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Abstract: Bread wheat (Triticum aestivum L.) is one of the world's most important staple food crop providing 20% global energy and dietary proteins. It is widely grown in sub-tropical and tropical areas and as such exposed to heat-stress especially at grain filling period (GFP). Global warming has further affected its production and productivity in these heat-stressed environments. We examined the effect of heat-stress on 18 morpho-physiological and yield-related traits in 96 bread wheat accessions. Heat susceptibility index (HSI <0.60) and yield stability (βi <0.55) used as criteria for selecting the tolerant accessions. Heat-stress, imposed by delayed sowing, decreased crop growth and GFP, and as a result reduced morphological and yield-related traits, namely days to 50% anthesis, plant height, peduncle length, flag-leaf area, spike length, spikelets per spike, grain length and width, thousand grain weight (TGW), harvest index and yield. The reduction in the trait values was severe in susceptible accessions (48.2% yield reduction in IC277741) than the tolerant. Physiological traits like chlorophyll content, canopy temperature depression (CTD), normalized difference vegetation index (NDVI), plant waxiness and leaf rolling showed higher expression in the tolerant accessions under heat-stress. Scanning electron microscopy of matured wheat grains revealed ultrastructural changes in endosperm and aleurone cells caused due to heat-stress. The reduction of size and density of large starch granules is the major cause of yield and TGW decrease in the heat-stress susceptible lines. The most stable and high-yielding accessions namely IC566223, IC128454, IC335792, EC576707, IC535176, IC529207, IC446713 and IC416019 were identified as the climate-smart germplasm lines. Germplasm lines possessing desirable traits were selected as potential parents for the development of bi-parental and multi-parental populations.

Keywords: bread wheat; climate-smart germplasm; adaptation; global warming; heat tolerance; yield stability

1. Introduction

Bread wheat (Triticum aestivum L., 2n = 6X = 42, BBAADD), an important cereal crop, is a staple food for 40% of the world's population [1]. It provides 20% of the total dietary calories consumed globally [2], and contributes proteins, vitamins, dietary fiber and phytochemicals to human diet and for health benefits [3]. Wheat was cultivated on 219.0 million ha land with a global production of 760.9 million tonnes during 2020 and it contributed 8% to the world's food basket [4]. The demand of wheat is expected to rise by 60% from today's level by 2050 and its production is expected to decrease by ~30% during this period due to extreme weather events [5]. Climate changes have impacted the

agriculture production and productivity globally during the past decades and seriously threatened the food supply [6,7]. Therefore, major advances in global food systems are required to ensure food security to burgeoning human population, which is expected to reach 10 billion by 2050 and for sustainable development [8]. With increasing climate instability due to global warming, agricultural productivity will continue to be negatively impacted [9,10]. Inter-Governmental Panel on Climatic Change (IPCC) 2018 report has predicted an increase in the global mean surface temperature between 3-4°C by 2100 relative to 1986-2005 if carbon emissions continue at the current rate [11]. Studies using global 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% worldwide and 8.0% in India [12].

The narrow genetic base of elite cultivars further emphasizes the need to utilize the available germplasm in genebanks to enhance resilience to abiotic and biotic stresses [13]. Efficient utilization of germplasm resources is crucial for enhancing the genetic gains to address the challenges posed by global warming [14]. Genetic diversity is paramount for genetic improvement of cultivated crops, and for bread wheat this resides in three genomes, which were constituted by polyploidization of ancestral diploid species [15]. The bread wheat was originated in Fertile Crescent after few events of initial allopolyploidization and its cultivation spread to all continents except Antarctica [16]. During the course of evolution and later on its world-wide cultivation, bread wheat has adapted to various agro-ecologies from temperate (cold) to sub-tropical (hot and dry) and tropical (hot and humid) environments. The widely adapted germplasm with tolerance to abiotic and biotic stresses is being conserved in genebanks of national and international research institutes (e.g. World Wheat Collection housed at CIMMYT, Mexico), which needs to be utilized in breeding programmes for further enhancing the genetic gains in wheat [17,18].

Late sowing of bread wheat is common in western part of Indo-Gangetic Plain (IGP) region of India, where popular rice-wheat, potato-wheat and sugarcane-wheat cropping systems are followed, which delays sowing of bread wheat exposing it to terminal-heat stress. A large area in eastern and central part of India is also under late-sown condition due to delayed harvesting of Kharif crops and in such areas the wheat crop is exposed to terminal-heat stress resulting in significant yield losses [19,20]. IGP contributes about 15% of global wheat production, however, ~51% of its area might be re-classified as a heat-stressed (HS), short season production mega- environment by 2050 [21]. The significant part of eastern IGP, peninsular and central part of India are under severe heat-stress, but it is moderate in north-western parts of IGP [22,23]. Globally, high temperatures during grain filling period (GFP) is a major yield reducing factor in many wheat growing areas [24]. High temperature-stress during GFP, referred to as terminal-heat stress, adversely affects plant growth and grain yield. Every 1°C rise in temperature above the optimum of 28°C during GFP has resulted in yield losses of 3-17% in Great Plains of USA and the eastern Gangetic Plains of India [25,26]. Hence, study on impact of heat-stress on productivity of wheat in these regions has emerged as top priority in the climate change scenario [22,27] and as a novel strategy in wheat breeding for HS environment should focus on the ideotype development [28].

Several adaptive morpho-physiological traits like early ground cover, epicuticular wax, leaf rolling, stay-green, biomass, and flag-leaf area contribute toward heat-stress tolerance ability of wheat plant [29,30]. The adaptive physiological traits such CTD, cell membrane thermo-stability, NDVI, chlorophyll content and fluorescence are associated with heat-stress tolerance and significantly contribute to the performance of the tolerant wheat lines in HS environment during GFP [31–33]. Heat-stress during crop growth and grain development stage significantly reduces morphological and yield-realated traits viz., plant height, tillers' number, peduncle length, spike length, spikelets and grains per spike, TGW and yield in wheat [34,35]. Endosperm shrinkage in the wheat grains of heat-stressed plants is a major cause of yield and TGW reduction [36,37]. Heat-stress causes damage to cellular structure and affects various metabolic pathways, mainly those related to membrane thermostability, photosynthesis and starch synthesis [38–40]. Each genotype responds to changed environment differently due to its genetic makeup and interaction with the environment [19,41]. The genotypes, which maintain high TGW and yield under HS environment, seem to possess higher tolerance to hot environments [42,43]. Exposure of wheat plants at anthesis and during GFP to higher

than the optimal temperature affects the seed development and as a result reduces productivity [44,45]. The knowledge of source-sink relationships during grain development is critical for the selection of germplasm that can produce better yield and quality under global warming [46]. Thus, understanding of sink-source relationship and plant metabolism under heat- stress in a wide range of wheat germplasm could be useful for selecting tolerant lines.

Genotype-Environment (G × E) interactions are important factors in the expression of quantitative traits such as yield and its component traits, and are major bottlenecks in selection of superior breeding lines. Stability analysis has been used to identify the varieties with superior performance and yield stability under different environments [47]. Germplasm offers the best opportunity to develop varieties which show small G × E interaction. Climate-smart varieties tolerate negative effects of climate change better and produce higher yield and better quality in unfavourable environments such as heat, cold, salinity, drought etc. Therefore, selection of climate-smart germplasm by screening a large genebank collection is a right approach to develop climate-resilience [13]. The evaluation of diverse germplasm under HS environment would be useful in understanding the complexity of plant responses to heat-stress and identification of superior lines for the development of mapping populations, which might be utilized further in development of heat-stress tolerant cultivars [48]. The morpho-physiological traits have been used to screen germplasm for heat stress tolerance in numerous studies world-wide [24,49]. Thus, aims of the study were to identify superior germplasm based on morpho-physiological traits and yield stability, and to select desirable parents for the creation of bi-parental and MAGIC (Multi-parent Advanced Generation Inter-Cross) populations.

2. Materials and Methods

2.1. Plant Materials

Plant materials for the present study consisted of a subset of 96 bread wheat accessions (79 indigenous and 17 exotic collections), selected from a large genebank collection of wheat [50], which included some of released varieties for late sown condition, trait-specific germplasm lines and genetic stocks. Seeds of each accession were obtained from the working collection of National Gene Bank (NGB) at ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi. Passport data of wheat accessions used for this study are provided in Supplementary Table S1. Geo-referencing of bread wheat accessions on the world map was done using software 'DIVA-GIS' [51]. The germplasm accessions chosen for the present study were genetically diverse and represented all wheat growing zones of India (Supplementary Figure S1).

2.2. Experimental Site, Design and Weather Conditions

Field experiments were conducted at Experimental Farm, ICAR-NBPGR, New Delhi, situated at latitude 28.649°N, longitude 77.152°E and altitude 220 m, during two successive years 2018-2019 and 2019-2020. The farm area is in north western plain zone (NWPZ) of wheat production with semi-arid and sub-tropical climate and sandy-loam alluvial soil, slightly alkaline in pH and low in organic matter content. In each crop season, two sowing dates were i) Normal (sown in 1st week of Dec.; NS environment) and ii) late sown wheat (sown in 1st week of Jan.; HS environment). Thus, late sown wheat accessions were exposed to high temperature stress during GFP. Combination of year and sowing dates made our field experiments with four environments, that included two NS and two HS environments for testing the stability of germplasm for heat-stress tolerance. The field trial was laid out in augmented block design (ABD) with five blocks, where four checks namely Raj3765, HD2932, WR544 and HD2967 were randomized and replicated in each block. Each experimental plot consisted of three rows of 2 m length with 25 cm spacing between rows (1.5 m²). The standard crop management practices for irrigated ecology followed for raising the healthy wheat crop. Weather parameters namely temperature and rainfall recorded during entire crop period from sowing to maturity for two crop seasons during 2018-19 and 2019-20. Crop duration expressed in standard meteorological weeks (SMW).

2.3. 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 [52]. Phenological parameters were recorded using Zadoks scale observing the phenotype of whole plot [53]. Eighteen traits were recorded during different growth phases of the bread wheat crop trials (Table 1).

2.4. Scanning Electron Microscopy

Mature grains from heat-stress tolerant and susceptible accessions of bread wheat were transverse sectioned into three small pieces. The middle sections were mounted on an aluminum stub using double side adhesive carbon tape. The specimens were uniformly coated with thin layer of gold-palladium using Emitech SC7620 sputter coater. Specimens were examined under Scanning Electron Microscope (Model Tescan Vega3, Tescan Analytics, France) operated at 10.0 kV using secondary electron detector. Aleurone layer, endosperm cells and starch granules were observed and their images captured.

Table 1. Morpho-physiological and yield-related traits studied in 96 bread wheat accessions under non-stressed and heat-stressed environments during two crop seasons of 2018-19 and 2019-20.

Sl.No.	Traits studied	Code	How was the trait measured?
1.	Chlorophyll Content	CC	Estimated on five flag leaves of main tillers in each accession by non-destructive method using hand-held Chlorophyll Content Meter (Model-CCM-200 plus, Opti- Science, USA) and expressed as chlorophyll content index (CCI) [52].
2.	Canopy Temp. Depression (°C)	CTD	Measured on warm, sunny and cloudless day using portable Infrared Thermometer (Fisher Scientific, England) [54].
3.	Normalized Difference Vegetation Index	NDVI	NDVI was recorded using hand-held crop sensor (Green Seeker®, Trimble, USA) covering entire plot. The value for crop canopy ranged from 0 to 1; where 0 represents no green area and 1 represent maximum greenness [55].
4.	Membrane Stability Index (%)	MSI	MSI estimated as per procedure of Sairam et al. [56]. Leaf samples (0.1 g) from each plot were taken and cut into uniform small discs. MSI was calculated using formula: MSI = $[1 - (C1/C2)] \times 100$, where C1 and C2 represent reading of EC recorded using digital conductivity meter at 45 °C and 100 °C, respectively.
5.	Plant Waxiness (0-10 scale)	PW	Plant waxiness measured by visual observations of whole plot during mid of GFP and scored using the scale from 0 (0%) to 10 (100%) in increment of 10 %.
6.	Leaf Rolling (0-10 scale)	LR	Leaf rolling measured at mid grain filling period by visual observation of whole plot and rated the proportion of the leaves showing rolling effect using a scale from 0 (0 %) to 10 (100 %) in increments of 10% [52].
7.	Days to 50 % Anthesis	DA	Recorded as the period between date of sowing and the date at which 50% of spikes start to extrude their anthers [53].
8.	Grain Filling Period (days)	GFP	GFP calculated as the difference between days to 50% anthesis and days to physiological maturity.
9.	Plant Height (cm)	PH	Plant height was measured from base of the plant to top of the spike excluding awns of the main tiller at maturity.
10.	Peduncle Length (cm)	PL	Measured from uppermost node to the spike collar of the main tiller at maturity in three plants per accession.
11.	Flag Leaf Area (cm²)	FLA	Derived on five randomly selected plant's flag leaves using following equation [57]: Leaf area = Length × Breadth × 0.75.

12.	Spike Length (cm)	SL	Measured from the spike collar to tip of spike excluding awns of the main tiller in three plants per accession.
13.	Number of Spikelets per Spike	NSS	Spikelets per spike were counted on the main tiller spike of three plants per accession.
14.	Grain Length (mm)	GL	Measured on five grains per accession by Digimatic Caliper (Model-CD-6"ASX, Mitutoyo Corporation, Japan).
15.	Grain Width (mm)	GW	Grain width was measured from randomly selected five grains per accession by Digimatic Caliper.
16.	1000-Grain Weight (g)	TGW	TGW recorded on randomly selected 1000 grains from plot yield and weighted using sensitive electronic balance (d = 0.1 mg, Sartorius, model CPA64, Germany).
17.	Harvest Index (%)	HI	HI calculated using the following formula: HI = (Grain yield per plant/ Biological yield per plant) × 100.
18.	Grain Yield (g/m²)	GY	Plot yield of individual accession harvested at maturity and threshed manually. Weight of grains recorded using electronic balance and expressed as grain yield per unit area.

2.5. Statistical Analysis

Adjusted mean values were used for the statistical analysis of ABD trial data for 18 morpho-physiological and yield related traits of both NS and 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 [58]. Pearson's correlation coefficients (r) were derived using IBM SPSS statistics software version 20.0 [59] for both NS and HS environments. Cluster analysis was performed with Ward's minimum variance using Euclidean distance matrices and dendrogram was constructed using unweighted paired group method of arithmetic averages (UPGMA) algorithm with SAS software. We performed boxplot analysis for 18 quntitative traits in tolerant and susceptible accessions. The bread wheat accessions were categorized as heat-stress tolerant or susceptible based on heat susceptibility index (HSI) values calculated on grain yield (GY) data using following formula [60].

$$\text{HSI} = (1 - X_a/X_b)/(1 - Y_a/Y_b) \,,$$

Where, X_a and X_b are mean value of GY of individual accession under HS and NS environment, respectively and Y_a and Y_b are mean value of GY of all accessions under HS and NS environment, respectively. HSI values were used to categorize bread wheat accessions as highly tolerant (< 0.50), tolerant (0.51-1.0), susceptible (1.0–1.50) and highly susceptible (>1.50). Phenotypic variance (σ^2_{ph}), genotypic variance (σ^2_{g}), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (H') in broad sense and genetic advance (GA) were calculated on mean data of four checks using analyzed using SAS software version 9.4.

$$\text{Heritability (H) \%} = (\sigma^2_{g}/\sigma^2_{ph}) \times 100 \,, \text{ and Genetic advance (GA)} = H \times k \times \sigma_{ph}$$

Where, σ_{ph} is phenotypic standard deviation and k is constant, 2.06 at 5% selection intensity. The diversity estimates based on quantitative traits were derived utilizing Shannon-Weiner index [61]. The formula for calculating Shannon-Weiner index is as follows.

$$\text{Shannon-Weiner index (H')} = -\sum_{i=1}^{n} P_i \ln P_i$$

Where n is the number of phenotypic classes for a character, P_i is the relative frequency in the ith class of the jth trait and ln is the natural logarithm of P_i. The extent of diversity was interpreted as <0.50 value of H': low diversity, 0.50 to 1.00 value of H': high and >1.00 value of H': very high diversity. The traits, which showed H' >1.00, revealed the existence of great genetic diversity among the accessions.

The stability analysis for grain yield and 1000-grain weight was carried out using Eberhart and Russell (1966) model [62]. We performed statistical analyses using Windostat software version 9.2 (IndoStat Services, Hyderabad, India). The stability model used is as follows: $Y_{ij} = \mu_i + \beta_i I_j + \delta_{ij}$

Where, Y_{ij} is accession mean of ith accession in j^{th} environment, μ_i is i^{th} accession mean over all environments, β_i is regression coefficient of ith accession to varying environments, I_j is environmental index, and δ_{ij} is deviation from regression of ith accession at j^{th} environment. According to this model, an ideal (stable) accession has higher mean than the population mean, regression coefficient ($\beta_i = 1$) and deviations from the regression ($S^2 d_i$) as small as possible. These accessions are well adapted to all environments. The accessions having higher mean value than the population mean, $\beta_i > 1$ and $S^2 d_i$ as small as possible, are specifically adapted to favorable (NS) environment. The accessions exhibiting higher mean than the population mean, $\beta_i < 1$ and $S^2 d_i$ as small as possible, are specially adapted to unfavourable (HS) environment. Bread wheat accessions classified into four categories namely highly tolerant, tolerant, susceptible and highly susceptible on the basis of mean performance, stability and HSI.

3. Results

3.1. Weather Conditions during Crop Seasons

The maximum and minimum temperatures and rainfall occurred during GFP of 2018-19 and 2019-20 crop growing seasons are presented in Figure 1.
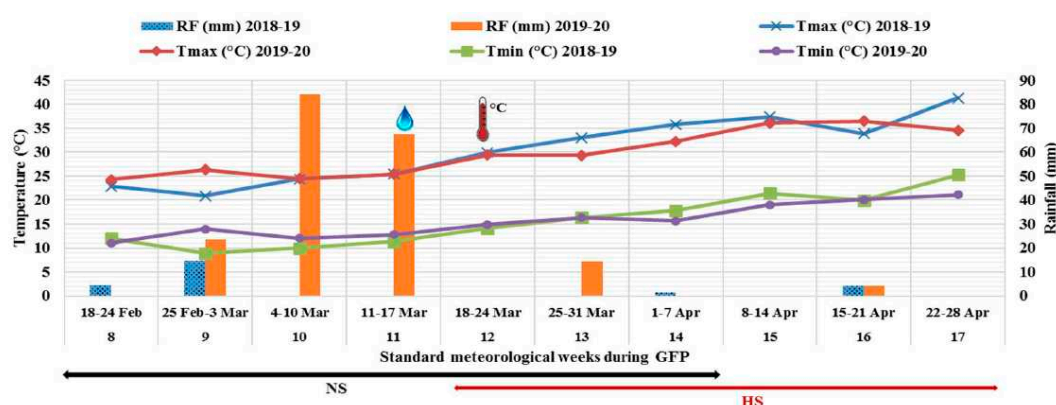

Figure 1. Temperature and rainfall variation during grain filling period (GFP; from anthesis to physiological maturity) in both non-stressed (NS) and heat-stressed (HS) environments in crop seasons of 2018-19 and 2019-20. RF-rainfall, Tmax-maximum temp., Tmin - minimum temp.

Grain filling initiated from 8 SMW (18-24 Feb.) and completed during 14 SMW (1-7 Apr.) in normal sown crop (NS environment), while the GFP was from 12 SMW (18-24 Mar.) to 17 SMW (22-28 Apr.) for the late sown crop (HS environment) of both the seasons. Light to moderate rainfall occurred during GFP under NS environment in 2018-19, but it was moderate to heavy rainfall during 2019-20. The light rainfall during GFP slightly pulled down the rising day and night temperatures under HS environment in both the crop seasons. The HS environment (sown in January) was exposed to day temperatures of 35°C in 2018-19 and 33ºC in 2019-20 during the grain filling stage. Similarly, night temperature was also higher during GFP under the HS environment than the NS environment in both the crop seasons. On average, the maximum temperature was 7.7°C higher in 2018-19 and 5.7°C in 2019-20 under HS environment. Thus, the prevailing air temperature during GFP in late sown crop was significantly higher than the temperature needed for the normal grain filling (22-25°C). Hence, the late sown crops were exposed to the heat-stress during the grain filling stage.

3.2. Crop Growth and Genetic Variability

The differences in the climatic conditions in the environments were reflected by the variations observed for the phenological and agronomic traits as presented in Table 2. Significant differences

observed for DA, PH, PL, FLA, SL, NSS and GFP between environments. Bread wheat accessions showed significant differences for all the traits except CTD, under both NS and HS environments (Supplementary Table S2). CTD showed large variation due to year effect and hence exhibited the non-significant variation for the genotype. The other physiological traits like NDVI and MSI also showed greater variation due to year than the genotype. Thus, the variation due to year was significant for almost all traits except CC, LR, NSS, HI and YPP, under both NS and HS environments. Genotype-year (G×Y) interaction showed non-significant differences in most of the traits.

Table 2. Descriptive statistics of pooled data of 18 morpho-physiological and yield traits recorded in 96 accessions of bread wheat in NS and HS environments during two crop seasons of 2018-2020.

Trait	Environment	Range		Mean ± S.E.	SD	CV (%)	PCV (%)	GCV (%)	H (%)	GA (%)
		Min.	Maxi.							
CCI	NS	17.4	38.5	26.9±0.43	4.21	15.69	18.1	15.0	68.3	25.5
	HS	13.5	37.2	22.7±0.49	4.10	18.10	18.6	16.2	75.9	29.0
CTD (°C)	NS	2.6	9.7	6.2±0.18	1.80	29.19	23.3	10.5	20.3	9.7
	HS	4.4	12.0	6.9±0.15	1.47	21.24	26.1	11.0	17.8	9.6
NDVI (0 - 1)	NS	0.54	0.72	0.62±0.01	0.04	6.03	7.5	5.1	42.9	6.6
	HS	0.32	0.64	0.48±0.01	0.06	12.61	10.9	8.5	63.8	14.3
MSI (%)	NS	50.0	77.5	66.6±0.70	6.89	10.35	10.5	6.0	33.0	7.2
	HS	43.7	72.6	59.4±0.68	6.70	11.29	8.5	2.5	8.7	1.5
PW (0 - 10)	NS	1.0	10.0	6.2±0.20	1.94	31.35	23.8	19.6	67.6	33.2
	HS	2.0	10.0	6.9±0.17	1.68	24.47	21.4	17.2	64.6	28.5
LR (0 - 10)	NS	2.5	9.5	5.8±0.13	1.28	22.04	20.8	17.5	71.4	30.5
	HS	4.0	10.0	6.5±0.13	1.26	19.29	19.1	15.0	61.4	24.1
Days to 50% anthesis	NS	83.4	119.0	91.9±0.48	4.72	5.13	6.1	5.6	84.9	10.6
	HS	66.6	94.0	74.6±0.40	3.88	5.20	6.8	6.3	85.4	11.9
GFP (days)	NS	24.0	39.5	34.2±0.25	2.45	7.16	19.9	10.7	29.0	11.9
	HS	23.5	32.0	28.1±0.20	1.94	6.91	8.7	5.7	43.0	7.7
Plant height (cm)	NS	84.9	150.9	106.6±1.31	12.83	12.03	5.9	2.5	17.8	2.2
	HS	73.2	127.0	96.9±1.25	12.23	12.63	7.1	4.4	39.1	5.7
Peduncle length (cm)	NS	29.4	60.1	39.2±0.61	6.00	15.33	14.7	12.1	67.7	20.6
	HS	27.0	53.8	35.1±0.54	5.27	15.04	14.5	12.9	79.4	23.7
Flag leaf area (cm²)	NS	21.9	77.0	37.4±0.88	8.61	23.01	20.4	16.1	62.4	26.2
	HS	14.2	51.7	23.8±0.61	5.95	24.96	17.8	14.5	66.3	24.3
Spike length (cm)	NS	8.7	15.6	11.7±0.12	1.18	10.14	6.8	3.0	19.4	2.7
	HS	8.1	13.5	10.6±0.11	1.10	10.38	4.8	1.3	7.1	0.7
Spikelets per spike	NS	16.0	24.0	20.2±0.15	1.46	7.23	5.9	2.1	12.7	1.6
	HS	15.5	22.7	18.7±0.15	1.49	7.98	5.9	2.4	16.3	2.0
Grain length (mm)	NS	5.97	8.76	6.90±0.04	0.39	5.59	5.6	4.9	77.9	9.0
	HS	5.85	8.62	6.68±0.04	0.38	5.63	6.1	5.8	89.3	11.3
Grain width (mm)	NS	2.84	4.02	3.49±0.02	0.19	5.31	3.5	2.0	33.0	2.4
	HS	2.64	3.68	3.27±0.02	0.18	5.52	3.3	1.4	17.9	1.2
1000-grain weight (g)	NS	30.0	52.2	41.5±0.53	5.19	12.51	6.0	3.0	24.1	3.0
	HS	24.9	46.6	35.5±0.48	4.73	13.34	8.0	3.3	16.7	2.8
Harvest index (%)	NS	21.6	50.6	39.7±0.49	4.84	12.19	9.4	1.7	13.3	0.6
	HS	23.4	48.0	34.4±0.45	4.38	12.76	9.3	3.2	11.9	2.3
Grain yield (g/m²)	NS	300.0	802.5	562.2±8.77	86.00	15.30	11.3	7.3	42.1	9.8
	HS	176.7	598.1	423.6±7.34	71.95	16.97	10.5	5.8	30.2	6.6

NDVI had significant G×Y interaction in both NS and HS environments, while TGW showed significant G×Y interaction in NS environment. Morphological traits, namely PH, PL, SL and GL showed significant G×Y interaction under heat-stress. Thus the morpho- morphological and yield-related traits showed a wide-range of phenotypic variability under both NS and HS environments.

Check variety WR544 (IC296383) had earliest anthesis on 83.4 and 66.6 days in normal and late-sown trials, respectively. Accessions 89 (IC393878) and 7 (IC252725) showed the highest GFP of 39.5 and 32.0 days under NS and HS environments, respectively. Accession 34 (EC577013) was the tallest (150.9 cm) under NS, while accession 84 (EC576585) was the tallest (127.0 cm) under HS environment. However, accession 38 (IC335792 produced the shortest plant (84.9 cm) under NS and accession 64 (IC443653) developed the shortest plants (73.2 cm) under heat-stress. Accession 9 (IC277741) produced the highest grain yield (802.5 g) in NS environment, while accession 91 (IC566223) produced the highest yield (598.1g) under HS environment. The lowest grain yield (300.1 g) produced in accession 26 (IC542509) under NS, whereas accession 15 (IC539287) produced the lowest yield (176.7 g) under HS environment. Accessions 58 (IC573461) produced bold grains (TGW = 52.2 g) under NS, while accession 14 (IC539221) produced bold grains (TGW=46.6 g) under HS environment. Accession 64 (IC443653) showed highest harvest index (48.0%) under HS environment.

Bread wheat accessions exhibited significant variation for most of the physiological traits. They showed high variability in physiological traits such as plant waxiness and leaf rolling (Figure 2). Accessions 43 (IC528965) and 60 (IC529207) developed the highest plant waxiness (score 10) under both the environments. Accession 22 (IC416019) showed the highest leaf rolling (score 9.5) in NS environment, whereas accessions 22 (IC416019) and 30 (IC416055) expressed the highest leaf rolling trait (score 10) under HS condition.

Figure 2. Adaptation to terminal heat-stress by highly tolerant accessions of bread wheat germplasm during grain filling period. Comparisons of accession IC529207 (highly tolerant) with highest plant waxiness (score 10) **(a)** vs. accession IC252431 (highly susceptible) showing the least plant waxiness (score 1) **(b)**, and accession IC416019 (highly tolerant) showing the highest leaf rolling (score 10) **(c)** vs. IC553599 (highly susceptible) showing the least leaf rolling (score 4) **(d).**

For chlorophyll content, accession 58 (IC573461) showed the highest value (38.5) under NS, while accession 59 (IC252444) had the highest CC (37.2) under HS condition. Accession 80 (IC542547) showed the lowest CC in both the environments. Accessions 26 (IC542509) and 91 (IC566223) showed the highest value of NDVI in both NS (0.72) and HS (0.64) environments. CTD was higher in HS than the NS environment. Accession 81 (EC190962) showed highest value of CTD (9.7°C) under NS environment, while accession 26 (IC542509) exhibited the highest CTD (12.0°C) in HS environment. The accessions 9 (IC277741) and 83 (EC573527) exhibited the highest value for MSI under NS (77.5%) environment, while accession 90 (IC542544) showed the highest value of MSI (72.6%) under HS environment. Detailed data for the morpho-physiological and yield-related traits of all accessions are shown in Supplementary Tables S3 and S4.

A wide-range of genetic variability was recorded for most of the traits (Table 2). PCV was higher than GCV for all the quantitative traits studied. The highest PCV was observed for CTD (26.1%) under HS environment followed by plant waxiness (23.8%) under NS environment. The highest GCV recorded for plant waxiness (19.6%) followed by leaf rolling (17.5 %) under NS environment. The estimates of high heritability (>70%,) were recorded for days to 50% anthesis (84.9% under NS and 85.4% under HS), grain length (77.9% under NS and 89.3% under HS) in both the environments, CC (75.9%) and PL (79.4%) under HS environment, and LR (71.4%) under NS environment. Genetic advance was the highest for PW (33.2 %) followed by LR (30.5%) under the NS environment. Shannon-

Weiner diversity index (H′) revealed a high morpho- physiological diversity among studied bread wheat accessions considering all traits under NS (H′=1.56) and HS environments (H′=1.47). The most polymorphic traits were PW (H′=1.90), CTD (H′=1.88), NSS (H′=1.79) and NDVI (H′=1.78) under NS environment, while under the HS environment, the traits displaying high diversity were NSS (H′=1.78), PW (H′= 1.77), MSI (H′=1.70) and CTD (H′ =1.69). Grain length showed the least variability under both the NS (H′=1.19) and HS (H′= 1.14) environments. Frequency distribution of wheat accessions for 12 important phenological, physiological and yield-related traits, namely days to 50% anthesis, GFP, chlorophyll content, CTD, NDVI, MSI, plant waxiness, leaf rolling, plant height, peduncle length, TGW and grain yield also supported the existence of wide-range of genetic variability in the germplasm (Supplementary Figure S2).

3.3. Genetic Relationship in Wheat Accessions

UPGMA dendrogram grouped all the 96 bread wheat accessions into 6 major clusters using the data of 18 morpho-physiological and yield-related traits in HS environment (Figure 3). Most of the accessions (80 acc.) included in first three clusters with cluster-I, -II and -III consisted of 21, 42 and 17 accessions, respectively. Remaining three clusters grouped 16 accessions with cluster-IV, -V and -VI comprised of 10, 5 and 1, accessions, respectively. Most of the accessions adapted to heat-stress viz., accession number 91 (IC566223), 60 (IC529207), 12 (IC535176), 18 (EC534487), 27 (IC252348), 13 (IC401976), 25 (IC075240), 16 (IC539531) and 5 (EC574731) grouped in cluster-III along with two national check varieties, namely 2 (HD2932) and 4 (HD2967). Accession 26 (IC542509) took the longest time to 50% anthesis (119 days in NS and 94 days in HS environment), produced the lowest yield in both the NS and HS environments and formed a solitary accession in cluster VI. The accessions 84 (EC576585), 68 (IC536162), 70 (IC252999), 85 (EC190899), 56 (IC252867), 69 (IC536050), 20 (IC416075), 55 (IC572925), 64 (IC443653) and WR544 flowered early (<69 days) in HS environment and were grouped in cluster-I.
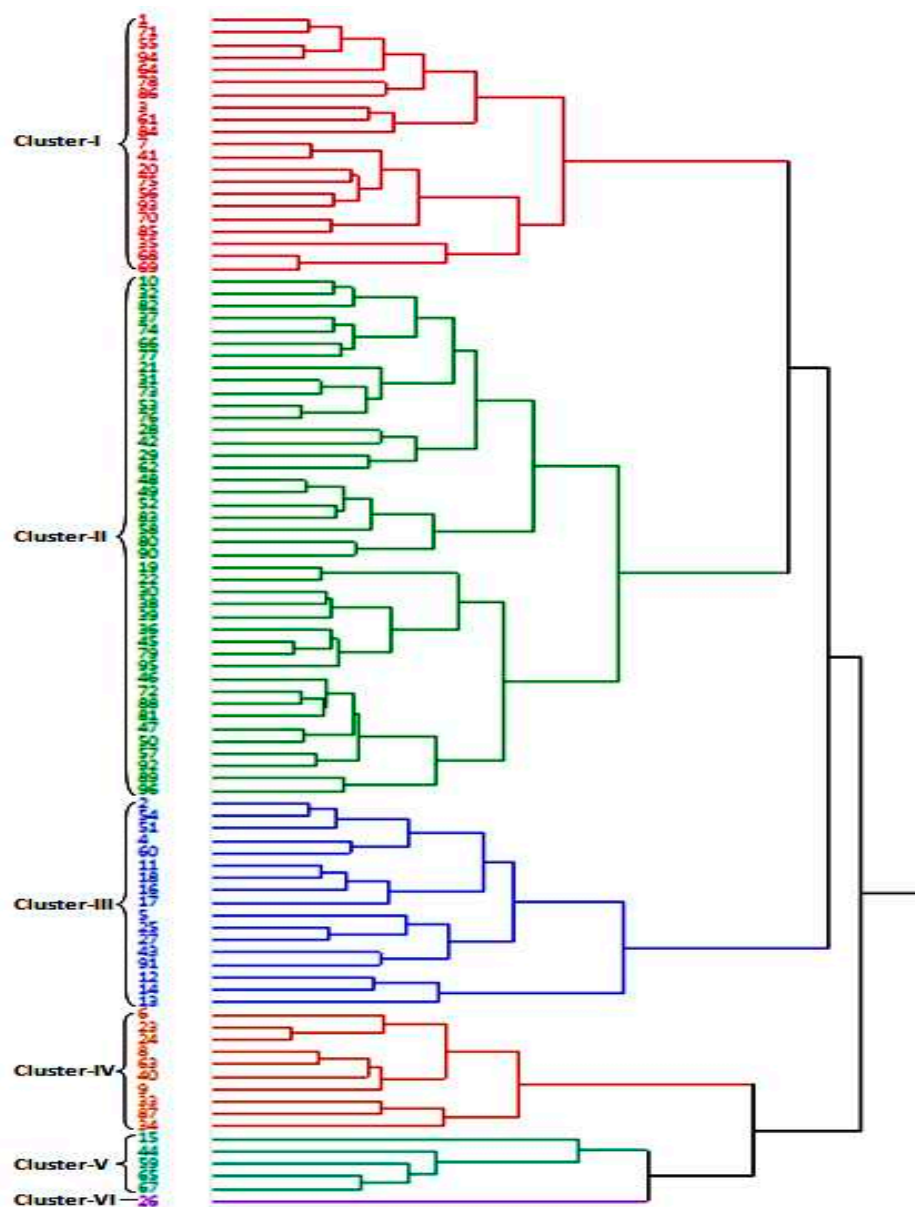
Figure 3. UPGMA dendrogram constructed with Ward's minimum variance method for 96 bread wheat accessions using data of 18 morpho-physiological and yield-related traits recorded under heat-stress environment. Six clusters are marked on the left side of the dendrogram.

3.4. Correlations with Grain Yield

Grain yield associated positively with plant waxiness, leaf rolling, GFP, GW and HI under HS environment (Figure 4a). However, under NS environment, yield associated positively with CTD, TGW, GFP, GW and HI, but negatively with NDVI and DA (Figure 4b). TGW showed positive association with GFP, GL, GW and HI under both the environments, whereas under the heat-stress, TGW also associated positively with PH, PL and FLA, but negatively with MSI, LR and DA. The strong positive correlations recorded between PH and PL, and SL and NSS under both the environments. GFP associated positively with GW, TGW and HI, but negatively with DA under both the environments. Similarly, DA also associated negatively with HI, TGW and GW under both environments.
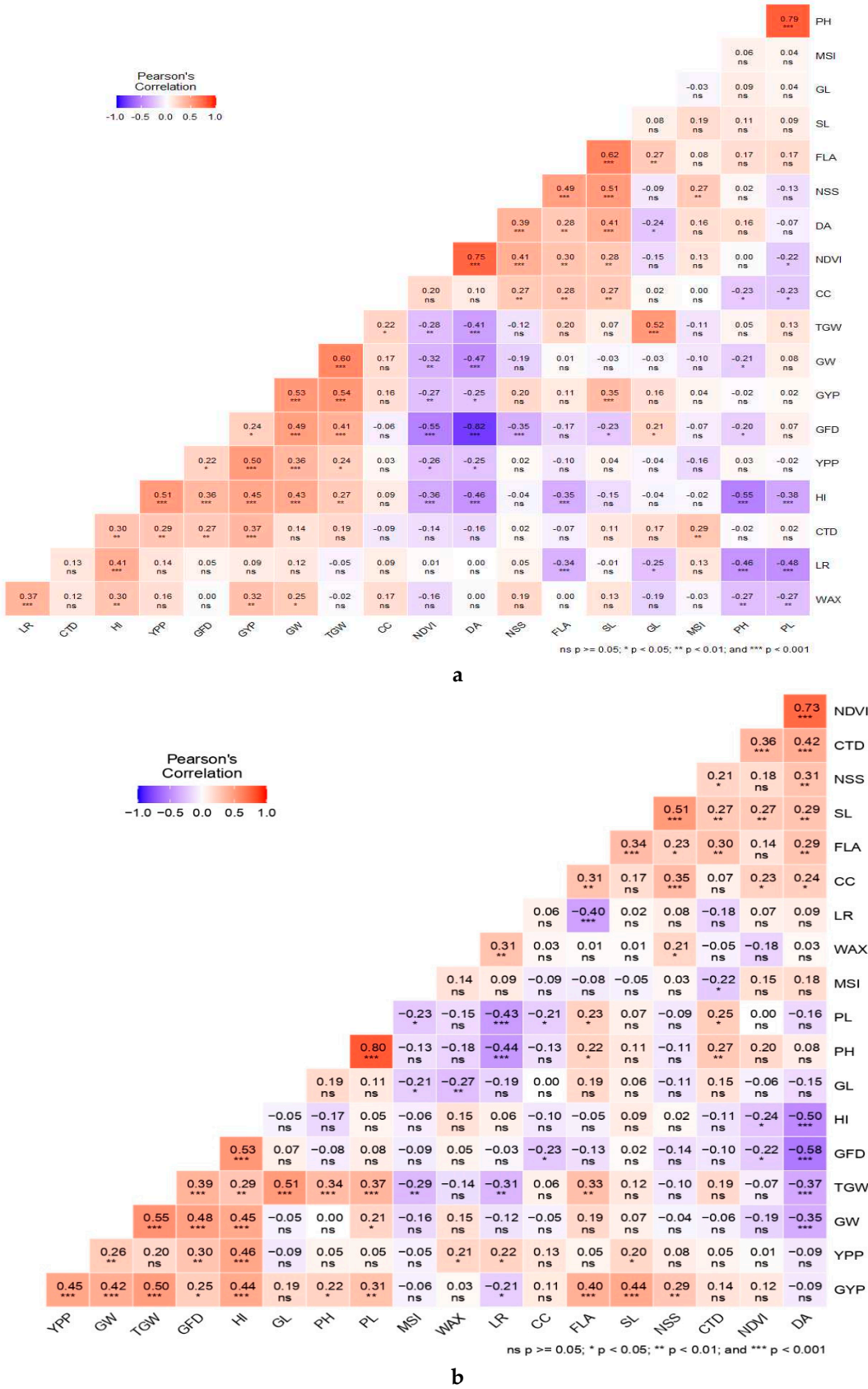
a

b

Figure 4. Pearson's correlations coefficients derived between 18 morpho-physiological and yield traits under non-stressed (**a**) and heat-stressed (**b**) environments in 96 bread wheat accessions.

Among physiological traits, positive association was observed between plant waxiness and leaf rolling under both the environments. NDVI associated positively with both chlorophyll content and

CTD under heat-stress. The cooler canopy (CTD) showed positive correlation with DA, PH, PL, FLA, SL and NSS under the HS environment. However, CTD associated positively with MSI under NS environment and negatively under the HS environment.

3.5. Impact of Heat-stress on Yield and Morpho-Physiological Traits

Late showing of bread wheat reduced the optimal period for crop growth and grain development. The unfavorable weather conditions under HS environment had an adverse effect on yield and its component traits, which showed reduced expression (Table 2). However, physiological adaptive traits namely CTD, plant waxiness and leaf rolling showed increased expression in HS environment. Phenological trait like days to 50% anthesis was reduced by 17 days under HS environment. Plant height, peduncle length and GFP traits were decreased in HS environment by 9.7 cm, 4.1 cm and 6 days, respectively. Likewise, heat-stress reduced flag leaf area by 13.6 cm².

High-temperature stress prevailed at the onset of reproductive phase and during grain development stage decreased spike length, number of spikelets per spike, and grain length and width. Furthermore, the heat-stress condition during GFP had adverse effect on grain yield and TGW, and consequently reduced these traits by 138.6 g and 6.0 g, respectively, under HS environment. The harvest index was decreased from 39.7 to 34.4 under HS environment. Among the physiological traits, chlorophyll content, NDVI and MSI were reduced by 4.2, 0.14 and 7.2%, respectively, under the HS environment. However, CTD was slightly increased from 6.2 to 6.9 °C under HS environment to maintain optimal sub-cellular temperature during GFP. Plant waxiness and leaf rolling were also increased from 6.2 to 6.9 and 5.8 to 6.5, respectively, under HS environment.

Boxplot analysis clearly showed differential effect of heat-stress on the morpho- physiological traits in tolerant and susceptible accessions (Figure 5). All the traits were reduced under the HS environment in both tolerant and susceptible accessions except CTD, PW and LR. However, mean values of CTD, PW and LR were higher under the HS environment in tolerant accessions. The heat-stress tolerant accessions showed higher mean values for all traits as compared to heat susceptible ones under HS environment except MSI. The higher MSI was observed in the susceptible accessions because of more leakage of cell contents at 100 °C and showed higher electrical conductivity as compared to the tolerant bread wheat accessions.

The effect of heat-stress on important morpho-physiological and yield- related traits was also investigated in five classes of accessions, namely highly tolerant, tolerant, susceptible and highly susceptible accessions along with check varieties (Figure 6). Under heat-stress, reduction of chlorophyll and NDVI was more evident in susceptible accessions as compared to the tolerant. However, under the HS environment, MSI showed relatively higher values in susceptible accessions than the tolerant accessions. CTD, PW and LR increased in the tolerant accessions as compared to the susceptible ones under HS environment. DA, PH, PL, FLA, GFP, SL, TGW, HI and GY all decreased in both tolerant and susceptible accessions under the heat-stress. The tolerant accessions produced higher yield and bold grains than the susceptible under the heat-stress mainly due to reduced number of grains per spike and faster grain developmentin under the HS environment.
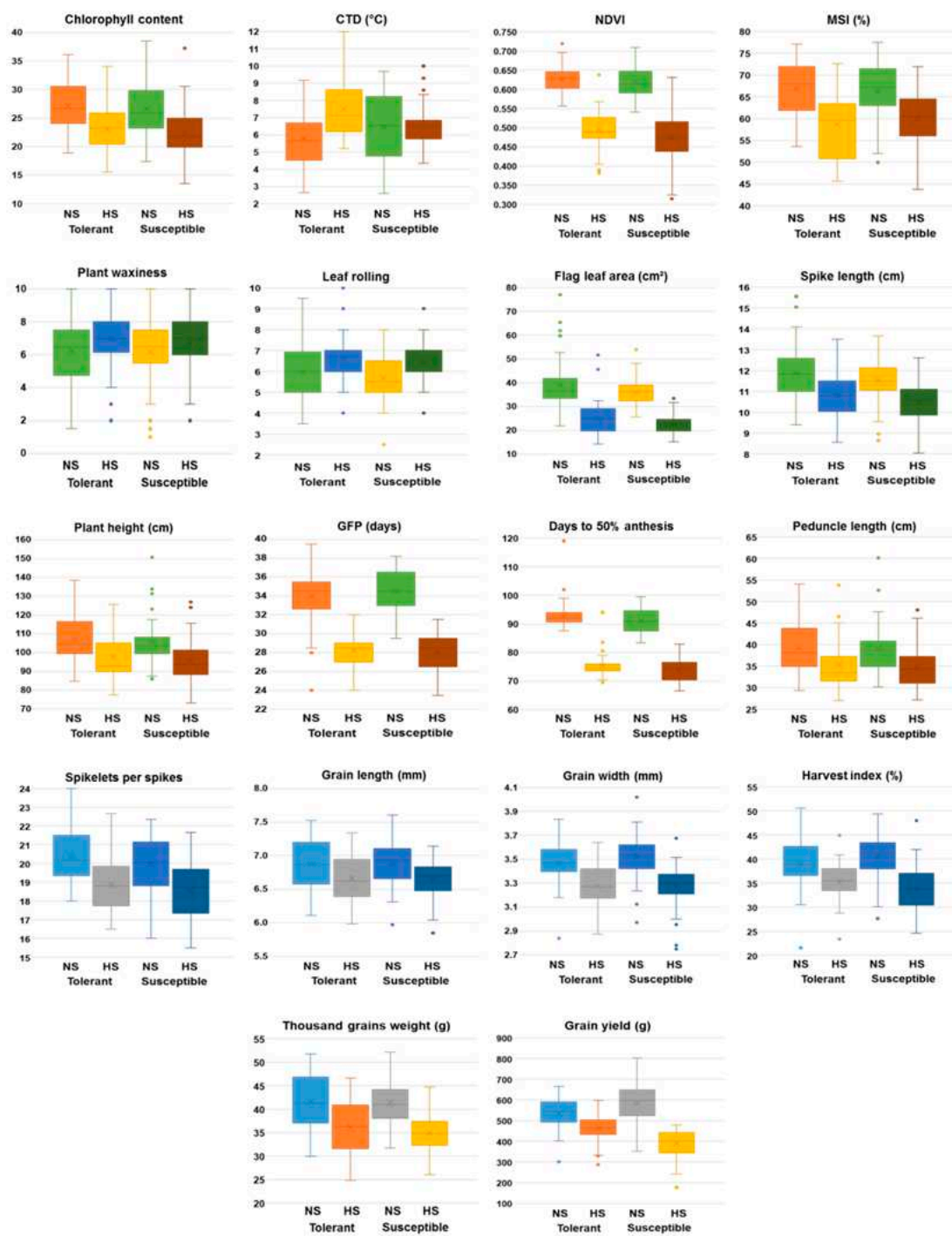
Figure 5. Boxplot distribution of variability assessed for 18 morpho-physiological and yield contributing traits under non-stressed (NS) and heat-stressed (HS) environments in 96 bread wheat accessions categorized based on HSI values as tolerant (HSI <1.0).
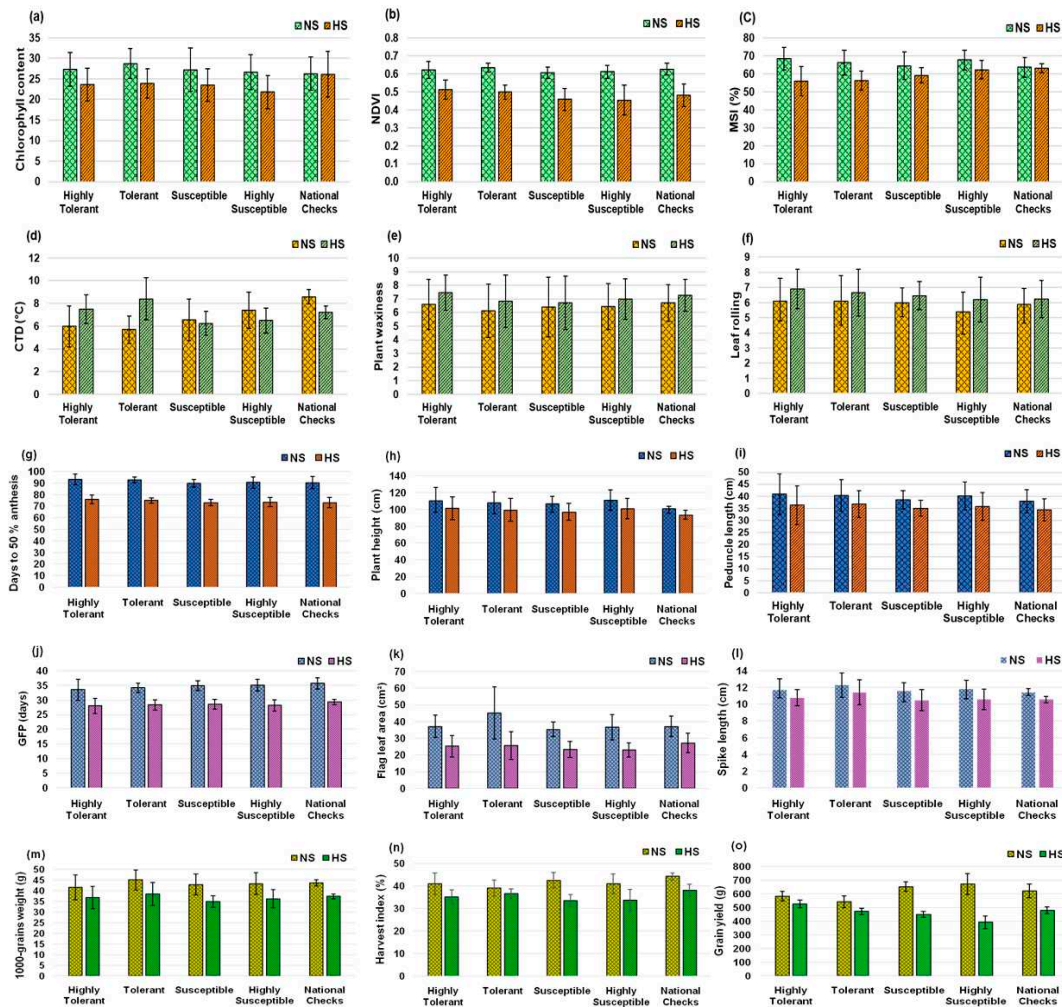
Figure 6. Effects of heat-stress on important morpho-physiological and yield-traits on highly tolerant, tolerant, susceptible and highly susceptible accessions with national checks. Reduction of CC and NDVI was more evident in highly susceptible and susceptible accessions compared to highly tolerant and tolerant accessions under HS (**a-b**). MSI was more in highly susceptible and susceptible accessions than the tolerant accessions in HS environment (**c**). CTD, plant waxiness and leaf rolling increased in the tolerant accessions as compared to the susceptible ones under HS environment (**d-f**). Under heat-stress, days to 50% anthesis, plant height and peduncle length were reduced in both the tolerant and susceptible accessions (**g-i**). GFP, flag-leaf area and spike length all decreased in both tolerant and susceptible accessions under heat-stress (**j-l**). TGW, HI and grain yield were higher in the tolerant accessions than the susceptible ones in HS environment (**m-o**).

3.6. Impact of Heat-stress on Wheat Grains

Heat-stress reduced grain width, length and TGW in all the accessions. However, the effect of the heat-stress during GFP on wheat grain traits (GL, GW and TKW) was more evident in susceptible than the tolerant accessions (Figures 5 and 7). The reduction in GW was significantly high in the susceptible than the tolerant accessions in heat-stress (Figure 7a). Scanning electron microscopy of aleurone layer and endosperm was carried out in mature grains of heat tolerant and susceptible accessions of bread wheat grown under NS and HS environments (Figure 7b). Heat-stress during GFP adversely affected aleurone layer and endosperm of wheat grains.
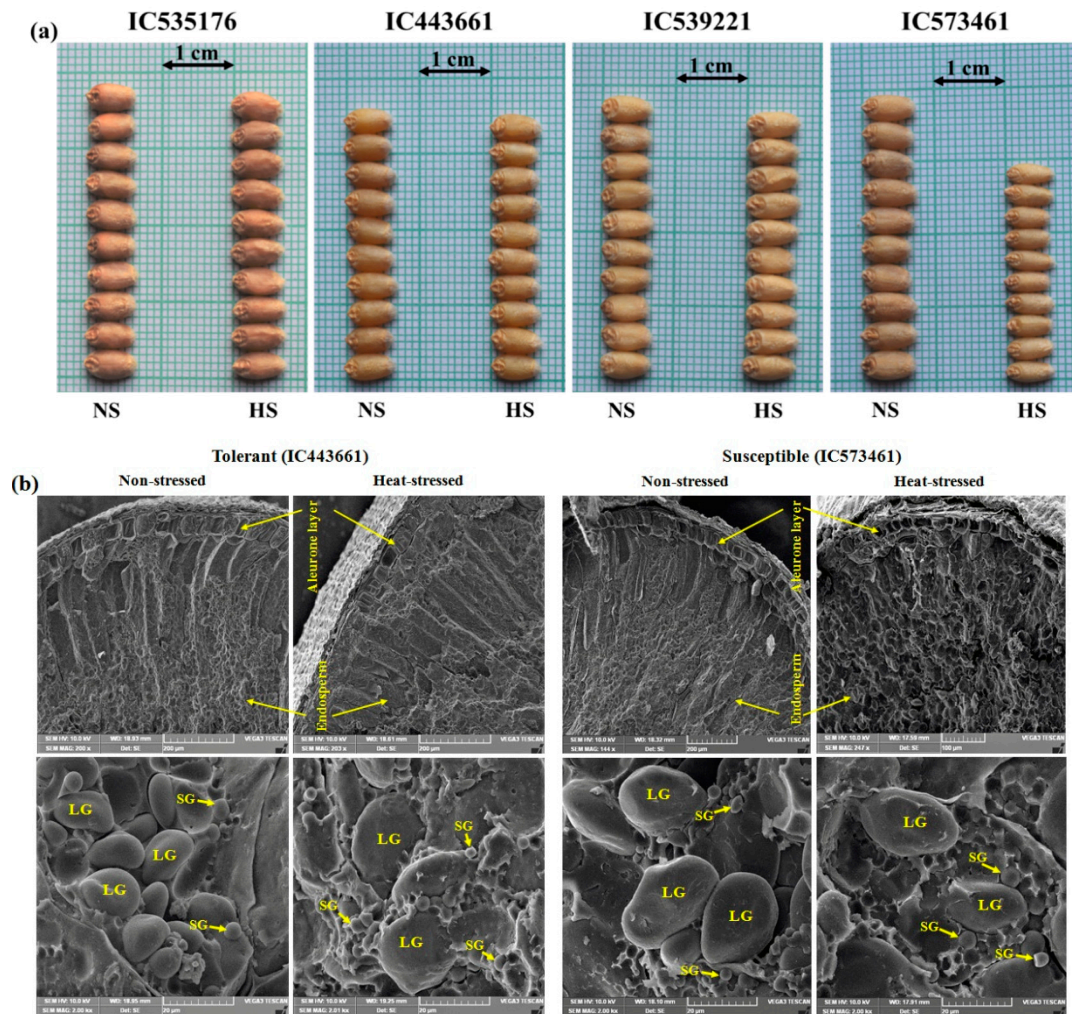
Figure 7. Impact of heat-stress on grain morphology and ultrastructure as visualized by scanning electron microscopy (SEM) in tolerant and susceptible accessions of bread wheat. Reduction of grain width due to heat-stress was more evident in susceptible accession IC573461 as compared to three tolerant accessions IC535176, IC443661 and IC539221(**a**). Ultrastructural changes caused by heat-stress in endosperm and aleurone layer of grains in very late sown bread wheat accessions (**b**). SEM revealed ultrastructure of matured wheat grains showing aleurone layer and endosperm (**b,** upper panel; low magnification) and packing of starch granules (structure and density as seen in close-up view) in the endosperm (**b,** Lower panel; high magnification**)** in tolerant (IC443661) and susceptible (IC573461) accessions in non-stressed (NS) and heat-stressed (HS) environments**.**

The concentration and morphology of starch granules changed under heat stress. The size, shape and structure of aleurone layer and starch granules of heat-stress tolerant grains were quite different from the heat-stress susceptible genotypes. The aleurone layer was de-structured in susceptible accessions as compared to tolerant ones (Figure 7b, upper panel). Heat-stress showed adverse effects on structure and packing of starch granules. Robust, bold and well-structured large starch granules (LG) were observed in both heat-stress tolerant and susceptible accessions in NS environment, whereas un-structured, shrivelled LG with prominence of small starch granules (SG) were present in the wheat grains developed under HS environment. Density of LG (A-type; 15-35 μm) was higher in both heat-stress tolerant and susceptible accessions in NS environment, whereas, in HS environment the density of LG slightly reduced in tolerant accessions while it was considerably reduced in the susceptible accessions. The density of SG (B-type; 2-8 μm) was higher in both tolerant and susceptible accessions in the HS environment as compared to NS environment.

3.7. Selection of Heat-stress Adapted Germplasm

Grain yield and TGW were used for stability analysis using four environment datasets. ANOVA showed significant differences (p <0.01) among accessions (= Genotype, G) and environments (E) for both the traits (Table 3). The yield exhibited significant interactions for G × E, G × E (Linear), E + (G × E) and E (Linear). However, TGW revealed significant interactions for E + (G × E) and E (Linear), but non- significant for G × E and G × E (Linear). On the basis of mean performance, regression coefficient (βi) and HSI, wheat accessions were classified as adapted to unfavorable (HS) environment, favorable (NS) environment or both environments (Table 4). Accession number 91 (IC566223), 60 (IC529207), 38 (IC335792), 12 (IC535176), 6 (EC576707), 29 (IC128454), 22 (IC416019), 23 (IC446713), 41 (IC265318), 27 (IC252348), 13 (IC401976), 25 (IC075240) and 16 (IC539531) performed well under HS environment and were identified as highly tolerant genotypes. Accessions 65 (IC252431), 9 (IC277741), 85 (EC190899), 96 (CUO/79/Pru 11A), 95 (EC277134), 57 (IC524299), 94 (IC553599), 84 (EC576585), 58 (IC573461), 82 (EC576066) performed well in NS environment but poor in HS environment, were referred as highly susceptible ones. Accessions 89 (IC393878), 19 (IC416018), 14 (IC539221) and 17 (IC443661) performed well under both NS and the HS environments were called as general adapters.

Table 3. ANOVA for TGW and grain yield over four environments for stability analysis using Eberhart and Russell model.

Sources of variation	D.F.	TGW (MSS)	Grain Yield (MSS)
Genotypes	95	89.54**	19206.70**
Environment + (G × E)	288	28.00**	9164.46**
Environment	3	1589.07**	614125.02**
Environment G × E	285	11.57	2796.45**
Environment (Linear)	1	4767.22**	1842375.06**
G × E (Linear)	95	9.53	18197.54**
Pooled deviation	192	12.46	148.88
Total	383	43.27	11655.34

** significant at 0.01 level of probability; **TGW**: 1000-grain weight, **MSS**: Mean sum of squares.

Likewise, stability for TGW revealed that some accessions were well adapted to HS, NS or both environments (Table 4, Figure 8). The detail of mean performance and stability parameters of 96 bread wheat accessions for TGW and grain yield are presented in Supplementary Table S5. The accessions showing higher mean than the population mean, regression coefficient (β_i <1) and HSI < 0.5 and producing more than 500 g grain yield were considered as highly tolerant genotypes (Supplementary Table S6). The top 10 accessions were screened out for various morphological, physiological, yield and its contributing traits under both the NS and HS environments (Supplementary Table S7).

Table 4. Grouping of different accessions based on Eberhart and Russell stability model, their mean performance under NS and HS environments along with heat susceptibility index. **.**

Adap-tation	Accession	TGW			Grain Yield			TGW		Grain Yield		HSI
		μ	β_i	S^2D_i	μ	β_i	S^2D_i	NS	HS	NS	HS	
Un-favourable (heat-stressed) environment	IC543425	37.4	1.32	0.09	487.2	- 0.56	319.85	39.7	31.9	508.0	498.0	0.08
	IC128454	33.6	1.05	1.91	543.6	- 0.22	376.52	34.9	29.1	546.0	538.1	0.05
	IC265318	39.1	0.71	11.30	500.7	- 0.15	363.40	38.5	36.4	508.0	504.1	0.03
	IC252348	46.0	1.38	16.61	480.1	- 0.15	529.35	46.9	41.9	488.0	476.7	0.09
	IC566223	36.3	0.76	23.13	589.5	0.04	119.09	40.4	34.7	604.3	598.1	0.04
	IC335792	33.6	1.02	7.37	559.7	0.20	446.45	34.0	29.9	593.4	539.4	0.37
	IC290191	37.3	1.12	1.93	500.5	0.26	9.76	39.7	34.5	522.2	482.7	0.31
	IC401976	48.0	0.73	4.67	493.3	0.28	21.89	49.7	45.2	510.5	478.1	0.26

Category	Genotype										
	IC446713	44.7	0.62	1.02	522.7	0.32	11.41	46.6	41.7	545.2	505.4	0.30
	EC576707	33.7	0.15	2.20	552.1	0.36	37.58	33.8	32.4	579.2	534.7	0.37
	IC075240	42.9	0.88	6.98	474.5	0.33	63.50	44.7	39.9	501.9	460.1	0.34
	IC416019	43.6	1.71	1.29	510.0	0.41	55.05	49.1	36.8	542.5	490.1	0.40
	EC574731	42.0	0.99	0.22	520.7	0.43	62.51	45.2	37.6	549.2	500.7	0.36
	IC535176	46.7	0.19	0.94	556.0	0.47	61.91	47.1	45.0	593.9	532.1	0.43
	IC539531	46.1	0.75	2.11	478.3	0.50	49.48	48.5	42.6	517.2	452.1	0.52
	IC529207	37.7	1.06	26.18	563.5	0.55	5.10	39.3	35.7	604.9	527.4	0.52
Both environments	IC416018	43.0	0.89	13.29	553.8	0.78	36.99	47.1	37.6	605.9	508.1	0.66
	IC443661	40.8	- 0.06	1.18	463.9	0.85	24.38	41.1	39.2	523.2	413.4	0.86
	EC534487	43.2	1.24	2.62	551.6	0.92	80.85	47.7	37.6	609.9	496.1	0.76
	IC539221	49.8	0.86	3.33	529.0	0.94	12.34	51.8	46.6	593.2	468.7	0.86
	IC393878	45.3	1.21	3.85	572.2	0.96	109.62	49.3	43.9	633.7	516.7	0.75
Favourable (non-stressed) environment	IC573461	41.8	2.86	46.15	558.5	1.39	34.86	52.2	31.1	654.9	462.7	1.20
	IC535717	39.4	0.69	17.82	553.6	1.45	104.97	43.4	38.0	649.0	464.1	1.17
	IC144911	33.3	0.81	0.05	537.0	1.56	79.27	35.0	31.1	648.9	425.4	1.41
	EC576175	40.0	1.32	3.41	557.9	1.62	176.60	44.4	38.1	661.7	456.7	1.27
	EC277134	40.4	1.32	71.39	570.8	1.69	154.00	48.0	35.3	680.4	464.7	1.30
	IC252619	35.0	1.39	1.74	545.9	1.70	112.16	40.2	32.2	659.5	441.7	1.35
	IC529242	34.3	1.56	2.87	482.2	1.82	169.29	40.5	30.7	600.9	369.1	1.58
	IC443694	33.6	0.89	5.34	505.4	1.93	240.81	37.7	31.9	630.9	387.7	1.58
	IC553599	39.6	0.66	2.56	550.5	1.98	57.60	42.4	39.3	685.7	424.7	1.56
	IC524299	42.5	1.56	117.81	565.8	2.02	114.97	50.5	34.1	709.5	422.7	1.65
	IC252431	34.1	0.76	1.61	607.9	2.05	269.05	37.7	33.0	739.5	478.4	1.44
	EC190899	35.8	1.81	3.98	588.3	2.18	155.19	41.1	33.1	732.4	448.7	1.58
	EC576585	45.4	0.75	7.26	492.9	2.19	178.97	48.5	44.7	637.5	353.7	1.82
	CUO/79/Pru 11A	45.1	1.10	75.79	580.5	2.38	102.43	52.1	40.7	741.0	423.4	1.75
	IC277741	37.5	0.68	42.16	597.6	2.74	76.59	38.0	35.8	802.5	416.1	1.97
National check	RAJ3765	40.8	0.80	4.03	514.2	0.61	35.67	43.0	38.7	552.5	472.0	0.60
	HD2932	39.2	1.02	7.19	538.1	1.14	49.90	41.7	36.6	614.7	458.8	1.04
	WR544	41.1	0.99	3.10	562.8	1.31	25.24	44.6	37.7	655.1	472.1	1.14
	HD2967	40.5	1.25	3.38	592.7	1.03	46.12	44.9	36.2	665.2	518.7	0.90
Population Mean		38.6	-	-	489.4	-	-	41.5	41.5	562.2	423.6	1.00
LSD (5%)		-	-	-	-	-	-	2.1	2.6	49.0	21.1	-

Abbreviations: TGW: 1000-grain weight, **HSI**: Heat susceptibility index, **NS**: Non-stressed environment, **HS**: Heat-stressed environment, μ: Mean, β_i: Regression coefficient, **S²d$_i$**: Deviations from the regression.
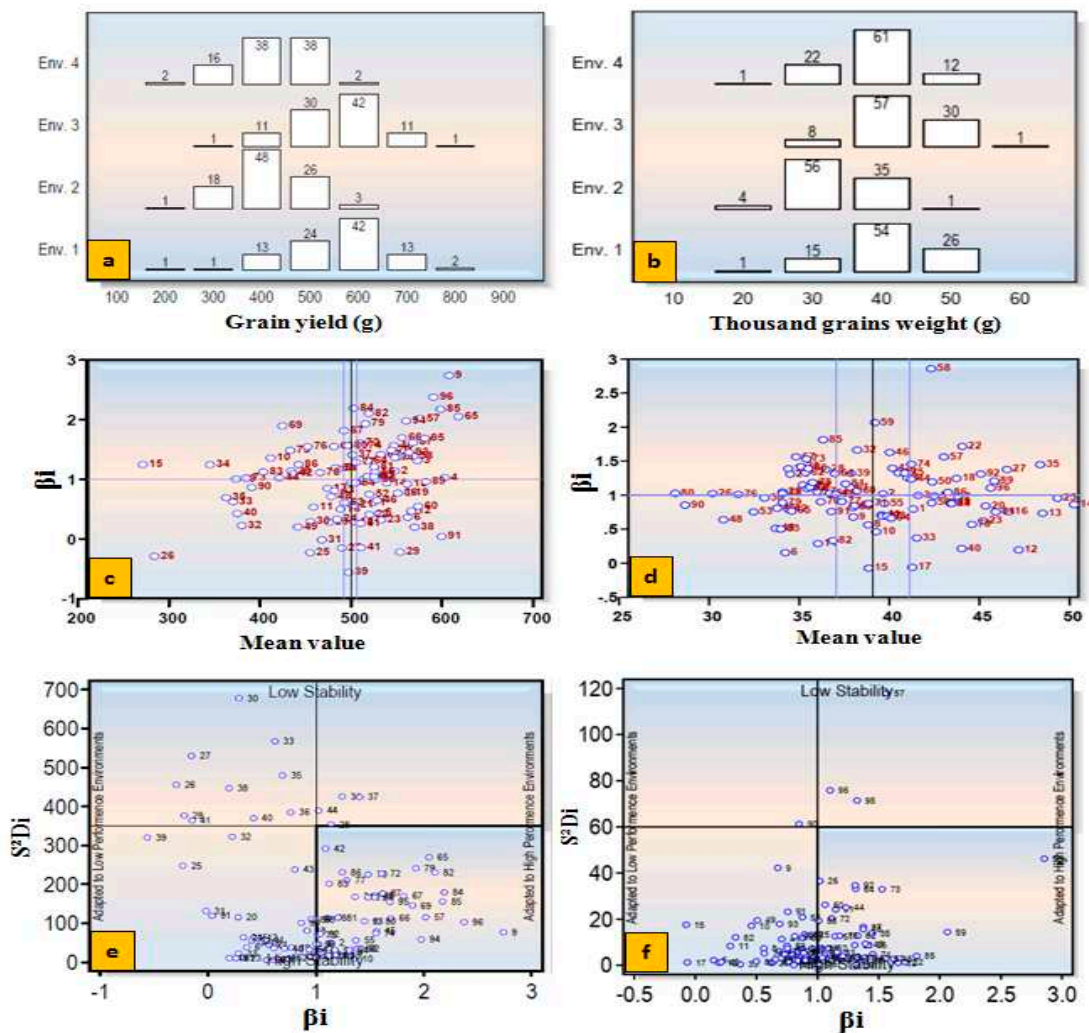
Figure 8. Stability parameters based on Eberhart and Russell model. Frequency distribution of accessions for grain yield per plot and thousand grain weight **(a-b).** Mean performance of accessions and their regression value (β_i) for grain yield and thousand grain weight **(c-d).** Stability of accessions based on deviation from linearity (S^2D_i) for grain yield and TGW **(e-f).**

We selected germplasm lines based on their performance under HS environment as potential parents for the development of bi-parent and MAGIC populations (Table 5). The germplasm accessions, which showed extreme phenotype under HS environment, were chosen as the potential parents for the creation of trait-specific bi-parental mapping populations. However, for the selection of promising parents for 4-parental and 8-parental MAGIC populations, accessions showing the highest expression of yield and its associated morpho-physiological traits were considered.

Table 5. Identification and selection of parents for the development of mapping populations for heat-stress tolerance in bread wheat.

Sl. No.	Traits for mapping population	Parents with desirable traits for heat-stress tolerance	
a).	**Bi-parental population**	**Parent (Higher value)**	**Parent (Lower value)**
1.	Plant waxiness	IC529207, IC528965	IC252431, IC252444
2.	Leaf rolling	IC416019, IC416055	IC553599, IC252816
3.	Earliness	IC296383	IC542509
4.	Grain filling period	IC252725	EC577013

5.	Grain width	IC401976	IC112258
6.	1000-grain weight	IC539221	IC542544
7.	Harvest index	IC443653	IC542509
8.	Grain yield	IC566223	EC577013
b)	**MAGIC Population**	**Parents with desirable traits**	
1.	4-parent MAGIC	IC566223, IC529207, IC416019, IC296383	
2.	8-parent MAGIC	IC128454, IC519900, IC528965, IC416055,	
		IC539221, IC401976, IC535176, IC566223	

4. Discussion

The production and productivity of bread wheat is adversely affected due to terminal-heat stress as a result of delayed sowing in many parts of India [23]. In the present study, we have evaluated a diverse set of 96 accessions of bread wheat to analyse plant responses in terms of effect of heat-stress on morpho-physiological and yield-related traits. Proper endosperm development under heat-stress is the key to maintain TGW, yield level, grain quality in wheat, and to facilitate the selection of superior and stable genotypes under HS environment [42,63]. The heat-stress imposed by sowing the wheat trial in a very late condition during first week of January in both years, created a unique environment to test germplasm for heat-stress tolerance. The delayed sown wheat is exposed to higher temperatures at reproductive and grain filling stages, and is a widely used strategy to screen germplasm for yield and other traits under heat-stress [28,64]. Yield, a complex quantitative trait, is the 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 [65].

4.1. Trait Variability and Impact of Heat-stress

Bread wheat accessions showed varied response to heat-stress and provided ample scope for further selection of trait-specific accessions for terminal heat-tolerance. The high genotypic variability obtained in present study is due to selection of diverse germplasm accessions for different traits related to heat-stress tolerance. Non-significant differences observed for genotype × year (G × Y) interaction for most of the traits reveal that accessions had similar expressions in both the years for these traits. Significant G × Y interactions for NDVI in both environments, TGW under NS, and plant height, peduncle length, spike length and grain length under HS environment indicate that accessions performed differently over the years for these traits. Our results corroborate with earlier studies [41,66], which also reported varied responses of different traits in different genotypes and their significant interactions with environment under the heat-stress. However, minor differences in the range of quantitative traits are expected in these studies due to differences in the genetic make-up accessions used and test environments. The heat-adapted genotypes with best yielding ability also possessed high early biomass, high grain filling rates and low canopy temperatures [67].

The response to heat stress involves physiological adaptations that are required to protect the cellular functions including photosynthesis, assimilate-partitioning and accumulation of secondary metabolites [65,68]. Heat-stress showed an adverse effect on crop growth and development, and consequently it negatively affected morphological and yield-related traits such as days to 50% anthesis, plant height, peduncle length, flag-leaf area, GFP, spike length, spikelets per spike, grain length and width, TGW, harvest index and yield. The physiological traits, namely chlorophyll content, NDVI and MSI were reduced, whereas CTD, PW and LR showed enhanced expressions under the HS environment. The reduction in the expression of traits was more prominent in susceptible accessions as compared to the tolerant ones. Under heat-stress, reduced expression of morphological and yield-related traits was also reported in several studies conducted across the globe [28, 34, 42, 43). CTD found to be a reliable and non-invasive method for selecting heat-stress tolerant

lines and it showed the best results for evaluation of wheat genotypes under terminal heat stress condition [69].

Significantly reductions in the days to heading, GFP, TGW, harvest index and yield in late sown wheat were observed earlier [42] and well-supported our results in these traits. Similarly, reduction in days to heading, GFP, yield, plant height, harvest index and TGW under heat-stress was also reported in a previous study [34]. The extent of reduction of the traits varied in these studies due to use of different set of genotypes and test conditions. The yield and its component traits are more severely affected with the increase of heat-stress [43]. High temperature during post-anthesis stage reduced grain filling duration, yield and TGW [70]. The yield parameters were also reduced in plants exposed to high temperatures in wheat landraces [71]. Recently in India, the reduction of days to anthesis, MSI and yield traits was reported in late sown wheat compared to normal [72]. They also observed that HSI was the lowest in the heat tolerant germplasm lines and supports our observations on HSI. The heat-stress reduced plant height, days to anthesis and maturity, grain weight and yield between 4-7% with every 1°C rise in mean maximum temperature above the optimum of 25°C [28]. The higher leaf waxiness under HS condition reported earlier [30] supports our results of increased waxiness under heat-stress, which protects plant against excess radiation and water loss through reflection of visible and infrared wavelengths [73]. Heritability of waxiness was low because of significant environment interactions as also reported in previous study [74]. Genotypes with stay green traits performed better under heat stress and donor genotypes were identified for utilization in heat-stress tolerance breeding [75].

4.2. Association of Grain Yield with Other Traits

Yield, being a complex quantitative character, is determined by interaction between morphological, physiological and other related parameters. Under HS environment, yield showed positive correlations with GFP, HI, GW, PW and LR. The positive correlation of yield with GFP reveals that the longer grain development period is an important contributing factor for improving yield under HS environment. In a previous study [42], grain yield and HI showed positive correlation with TGW under both normal and terminal-heat stress conditions, whereas GFP positively associated with TGW only under heat-stress. This study advocates that the selection for low TGW reduction as an indirect criterion to identify high yielding lines under terminal heat-stress. In the present study, TGW was associated with yield only under the NS environment. However, another study [76] reported the association of TGW with yield under both optimal and HS environments. The positive association of yield with GFP and TGW was also reported under the heat-stress [77]. There was non-significant association of grain yield with days to 50% anthesis, plant height and TGW in present study under HS environment, which confirms the results of an earlier study [28]. Hence, to increase yield in wheat under HS environment, the focus should be given on traits which have high and significant association with yield for the selection of heat tolerant lines.

In our study, there was non-significant correlation between chlorophyll content and grain yield under both NS and HS environments, whereas, CTD was significant associated with grain yield only under NS environment. Similarly, Elbasyoni [41] also observed no correlation between chlorophyll content and grain yield. However, a high correlation of both CTD and chlorophyll content with yield under heat-stress environment was reported earlier [31,78]. The high genetic variation for chlorophyll retention and seed weight along with positive association between chlorophyll content and seed weight was found under heat stress condition in diverse elite winter wheat lines [79]. Similarly, association of canopy temperature with grain yield was also observed under both NS and HS environments [26]. Under heat-stress, the grain yield showed a strong positive correlation with both CTD and days to heading [80–82]. It was suggested that the longer time before heading enables the development of larger spikes and more numbers of spikelets producing enhanced grain numbers per plant [82]. The present and earlier association studies revealed that grain yield in bread wheat correlates positively with its contributing and physiological traits under HS environment. Therefore, selection of these traits could be valuable in wheat breeding programme designed for heat-stress tolerance.

4.3. Grain Development under Heat Stress

Heat stress during GFP adversely affects the grain size in bread wheat. In our study, the effect of heat-stress on grain width was more severe as compared to length, which resulted into shrivelled grains in susceptible accessions. The development of shrivelled grains in the susceptible accessions was due to changes in ultrastructure of aleurone cells and starch granules in endosperm. Our findings on grain development under heat-stress corroborate with a recent study [83], wherein they observed severe effect of heat stress on grain characters like grain length, width and area along with starch synthesis. The effect of heat-stress shows a significantly reduced grain width and perimeter. The endosperm of mature wheat grain contains two types of starch granules: large (10–35 μm) A-type and small (1–10 μm) B-type [84,85]. The density and size of large type of starch granules were slightly reduced in heat-stress tolerant accessions while it was considerably reduced in heat susceptible ones under HS environment. The concentration of small type of starch granules was higher in both heat tolerant and susceptible accession under HS environment as compared to NS environment. Our findings on the size and density of starch granules are well supported by previous study [86], where it reported differences in size, shape and structure of starch granules in wheat grain after heat-shock. They also observed that the ratio of large and small type of starch granules decreases significantly under heat-stress and this limits the potential sink size for dry matter deposition in grain. Dias et al. [37] also reported that the heat-stress during GFP triggers ultrastructural changes in the aleurone layer and endosperm cells and causes disordered cells, grain shrinkage and reduced weight in heat-stress susceptible genotypes.

Grain filling is influenced by various metabolic processes occurring in leaves, mainly the production and translocation of photoassimilates and importing of precursors for biosynthesis of grain reserves, minerals and other functional constituents [87]. It is, therefore, important to know the physiological, biochemical and genetic mechanisms, which govern the grain filling events under heat-stress to devise strategies for yield enhancement in wheat. Identification and selection of germplasm lines with higher yield and grain weight along with early maturity, semi-tall plant height, higher NDVI during grain filling and higher content of chlorophyll at milky stage should be the hallmark of breeding strategies for heat-stress tolerance [28,76]. Harnessing the genetic variability for these traits in the germplasm of bread wheat is vital for breeding of heat-stress tolerant cultivars [14].

4. Yield Stability and Selection of Accessions Adapted to Heat-stress

Heat-stress adaptation is a complex phenomenon and is influenced by several factors such as genotype and its interaction with environment over a period of time from decades to centuries. Wheat plant achieves adaptation through variation in phenology and related traits determining plant architecture [49]. Hence, it is crucial to understand genes that underpin the variations in plant phenology and their interactions with other genes, morpho-physiological traits and the environment. The yield stability across the environments is a reliable criterion for the selection of heat-stress adapted germplasm [47,88]. Climate change associated global warming has severely affected yield stability in cereal crops [89]. Genotype – environment interactions are of major importance to the plant breeder for developing improved varieties. We used stability analysis [62] to identify stable and better performing accessions under HS environment. The excellent performing accessions in HS environment may be used as donor parents for heat-stress adaptation breeding programme. Similar study was also carried [41] to select superior yielding lines under heat-stress. However, G × E interaction biplots for yield were used to select genotypes with stable performance across all environments [19]. In a similar study [35], genotype by environment interaction was used to identify superior yielding genotypes with heat stress tolerance. A selection strategy was also suggested to improve adaptation to heat stress in the bread wheat [28]. Heat stress affects significantly all the yield contributing traits and TGW an important trait for selection of tolerant lines. The heat tolerant lines with high grain yield could be selected using heat tolerance index and both AMMI and GGE biplot stability analyses [90]. The stable and higher yielding accessions identified under HS environment could be utilized in the breeding programme for the development of terminal heat-stressed tolerant cultivars.

5. Conclusions

Wheat genetic resources used in the present study represent a part of the reference set for heat-stress tolerance and hence exhibited high extent of genetic variability for morpho-physiological and yield-related traits. The terminal heat-stress during late sown bread wheat crop negatively affected yield and its contributing traits. The higher than the optimum temperature during GFP reduced the grain size, and eventually decreased TGW and yield under heat-stressed environment. Ultrastructural analysis of wheat grains showed that the decrease in size and density of large starch granules in the endosperm is the main cause of yield and TGW reduction in the heat-stress susceptible germplasm. The better yielding accessions possessing desirable morpho-physiological traits and adaptation to heat-stress identified, could be utilized for the development of mapping populations and genetic improvement of bread wheat.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Supplementary Figure S1 to S2: Supplementary Table S1 to S7.

Data availability statement: The original contributions presented in the study are included in the article/ Supplementary Materials. Further queries can be directed to the corresponding author.

Author Contributions: Mahesh C. Yadav conceptualized and validated the study. Anil Patidar performed the experiments and recorded field and lab data. Jyoti Kumari provided germplasm lines and analysed quantitative data. Shailesh Tiwari performed data recording and crop management. Gautam Chawla performed ultrastructural analysis of wheat grains. MCY and AP wrote and prepared the original draft and finalized the manuscript. JK and Vijay Paul reviewed and edited the manuscript. MCY supervised the study, was in charge of the project administration, and acquired the funding for the study. All authors contributed to the research article and approved the submitted version.

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