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Genetic variability and traits association in wheat under terminal heat stress in the Haryana environment

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Abstract
Numbers of environmental stresses influence plant growth and development, which limits agricultural production and productivity, globally. High heat stress is a major factor that significantly reduced the wheat grain yield. Terminal heat stress adversely affects wheat plants resulting in low productivity. The ability of the plant material to mitigate the heat stress on yield may depend on the association between yield and other traits, and the variability of a trait will help identify desirable genotypes in a breeding program. For this purpose, sixty-four bread wheat genotypes were evaluated to assess genetic variability, heritability, correlations and path coefficient analysis of the traits. As result, highly significant differences in genotypes for all 15 morpho-physiological traits were observed. GCV and PCV exceeded 42% for total chlorophyll content and 16% for Main spike weight. Grain yield/plot was significantly and positively associated with spike length, spike weight, number of grains/spike, number of spikelets/spike, 1000-grain weight, biological yield/plot, GGR, total chlorophyll content and number of productive tillers/meter. As conclusion, the production of wheat in heat-stressed environment is greatly enhanced by the selection of key morphological and physiological features associated with grain yield. Five genotypes, P13833, P13828, P13031, P13723, and P13726, had high yields under heat stress condition and ought to be utilized as parents in breeding programs.

Keywords: Bread wheat, terminal heat stress, genetic diversity, canopy temperature, heritability, genetic advance

Introduction

Wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) is a self-pollinated, and most popular cereal crop for human food and livestock feed globally. Wheat belongs to the subtribe Triticinae of the tribe Triticeae (Hordaceae) within the Poaceae family. Wheat is adapted to wide range of environments from temperate, irrigated areas to tropical, high-rainfall areas and from warm, humid conditions to cold, dry conditions. Wheat can be grown at latitudes of 30°- 60° N and 27°- 40° S and 3000 to 4570 mean sea level (Nuttonson, 1955; Percival, 1921) [1, 2].

Wheat is the second major cereal crop after maize in the world with area, production and productivity of 214.29 m ha, 734.05 m tonnes and 3.43 tonnes per hectare, worldwide (FAO, 2018) [3]. Wheat is a staple food over one-third (40%) of the world population and covers over 17% of crop acreage worldwide. India is the second largest producer of wheat after China with annual production above 100 million tonnes consistently. According to latest estimate, India's total wheat production is 109.52 million tonnes from 31.35 mha-1 cultivated area with a productivity of 34.4 qha-1, while, Haryana produced 11.87 million tonnes of wheat from 2.53 mha-1 of cultivated area and productivity of 4.68tonnes per hectare (Anonymous, 2021).

It is estimated that wheat production must be increased by 60% to meet out the demand of the growing population (Rosegrant and Agcaoili, 2010) [4]. Annually, the yield must be increased at least by 1.6% along with tolerance to abiotic and biotic stresses (Narayanan, 2018) [5]. Several environmental stresses influence the plant growth and development, which limits the agricultural production and productivity, globally. Among the abiotic stresses, heat stress is a major factor that significantly reduces the wheat grain yield worldwide especially in arid, semi-arid, tropical, and sub-tropical regions (Okechukwu *et al.*, 2016) [28]. The rise in temperature beyond a certain threshold level is sufficient to induce irreversible damage to plant growth and development (Wahid *et al.*, 2007) [7].

High temperature decreases the yields by to 3-5 per cent for each 1 °C increase in temperature above 15 °C under control condition (Elbashier *et al.*, 2019) [8]. Terminal heat stress impose many changes in plant such as reduced crop stand, shorter life cycle, reduced tillering, less biomass production, reduced seed size, reduction in number of spikes per plant, number of grains per spike and grain weight which ultimately reduce of grain yield (Saha *et al*., 2020) [9] and also affects photosynthetic activity during grain filling period and grain weight (Narayanan, 2018) [5]. Heat stress also affects chlorophyll accumulation that affects photosynthesis in plant.

Thus, breeding for heat tolerance in wheat is a key global concern and development of heat-tolerant genotypes is of prime importance in wheat breeding programs. The suitable plant type is to be selected from diverse gene pool for utilization in hybridization programme further; success in crop improvement generally depends on the magnitude of genetic variability and the extent to which the desirable characters are heritable and associated with grain yield. Therefore, the present investigation was undertaken to study the genetic variability for yield and yield contributing traits of wheat under terminal heat stress conditions, using the genetic material available in the gene bank at CCSHAU, Hisar.

Materials and Methods

The study was conducted at CCS HAU, Hisar, India during the Rabi season of 2019-20 under late sowing conditions for the exposing genotype to high temperatures. The maximum temperature during crop season ranged from 15 °C (December) to 39 °C (April) while the minimum temperature varied from 2 °C (January and February) to 18 °C (April) (Fig. 1). A total of 64 genotypes were grown in a randomized complete block design (RCBD) with three replications with a plot size of 6.48 m^2 and recommended agronomic practices were followed to raise the healthy crop. Observations were recorded on grain yield (GY) and its related traits, *viz*., days to heading (DH), number of productive tillers per meter (NPTM), main spike length (MSL), number of spikelets per spike (NSPS), main spike weight (MSW), number of grains per spike (NGPS), days to maturity (DM), 1000 grain weight TGW), biological yield (BY), grain yield per plot (GY), grain growth rate (GGR), canopy temperature (CT) and total chlorophyll content (TCC). The grain growth rate was measured at 14, 21, and 28 days after anthesis by weighing the kernel from main spikes and expressed as g/grain/day. Canopy temperature was recorded at 7 days after anthesis (DAA) with the help of a handheld infrared thermometer. Chlorophyll 'a' and chlorophyll 'b' and total chlorophyll content were extracted as per standard procedure described by Sawhney and Singh (2002) [26] at 28 DAA. The data was analyzed using OPSTAT (software available at http: //www.hau.ernet.in.), INDOSTAT (version 8.0), SPSS (version 24.0), and STAR (Statistical Tool for Agricultural Research) software.

Results and Discussion

Wheat breeding for resistance to terminal heat stress is becoming more and more crucial due to a result of global warming. Due to its complex character, which cannot be measured on its own, breeding for heat tolerance is more difficult than breeding for biotic stresses. It needs to be evaluated in terms of how it affects the genotype's ability to perform differently for a particular trait. Knowing that stress quickens the plant's phonological development, which then affects all of the plant's characteristics. In this study, some key characteristics that are crucial in the selection of genotypes that are resistant to heat stress are discussed.

The results showed that variation due to genotypes was highly significant (p<0.01) for all the traits indicating highly diverse genotypes used for the study (Table 1). The mean values for the 15 morpho-physiological traits are presented in Table 2. Genotypes mean ranged from 74.00-88.00 days for DH, 35.00 to 65.00 for NGPS and 15.00 - 23.00 for NSPS, 119.00 - 127.00 days for DM, 4.27-8.21 for GGR14, 8.19-16.67 GGR21, 12.61-21.08 for GGR28, 19.00 - 31.00 CT and 14.30 - 44.10 for TTC. Grain yield per plot ranged from 2.37 to 3.60 kg/plot with an overall mean of 2.94 kg plot-^{1}. Top five high yielding (Highest 3.60 to 3.53 kg plot-^{1}) genotypes *viz*., P13726 (3.53 kg plot-^{1}), P13723 (3.57 kg plot-^{1}), P13833 (3.60 kg plot-^{1}), P13828 (3.60 kg plot-^{1}) and P13031 (3.60 kg plot-^{1}) performed better than the best check HD 3059 (3.13 kg plot-^{1}).

The data on physiological traits i.e. total chlorophyll content, GGR14, GGR21, GGR28 and canopy temperature compared to grain yield and grain growth rate vs 1000 grain weight based on top 5 genotypes *viz*., P1 38 33, P1 38 28, P1 30 31, P1-37 23 and P1-37 26 is presented in Fig 2 and 3. The extremely low CT, high rate of GGR (14,21 and 28) and TCC are impotent physiological traits that highly contribute to grain yields and increase the 1000 grain weight in heat-stressed environments (35-45ºC) and the selection based on these traits is exceptionally important for the development of heat tolerant variety.

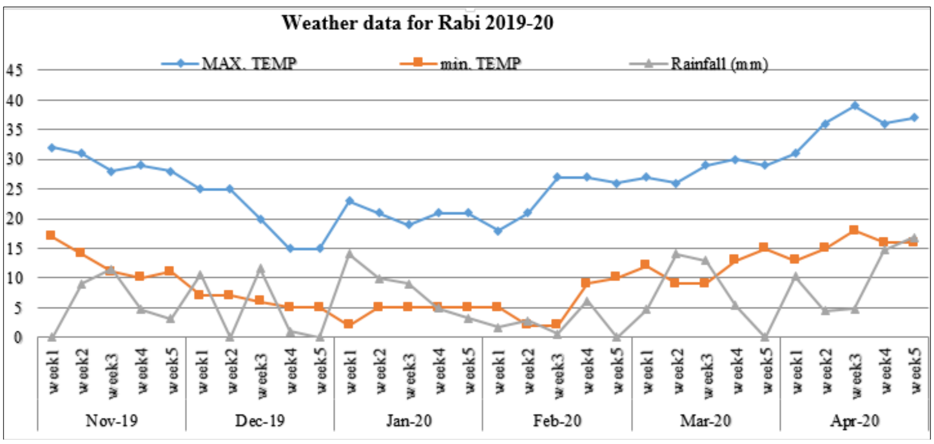

Fig 1: Weekly base weather data for *Rabi* crop season 2019-2020

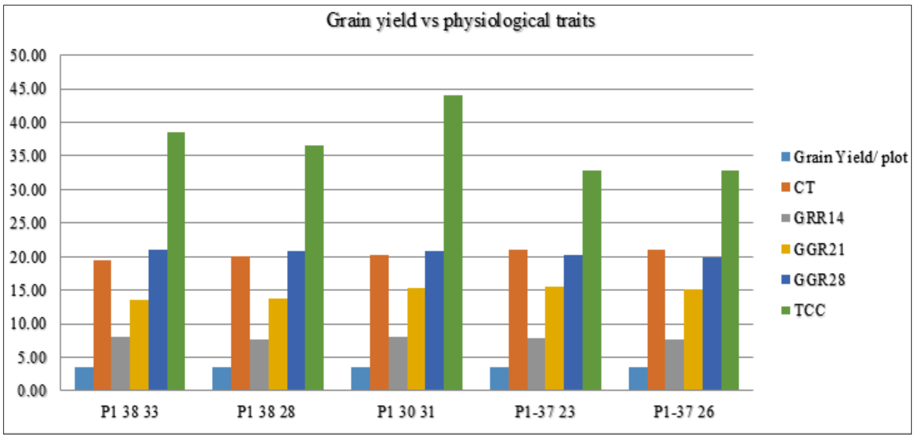

Fig 2: Variability for grain yield/plot, canopy temperature, grain growth rate, and total chlorophyll content in 64 wheat genotypes under late sowing conditions

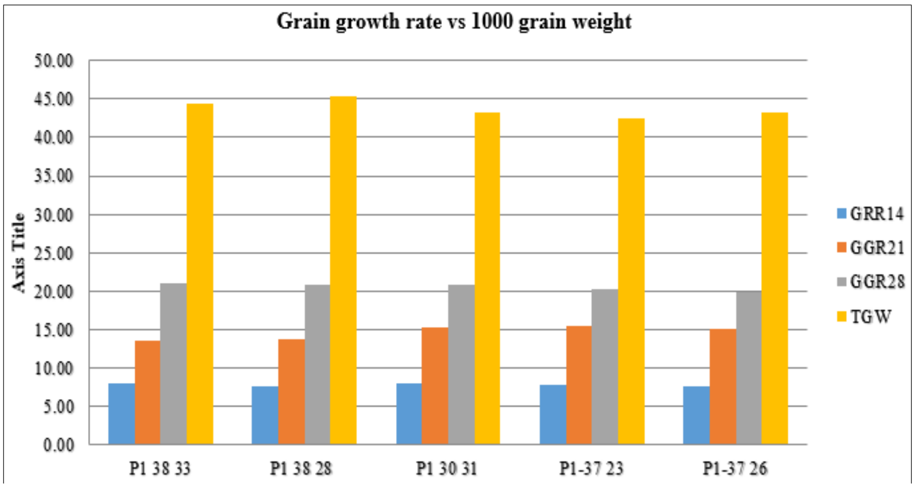

Fig 3: Graphical representation of grain growth rate vs 1000-grain weight

Table 1: Analysis of variance for different traits of wheat under late sown conditions

		Mean Sum Square (MSS)								
Traits	**DF**	**FE**	**DH**	**PH**	**NPTP**	**PL**	**MSL**	**MSW**	**NGPS**	**NSPS**
Genotypes	63	2.64**	16.20**	84.88**	658.20**	22.29**	3.83**	0.66**	122.03**	7.36**
Replication	2	1.05	2.54	26.69	305.43	5.82	1.41	0.24	1.31	1.05
Error	211	0.13	0.79	34.88	44.38	1.72	0.29	0.08	27.31	1.04
C.V.%		3.07	1.06	6.32	4.61	9.18	5.15	10.65	10.45	5.57
C.D.		0.59	1.44	9.55	10.78	2.12	0.88	0.46	8.46	1.65
		Mean Sum Square (MSS)								
Traits	**DF**	**GGR14**	**GGR21**	**GGR28**	**CT**	**TCC**	**DM**	**TGW**	**BY**	**GY**	**HI**
Genotypes	63	0.02**	0.02**	0.04**	14.99**	176.99**	11.72**	25.61**	1.68**	0.28**	46.34**
Replication	2	0.01	0.01	0	2.94	8.04	0.8	0.76	0.44	0.12	7.99
Error	211	0.01	0	0.01	2.25	1.57	1.64	0.24	0.32	0.06	14.79
C.V.%		8.87	4.7	5.68	5.9	6.89	1.12	1.3	7.26	7.94	10.04
C.D.		0.11	0.09	0.11	2.43	2.03	2.07	0.79	0.91	0.38	6.22

** Significant at (*p*< 0.01) level of significance, C.V= coefficient of variation of experimental error at general mean level. C.D= critical difference at (? 5%). DF= degrees of freedom.

FE: Field emergence, DH: Days to heading, PH: Plant height (cm), NPTP: Number of productive tillers/meter, PL: Peduncle length (cm), MSL: Main spike length (cm), MSW: Main spike weight (g), NGPS: Number of grains/spike, NSPS: Number of spikelets/spike, DM: Days to maturity, BY: Biological yield /plot (kg), GY: Grain yield /plot (kg), HI: Harvest index (%), TGW: 1000-grain weight (g), GGR14: Grain growth rate at 14^{th} days after anthesis, GGR21: Grain growth rate at 21^{st} days after anthesis, GGR28: Grain growth rate at 28^{th} days after anthesis, CT: Canopy temperature (˚C), TCC: Total chlorophyll content (%)

Estimation of variability components

Phenotypic and genotypic coefficients parameters were estimated for all the traits under late-sown conditions (Table 2). Across all the traits (19), the genotypic coefficient of variation (GCV) varied from 1.64% to 42.07% and the phenotypic coefficient of variation (PCV) from 1.95% to 42.63%. The small difference existing between PCV and GCV for all the characters indicated the low contribution of environmental factors on the expression of traits. High phenotypic and genotypic coefficients of variation were observed for TCC (42.07 and 42.63%) followed by MSW (19.54 and 16.70%), NGPS (15.20 and 10.97%), MSL (11.62 and 10.47%), GY (12.23 and 9.31%) and NPTM (10.92 and 9.90%). Selection will be effective for these traits in the breeding program. Earlier researchers also reported significant GCA and SCA for grain yield per plant, length of main spike, 100-grain weight, number of effective tillers per plant, chlorophyll (Ramanuj *et al*., 2018) [10], main spike length, number of grains per spike (Chimdesa *et al.* (2017) [11] and grain weight per spike (Balkan *et al*., 2018) [12]. The significance of GCA and SCA variances recommended both additive and non-additive types of gene action played an important role in the inheritance of the traits and highly responded to selection (RAM *et al*., 2014) [13]. This genetic variability of impotent traits among wheat germplasms is highly usable in heat tolerance breeding programs**.**

Heritability and genetic advance (%)

The heritability (bs) estimates ranged from 27.82% (GGR21) to 98.47% (1000 grain weight). Heritability is quite useful in deciding the traits while making a selection, these traits could be improved directly through selection as these traits are least influenced by environment as there would be high correspondence between genotype and phenotype. High (>60%) heritability was observed for TGW (98.47%), TTC (97.39%), DH (86.49%), NPTM (82.17%), MSL (81.19%), MSW t (73.06%), DM (71.25%), NSPS (66.20%) and CT (65.35%). Zeeshan *et al.* (2014) [14] reported high heritability (> 60%) for days to heading, physiological maturity and number of spikelets/spike, Raaj *et al.* (2018) [15] for spike length, chlorophyll content and 1000- grain weight, Thapa *et al*. (2019) [27] for canopy temperature depression, and Haydar *et al*. (2020) [17] for plant height and spike length.

While, the traits *viz*. GY (57.91%), GGR28 (44.55%), GGR14 (32.2%) and GGR21 (21.82) had moderate heritability. The results were in consistence with the former researchers like Iqbal *et al.* (2017) [18], Ramanuj *et al*. (2018) [10] and Raaj *et al*. (2018) [15] for grain yield per plant, biological yield per plant, and number seed/spike. Therefore, the high heritable traits are highly responsive to selection.

There are limitations of using broad sense heritability because it includes both additive and non-additive gene effects. High heritability together with genetic advance is an indication of additive gene effect, whereas, high heritability with low genetic advance is an indication of dominance and epistatic effect. High heritability coupled with high genetic advance as percent of mean was observed for TCC (85.52%), and MSL (29.40%). These traits will show a high response to selection due to the predominance of additive gene effects. A similar result was reported by Wahidy *et al*. (2016) [29] for spike length; Raaj *et al.* (2018) [15] for chlorophyll content and Thapa *et al*. (2019) [27] for grain weight per spike. The rest of the characters had low to the moderate genetic advance percent of the mean, showing the low scope of improvement by selection under terminal heat stress

Table 2: Range, genotypic and phenotypic coefficient of variance, heritability and genetic advance for morpho-physiological traits under late sown conditions

Sr. No.	Traits	Genetic Parameters					
		Mean	Range	GCV (%)	PCV (%)	h^2 (bs) (%)	% Genetic advance over mean (at 5% intensity of selection)
1	Days to heading	84.26	74.00-88.00	2.69	2.89	86.49	5.14
2	Number of productive tillers/meter	144.47	77.00 - 159.00	9.90	10.92	82.17	18.49
3	Main spike length (cm)	10.49	8.00 - 14.00	10.47	11.62	81.19	19.43
4	Main spike weight (g)	2.65	2.11 - 3.90	16.70	19.54	73.06	29.40
5	Number of grains/spike	49.87	35.00 - 65.00	10.97	15.20	52.09	16.31
6	Number of spikelets/spike	18.53	15.00 - 23.00	8.31	10.21	66.20	13.93
7	Grain growth rate at 14 DAA (g/gr x 10^{-4})	0.79	4.27-8.21	7.92	13.96	32.20	9.26
8	Grain growth rate at 21 DAA (g/gr x 10^{-4})	1.11	8.19-16.67	10.34	19.60	27.82	11.23
9	Grain growth rate at 28 DAA (g/gr x 10^{-4})	1.23	12.61-21.08	9.45	14.15	44.55	12.99
10	Canopy temperature (˚C)	25.43	19.00 - 31.00	8.10	10.02	65.35	13.49
11	Total chlorophyll content (%)	18.17	14.30 - 44.10	42.07	42.63	97.38	85.52
12	Days to maturity	114.80	119.00 - 127.00	1.64	1.95	71.25	2.86
13	1000 grain weight (g)	37.37	29.90 - 45.45	7.83	7.89	98.47	16.01
14	Biological yield /plot (kg)	7.75	6.13 - 9.70	8.70	11.32	58.99	13.76
15	Grain yield/plot (kg)	2.94	2.37 - 3.60	9.31	12.23	57.91	14.59

PCV: Phenotypic coefficient of variation; GCV: Genotypic coefficient of variation; h^2 (bs): Heritability (broad sense)

Table 3: Phenotypic correlation coefficient among grain yield and component traits in wheat under late sown conditions

Traits	DH	NPTP	MSL	MSW	NGPS	NSPS	GGR14	GGR21	GGR28	CT	TTC	DM	TGW	BY	GY
FE															
NPTP	0.018														
MSL	-0.084	0.177*													
MSW	-0.120	0.147*	0.593**												
NGPS	-0.073	0.111	0.505**	0.618**											
NSPS	-0.015	0.257**	0.558**	0.537**	0.473**										
GGR14	-0.283**	0.190**	0.511**	0.461**	0.368**	0.542**									
GGR21	-0.265**	0.179*	0.572**	0.525**	0.404**	0.568**	0.955**								
GGR28	-0.273**	0.185*	0.568**	0.516**	0.399**	0.569**	0.979**	0.993**							
CT	0.199**	-0.234**	-0.604**	-0.572**	-0.474**	-0.572**	-0.661**	-0.728**	-0.719**						
TTC	-0.214**	0.201**	0.663**	0.604**	0.518**	0.607**	0.724**	0.805**	0.792**	-0.794**					
DM	0.101	0.330**	0.181*	0.112	0.153*	0.178*	0.187*	0.158*	0.168*	-0.097	0.084				

TGW	-0.129	0.121	0.687**	0.636**	-0.599**	0.599**	0.614**	0.676**	0.672**	-0.693**	0.757**	0.143*			
BY	-0.193**	0.134	0.234**	0.247**	0.202**	0.284**	0.347**	0.382**	0.374**	-0.326**	0.348**	0.121	0.350**		
GY	-0.250**	0.162*	0.533**	0.470**	0.393**	0.537**	0.832**	0.865**	0.867**	-0.642**	0.748**	0.098	0.638**	0.379**	

Table 4: Path coefficient analysis showing direct (diagonal and bold) and indirect (off - diagonal) effects of different morphological traits on grain yield per plot under late sowing conditions

Traits	DH	NPTP	MSL	MSW	NGPS	NSPS	DM	TGW	BY	r with GY
DH	0.0127	0.0001	0.0005	-0.0025	0.0007	0.0001	-0.0019	-0.0015	-0.1448	-0.250**
NPTP	0.0002	0.0070	-0.0011	0.0030	-0.0011	-0.0017	-0.0063	0.0014	0.1004	0.162*
MSL	-0.0011	0.0012	-0.0064	0.0122	-0.0052	-0.0036	-0.0035	0.0077	0.1754	0.533**
MSW	-0.0015	0.0010	-0.0038	0.0206	-0.0063	-0.0035	-0.0021	0.0072	0.1849	0.470**
NGPS	-0.0009	0.0008	-0.0032	0.0127	-0.0103	-0.0031	-0.0029	0.0068	0.1513	0.393**
NSPS	-0.0002	0.0018	-0.0035	0.0111	-0.0049	-0.0065	-0.0034	0.0063	0.2126	0.537**
DM	0.0013	0.0023	-0.0012	0.0023	-0.0016	-0.0012	-0.0190	0.0016	0.0906	0.098
TGW	-0.0016	0.0009	-0.0044	0.0131	-0.0061	-0.0036	-0.0027	0.0113	0.2621	0.638**
BY	-0.0025	0.0009	-0.0015	0.0051	-0.0021	-0.0019	-0.0023	0.0039	0.7490	0.379**

Residual effect: 0.02489

Table 5: Path coefficient analysis showing direct (diagonal and bold) and indirect (off - diagonal) effects of different physiological traits on grain yield per plot under late sowing conditions

Traits	GGR14	GGR21	GGR28	CT	TTC	r with GY
GGR14	-0.2027	0.1701	0.1427	0.0083	-0.0008	0.832**
GGR21	-0.1935	0.1782	0.1447	0.0092	-0.0009	0.865**
GGR28	-0.1984	0.1768	0.1458	0.0090	-0.0009	0.867**
CT	0.1341	-0.1297	-0.1048	-0.0126	0.0009	-0.642**
TTC	-0.1469	0.1435	0.1155	0.0100	-0.0011	0.748**

Residual effect: 0.02458

GGR14: Grain growth rate at 14th days after anthesis, GGR21: Grain growth rate at 21st days after anthesis, GGR28: Grain growth rate at 28th days after anthesis, CT: Canopy temperature (˚C), TCC: Total chlorophyll content (%)

Traits association

At the phenotypic level, the grain yield/plot had positive and significant (p<0.01) correlation with, GGR28 (0.867), GGR21 (0.865), GGR14 (0.832), TCC (0.748), TGW (0.638), MSW (0.470), NGPS (0.393), BY (0.379), NPTM (0.162) and DM (0.098) (Table 3). The positive association of these traits indicates the prospect of improving grain yield by direct selection of these characters. Similar findings of grain yield association were also reported by Zeeshan *et al*. (2014) [14] for spike length, number of spikelets/spike and number of grains/spike; Sharma *et al*. (2018) [20] for spike weight; Bhanu *et al.* (2018) [21] for chlorophyll content; Baral *et al*. (2013) [22] for GGR14, GRR21 and GGR28 and canopy temperature depression. The adaptation of wheat cultivars to the typical field conditions in a given region is influenced by important traits like heading and maturity. Furthermore, early maturing genotypes are ideal for heat-stressed environments, but late maturing genotypes will work well in heat-favorable environments, according to the correlation. This suggests that days to maturity may be advantageously chosen for increased grain yield, with the exception of extreme and prolonged heat stress, particularly in the late growing season. Fast-maturing genotypes will perform best under extreme heat stress by using escape mechanisms to prevent the prolonged terminal heat stress that typically occurs, especially in the tropics and subtropics. DH and grain filling rate both influence grain growth rate. It is best to select medium HD (79-88 days) and medium DM (120 days) when creating high-yielding genotypes for the heat-stress environment. Grain yield per plot was negatively and significantly correlated with DH (-0.250) and CT (-0.642). It is indicated that the low CT has an effect on grain yield in a stressful environment and increases the grain yield.

Path coefficient analysis

Path analysis for morphological traits

The path coefficient analysis exhibited (Table 4) that the highest and positive direct effect on grain yield/plot was recorded by BY (0.9287), while the traits such as TGW (0.0293), MSW (0.0167), NSPS (0.0110), DH (0.0094), NPTM (0.0082), NGPS (0.0066), MSL (0.0061), were revealed low positive direct effect. DM showed a negative direct effect on grain yield per plot (-0.0199).

Path analysis for physiological traits

In respect to physiological traits (Table 5), the TCC (0.5912), GGR21 (0.0853), GGR14 (0.0451) and GGR28 (0.0147) had the highest and positive direct effect toward grain yield/plot and CT had a negative direct effect (-0.1209) on grain yield/plot while showed indirect positive effect through TCC (0.0961), GGR14 (0.0592), GGR28 (0.0562) and GGR21 (0.0328) toward grain yield/plant. Path coefficient analysis showed that residual effect is very low (0.02489). The findings are in accordance with the earlier reports where direct positive effects for biological yield/plant, grain growth rate, grains/splike and spikelets/spike (Shenarvar and Golparvar 2015) [23]; days to heading, plant height and chlorophyll (Ramanuj *et al*., 2018) [10]; number of effective tillers per plant and harvest index (Nagar *et al.*, 2018) [24]; hundred-grain weight (Sharma *et al*., 2018) [20]; protein content and days to flowering (Kumari *et al.*, 2020) [25] has been reported in bread wheat.

Conclusion

The study indicated the presence of a considerable amount of variability among different genotypes of bread wheat for all the traits. High GCV and PCV were observed for total chlorophyll content and peduncle length and moderate GCV and PCV for main spike weight, number of grains/spike, main spike length. High heritability coupled with high genetic advance as a percent of the mean was observed for total chlorophyll content and main spike weight. Grain yield/plot was significantly and positively correlated with main spike length, main spike weight, number of grains/spike, number of spikelets/spike, days to maturity, 1000-grain weight, biological yield/plot, GGR14, GGR21, GGR28, total chlorophyll content, and number of productive tillers/meter under stress environments. The grain yield under heat stress conditions can be improved through the direct selection of the previously mentioned traits. Biological yield/plot had the highest positive direct effect on grain yield per plot followed by harvest index, GGR21, field emergence, GGR28, main spike weight, days to heading, 1000-grain weight and number of productive tillers/meter. Most of the characters contributed to the grain yield/plot through biological yield/plot and harvest index. Therefore, these traits must be given high importance during breeding for heat tolerance in wheat.

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Conflict of interest
The authors declare that there is no conflict of interest

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