

RESEARCH ARTICLE

## Morpho-physiological Characterization of Bread Wheat Accessions for Heat Stress Tolerance under Late Sown Conditions of North-Western Plain Zone of India

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Bread wheat is grown in the sub-tropical and tropical regions world-wide. Terminal-heat stress is a major abiotic stress in wheat and global warming due to climate change has negatively impacted its production and productivity. We evaluated 96 accessions of bread wheat germplasm, which included 79 indigenous and 17 exotic collections, by investigating 16 morpho-physiological and yield-related traits during two successive *Rabi* seasons of 2018-19 and 2019-20 under non-stressed and heat-stressed (as a delayed sown crop) environments. Heat susceptibility index (HSI) was used to classify tolerant (HSI <1.0) and susceptible (HSI >1.0) accessions. The heat-stress showed negative impact on the phenotypic expression of 14 morpho-physiological and yield related traits by reducing the traits more prominently in susceptible accessions than the tolerant. However, early ground cover showed the increased expression in the tolerant accessions and higher variability in the germplasm under the heat-stress. Grain length/width ratio (LWR) also improved in the germplasm under heat-stress, while, there was a marked increase in LWR of the susceptible accessions mainly due to relatively higher decrease of grain width in these accessions under heat-stress. The plot yield associated positively with its contributing traits namely yield per plant, number of grains per plant, number of grains per spike, grain weight per spike and plant biomass under both the non-stressed and the heat-stressed environments, but its association with chlorophyll fluorescence was only under heat-stress. The germplasm accessions IC574476 (HD2967), IC393878 (WH157), IC296383 (WR544), EC534487 (PAU351), IC519900 (HD2932) and IC539221 {EIGN-I-(04-05)/149} produced high yield and were stable across the environments. The identified bread wheat germplasm lines could be used as potential parents for bi-parental and multi-parental populations.

**Key Words:** Bread wheat, Characterization, Germplasm accessions, Heat-stress, Morpho-physiological traits

### Introduction

Bread wheat (*Triticum aestivum* L.), an important cereal crop, is a staple food for 40% of the global population (Acevedo *et al.*, 2018). It provides 20% of the total dietary calories consumed world-wide (Shiferaw *et al.*, 2013). Wheat crop was cultivated on 215.9 million ha land with global production of 765.8 million tonnes during 2019 and contributed 8% to world's food basket (FAO, 2021). Global warming has negatively impacted the production and productivity of food crops in different

regions of world during the last decade (Wang *et al.*, 2018). Effective utilization of genetic resources conserved in the genebanks is vital for crop breeding to increase genetic gains and thus addressing the challenges posed by global warming (Reynolds *et al.*, 2015; Mondal *et al.*, 2016). Studies using global and country based models suggest that every 1°C rise in average global surface temperature will lead to decline in wheat yields from 4.1 to 6.4% world-wide and 8.0% in India (Liu *et al.*, 2016). High temperature during grain development

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stage is a major limiting factor in wheat production in many production environments (Farooq *et al.*, 2011). Late sown wheat is exposed to high temperature-stress at grain filling stage, commonly known as terminal-heat stress, and it adversely affects plant growth and consequently the grain yield.

Late sowing of the bread wheat is prevalent in the western part of the Indo-Gangetic Plain (IGP) region of India, where popular rice-wheat cropping system is followed. As a result, it delays sowing of wheat and leads to exposing the crop to terminal-heat stress. A large area in eastern and central parts of India is under late-sown condition due to delayed harvesting of *Kharif* crops, which exposes wheat crop to terminal-heat stress resulting in significant yield losses (Rane *et al.*, 2007; Kumar *et al.*, 2014). The substantial area of eastern IGP, peninsular and central parts of India are under severely heat-stressed, whereas it is moderate in north-western part of IGP (Joshi *et al.* 2007b,c). Hence, the assessment of impact of heat-stress on the productivity of wheat has emerged as top priority in the climate change scenario (Joshi *et al.* 2007a,b). Selection of stress tolerant germplasm lines by screening large genebank collections is a pragmatic approach to develop climate-resilience (McCouch *et al.*, 2020).

The morpho-physiological adaptive traits like early or rapid ground cover, stay-green, chlorophyll fluorescence, biomass and flag-leaf area contribute towards heat-stress tolerance ability of wheat plant (Reynolds *et al.*, 1994, 2001; Cossani and Reynolds, 2012; Latif *et al.*, 2020). High temperature during crop growth and grain filling stage in wheat significantly decreases morphological, yield and its component traits *viz.*, plant height, number of tillers per plant, spike length, number of spikelets per spike, number of grains per spike, grain weight and yield (Mondal *et al.*, 2015; Akter and Islam, 2017; Telfer *et al.*, 2018). However, Telfer *et al.* (2021) advocated that adaption to heat stress should be considered as the combination of total performance and responsiveness to heat stress. Each genotype responds to the changed environment differently due to its genetic makeup and its interaction with environment (Rane *et al.*, 2007; Elbasyoni, 2018; Yashavanthakumar *et al.*, 2021). The genotypes, which maintain high grain weight and yield under heat-stressed environment, seem to possess higher tolerance to hot environment (Sharma *et al.*, 2008; Fleitas *et al.*, 2020).

Genotype-environment interaction is an important factor in the expression of quantitative traits such as yield and its contributing traits. The  $G \times E$  interactions are major bottlenecks in selection of superior breeding lines. Therefore, stratification of the environment has been used to reduce the  $G \times E$  interaction (Eberhart and Russell, 1966). Germplasm being heterogeneous and heterozygous population offers the best opportunity to develop varieties, which might show small  $G \times E$  interaction. GGEbiplot is useful for ranking the cultivars based on their performance in a specific environment, comparing the performance of pair of cultivars in different environments and identifying the best cultivars in each environment (Yan and Kang, 2002). It is also used in evaluating the cultivars based on both average yield and stability and provides visual analysis of  $G \times E$  interactions (Frutos *et al.*, 2014). The aim of this study was to characterize bread wheat germplasm accessions based on morpho-physiological traits and to assess their yield stability in heat-stressed environment for identification of parents for the development of biparental and multiparental mapping populations, and their further utilization as donor lines in wheat breeding programmes.

## Materials and Methods

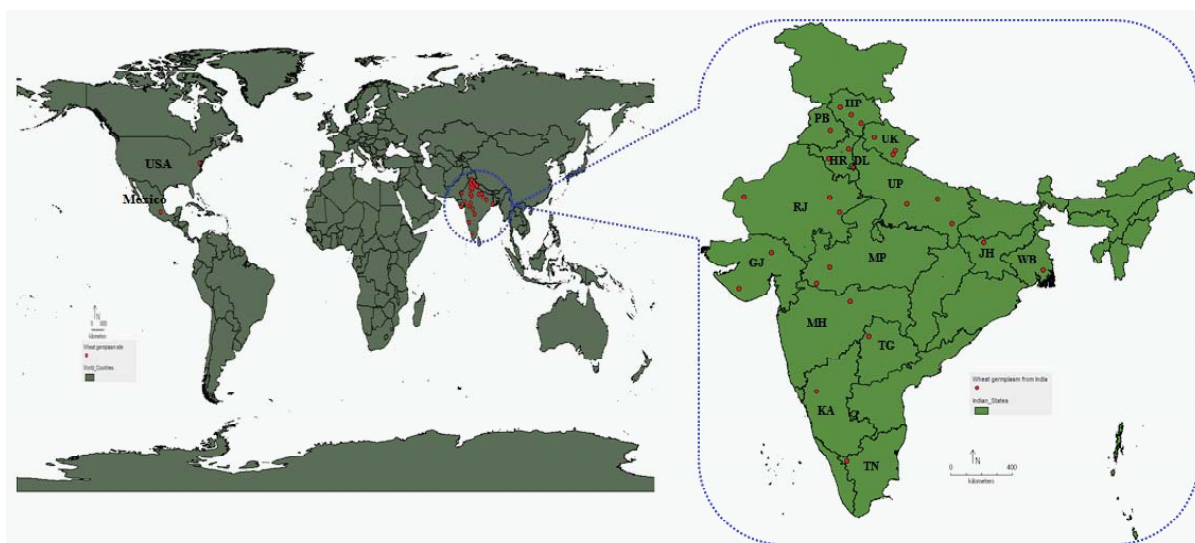
### *Plant Materials and Experimental Design*

A select set of 96 bread wheat accessions, which included released varieties, germplasm lines and genetic stocks, was used in the present experiment. The bread wheat accessions were acquired from the working collection of National Gene Bank (NGB), ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi. The details of these accessions are provided in Table 1. The geo-referencing of bread wheat accessions carried out using software 'DIVA-GIS' (Hijmans *et al.*, 2001) and are depicted in Figure 1. The germplasm lines selected were diverse in nature covering all the wheat growing zones of India. The experimental trials were conducted at New Area Research Farm of ICAR-NBPGR, New Delhi, located at latitude 28.649°N, longitude 77.152°E and altitude 220 m, during *Rabi* crop seasons of 2018-19 and 2019-20. The accessions were evaluated for two consecutive crop seasons using two sowing dates: Normal (sown in 1st week of December) and very late sown wheat (sown in 1st week of January) using Augmented block design (ABD) with five blocks, where four national checks namely Raj3765, HD2932, WR544 and HD2967 were randomized and replicated in each

**Table 1. Details of bread wheat accessions used in the study along with their IC/EC (Indigenous/Exotic Collection) number, alternate identity and original source of acquisition**

Accession Number	Alternate id	Original Source	Accession Number	Alternate id	Original Source
IC443766	RAJ3765	RARI, Jaipur	IC542652	PAU4061	PAU, Ludhiana
IC519900	HD2932	IARI, New Delhi	IC536468	K8415	CSUAT, Kanpur
IC296383	WR544	IARI, New Delhi	IC536483	CPAN2039	IIWBR, Karnal
IC574476	HD2967	IARI, New Delhi	EC574735	8842	CIMMYT, Mexico
EC574731	8832	CIMMYT, Mexico	IC531191	PBW103	PAU, Ludhiana
EC576707	E4828	CIMMYT, Mexico	IC333095	NKD/YSR2910	Barwani, MP
IC252725	HUW467	BHU, Varanasi	IC572925	HPW185	Malan, Kangra, HP
IC252816	KRL13	IIWBR, Karnal	IC252867	NW1014	NDUAT, Ayodhya
IC277741	Dharwad680	Dharwad, Karnataka	IC524299	HW2012	IARI RS, Wellington
IC536081	WL3226	PAU, Ludhiana	IC573461	GW11 (GW396)	Mehsana, Gujarat
IC279617	KHH416	Tehri, Uttarakhand	IC252444	BW/SH49	Kolkata, WB
IC535176	WON-D14	IARI, New Delhi	IC529207	VFW2150	VPKAS, Almora
IC401976	PHR1011	IIWBR, Karnal	IC290191	HW971	Wellington, TN
IC539221	EIGN-I-(04-05)/149	IIWBR, Karnal	IC112258	VL401	VPKAS, Almora
IC539287	MC2003-71	IIWBR, Karnal	IC627711	CAZ/JSM/AP/01	Jaisalmer, Rajasthan
IC539531	EIGN1(03-04)/112	IIWBR, Karnal	IC443653	HD2851	IARI, New Delhi
IC443661	PHR1017	IIWBR, Karnal	IC252431	BW/SH30	Kolkata, WB
EC534487	VEE/KOEL(PAU351)	USDA, USA	IC252619	HD2590	IARI, New Delhi
IC416018	PAU438 (W 7484)	PAU, Ludhiana	IC529242	VFW1290	VPKAS, Almora
IC416075	PAU495 (W 8067)	PAU, Ludhiana	IC536162	WL4996	PAU, Ludhiana
IC416078	PAU498 (W 8093)	PAU, Ludhiana	IC536050	WL1803	PAU, Ludhiana
IC416019	PAU439 (W 7485)	PAU, Ludhiana	IC252999	WH594	CCSHAU, Hisar
IC446713	PSR11489	Nizamabad, Telangana	IC443640	DWR240	IIWBR, Karnal
IC075240	C306	CCSHAU, Hisar	IC445365	7 <sup>th</sup> EGPSN78	IIWBR, Karnal
EC178071	NA	CIMMYT, Mexico	IC303071	RAJ3777	RARI, Jaipur
IC542509	PAU1453	PAU, Ludhiana	IC252414	BW1050	Kolkata, WB
IC252348	AKW2294	PDKV, Akola	IC372643	KRR/AK7	Mandi, HP
IC543293	PAU1218	PAU, Ludhiana	IC252620	HD2615	IARI, New Delhi
IC128454	HB602	Bhowali, Uttarakhand	IC240818	GW273	Mehsana, Gujarat
IC416055	PAU475 (W 7883)	PAU, Ludhiana	IC401940	K8962 (Indra)	CSUAT, Kanpur
IC111800	J1-7	Junagadh, Gujarat	IC443694	PBW527	PAU, Ludhiana
IC111931	K101	CSUAT, Kanpur	IC542547	PAU4091	PAU, Ludhiana
EC576317	E1804	CIMMYT, Mexico	EC190962	Cultivar No.30	CIMMYT, Mexico
EC577013	E8826	CIMMYT, Mexico	EC576066	410	CIMMYT, Mexico
EC414149	CHNQIRR 95S OCHN	CIMMYT, Mexico	EC573527	3850	CIMMYT, Mexico
IC252653	HI1433	IARI, RS, Indore	EC576585	E3228	CIMMYT, Mexico
IC252739	HW1058	Wellington, TN	EC190899	Cultivar No.37	CIMMYT, Mexico
IC335792	KAUZ+1B.1R	IIWBR, Karnal	EC574849	9008	CIMMYT, Mexico
IC543425	10TH HTWYT38	IARI, New Delhi	EC576175	E10906	CIMMYT, Mexico
IC402055	K20008 (KRL35)	IIWBR, Karnal	IC582706	HPW240	Malan, Kangra (HP)
IC265318	NKD2671	Sawai Madhopur, Raj	IC393878	WH157	CCSHAU, Hisar
IC445449	ET99745	IIWBR, Karnal	IC542544	PAU4088	PAU, Ludhiana
IC528965	VFW182	VPKAS, Almora	IC566223	HS492	IARI RS, Shimla
IC549437	PHR1032	IIWBR, Karnal	IC342668	VKG21/72	Chatra, Jharkhand
IC144911	VW120 (GW120)	Mehsana, Gujarat	IC535717	PBW139	PAU, Ludhiana
IC542578	PAU558	PAU, Ludhiana	IC535599	HI1544	IARI RS, Indore
IC535704	PBW124	PAU, Ludhiana	EC277134	713	CIMMYT, Mexico
EC542533	NA*	USDA, USA	NA	CUO/79/Pru 11A	CIMMYT, Mexico

\*NA-Not available; **RARI**-Rajasthan Agricultural Research Institute, Jaipur, Rajasthan; **IARI**-Indian Agriculture Research Institute, New Delhi; **CIMMYT**-International Maize and Wheat Improvement Center, Mexico; **BHU**-Banaras Hindu University, Varanasi, Uttar Pradesh; **IIWBR**-Indian Institute of Wheat and Barley Research, Karnal, Haryana; **PAU**-Punjab Agricultural University, Ludhiana, Punjab; **USDA**-United States Department of Agriculture, USA; **CCSHAU**-CCS Haryana Agricultural University, Hisar, Haryana; **PDKV**-Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra; **CSUAT**-Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh; **VPKAS**-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand; **NDUAT**-Acharya Narendra Deva University of Agriculture and Technology, Ayodhya, Uttar Pradesh.



**Fig. 1.** Geo-referencing map of bread wheat accessions comprised of exotic (17) and indigenous (79) collections used in the screening for terminal heat-stress tolerance

block. The very late sown trial was exposed to high temperatures during grain filling stage. The experimental plot consisted of three rows of 2 m length with 25 cm spacing covering an area of 1.5 m<sup>2</sup>. Standard agronomic and cultural practices were followed for raising the healthy wheat crops.

#### Field Phenotyping and Data Recording

The morpho-physiological and yield related traits for terminal heat-stress tolerance were recorded as per Manual on Physiological Breeding II: A field guide to wheat phenotyping (Pask *et al.*, 2012). The phenological parameters were recorded using Zadoks scale observing whole plot (Zadoks *et al.*, 1974). Sixteen traits were recorded during different growth phases of the bread wheat crop (Table 2).

#### Statistical Analysis

The adjusted mean values were used for the statistical analysis of ABD trial data for 16 morpho-physiological and yield contributing traits tested under non-stressed (NS) and heat-stressed (HS) environments over two years. Combined analysis of variance (ANOVA) was performed following a test of homogeneity of variances and applying Aitkin's transformation using SAS software version 9.4 (SAS Institute, 2013). The descriptive statistics and Pearson's correlation coefficients (*r*) among traits were derived using IBM SPSS statistics software version 20.0 (IBM SPSS, 2011) for both NS and HS environments. We also performed boxplot analysis of 16

morpho-physiological and yield-related traits for both NS and HS environments. The bread wheat accessions were categorized as heat-stress tolerant or susceptible based on heat susceptibility index (HSI) values calculated on grain yield per plot (YPP) data using following formula (Fischer and Maurer, 1978).

$$HSI = (1 - X_a/X_b)/(1 - Y_a/Y_b)$$

Where,  $X_a$  and  $X_b$  are mean value of YPP of individual accession under HS and NS environment, respectively, and  $Y_a$  and  $Y_b$  are mean value of YPP of all accessions under HS and NS environment, respectively. HSI values were used to categorize bread wheat accessions as tolerant ( $HSI < 1.0$ ) or susceptible ( $HSI > 1.0$ ).

**GGE biplot analysis:** The GGE biplot is based on the concept of the biplot (Gabriel, 1971), and provides graphical analysis of genotype (G) and genotype (G) × Environment (E) interaction of multi-environment trials (Yan *et al.*, 2000). The GGE biplot analysis was carried out using four environments (two non-stressed: E1, E3 and two heat-stressed: E2, E4) datasets of grain yield per plot using R software version 3.6.1, package GGE Biplot GUI (R Core Team, 2019). The interpretation of GGE biplots was carried out according to Yan and Tinker (2006). The model for constructing a GGE biplot (Yan, 2001) is as follows:  $Y_{ij} - \bar{Y}_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \epsilon_{ij}$

Where,  $Y_{ij}$  - average yield of  $i^{th}$  accession in  $j^{th}$  environment,  $\bar{Y}_j$  - average yield over all accession in  $j^{th}$  environment;  $\lambda_1$  and  $\lambda_2$  - singular values for PC1 and



**Table 2. Morpho-physiological and yield related traits studied in 96 bread wheat accessions grown in non-stressed and heat-stressed conditions during Rabi seasons of 2018-19 and 2019-20**

Trait studied	Code	How was the trait measured?
Early ground cover (0-10 scale)	EGC	EGC measured by visual observation of whole plot at 45 days after sowing and scored using the scale from 0 (0%) to 10 (100%) by estimating the percentage cover in increments of 10%.
Chlorophyll Fluorescence	CF	CF measured on three randomly selected flag leaves in each plot using hand-held chlorophyll fluorometer (Model OS-30p Chlorophyll Fluorometer, Opti-Science, USA) and dark adaptation leaf clips (Pask <i>et al.</i> , 2012).
Days to 50% heading	DH	DH recorded as the duration between date of sowing and date at which base of 50% of the spikes have emerged from the flag leaf sheath (Zadoks <i>et al.</i> , 1974).
Spike Bearing Tillers per Plant	SBT	Number of tillers with fully developed spikes counted each plant and expressed as spike bearing tillers per plant.
Plant Height (cm)	PH	Plant height measured from base of the plant to the top of spike excluding awns of the main tiller at maturity.
Flag Leaf Length (cm)	FLL	FLL measured from base to the tip of flag-leaves of five randomly selected plants on main tillers during grain filling period.
Flag Leaf Width (cm)	FLW	FLW measured from the widest part of the flag leaf of randomly selected five flag leaves of main tillers during grain filling period.
Spike Length (cm)	SL	SL measured from the spike collar to the tip of the spike excluding awns of the main tiller in three plants per accession.
Physiological maturity (days)	PM	PM recorded as the period between date of sowing and date when 50% peduncle in a plot were matured or turned yellow and at this point the glumes will also be losing their colour (Zadoks <i>et al.</i> , 1974).
Biological yield per plant (g)	BYP	Three plants per accession randomly selected, uprooted at maturity, adhering soil removed and the weight of total biomass recorded for individual plant.
Number of Grains per Plant	NGP	All spikes of each plant were threshed together, debris cleaned and all grains were counted.
Number of Grains per Spikes	NGS	NGS was calculated by dividing total number of grains per plant by number of spikes produced in that particular plant.
Grain weight per spikes	GWS	Total grains of each spike weighed using electronic balance.
Grain L/W Ratio	LWR	LWR was calculated by dividing the length of wheat grain with its width.
Grain Yield per Plant (g)	GYP	Five plants per accession were randomly selected, harvested at maturity and threshed separately. Weight of total grains per plant was taken using electronic balance.
Yield per Plot (g)	YPP	The whole plot (1.5 m <sup>2</sup> ) was harvested at maturity and thrashed manually. After threshing whole plot grains were weighted using electronic balance machine.

PC2, respectively;  $\xi_{i1}$  and  $\xi_{i2}$  - PC1 and PC2 scores, respectively for  $i^{\text{th}}$  accession;  $\eta_{j1}$  and  $\eta_{j2}$  - PC1 and PC2 scores, respectively, for  $j^{\text{th}}$  environment; and  $\varepsilon_{ij}$  - residual of the model associated with the  $i^{\text{th}}$  accession in  $j^{\text{th}}$  environment.

## Results

### Genetic Variability for Morpho-physiological Traits

Mean sum of squares estimated for year, genotype (= accession),  $G \times Y$  interaction and model from ANOVA for 16 morpho-physiological and yield traits in 96 accessions of bread wheat under NS and HS environments are presented in Supplementary Table 1. Accessions showed significant differences for most of the traits under NS environment except SBT, BYP, NGP and GYP. However, these traits showed significant differences due to year and model effects. All the traits except chlorophyll fluorescence showed significant differences due to genotype effect under HS

environment. This revealed that the accessions exhibited diverse responses under heat-stress and hence showed significant variability for these traits. The accessions revealed non-significant differences for chlorophyll fluorescence under both the environments, where the effect due to year was significant. Descriptive statistics derived for 16 morpho-physiological and yield-related traits recorded in 96 accessions of bread wheat under both NS and HS environments are presented in Table 3. Accessions showed remarkable variability for early ground cover and days to 50% heading traits. The terminal heat-stress exerted an adverse effect on most of the traits and the reduced values were observed for these traits except early ground cover and grain L/W ratio, which showed higher values under HS environment (Table 3). Phenological traits like days to 50% heading and physiological maturity were significantly reduced by 16.4 days and 23.4 days, respectively, under HS environment. Similarly, plant height and spike length also decreased due to heat-stress by 9.7 cm and 1.1 cm,

**Table 3. Descriptive statistics for 16 morpho-physiological and yield contributing traits recorded in 96 accessions of bread wheat grown in non-stressed and heat-stressed environments during two Rabi seasons of 2018-19 and 2019-20**

Characters	Environment	Range		Mean $\pm$ S.E.	Standard deviation	CV (%)
		Min.	Maxi.			
Early Ground Cover (0-10 scale)	Non-stressed	3.0	8.0	5.0 $\pm$ 0.11	1.12	22.20
	Heat-stressed	3.0	9.0	5.1 $\pm$ 0.13	1.23	24.21
Chlorophyll Fluorescence	Non-stressed	0.72	0.80	0.76 $\pm$ 0.01	0.02	2.05
	Heat-stressed	0.62	0.77	0.72 $\pm$ 0.01	0.03	3.80
Days to 50% Heading	Non-stressed	77.0	112.0	87.3 $\pm$ 0.50	4.94	5.66
	Heat-stressed	62.7	89.5	70.9 $\pm$ 0.41	3.98	5.62
Spike Bearing Tillers per Plant	Non-stressed	7.8	17.5	10.9 $\pm$ 0.17	1.65	15.16
	Heat-stressed	7.0	14.7	9.7 $\pm$ 0.15	1.49	15.39
Plant Height (cm)	Non-stressed	84.9	150.9	106.6 $\pm$ 1.31	12.83	12.03
	Heat-stressed	73.2	127.0	96.9 $\pm$ 1.25	12.23	12.63
Flag Leaf Length (cm)	Non-stressed	18.4	35.4	24.7 $\pm$ 0.34	3.33	13.48
	Heat-stressed	12.6	29.4	18.6 $\pm$ 0.31	3.03	16.29
Flag Leaf Width (cm)	Non-stressed	1.6	2.9	2.0 $\pm$ 0.02	0.23	11.55
	Heat-stressed	1.3	2.4	1.7 $\pm$ 0.02	0.18	10.55
Spike Length (cm)	Non-stressed	8.7	15.6	11.7 $\pm$ 0.12	1.18	10.14
	Heat-stressed	8.1	13.5	10.6 $\pm$ 0.11	1.10	10.38
Physiological Maturity (days)	Non-stressed	121.0	143.0	126.1 $\pm$ 0.31	3.04	2.41
	Heat-stressed	94.5	118.0	102.7 $\pm$ 0.32	3.18	3.10
Biological yield per Plant (g)	Non-stressed	32.8	93.6	55.8 $\pm$ 1.16	11.40	20.44
	Heat-stressed	27.6	74.1	43.8 $\pm$ 0.94	9.22	21.07
Number of Grains per Plant	Non-stressed	243.0	831.8	530.9 $\pm$ 10.56	103.45	19.49
	Heat-stressed	246.0	636.7	418.9 $\pm$ 8.46	82.86	19.78
Number of Grains per Spike	Non-stressed	27.9	71.2	48.9 $\pm$ 0.89	8.69	17.78
	Heat-stressed	25.6	70.6	43.8 $\pm$ 0.84	8.28	18.90
Grain Weight per Spike (g)	Non-stressed	0.9	3.6	2.0 $\pm$ 0.04	0.40	19.83
	Heat-stressed	0.9	3.2	1.6 $\pm$ 0.04	0.37	23.54
Grain L/W Ratio	Non-stressed	1.70	3.09	1.99 $\pm$ 0.02	0.18	8.82
	Heat-stressed	1.78	3.28	2.05 $\pm$ 0.02	0.19	9.06
Grain yield per plant (g)	Non-stressed	9.5	40.6	22.0 $\pm$ 0.52	5.08	23.02
	Heat-stressed	8.7	26.5	15.1 $\pm$ 0.37	3.62	24.06
Yield Per Plot (g)	Non-stressed	450.1	1203.8	844.0 $\pm$ 13.39	131.23	15.59
	Heat-stressed	265.1	897.1	635.2 $\pm$ 11.09	108.69	17.09

respectively. Flag-leaf length and width decreased under HS environment by 6.1 cm and 0.3 cm, respectively. Likewise, heat-stress significantly reduced spike bearing tillers from 10.9 to 9.7 and biological yield from 55.8 to 43.8 g. The numbers of grains per spike and grain weight per spike were also reduced in HS condition. Furthermore, the heat-stress condition showed adverse effects on number of grains per plant, grain yield per plant and yield per plot, and the decrease was from 530.9 to 418.9, from 22.0 to 15.1 g and from 844.0 to 635.2 g, respectively. The value of chlorophyll fluorescence was also reduced from 0.76 to 0.72 under HS environment. However, the values of early ground cover and grain L/W ratio were slightly increased from 5.0 to 5.1 and 1.99 to 2.05, respectively, under HS environment. The highest CV was observed for early ground cover

(24.21%) followed by grain yield per plant (24.02%) under HS environment. The lowest CV was recorded for chlorophyll fluorescence (2.05%) closely followed by physiological maturity (2.41%) under heat-stress.

Check variety 3 (WR544) showed the earliest heading on 77.0 and 62.7 days under NS and HS environment, respectively. Accession 34 (EC577013) was the tallest (150.9 cm) under NS environment, while the accession 84 (EC576585) was the tallest (127.0 cm) under HS environment. Similarly, the shortest plant height was recorded in accession 38 (IC335792; 84.9 cm) under NS and in accession 64 (IC443653; 73.2 cm) under HS condition. Accession 96 (CUO/79/PRU11A) exhibited the highest yield per plant (40.6 g) under NS, whereas, accession 86 (EC574849) showed the highest yield per plant (26.5 g) under HS condition.

Accession 15 (IC539287) produced the lowest yield per plant (9.5 g) under NS environment, while accession 34 (EC577013) produced the lowest yield per plant (8.7 g) under HS environment. Accession 86 (EC574849), which produced the highest yield/plant, also exhibited the highest biological yield (74.1 g) under heat-stress. Under NS environment, the highest yield per plot was recorded in accession 9 (IC277741; 1203.8 g), whereas accession 91 (IC566223) produced the highest yield (897.1 g) under HS environment. The lowest yield per plot (450.1 g) was produced by accession 26 (IC542509) under NS, whereas, accession 15 (IC539287), produced the lowest yield (265.1 g) under HS environment. The highest EGC recorded in accessions 24 (IC075240), 57 (IC524299) and 91 (IC566223). Accessions 85 (EC190899), 84 (EC576585), 17 (IC443661) and 60 (IC529207) showed early ear-heading.

Boxplot analysis showed diverse pattern of genetic variability for 16 morpho-physiological and yield related traits under NS and HS environments. Boxplot analysis clearly depicted minimum, maximum, mean, median and outliers' values under both the NS and HS environments (Fig. 2). All the traits, except EGC and grain L/W ratio, showed lower values of mean and median in boxplots under HS environment as compared to the NS environment. EGC showed more variable expression by different accessions under heat-stress than under non-stress condition. However, boxplot revealed almost similar mean and median for EGC under both environments. Some of the traits (CF, FLL, NGS, SL and PL) showed comparatively more variability, as depicted by spreads of boxplots, under the HS environment. The boxplot showed that the accessions took significantly less time for 50% heading and physiological maturity under heat-stress as compared to non-stress condition. It was interesting to note that the grain L/W ratio was increased mainly due to more reduction of grain width under heat-stress. Traits like DH, SBT, PM, BYP, NGP, GYP, GWS and YPP expressed more variability under non-stress condition.

### ***Correlation of Grain Yield with Other Traits***

Correlation coefficients were estimated among 16 morpho-physiological and yield-related traits in 96 bread wheat accessions under NS and HS environments (Table 4). Among the various traits studied, grain yield per plot (YPP) positively associated with GYP, NGP, NGS, GWS and BYP under both NS and HS environments.

However, YPP positively correlated with EBT under NS environment and chlorophyll fluorescence under HS environment only. YPP showed negative association with LWR under both the environments, and with DH and PM under NS environment only. The grain yield per plant (GYP) showed moderate positive association with its component traits such spike length, SBT, NGS and strong association with NGP, GWS and BYP under both NS and HS environments, while it showed moderate positive association with plant height, FLL and FLW only under HS environment. Early ground cover (EGC) was found to be positively associated with DH, PM, PH and FLL under both the environments, and with BYP under NS environment. Similarly, chlorophyll fluorescence (CF) exhibited positive correlation with FLL, SL and BYP under NS environment and with YPP under HS environment. Days to 50% heading (DH) showed strong positive association with physiological maturity (PM) under both the environments. Yield contributing traits like SL, NGP, NGS, GWS, GYP and BYP also showed moderate to strong positive association with each other. Spike length (SL) also showed moderate positive association with DH, PM, FLL and FLW under both the environments, but with CF only in NS environment.

### ***Impact of Heat Stress on Morpho-Physiological Traits in Tolerant and Susceptible Accessions***

The effect of heat-stress on 16 morpho-physiological traits was investigated after classifying the accessions into tolerant or susceptible one based on HSI values (Table 5). Most of the traits (CF, DH, SBT, PH, FLL, FLW, SL, PM, GYP, NGP, NGS, GWS, GYP and YPP) were negatively impacted by the heat-stress and showed reduced expression under HS environment (Fig. 3). There was marked reduction in the trait values in the susceptible accessions than the tolerant ones in CF, PH, FLL, SL, BYP, NGP, NGS, GWS, GYP and YPP under heat-stress condition. However, physiological trait like EGC showed increased expression under heat-stress specifically in the tolerant accessions. The grain trait namely LWR also exhibited increased value under heat-stress in both the tolerant and susceptible accessions but the susceptible accessions showed comparatively more increase in LWR.

### ***GGE Biplot Analysis and Selection of Heat-stress Adapted Wheat Germplasm***

Graphical representation of relationship between accessions and test environments based on grain yield

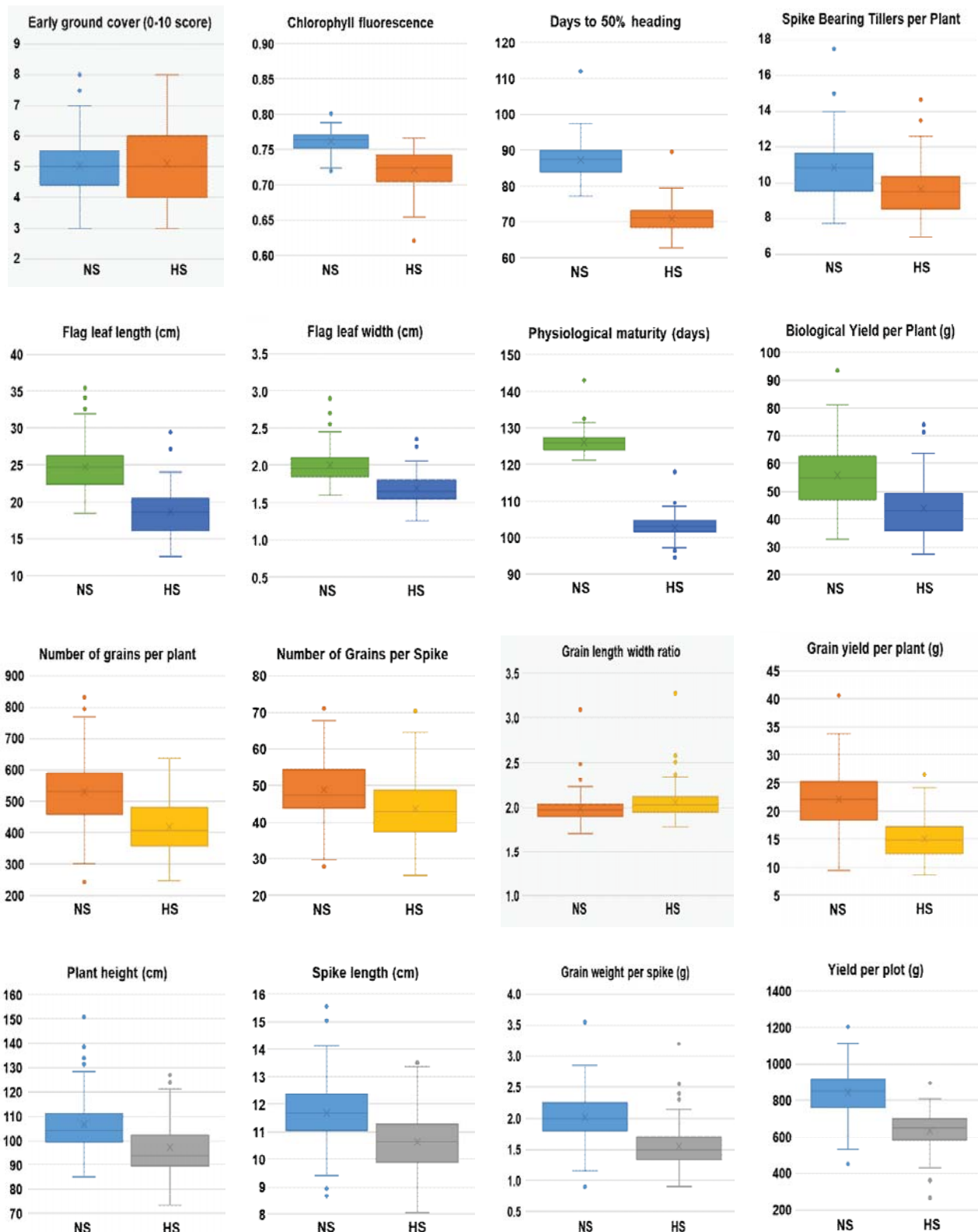


Fig. 2. Box plot analysis of variability in 16 morpho-physiological and yield contributing traits observed in 96 bread wheat accessions tested under non-stressed (NS) and heat-stressed (HS) environments



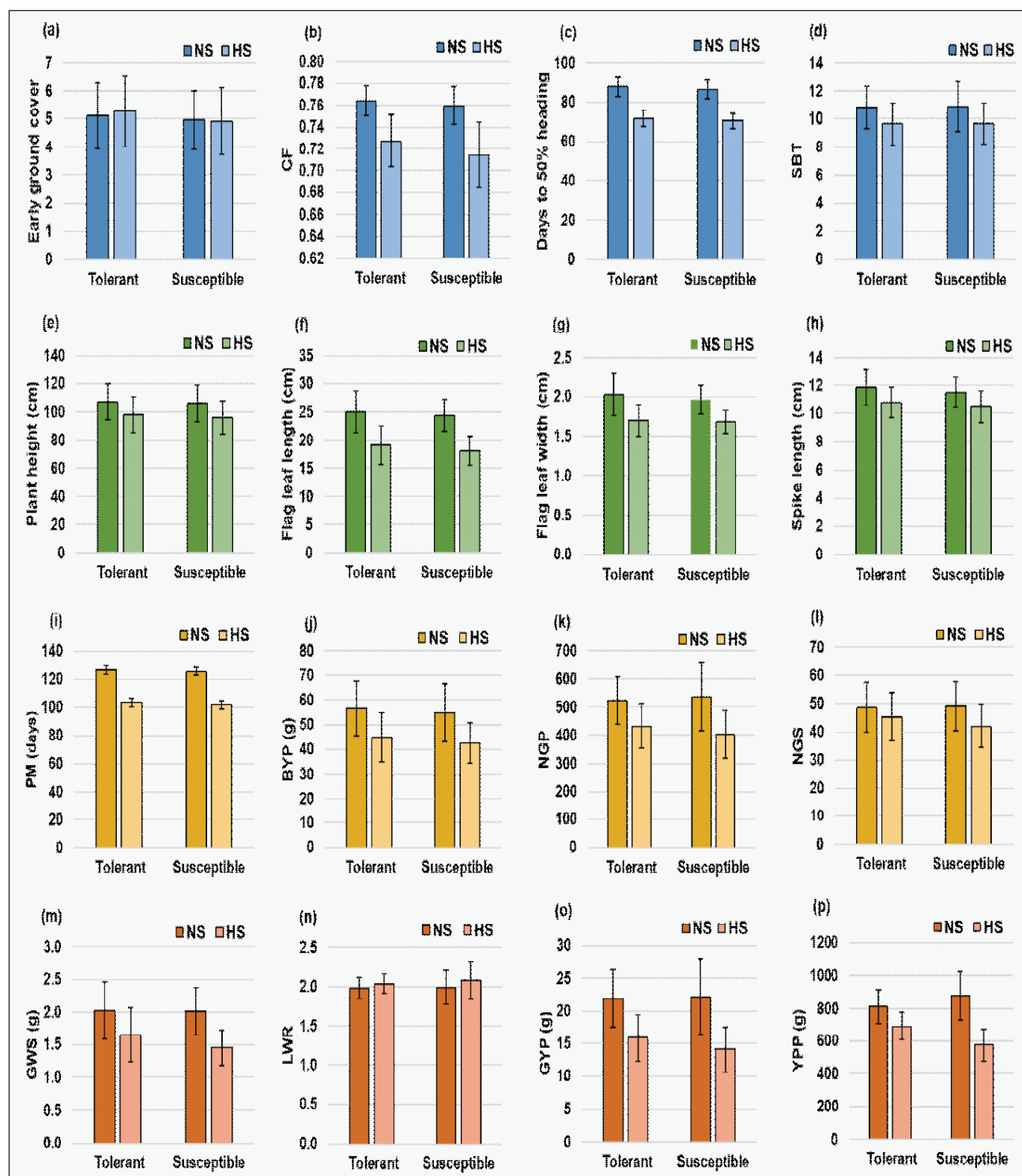


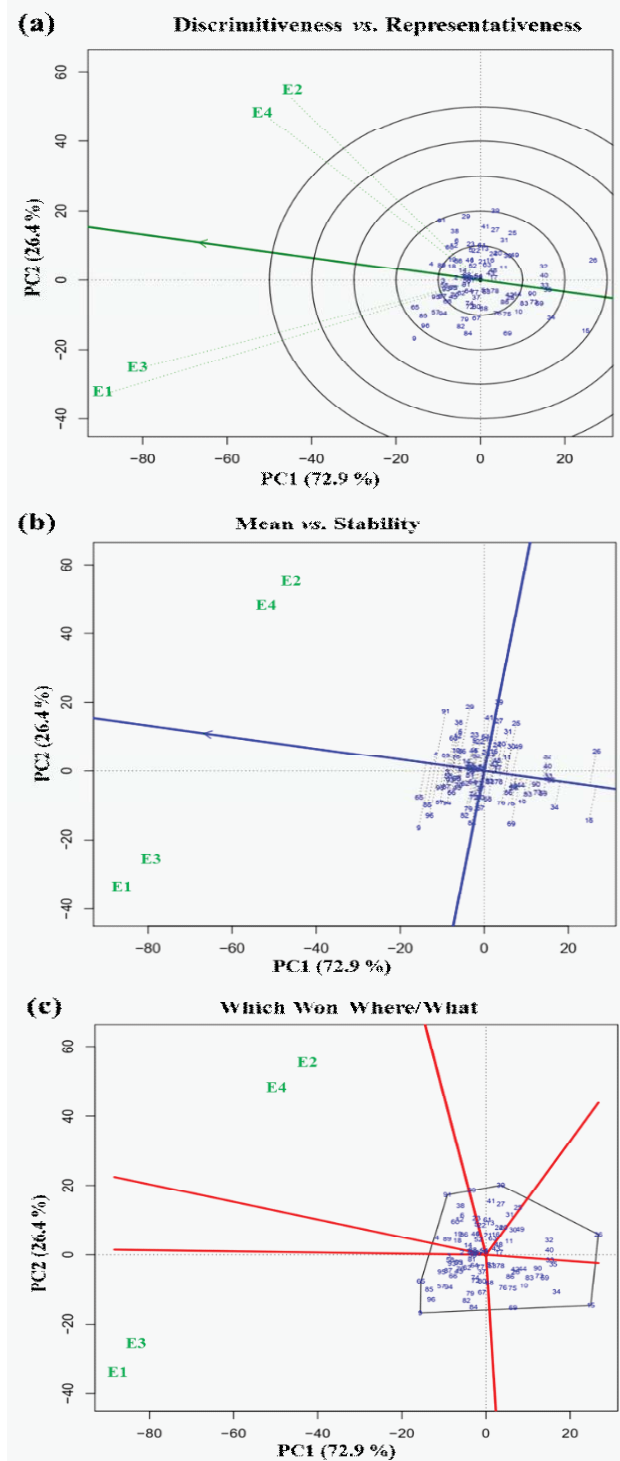
Fig. 3. Effects of heat-stress on different morpho-physiological and yield-related traits in tolerant and susceptible accessions (a-p). Early ground cover was higher in tolerant accessions under HS environment (a). Grain length width ratio (LWR) were relatively higher in susceptible accessions than the tolerant accessions under HS environment (n). CF-Chlorophyll fluorescence, SBT-Spike bearing tillers per plant, PM-Physiological maturity (days), BYP-Biological yield per plant (g), NGP-Number of grains per plant, NGS-Number of grains per spikes, GWS-Grain weight per spikes (g), LWR-Grain length width ratio, GYP-Grain yield per plant (g), YPP-Yield per plot (g)

per plot is presented in Figure 4. The principal component (PC) 1 and PC2 together explained 99.3 % of the total variation.

**Characterization of test environments:** The characterization of four test environments is explained by Figure 4a. The single-arrowed line represents the average environment axis (AEA). Highly positive correlations (acute angle) observed between E1 and E3, and between E2 and E4 environments. Environment E3 showed more representativeness because of the small angle with AEA followed by E4 and E1, while environment E2 was the least representative test environment. Among group 1, environment E1 was more discriminative (longest environment vector) than E3, whereas in group 2, E2 and E4 environments were of equal discriminative nature. Thus, E3 environment was more representative and discriminative test environment in comparison to others for selecting adapted genotypes.

**Mean performance and stability:** GGE biplot showing mean performance and stability of the bread wheat accessions for yield per plot are depicted by Figure 4b. An ideal genotype produces high yield with high stability across the environments. The direction of AEA points to higher mean yield across environments. Thus, accessions 65 (IC252431), 9 (IC277741) and 4 (HD2967) were the top yielding accessions followed by 91 (IC566223), 85 (IC566223), 96 (CUO/79/Pru 11A) and 89 (IC393878), whereas the accessions 15 (IC539287), 26 (IC542509), 34 (EC577013), 35 (EC414149) and 33 (EC576317) were the low yielding accessions across the environments. The vector or line which joined the accessions with the AEA showed stability in either direction. Thus, accessions having very small vector or positioned very near to the AEA showed high stability. Accordingly, the mean vs. stability biplot showed that accessions 4 (HD2967), 89 (IC393878), 3 (WR544), 18 (EC534487), 2 (HD2932) and 14 (IC539221) produced high yield and were stable across the environments. However, accessions 9 (IC277741) and 91 (IC566223) were the high yielder with low stability across the environments. Further, the accessions 15 (IC539287), 26 (IC542509) and 34 (EC577013) were low yielder with poor stability across the environments.

**Performance of genotypes to specific environments:** The which-won-where view of the GGE biplot (Fig. 4c) expressed which genotypes performed best in which



**Fig. 4.** GGE biplot showing genotype,  $G \times E$  interactions with discriminativness vs. representativeness view of test environments (a), the mean performance and stability of wheat accessions (b) and which-won-where pattern of genotypes (c). Two principal components, PC1 and PC2 showed genetic variability of 99.3%. E1: NS environment 2018-19, E2: HS environment 2018-19, E3: NS environment 2019-20, E4: HS environment 2019-20

**Table 4.** Pearson's correlation coefficients for 16 morpho-physiological and yield related traits in 96 bread wheat accessions in non-stressed (above diagonal) and heat-stressed (below diagonal) environments during two *Rabi* crop seasons of 2018-19 and 2019-20

Trait	EGC	CF	DH	PM	PH	FLL	FLW	SL	LWR	SBT	NGP	NGS	GYP	GWS	BYP	YPP
EGC	--	0.10	0.25*	0.25*	0.40**	0.24*	0.13	0.18	0.09	0.14	-0.01	-0.14	0.14	0.04	0.33**	-0.06
CF	0.02	--	0.11	0.14	-0.02	0.21*	0.18	0.23*	-0.09	0.07	0.15	0.11	0.20	0.18	0.26*	-0.05
DH	0.32**	0.14	--	0.87**	0.13	0.19	0.17	0.35**	0.17	-0.14	-0.03	0.11	-0.23*	-0.14	0.01	-0.28**
PM	0.30**	0.19	0.86**	--	0.08	0.27**	0.23*	0.45**	0.10	-0.09	-0.04	0.07	-0.18	-0.09	0.07	-0.23*
PH	0.35**	-0.14	0.08	0.05	--	0.34**	-0.03	0.11	0.21*	0.13	-0.06	-0.18	-0.01	-0.10	0.32**	0.03
FLL	0.22*	-0.05	0.19	0.19	0.37**	--	0.54**	0.53**	0.19	-0.23*	-0.06	0.18	0.03	0.28**	0.31**	-0.02
FLW	0.10	0.05	0.19	0.26*	-0.06	0.54**	--	0.53**	0.16	-0.22*	0.04	0.29**	0.17	0.46**	0.34**	-0.14
SL	0.13	0.07	0.27**	0.37**	0.12	0.28**	0.28**	--	0.06	0.04	0.34**	0.40**	0.35**	0.46**	0.49**	0.02
LWR	0.08	-0.08	0.14	-0.02	0.12	-0.01	-0.02	-0.03	--	-0.04	-0.31**	-0.33**	-0.26**	-0.30**	-0.12	-0.28**
SBT	0.08	0.01	-0.09	-0.04	0.17	0.10	0.03	0.01	0.02	--	0.53**	-0.30**	0.56**	-0.19	0.51**	0.21*
NGP	-0.08	0.11	0.05	0.13	0.03	0.23*	0.22*	0.44**	-0.21*	0.49**	--	0.63**	0.85**	0.53**	0.71**	0.46**
NGS	-0.15	0.11	0.14	0.21*	-0.12	0.18	0.22*	0.50**	-0.27**	-0.33**	0.65**	--	0.43**	0.78**	0.34**	0.32**
GYP	0.05	0.11	-0.16	0.01	0.21*	0.37**	0.36**	0.44**	-0.18	0.44**	0.83**	0.52**	--	0.68**	0.85**	0.50**
GWS	-0.02	0.10	-0.09	0.07	0.09	0.35**	0.38**	0.50**	-0.22*	-0.24*	0.53**	0.82**	0.74**	--	0.59**	0.39**
BYP	0.20	0.08	0.16	0.23*	0.35**	0.46**	0.46**	0.43**	0.01	0.49**	0.71**	0.36**	0.85**	0.55**	--	0.28**
YPP	0.05	0.23*	-0.10	0.07	0.05	0.05	0.04	0.20	-0.28**	0.12	0.39**	0.33**	0.42**	0.38**	0.22*	--

\*\*, \* significant at 0.01 and 0.05 probability level, respectively.

**Abbreviations:** EGC-Early ground cover (0-10 score), CF-Chlorophyll fluorescence, DH-Days to 50% heading, PM-Physiological maturity (days), PH-Plant height (cm), FLL-Flag leaf length (cm), FLW-Flag leaf width (cm), SL-Spike length (cm), LWR-Grain length width ratio, SBT-Spike bearing tillers per plant, NGP-Number of grains per plant, NGS-Number of grains per spike, GYP-Grain yield per plant (g), GWS-Grain weight per spike (g), BYP-Biological yield per plant (g), YPP-Yield per plot (g).

environments. A polygon was drawn on genotypes at the extreme from the biplot origin so that all other genotypes are enclosed within the polygon. The genotype at the vertex of the polygon performs best in the environment falling within the sectors. The hexagon was drawn which has eight accessions at the vertices viz. 91 (IC566223), 65 (IC252431), 9 (IC277741), 69 (IC536050), 15 (IC539287), 26 (IC542509), 39 (IC543425) and 29 (IC128454). Based on the which-won-where view of the GGE biplot the accessions 9 (IC277741), 65 (IC252431), 96 (CUO/79/ Pru 11A), 85 (EC190899), 57 (IC524299), 94 (IC553599), 84 (EC576585), 82 (EC576066) and 95 (EC277134) fall in E1 and E3 (Non-stressed) environment sectors, which were the superior performer in the favourable environment but highly susceptible for unfavourable (heat-stressed) environment. Similarly, accessions 91 (IC566223), 29 (IC128454), 38 (IC335792), 6 (EC576707), 12 (IC535176), 60 (IC529207), 23 (IC446713) and 22 (IC416019) were included in E2 and E4 (heat-stressed) environment and hence these accessions were the excellent performer in HS environment and were the highly tolerant to heat-stress. The winning accession for NS environment was 9 (IC277741), whereas accession 91 (IC566223) was the

winning accession for HS environment because these accessions were found to be located on the vertex of NS and HS environment, respectively.

## Discussion

Evaluation of diverse germplasm under heat-stress would help us in understanding the complexity of crop response to global warming and identification of superior lines for the development of mapping populations, which may be utilized for the development of heat tolerant cultivars. In present investigation, we have evaluated a diverse set of 96 accessions of bread wheat along with four national checks to analyse plant responses in terms of effect of heat-stress on morpho-physiological and yield related traits. The late sown wheat crop is exposed to higher temperatures at reproductive and grain filling stages and has been the widely used strategy to screen germplasm for yield and other traits under heat-stress (Ullah *et al.*, 2020). Grain yield is complex quantitative trait and is an end-product of many interactions between genes for physiological and yield component traits. High temperature stress has a wide range of effects on plants in terms of physiological, biochemical and gene regulation pathways (Bita and Gerats, 2013). Wheat

**Table 5. The mean yield and heat susceptibility index (HSI) of 96 accessions of bread wheat grown under non-stressed (NS) and heat-stressed environments during Rabi seasons of 2018-19 and 2019-20**

Accession	Yield per plot (g)		HSI	Accession	Yield per plot (g)		HSI
	NS	LS			NS	LS	
IC265318	762.1	756.1	0.03	IC536468	879.3	653.1	1.05
IC566223	906.4	897.1	0.04	IC401940	793.3	587.6	1.06
IC128454	819.0	807.1	0.06	IC252867	896.3	658.1	1.09
IC543425	762.0	747.0	0.08	IC443640	938.3	684.6	1.11
EC178071	694.1	681.1	0.08	IC240818	863.3	628.6	1.11
IC252348	732.1	715.1	0.09	WR544	982.6	708.2	1.14
IC111800	699.1	678.1	0.12	EC576317	634.1	455.1	1.15
IC542509	450.1	431.1	0.17	IC535717	973.6	696.1	1.17
IC416075	724.8	687.1	0.21	IC303071	662.3	470.6	1.18
IC401976	765.8	717.1	0.26	EC573527	694.3	492.6	1.19
IC542652	673.3	627.1	0.28	IC252999	941.3	665.6	1.20
IC446713	817.8	758.1	0.30	IC573461	982.3	694.1	1.20
EC576707	868.8	802.1	0.31	EC414149	633.1	447.1	1.20
IC290191	783.3	724.1	0.31	IC572925	951.3	669.1	1.21
IC075240	752.8	690.1	0.34	IC531191	836.3	586.1	1.22
EC574731	823.8	751.1	0.36	IC536483	846.3	592.1	1.23
IC335792	890.1	809.1	0.37	IC443653	899.3	626.1	1.24
IC416019	813.8	735.1	0.40	EC574849	760.6	530.1	1.24
IC535176	890.8	798.1	0.43	EC576175	992.6	685.1	1.27
IC529207	907.3	791.1	0.52	IC112258	931.3	637.1	1.29
IC539531	775.8	678.1	0.52	IC252444	645.3	441.1	1.29
IC279617	723.8	630.1	0.53	EC277134	1020.6	697.1	1.30
IC416078	795.8	693.1	0.53	IC252619	989.3	662.6	1.35
IC111931	603.1	521.1	0.56	IC549437	752.1	500.1	1.37
Raj3765	828.8	708.0	0.60	IC445365	902.3	600.6	1.37
IC416055	736.1	628.1	0.60	IC252414	908.3	604.6	1.37
IC416018	908.8	762.1	0.66	IC445449	776.1	515.1	1.38
IC627711	787.3	661.1	0.66	IC542547	887.3	586.6	1.39
IC542578	850.3	711.1	0.67	IC536162	856.3	564.6	1.39
IC393878	950.6	775.1	0.75	IC144911	973.3	638.1	1.41
EC534487	914.8	744.1	0.76	IC543293	784.1	512.1	1.42
EC574735	848.3	688.1	0.77	IC252431	1109.3	717.6	1.44
EC542533	786.3	629.1	0.82	IC372643	778.3	499.6	1.46
IC402055	619.1	493.1	0.83	IC252620	813.3	521.6	1.47
IC539221	889.8	703.1	0.86	IC252739	913.1	580.1	1.49
IC582706	872.6	689.1	0.86	IC536081	748.8	474.1	1.50
IC443661	784.8	620.1	0.86	IC553599	1028.6	637.1	1.56
IC252653	920.1	722.1	0.88	IC443694	946.3	581.6	1.58
HD2967	997.8	778.0	0.90	IC529242	901.3	553.6	1.58
IC252816	831.8	647.1	0.91	EC190899	1098.6	673.1	1.58
IC342668	873.6	676.1	0.92	IC524299	1064.3	634.1	1.65
IC252725	897.8	689.1	0.95	EC576066	969.3	564.6	1.71
IC542544	653.6	497.1	0.98	CUO/79/ PRU 11A	1111.6	635.1	1.75
IC333095	850.3	645.1	0.99	EC576585	956.3	530.6	1.82
IC528965	812.1	613.1	1.00	EC577013	662.1	363.1	1.85
IC535704	865.3	652.1	1.01	IC536050	808.3	438.6	1.87
EC190962	892.3	666.6	1.03	IC277741	1203.8	624.1	1.97
HD2932	922.0	688.2	1.04	IC539287	529.8	265.1	2.04

HSI value <0.50 considered as highly tolerant, HSI value = 0.51 to 1.04 as tolerant, HSI value = 1.05 to 1.50 as susceptible and HSI value of >1.51 as highly susceptible accessions of bread wheat.



achieves adaptation through variation in phenology and related traits determining plant architecture (Hyles *et al.*, 2020). Hence, it is crucial to understand the genes that underpin the variations in plant phenology and their interactions with other genes, morpho-physiological traits and the environment.

### ***Trait Variability in Germplasm and Impact of Heat-stress***

The ANOVA revealed that the bread wheat accessions possess significant differences for most of the traits under both NS and HS environments, which shows that the accessions have varied response to heat-stress and it provides ample scope for further selection of positively associated traits for terminal heat-tolerance. The heat-stress showed an adverse effect on crop growth, development, morpho-physiological and yield traits. Most of the traits showed reduced values under HS environment except early ground cover and grain length/width ratio, which were increased under heat-stress. The similar results were also reported by Gowda *et al.* (2011) and Elbasyoni (2018). According to Pinto *et al.* (2017), the heat-adapted genotypes with best yielding ability also showed high early biomass and high grain filling rates. In most of the traits, the reduction was more prominent in susceptible accessions as compared to the tolerant. Heat-stress reduced plant height, days to heading, spike length, maturity, grain weight and yield between 4-7% with every 1°C rise in average maximum temperature above the optimal 25°C (Ullah *et al.*, 2020). Mondal *et al.* (2015) also observed that the values of days to heading, plant height, grain weight and yield were reduced in HS environment. Sharma *et al.* (2008) reported significantly lower values for days to heading, grain weight, and yield in late sown wheat. Similarly, Fleitas *et al.* (2020) found that the grain yield and yield component traits were more severely affected as the heat-stress increases. High temperature applied at post-anthesis shortened maturity and grain filling duration, and reduced grain weight and yield (Kaur and Behl, 2010). Agarwal *et al.* (2021) reported that days to anthesis and yield traits were reduced in late sown wheat as compared to normal. These previous studies support our finding of reduction in 14 out of the 16 morpho-physiological and yield traits analysed under the heat-stress.

### ***Association of Grain Yield with Other Traits***

Yield is complex character and determined by many morphological, physiological and yield related parameters. Under heat-stress, yield showed significantly positive association with yield per plant and yield contributing traits like number of grains (NGP, NGS), grain weight (GWS) and plant biomass (BYP). Sharma *et al.* (2008) reported significantly positive association of grain yield with grain weight under both normal and terminal-heat stress conditions, and corroborates our results. Yashavanthakumar *et al.* (2021) reported the positive association of yield with TGW and GFP, and negative association with days to heading and maturity. However, there was no association of yield with days to 50% heading and plant height in the present study under HS environment, which also confirmed by Ullah *et al.* (2020). Hence, to increase the yield in wheat under heat-stress, the focus should be given on traits which have high and significant association with grain yield for the selection of heat tolerant lines. Lordkaew *et al.* (2019) reported positive correlation between days to heading and grain yield and opined that longer time before heading enables the development of larger spikes and more numbers of spikelets producing enhanced grain numbers per plant. Positive association of grain yield with number of grains per spike and grain weight per spike was also reported by Sharma *et al.* (2016). They found that the phenological characters such as days to heading and days to maturity showed strong positive correlation (0.96) and validates our finding. The present and earlier association studies revealed that yield is positively correlated with its contributing traits under HS environment. Therefore, selection for these traits could be valuable in wheat breeding programme designed for heat-stress tolerance.

### ***Yield Stability and Identification of Accessions Adapted to Heat-stress***

Yield stability analysis is used to identify the accessions exhibiting superior performance and stability in one or more groups of environments. The graphical presentation of GGE biplots have been used to identify and select the accessions which were adapted to unfavorable HS environment, favorable NS environment and both environments (Yan and Tinker, 2006). Yield stability across the environments is a reliable criterion for the selection of heat-stress adapted germplasm (Kang

*et al.*, 2004). The GGE biplot showed that accessions HD2967, IC393878, WR544, EC534487, HD2932 and IC539221 produced high yield and were stable across environments and may be used as donor parents in heat-stress adaptation breeding programme. Similar studies were also carried by Elbasyoni (2018) and Fleitas *et al.* (2020) using GGE biplot to select superior yielding lines under heat-stress. The G×E interaction biplots for grain yield showed that genotypes Raj3765 and Raj4027 were more stable across all environments (Rane *et al.*, 2007). The significant influence of environment, genotype and G × E interactions on the grain yield was reported by Yashavanthakumar *et al.* (2021). They selected three superior genotypes namely MACS 6729, HD 2932, and MACS 6733 based on yield and stability performance across the environments. The stable and higher yielding accessions identified under HS environment in our study could be utilized for the development of terminal heat-stressed tolerant cultivars in bread wheat.

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\*Supplementary Table or Figure mentioned in the article are available in the online version.

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