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Research Note

Estimation of stress tolerance indices for identification of heat tolerant genotypes in barley

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Abstract
The objective of present study was to assess the heat tolerance of barley genotypes employing a set of 12 heat stress indices namely SSI, TOL, STI, SSPI, YI, YSI, RSI, MP,GMP, HM, MRP and RED, estimated using grain yield. A total of 29 barley genotypes were evaluated under normal (non-stress) and heat stress conditions during 2021-22 crop season with four replications in randomized block design (RBD) at Research Area, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar (Haryana). The genotypes, DWRB 91, BH 20-40, BH 20-05, BH 20-07 and BH 19-15 were found to be heat tolerant based on average rank of SSI for different traits. However, based on overall rank of stress indices employed on grain yield, BH 19-13, BH 20-40, BH 393, BH 19-15, BH 20-02 and BH 946 were found prominent with tolerance to heat stress. Grain yield (Y_s) showed negative association with SSI, RSI and RED and significant positive correlation with the indices *viz*., STI, YI, MP, GMP and HM. Hence these indices could be regarded as the best selection indicators for heat stress tolerance. PCA study considered second principal component (PC 2) as heat tolerant component based on strong correlation with STI, YI, YSI, MP, GMP, HM, MRP and Y_s under stress condition. The genotypes of cluster I exhibited better performance under stress condition for grain yield (Y_s) and SSI, TOL, SSPI, RSI, RED, YI, YSI, and MRP. The genotypes from this group could be utilized as promising breeding material for development of new heat tolerant barley cultivars.

Keywords: Barley, correlation, cluster analysis, PCA, stress indices, heat tolerance

Barley (*Hordeum vulgare* L.) is an economically important crop exhibiting excellent adaptation capability to a wide range of environments, and as a result, can be used as an excellent model for deciphering crop response to climate change (Dawson *et al*., 2015). Barley is mainly cultivated for feeding livestock (Hassan *et al*., 2021) and is a good source of food (Jamshidi and Javanmard, 2018). Alternatively, it is also used in the field of brewing and medicine industries, hence is an important crop of present era. This crop offers various health benefits in terms of lowest glycemic index (28) among cereals, higher quantity of beta glucan (5-10%), low amount of anti-nutritional factor *i.e.* phytates (386 mg) and contains almost half of gluten than that of wheat.

It has potential to alleviate food shortage as well as malnutrition (Vinesh *et al*., 2018). This nutri-rich cereal holds fourth position in terms of harvested area (46.90 million hectares) and global production (142.64 million tonnes) during 2022-23 (ICAR-IIWBR, 2023). In India, the total barley production during 2022-23 was 1.69 million tonnes from 0.62 million hectare with average national productivity of 27.33 q/ha. Despite the huge importance of cultivated barley (*Hordeum vulgare* ssp. *vulgare*) as a global cereal, its thermo-tolerance mechanisms are not well known (Bahrami *et al*., 2019).

The environmental stresses particularly water and temperature plays a major role in determining the yield (Rakavi The environmental stresses particularly water and temperature plays a major role in determining the yield (Rakavi *et al.,* 2018). High temperature is considered as a major abiotic stress with detrimental impact on crop productivity globally and has terrible consequences on food security (Abou-Elwafa and Amein, 2016). Approximately 90% of total arable land is estimated to be prone to one or more abiotic stresses, leading to considerable losses in the quality and quantity of major food crops (He *et al*., 2018). It is predicted in a metadata analysis that the crops will be seriously challenged by increasing the global average temperatures, which may rise by 2.0-4.9 °C till the end of this century (Raftery *et al*., 2017). It is expected that a change in temperature by 1◦C may adversely affect the biochemical and physiological activities of plants (Kumar *et al*., 2022). Plant growth and development owing to vulnerability of plant architecture and its physiological and reproductive processes can be affected by high temperatures (Driedonks *et al.,*2016). Furthermore, heat stress may reduce the crop yield at elevated temperatures (35 °c day/25 °c night) by inducing pollen sterility and seed abortion during the reproductive growth stage (Barnabas *et al*., 2008 and Klink *et al.,* 2014). High temperatures have been reported to shorten the grain-filling period leading to substantial reduction in seed yield (Prasad and Djanaguiraman, 2014). Further, delayed sowing has been reported to reduce grain yield as compared to normal sowing. However, the genotypes may differ for productivity (Joshi *et al.,* 2016). Kumar *et al*. (2022) also highlighted that early stages of grain development in barley are more sensitive to heat stress as compared to the later ones. In addition, it was also observed that photosynthesis is significantly affected by heat stress by decreasing the Rubisco activation state *via* inhibition of Rubisco activase. (Wang *et al*., 2015). The physiological aspects of heat tolerance in barley need to be dissected in comprehensive details at the genetic level before their exploitation in introgression breeding.

The heat tolerance studies in crop cultivars require reliable criteria to be established for screening thermo-tolerant wild germplasm. The wild barley can contribute tremendously to improve the carbon sequestration in cultivated barley by exploiting genetic and breeding tools (Bahrami *et al.,* 2019). Effective utilization of wild type resources as compared to the cultivated ones were also supported by Bahrami *et al*. (2021) for introgression of stress tolerance trait. The first essential step toward enhancing the stress tolerance of crop plants is to sort the suitable genetic donors for being to incorporate tolerance to elite genotypes (Javed *et al*., 2022). In this context, evaluation and exploitation of germplasm resources can be done to develop promising genotypes with desired levels of yield and quality.

Heat tolerance is a complex polygene controlled trait that involves inter-allelic as well as genotype × environment interactions (Abou-Elwafa and Amein, 2016). Hence, to cope up with the alarming threat of heat stress, it is vital to elucidate the molecular/ genetic basis of heat stress tolerance for development of superior crop plants through conventional or molecular breeding techniques to evade the challenges of climate change (Anwar and Kim, 2020). Consequently, this research work aimed to assess the responses of 29 barley genotypes to heat stress using a set of 12 stress indices including SSI, TOL, STI, SSPI, YI, YSI, RSI, MP, GMP, HM, MRP and RED, intended to develop heat tolerant varieties.

An experiment was performed with a set of 29 barley genotypes during 2021-22 crop season at Research Area of the Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar. The experiment was planted under two environments *i.e.* non-stress (Timely/normal sown) and stress (Heat/Late sown) conditions. Under non-stress, the materials were sown on 15th November, 2021 and for stress on 14th December, 2021. It was expected that the material sown in mid of December may encounter terminal heat stress during grain filling stage. The experimental material consisted of 20 two-rowed and nine six-rowed genotypes, evaluated in Randomized Block Design (RBD) with four replications. Each genotype occupied a plot size of 6.9 m^2 (six rows of 5 m length spaced at 23 cm apart). The package of practices recommended for both environments were followed to raise the crop.

The traits *viz*., days to heading, days to maturity, plant height (cm), number of effective tillers per meter, spike length (cm), number of grains per spike, 1000-grain weight (g), biological yield (q/ha), harvest index (%), and grain yield (q/ha) were studied under both non-stressed and stress environments. The different stress indices used in the present study for heat tolerance are furnished in **Table 1** with their formulae. The recorded data were subjected to statistical analysis using different formulae in Microsoft Excel for calculation of stress indices. R studio version 2023.12.1.402 was used for correlation coefficient and cluster analyses.The Principal Component Analysis (PCA) was carried out using SPSS Statistics version 27 software and biplots were drawn. The weather parameters for crop season 2021-22 are presented in **Fig. 1**. It revealed the maximum and minimum temperatures as 37.5°C and 17.2°C, respectively in the standard week 13thand 12th(2022). Further, the weather data (**Fig. 2**) for post heading phase of the crop indicated that the average minimum and maximum temperature under non-stress condition was 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 environments comparably.

The mean sum of squares for different traits under study is presented in **Table 2**. The results indicated that mean sum of squares due to genotypes and environments were highly significant for all the characters studied, except for days to maturity for genotypes. Similarly, mean sum of squares were also observed significant due to

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Table 1. Stress indices used in the study

S. No.	Stress indices	Abbreviations	Formulae	References
1	Stress susceptibility index	SSI	$SSI = (1 - Xh/X)/(1 - Yh/Y)$	Fischer and Maurer (1978)
2	Stress tolerance	TOL	$TOL = Yp - Ys$	Rosielle and Hamblin (1981)
3	Stress tolerance index	STI	$STI = (Yp \times Ys) / Xp^2$	Fernandez (1992)
4	Stress susceptibility percentage index	SSPI	$SSPI = \frac{Yp-Ys}{2\ (Xp)} \times 100$	Moosavi *et al.* (2008)
5	Yield index	YI	$YI = Ys / Xs$	Gavuzzi *et al.* (1997)
6	Yield susceptibility index	YSI	$YSI = Ys / Yp$	Bouslama and Schapaugh (1984)
7	Relative stress index	RSI	$RSI = \frac{(Yp/Ys)}{Xs/Xp}$	Fischer and Wood (1979)
8	Mean productivity	MP	$MP = (Yp + Ys) / 2$	Rosielle and Hamblin (1981)
9	Geometric mean productivity	GMP	$GMP = \sqrt{Ys} \times Yp$	Fernandez (1992)
10	Harmonic mean	HM	$HM = 2(Yp \times Ys) / (Yp + Ys)$	Bidinger *et al.* (1987)
11	Mean relative performance	MRP	$MRP = (Ys/Xs) + (Yp/Xp)$	Ramirez and Kelly (1998)
12	Reduction	RED	$RED = (Yp - Ys) / Yp \times 100$	Farshadfar and Javadinia (2011)

X = Grain yield of individual genotype under non-stress condition; Y = Mean of all genotypes under non-stress condition; Xh = Grain yield of individual genotype under stress condition; Yh = Mean of all genotypes under stress condition; Yp =Grain yield of genotype under non-stress condition; Ys= Grain yield of genotype under stress condition; Xp =Mean yield of all genotypes under non-stress condition; Xs =Mean yield of all genotypes under stress condition

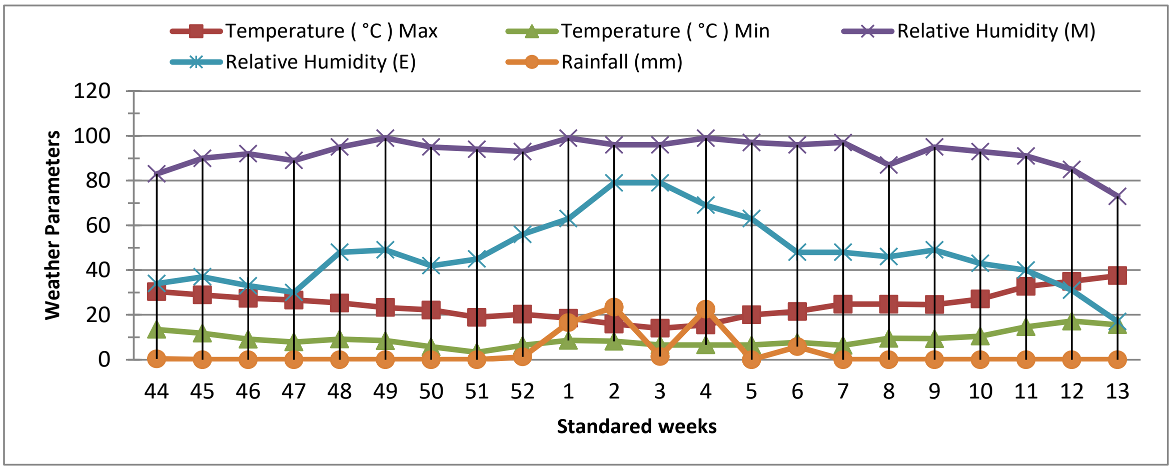

Fig. 1 Mean meteorological data during 2021-2022crop season

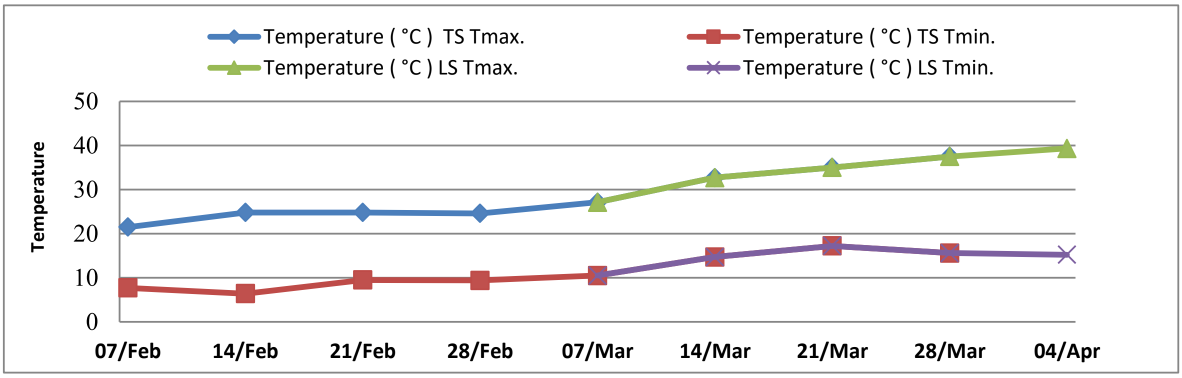

Fig. 2 Post heading maximum and minimum temperature under normal and late sown condition during 2021-22

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Table 2. Mean sum of squares for different traits of barley genotypes

Source of Variation	d. f.	Mean sum of squares									
		DH	DM	PH	ETM	SL	GPS	TGW	BY	HI	GY
Genotypes	28	36.23*	31.18	946.04**	4248.62**	11.88**	2306.12**	339.91**	397.27**	82.43**	135.98**
Replications	3	402.14	768.38	898.79	2067.01	11.09	46.13	808.10	1703.32	78.53	482.96
Environments	1	4380.36**	13904.12**	2048.21**	53286.12**	46.26**	534.13**	1562.19**	10106.83**	82.94**	1501.92**
Genotypes: Environments	28	14.56	14.22	18.82	1076.15**	0.67**	12.60**	27.37**	78.54**	5.83**	10.19*
Residuals	171	25.74	57.37	21.60	81.09	0.21	6.60	6.30	30.21	1.52	5.90

DH: Days to heading, DM: Days to maturity, PH: Plant height, ETM: Number of effective tillers per meter, SL: Spike length, GPS: Number of grains per spike, TGW: 1000-grain weight, **BY**: Biological yield (q/ha), **HI**: Harvest index, **GY**: Grain yield (q/ha), *, **Significance at 0.05 & 0.01, respectively.

interaction of genotypes and environments for all traits except for days to heading, maturity and plant height. This indicated a wide range of genetic variability, which could be exploited for selection of genotypes for heat tolerance breeding.

Analysis of the difference in performance of genotypes for different traits revealed wide variation in performance under both the conditions (**Table 3**). The genotypes BH 20-38 (46.58 q/ha), BH 19-44 (42.53 q/ha) and BH 19-52 (42.48 q/ha) were found to record maximum grain yield and high harvest index under non-stress condition. Similarly the genotypes *viz*., BH 946 (146.94 q/ha) and BH 20-40 (136.88 q/ha) exhibited highest biological yield. The minimum plant height was recorded in the genotypes BH 20-16 (85 cm) and BH 20-15 (86 cm). Highest number of effective tillers per meter was observed in BH 20-09 (194) and BH 19-02 (178). The genotype BH 20-04 (12.7 cm) among two rowed and BH 19-44 (8.3 cm) among six rowed barley showed longest spikes with highest number of grains per spike. Likewise, BH 20-14 (56.4 g) and BH 20-04 (53.8 g) revealed highest 1000-grain weight under normal environment. Under stress condition, BH 20-38 (38.59 q/ha) registered highest grain yield and also showed highest harvest index (33.07%). Highest biological yield was recorded by BH 946 (138.97 q/ha) followed by BH 20-40 (131.16 q/ha). Highest number of effective tillers was observed in DWRB 182 (166) and

Table 3. Performance of barley genotypes for different traits under non-stress and stress conditions

S. No.	Genotypes	RT	E	DH	DM	PH	ETM	SL	GPS	TGW	BY	HI	GY
1	BH 20-02	2	NS	96	137	101	155	8.2	28	49.2	130.67	28.13	36.76
			S	81	120	96	124	6.5	23	46.3	120.94	27.50	33.26
2	BH 20-11	2	NS	92	139	96	129	7.4	26	51.3	126.81	27.65	35.06
			S	84	122	95	126	6.4	25	46.3	98.48	26.93	26.52
3	BH 20-10	2	NS	96	139	97	170	9.0	28	42.4	125.60	28.88	36.27
			S	85	123	93	154	6.7	28	33.9	106.52	24.46	26.05
4	BH 20-39	2	NS	90	137	107	173	8.9	30	52.3	127.62	25.44	32.47
			S	83	118	99	104	7.3	25	42.0	110.26	25.19	27.78
5	BH 20-04	2	NS	94	135	126	158	12.7	33	53.8	136.07	21.86	29.75
			S	85	122	119	135	11.2	29	37.8	112.03	21.57	24.17
6	BH 946	6	NS	90	136	116	119	7.3	68	39.7	146.94	27.88	40.97
			S	80	119	108	108	6.4	62	32.5	138.97	26.20	36.41
7	BH 20-06	2	NS	92	140	104	164	9.5	28	50.9	115.94	25.52	29.59
			S	84	119	103	86	7.7	26	46.7	107.97	23.26	25.11
8	DWRB 101	2	NS	90	134	97	174	6.4	25	43.6	130.84	28.06	36.71
			S	82	119	91	110	6.4	24	38.4	110.49	26.76	29.57
9	BH 20-40	6	NS	88	135	116	93	7.5	71	40.7	136.88	26.56	36.35
			S	82	122	113	80	6.7	68	30.5	131.16	25.47	33.41
10	BH 20-17	2	NS	91	135	115	135	7.6	24	52.2	132.85	23.28	30.93
			S	77	120	108	94	7.6	22	51.0	118.45	23.03	27.28

Table 3. Continued..

S. No.	Genotypes	RT	E	DH	DM	PH	ETM	SL	GPS	TGW	BY	HI	GY
11	BH 20-36	2	NS	91	134	98	106	8.2	29	48.7	122.79	26.18	32.14
			S	80	120	97	89	7.7	28	41.8	113.04	24.97	28.23
12	BH 20-15	2	NS	89	137	86	129	7.7	28	44.9	126.01	29.46	37.12
			S	81	121	79	112	6.4	23	44.5	110.58	28.47	31.49
13	BH 20-13	2	NS	90	135	95	108	8.4	27	53.2	131.64	26.84	35.33
			S	81	119	88	98	7.3	27	44.4	115.41	26.59	30.69
14	BH 20-38	2	NS	90	134	105	155	8.1	27	39.7	134.46	34.64	46.58
			S	78	121	98	140	7.5	26	38.7	116.67	33.07	38.59
15	BH 20-14	2	NS	95	134	96	112	8.3	26	56.4	128.42	21.91	28.14
			S	84	123	89	105	8.1	24	50.4	114.57	21.09	24.17
16	BH 20-05	2	NS	90	136	94	99	8.2	26	51.6	114.73	25.00	28.68
			S	82	118	92	94	7.2	23	49.7	110.15	24.62	27.12
17	BH 20-09	2	NS	91	138	91	194	7.2	27	45.9	133.65	28.13	37.60
			S	83	122	85	152	6.5	26	45.2	112.32	27.65	31.05
18	BH 20-07	2	NS	90	137	87	136	8.4	26	48.2	121.18	24.15	29.26
			S	82	121	83	89	8.2	24	43.1	115.94	23.30	27.01
19	BH 20-16	2	NS	87	137	85	174	6.3	23	51.3	117.95	29.83	35.19
			S	79	114	76	96	5.8	23	47.0	106.88	29.46	31.49
20	DWRB 182	2	NS	88	134	88	174	5.2	25	39.4	119.56	29.56	35.35
			S	84	117	83	166	4.9	22	28.0	105.30	29.25	30.80
21	BH 393	6	NS	83	129	93	125	7.6	52	37.0	118.36	29.69	35.14
			S	78	113	90	102	6.2	48	33.7	100.58	32.96	33.15
22	BH 19-15	6	NS	87	135	114	115	7.7	62	40.1	126.01	32.82	41.36
			S	80	121	109	106	6.3	58	32.9	116.67	31.96	37.28
23	BH 19-52	6	NS	90	133	118	174	6.8	60	37.2	124.40	34.15	42.48
			S	84	120	108	124	6.3	58	34.6	117.03	28.92	33.84
24	BH 19-49	6	NS	90	137	114	172	7.4	64	34.4	130.84	28.06	36.71
			S	84	122	100	104	6.2	54	32.7	112.61	27.93	31.45
25	BH 19-02	2	NS	90	132	102	178	6.3	27	43.4	119.56	31.52	37.68
			S	85	120	96	146	5.6	24	38.3	103.12	30.60	31.56
26	BH 19-44	6	NS	90	134	119	130	8.3	76	39.8	125.60	33.86	42.53
			S	81	120	113	90	7.4	68	36.9	116.30	28.22	32.83
27	BH 19-13	6	NS	89	136	102	154	6.3	58	34.3	127.17	31.71	40.33
			S	80	121	97	120	5.3	50	30.8	120.14	30.56	36.71
28	DWRB 91	2	NS	90	132	95	148	8.0	28	44.1	124.32	26.60	33.07
			S	80	119	91	138	7.7	28	42.8	115.44	26.13	30.16
29	RD 2794	6	NS	91	133	112	114	7.1	62	33.5	125.33	25.88	32.44
			S	79	119	101	96	6.7	60	27.8	121.38	22.48	27.28
		Mean	**NS**	**90.3**	**135.3**	**102.5**	**143.6**	**7.8**	**38.4**	**44.8**	**127.0**	**28.0**	**35.6**
			S	**81.7**	**119.8**	**96.5**	**113.4**	**6.9**	**35.4**	**39.6**	**113.8**	**26.8**	**30.5**
		Max.	**NS**	**96**	**140**	**126**	**194**	**12.7**	**76**	**56.4**	**146.94**	**34.64**	**46.58**
			S	**85**	**123**	**119**	**166**	**11.2**	**68**	**51.0**	**138.97**	**33.07**	**38.59**
		Min.	**NS**	**83**	**129**	**85**	**93**	**5.2**	**23**	**33.5**	**114.73**	**21.86**	**28.14**
			S	**77**	**113**	**76**	**80**	**4.9**	**22**	**27.8**	**98.48**	**21.09**	**24.17**

RT: Row type, E: Environment/condition, NS: Non-stress, S: Stress, DH: Days to heading, DM: Days to maturity, PH: Plant height (cm), ETM: Number of effective tillers per meter, SL: Spike length (cm), GPS: Number of grains per spike, TGW: 1000-grain weight (g), BY: Biological yield (q/ha), HI: Harvest index, GY: Grain yield (q/ha)

BH 20-10 (154). Similarly, the genotype BH 20-04 (11.2 cm) among two rowed and BH 19-44 (7.4 cm) among six rowed barley exhibited longest spikes with high number of grains per spike. Among the genotypes, BH 393 (129 & 113 days) was found early maturing under both environments.

In order to examine the heat tolerance of genotypes exploiting all the studied traits, SSI was calculated and presented in **Table 4**. The grain yield recorded under non-stress and stress conditions are indicated as Yp and Ys, respectively. The genotype BH 20-38 was found with maximum (46.58 and 38.59 q/ha) and BH 20-14 with minimum (28.14 and 24.17 q/ha) grain yield under normal and stress conditions, respectively. The results also showed a reduction in mean grain yield under stress condition by 14.3 percent compared to non-stress condition, which indicated the influence of high temperature on grain yield. Parashar *et al*. (2019) reported grain yield decline of 32.15 percent due to heat stress. Similar yield reduction was also observed by Bhagat *et al*. (2023), This might be a resultant of forced maturity and shortening of grain filling period with delayed sowing. Higher SSI values represent the susceptibility

Table 4. Grain yield and stress susceptibility index (SSI) of barley genotypes for different traits

S. No.	Genotypes	Yp	Ys	DH	DM	PH	ETM	SL	GPS	TGW	BY	HI	GY	R
1	BH 20-02	36.76	33.26	1.62	1.08	0.80	0.94	1.85	2.41	0.51	0.72	0.53	0.67	29
2	BH 20-11	35.06	26.52	0.61	0.86	0.05	0.16	0.89	0.18	0.68	1.56	0.18	1.70	11
3	BH 20-10	36.27	26.05	0.80	0.80	0.31	0.64	1.79	0.00	1.40	1.06	1.07	1.97	27
4	BH 20-39	32.47	27.78	0.54	0.97	0.48	2.79	1.26	1.15	1.38	0.95	0.07	1.01	28
5	BH 20-04	29.75	24.17	0.67	0.67	0.42	1.02	0.82	0.71	2.08	1.24	0.09	1.31	25
6	BH 946	40.97	36.41	0.78	0.87	0.48	0.62	0.86	0.62	1.27	0.38	0.42	0.78	21
7	BH 20-06	29.59	25.11	0.61	1.05	0.07	3.31	1.28	0.58	0.58	0.48	0.62	1.06	22
8	DWRB 101	36.71	29.57	0.62	0.78	0.46	2.57	0.04	0.37	0.83	1.09	0.32	1.36	26
9	BH 20-40	36.35	33.41	0.48	0.67	0.20	0.98	0.77	0.33	1.75	0.29	0.29	0.57	2
10	BH 20-17	30.93	27.28	1.08	0.78	0.44	2.12	-0.06	0.67	0.16	0.76	0.07	0.82	10
11	BH 20-36	32.14	28.23	0.85	0.73	0.05	1.12	0.43	0.16	0.99	0.55	0.32	0.85	6
12	BH 20-15	37.12	31.49	0.63	0.82	0.59	0.92	1.21	1.10	0.06	0.86	0.23	1.06	22
13	BH 20-13	35.33	30.69	0.70	0.83	0.49	0.65	0.97	0.00	1.16	0.86	0.06	0.92	16
14	BH 20-38	46.58	38.59	0.93	0.68	0.45	0.66	0.52	0.34	0.18	0.93	0.32	1.20	13
15	BH 20-14	28.14	24.17	0.81	0.57	0.53	0.44	0.17	0.54	0.74	0.75	0.26	0.99	9
16	BH 20-05	28.68	27.12	0.62	0.93	0.15	0.35	0.91	0.82	0.26	0.28	0.10	0.38	3
17	BH 20-09	37.60	31.05	0.61	0.81	0.46	1.51	0.68	0.17	0.11	1.12	0.12	1.22	14
18	BH 20-07	29.26	27.01	0.62	0.82	0.35	2.42	0.17	0.62	0.74	0.30	0.25	0.54	4
19	BH 20-16	35.19	31.49	0.64	1.17	0.74	3.13	0.56	0.00	0.59	0.66	0.09	0.74	17
20	DWRB 182	35.35	30.80	0.32	0.89	0.40	0.32	0.40	0.84	2.02	0.83	0.07	0.90	8
21	BH 393	35.14	33.15	0.42	0.87	0.25	1.26	1.29	0.58	0.62	1.05	-0.77	0.40	7
22	BH 19-15	41.36	37.28	0.56	0.73	0.33	0.55	1.27	0.45	1.26	0.52	0.18	0.69	4
23	BH 19-52	42.48	33.84	0.47	0.68	0.63	2.01	0.52	0.27	0.49	0.41	1.07	1.42	12
24	BH 19-49	36.71	31.45	0.47	0.77	0.86	2.76	1.13	1.06	0.35	0.97	0.03	1.00	20
25	BH 19-02	37.68	31.56	0.39	0.64	0.41	1.26	0.78	0.78	0.82	0.96	0.20	1.14	14
26	BH 19-44	42.53	32.83	0.70	0.73	0.39	2.15	0.79	0.71	0.51	0.52	1.16	1.60	24
27	BH 19-13	40.33	36.71	0.71	0.77	0.36	1.54	1.11	0.96	0.71	0.39	0.25	0.63	18
28	DWRB 91	33.07	30.16	0.78	0.69	0.29	0.47	0.26	0.00	0.21	0.50	0.12	0.62	1
29	RD 2794	32.44	27.28	0.92	0.74	0.71	1.10	0.39	0.23	1.19	0.22	0.92	1.11	19
	Mean	**35.59**	**30.50**	**0.69**	**0.81**	**0.42**	**1.37**	**0.79**	**0.57**	**0.82**	**0.73**	**0.30**	**0.99**	
	Max.	**46.58**	**38.59**	**1.62**	**1.17**	**0.86**	**3.31**	**1.85**	**2.41**	**2.08**	**1.56**	**1.16**	**1.97**	
	Min.	**28.14**	**24.17**	**0.32**	**0.57**	**0.05**	**0.16**	**-0.06**	**0.00**	**0.06**	**0.22**	**-0.77**	**0.38**	

Y_p: Grain yield (q/ha) under non-stress condition, Y_s: Grain yield (q/ha) under stress condition, DH: Days to heading, DM: Days to maturity, PH: Plant height, ETM: Number of effective tillers per meter, SL: Spike length, GPS: Number of grains per spike, TGW: 1000-grain weight, BY: Biological yield, HI: Harvest index, R: Overall Rank of genotypes to higher temperature and *vice versa* (Fischer and Maurer 1978). Based on SSI values of traits, DWRB 91, BH 20-40, BH 20-05, BH 20-07 and BH 19-15 were identified as heat tolerant among the 29 studied genotypes. Though these genotypes (DWRB 91, BH 20-05 and BH 20-07) exhibited high heat tolerance, their yield potential was not up to the mark and hence these were not identified as promising genotypes. The genotypes with low SSI (≤0.5) for a particular trait is suitable for climate resilience by exhibiting stable performance for that trait under heat stress compared to non-stress condition (Thakur *et al*., 2020). Heat susceptibility index (HSI) of various traits in barley were also used by Ram and Shekhawat (2017), Yadav *et al.* (2023) in barley and Kiranakumara *et al.* (2024) in wheat for selection and utilization of heat tolerant genotypes in future breeding programme.

Efficient screening techniques for heat tolerance assessment are still lacking in barley. Hence, in order to select the tolerant genotypes, 12 stress indices calculated on the basis of grain yield were employed. Among the indices utilized, the higher estimates of SSI, TOL, SSPI, RSI, and RED reflected susceptibility of genotypes to heat, while higher STI, YI, YSI, MP, GMP, HM and MRP estimates indicated heat tolerance. The suitability of

Table 5. Stress indices of barley genotypes

S. No.	Genotypes	SSI	TOL	STI	SSPI	YI	YSI	RSI	MP	GMP	HM	MRP	RED	R
1	BH 20-02	0.67	3.50	0.97	4.92	2.76	0.90	1.29	35.01	34.97	34.92	1.06	0.10	5
2	BH 20-11	1.70	8.54	0.73	12.00	-3.98	0.76	1.54	30.79	30.50	30.20	0.88	0.24	27
3	BH 20-10	1.97	10.22	0.75	14.36	-4.45	0.72	1.62	31.16	30.74	30.32	0.84	0.28	29
4	BH 20-39	1.01	4.69	0.71	6.59	-2.72	0.86	1.36	30.13	30.03	29.94	1.00	0.14	22
5	BH 20-04	1.31	5.58	0.57	7.85	-6.33	0.81	1.44	26.96	26.81	26.67	0.95	0.19	28
6	BH 946	0.78	4.55	1.18	6.40	5.92	0.89	1.31	38.69	38.62	38.56	1.04	0.11	6
7	BH 20-06	1.06	4.48	0.59	6.30	-5.39	0.85	1.38	27.35	27.26	27.17	0.99	0.15	25
8	DWRB 101	1.36	7.15	0.86	10.05	-0.93	0.81	1.45	33.14	32.95	32.75	0.94	0.19	26
9	BH 20-40	0.57	2.95	0.96	4.14	2.91	0.92	1.27	34.88	34.85	34.82	1.07	0.08	2
10	BH 20-17	0.82	3.65	0.67	5.12	-3.22	0.88	1.32	29.11	29.05	28.99	1.03	0.12	16
11	BH 20-36	0.85	3.91	0.72	5.49	-2.27	0.88	1.33	30.19	30.12	30.06	1.02	0.12	13
12	BH 20-15	1.06	5.63	0.92	7.92	0.99	0.85	1.38	34.30	34.19	34.07	0.99	0.15	17
13	BH 20-13	0.92	4.64	0.86	6.52	0.19	0.87	1.34	33.01	32.93	32.85	1.01	0.13	15
14	BH 20-38	1.20	7.99	1.42	11.23	8.09	0.83	1.41	42.58	42.40	42.21	0.97	0.17	11
15	BH 20-14	0.99	3.97	0.54	5.58	-6.33	0.86	1.36	26.15	26.08	26.00	1.00	0.14	23
16	BH 20-05	0.38	1.56	0.61	2.19	-3.38	0.95	1.23	27.90	27.89	27.88	1.10	0.05	9
17	BH 20-09	1.22	6.55	0.92	9.20	0.55	0.83	1.41	34.33	34.17	34.01	0.96	0.17	20
18	BH 20-07	0.54	2.25	0.62	3.16	-3.49	0.92	1.26	28.14	28.11	28.09	1.08	0.08	10
19	BH 20-16	0.74	3.70	0.87	5.20	0.99	0.89	1.30	33.34	33.28	33.23	1.04	0.11	8
20	DWRB 182	0.90	4.55	0.86	6.39	0.30	0.87	1.34	33.07	32.99	32.92	1.02	0.13	12
21	BH 393	0.40	1.99	0.92	2.80	2.65	0.94	1.24	34.15	34.13	34.12	1.10	0.06	2
22	BH 19-15	0.69	4.08	1.22	5.73	6.78	0.90	1.29	39.32	39.27	39.22	1.05	0.10	4
23	BH 19-52	1.42	8.64	1.14	12.14	3.34	0.80	1.46	38.16	37.92	37.67	0.93	0.20	19
24	BH 19-49	1.00	5.27	0.91	7.40	0.95	0.86	1.36	34.08	33.98	33.88	1.00	0.14	13
25	BH 19-02	1.14	6.12	0.94	8.60	1.06	0.84	1.39	34.62	34.48	34.35	0.98	0.16	18
26	BH 19-44	1.60	9.71	1.10	13.64	2.33	0.77	1.51	37.68	37.37	37.05	0.90	0.23	21
27	BH 19-13	0.63	3.62	1.17	5.09	6.21	0.91	1.28	38.52	38.48	38.43	1.06	0.09	1
28	DWRB 91	0.62	2.91	0.79	4.09	-0.34	0.91	1.28	31.62	31.58	31.55	1.06	0.09	7
29	RD 2794	1.11	5.16	0.70	7.25	-3.22	0.84	1.39	29.86	29.75	29.64	0.98	0.16	24
	Mean	**0.99**	**5.09**	**0.87**	**7.15**	**0.00**	**0.86**	**1.36**	**33.04**	**32.93**	**32.81**	**1.00**	**0.14**	
	Max.	**1.97**	**10.22**	**1.42**	**14.36**	**-8.09**	**0.95**	**1.62**	**42.58**	**42.40**	**42.21**	**1.10**	**0.28**	
	Min.	**0.38**	**1.56**	**0.54**	**2.19**	**6.33**	**0.72**	**1.23**	**26.15**	**26.08**	**26.00**	**0.84**	**0.05**	

SSI: Stress susceptibility index, TOL: Stress tolerance, STI: Stress tolerance index, SSPI: Stress susceptibility percentage index, YI: Yield index, YSI: Yield stability index, RSI: Relative stress index, MP: Mean productivity, GMP: Geometric mean productivity, HM: Harmonic mean, MRP: Mean relative performance, RED: Reduction, R: Overall Rank

MP, GMP, STI, SSI and TOL indices for isolation of heat tolerant genotypes in barley was also corroborated by Pathak *et al*. (2017).Various researchers also applied different stress indices in barley for sorting the heat tolerant genotypes(Subhani *et al*., 2015; Bahrami *et al*., 2020).All the genotypes were ranked based on overall rank, calculated based on all indices (**Table 5**). The most promising genotypes tolerant to heat thus identified included, BH 19-13, BH 20-40, BH 393, BH 19-15, BH 20-02 and BH 946 among the entries evaluated.

Further, correlation coefficient analysis was performed among stress indices including grain yield (Y_p and Y_s) and the findings are illustrated in **Table 6**. Grain yield (Y_s) showed negative association with SSI, RSI and RED and significant positive correlation with the indices *viz*., STI, YI, MP, GMP and HM, indicating the importance of these indices for heat tolerance under stress. Hence, STI, YI, MP, GMP and HM could be regarded as the best selection indicators for heat stress tolerance. The negative correlation of SSI with grain yield under stress condition was also substantiated by Bhagat *et al*. (2023). Significant positive correlation was observed for grain yield under non-stress and stress situations. This finding confirms the finding of Nazari and Pakniyat (2010). Likewise, among the stress indices, significant positive associations were observed for SSI with TOL, SSPI, RSI and RED; TOL with SSPI, RSI and RED; STI with YI, MP, HM and GMP; SSPI with RSI and RED; YI with MP, HM and GMP; YSI with MRP; RSI with RED; and MP and GMP with HM. The results also depicted significant negative association of SSI, TOL and SSPI with YSI and MRP; YSI with RSI and RED; and MRP with RSI and RED. These results corroborate the association among two or more variables with the findings of Nazari and Pakniyat (2010), Subhani *et al*. (2015) and Ghomi *et al*. (2023).

Principal Component Analysis (PCA) represents the association between all the traits at once with reduced number of variables contributing to the maximum percentage of total variation, is better criterion over 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 7** revealed that the first two components with eigen value > 1.00 accounted for about 99.8 percent of the total variation present in the studied genotypes. The results are in line with observation of Ghomi *et al*. (2023); explained 99.2 percent of variation by first two PCs. The first PC accounted for 52.1 percent of the total variance, regarded as heat susceptible component as it showed strong association with Y_p, SSI, TOL, SSPI, RSI and RED. Similarly, second PC explained 47.7 percent of the total variability and exhibited strong correlation with STI, YI, YSI, MP, GMP, HM, MRP and $Y_{s,}$ therefore considered as heat tolerant component. Thus, the selection of genotypes with high PC2 and low PC1 are suitable for both environments. These results are in agreement with the finding of Subhani *et al*. (2015). The genotypes BH 19-15, BH 19-13, BH 946, BH 393, BH 20-40 and BH 20-02 were found with high PC2 and low PC1 and could be regarded as superior genotypes for both stress and non-stress conditions. Similar kind of approach was also used by Dorostkar *et al*. (2015) to classify the components.

Table 6. Correlation between grain yield (Y_p and Y_s) and different stress indices of barley genotypes

Stress indices	Yp	Ys	SSI	TOL	STI	SSPI	YI	YSI	RSI	MP	GMP	HM	MRP	RED
Yp	1													
Ys	0.872**	1												
SSI	0.259	-0.244	1											
TOL	0.519**	0.035	0.954**	1										
STI	0.965**	0.965**	0.008	0.286	1									
SSPI	0.52**	0.035	0.955**	1.000**	0.286	1								
YI	0.872**	1.000**	-0.244	0.035	0.965**	0.035	1							
YSI	-0.258	0.244	-0.999**	-0.952**	-0.007	-0.953**	0.244	1						
RSI	0.244	-0.258	0.997**	0.949**	-0.008	0.949**	-0.258	-0.996**	1					
MP	0.972**	0.962**	0.028	0.306	0.997**	0.306	0.962**	-0.027	0.013	1				
GMP	0.967**	0.968**	0.005	0.284	0.997**	0.284	0.968**	-0.004	-0.01	1.000**	1			
HM	0.961**	0.974**	-0.018	0.261	0.997**	0.261	0.974**	0.019	-0.033	0.999**	1.000**	1		
MRP	-0.25	0.252	-0.999**	-0.951**	0.002	-0.951**	0.252	0.998**	-0.996**	-0.019	0.003	0.026	1	
RED	0.258	-0.244	0.999**	0.952**	0.007	0.953**	-0.244	-1.000**	0.996**	0.027	0.004	-0.019	-0.998**	1

Y_p: Grain yield under non-stress condition, Y_s:Grain yield under stress condition, SSI: Stress susceptibility index, TOL: Stress tolerance, STI: Stress tolerance index, SSPI: Stress susceptibility percentage index, YI: Yield index, YSI: Yield stability index, RSI: Relative stress index, MP: Mean productivity, GMP: Geometric mean productivity, HM: Harmonic mean, MRP: Mean relative performance, RED: Reduction, *, ** Significant at 0.05 and 0.01 levels of probability, respectively

Table 7. Principal component analysis based on grain yield and stress indices in barley genotypes

Components	PC 1	PC 2
Eigen value	7.293	6.675
Proportion of total variation (%)	52.094	47.679
cumulative percentage of variance	52.094	99.773
Yp	0.848	0.53
Ys	0.481	0.876
SSI	0.732	-0.681
TOL	0.891	-0.448
STI	0.685	0.725
SSPI	0.891	-0.448
YI	0.481	0.876
YSI	-0.73	0.681
RSI	0.72	-0.69
MP	0.701	0.713
GMP	0.685	0.728
HM	0.668	0.744
MRP	-0.725	0.687
RED	0.73	-0.681

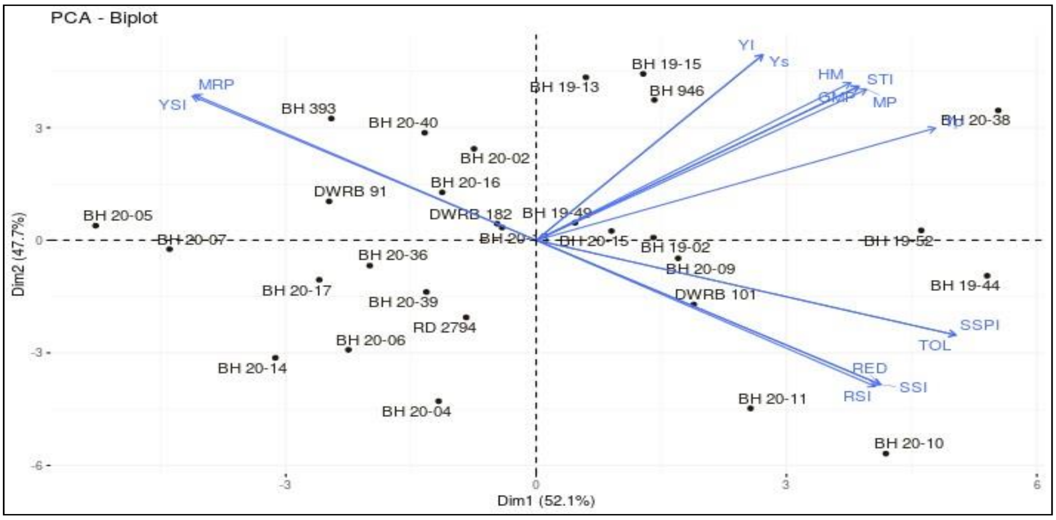

Fig. 3. Biplot based on PCA showing correlation among stress indices

In the biplot analysis, when the angle between their vectors is < 90 (acute)degrees, it indicates the positive association of indices and when the angle is > 90 (obtuse) degrees it indicated negative correlation among the indices (Devi *et al*., 2021). The first two components were used to construct biplot (**Fig. 3**) for comparing relationship between genotypes and stress indices. The biplot displayed positive association of Yp and Ys with all stress indices except of YSI and MRP as indicated by the acute and obtuse angles between their vectors, respectively. Likewise, SSI, TOL, STI, SSPI, YI, RSI, MP, GMP, HM and RED exhibited negative correlation with YSI and MRP, and positively associated among themselves.

The cluster analysis was performed based on stress indices which grouped the genotypes into four clusters. The findings of cluster analysis are illustrated in **Table 8** along with genetic distances between clusters. The clustering pattern identified cluster I as the largest with 10 genotypes, followed by cluster III (9) and IV (8) cluster, while the cluster II being smallest one containing two genotypes. The estimates of various stress indices were also used by Lamba *et al.* (2023) for clustering of genotypes evaluated under stress condition. Several other genetic studies for classification of genotypes into different tolerance categories have also been conducted in barley using stress indices (Ghomi *et al*., 2023).

Table 8. Clustering of barley genotypes based on stress indices and their genetic distances

Cluster Members	Number of Genotypes	Clusters	Cluster Distances			
			Cluster I	Cluster II	Cluster III	Cluster IV
BH 20-02, BH 946, BH 20-40, BH 20-13, BH 20-16, DWRB 182, BH 393, DWRB 91, BH 19-15, BH 19-13	10	Cluster I	**18.75**	24.72	32.27	27.44
BH 20-11, BH 20-10	2	Cluster II	24.72	**3.25**	17.62	29.16
BH 20-39, BH 20-04, BH 20-06, BH 20-17, BH 20-36, BH 20-14, BH 20-05, BH 20-07, RD 2794	9	Cluster III	32.27	17.62	**9.91**	40.05
DWRB 101, BH 20-38, BH 19-52, BH 19-44, BH 20-15, BH 20-09, BH 19-49, BH 19-02	8	Cluster IV	27.44	29.16	40.05	**23.03**

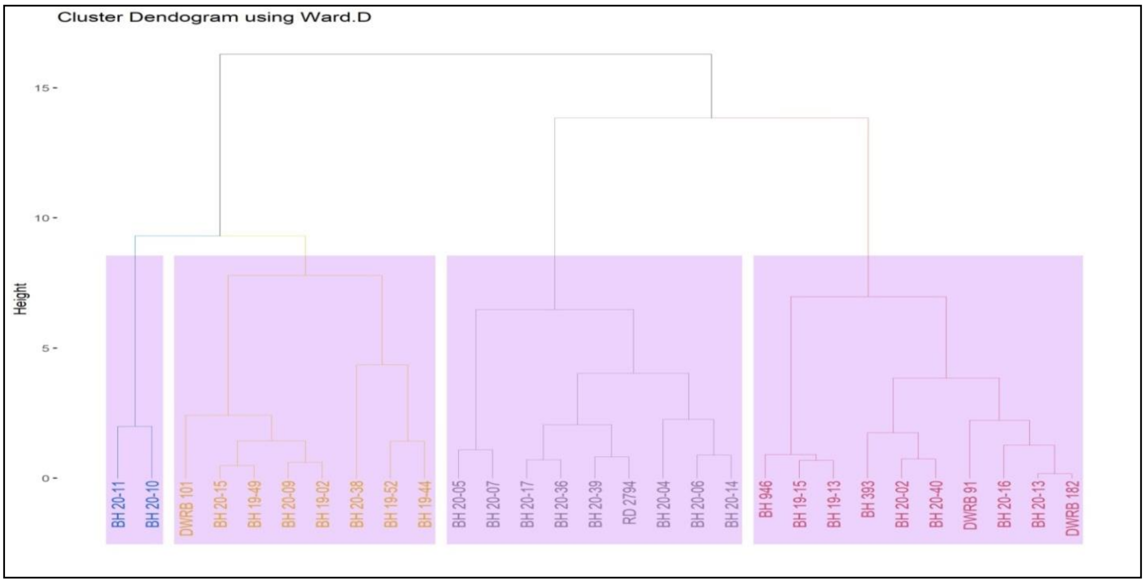

Fig. 4. Dendrogram portraying clustering pattern of 29 barley genotypes

The genetic distances (intra and inter-cluster) were also calculated (**Table 7**), indicating the magnitude of genetic diversity among the genotypes and the diversity and the same is depicted in **Fig. 4**. The results revealed maximum intra-cluster distance for cluster IV followed by cluster I. This indicates the grouping of genotypes with relatively more diversity compared to genotypes belonging to other clusters. Similarly, the genotypes of cluster II showed more similarity as deciphered by minimum intra-cluster distance. The results in addition also revealed that cluster III was placed most distantly from cluster IV as exhibited by maximum inter-cluster distance among all cluster combinations, followed by clusters I and III. However, cluster II was closest to cluster III as observed based on minimum distance among clusters. Similar grouping of barley genotypes based on heat tolerance was reported by Abou-Elwafa and Amein (2016).

The average performance of genotypes in relation to grain yield (Y_p and Y_s) and stress indices under study is portrayed in **Table 9**. The observations indicated cluster I with minimum SSI, TOL, SSPI, RSI, RED and higher YI, YSI, MRP and Y_s. Similarly, cluster IV was characterized by higher STI, MP, GMP, HM and Y_p. Hence, cluster I and IV could be considered as promising for stress and non-stress conditions, respectively. The genotypes from cluster I could serve as genetic resource for developing cultivars suitable for high terminal temperatures. The genotypes from cluster IV were recognized as ideal for utilisation in breeding for developing varieties needed in normal temperatures.

The current study concluded that the stress indices employed were recognized as vital for identification and isolation of promising cultivars with high tolerance to heat. Based on the average rank of SSI of different traits, the genotypes DWRB 91, BH 20-40, BH 20-05, BH 20-07 and BH 19-15 were found heat tolerant among the 29 genotypes studied. However, based on the overall rank of stress indices employed on grain yield, BH 19-13, BH

Table 9. Performance of clusters for grain yield of genotypes and stress indices

	Yp	Ys	SSI	TOL	STI	SSPI	YI	YSI	RSI	MP	GMP	HM	MRP	RED
Cluster I	36.99	33.34	0.69	3.65	0.98	5.13	2.84	0.90	1.29	35.16	35.11	35.06	1.05	0.10
Cluster II	35.67	26.29	1.84	9.38	0.74	13.18	-4.22	0.74	1.58	30.98	30.62	30.26	0.86	0.26
Cluster III	30.38	26.46	0.90	3.92	0.64	5.50	-4.04	0.87	1.34	28.42	28.34	28.27	1.02	0.13
Cluster IV	39.68	32.55	1.25	7.13	1.03	10.02	2.05	0.82	1.42	36.11	35.93	35.75	0.96	0.18

20-40, BH 393, BH 19-15, BH 20-02 and BH 946 were found most promising exhibiting tolerant to heat stress. Grain yield (Y_s) showed negative association with SSI, RSI and RED and significant positive correlation with the indices *viz*., STI, YI, MP, GMP and HM, signifying importance of these indices for heat tolerance under stress. The second principal component (PC2) exhibited strong correlation with STI, YI, YSI, MP, GMP, HM, MRP and Y_s and hence could be considered as heat tolerant component. The genotypes of cluster IV indicated minimum SSI, TOL, SSPI, RSI, RED and higher YI, YSI, MRP and Y_s, resultantly could be utilized as elite donor for heat tolerance barley breeding.

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