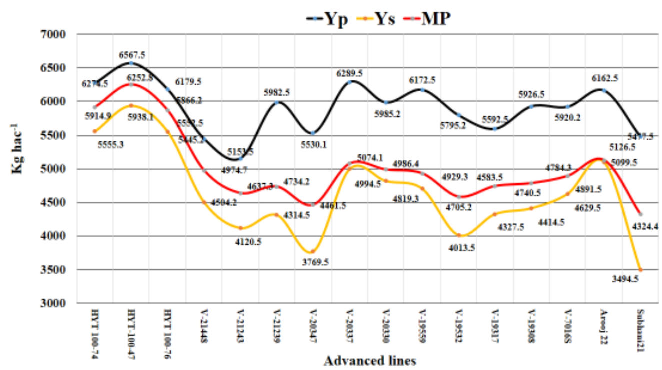

Figure 2. Mean performance of Yp, Ys and MP over advanced wheat lines.

***Multivariate Analysis Principal component analysis*:** Principal component analysis (PCA) is a useful tool for reducing large data tables and develop an inference about associations among plant parameters (Ali *et al.*, 2015). In order to identify patterns of variation, PCA was performed on all variables simultaneously. Table 2 presents the Eigen values, variability percentages, and cumulative percentages. The first two principal components explain the concentrated unevenness. Overall, the first two PCs account for 99.7% of the evidence presented in the figures. Out of the five traits studied, one trait was found to have an Eigen value greater than 1, indicating that it has more variance than the average. The PCI accounted for 93.1% of the total inconsistency of the data, mainly attributed to YDI, YDSI, and MNP, with positive factor loadings, except for RHSI and %YDR, which had the same percentage. PCII explained 6.6% of the total variability and was depicted mainly by YDSI's positive loading only. These results are consistent with the findings of Lamba *et al.* (2023), who also demonstrated positive and negative factor loadings for the same traits. The PCIII contributed 0.03% to the total variability and was mainly associated with YDI, RHSI, and RHSI, with positive loadings, and negative loadings with MNP and %YDR.

Table 2. PCs values based on five traits

Variables	YDI	RHSI	YDSI	MNP	%YDR
Eigenvalue	4.654	0.323	0.017	0.001	-0.000
Proportion	0.931	0.066	0.003	0.000	-0.000
Cumulative	0.931	0.997	1.000	1.000	1.000
PC-1	0.453	-0.450	0.455	0.423	-0.455
PC-2	-0.365	-0.382	0.325	-0.714	-0.325
PC-3	0.136	0.805	0.396	-0.141	-0.396
PC-4	-0.802	0.056	0.177	0.540	-0.177
PC-5	0.000	0.000	0.707	-0.000	0.707

Figure 3 shows the relationship between different attributes and advanced lines through a biplot. To compare heat tolerance indices with advanced lines, a biplot was constructed using the first two principal components. In biplot analysis, correlations among the indices and advanced lines can be judged by the cosine of the angle between their vectors. An obtuse angle designates a positive correlation, an acute angle designates a negative correlation, and a perpendicular angle designates no correlation between them (Yan and Tinker, 2006). The biplot shows that %YDR is positively associated with RHSI (obtuse angle) and negatively correlated with MNP and YDI (acute angle). Currently, the largest incline (slightly greater than 90°) of YDSI with RHSI and %YDR suggests a moderately larger interaction. The ranks of the advanced lines based on index performance in both environments can be seen. Advance line V-21448 had lower than average RHSI and %YDR, while V-21243 had near average, and all others had higher than average. V-19532 and HYT 100-47 remained with the highest and lowest average, respectively, for these indices under normal and heat stress conditions. Similarly, HYT 100-74 and HYT 100-76 had the highest average for MNP and YDI under heat stress conditions. V-21448 and V-19317 were highly unstable due to lower-than-expected environmental conditions, while HYT 100-47, HYT 100-74, and HYT 100-76 exhibited high stability in high-temperature environments.

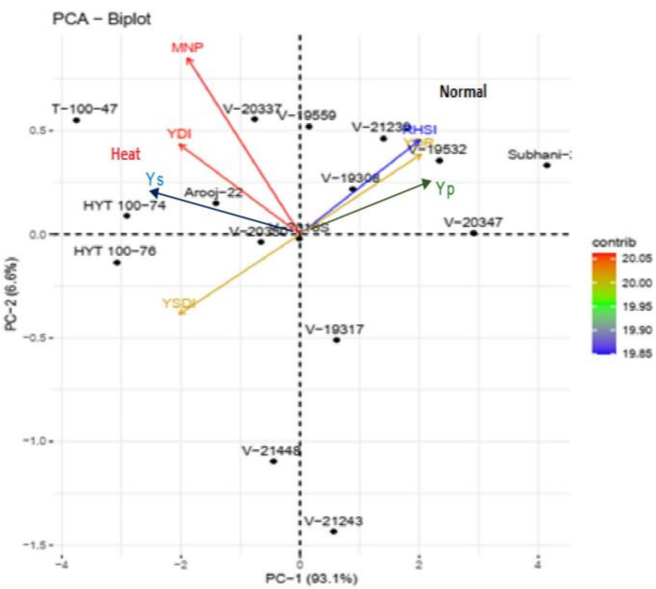

Figure 3. PCs biplot based dissemination of studied advanced lines and traits.

Assortment of ranked clusters**:** The five measured traits were separated into four clusters (see Table 3 and Figure 4). Cluster II contained the maximum number of lines (6), while clusters I and IV contained the minimum number of lines (3), and cluster III contained 4 lines. Clusters I and IV indicated more similarities, while the lines among clusters I and II showed higher variations in the studied trait values. One group possessed a higher response of RHSI and YDR%, while the second group yielded a higher response on MNP and YDI. The MNP, YDI, and YDSI responses are higher in the 3rd group varieties, while the 4th group exhibits average responses. This grouping is also evident in Figure 3.

Table 3. Members of clusters based on five traits.

Cluster	No. of lines	Members
1	3	HYT100-74, HYT100-76, HYT100-47
2	6	V-21448, V-19317, V-19532, V-21239, V-19308, V-7016S
3	4	V-20337, Arooj-22, V-20330, V-19559
4	3	V-21243, V20347, Subhani-21

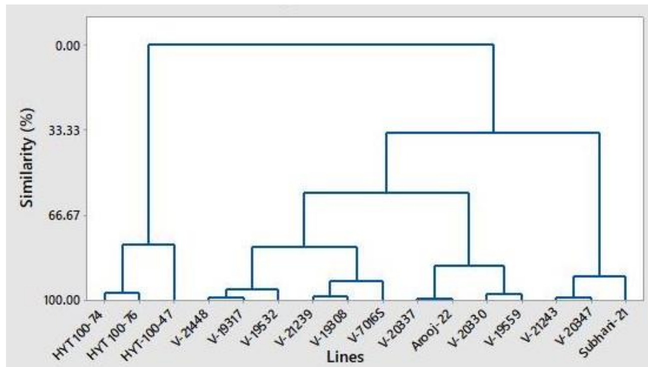

Figure 4. Clustering decoration of advanced wheat lines founded on heat stress indices.

Correlation analysis**:** The character associations through correlation matrix have been largely used to study and aid the development of stress tolerant and high yield crop varieties (Ali *et al.*, 2009). To evaluate and calculate the strength of the association between stress-tolerant measures, correlations were drawn between Ys, Yp, and the studied heat tolerance indices (Table 4). A positive correlation was found between Yp and Ys (0.568), indicating that they can be used to identify high-yielding advanced lines under both stress and normal conditions. Under normal conditions, YDI (0.431), RHSI (0.654), and % YDR (0.634) showed a positive correlation, while under heat stress conditions, the same traits were negatively correlated (-0.633, -0.545, and -0.498, respectively). Therefore, selecting based on these indices will increase yield under normal conditions but decrease it under heat stress.

The YDSI index is useful for identifying vulnerable and heat-tolerant advanced plant lines. It distinguishes between inferior and superior lines under heat stress conditions. YDSI showed a negative correlation with Yp (-0.632) but a positive correlation with Ys (0.499). MNP showed a high positive correlation with yield under both stress and normal conditions (0.871 and 0.923, respectively). This suggests that MNP is a superior trait for detecting advanced lines with elevated yield under both conditions. This correlation was also reported by

Table 4. Correlation pattern of stress tolerance indices.

Traits	YDI	RHSI	YDSI	MNP	%YDR	Ys	Yp
YDI	1.000						
RHSI	-0.901	1.000					
YDSI	0.921	-0.987	1.000				
MNP	0.977	-0.796	0.817	1.000			
%YDR	-0.921	0.987	-1.000	-0.817	1.000		
Ys	0.633	-0.545	0.499	0.817	-0.498	1.000	
Yp	0.431	0.654	-0.632	0.923	0.634	0.568	1.000

Chugh *et al.* (2022) while working on mustard genotypes under diverse conditions. On the other hand, MP showed a negative correlation with % YDR (-0.817), while % YDR (-0.498) showed an adverse link with Ys. Therefore, both indices are inversely proportional to each other because greater YDR percentage decreases Ys under heat stress, leading to a decline in the MP value.

Conclusion: Manipulation of stress tolerance indices was carried out on advanced wheat lines sown under normal and heat stress during the post-anthesis phase. This was done to evaluate their ability to withstand heat and contribute to the country's food security as genetic resources and donors for further wheat enhancement. Stress tolerance indices are a better approach for selecting heat-resistant wheat lines. The heat tolerant lines (HYT 100-74, HYT 100-47, and HYT 100-76) were recognized as having the highest values for YDI and MP, and were found to be highly stable in a heat environment. Conversely, the lines with the lowest values for these indices (Subhani-21, V-19532, and V-20347) were treated as heat susceptible. It was observed that the lines which displayed inferior values for %YR were able to withstand heat stress and are therefore considered heat stress lenient, while the opposite is true for lines with superior values for %YR. The at par %YR was established in lines V-7016 S and V-20337, which showed identical influence under heat stress conditions. The improvement in wheat for heat tolerance could be achieved by using these indices in future that will ultimately lead to development of heat tolerant source material to be used in wheat breeding programs.

Author's contributions: Zarqa Hassan, Adil Zahoor and Ikhlas Shafique mainly worked on the project and develop initial draft, Muhammad Jabran, Saman Shahzadi, Kinza Ahsan, and Nafeesa Aman helped in different analyses and validation, Muhammad Burhan and Amjad Abbas reviewed the manuscript and Muhammad Amjad Ali supervised the study.

Conflicts of Interest: The authors proclaim that they have no conflicts of interest.

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