

## Identification of heat tolerant barley genotypes based on heat susceptibility index

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### Abstract

The present experiment was planned to understand the impact of high temperature on grain yield and its component traits in order to select heat tolerant genotypes for future breeding programmes. Fifty barley genotypes were evaluated under two environments created by different dates of sowing *i.e.* timely sown and late sown, during *Rabi* 2016-17. The mean sum of squares due to genotypes based on heat susceptibility index (HSI) revealed the presence of significant variation for all the traits except plant height, spike length and grains per spike. Correlation among HSI of different traits indicated significant positive association of grain yield per plot with harvest index, biological yield, 1000-grain weight, plant height and days to maturity. Out of 50 genotypes studied, HUB 242 exhibited lowest HSI and percent reduction in grain yield under heat stress conditions. The genotypes namely HUB 242, DWRUB 52, RD 2904, BH 902 and IBYT-HI-13 were found most promising based on heat susceptibility index (HSI) of grain yield. However, based on overall rank of HSI of all the traits, IBON-HI-13, IBYT-HI-13, RD 2904, HUB 242 and IBON-HI-3 were identified heat tolerant genotypes. These genotypes could be utilized as promising breeding material for the development of new heat tolerant barley varieties.

**Key words:** Barley, heat stress, HSI, tolerance

## 1. Introduction

Barley (*Hordeum vulgare* L.) is an important *rabi* cereal crop grown throughout the temperate and sub-tropical regions of the world. This crop occupied fourth position in total cereal production in the world after wheat, rice and maize (USDA, 2020). Nationally, it is cultivated on an area of 0.62 million hectare producing 1.59 million tonnes grain with productivity of 25.73 q/ha during the crop season 2019-20 (ICAR-IIWBR, 2020). In Haryana state, 44000 tonnes barley was produced from 12,200 hectare area with average productivity of 36.07 q/ha which ranks second after Punjab (37.67 q/ha). Barley can be cultivated in diverse landforms for its tolerance against heat, drought, frost and alkaline soils (Mishra and Shivakumar, 2000).

Its cultivation in India is now becoming oriented towards industrial utilization. There is challenge for the breeders to develop genotypes for high yield potential with high malt content and greater stability. Such genotypes with short maturity and good tillering can further overpass the yield gap and can be helpful to meet the demand of quality grain for malting purpose. Generally long duration genotypes are high yielding. But under late sown or terminal heat condition, early maturing/ short duration genotypes may perform better with minimum reduction in grain yield.

Temperature is an important environmental factor influencing the growth and development, and finally the yield of crop plants. Increased ambient temperature as a



result of global warming and climate changes is emerging as a great threat to the growth and development of most crop plants. When temperatures are elevated from anthesis to grain maturity, grain yield is reduced because of the reduced time for sink (grain) development. The drastic reduction in morphological and yield contributing traits *i.e.*, plant height, number of tillers/plant, spike length, 1000-grain weight and plant yield under heat stress conditions could be due to the inhibition of photosynthesis which is reflected by the loss of chlorophyll content of the leaves. Vaezi *et al.* (2010) reported reduction in number of spikes per square meter, grain number per spike and 1000-grain weight in barley due to delayed sowing and also observed reduction in grain yield by 39.59% and 31.39%, respectively in two- and six- row genotypes. Modhej *et al.* (2015) also reported an average grain yield reduction in barley and bread wheat genotypes by 17% and 23%, respectively, when these crops were exposed to heat stress after anthesis.

Recent climate change gained the attention of plant breeders due to its adverse effect on crop production. The increased temperature at the far ahead phases of crop period starting from pre-heading to post-anthesis must be understood as chief yield limiting feature (Farooq *et al.*, 2011). The most favorable temperature for barley at grain filling stage is 20°C in sub-tropical regions as depicted by various researchers (Dwivedi *et al.*, 2017). Effect of high temperature on barley growth and development becomes complex after anthesis, as with high temperature (>32°C), period of assimilate accumulation becomes short which results in lower yields (Funaba *et al.*, 2006). It was also observed that even slight increase of 1° C from the optimum ranges of temperature during grain filling had adverse effects on grain yield (Narayanan, 2018). Terminal heat stress, in particular, at post-heading stage causes considerable yield reduction due to stress at critical stages, *i.e.*, anthesis and grain filling (Rehman *et al.*, 2009). At flowering, it causes negative effect on pollen fertility and seed setting which lead to low grain number per spike (Ferris *et al.*, 1998). Furthermore, it shortens the period of grain filling and reduces individual grain weight (Dias and Lidon, 2009; Kaur and Behl, 2010).

The development of barley cultivars with stable performance and higher economic yield under different environments is a primary prerequisite of any breeding

program to cope with adverse (abiotic and biotic stress) conditions. Incorporation of heat tolerance in the variety development process is an essential task that breeders would like to achieve by exploring new sources of genetic variability and their utilization (Verma *et al.*, 2021). The adverse effects of heat stress can be mitigated by developing crop plants with improved thermo tolerance using various genetic approaches. The genotypes may display different ability to produce acceptable yield under heat stress. In order to exploit heat tolerance in breeding programmes, a thorough understanding of physiological responses of plants to high temperature, mechanisms of heat tolerance and possible strategies for improving crop thermo tolerance is imperative. The heat susceptibility index (HSI) may be used as an indicator of yield stability and a proxy for heat tolerance (Kavita *et al.*, 2016). Hence, the fifty genotypes of barley including six and two-rowed were evaluated for heat tolerance using.

## 2. Materials and Methods

The experimental material consisted of 50 diverse genotypes of barley including BH 946 and DWRB 101 as check varieties. The material was grown under two different conditions *i.e.* timely (15<sup>th</sup> November) and late sown (14<sup>th</sup> December) at Barley Research Area, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar during *rabi* 2016-17 under irrigated condition. The experimental location is situated at latitude of 29° 10' N, longitude of 75° 46' E and at an altitude of 215.2 m above mean sea level. The experimental material represented both two (17) and six (33) row types and evaluated in RBD with three replications. Each genotype was grown in a plot size of 3.0 x 0.69 m<sup>2</sup> per replication and the recommended cultural practices were adopted to raise the crop. Observations were recorded on 10 quantitative characters *viz.*, days to heading, days to maturity, plant height (cm), spike length (cm), tillers per meter row, grains per spike, 1000-grain weight (g), biological yield (g/plot), grain yield (g/plot) and harvest index (%). Five randomly selected competitive plants in each replication were recorded for all the traits under study except days to heading, days to maturity, biological yield and grain yield which were recorded on plot basis. Further, the value of harvest index was calculated as per the formula given by Donald and Humblin (1976). The weather parameters



during the crop season are presented in Fig. 1. Weekly mean maximum temperature varied between 16.9 to 42.9 °C, whereas, the weekly mean minimum temperature was between 3.2 to 24.6 °C. Morning RH varied from 46 to 100% while evening RH was highly variable with a range from 16 to 81%. Total amount of rainfall received during the season at Hisar was 59.2 mm.

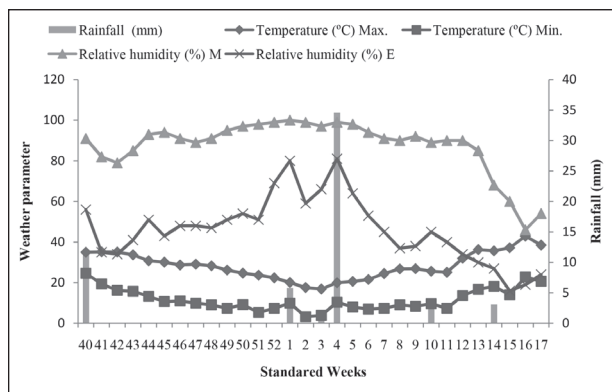


Fig. 1 Weather parameters during the crop season (2016-17)

The recorded data was subjected to analysis using OPSTAT Software (Sheoran *et al.*, 1998). HSI was

calculated for grain yield and all other traits by using the formula as suggested by Fischer and Maurer (1978).  $HSI = [1 - YD/YP]/D$  Where, YP = Mean of genotypes under timely sown, YD = Mean of genotypes under late sown and  $D = 1 - \text{Mean YD of all genotypes} / \text{Mean YP of all genotypes}$ .

### 3. Results and Discussion

The reduction in the performance of barley genotypes under terminal heat condition was expressed in terms of HSI. The HSI of ten quantitative traits were subjected to analysis of variance and mean sum of squares has been presented in Table 1. This table describes the significance of genotypes for HSI of different traits which is prerequisite for further analysis. The results revealed the presence of significant variance among genotypes for the HSI of all the traits except plant height, spike length and grains per spike. This implies that the magnitude of differences in genotypes was enough to provide scope for selection with improved heat stress tolerance. These results corroborate with the findings of Shehrawat *et al.* (2020) for plant height and spike length.

**Table 1.** Mean sum of squares for HSI of different traits in barley genotypes

Source of Variation	d.f.	Mean Sum of Squares									
		DH	DM	PH	SL	T/M	G/S	TGW	BY	GY	HI
Replication	2	1.090	0.050	1177.8735	3011224.6615	6.541	20.8315	0.008	5.209	0.324	1.971
Treatment	49	12.138**	0.259**	8.014	17778.833	1.972**	1.983	1.229**	2.216**	0.7099**	3.471**
Error	98	0.426	0.015	6.551	19778.941	0.748	1.658	0.109	0.467	0.167	0.753

DH: Days to heading, DM: Days to maturity, PH: Plant height, SL: Spike length, T/M: Tillers per meter, G/S: Grains per spike, TGW: 1000-grain weight, BY: Biological yield per plot, GY: Grain yield per plot, HI: Harvest index, \*\*: Significant at 1%

The of different traits for 50 barley genotypes have been depicted in Table 2. The genotypes with high positive HSI values are susceptible to high temperature and *vice versa* (Fischer and Maurer, 1978). The estimates of HSI for the important traits under study can be utilized for selection of tolerant genotypes. The HSI for grain yield revealed that the genotype HUB 242 (-0.37) followed by DWRUB 52 (-0.01), RD 2904 (0.11), BH 902 (0.13), IBYT-HI-13(0.18) and IBON-HI-13 (0.18), exhibited minimum HSI, therefore, these entries possessed low heat susceptibility and high yield stability under heat stress condition. In contrast, BH 14-42 (1.79) followed

by MGL 105 (1.64) recorded with maximum HSI for grain yield and were identified as highly susceptible to heat. Bahrami *et al.* (2020) also assessed tolerance to terminal heat stress in cultivated (*Hordeum vulgare ssp. vulgare* L.) and wild (*H. vulgare ssp. spontaneum* L.) barley genotypes using phenological and agronomic traits as well as selection indices based on grain yield. Four genotypes namely, 2<sup>nd</sup> GSBSN-15-35 (-5.81, 0.13), IBON-HI-13 (-3.40, 0.45), IBON-HI-37 (-3.40, 0.52) and 2<sup>nd</sup> GSBSN-15-8 (-2.16, 0.60) exhibited superiority for days to heading and maturity based on lowest HSI. These genotypes showed low reduction for days



to maturity as well as grain yield under late sown as compared to timely sown. Under stress condition, no reduction in plant height was recorded in IBON-HI 37 (-4.08), UPB 1059 (-3.80), IBON-HI-13 (-3.71), 2<sup>nd</sup> GSBSN-15-8 (-2.06) and IBON-HI-3 (-2.00). The estimates of HSI for spike length highly varied from -188.87 to 215.88. Out of fifty genotypes studied,

fourteen showed negative HSI and BH 15-30 (-188.87), MGL-64 (-157.74), MGL-117 (-96.07), BH 15-17 (-80.18) and DWRB 101 (-78.63) genotypes were found highly heat tolerant for this trait. Various morpho-physiological traits were also used by Sallam *et al.* (2018) in order to identifying the tolerant genotypes for heat tolerance improvement in barley through breeding.

**Table 2.** HSI of different traits in barley genotypes

Sr. No.	Genotypes	RT	DH	DM	PH	SL	T/M	G/S	TGW	BY	GY	HI	Rank based on GY	Overall Rank
1	IBYT-HI-19	6	1.20	1.39	2.97	-64.88	-1.30	0.57	1.29	0.35	1.23	2.11	32	29
2	IBYT-HI-13	6	1.77	0.95	1.58	-4.37	-0.05	0.74	0.32	-0.42	0.18	0.70	5	2
3	IBYT-HI-17	2	1.46	0.94	-0.45	22.46	-0.27	1.97	1.10	-0.63	0.56	1.54	13	11
4	IBYT-HI-16	6	0.12	1.21	0.26	121.43	0.82	0.99	1.50	0.05	1.03	1.83	25	31
5	IBYT-HI-18	6	0.36	1.27	0.40	61.80	1.90	1.08	1.27	1.00	1.10	1.15	30	42
6	IBYT-HI-23	2	2.15	1.25	1.23	-10.18	0.28	1.13	0.50	0.96	1.24	1.52	33	28
7	IBYT-HI-15	6	2.33	1.00	0.73	43.78	1.38	0.63	0.98	0.58	0.46	0.25	10	20
8	IBYT-HI-20	6	0.88	0.82	-0.34	77.58	1.07	1.18	1.77	0.78	1.26	1.81	35	37
9	BH 959	6	2.94	1.25	-0.64	105.20	0.88	1.94	0.92	0.74	1.30	1.93	36	47
10	DWRB 123	2	2.56	1.34	0.07	-16.89	1.37	1.28	1.07	1.07	0.91	0.71	21	33
11	DWRB 137	6	2.43	0.75	1.08	-3.36	0.26	0.35	0.34	0.58	1.06	1.53	27	10
12	MBGSN 145	2	2.32	0.66	0.46	10.18	0.63	2.06	-0.34	1.19	0.75	0.14	17	13
13	RD 2904	2	2.61	0.94	-0.09	19.05	-0.19	1.12	0.37	-0.38	0.11	0.57	3	3
14	RD 2909	6	2.20	1.04	0.41	49.63	3.00	0.29	0.05	1.00	0.42	-0.33	8	17
15	UPB 1059	6	1.53	0.73	-3.80	28.58	1.87	2.04	0.49	0.04	0.63	1.19	14	13
16	HUB 242	6	2.49	1.06	-1.33	68.40	0.62	0.02	-0.38	1.05	-0.37	-2.41	1	4
17	2 <sup>nd</sup> GSBSN-28 (2015)	6	2.33	1.16	-0.11	58.72	1.88	0.52	0.48	0.61	0.44	0.16	9	19
18	2 <sup>nd</sup> GSBYT-23 (2015)	6	1.18	0.80	-0.22	97.06	1.09	2.43	0.79	0.57	1.00	1.38	24	25
19	K 560	6	2.45	0.97	1.76	70.19	1.75	-0.12	0.37	1.22	0.42	-0.92	7	23
20	JB 481	6	0.63	0.66	2.28	21.05	1.78	-0.09	0.90	1.35	1.48	1.70	45	35
21	2 <sup>nd</sup> GSBSN-60 (2015)	6	1.03	0.96	1.50	34.30	0.47	1.24	0.64	0.88	0.72	0.55	15	15
22	2 <sup>nd</sup> GSBYT-02 (2015)	2	0.88	0.85	0.95	69.16	1.46	0.83	1.54	1.22	0.53	-0.45	11	24
23	MGL-58	6	0.63	0.96	1.63	62.95	1.66	0.78	0.63	1.49	1.45	1.63	44	44
24	MGL-62	2	-0.79	1.33	1.67	-33.24	1.62	2.15	0.38	0.77	1.44	2.15	43	39
25	MGL-64	6	1.11	1.02	0.33	-157.74	1.48	0.22	0.07	1.89	0.74	-1.20	16	8
26	DWRB 101	2	2.01	0.63	0.32	-78.63	1.25	1.47	1.04	1.23	0.81	0.08	18	12



27	MGL 105	6	-1.54	0.94	1.43	46.56	0.73	1.18	1.34	0.56	1.64	2.67	49	35
28	MGL-117	6	2.69	0.89	-0.79	-96.07	-0.14	0.55	0.70	-0.46	0.98	2.13	23	6
29	IBON-HI-1 (2015-16)	6	6.06	1.51	-0.72	215.88	0.15	0.16	1.17	1.00	1.49	2.08	47	46
30	IBON-HI-3 (2015-16)	2	-0.13	0.87	-2.00	6.15	-0.09	0.39	1.65	0.09	0.92	1.68	22	5
31	IBON-HI-13 (2015-16)	6	-3.40	0.45	-3.71	192.24	-0.01	4.03	0.62	-0.85	0.18	0.95	6	1
32	IBON-HI-37 (2015-16)	6	-3.40	0.52	-4.08	60.21	0.95	1.66	1.49	-0.68	1.07	2.42	28	16
33	IBON-HI-67 (2015-16)	6	2.09	1.37	-1.00	199.43	0.72	2.24	0.70	0.55	1.21	1.82	31	41
34	BH 902	6	0.13	1.04	0.43	86.39	0.08	1.09	0.93	0.82	0.13	-0.80	4	9
35	2nd GSBSN-15-8	6	-2.16	0.60	-2.06	50.11	0.73	1.02	1.31	-0.99	0.83	2.24	20	7
36	INBON-15-16	6	5.34	1.49	0.55	68.58	1.66	0.81	-0.98	1.08	1.07	1.09	29	45
37	INBON-15-22	6	0.12	0.85	0.17	4.42	2.01	-0.17	1.26	1.41	1.25	1.18	34	22
38	2nd GSBSN-15-35	2	-5.81	0.13	2.17	59.24	0.95	1.86	1.95	0.28	0.54	0.75	12	21
39	AZAD	6	-0.65	1.22	3.40	-22.92	2.18	1.48	1.11	2.06	1.49	0.82	46	48
40	DWRB 143	2	0.66	1.03	-0.22	54.76	1.66	1.99	1.15	2.42	1.39	0.02	40	43
41	BH 13-20	2	2.06	0.56	2.28	1.39	1.12	0.59	1.49	1.08	1.04	0.89	26	25
42	BH 13-22	6	2.89	1.40	1.69	25.03	0.66	1.77	1.14	2.09	1.41	0.52	41	49
43	BH 13-26	2	0.51	1.03	2.09	-78.55	1.56	0.08	1.85	2.34	1.42	0.11	42	38
44	BH 14-25	2	-0.27	0.86	1.41	58.42	0.11	1.55	1.65	1.73	1.51	1.44	48	40
45	BH 14-42	6	-1.13	1.08	1.86	47.72	1.46	1.28	2.04	2.26	1.79	1.67	50	50
46	BH 15-17	2	1.05	0.92	2.28	-80.18	0.11	1.55	1.63	2.37	1.32	-0.18	37	32
47	BH 15-30	6	0.48	1.29	-0.03	-188.87	1.17	1.31	1.70	1.73	1.32	0.69	38	34
48	BH 946	6	0.76	1.52	1.75	14.72	1.34	-0.06	1.08	1.29	0.82	0.19	19	25
49	BH 885	2	0.12	1.03	-0.38	9.86	1.36	1.07	1.63	1.87	1.34	0.76	39	30
50	DWRUB 52	2	1.60	1.19	2.21	-28.45	-0.04	1.57	1.48	0.97	-0.01	-1.33	2	18

RT: Row type, DH: Days to heading, DM: Days to maturity, PH: Plant height, SL: Spike length, T/M: Tillers per meter, G/S: Grains per spike, TGW: 1000-grain weight, BY: Biological yield per plot, GY: Grain yield per plot, HI: Harvest index

The lowest HSI for tillers per meter was found in IBYT-HI-19 (-1.30) followed by IBYT-HI-17 (-0.27), RD 2904 (-0.19), MGL-117 (-0.14) and IBON-HI-3 (-0.09), whereas, for grains per spike the genotypes *i.e.* INBON-15-22 (-0.17), K 560 (-0.12), JB 481 (-0.09), BH 946 (-0.06) and HUB 242 (0.02) showed minimum HSI. The HSI for 1000-grain weight ranged from -0.98 (INBON-15-16) to 2.04 (BH 14-42). The genotypes showed negative values of HSI for a particular trait signifies the better performance of genotype under heat stress than the non-stress condition for that trait, is suitable for climate resilience (Thakur *et al.*, 2020).

The heat tolerant genotypes identified for biological yield per plot were 2<sup>nd</sup> GSBSN-15-8 (-0.99), IBON-HI-13 (-0.85), IBON-HI-37 (-0.68), IBYT-HI-17 (-0.63) and MGL-117 (-0.46) while for harvest index, HUB 242 (-2.41) followed by DWRUB 52 (-1.33), MGL-64 (-1.20), K 560 (-0.92) and BH 902 (-0.80) exhibited superiority. All the genotypes with low HSI mentioned above showed their superiority for tolerance to high temperature than other genotypes. Ram and Shekhawat (2017) also calculated HSI for various traits in barley in order to select heat tolerant genotypes for future breeding programme. Top ranked genotypes based on HSI of grain yield were HUB



242, DWRUB 52, RD 2904, BH 902 and IBYT-HI-13, whereas, the genotypes *viz.*, IBON-HI-13, IBYT-HI-13, RD 2904, HUB 242 and IBON-HI-3 were identified heat tolerant based on overall rank of HSI of all the traits studied. HSI was also used by Parashar *et al.* (2019) to study the impact of high temperature on yield and its attributing traits for selection of heat tolerant parents and cross combinations in barley. The study by Suresh *et al.* (2017) corroborates our results for identification and /or selection of genotypes based on HSI values.

Correlation coefficients were worked out based on HSI of different traits, to estimate the degree of association among various characters for heat tolerance (Table 3). Grain yield per plot exhibited significant positive association with HI, biological yield, 1000-grain weight, plant height and days to maturity, showing the

importance and effectiveness of these traits for detection and screening of high yielding thermo-tolerant genotypes under stress condition. Significant positive correlation was also observed for days to heading with days to maturity and biological yield; days to maturity with biological yield; plant height with spike length and biological yield; tillers per meter with biological yield; and 1000-grain weight with harvest index. Similarly, significant negative correlation was recorded for days to heading with grains per spike, 1000-grain weight and harvest index; plant height with grains per spike; spike length with tillers per meter; grains per spike with biological yield; and HI with biological yield. Correlation among HSI of different characters were also worked out by Shehrawat *et al.* (2020) to estimate the degree of association for heat tolerance.

**Table 3.** Correlation among HSI of different traits in barley genotypes

Traits	DH	DM	PH	SL	T/M	G/S	TGW	BY	GY	HI
DH	1.000	0.514**	0.024	-0.024	-0.024	-0.234**	-0.441**	0.166*	-0.036	-0.166*
DM	0.514**	1.000	0.131	0.053	-0.032	-0.122	-0.140	0.257**	<b>0.165*</b>	-0.039
PH	0.024	0.131	1.000	0.622**	-0.125	-0.376**	0.042	0.334**	<b>0.221**</b>	-0.060
SL	-0.024	0.053	0.622**	1.000	-0.207*	-0.091	0.017	-0.086	-0.013	0.062
T/M	-0.024	-0.032	-0.125	-0.207*	1.000	-0.052	-0.071	0.297**	0.150	-0.084
G/S	-0.234**	-0.122	-0.376**	-0.091	-0.052	1.000	0.021	-0.211**	-0.111	0.071
TGW	-0.441**	-0.140	0.042	0.017	-0.071	0.021	1.000	0.129	<b>0.309**</b>	0.193*
BY	0.166*	0.257**	0.334**	-0.086	0.297**	-0.211**	0.129	1.000	<b>0.478**</b>	-0.366**
GY	-0.036	0.165*	0.221**	-0.013	0.150	-0.111	0.309**	0.478**	1.000	0.619**
HI	-0.166*	-0.039	-0.060	0.062	-0.084	0.071	0.193*	-0.366**	<b>0.619**</b>	1.000

DH: Days to heading, DM: Days to maturity, PH: Plant height, SL: Spike length, T/M: Tillers per meter, G/S: Grains per spike, TGW: 1000- grain weight, BY: Biological yield per plot, GY: Grain yield per plot, HI: Harvest index, \*, \*\*: Significant at 5 and 1%, respectively

The high temperature during the reproductive phase of barley poses detrimental effect to the growth and development. But, the genotypes performed differently under heat stress conditions. Some of the genotypes were adversely affected while some could combat with the stress. *Fig. 2* depicts a radar graph representing the genotypes with per cent reduction in grain yield. One of the promising genotype showing no reduction in grain

yield was HUB 242 (-12%). Other tolerant genotypes having minimum reduction in grain yield were RD 2904 (4%), DWRUB 52 (5%), IBON-HI-13 (2015-16) (6%), BH-902 (8%) and IBYT-HI-13 (9%). Pathak *et al.* (2017) also reported reduction in grain yield, spike length, grains per spike and 1000-grain weight in barley under stress condition.



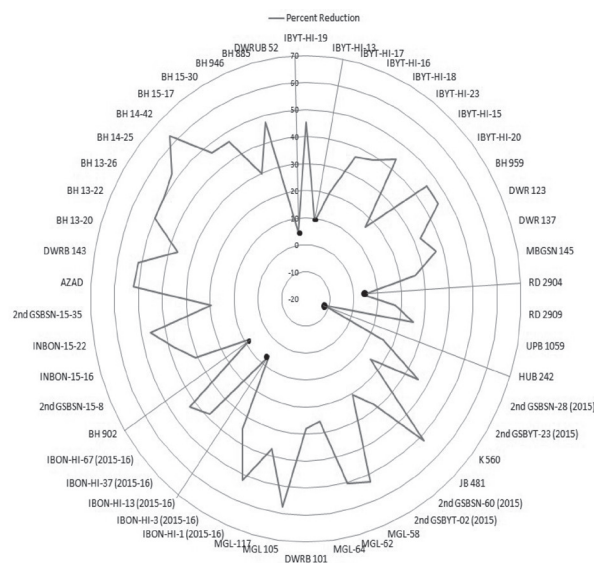


Fig. 2 Per cent reduction in grain yield in barley genotypes

#### 4. Conclusion

From this study, it could be concluded that HSI used under study was recognized as paramount for identifying cultivars with high tolerance to heat stress. The genotypes HUB 242, DWRUB 52, RD 2904, BH 902 and IBYT-HI-13 were found promising based on HSI of grain yield. Likewise, based on overall rank of HSI of all the traits studied, the genotypes *viz.*, IBON-HI-13, IBYT-HI-13, RD 2904, HUB 242 and IBON-HI-3 were identified heat tolerant. Hence, study gives an ample opportunity for selection of material to be incorporated in elite cultivars of barley to tolerate the terminal heat stress under North Indian conditions.

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#### Conflict of Interest

Authors declare that they have no conflict of interest.

#### Ethical Compliance Statement

NA

#### Author's Contribution

Conceptualization of research (YK); Designing of the experiments (YK, SD); Contribution of experimental

materials (YK); Execution of experiments and data collection (SD, YK); Analysis of data and interpretation (YK, SS); Preparation of the manuscript (YK, SD)

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