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Research Article

Exploring heat stress tolerance indices in barley genotypes based on grain yield

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Abstract

The objective of the present study was to assess the heat tolerance of barley genotypes using a set of six heat stress indices *i.e.* HSI, HRI, HTI, TOL, MP and GMP, estimated using grain yield. A panel of 44 barley genotypes was evaluated under normal (non-stress) and heat stress conditions during 2021-22 crop seasons with three replications in randomized block design (RBD) at Experimental Area, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar (Haryana). The genotypes, IBON-HI-2021-102, BH 1018, IBON-HI-2020-51, DWRB 123 and 7th GSBON-2020-101 were found to be heat tolerant based on average rank of stress indices employed on grain yield. Grain yield under stress condition (Y_s) showed significant positive correlation with the indices *viz*., HRI, MP and GMP while, it was negatively correlated with HSI. The association study signifies HRI, MP, GMP and HSI as the best selection criterion for heat stress tolerance. PCA study considered second principal component (PC 2) as heat tolerant component based on strong correlation with HRI, HTI and grain yield (Y_s) under stress condition. The genotypes of cluster IV exhibited better performance under stress condition for grain yield and other stress indices. The genotypes from this group could be utilized as promising breeding material intended to develop new heat-tolerant barley varieties.

Key words: Barley, correlation, cluster analysis, PCA, stress indices, heat tolerance

1. Introduction

Barley, one of the earliest domesticated cereal crops, has been consumed in various ways, including as human food, livestock feed, and in malt production (Kumar *et al.*, 2020). Its products are readily digestible as compared to wheat because of their low gluten content, have the ability to control the blood cholesterol level due to high -glucan content and its low glycemic index makes barley an attractive nutritious source. There are many reasons for which barley has been neglected in our country, resulting in the declined acreage, crop harvest, and production. Recently, barley has occupied an area of 0.62 million hectares in India, producing 1.69 million tons of grain with a national productivity of 2733 kg/ha. It was cultivated on 15,300 hectares with a production of 53,300 tons in Haryana and a productivity of 3486 kg/ha during the 2022-23 crop season (ICAR-IIWBR, 2023).

Globally, abiotic stresses are major limiting factors in attaining optimum crop production and productivity (Rani *et al.*, 2018). Plant responses to abiotic stresses nowadays have been the subject of numerous studies worldwide (Dos Santos *et al.*, 2022). Barley could be considered as a model crop for observing these responses to climate change (Dawson *et al.*, 2015). Barley shows more adaptive potential against abiotic stresses such as heat, drought, and salinity (Bhagat *et al.,* 2023) as compared to wheat, rice, and maize. In the present global warming scenario, heat stress is the key factor causing yield reductions in cereal crops. Additionally, it is the most prevalent type of abiotic stress in the world, and is a bottleneck in the production of sustainable crop yields, including barley (Ghomi *et al.*, 2023). The grain filling rate and grain development duration are highly sensitive phenomena to heat stress (Mariey *et al.*, 2021). Photosynthesis, among the physiological processes, is also influenced by heat stress (Aneja *et al.*, 2022). Depending on the climatic regions, heat stress can occur at any growth stage; hence growth stages should be carefully considered (Sallam *et al.*, 2018, Dawood *et al.*, 2020; Aneja *et al.*, 2022). Some researchers also cited the harmful effect of terminal heat stress on barley malt quality (Kumar *et al.*, 2020). The climate change, especially the high temperature, is expected to influence the modern and traditional barley varieties differentially. Barley wild relatives may be exploited in conventional breeding approaches for component traits associated with abiotic stress tolerance (Bahrami *et al.*, 2019; Barati *et al.*, 2019; Ebrahim *et al.*, 2020). Wild and domesticated barley could be utilized in hybridization to improve stress adaptability in modern cultivated genotypes (El-Hashash and El-Absy, 2019). In addition, for heat tolerance incorporation, physiological mechanisms related to high temperatures and reliable screening approaches may also be considered for the development of heat-tolerant varieties with suitable agronomic and quality traits (Arzani and Ashraf, 2016). Furthermore, the genotypes may be screened under stress and non-stress conditions at multilocation to overcome the complexity of phenotyping this complex trait (Jha *et al.*, 2014).

A combination of tolerance indices could be employed instead of a single index using various agronomic attributes for accurate and reproducible identification of high-yielding stable genotypes (Bahrami *et al.*, 2020). Stress tolerance indices are among the most useful tools and indicators of plant responses under stress (Jamshidi and Javanmard, 2018). To cope with the alarming threat of rising temperatures, it is quite important to understand the genetic basis of tolerance breeding, either through conventional or molecular approaches, to evade the challenges of climate change (Anwar and Kim, 2020). Consequently, this research work is intended to assess the responses of 44 genotypes to heat stress using a set of six stress indices, including HSI, HTI, HRI, TOL, MP and GMP, with respect to the grain yield of barley genotypes.

2. Material and Methods

The field trial was conducted during the 2021-22 crop season at the Experimental Area of the Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, situated in the subtropical region of the North Western Plain Zone of India. The experimental material included 44 barley genotypes, comprising 23 two-rowed and 21 six-rowed barley including four check varieties, DWRB 123 and BH 946 (timely sown), and DWRB 91 and DWRUB 64 (late sown genotypes). Randomized Block Design (RBD) was used to evaluate the experimental material which was replicated thrice. Each genotype was planted in a paired row of 2.5 m length spaced 23 cm apart. The experimental material was grown under normal (non-stress) and late sown (heat stress) conditions on 20th November and 20th December, 2021, respectively. It was expected that late sown material may encounter terminal heat stress during grain filling stage. The package of practices recommended for both environments were accordingly followed to raise the crop.

Grain yield per plant of each genotype was measured under non-stress and stress conditions denoted as Y_p and Y_s, respectively. The grain yield of all genotypes was used to calculate stress indices. Heat susceptibility index (HSI) and heat response index (HRI) were calculated using the equations given by Fischer and Maurer (1978) and Bidinger *et al.* (1987), respectively. Heat tolerance index (HTI) and geometric mean productivity (GMP) were worked out as suggested by Fernandez (1992). Likewise, stress tolerance (TOL) and mean productivity (MP), as described by Rosielle and Hamblin (1981), were estimated. The recorded data was subjected to statistical analysis using Microsoft Excel for calculation of stress indices. R studio version 2023.12.1.402 was used for correlation coefficient and cluster analysis. SPSS Statistics version 27 was exploited for principal component analysis (PCA) and to draw the biplot diagram.

The weather parameters for crop season 2021-22 are presented in Fig. 1, revealing the highest maximum and minimum temperatures as 37.5°C and 17.2°C, respectively, in the standard weeks 13th and 12th (2022). Further, the weather data (Fig. 2) for the post heading phase of the crop unveiled average minimum and maximum temperature under non-stress condition as 11.4 and 28.50°C, whereas, under stress condition, it was 13.5 and 31.4°C, respectively. These observations indicated the occurrence of heat stress during post heading as evident by an average rise of minimum (2.1°C) and maximum temperatures (2.9°C) under stress to non-stress environment comparably.

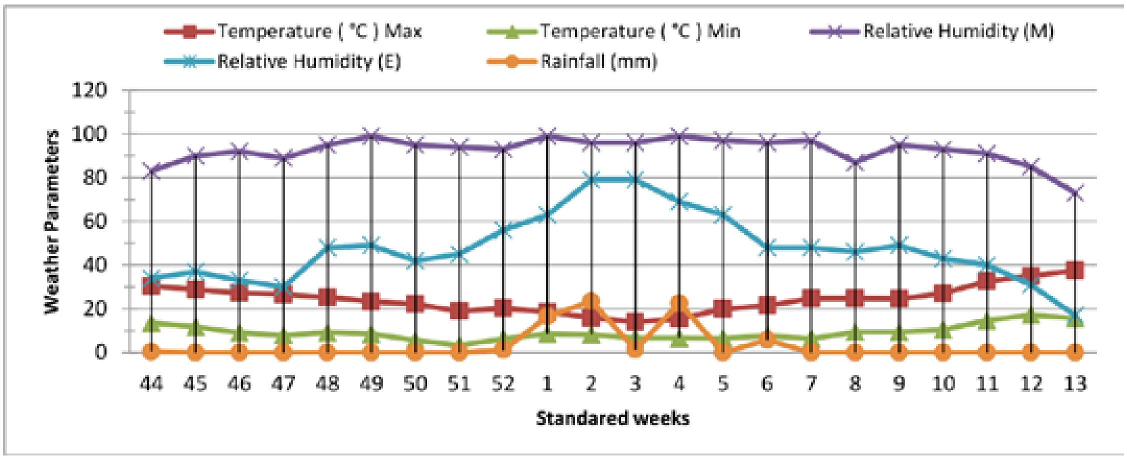

Fig. 1: Mean meteorological data during 2021-2022 crop season

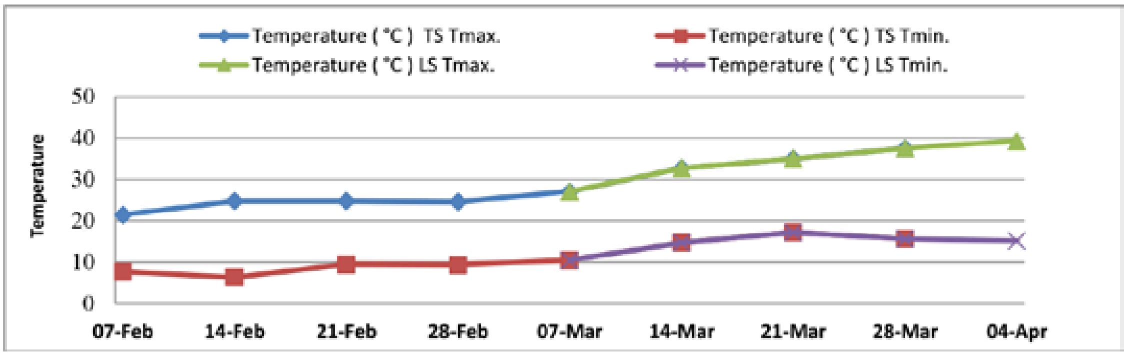

Fig. 2: Post heading maximum and minimum temperature under normal and late sown condition during 2021-22

3. Results and Discussion

The performance of genotypes for grain yield and six stress indices of heat tolerance are presented in Table 2. The results revealed differences in the performance of genotypes under both conditions. The genotypes BH 1038 and IBYT-HI-2020-11 were found to have maximum (39.60 g and 26.13 g) and minimum (18.67 g and 15.27 g) grain yields per plant under normal and stress conditions, respectively. The reduction of 24.74% in mean grain yield under stress conditions as compared with non-stress conditions indicated the influence of high temperatures on grain yield.

3.1 Stress indices

The efficient screening techniques for heat tolerance evaluation are still lacking in barley. Hence, an attempt was made to select the tolerant genotypes employing six stress indices, *viz*., HSI, HRI, HTI, TOL, MP, and GMP, calculated on the basis of grain yield. All the genotypes were ranked first on the basis of their respective indices and then, based on the average of ranks of all the stress indices, the genotypes were ranked for reaching the overall rank (Table 1). Among the six stress indices utilized, the higher estimates of HSI and TOL indicate the susceptibility of genotypes to heat; however, higher HRI, HTI, MP, and GMP estimates reveal heat tolerance.

The findings revealed minimum HSI for the genotype BH 1018 (0.09), followed by IBON-HI-2021-52 (0.23), BH 1036 (0.42), DWRUB 64 (0.43), 7th GSBON-2020-101 (0.47), IBON-HI-2021-102 (0.51), IBON-HI-2021-85 (0.53), and DWRB 123 (0.56), indicating the genotypes with stable performance and high heat tolerance under stress conditions. In contrast, IBON-HI-2020-155 (1.86)

Table 1: Grain yield and different stress indices with ranks of barley genotypes

Sr. No.	Genotypes	Y_p	Y_s	HSI	R	HRI	R	HTI	R	TOL	R	MP	R	GMP	R	OR
1	BH1029	31.20	18.47	1.71	42	1.31	41	0.59	42	12.73	39	24.84	18	24.01	18	41
2	BH1034	21.20	17.60	0.71	14	2.47	33	0.83	14	3.60	12	19.40	41	19.32	41	27
3	BH 1035	25.93	20.87	0.82	20	3.95	14	0.80	20	5.06	21	23.40	25	23.26	21	17
4	BH 1036	20.00	18.00	0.42	3	2.96	27	0.90	3	2.00	3	19.00	42	18.97	42	16
5	BH 1038	39.60	26.13	1.42	34	5.27	5	0.66	34	13.47	42	32.87	1	32.17	1	14
6	BH 1039	27.07	20.73	0.98	25	3.65	18	0.77	24	6.34	26	23.90	19	23.69	19	21
7	BH 1018	25.13	24.60	0.09	1	6.74	**2**	0.98	**1**	0.53	**1**	24.87	**17**	24.86	**16**	**2**
8	BH 1025	26.53	20.60	0.94	23	3.65	19	0.78	22	5.93	24	23.57	23	23.38	20	22
9	BH 1026	28.67	18.87	1.43	36	2.04	37	0.66	35	9.80	34	23.77	20	23.26	22	38
10	BH 1027	23.80	19.73	0.72	16	3.52	22	0.83	15	4.07	17	21.77	29	21.67	29	20
11	IBYT-HI- 2021-3	26.73	19.80	1.09	29	3.05	25	0.74	29	6.93	28	23.27	26	23.01	25	30
12	IBYT- HI-2021-9	27.87	19.40	1.27	32	2.56	31	0.70	30	8.47	31	23.64	22	23.25	23	33
13	IBYT- HI- 2021-15	30.80	21.60	1.25	30	3.61	20	0.70	31	9.20	32	26.20	10	25.79	10	23
14	IBYT-HI-2021-17	36.20	22.87	1.54	38	3.55	21	0.63	38	13.33	41	29.54	3	28.77	3	26
15	IBYT-HI-2021- 18	31.07	23.53	1.02	27	4.93	8	0.76	26	7.54	30	27.30	7	27.04	7	11
16	IBON-HI-2021-27	32.47	20.13	1.59	39	2.27	35	0.62	39	12.34	38	26.30	9	25.57	11	34
17	IBON-HI-2021-31	29.47	18.07	1.62	41	1.33	40	0.61	40	11.40	36	23.77	21	23.08	24	42
18	IBON-HI-2021-33	27.47	22.80	0.71	15	5.05	6	0.83	16	4.67	18	25.14	16	25.03	15	6
19	IBON-HI-2021-38	24.40	18.47	1.02	28	2.52	32	0.76	27	5.93	25	21.44	32	21.23	32	36
20	IBON-HI-2021-52	21.00	19.87	0.23	2	4.11	13	0.95	2	1.13	2	20.44	36	20.43	36	8
21	IBON-HI-2021-56	31.80	22.27	1.25	31	3.91	15	0.70	32	9.53	33	27.04	8	26.61	8	19
22	IBON-HI-2021-81	24.47	20.80	0.63	12	4.16	11	0.85	11	3.67	13	22.64	27	22.56	27	10
23	IBON-HI-2021-85	20.87	18.23	0.53	7	2.97	26	0.87	7	2.64	6	19.55	39	19.51	38	18
24	IBON-HI-2021-99	20.73	15.80	1.00	26	1.27	42	0.76	28	4.93	20	18.27	43	18.10	43	43
25	IBON-HI-2021-102	29.20	25.67	0.51	6	6.78	**1**	0.88	**6**	3.53	**11**	27.44	**6**	27.38	**6**	**1**
26	RD 3002	23.27	19.87	0.61	11	3.71	17	0.85	12	3.40	8	21.57	30	21.50	30	12
27	DWRB 197	28.33	22.87	0.81	19	4.95	7	0.81	19	5.46	23	25.60	13	25.45	13	9
28	DWRB 209	21.47	17.52	0.77	18	2.36	34	0.82	17	3.95	15	19.50	40	19.39	40	32

29	BCLA 11-6	28.47	18.47	1.47	37	1.79	38	0.65	37	10.00	35	23.47	24	22.93	26	39
30	IBON-HI-2020-71	23.33	18.53	0.86	21	2.75	28	0.79	21	4.80	19	20.93	34	20.79	34	28
31	7th GSBON-2020-90	23.53	16.20	1.30	33	1.06	44	0.69	33	7.33	29	19.87	37	19.52	37	44
32	IBON-HI-2020-6	22.20	19.13	0.58	9	3.38	23	0.86	9	3.07	7	20.67	35	20.61	35	15
33	IBON-HI-2020-51	28.13	24.13	0.60	10	5.88	**3**	0.86	**10**	4.00	**16**	26.13	**11**	26.05	**9**	**3**
34	7th GSBON-2020-140	36.40	22.33	1.62	40	3.13	24	0.61	41	14.07	43	29.37	4	28.51	4	29
35	IBON-HI-2020-55	21.27	17.87	0.67	13	2.64	30	0.84	13	3.40	9	19.57	38	19.50	39	25
36	7th GSBON-2020-101	23.13	20.56	0.47	5	4.23	**10**	0.89	**5**	2.57	**5**	21.85	**28**	21.81	**28**	**5**
37	IBYT-HI-2020-6	24.00	18.67	0.93	22	2.73	29	0.78	23	5.33	22	21.34	33	21.17	33	31
38	7th GSYT-HI-2020-20	31.73	18.67	1.72	43	1.36	39	0.59	43	13.06	40	25.20	15	24.34	17	40
39	IBYT-HI-2020-11	18.67	15.27	0.76	17	1.26	43	0.82	18	3.40	10	16.97	44	16.88	44	37
40	IBYT-HI-2020-155	38.77	21.53	1.86	44	2.14	36	0.56	44	17.24	44	30.15	2	28.89	2	35
41	DWRB 123 (**c**)	27.27	23.60	0.56	8	5.65	**4**	0.87	**8**	3.67	**14**	25.44	**14**	25.37	**14**	**4**
42	BH 946 (**c**)	29.10	22.33	0.97	24	4.43	9	0.77	25	6.77	27	25.72	12	25.49	12	13
43	DWRB 91 (**c**)	35.00	23.07	1.43	35	3.91	16	0.66	36	11.93	37	29.04	5	28.42	5	24
44	DWRUB 64 (**c**)	22.67	20.33	0.43	4	4.15	12	0.90	4	2.34	4	21.50	31	21.47	31	7

Yp: Grain yield under non-stress condition, Ys: Grain yield under stress condition, HSI: Heat susceptibility index, HRI: Heat response index, HTI: Heat tolerance index, TOL: Stress tolerance, MP: Mean productivity and GMP: Geometric mean productivity, R: Rank; OR: Overall rank

Table 2: Correlation between grain yield (Y_p and Y_s) of barley genotypes and different stress indices

Stress indices	**HSI**	**HRI**	**HTI**	**TOL**	**MP**	**GMP**	**Y_p**	**Y_s**
HSI	1.000	-0.525**	-0.999**	0.968**	0.475**	0.440**	0.714**	-0.054
HRI		1.000	0.528**	-0.316*	0.431**	0.473**	0.155	0.848**
HTI			1.000	-0.967**	-0.471**	-0.436**	-0.710**	0.059
TOL				1.000	0.650**	0.623**	0.842**	0.170
MP					1.000	0.996**	0.945**	0.822**
GMP						1.000	0.926**	0.851**
Y_p							1.000	0.613**
Y_s								1.000

HSI: Heat susceptibility index, **HRI:** Heat response index, **HTI:** Heat tolerance index, **TOL:** Stress tolerance, **MP:** Mean productivity and **GMP:** Geometric mean productivity, **Y_p**: Grain yield under non-stress condition, **Y_s**: Grain yield under stress condition, *, ** Significant at 0.05 and 0.01 levels of probability, respectively was found highly susceptible genotype to heat as observed by highest HSI.

The genotypes with low HSI for a particular trait are suitable for climate resilience by exhibiting stable performance for that trait under heat stress as compared with non-stress conditions (Thakur *et al.*, 2020). The estimates of TOL indicated the highest grain yield reduction in IBON-HI-2020-155 (17.24), and the promising genotypes thus identified based on TOL index included BH 1018 (0.53), IBON-HI-2021-52 (1.13), BH 1036 (2.0), DWRUB 64 (2.34), 7th GSBON-2020-101 (2.57), IBON-HI-2021-85 (2.64), IBON-HI-2020-6 (3.07), RD 3002 (3.40) and IBON-HI-2020-55 (3.4). Bahrami *et al.* (2020) assessed heat tolerance using phenological and agronomic traits in cultivated and wild barley genotypes and also selected tolerant genotypes employing stress indices. Munjal and Dhanda (2016) viewed HRI as more useful criteria of genotypic categorization concluded based on escape, resistance or tolerance mechanisms of heat tolerance. The promising genotypes based on high HRI were IBON-HI-2021-102 (6.78), BH 1018 (6.74), IBON-HI-2020-51 (5.88), DWRB 123 (5.65), BH 1038 (5.27), and IBON-HI-2021-33 (5.04). HRI was also exploited earlier by Suresh *et al.* (2018) for heat tolerance in wheat and triticale cultivars.

The genotypes BH 1018 (0.98), IBON-HI-2021-52 (0.95), BH 1036 (0.90), DWRUB 64 (0.90) and 7th GSBON-2020-101 (0.89) indicated high estimation with respect to HTI, denoting better cultivars in experimental material. The most susceptible genotype was IBON-HI-2020-155 (0.56) as displayed by low HTI. Likewise, MP and GMP indices specify BH 1038, IBON-HI-2020-155, IBYT-HI-2021-17, 7th GSBON-2020-140, DWRB 91, IBON-HI-2021-102, IBYT-HI-2021-18 and IBON-HI-2021-56 as better performing entries in the material evaluated. The genotypes thus identified based on different stress tolerance indices confirmed huge potential to be availed in heat tolerance breeding. The suitability of MP, GMP, STI, SSI and TOL indices for isolation of heat tolerant genotypes in barley was also corroborated by Pathak *et al.* (2017).

The check varieties DWRUB 64 and DWRB 91, recommended for late condition, performed well for HSI, HRI, HTI and TOL; and MP and GMP, respectively. Hence, they could be used for heat tolerance study employing respective stress indices. Subhani *et al.* (2015) also applied different stress indices in barley for sorting the heat tolerant genotypes. The results based on overall rank identified IBON-HI-2021-102, BH 1018, IBON-HI-2020-51, DWRB 123 and 7th GSBON-2020-101 genotypes promising with tolerance to heat among forty-four entries evaluated.

3.2 Correlation between grain yield and stress indices:

Correlation coefficient analysis was performed among stress indices, including grain yield (Y_p and Y_s), and the results are illustrated in Table 2. Grain yield (Y_s) showed a negative association with HSI and a significant positive correlation with the indices HRI, MP and GMP, suggesting the significance of higher HRI, MP and GMP, and lower HSI for heat tolerance. Bhagat *et al.* (2023) observed correlation between stresses tolerance indices and grain yield in barley and also reported negative correlation of SSI with grain yield under stress conditions. The stress indices HRI, MP and GMP exhibited the maximum positive correlation with grain yield (Y_s); hence, they could be regarded as the best selection criterion for heat stress tolerance. A positive correlation was found for grain yield under non-stress and stress situations, as substantiated by Nazari and Pakniyat (2010). Under non-stress condition, grain yield (Y_p) recorded significant positive correlation with all the indices except HRI and significant negative association with HTI. Likewise, among the stress indices, significant positive associations were observed for HSI with TOL, MP and GMP; HRI with HTI, MP and GMP; TOL with MP and GMP; and MP with GMP. The results also revealed a significant negative association of HSI with HRI and HTI; HRI with TOL; HTI with TOL, MP and GMP. These results are in congruence with the findings of Nazari and Pakniyat (2010) and Ghomi *et al.* (2023) about association between two or more variables.

3.3 Principal component and biplot analysis:

Principal Component Analysis (PCA) represents the association between all variables at once with decreased number of variables that contribute to the maximum percentage of total variation, which is a better criterion than the correlation coefficient for assorting promising genotypes in different environments (Nouri *et al.*, 2011). PCA based on grain yield and stress indices as depicted in Table 3 revealed that the first two components with Eigen value $>$ 1.00 accounted for about 99.7% of the total variation present in the studied genotypes. The results are in line with the observations of Nazari and Pakniyat (2010) and Ghomi *et al.* (2023); they explained 97.9% and 99.2% of the variation by the first two PCs, respectively. The first PC accounted for 63.45% of the total variance, regarded as heat susceptible component as it showed strong association with Y_p, HSI, TOL, MP and GMP. Similarly, the second PC explained 36.24% of the total variability and exhibited a strong correlation with HRI, HTI, and Y_s, therefore being considered a heat tolerant component. Thus, the selection of genotypes with high PC 2 and low PC 1 is suitable for both environments. Consequently, genotypes BH 1018, IBON-HI-2021-102, IBON-HI-2020-51and DWRB 123 were found to have high PC2 and low PC1, therefore, regarded as superior genotypes for both stress and non-stress conditions. A similar kind of approach was also used by Dorostkar *et al.* (2015) to classify the components.

Table 3: Principal component analysis based on grain yield and stress indices in barley genotypes

Components	**Eigen value**	**Proportion of total variation (%)**	**Variables**							
			$\mathbf{Y_p}$	$\mathbf{Y_s}$	**HSI**	**HRI**	**HTI**	**TOL**	**MP**	**GMP**
PC 1	5.08	**63.45**	0.996	0.558	0.794	0.191	-0.792	0.906	0.932	0.906
PC 2	**2.90**	**36.24**	0.178	0.830	-0.603	0.996	0.606	-0.417	0.361	0.422

PC1: First principal component, **PC2**: Second principal component, **HSI:** Heat susceptibility index, **HRI:** Heat response index, **HTI:** Heat tolerance index, **TOL:** Stress tolerance, **MP:** Mean productivity and **GMP:** Geometric mean productivity, $\mathbf{Y_p}$: Grain yield under non-stress condition, $\mathbf{Y_s}$: Grain yield under stress condition

In the biplot analysis, when the angle between their vectors is < 90 degrees, it indicates the positive association of indices, and when the angle is > 90 degrees, it shows a negative correlation among indices (Devi *et al.*, 2021). The first two components were used to generate a biplot (Fig. 3) for comparing the relationship between genotypes and stress indices. The biplot displayed positive associations of Yp and Ys with HRI, MP, GMP, HSI, and TOL, while negatively correlated with HTI, as indicated by the acute and obtuse angles between their vectors, respectively. Likewise, HSI and TOL exhibited a negative correlation with HTI and HRI, and were positively associated with MP and GMP.

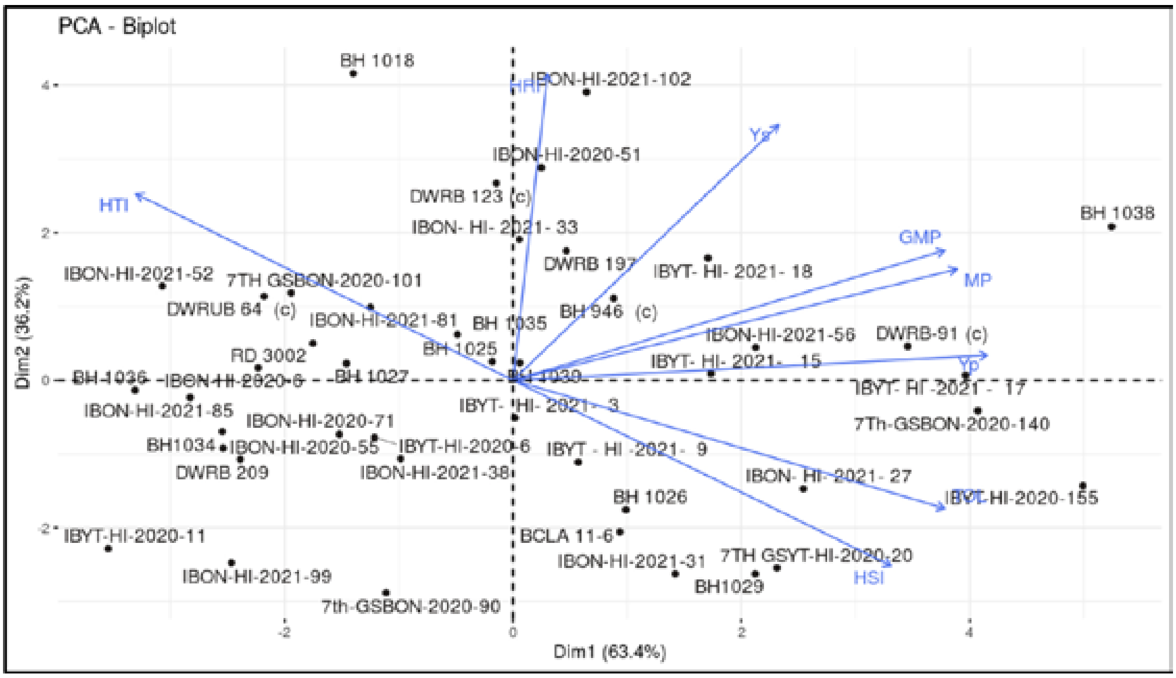

Fig. 3. Biplot based on PCA showing correlation among stress indices and grain yield

PC1 (Dim1): First principal component, PC2 (Dim2): Second principal component, **HSI:** Heat susceptibility index, **HRI:** Heat response index, **HTI:** Heat tolerance index, **TOL:** Stress tolerance, **MP:** Mean productivity and **GMP:** Geometric mean productivity, $\mathbf{Y_p}$: Grain yield under non-stress condition, $\mathbf{Y_s}$: Grain yield under stress condition

3.4 Cluster analysis

Cluster analysis based on stress indices was performed, classifying the genotypes into four clusters. The findings of cluster analysis are illustrated in Table 4, along with genetic distances between clusters. The clustering pattern identified cluster II as the largest one with 21 genotypes, followed by cluster IV (10), and cluster I (8), while cluster III was the smallest with five genotypes. The estimates of various stress indices were also used by Lamba *et al.* (2023) for clustering genotypes evaluated under stress conditions. Several other genetic studies for the classification of genotypes into different tolerance categories have also been conducted in barley using stress indices (Ghomi *et al.*, 2023).

The relationship among the studied genotypes is presented in Fig. 4 in the form of a circular dendrogram displaying the serial number of genotypes that may be decoded by conferring Table 1. The genetic distances (intra- and inter-cluster) were also calculated (Table 4), indicating the magnitude of genetic diversity among the genotypes. The results revealed the maximum intra-cluster distance for cluster II, followed by cluster IV, implying that the genotypes have relatively more diversity as compared to genotypes belonging to other clusters. Similarly, the genotypes in cluster III showed more similarity as deciphered by the minimum intra-cluster distance. In addition, The results also revealed that cluster II was placed most distantly from cluster III, as exhibited by maximum inter-cluster distance among all cluster combinations, followed by clusters III and IV. However, cluster I is most closely placed to cluster IV, as observed based on the minimum distance among inter cluster distances. The cluster analysis of 326 genotypes constituted two distinct classes in relation to heat tolerance in barley, which was also substantiated by Abou-Elwafa and Amein (2016).

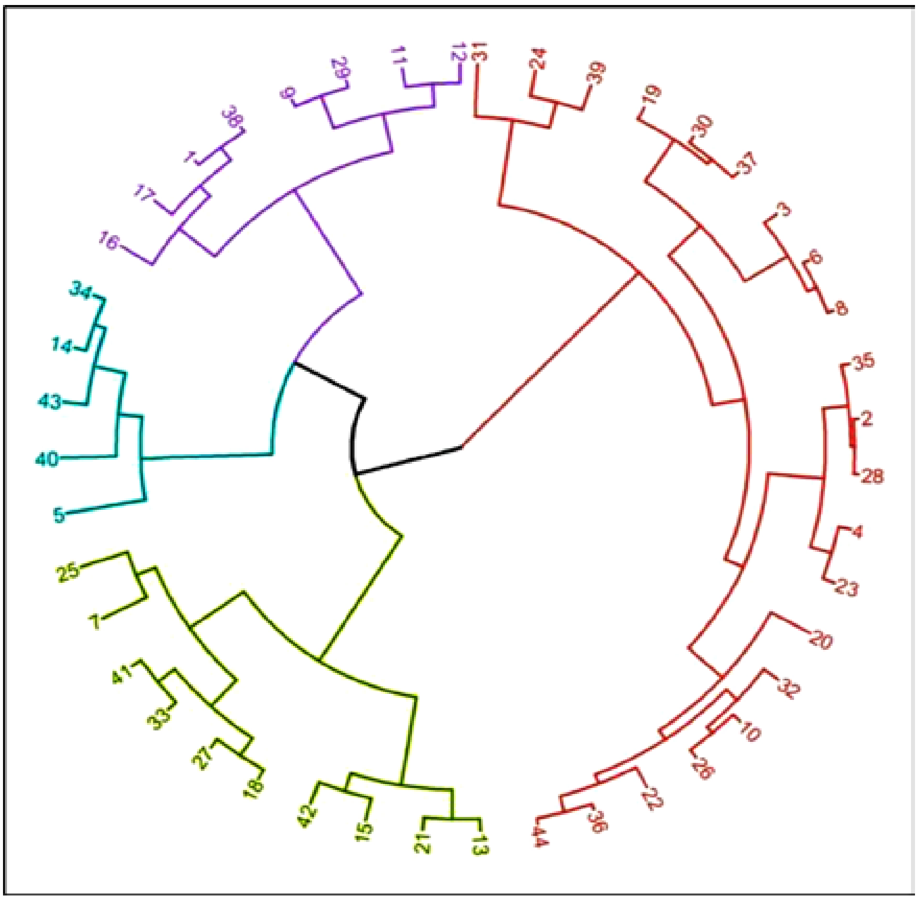

Fig. 4. Dendrogram portraying clustering pattern of 44 barley genotypes
(For detail of genotypes with Sr. No., refer to Table 1)

Table 4: Clustering of barley genotypes based on stress indices and genetic distances

Cluster Members	Number of Genotypes	Clusters	Cluster Distances			
			Cluster I	Cluster II	Cluster III	Cluster IV
BH 1029, BH 1026, IBYT- HI-2021- 3, IBYT- HI-2021-9, IBON-HI-2021-27, IBON-HI-2021-31, BCLA 11-6, 7th GSYT-HI-2020-20	8	Cluster I	**8.88**	21.41	20.73	16.37
BH 1034, BH 1035, BH 1036, BH 1039, BH 1025, BH 1027, IBON-HI-202-38, IBON-HI-2021-52, IBON-HI-2021-81, IBON-HI-2021-85, IBON-HI-2021-99, RD 3002, DWRB 209, IBON-HI-2020-71, 7th GSBON-2020-90, IBON-HI-2020-6, IBON-HI-2020-55, 7th GSBON-2020-101, IBYT-HI-2020-6, IBYT-HI-2020-11, DWRUB 64	21	Cluster II	21.41	**14.46**	34.07	21.67
BH 1038, IBYT- HI -2021 -17, 7th GSBON-2020-140, IBYT-HI-2020-155, DWRB 91	5	Cluster III	20.73	34.07	**8.01**	23.31
BH 1018, IBYT- HI-2021-15, IBYT- HI-2021-18, IBON-HI-2021-33, IBON-HI-2021-56, IBON-HI-2021-102, DWRB 197, IBON-HI-2020-51, DWRB 123, BH 946	10	Cluster IV	16.37	21.67	23.31	**12.17**

Table 5: Performance of clusters for grain yield of genotypes and stress indices

	HSI	HRI	HTI	TOL	MP	GMP	Y_p	Y_s
Cluster I	1.49	1.96	0.65	10.59	24.28	23.68	29.58	18.99
Cluster II	**0.73**	3.02	**0.83**	**4.04**	20.82	20.70	22.84	18.79
Cluster III	1.57	3.60	0.62	14.01	**30.19**	**29.35**	**37.19**	23.19
Cluster IV	**0.78**	**5.19**	**0.82**	**5.49**	26.09	25.91	28.83	**23.34**

HSI: Heat susceptibility index, **HRI:** Heat response index, **HTI:** Heat tolerance index, **TOL:** Stress tolerance, **MP:** Mean productivity and **GMP:** Geometric mean productivity, $\mathbf{Y_p}$: Grain yield under non-stress condition, $\mathbf{Y_s}$: Grain yield under stress condition

The average performance of genotypes in relation to grain yield (Y_p and Y_s) and stress indices under study is portrayed in Table 5. The observations indicated cluster II with minimum HSI and TOL and high HTI. These indices of cluster II are at par with cluster IV. Hence, cluster IV could be considered a promising one for these indices. In addition, cluster IV also performed the best for HRI and grain yield (Y_s) under stress conditions. Similarly, cluster III was characterized by higher MP and GMP with high yield potential (Y_p) under non-stress conditions. So genotypes to be improved as per breeding target should undergo for crossing with parents from these clusters. Cluster IV has the genetic resources to meet the need for heat tolerance.

Conclusion

The current study concluded that the stress indices employed were recognized as vital for the identification and isolation of promising cultivars with high tolerance to heat. Among the forty four genotypes included in the study, namely IBON-HI-2021-102, BH 1018, IBON-HI-2020-51, DWRB 123, and 7th GSBON-2020-101, elite genotypes were found based on the average rank of all six stress indices employed on grain yield in relation to heat tolerance. The stress indices, HRI, MP**,** and GMP exhibited the maximum positive correlation with grain yield (Y_s), while HSI showed a negative relationship with grain yield under stress conditions; therefore, these indices can be regarded as the best selection criteria for heat tolerance. The second principal component (PC2) exhibited a strong correlation with HRI, HTI and Y_s and, hence, could be considered a heat tolerant component. The genotypes in cluster IV exhibited better performance under stress for grain yield and other stress indices, which consequently could be incorporated in barley cultivars intended to develop new heat-tolerant varieties.

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Author's Contribution

Conceptualization of research (YK), Designing of experiment (YK), Execution of experiment and data recording (A, YK), Data analysis (SD, A), Manuscript writing (YK, DP). All authors read, edited and approved the final manuscript.

Conflict of interest

No

Ethical Compliance Statement

NA

Declaration

The authors declare no conflict of interest.

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