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PERFORMANCE OF WHEAT GENOTYPES FOR MORPHO-PHYSIOLOGICAL TRAITS USING MULTIVARIATE ANALYSIS UNDER TERMINAL HEAT STRESS

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Abstract

High temperature adversely affects the development and growth of wheat crop. It disturbs photosynthesis and transpiration due to canopy temperature and senescence of leaves that cause reduction in wheat grain yield. Genetic divergence among genotypes and suitable selection criteria is imperative for efficient breeding programs to develop tolerance against heat stress. Therefore, 158 wheat genotypes were evaluated under normal and delayed planting mediated heat stress. Current study revealed reduction in grain yield (42.41%), grains per spike (16.51%), photosynthetic rate (39.84% and 48.52%), transpiration rate (55.88% and 51.85%) at vegetative and reproductive stage respectively. Furthermore, cell membrane injury (11.4-52.0% and 10.4-32%), canopy temperature depression (15.5-10.8ºC and 11.3-6.1ºC) at vegetative and reproductive stage, leaf angle (6º-42º) and stay green (68.8-17.5) were observed from heat tolerant to susceptible genotypes. Principal component analysis indicated highest negative loading components viz., stay green followed by grain yield and canopy temperature depression at reproductive stage whereas positive loading component such as heat susceptibility index for grain yield that would be used as effective selection criteria against heat stress. Correlation analysis suggested that high values of stay green, thousand grain weight, grains per spike, photosynthetic rate, transpiration rate and proline content while low values of cell membrane injury, heat susceptibility index for grain yield and canopy temperature can improve grain yield. Cluster analysis characterized 158 wheat genotypes into three groups based on different physio-morphic traits. Cluster-III retained genotypes with high value for heat tolerance indices such as stay green, grain yield, canopy temperature depression at reproductive stage while least value of heat susceptibility index for grain yield those were selected in PCA. These results would enhance the efficiency and precision as selection criteria for improvement in wheat against heat stress.

Key words: Heat stress, Heat susceptibility index, PCA, cluster analysis, Genotypic correlation.

List of Abbreviations

TGW: 1000-grain weight, BM: Biomass per plant, FLA: Flag leaf area, GY: Grain yield per plant, GPS: Grains per spike, PH: Plant height, SL: Spike length, SPS: Spikelet per spike, TP: Tillers per plant, DH: Days to 50% heading, DA: Days to anthesis, DM: Days to 50% physiological maturity, GFD: Grain filling duration, PNS: Photosynthetic rate at vegetative stage, ES: Transpiration rate at vegetative stage, PROS: Proline content at vegetative stage, PNR: Photosynthetic rate at reproductive stage, ES: Transpiration rate at reproductive stage, PROS: Proline content at reproductive stage, Cell membrane injury at vegetative stage, CTDS: Canopy temperature depression at vegetative stage, Cell membrane injury at reproductive stage, CTDS: Canopy temperature depression at reproductive stage, LA: Leaf angle, SG: Stay green, HSI: Heat susceptibility index for grain yield, EV: Eigen value, %TV: Percentage of total variability, CEV: Cumulative Eigen value, C%: Cumulative percentage, RP: Relative performance, PCA: Principal component analysis.

Introduction

Wheat is imperative cereal crop possess vital source of calories and protein in human diet. Food security requires developing high yielding cultivars with tolerance to abiotic and biotic stresses. Heat stress is major limiting factor among abiotic stresses that adversely affects the productivity of wheat. In Pakistan, 80% wheat planted delayed due to late physiological maturity of rice and picking of cotton whereas 20% planted at normal time (Laghari *et al.*, 2012). Due to delayed sowing wheat plant completes its growing degree days earlier and shortens the life cycle that leads towards terminal heat stress at anthesis and grain filling duration (Aslam *et al.*, 2017). Delayed planting expose the plant to complete its vegetative stage in short period and enter in reproductive stage. Vernalization and photoperiodic sensitive genes regulate the developmental phases at high temperature that cause earliness and shorten the different growth phases in wheat (Slafer, 1996). Heat stress due to delayed planting inhibits the photosynthetic apparatus and translocation of different photosynthates to the grains (Bita & Gerats, 2013). Furthermore, it develops the abnormal gametes due to disturbance in microsporogenesis and microgametogeneis consequently reduction in grains formation and ultimately grain yield (Thakur *et al.*, 2010). Thus physiological stability through cooler canopy temperature (Munjal & Rana, 2003; Ray & Ahmed, 2015) enhanced proline content and stays green (Lopes & Reynolds, 2012; Adu *et al.*, 2011) can mitigate the adverse effects of heat stress. High temperature 3-4°C above the optimum temperature during grain filling duration can reduce wheat yield 25-35% in Middle East and 20-35% in Asia (Ortiz *et al.*, 2008). Similarly, Mondal *et al.*, (2013) observed 3-15% reduction in wheat yield with each degree increased temperature above cardinal temperature. Furthermore, Joshi *et al.*, (2016) observed up to 45% reduction in wheat yield due to delayed planting mediated heat stress.

Multivariate analysis helps to identify the groups of genotypes with desirable traits for breeding programs against heat stress. Diversity of wheat genotypes by principal component analysis (PCA) and cluster analysis explains the variation among genotypes. PCA has ability to reduce large number of variables into least variables with maximum information (Ahmad *et al*., 2014). It enhances the efficiency of breeders to select heat tolerant germplasm rather than selection considering various physio-morphic traits. Grain yield is ultimate objective of breeders that is complex quantitative trait. So, genetic and phenotypic association of these traits with grain yield will further help the breeders to improve heat tolerance in wheat. Cluster analysis classifies heat tolerant and heat susceptible wheat genotypes for desirable traits based on genetic similarity. Classification of wheat genotypes in different clusters have also been studied by Hailegiorgis *et al*., (2011) and Dhanda & Munjal (2017).

Thus there is need to identify the suitable selection criteria that will enhance the efficiency of breeders to select heat tolerant genotypes from diverse populations. Current study designed to evaluate diverse wheat genotypes for various phenological, morphological and physiological traits under normal and heat stress conditions. The aim of this study was to determine suitable traits for selecting thermotolerant genotypes, genotypic and phenotypic association among trait and classification of wheat genotypes for thermotolerance.

Materials and Methods

Plant material: Experiment was performed at PMAS-Arid Agriculture University research farm (33.11 °N, 73.01 °E) Pakistan during 2016-17 and 2017-18. One hundred and fifty eight wheat genotypes were collected from Pakistan viz., National Agricultural Research Center Pakistan, Barani Agricultural Research Institute, Ayub Agricultural Research Institute, Arid Zone Research Institute and CIMMYT Mexico (23rd semi-arid wheat yield trial and 24th semi-arid wheat yield trial). Genotypes were planted in field using augmented complete block design with thirteen blocks and two checks (AAS-11 & AS-2002) with three replications. Length of row was 10 meter with row to row and plant to plant distance maintained at 30 cm and 15 cm respectively. Before planting, soil sample were collected for basic soil analysis. Soil had 3.14% moisture, sandy clay soil texture, 7.65 pH, 0.192 dS/m electrical conductivity, 0.38 mg/g total nitrogen, 9.62 ppm extractable phosphorous, 83.88 ppm potassium and 0.71% organic matter. Urea and Diamonium phosphate fertilizer were applied @ 120 and 60 Kg per hectare at the time of sowing. All recommended agronomic practices such weeding and hoeing was done as per requirement. Ten plants were selected randomly for data collection. Data were collected for different morphological and physiological traits under normal and heat stress conditions.

Phenological and morphological traits: Phenological traits were recorded for days to 50% heading, days to anthesis and days to 50% physiological maturity grain filling duration. Morphological traits were recorded for flag leaf area, plant height, spikelet per spike, spike length, fertile tillers per plant, 1000-grain weight, grains per spike, grain yield per plant and biomass per plant were measured at physiological maturity Zadoks scale 90 (Zadoks *et al*., 1974) whereas leaf angle (Simon, 1999) were measured at vegetative stage (Zadoks scale 39).

Physiological traits: Physiological traits were measured at vegetative stage (Zadoks scale 39) and reproductive stage (Zadoks scale 69). Photosynthetic and transpiration rate were measured with Infrared Gas Analyzer (IRGA, LCA-4, ADC, Hoddesdon, UK) on clear day between 10:00am to 12:00pm at both vegetative stage and reproductive stage. The measurement conditions were photosynthetically active radiation at 1600 mmol m^{-2} s^{-1} and carbon dioxide at 360 mmol mol^{-1} at constant (Long & Bernacchi, 2003).

Cell membrane injury was estimated from flag leaf with normal and heat treatment at 40ºC in test tubes as described by Deshmukh *et al*., (1991). Canopy temperature depressions was recorded according to method described by Ray & Ahmed (2015) at vegetative (before anthesis) and grain filling stage (post anthesis) with Infrared thermometer Model AG-42, tela-temp crop, Fullerton, CA. Air temperature was also noted after and before recording of reading on thermometer. Mean canopy temperature depression reading was computed by subtracting canopy temperature from air temperature. Proline content was estimated following Bates *et al*., (1973) at vegetative and reproductive stage. Stay green was measured according to LAUG (leaf area under greenness) approach (Joshi *et al*., 2007).

Statistical analysis

Data of physio-morphic traits were analyzed by Augmented design using PROC Mixed with entries fixed and block random in statistical software SAS (Scott & Milliken, 1993). Relative performance of collected data were estimated for different physio-morphic traits following Asana & Williams (1965) and subjected to principal component analysis (Harman, 1976), phenotypic and genotypic correlation (Kown & Torrie, 1964) and Mahalonobis D^2 statistics using Tocher's method (Rao, 1952) cluster analysis utilizing Mahalonobis D^2 in statistical software STATISTICA version 7.0.

Results

Delayed planting mediated heat stress forced the plant to complete its vegetative phase and entered in reproductive phase due to earlier completion of growing degree days. Normal planting exposed the wheat plant to optimimum temperature at anthesis stage (25-27ºC) and grain filling phase (30-32ºC) that was below the threshold level. When wheat planted delayed then high temperature 29-31ºC was observed during anthesis and 35-36ºC during grain filling duration that was 3-4ºC above the threshold level as shown in Fig. 1. However, wheat genotypes performance for 1000-grain weight (37.8-30.8g), biomass (56.5-42.0g), grains per spike (54.3-45.3), plant height (85.7-63.7cm), tillers per plant

(6.7-4.8), days to 50% heading (108.7-90.7 days), days to anthesis (125.1-109.1 days), days to 50% physiological maturity (138.6-125.3 days), grain filling duration (33.5-29.6 days) and grain yield per plant (9.9-5.7g) under normal and heat stress conditions were displayed in Table 1. Furthermore, photosynthetic rate at vegetative and reproductive stage (19.6-11.8 µmol $m^{-2}s^{-1}$ and 16.6-8.5 µmol $m^{-2}s^{-1}$), transpiration rate at vegetative and reproductive stage (0.34-0.15 mmol $m^{-2}s^{-1}$ and 0.27-0.13 mmol $m^{-2}s^{-1}$) under heat stress conditions as compared to normal conditions whereas proline content was increased at vegetative and reproductive stage (0.44-0.54 µmoles/g and 0.54-0.68 µmoles/g). Mean performance for cell membrane injury was ranged from 11.4-52.0% at vegetative and 10.4-32.0% at reproductive stage, canopy temperature depression 15.5-10.8°C at vegetative stage and 11.3-6.1°C at reproductive stage, leaf angle 6°-42° and stay green 68.8-17.5 in wheat genotypes from thermotolerant to heat susceptible.

Principal component analysis: PCA was performed to identify imperative variables for selection under heat stress. In our study, first nine components indicated more than one Eigen value and 68.23% variability for heat tolerance. PC1 indicated 15.07% of total variation and constituted high negative loading component for stay green followed by grain yield and canopy temperature depression at grain reproductive stage whereas high positive loading of heat susceptibility index for grain yield as shown in Table 2. Negative and positive loading indicated the presence of negative and positive association trends among variables. PC2 exhibited 11.05% of total variability and contained highest positive loading components for proline content at vegetative stage. PC3 showed 8.35% variation and exhibited proline content at reproductive stage whereas PC4 retained 7.46% of total variation and spike length was high positive loading components. Fifth component revealed that negative loading component leaf angle had contribution of 6.16% variability in total variation.

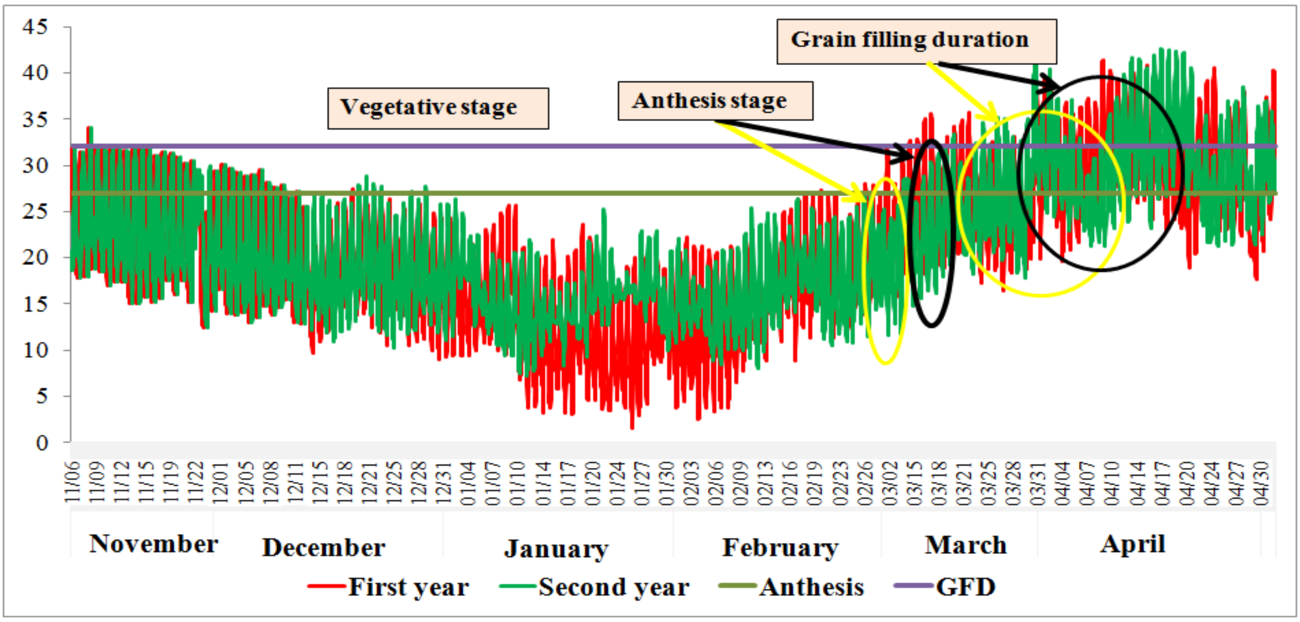

Fig. 1. Temperature data during wheat life cycle during 2016-17 and 2017-18
Source: Department of Environmental Science, PMAS-AAUR

Correlation

Genotypic and phenotypic significant correlation among physio-morphic indices indicated the significant negative association of grain filling duration with days to heading whereas positively correlated with days to maturity as displayed in Tables 3 & 4. Spike length was significant positively associated with grains per spike while spikelet per spike with transpiration rate at vegetative and biomass per plant with tillers per plant. Proline content at vegetative stage indicated significant positive association with proline content, photosynthetic rate and transpiration rate at reproductive stage whereas proline content at reproductive with photosynthetic rate and transpiration rate at reproductive stage. Photosynthetic rate at reproductive stage was positively associated with photosynthetic rate at vegetative and transpiration rate at reproductive stage.

Cell membrane injury at reproductive stage was positively correlated with cell membrane injury at vegetative stage and tillers per plant whereas canopy temperature depression at reproductive stage was positively associated with canopy temperature depression and transpiration rate at vegetative. Stay green was positively associated with grain yield, transpiration rate at vegetative and reproductive stage while negatively correlated with canopy temperature depression at reproductive stage. Grain yield was positively correlated with spikelet per spike, tillers per plant, thousand grain weight and biomass. Heat susceptibility index was positively correlated with cell membrane injury at reproductive stage while negatively correlated with thousand grain weight, biomass, transpiration rate at vegetative stage, stay green and grain yield.

Table 1. Descriptive statistic for physio-morphic traits.

Traits	Normal			Late			
	Mean	Min	Max	Mean	Min	Max	RP%
TGW	37.82 ± 0.15	31.8	41.8	30.81 ± 0.17	24.6	36.8	18.53
BIO	56.47 ± 0.31	44.7	65.3	41.98 ± 0.18	37.0	49.1	25.65
FLA	32.78 ± 0.15	21.8	46.0	17.46 ± 0.24	11.8	24.4	46.74
GY	9.95 ± 0.09	6.8	12.3	5.73 ± 0.12	3.4	8.0	42.41
GPS	54.28 ± 0.24	41.6	65.7	45.32 ± 0.24	34.7	52.0	16.51
PH	85.73 ± 0.25	77.6	95.6	63.69 ± 0.27	52.5	74.9	25.71
SL	12.24 ± 0.07	10.1	14.6	10.71 ± 0.08	7.8	13.6	12.50
SPS	20.50 ± 0.11	16.4	23.8	18.59 ± 0.13	14.2	22.2	9.93
TP	6.68 ± 0.07	5.0	9.2	4.84 ± 0.07	3.2	8.2	27.55
DH	108.7 ± 0.33	101.1	118.1	90.66 ± 0.28	83.9	99.4	16.57
DA	125.1 ± 0.26	117.5	130.3	109.1 ± 0.21	103.2	113.7	12.79
DM	138.6 ± 0.21	130.0	142.6	125.3 ± 0.19	121.6	141.1	9.62
GFD	33.52 ± 0.35	24.4	44.2	29.64 ± 0.22	22.9	35.4	11.58
PNS	19.63 ± 0.26	13.2	30.0	11.81 ± 0.17	8.1	19.9	39.84
ES	0.34 ± 0.01	0.1	0.7	0.15 ± 0.01	0.0	0.4	55.88
PROS	0.44 ± 0.01	0.2	0.6	0.54 ± 0.01	0.4	0.8	+185.2
PNR	16.59 ± 0.20	11.8	22.7	8.54 ± 0.18	4.6	17.0	48.52
ER	0.27 ± 0.01	0.1	0.5	0.13 ± 0.01	0.0	0.3	51.85
PROR	0.52 ± 0.01	0.3	0.7	0.68 ± 0.01	0.5	0.9	+235.2
CMIS				29.41 ± 0.74	11.4	52.0	
CTDS				13.47 ± 0.07	10.8	15.5	
CMIR				19.76 ± 0.44	10.4	32.0	
CTDR				8.17 ± 0.10	6.1	11.3	
LA				17.3 ± 0.62	6.0	42.0	
SG				46.57 ± 1.14	17.5	68.8	
HSI				0.99 ± 0.03	0.3	1.7	

Table 2. Principal component analysis for Physio-morphic indices among wheat genotypes.

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
DAH	-0.396	0.102	-0.258	-0.119	0.095	0.130	0.336	0.249	0.085
DAA	-0.168	-0.401	-0.438	-0.369	0.103	-0.150	-0.091	-0.277	-0.101
DM	0.070	-0.446	0.432	-0.235	0.517	0.182	-0.249	-0.091	0.083
GFD	0.187	-0.426	0.473	-0.170	0.449	0.108	-0.231	-0.029	0.267
ProS	-0.280	0.511	0.431	-0.032	-0.154	-0.351	-0.085	0.030	0.035
ProR	-0.280	0.484	0.680	-0.011	-0.084	-0.293	-0.094	0.032	0.000
PnS	-0.376	0.298	-0.265	-0.218	-0.130	0.469	-0.167	-0.263	-0.079
PnR	-0.426	0.316	0.137	-0.335	-0.219	0.461	-0.201	-0.210	-0.062
ES	-0.443	-0.079	0.101	0.071	0.284	-0.064	0.281	-0.193	-0.174
ER	-0.163	0.359	0.158	-0.429	-0.102	0.397	0.023	-0.166	-0.096
CMIS	0.313	0.281	-0.233	0.486	0.171	0.042	-0.580	0.098	-0.268
CMIR	0.386	0.434	-0.151	0.360	0.097	0.094	-0.586	-0.017	-0.212
CTDS	0.042	-0.243	0.440	0.133	-0.108	0.225	0.193	0.134	-0.565
CTDR	-0.551	-0.334	0.260	-0.018	-0.260	0.263	0.062	0.068	-0.112
LA	0.091	-0.144	0.070	0.008	-0.621	-0.278	0.010	-0.123	0.088
SG	-0.725	0.325	-0.128	0.002	0.117	-0.101	-0.005	0.086	-0.070
PH	-0.153	0.217	0.131	0.221	0.114	0.266	-0.222	0.205	0.309
FLA	-0.432	0.164	-0.025	-0.169	0.377	-0.054	0.084	0.302	-0.303
SL	-0.067	0.042	0.060	0.554	0.076	-0.058	0.160	-0.435	0.021
SPS	-0.492	-0.016	0.064	0.319	0.269	-0.128	0.055	-0.605	0.026
TP	-0.454	-0.491	-0.148	0.157	-0.248	0.069	-0.248	0.029	0.142
GPS	0.195	-0.057	0.240	0.423	0.027	0.551	0.459	-0.019	-0.178
TGW	-0.205	-0.434	0.037	-0.205	0.009	-0.345	-0.206	0.032	-0.527
BM	-0.432	0.047	-0.160	0.270	0.137	0.077	0.189	0.313	0.173
GY	-0.691	-0.462	0.114	0.288	-0.216	0.127	-0.202	0.113	-0.005
HSI	0.691	0.462	-0.114	-0.287	0.218	-0.127	0.203	-0.113	0.005
EV	3.918	2.872	2.171	1.939	1.602	1.565	1.425	1.196	1.052
% TV	15.07	11.05	8.35	7.46	6.16	6.02	5.48	4.60	4.05
CEV	3.92	6.79	8.96	10.90	12.50	14.07	15.49	16.69	17.74
C%	15.07	26.11	34.46	41.92	48.08	54.10	59.58	64.18	68.23

Table 3. Phenotypic (lower diagonal) and genotypic (upper diagonal) correlation among different physiological and morphological traits under heat stress conditions.

Traits	DAH	DAA	DM	GFD	PH	FLA	SL	SPS	TP	GPS	TGW	BM	GY
DH	1.000	0.134	-0.112	-0.157*	0.038	0.179*	-0.073	0.069	0.037	-0.007	-0.042	0.253*	0.139
DA	0.594*	1.000	0.159*	-0.047	-0.156	0.014	-0.083	0.108	0.232*	-0.287*	0.318*	-0.018	0.074
DM	0.067	-0.029	1.000	0.720*	-0.002	-0.008	-0.052	0.008	0.010	0.003	0.153	-0.063	0.093
GFD	-0.448*	-0.819*	0.629*	1.000	-0.023	-0.047	-0.077	-0.004	0.033	0.045	0.072	-0.157	0.021
PH	-0.290*	-0.127	-0.249*	-0.003	1.000	0.066	0.001	0.100	0.005	0.021	-0.157	0.090	0.099
FLA	0.186	0.237*	-0.080	-0.283*	0.390*	1.000	-0.140	0.156*	0.005	-0.043	0.137	0.148	0.111
SL	-0.059	-0.160	0.048	0.159	0.056	0.305*	1.000	0.328*	-0.015	0.221*	-0.057	0.101	0.085
SPS	-0.131	-0.170	0.305*	0.305*	-0.019	-0.063	0.542*	1.000	0.200*	0.030	0.033	0.156	0.288*
TP	0.017	-0.014	0.327*	0.115	0.044	0.271	0.256*	0.064	1.000	-0.080	0.174*	0.172*	0.562*
GPS	-0.136	-0.076	0.202*	0.189	0.175	-0.049	0.302*	0.557*	0.169	1.000	-0.209*	-0.007	-0.002
TGW	-0.061	-0.171	0.168	0.184	0.242*	0.113	0.100	0.080	-0.100	-0.085	1.000	-0.070	0.263*
BM	0.020	0.087	0.066	-0.043	0.059	0.334*	0.087	0.025	0.227*	0.073	0.563*	1.000	0.252*
GY	-0.103	-0.250*	0.378*	0.358*	0.053	0.161	0.259*	0.229*	0.452*	0.314*	0.782*	0.480*	1.000
ProS	0.001	0.114	0.381*	0.148	0.075	0.108	0.050	0.140	-0.152	-0.109	-0.025	0.018	0.031
ProR	0.031	0.119	0.346*	0.128	0.163*	0.140	0.063	0.129	-0.160	-0.064	0.001	0.013	0.052
PnS	-0.049	-0.185	0.589*	0.384*	0.039	0.131	-0.001	0.067	0.037	-0.100	-0.090	0.054	0.140
PnR	-0.082	-0.175	0.278*	0.221*	0.170*	0.130	-0.096	0.083	0.145	-0.074	-0.062	0.020	0.128
ES	-0.137	-0.275*	0.351*	0.400*	0.072	0.243*	0.141	0.296*	0.082	0.032	0.101	0.147	0.211*
ER	-0.067	-0.329*	0.229*	0.392*	0.028	0.101	-0.066	-0.049	-0.158	-0.020	-0.094	0.009	-0.068
CMIS	0.105	-0.018	-0.272*	-0.117	0.087	-0.052	0.138	-0.092	-0.121	0.047	-0.101	0.010	-0.150
CMIR	0.056	-0.025	-0.290*	-0.136	0.127	-0.144	0.049	-0.088	-0.218*	0.030	-0.213*	-0.116	-0.299*
CTDS	-0.018	0.032	0.137	0.003	-0.036	-0.082	-0.039	0.024	-0.001	0.290*	0.100	-0.018	0.135
CTDR	0.138	0.263*	-0.065	-0.249*	-0.037	-0.285*	-0.060	-0.359*	-0.062	0.275*	0.050	-0.208*	0.145
LA	-0.055	0.051	-0.066	-0.050	-0.110	-0.211*	0.004	-0.098	0.104	-0.079	0.021	-0.135	0.058
SG	-0.561*	-0.629*	0.360*	0.719*	0.117	0.404*	-0.001	0.360*	0.175	-0.203*	0.058	0.383*	0.262*
HSI	0.171	0.130	-0.318*	-0.257*	0.036	0.020	0.033	-0.148	-0.200	-0.077	-0.591*	-0.253*	-0.589*

Correlation significance at 5% probability

Table 4. Phenotypic (lower diagonal) and genotypic (upper diagonal) correlation among different physiological and morphological traits under heat stress conditions

Traits	ProS	ProR	PnS	PnR	ES	ER	CMIS	CMIR	CTDS	CTDR	LA	SG	HSI
DH	0.014	-0.035	0.186*	0.114	0.140	0.100	-0.118	-0.178*	-0.045	-0.297*	-0.103	0.276	-0.138
DA	-0.308*	-0.314*	0.061	0.019	0.086	-0.040	-0.140	-0.210*	-0.119	-0.100	0.019	0.018	-0.075
DM	-0.085	-0.005	-0.152	-0.024	0.097	0.060	-0.097	-0.092	0.188*	0.158*	-0.137	-0.178*	-0.093
GFD	-0.023	-0.029	-0.195*	-0.101	-0.033	-0.089	-0.094	-0.117	0.108	0.185*	-0.075	-0.264*	-0.021
PH	-0.006	-0.045	0.184	0.164	-0.100	0.068	-0.003	-0.052	0.308*	0.264*	-0.149	-0.022	-0.098
FLA	-0.131	-0.081	0.242*	0.352*	-0.225*	-0.088	-0.041	-0.055	0.341*	0.699*	0.192	-0.709*	-0.110
SL	0.131	0.117	-0.060	-0.065	0.043	0.077	-0.044	-0.032	0.227*	0.164	0.143	-0.126	-0.084
SPS	0.183	0.197	0.096	0.067	0.252*	0.244*	-0.090	-0.101	0.269*	-0.051	-0.007	0.169	-0.286*
TP	0.209*	0.170	0.366*	0.306*	0.200	0.162	-0.327*	-0.318*	0.278*	0.142	0.199	-0.062	-0.562*
GPS	0.248*	0.214*	0.113	0.084	0.085	0.133	-0.018	-0.126	0.108	-0.204	-0.148	0.210*	0.003
TGW	0.052	0.051	0.670*	0.339*	0.342*	0.374*	-0.030	-0.052	0.091	-0.191	-0.033	0.232*	-0.263*
BM	-0.011	-0.006	0.416*	0.383*	0.144	0.089	-0.152	-0.147	0.179	0.032	0.119	-0.100	-0.251*
GY	0.114	0.102	0.419*	0.332*	0.376*	0.371*	-0.203*	-0.221*	0.226*	-0.180	0.033	0.236*	-0.899*
ProS	1.000	0.897*	-0.019	0.255*	0.030	0.187	-0.072	-0.005	0.043	-0.206*	0.081	0.272*	-0.032
ProR	0.661*	1.000	-0.014	0.256*	0.123	0.177	-0.067	-0.012	0.051	-0.151	-0.002	0.221*	-0.052
PnS	0.620*	0.586*	1.000	0.526*	0.047	0.292*	-0.014	0.022	-0.157	-0.234*	-0.120	0.288*	-0.141
PnR	0.261*	0.279*	0.930*	1.000	0.107	0.431*	-0.173	-0.016	0.014	-0.104	-0.055	0.303*	-0.129
ES	0.376*	0.348*	0.914*	0.501*	1.000	-0.037	-0.190	-0.189	0.060	-0.186*	-0.049	0.280*	-0.211*
ER	0.286*	0.304*	0.686*	0.551*	0.903*	1.000	-0.127	-0.041	0.007	-0.055	-0.049	0.164	0.068
CMIS	-0.178	-0.161	-0.222	-0.159	-0.275*	-0.172	1.000	0.679*	-0.072	-0.005	-0.109	-0.087	0.150
CMIR	-0.136	-0.103	-0.182	-0.021	-0.241*	-0.137	0.677*	1.000	-0.051	-0.009	-0.037	-0.080	0.299*
CTDS	0.157	0.169	0.332*	0.266*	-0.027	-0.083	-0.082	-0.052	1.000	0.235*	0.070	-0.098	-0.134
CTDR	0.106	0.100	0.142	0.200	-0.304*	-0.186	-0.016	-0.016	0.224*	1.000	0.137	-0.563*	0.145
LA	-0.113	-0.118	-0.106	0.051	-0.151	-0.105	-0.108	-0.034	0.069	0.133	1.000	-0.108	-0.059
SG	0.180	0.164	0.173	0.099	0.482*	0.425*	-0.087	-0.080	-0.094	-0.571*	-0.103	1.000	-0.261*
HSI	-0.150	-0.117	-0.252*	-0.139	-0.281*	-0.178	0.147	0.298*	-0.140	0.134	-0.060	-0.261*	1.000

Correlation significance at 5% probability

Table 5. Cluster mean value and Mahalonobis statistic for physio-morphic indices.

Traits	Cluster-I	Cluster-II	Cluster-III
DAH	84.279	84.206	84.524
DAA	88.332	88.228	87.527
DM	90.187	90.130	90.118
GFD	84.535	84.474	84.216
ProS	106.715	129.460	153.676
ProR	123.908	141.815	156.214
PnS	61.338	60.538	63.598
PnR	53.723	55.374	61.231
ES	62.927	61.885	66.139
ER	43.341	43.446	50.700
CMIS	29.636	30.963	28.197
CMIR	19.771	21.178	19.889
CTDS	10.236	10.157	10.349
CTDR	9.030	9.347	9.569
LA	71.815	75.050	71.489
SG	43.993	47.158	56.865
PH	75.536	75.591	76.649
FLA	48.445	49.831	52.604
SL	84.959	84.749	85.608
SPS	86.911	88.339	88.604
TP	78.979	78.977	76.007
GPS	85.580	85.241	84.846
TGW	88.127	88.608	87.565
BM	86.627	86.229	87.402
GY	68.593	70.140	70.662
HSI	1.037	0.986	0.969
Mahalonobis distance			
Cluster-I	0.00000	33.60283	137.7059
Cluster-II	5.79679	0.00000	40.2487
Cluster-III	11.73482	6.34419	0.0000

Cluster analysis

Cluster analysis classified wheat genotypes into three clusters. D^2 statistics (Mahalonobis distance) revealed highest distance among cluster-I and cluster-III (137.71) followed by cluster-II and cluster-III (40.25) and cluster-I and cluster-II (33.60) that indicated the highest genetic diversity of genotypes among these clusters. Least genetic diversity was observed among the members of cluster-I and cluster-II as shown in Table 5. Cluster-III retained wheat genotypes with highest values for grain yield, flag leaf area, stay green, biomass, spikelet per spike, plant height, spike length, canopy temperature depression at reproductive, proline content, photosynthetic and transpiration rate at both stages. Whereas, least values were observed for cell membrane injury at vegetative stage, tillers per plant and heat susceptibility index for grain yield.

Cluster-I had highest mean value for heat susceptibility index for grain yield, maturity period and grain filling duration among clusters. Whereas, least values were noticed for grain yield, stay green, photosynthetic and transpiration rate at reproductive stage, spikelet per spike, and canopy temperature depression at reproductive stage, proline content at both vegetative and reproductive stage. Furthermore, cluster analysis differentiated the wheat genotypes into three clusters viz., cluster-I (sixty seven genotypes), cluster-II (forty seven genotypes) and cluster-III (forty four genotypes) as shown in Table 6.

PCA based traits indicated the highest and lowest values in these clusters. Characterization of these wheat genotypes in Cluster-III as thermotolerant genotypes due to high values for stay green, grain yield, canopy temperature depression at reproductive stage and least value for heat susceptibility index for grain yield. Members in Cluster-I were considered as temperature sensitive due to least value of these traits except heat susceptibility index for grain yield which is negatively correlated with grain yield, it indicated the highest value in Cluster-I. Members in Cluster-II were considered as moderately heat tolerant.

Discussion

High temperature above the cardinal temperature for wheat can causes reduction in growth of wheat. Cardinal temperature for wheat growth at heading (16-20ºC), anthesis (22-25ºC) and grain filling duration (26-28ºC) are required that maintains the stability of metabolic activities and physiological process (Asseng *et al.*, 2015; Tack *et al.*, 2015; Farooq *et al.*, 2011; Al-Khatib & Paulsen 1999). In our study, we delayed planting that exposed wheat genotypes to terminal heat stress during grain filling duration. Furthermore, temperature reached up to 35ºC during grain filling duration which reduced the translocation of carbohydrates into grain and ultimately reduced its weight. High temperature above the 25ºC was also observed during anthesis that reduced the grain formation as shown in Fig. 1. Incessant enhanced temperature between 30-35ºC during grain filling duration can causes 20-50% reduction in yield (Tewolde *et al.*, 2006).

Table 6. Genotypes classified in different clusters based on physio-morphic indices.

Clusters	Genotypes	Genotypes with genetic distance
Heat tolerant	44	Pakistan-13 (9.574), Millet-11 (7.777), Chakwal-50 (8.026), AARI-10 (8.729), Miraj-08 (7.789), 306 (7.643), 307 (8.367), 312 (7.883), 317 (9.086), 349 (9.271), 407 (8.239), 409 (11.684)
Moderately heat tolerant	47	Lasani-08 (9.122), Inqlab-91 (8.186), Bahawalpur-2000 (8.144), Chakwal-86 (8.066), Pak-81 (7.705), Blue silver (9.245), Fakhre-sarhad (7.775), Shalimar-88 (7.674), Wafaq-2001 (8.719), FSD-08 (7.783), AAS-11 (7.572), 304 (7.719)
Heat susceptible	67	Anmol-91 (10.110), Bhakhar-02 (8.512), Barani-83 (10.135), Sonalika (7.949), SUSSUI (9.527), Attila © (8.735), 322 (8.940), 348 (9.443), 350 (8.466), 414 (7.871), 417 (7.973)

Heat stress force the plant to complete it vegetative phase and enter in reproductive phase early than normal by completing its growing degree days with compensating its growth and development. Our results indicated the reduction in relative performance of physio-morphic traits under heat stress as compared to normal conditions. Reduction in performance of heading, maturity period, anthesis period, grain filling duration (Mondal *et al.*, 2016) flag leaf area, spike length, spikelet per spike, grains per spike, plant height, 1000-grain weight, biomass, grain yield (Elbashir *et al.*, 2017; Khan *et al.*, 2018; Hussain *et al.*, 2018), proline content, photosynthetic rate, transpiration rate in wheat were also observed under heat stress conditions. Cooler canopy and stability of membrane under heat stress improve the yield in wheat (Bilge *et al.*, 2008). Stay green maintained greenness of plants under heat stress and stabilized the production and translocation of photosynthates that ultimately increased grain yield (Adu *et al.*, 2011). In our study cooler canopy temperature along with high proline content, stay green, photosynthetic and transpiration rate were observed in tolerant genotypes under heat stress conditions.

PCA analysis revealed the 68.23% variation in first nine components under heat stress conditions. Similarly, Tian *et al.*, (2015) found first four component retained 88.35% variation based on physiological traits. In our study, highest loading component were stay green, grain yield, canopy temperature depression at reproductive stage and heat susceptibility index which suggested these traits are effective selection criteria for selecting tolerant genotypes against heat stress conditions. Nagar *et al.*, (2015) performed PCA for physio-morphic traits and identified grain yield, photosynthetic rate and membrane stability index under heat stress conditions. Furthermore, others traits such as plant height, seeds per spike (Khodadadi *et al.*, 2011), days to anthesis (Fahim, 2014), seeds per spike (Beheshtizadeh *et al.*, 2013), cell membrane injury (Ahmad *et al.*, 2019) 1000-grain weight (Rymuza *et al.*, 2012), grain yield and biomass (Hailegiorgis *et al.*, 2011; Mishra *et al.*, 2015; Ahmad *et al.*, 2014) were found efficient selection criteria to identify genetic divergence among wheat genotypes.

Genotypic and phenotypic correlation analysis in our study suggested that increased 1000-grain weight, stays green, grains per spike, photosynthetic rate, transpiration rate, cooler canopy, proline content at both vegetative and reproductive phase can improve the grain yield in wheat under heat stress conditions. Negative association indicated that reduced cell membrane injury and heat susceptibility index for grain yield can enhance the yield under heat stress. Similar association among physio-morphic traits with grain yield were also observed by Ahmad *et al.*, (2010), Sunita *et al.*, (2017), Khan & Kabir (2014), Dhanda & Munjal (2017) and Kamrani *et al.*, (2017) under heat stress conditions.

Cluster analysis categorized wheat genotypes into three clusters. Mahalonobis distance revealed the highest genetic difference among cluster-I and cluster-III. The higher genetic distance indicated the genetic diversity among members retaining in these clusters which would be useful in further breeding programs for developing variation against different environmental conditions. PCA identified imperative traits viz., grain yield, stay green, canopy temperature depression and heat susceptibility index for grain yield. So we identify the heat tolerant genotypes in cluster-III having high value of grain yield, stay green, canopy temperature depression and least value of heat susceptibility index. Wheat genotypes in cluster-I were designated as heat susceptible due to low values of these traits selected in PCA that would be useful in further breeding program for substantial variability against heat stress conditions.

Conclusion

High temperature adversely reduced the growth and development of wheat by inhibiting physiological process and metabolic activities. Stay green, grain yield, canopy temperature depression at reproductive stage and heat susceptibility index for grain yield can be utilize as effective criteria for selecting tolerant germplasm against heat stress. Genotypic and phenotypic correlation indicated that these traits exhibited significant positive association with grain yield except heat susceptibility index for grain yield. These results suggested that increased stay green, canopy temperature depression at reproductive stage and reduced heat susceptibility index can improve the grain yield in wheat. Furthermore, cluster analysis discriminated heat tolerant and susceptible genotypes based on traits selected through PCA that could be used in further breeding programs for developing variations against heat stress conditions.

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