

RESEARCH ARTICLE

Evaluation of Barley (*Hordeum vulgare* L.) Germplasm for Heat Tolerance Using Germination Indices and Seedling Traits

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Abstract

Heat stress is a major constraint in barley production. In the present study, 92 genotypes of barley along with 8 checks were evaluated for heat tolerance at the seedling stage under *in vitro* conditions at three temperature regimes viz., 25°C (control), 30°C and 35°C. The genotypes were assessed for germination indices viz., germination percentage, speed of germination and seedling traits viz., root length, shoot length, seedling vigour index I and II. Tolerant genotypes exhibited higher germination percentage, root length, shoot length, seedling vigour index I and II at 30 and 35°C. Based on the hierarchical cluster analysis, nine heat-tolerant genotypes viz., BL 1515, BL 1729, BL 1780, BL 1784, BL 1786, BL 1792, BL 1794, BL 1797 and IBYT-E24 and three heat-sensitive genotypes viz., BL 1723, IBON-23 and IBYT-E15 were identified. These heat-tolerant genotypes can be used as potential donors for developing heat-tolerant barley varieties in future breeding programs.

Keywords: Germination, Heat stress, Screening, Seedling vigour, Tolerant genotypes

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Received: 01/04/2025

Revised: 22/03/2026

Accepted: 17/04/2026

How to cite this article: Bhagat MA, N Kaur, P Goyal and S Kaur (2026) Evaluation of Barley (*Hordeum vulgare* L.) Germplasm for Heat Tolerance Using Germination Indices and Seedling Traits. *Indian J. Plant Genet. Resour.* 39(2): 58-66.

DOI: <https://doi.org/10.56093/ijpgr.v39i2.178163>

Introduction

The average temperature of the Earth has risen between 0.5 and 0.8°C over the past 100 years due to global warming (Ju *et al.*, 2013). Scientists of the Intergovernmental Panel on Climate Change (IPCC) have predicted that the global annual temperature increased at an average rate of 0.08°C per decade since 1880 and over twice that rate (0.18°C/0.32°F) since 1981 (IPCC, 2022). There have been considerable changes in weather events since the onset of the middle of the 20th century, which have put global food security in great threat (Lesk *et al.*, 2016). Greenhouse gases are major causes of global warming. Over the last 250 years, 150% and 300% rise in the concentration of CH₄ and CO₂, respectively, have been documented (Friedlingstein *et al.*, 2010). It has been predicted that for every 1°C rise in temperature, there is 6% decline in global wheat production (Asseng *et al.*, 2015). In barley, grain yield and grain protein reduction of up to 29% and 22%, have been reported under high temperature stress (Ingvordsen *et al.*, 2015). This emphasizes the need to frame breeding programs that can target the development of new genotypes by incorporating the traits for heat stress tolerance (Rollins *et al.*, 2013).

Barley (*Hordeum vulgare* L.) is the fourth major cereal grain crop in the world after maize, rice and wheat, belonging to the family Poaceae (To *et al.*, 2020). It is commonly used for animal feeding, human food and malting purposes due to the

presence of important nutrients such as proteins, niacin, vitamins, fibres, and minerals (Huang *et al.*, 2020). As compared to other cereals such as maize, rice and wheat, barley shows promising adaptive capabilities under heat, drought and salinity stresses (Munns *et al.*, 2006). Therefore, barley is considered an important staple food crop in those regions of the earth that are sensitive to climate change. Due to such features, barley has become a model crop for the researchers working on stress-responsive genes (Li *et al.*, 2013).

Heat stress is one of the abiotic stresses that limits the production and productivity of barley (Sallam *et al.*, 2018; Bhagat *et al.*, 2025). The metabolic processes of plants are affected by high temperatures, which ultimately determine the crop's productivity. Information regarding the genetic variations, changes in physiological processes and level of existing genetic diversity in barley germplasm is needed to produce new cultivars having high tolerance to heat stress. Heat stress significantly reduces barley growth and yield by decreasing kernel weight, accelerating grain filling activities, and inducing early senescence (Sehgal *et al.*, 2018). Genetic improvement is considered the best tool for developing high-yielding barley cultivars with increased heat tolerance. Hawker and Jenner (1993) have reported that high temperature caused the inhibition of seed germination and seedling growth in barley by causing hindrance in the availability of energy and nutrients by preventing the synthesis and activity of hydrolytic enzymes like α -amylase. Lachno and Baker (1986) have reported that an increase in temperature results in ABA accumulation in seeds, which inhibits the activity of α -amylase.

Understanding the response mechanism to heat stress at seed germination stage may partially help to address some of the concerns for improving tolerance to heat stress and minimize consequent impacts. The genotypes exhibiting heat tolerance at the seedling stage have been reported to possess heat tolerance at the flowering and grain-filling stages as well. Wu (2013) reported that screening for heat stress tolerance at the seedling stage under controlled conditions in the growth chamber can be used for selecting heat-tolerant genotypes in cotton (*Gossypium hirsutum* L.). Screening germplasm for heat stress tolerance at seedling stage under *in-vitro* conditions is a simple, rapid, economical and time-effective methodology, as screening large germplasm under field conditions puts constraints in terms of time, labour and field resources. Keeping these in view, the present study was conducted to screen the advanced breeding lines of barley at seedling stage for selecting the heat-tolerant lines that can be used as

heat-tolerant donors in future barley breeding programs for the development of heat-tolerant varieties.

Materials and Methods

Experimental area and plant material

A set of ninety-two genotypes of barley was screened for heat tolerance at the seedling stage. For comparative studies, eight checks viz., DWRB-123, DWRUB 52, PL 807, PL 891, DWRB-92, PL 426, DWRB-137 and DWRB-101 were also evaluated alongwith the complete set. These genotypes were procured from the Department of Plant Breeding and Genetics (PBG), Punjab Agricultural University (PAU), Ludhiana. The genotypes from 1 to 57 were advanced breeding lines developed through pedigree method by the Department of PBG (Table 1). The genotypes from 58 to 92 were high-yielding and introduced from Institute National de la Recherche Agronomique du Maroc, Morocco (ICARDA-Morocco). The study was carried out at the Seed Physiology Laboratory, Office of the Director (Seeds), PAU, Ludhiana, India.

Table 1: Pedigree of one hundred genotypes of barley

Sr. no.	Entry name	Pedigree	Nomenclature in ICARDA, Morocco Nursery
1	BL1510	IEBON-33 (2010-11)/ PL426	-
2	BL1515	BK9816/DWRUB52	-
3	BL1521	BCU323/PL807	-
4	BL1523	PL830/DWR91	-
5	BL1531	PL807/PL861	-
6	BL1534	PL172/BCU347	-
7	BL1537	DWR83/RD2798	-
8	BL1539	PL840/PL833	-
9	BL1540	PL840/PL833	-
10	BL1544	BL71/13TH-HBSN-4	-
11	BL1566	VJM315/31TH-IBON-181	-
12	BL1594	BISON 87/CANELA	IBON-(2014-15)-E57
13	BL1607	LIGNEE527/GERBEL/3/ BOY-B*2/SURB//CI12225.2D/ 4/M104/6/LEGACY/4/TOCTE// GOB/HUMAI10/3/ATAH92/ ALELI/5/ESMERALDA	IBON-(2014-15)- E131
14	BL1608	ESMERALDA/3/LEGACY// PENCO/CHEVRON-BAR/4/ LA MOLINA 96	IBON-(2014-15)- E133
15	BL1610	E.ACACIA/DEFRA/3/ SVANHALS-BAR/MSEL// AZAF/GOB24DH	IBON-(2014-15)- E147

Sr. no.	Entry name	Pedigree	Nomenclature in ICARDA, Morocco Nursery	Sr. no.	Entry name	Pedigree	Nomenclature in ICARDA, Morocco Nursery
16	BL1649	GLORIA-BAR/COPAL// PM5/BEN/3/SEN/4/ PETUNIA 1/5/PETUNIA 2// PENCO/CHEVRON-BAR/ 4/PETUNIA 2/3/CHAMICO/ TOCTE//CONGONA	IBON-(2015-16)- E132	55	BL1808	PL807/PL841	-
17	BL1712	DWRB116/PL172	-	56	BL1810	PL807/PL61	-
18	BL1714	PL807/ BCU 361	-	57	BL1812	PL838/BH918	-
19	BL1715	PL807/ BCU 361	-	58	DWRB123	CHAMICO/TOCTE// CONGONA/6/P.STO/3/ LBIRAN/UNA80//LIGNEE640/ 4/BLLU/5/PETUNIA 1	-
20	BL1717	PL807/ BCU 361	-	59	IBON-4 (2018-19)	DWRUB54/DWR51	IBON-4 (2018-19)
21	BL1719	PL807/ BCU 361	-	60	IBON-5 (2018-19)	CANELA//E.QUEBRACHO/ W9338	-
22	BL1723	PL807/ BCU 361	-	61	IBON-8 (2018-19)	CANELA//LIMON/ BICHY2000	-
23	BL1724	PL807/ BCU 361	-	62	IBON-14 (2018-19)	PFC92126/ BICHY2000	-
24	BL1725	PL807/ BCU 361	-	63	IBON-23 (2018-19)	GLORIA-BAR/COPAL/ 6/P.STO/3/LBIRAN/ UNA80//LIGNEE640/4/ BLLU/5/PETUNIA 1	-
25	BL1728	PL807//PL426/BL10	-	64	IBON-39 (2018-19)	BLLU/6/P.STO/3/LBIRAN/ UNA80//LIGNEE640/4/ BLLU/5/PETUNIA 1	-
26	BL1729	PL830//PL426/BL10	-	65	IBON-55 (2018-19)	LBIRAN/UNA80//LIGNEE 640/3/PUNGSANCH APSSALBORI	-
27	BL1730	DWR96//PL426/BL10	-	66	IBON-58 (2018-19)	LBIRAN/UNA80//LIGNEE 640/3/PUNGSANCH APSSALBORI	-
28	BL1733	DWRUB 73//PL426/BL10	-	67	IBON-70 (2018-19)	J09072 F3 10/030575	-
29	BL1735	DWRUB 73//PL426/BL10	-	68	IBON-75 (2018-19)	P.STO/3/LBIRAN/ UNA80//LIGNEE640/4/ BLLU/5/PETUNIA1/6/ P.STO/3/LBIRAN/UNA80// LIGNEE640/4/BLLU/5/ PETUNIA 1	-
30	BL1736	DWRUB 73//PL426/BL10	-	69	IBON-78 (2018-19)	GLORIA-BAR/IAR.H.485// ALOE/3/CABUYA	-
31	BL1740	INBON-15 (2010-11)/PL426	-	70	IBON-88 (2018-19)	CANELA//ATAH92/ GOB	-
32	BL1743	INBON-15 (2010-11)/PL426	-	71	IBON-89 (2018-19)	CANELA//LIMON/BICHY 2000	-
33	BL1745	INBON-15 (2010-11)/PL426	-	72	IBON-90 (2018-19)	BISON 136/CANELA	-
34	BL1748	INBON-15 (2010-11)/PL426	-	73	IBON-91 (2018-19)	OPS66/CANELA	-
35	BL1750	INBON15 (2010-11)/DWRUB 73	-	74	IBON-107 (2018-19)	CIRU/3/AGAVE/ SUMBARD400//MARCO	-
36	BL1753	IEBON-33 (2010-11)/PL426	-	75	IBON-108 (2018-19)	MAHIGAN/H00058	-
37	BL1754	IEBON-33 (2010-11)/PL426	-				
38	BL1756	IEBON-33 (2010-11)/PL426	-				
39	BL1761	IEBON-33 (2010-11)/PL426	-				
40	BL1766	IEBON-33 (2010-11)/PL426	-				
41	BL1770	Moroc-9-73/A.Aswad//DWR96	-				
42	BL1771	Moroc-9-73/A.Aswad//DWR96	-				
43	BL1776	Moroc-9-73/A.Aswad //DWR96	-				
44	BL1780	BK9816/DWRUB52	-				
45	BL1781	BK9816/DWRUB52	-				
46	BL1784	EC492253/PL807	-				
47	BL1786	EC492253/PL807	-				
48	BL1787	EC492253/PL807	-				
49	BL1792	EC492253/PL838	-				
50	BL1794	BCU323/PL807	-				
51	BL1797	BCU323/PL807	-				
52	BL1801	BCU323/PL807	-				
53	BL1804	PL830/DWR94	-				
54	BL1807	PL807/PL841	-				

Sr. no.	Entry name	Pedigree	Nomenclature in ICARDA, Morocco Nursery
76	IBYT-E1 (2018-19)	P.STO/3/LBIRAN/UNA80 //LIGNEE640/4/BLLU/5/PETUNIA1/6/P.STO/3/LBIRAN/UNA80//LIGNEE 640/4/BLLU/5/PETUNIA 1	-
77	IBYT-E2 (2018-19)	P.STO/3/LBIRAN/UNA80// LIGNEE640/4/BLLU/5/PETUNIA1/6/P.STO/3/LBIRAN/UNA80//LIGNEE 640/4/BLLU/5/PETUNIA 1	-
78	IBYT-E5 (2018-19)	CIRU/ZIGZIG	-
79	IBYT-E6 (2018-19)	CIRU/ZIGZIG	-
80	IBYT-E7 (2018-19)	CANELA//LIMON/ BICHY2000	-
81	IBYT-E8 (2018-19)	CIRU/TOCTE	-
82	IBYT-E11 (2018-19)	S95031002008N/ H87010008N	-
83	IBYT-E12 (2018-19)	J09049 F3 10/030552	-
84	IBYT-E13 (2018-19)	MSEL/LOGAN-BAR	-
85	IBYT-E15 (2018-19)	MSEL/LOGAN-BAR	-
86	IBYT-E16 (2018-19)	LEGACY/CHAMICO// ATAH92/GOB	-
87	IBYT-E17 (2018-19)	AJO 61/6/Vmorales	-
88	IBYT-E19 (2018-19)	VMorales/6/ORGE618	-
89	IBYT-E20 (2018-19)	ALELI/SCARLETT	-
90	IBYT-21 (2018-19)	GLORIABAR/COPAL// PM5/BEN/3/SEN/4/PETUNIA1/5/PETUNIA2//PENCO/CHEVRONBAR/4/PETUNIA2/3/CHAMICO/TOCTE//CONGONA	-
91	IBYT-E22 (2018-19)	BBSC/CONGONA// FRESA	-
92	RIHANE-03 (IBYTE-24)	As46//Avt/Aths	IBYT-E24 (2018-19)
93	DWRB-123	DWRUB54/DWR51	
94	DWRUB52	DWR17/K551	-
95	PL807	LENT/BLLU//PINON	-
96	PL891 (IBYTE-24)	IBON 343 /12TH HSBN-176	-

Sr. no.	Entry name	Pedigree	Nomenclature in ICARDA, Morocco Nursery
97	DWRB-92	DWR28/DWR45	-
98	PL426	KARAN92/PL101	-
99	DWRB-137	DWR28/DWRUB64	-
100	DWRB-101	DWR28/BH581	-

*IBON, International Barley Observation Nursery; IBYT, International Barley Yield Trial; BL, Barley Ludhiana; DWRB, Directorate Of Wheat Research Barley; DWRUB, Directorate Of Wheat Research United Breweries.

Experimental design

The experiment was conducted in a completely randomized block design (CRBD) in four replications. One hundred seeds of each genotype in each replication of each treatment were sown using paper method as described by ISTA (2019). The seeds were surface sterilized using 0.5% sodium hypochlorite solution for 1-2 minutes, followed by washings thrice with autoclaved distilled water. These seeds were air-dried and placed on moistened germination paper. The paper was folded vertically. The seeds were allowed to germinate in a BOD incubator (REMI CI-10S). For heat stress, 30°C and 35°C temperature was maintained in BOD incubator. An optimum temperature 25°C was used as control. Twenty representative seedlings were used to measure seedling traits at 7-days after sowing (DAS).

Germination percentage (GP): The seeds with visible radicle protrusion were considered as germinated seeds. Per cent germination was calculated using the following formula (ISTA, 2019):

$GP = (\text{Total no. of seeds germinated} / \text{Total no. of seeds placed}) \times 100$

Root and shoot length (RL and SL): Root and shoot length of twenty seedlings of each genotype of each replication and each treatment were measured using centimeter scale at 7 DAS. The mean RL and SL were recorded in centimetres. Seedling length was computed by adding SL and RL, which was used for the calculation of the seedling vigour index I.

Seedling dry weight (DW): Twenty seedlings of each genotype from each replication and each treatment were dried in an oven at $65 \pm 2^\circ\text{C}$ until a constant weight was obtained. The mean seedling dry weight was recorded in grams. Seedling dry weight was further used for the calculation of seedling vigour index II.

Vigour index (VI): Seedling vigour index I (SVI I) and seedling vigour index II (SVI II) were calculated using the following formula (Abdul-Baki and Anderson, 1973):

SVI I = Germination(%) × mean seedling length (cm)

SVI II = Germination(%) × mean seedling dry weight (g)

Speed of germination: Germination counts were made on 4th, 5th, 6th and 7th days after the start of the experiment, with the criterion for germination being visible protrusion of the radicle from seed. Speed of germination (SG) was calculated using the following formula as suggested by ISTA (2019):

$$\frac{n_1}{d_1} + \frac{n_2}{d_2} + \frac{n_3}{d_3} + \dots$$

where, n = number of seeds germinated, d = number of days.

Statistical analysis

The variations in germination and seedling traits of one hundred genotypes were represented using a colour gradient heat map in Prism Graphpad (v.9.0, Prism Inc, San Diego, CA, USA). The data were subjected to two-way analysis of variance (ANOVA) to find differences among genotypes and treatments at 1% and 5% level of significance using Tukey's test (Tukey 1949).

The variation of responses according to the temperature differences was evaluated using the Ward's algorithm and the Euclidean distance under the Hierarchical Cluster Analysis (HCA) (Cha-um *et al.*, 2010). Cluster analysis of the data was done using SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). The cluster ranking for the groups was obtained based on Ward's minimum variance cluster analysis using the differences in the values of morpho-physiological parameters viz., germination percentage (%), speed of germination, root and shoot length, seedling dry weight and seedling vigour index I and II (Zeng *et al.*, 2002; Ravikiran *et al.*, 2018).

Results and Discussion

Effects of heat stress on germination indices and seedling traits of barley genotypes

The morpho-physiological characteristics viz., germination percentage, speed of germination, root length (RL), shoot length (SL), seedling dry weight, seedling length and seedling vigour index I and II, significantly varied ($p < 0.01$) among genotypes and treatments. ANOVA revealed that temperature, genotypes and their interaction significantly contributed to germination percentage, speed of germination, root length, shoot length, seedling length, seedling dry weight and vigour (Table 2).

Germination percentage

There were significant differences in germination percentage among the genotypes and treatments at 1% level of significance. The per cent germination ranged from 45-97.7% with a mean value 82.8% at 25°C (control), 2.5%-94.7% with a mean value 52.3% at 30°C and 1.3-86.3% with mean value 37.6% at 35°C (Table 3). Mean germination percentage decreased by 36% and 55% at 30°C and 35°C, respectively, as compared to mean germination percentage at 25°C. Heat-tolerant barley genotypes exhibited minor reduction in germination percentage (12.7%) as compared to heat-sensitive genotypes (87.6%) at 35°C (Fig.1a). Temperature showed considerable effects on germination characteristics, including the initiation and the final germination percentage. It is therefore the most critical factor in the determination of the success or failure of plant establishment (Al-Ahmadi and Kafi, 2007). Reduction in germination due to heat stress in wheat has been attributed to impairments in the activities of α -amylase, which hampers starch breakdown and abolishes the delivery of nutrients to developing embryos (Essemine *et al.*, 2010). Reduction in germination is also attributed to a reduction in

Table 2: F-values of ANOVA for germination and seedling traits in one hundred genotypes of barley

Source of variance	GP	SOP	RL	SL	TSL	DW	SVI I	SVI II
Temperature	263.3 ^{**}	271.8 ^{**}	141.3 ^{**}	103 ^{**}	167.9 [*]	410.3 ^{**}	299.6 ^{**}	109.2 [*]
Genotype	669.4 ^{**}	54.88 ^{**}	20.36 ^{**}	8.9 ^{**}	13.05 ^{**}	16.03 ^{**}	141.94 ^{**}	76.42 [*]
T×G	85.35 ^{**}	10.16 ^{**}	5.79 ^{**}	5.25 ^{**}	5.61 ^{**}	5.27 ^{**}	36.93 ^{**}	12.38 ^{**}

^{**}significant at 0.01 level: GP, germination percentage; SOP, Speed of germination; RL, Root length; SL, Shoot length; TSL, Total seedling length; DW, Dry weight; SVI I, Seedling vigour index I; SVI II, Seedling vigour index II.

metabolic efficiency at high temperature than optimum temperature in *Eurotia lanata* (Thygersen et al., 2002). Heat stress caused inhibition of seed germination through the synthesis of ABA in wheat (Hasanuzzaman et al., 2013). Heat and salt stress induced decrease in germination has also been documented in other plant species, viz., *Triticum aestivum* (Hakim et al., 2012; Hossain and Teixeira da Silva, 2012; Singh et al., 2025a, 2025b), *Zea mays* (Chhatwal et al., 2025), *Moringa peregrine* (Sardooi et al., 2019) and *Brassica juncea* (Toosi et al., 2014).

Speed of germination

The increment in speed of germination of barley genotypes was observed at 30°C; but a reduction was recorded at 35°C as compared to control temperature (25°C). It ranged from 0.8-17.8 at 25°C, 1.9-18.2 at 30°C and 0.3-8.7 at 35°C (Fig. 1b). The interaction effect of temperature and genotypes was significant for speed of germination in barley genotypes. The F-value observed

for temperature on speed of germination was 271.8 and 54.88 for genotypes indicating higher contribution of temperature towards reducing the speed of germination (Table 2). These results showed agreement with findings of Toosi et al., (2014) in *Brassica juncea* and Lima et al., (2021) in *Dalbergia spruceana*, who also observed that increase in temperature up to 30°C caused an increase in speed of germination and declined with the further increase in temperature to 35°C. With an increase in temperature, the activities of genes for production of GA are upregulated and thus speed of germination is hastened in *Brassica* (Finch-Savage and Footitt, 2012).

Root and shoot length

Seedlings RL and SL varied from 10.35-21.55 cm and 11.7-19.85 cm, respectively at 25°C, 8.0-19.6 cm and 6.3-22.13 cm, respectively at 30°C and 0.5-5.85 cm and 2.5-6.8 cm, respectively at 35°C (Fig. 2a & b). RL and SL of seedlings decreased significantly at 35°C but meagre

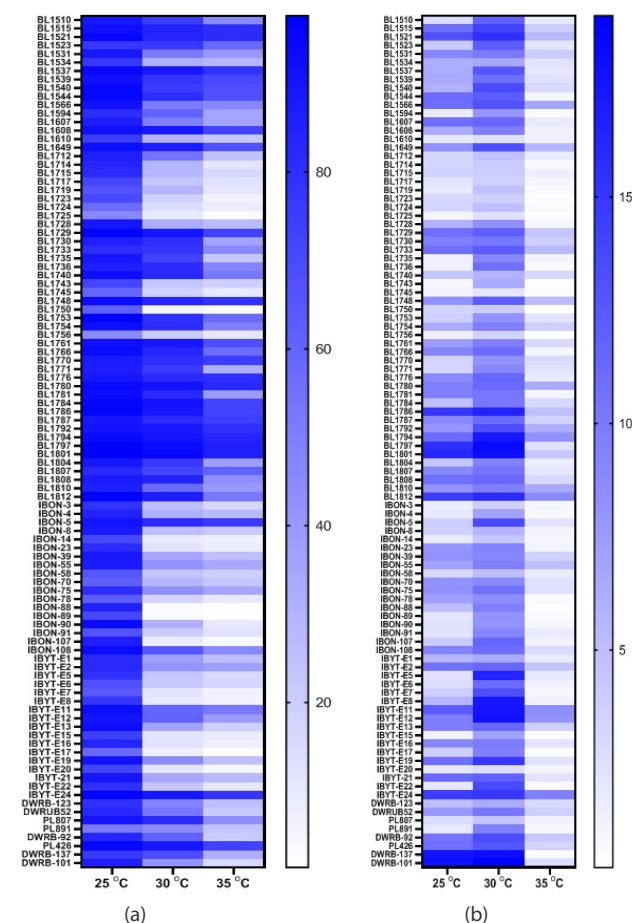


Fig. 1: Heat maps depicting effect of three temperature regimes (25, 30 and 35°C) on (a) germination percentage and (b) speed of germination in one hundred genotypes of barley. Higher colour intensity corresponds to higher germination percentage and speed of germination in figures (a) and (b) respectively and vice-versa.

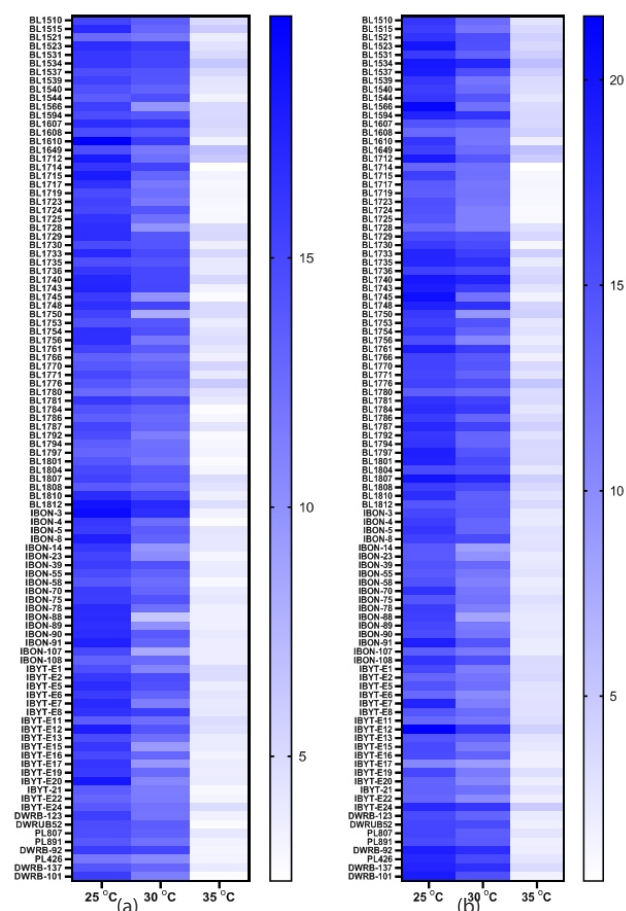


Fig. 2: Heat maps depicting effect of three temperature regimes (25, 30 and 35°C) on (a) shoot length (cm) and (b) root length (cm) in one hundred genotypes of barley. Higher colour intensity corresponds to higher shoot length and root length in figures (a) and (b) respectively and vice-versa.

reduction in RL and SL of seedlings was observed at 30°C. The RL ($F=141.3$) of seedlings showed more reduction as compared to SL ($F=103.0$) with the effects of heat stress being significant for both parameters ($p<0.01$). Reduction in shoot and root length in seedlings of wheat germplasm under heat stress environment was also reported by Lu *et al.*, (2022) and Hasan *et al.*, (2024).

Seedling vigour index I and II

Seedling vigour index I and II varied from 1419.8–3550.5 with mean value of 2635.0 and 10.3–23.4 with an average value of 16.4 respectively, under control conditions (25°C) (Table 3). Mean SVI I decreased by 89.2% at 35°C as compared to 25°C. The highest mean SVI II (16.3) was reported at 25°C trailed by at 30°C (7.8) and 35°C (3.7) (Fig. 3a, Table 4). The interaction effect between temperature and genotypes was also reported to be significant for both SVI I and II.

The results indicated that the SVI I ($F=299.6$) witnessed a higher reduction as compared to SVI II ($F=109.2$), with the effects of heat stress being significant for both parameters ($p<0.01$) (Table 2). Among selected barley genotypes, SVI II of sensitive genotypes ranged from 0.4 to 1.2 and tolerant genotypes from 6.1 to 11.2 at 35°C (Fig. 3b, Table 4). SVI II

Table 3: Mean values of morpho-physiological parameters at three temperature regimes of one hundred genotypes of barley

Trait	Temperature		
	25°C	30°C	35°C
Germination percentage	82.8	52.3	37.6
Speed of germination	6.6	10.0	72.5
Shoot length (cm)	15.5	12.8	4.0
Root length (cm)	16.3	13.4	2.9
Seedling length (cm)	31.8	26.3	7.0
Seedling vigour index I	2635.0	1436.5	285.7
Seedling vigour index II	16.3	7.8	3.7

Table 4: Mean values of germination and seedling parameters at three temperature regimes in heat-tolerant and heat sensitive genotypes of barley

Trait	Temperature					
	25°C		30°C		35°C	
	Heat-tolerant genotypes	Heat-sensitive genotypes	Heat-tolerant genotypes	Heat-sensitive genotypes	Heat-tolerant genotypes	Heat-sensitive genotypes
Germination percentage	95.0	74.9	91.8	14.3	82.9	9.3
Speed of germination	11.4	3.5	14.6	1.7	5.8	0.4
Seedling vigour index I	2922.3	2271.4	2558.8	297.3	623.0	50.7
Seedling vigour index II	18.2	15.1	13.6	1.7	8.5	0.8

of tolerant barley genotypes decreased by 37.5% at 35°C as compared to 30°C; while SVI II of sensitive genotypes decreased by 52.9% at 35°C as compared to 30°C. SVI I determines the fate of seedling establishment and incorporates the combined effects of germination and seedling growth. The higher vigour index of heat-tolerant genotypes could be attributed to higher germination rate and seedling length. On the

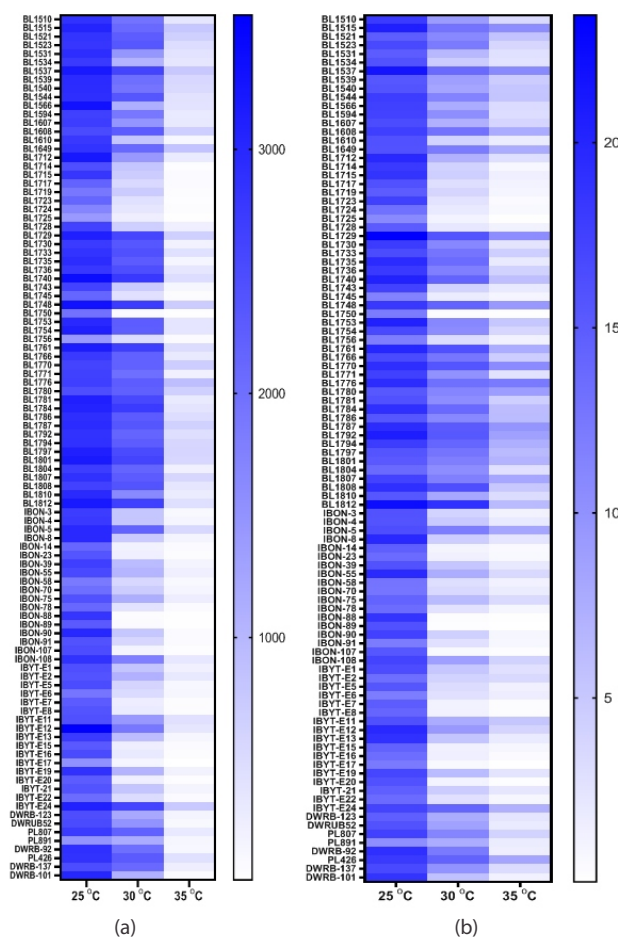


Fig. 3: Heat maps depicting effect of three temperature regimes (25, 30 and 35°C) on (a) seedling vigor index I and (b) seedling vigor index II in one hundred genotypes of barley. Higher colour intensity corresponds to higher seedling vigor index I and II in figures (a) and (b) respectively and vice-versa.

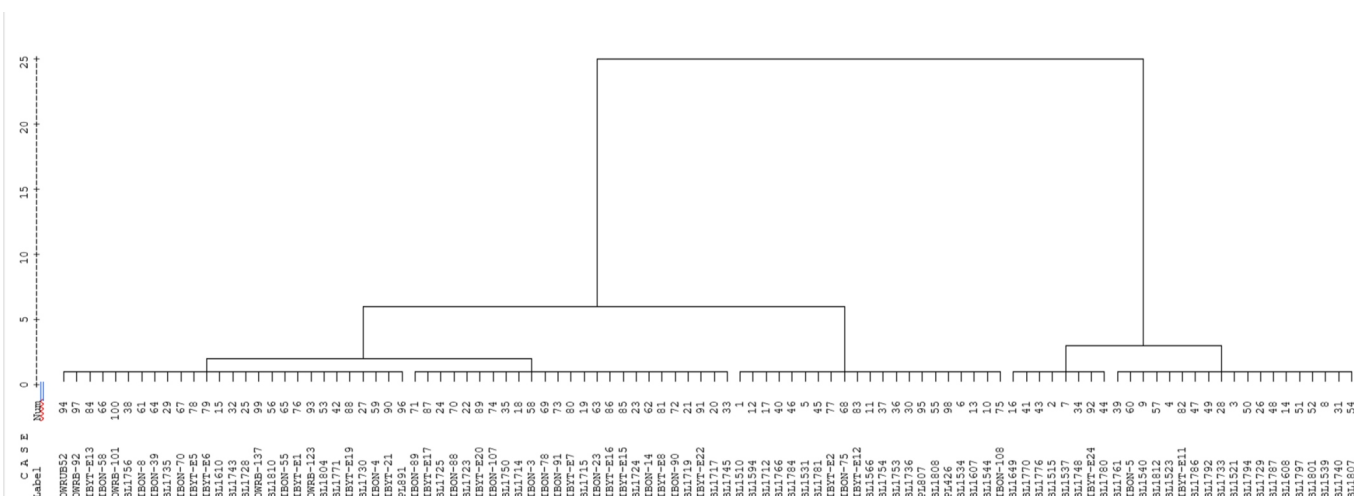


Fig. 4: Clustering of one hundred genotypes of barley using cluster dendrogram based on the furthest neighbour to determine the heat tolerance potential.

other hand, the sensitive ones had a lower germination rate and seedling length, and ultimately had a lower vigour index. The decline in germination and in turn, seedling vigour has also been observed due to high temperature stress during early seedling growth in wheat (Hasan *et al.*, 2013). Reduction in the seedling dry weight and vigour was a result of reduction in seed reserve mobilization due to heat stress in wheat (Essemine *et al.*, 2010).

Hierarchical clustering

Cluster ranking based on morphological and physiological changes in barley seedlings under heat stress classified the one hundred barley genotypes into three groups: highly heat tolerant (21), moderately heat tolerant (27) and heat sensitive (52) (Fig. 4). Out of these categorized genotypes, nine heat tolerant genotypes viz., BL1515, BL1729, BL1780, BL1784, BL1786, BL1792, BL1794, BL1797 and IBYT-E24 can be used as potential donors for developing heat-tolerant barley varieties in future breeding programs.

Conclusion

Temperature is a detrimental factor for plant growth and productivity worldwide. In the present study, one hundred barley genotypes were evaluated for their heat tolerance at seedling stage on the basis of morpho-physiological parameters, i.e. speed of germination, root length, shoot length, seedling dry weight, germination percentage, seedling vigour index I and II. Further, HCA (hierarchical clustering analysis) segregated the barley genotypes into three categories, i.e. highly heat tolerant (21), moderately heat tolerant (27) and heat sensitive (52) based on their tolerance to heat stress. Seedling vigour index was recognized as

promising trait for selection of heat-tolerant genotypes. Selected heat-tolerant barley genotypes in the present study could be used for future breeding programmes for the introgression of heat tolerance in cultivated barley varieties.

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