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GENETIC MANIFESTATION OF PHYSIO-MORPHIC AND YIELD RELATED TRAITS CONFERRING THERMOTOLERANCE IN WHEAT

ADEEL KHAN[1*], MUNIR AHMAD[1], MUHAMMAD KAUSAR NAWAZ SHAH[1] AND MUKHTAR AHMED[2]

[1]Department of Plant Breeding & Genetics, PMAS-Arid Agriculture University Rawalpindi (46300), Pakistan
[2]Department of Agronomy, PMAS-Arid Agriculture University Rawalpindi (46300), Pakistan
**Corresponding author's email: adeelkhanbreeder@gmail.com*

Abstract

High temperature threatens the sustainability of wheat productivity. Knowledge of gene action and combining ability of parents assist the breeders to develop heat resilient wheat cultivars. Therefore, present study was designed to ascertain the combining ability of parents, nature of gene action and heritability under delayed planting mediated heat stress. Initially five heat tolerant genotypes were selected form 158 wheat genotypes on the basis of least cell membrane injury, high rate of photosynthesis, proline content, transpiration rate and cooler canopy temperature at seedling stage. Heat tolerant genotypes as male parents were crossed with ten high yielding genotypes as female parents following line × tester mating design. Fifty F_1 hybrids along with 15 parents were evaluated under normal and heat stress for physio-morphic traits. Results revealed that all traits except stay green, plant height, leaf area, spike length and tillers per plant were controlled by additive genetic effects under both normal and heat stress conditions. High narrow sense heritability was noticed for canopy temperature depression at reproductive stage (65%), transpiration rate at seedling stage (73%), photosynthetic rate at seedling and reproductive (83%, 73%), proline content at seedling and reproductive (64%, 62%). High heritability with additive genetic effects suggested the utilization of these traits with modified pedigree selection for developing thermotolerant varieties.

Key words: Gene action, Combining ability, Heritability, Photosynthetic rate, Canopy temperature depression, Wheat.

Abbreviations: D: Additive genetic effects, H: Dominance genetic effects, E: Environmental variation, $h^2_{(n.s)}$: Narrow sense heritability, DH: Days to heading, DA: Days to anthesis, DM: Days to maturity, GFD: Grain filling duration, PH: Plant height, FLA: Flag leaf area, TP: Tillers per plant, SL: Spike length, SPS: Spikelet per spike, GPS: Grains per spike, TGW: Thousand grain weight, BM: Biomass per plant, GY: Grain yield per plant, ProS: Proline content at seedling stage, ProR: Proline content at reproductive stage, PnS: Photosynthetic rate at seedling stage, PnR: Photosynthetic rate at reproductive stage, ES: Transpiration rate at seedling stage, ER: Transpiration rate at reproductive stage, CMIS: Cell membrane injury at seedling stage, CMIR: Cell membrane injury at reproductive stage, CTDS: Canopy temperature depression at seedling stage, CTDR: Canopy temperature depression at reproductive stage, SG: Stay green.

Introduction

Wheat is imperative edible food crop for the population of world and cheapest energy source that provides 65-76% calories and 8-15% proteins in average diet (Day, 2013). Wheat productivity is highly affected by increasing threats of high temperature around the globe due to altering climatic conditions (Christensen & Christensen, 2007) and it is predicted to increase by 1.5-4.5ºC at the end of 21st century (Gao *et al.*, 2017).

Delayed sowing mediated heat stress is major concern about 40% in both developing and developed countries that covers 35 million hectares (Moshatati *et al.*, 2017). It occurs during the heading, anthesis and grain filling phase that drastically reduces the grains formation (Modhej *et al.*, 2012; Afshan & Farooqi, 2020) and grain development (Mohammadi, 2012; Hussain *et al.*, 2018). High temperature reduces the grain filling duration by completing the growing degree days earlier and diminishes the duration to capture reserves for grain development (Khan *et al.*, 2018). Longer grain filling duration facilitates the longer time to capture available resources and improve the grain weight (Talukder *et al.*, 2014). It also reduces the viability of pollen and ovaries that inhibits the formation of grains in spikes (Calderini *et al.*, 1999). Heat stress promotes unsaturation of fatty acids in plasma membrane that destabilizes the photosynthetic activity (Almeselmani *et al.*, 2012). Furthermore, high canopy temperature also disturbed the photosynthetic process and metabolic activities in wheat (Ahmad *et al.*, 2019; Ray & Ahmed, 2015).

Genetic potential exist in wheat germplasm for heat tolerance that can be exploited by exploring best combiners. General combining ability (GCA), specific combining ability (SCA) and gene action aid to develop thermotolerance in wheat. Line × tester is an important tool to study the combining ability and nature of gene action. This technique helps to isolate segregating genotypes and select best genotypes on the basis of their hybrids performance (Saeed & Khalil, 2017). GCA indicates main effects whereas SCA represents the interaction effects (Fasahat *et al.*, 2016). Gene action and combining ability in wheat have been observed for heading and maturity time (Tsenov & Tsenova, 2011), grain filling duration (Singh *et al.*, 2007), grains per spike, plant height, grains yield, thousand grain weight, biomass (Cifci & Yagdi, 2010; Irshad *et al.*, 2014b; Singh *et al.*, 2007; Raj & Kandalkar, 2013; Sprague & Tatum, 1942), leaf area, spike length (Aslam *et al.*, 2014; Mahpara *et al.*, 2008; Srivastava *et al.*, 2012), cell membrane injury, canopy temperature depression, proline content (Dhanda & Munjal, 2009; Farooq *et al.*, 2011; Irshad *et al.*, 2014a; Punia *et al.*, 2011b; Ram *et al.*, 2014; Yildirim *et al.*, 2009) under heat stress conditions.

Hence, this study aims to assess the good combiner testers, lines and crosses as well as nature of gene action and narrow sense heritability for cell membrane injury, canopy temperature depression, proline content, photosynthesis, transpiration rate, yield and its related components under heat stress conditions.

Materials and Methods

Research work was conducted to explore genetic potential of wheat for heat tolerance at University Research Farm of PMAS-Arid Agriculture University Rawalpindi (33.11°N, 73.01°E) Pakistan during 2016-2018. Experimental material comprised of wheat varieties/ genotypes from Pakistan and CIMMYT (23rd semi-arid wheat yield trial and 24th semi-arid wheat yield trial).

Identification of heat tolerant parents for physiological traits: Diverse germplasm 158 wheat genotypes from Pakistan and CIMMYT were evaluated under normal (1st week of November) and delayed (1st week of December) planting during 2016-17. Pre-basic soil analysis was conducted before sowing. Soil was sandy clay, organic matter 0.7%, pH 7.6, moisture 3.1%, E.C 0.19 desi siemens/meter, Nitrogen 0.4 mg/g, Phosphorous 9.6 mg/L, Potassium 84 mg/L. Urea @ 120Kg/ha and DAP @ 60Kg/ha were applied before sowing. Genotypes were sown in augmented complete block design with three replications. Row length was kept 10m whereas plant to plant distance was maintained 15cm and row to row distance 25cm. All other recommended agronomic practices viz., weeding, hoeing, pest scouting were done as per requirement. Ten plants were selected randomly based on physiological traits during vegetative phase at Zadoks scale 39 (Zadoks *et al.*, 1974) viz., cell membrane injury and proline content in labortary whereas canopy temperature depression, photosynthetic rate and transpiration rate in field conditions. Five heat tolerant genotypes (Pakistan-13, Aas-11, Miraj-08, genotype 307 from 23rd SAWYT and 409 from 24th SAWYT) were selected on the basis of performance against heat stress whereas ten high yielding genotypes (Punjab-11, Chakwal-50, line AUR-09, line AUR-10, Inqalab-91, Dharabi-11, Pirsabak-05, Pirsabak-08, Pirsabak-15 and Ihsan-16) were selected on the basis of high grain yield in rain-fed conditions.

Development of population: Five heat tolerant genotypes as male parent and ten high yielding female parents were planted in glass house. Recommended dose of fertilizer was applied N@120 Kg/acre and P@60 Kg/acre. Irrigation was applied at the interval of 3-4 weeks along with recommended agronomic practices i.e. weeding and hoeing. Ten high yielding (female parents) were crossed with five heat tolerant genotypes (male parents) in line × tester mating design. At maturity, these plants were threshed and collected seeds were preserved for further evaluation.

Evaluation of genetic material: Fifty F_1 hybrids along with fifteen parents were planted under normal (1st week of November, 2017) and delayed planting mediated heat stress (1st week of December, 2017) conditions following RCBD with three replications. Row length was kept at 3m while plant to plant distance was maintained at 15cm and row to row at 30cm. All agronomic practices, weeding, hoeing, irrigation and recommended rate of fertilizer were applied. Ten plants were selected randomly from each replication for data collection.

Data collection

Data was collected for 50% heading (Zadoks scale 55), anthesis (Zadoks scale 64), grain filling duration (anthesis to physiological maturity) and 50% physiological maturity period (Zadoks scale 88). Morphological traits were recorded at Zadoks scale 90 for plant height, spikelet per spike, spike length, thousand grain weight, grains per spike, tillers per plant, biomass per plant and grain yield per plant whereas flag leaf area at Zadoks scale 39 and leaf angle between flag leaf and main stem at Zadoks scale 61.

Physiological traits were measured at both seedling (Zadoks scale 39) and reproductive stage (Zadoks scale 69). Cell membrane injury was measured according to Deshmukh *et al.*, (1991) from two sets of leaf sample soaked in 4 ml deionized water and exposed the first sample to 40ºC heat stress. Deionized water (16 ml) was added to make the volume 20 ml subsequently incubation at 10ºC for 24 hours and first electric conductivity (EC) was measured (T1 and C1). These tubes were autoclaved and second EC measured (T2 and C2). CMI was measured using formula:

$$\text{CMI (\%)} = 1- [\{1- (T1/T2)\}/\{1-(C1/C2)\}] \times 100$$

Proline content were estimated using method described by Bates *et al.*, (1973). Briefly, 0.5g leaf was ground in 3% sulphosalicyclic acid follwed by centrifugation for 10 min @10K and 2 ml supernatant was transferred in test tubes. Then 2 ml glacial acetic acid was added along with 2 ml acid ninhydrin and boiled in water bath at 100ºC for 1 hour. After cooling 4 ml toluene was added in tubes and the absorbance was checked at 520 nm on spectrophotometer after 30 minutes. Proline content was calculated using formula:

$$\mu\text{moles proline/g of plant sample} = [\{(\mu\text{g proline/ml} \times \text{ml toluene})/115.5\ \mu\text{g}/\mu\text{moles}\}/ (\text{g sample}/5)]$$

Transpiration and photosynthetic rates were recorded according to Long & Bernacchi (2003) on sunny day between 10:00am to 12:00pm with IRGA (Infrared gas analyzer), Model LCA-4, ADC, Hoddesdon, UK by fixing PAR at 1600 mmol m^{-2} s^{-1} and CO_2 at 360 mmol mol^{-1}.

Canopy temperature was measured using Infrared thermometer (AG-42, tela-temp crop, Fullerton, CA) during day time 10:00am to 12:00pm that was subtracted from air temperature to calculate the canopy temperature depression (Amani *et al.*, 1996). Stay green was recorded using LAUG approach by scoring 0-9 for green area of flag leaf and spike form anthesis to physiological maturity at interval of 3-5 days (Joshi *et al.*, 2007). Stay green was calculated using formula:

$$\text{LAUG} = \sum_{i=1}^{a}[\left\{\frac{Yi+Y(i+1)}{2}\right\} times\ (t(i+1)-ti)]$$

Yi = difference between spike and flag leaf greenness score (scale 0-9) while $t_{(i+1)}$-t_i= time interval between two readings

Table 1. Mean and relative performance of selected five heat tolerant and ten high yielding genotypes during 2016-17 trial.

Genotypes	Pedigree	ProSN	ProSH	RP	PnSN	PnSH	RP	ESN	ESH	RP	CMIS	CTDS	LA	GY
Pakistan-13	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN	0.431	0.573	133	26.99	22.69	84	0.48	0.43	90	16.24	15.33	15	9.1
Aas-11	PRL/PASTOR//2236(V6550/SUTLEH-86)	0.392	0.562	143	24.33	21.77	89	0.48	0.37	78	15.02	14.62	9	9.2
Miraj-08	SPARROW/INIA//V.7394/WL711/13/BAUS	0.378	0.529	140	25.80	21.96	85	0.50	0.45	90	16.83	15.45	9	8.7
307	PFUNYE #1/KINGBIRD #1	0.418	0.577	138	25.06	21.98	88	0.48	0.40	83	12.60	14.80	14	8.4
409	WAXWING/7/TNMU/6/CEP80111/CEP81165/5/IAC5/4/YKT406/3/AG/ASN//ATR/8/ATTILA/3*BCN//BAV92/3/TILHI/4/SHA7/VEE#5//ARIV92	0.411	0.565	137	25.11	22.47	89	0.54	0.47	87	16.71	14.90	12	8.9
Punjab-11	AMSEL/ATTILA//INQ-91/PEW'S'	0.410	0.433	106	20.68	10.92	53	0.30	0.17	57	17.94	13.10	12	9.8
Chakwal-50	ATTILA/3/HUI/CARC//CHEN/CHTO/4/ATTILA	0.315	0.323	102	20.86	8.70	42	0.37	0.28	76	37.84	14.13	18	9.6
AUR-09	SUNSU/CHIBIA CMSS00M02326S-030M-0.30WGY-030M-6M-0Y	0.351	0.362	103	21.05	16.17	77	0.36	0.26	72	22.83	13.48	12	9.7
AUR-10	WBLL1*2/4/YACO/PBW65/2KAUZ*2/TRAP/KAUZ CGSS01Y00054T-099M-099Y-099M-099M-20Y-0B	0.344	0.412	120	23.31	13.26	57	0.42	0.34	81	17.38	15.03	16	9.8
Inqalab-91	WL 711/CROW "S"	0.417	0.505	121	16.26	12.86	79	0.31	0.21	68	16.18	14.03	14	9.8
Pirsabak-05	MUNIA/CHTO//AMSEL	0.341	0.411	121	16.27	8.24	51	0.31	0.15	48	17.64	13.78	15	10.0
Pirsabak-08	SPARROW/INIA//V.7394/WL711/13/BAUS	0.336	0.449	134	17.19	11.51	67	0.26	0.15	58	15.38	14.25	14	10.2
Pirsabak-15	CS/TH.SC//3*PVN/3/MIRLO/BUC/4/MILAN/5/TILHI	0.244	0.269	110	23.16	11.06	48	0.49	0.33	67	18.02	13.33	18	9.9
Dharabi-11	HXL7573/2*BAU//PASTOR	0.316	0.393	124	22.21	10.79	49	0.48	0.35	73	17.20	12.58	17	10.5
Ihsan-16	PASTOR/3/ALTAR84/AE.SQ//OPATA	0.321	0.419	131	23.89	12.13	51	0.41	0.23	56	23.81	15.23	8	9.6

Statistical analysis

Data was analyzed following Augmented complete block design using PROC Mixed with entries and treatments fixed and block random in statistical software SAS (Scott & Milliken, 1993) to identify the heat tolerant genotypes. Relative performance was calculated according to Asana & Williams (1965). Combining ability analysis was performed using the method described by Kempthorne (1957) and narrow sense heritability $h^2_{(n.s)}$ was estimated following Kown & Torrie (1964).

Results

Selection of parents: Relative performance indicated the overall reduction in photosynthetic rate (37.6%) and transpiration rate (31.7%) among studied wheat genotypes whereas proline content (117.7%) was enhanced under heat stress conditions (Table 1). Furthermore, performance of wheat genotypes for proline content was 0.199-0.631 µmole/g and 0.362-0.769 µmole/g, photosynthetic rate 13.2-29.9 µmol $m^{-2}s^{-1}$ and 8.1-19.9 µmol $m^{-2}s^{-1}$, transpiration rate 0.19-0.60 mmol $m^{-2}s^{-1}$ and 0.08-0.41 mmol $m^{-2}s^{-1}$ under normal and heat stress conditions respectively. Cell membrane injury 11.4%-51.9%, canopy temperature depression 15.5-10.8ºC and leaf angle 6º-42º from tolerant to susceptible genotypes were observed. Five heat tolerant parents selected on the basis of heat tolerant indices were crossed with ten high yielding genotypes to develop heat tolerant high yielding genotypes.

Genetic estimates of F_1 along parents: Higher general combining ability estimates for lines were observed for Pirsabak-05, AUR-09 and AUR-10 for photosynthetic rate, cell membrane injury, canopy temperature depression, grains per spike, thousand grain weight, flag leaf area and tillers per plant whereas Dharabi-11 for grains yield and spikelet per spike under heat stress conditions (Table 2). Furthermore, higher GCA estimates were observed by Pirsabak-15 for biomass, days to heading and days to anthesis, Inqalab-91 for grain filling duration and Chakwal-50 for stay green.

High GCA estimates among testers for photosynthetic rate, spikelet per spike, grain yield, thousand grain weight and tillers per plant were observed by Pakistan-13 whereas days to heading, anthesis, maturity, leaf area and biomass were observed by Aas-11. Physiological traits such as proline content, canopy temperature depression, cell membrane injury and stay green were observed by genotype 307 and Miraj-08.

Specific combining ability (SCA) results revealed the higher SCA estimates for Pirsabak-15 × Pakistan-13 for days to maturity, plant height and proline content, Pirsabak-05 × Miraj-08 for tillers per plant and thousand grain weight, spike length and spikelet per spike for AUR-10 × 307 and days to anthesis and grain yield for Chakwal-50 × Pakistan-13. Furthermore, high estimates of SCA were observed by Pirsabak-08 × Aas-11 for photosynthetic rate, Inqalab-91 × 409 for transpiration rate and Inqalab-91 × Pakistan-13 for stay green.

Table 2. Parents and Crosses exhibited high GCA and SCA estimates for traits under normal and heat stress.

Traits	Lines		Testers		Crosses	
	Normal	Heat	Normal	Heat	Normal	Heat
Days to 50% heading	Punjab-11, Pirsabak-08	Pirsabak-15, AUR-10	Pak-13, Aas-11	Miraj-08, Aas-11	Ihsan-16 × 409, Pirsabak-05 × 409	Ihsan-16 × Aas-11, Pirsabak-05 × Pakistan-13
Days to anthesis	Chakwal-50, Pirsabak-15	Dharabi-11, Punjab-11	Aas-11, Pakistan-13	307, Pakistan-13	Chakwal-50 × Miraj-08, Chakwal-50 × Pakistan-13	Chakwal-50 × Pak-13, Chakwal-50 × 307
Days to 50% physiological maturity	Pirsabak-05, Dharabi-11	Pirsabak-15, Dharabi-11	Aas-11, Miraj-08	409, 307	Pirsabak-15 × Pakistan-13, Pirsabak-05 × 307	Punjab-11 × Miraj-08, Chakwal-50 × 307
Grain filling duration	Chakwal-50, Dharabi-11	Dharabi-11, AUR-10	Aas-11, Miraj-08	307, 409	Chakwal-50 × Miraj-08, Punjab-11 × Pakistan-13	Chakwal-50 × Miraj-08, Pirsabak-08 × Aas-11
Plant height	Inqalab-91, Pirsabak-08	Inqalab-91, Punjab-11	Pakistan-13, Miraj-08	Pakistan-13, Miraj-08	Punjab-11 × Pakistan-13, Pirsabak-15 × Pakistan-13	Pirsabak-15 × Pakistan-13, Punjab-11 × Pakistan-13
Flag leaf area	Pirsabak-05, Punjab-11	Dharabi-11, AUR-09	Pakistan-13, Miraj-08	409, 307	AUR-09 × 307, AUR-09 × Miraj-08	Dharabi-11 × Aas-11, Pirsabak-05 × Pakistan-13
Tillers per plant	Chakwal-50, Pirsabak-05	AUR-10, Punjab-11	Aas-11, Pakistan-13	Aas-11, Pakistan-13	Pirsabak-05 × Aas-11, AUR-10 × Miraj-08	Pirsabak-05 × Miraj-08, Punjab-11 × Miraj-08
Spike length	AUR-10, Inqalab-91	AUR-10, Inqalab-91	Miraj-08, Pakistan-13	Pakistan-13, Miraj-08	Pirsabak-08 × 409, AUR-09 × Miraj-08	Pirsabak-08 × 409, AUR-09 × Miraj-08
Spikelet per spike	Pirsabak-15, Dharabi-11	Pirsabak-05, Chakwal-50	Miraj-08, Pakistan-13	Aas-11, 409	Pirsabak-15 × 307, Pirsabak-15 × Aas-11	AUR-10 × 307, Pirsabak-08 × 409
Grains per spike	AUR-09, AUR-10	AUR-09, AUR-10	Pakistan-13, Aas-11	Pakistan-13, Aas-11	Ihsan-16 × Miraj-08, Pirsabak-15 × Miraj-08	Inqalab-91 × Aas-11, Pirsabak-05 × Aas-11
Thousand grain weight	Pirsabak-05, Pirsabak-15	Dharabi-11, Pirsabak-05	Miraj-08, Pakistan-13	Miraj-08, Pakistan-13	Pirsabak-08 × Pakistan-13, Pirsabak-08 × Miraj-08	Pirsabak-05 × Miraj-08, Pirsabak-15 × 307
Biomass per plant	AUR-09, Dharabi-11	Pirsabak-05, AUR-09	Pakistan-13, 307	Pakistan-13, Aas-11	Ihsan-16 × 409, Pirsabak-08 × Aas-11	Ihsan-16 × 409, AUR-10 × 409
Grain yield per plant	Punjab-11, Dharabi-11	Dharabi-11, Pirsabak-08	Pakistan-13, Miraj-08	Aas-11, Pakistan-13	AUR-09 × Miraj-08, Punjab-11 × Miraj-08	Chakwal-50 × Pakistan-13, Inqalab-91 × Aas-11
Proline content at seedling stage	Pirsabak-08, Inqalab-91	Punjab-11, Inqalab-91	Aas-11, Pakistan-13	Aas-11, Pakistan-13	Chakwal-50 × Aas-11, AUR-10 × 409	Ihsan-16 × Miraj-08, Ihsan-16 × 409
Photosynthetic rate at seedling stage	Dharabi-11, AUR-10	AUR-09, Inqalab-91	Pakistan-13, Aas-11	Aas-11, Pakistan-13	Chakwal-50 × 307, Pirsabak-15 × Pakistan-13	Punjab-11 × Pakistan-13, Pirsabak-08 × Aas-11
Transpiration rate at seedling stage	AUR-10, Punjab-11	Punjab-11, Chakwal-50	Pakistan-13, Aas-11	409, 307	Pirsabak-08 × Pakistan-13, Chakwal-50 × 307	Inqalab-91 × 409, Chakwal-50 × Miraj-08
Proline content at reproductive stage	Pirsabak-05, Inqalab-91	AUR-09, Dharabi-11	307, 409	Miraj-08, Pakistan-13	Punjab-11 × 307, Pirsabak-08 × Aas-11	Pirsabak-05 × 307, Pirsabak-05 × Pakistan-13
Photosynthetic rate at reproductive stage	AUR-10, AUR-09	Dharabi-11, AUR-09	Miraj-08, Pakistan-13	409, Pakistan-13	Pirsabak-15 × Pakistan-13, Pirsabak-08 × Pakistan-13	Chakwal-50 × 307, Pirsabak-08 × Aas-11
Transpiration rate at reproductive stage	AUR-10, Dharabi-11	AUR-09, Dharabi-11	Pakistan-13, Aas-11	Aas-11, Pakistan-13	Inqalab-91 × Miraj-08, Punjab-11 × Pakistan-13	Inqalab-91 × 409, Pirsabak-08 × Pakistan-13
Cell membrane injury at seedling stage	Chakwal-50, Pirsabak-05		307, 409		Inqalab-91 × 307, AUR-10 × Miraj-08	
Cell membrane injury at reproductive stage	AUR-09, Punjab-11		Miraj-08, 307		AUR-09 × Aas-11, Pirsabak-08 × 307	
Canopy temperature depression at seedling	AUR-10, Pirsabak-05		Miraj-08, Aas-11		Punjab-11 × 409, AUR-10 × Miraj-08	
Canopy temperature depression at reproductive	Pirsabak-05, Dharabi-11		409, Pakistan-13		Pirsabak-15 × Aas-11, Pirsabak-15 × Pakistan-13	
Stay green	AUR-10, Chakwal-50		307, Aas-11		Inqalab-91 × Pakistan-13, Dharabi-11 × Pakistan-13	

Table. 3. Genetic estimates for yield and yield related components under normal and heat stress.

	Conditions	DH	DA	DM	GFD	PH	FLA	TP	SL	SPS	GPS	TGW	BM	GY
D	Normal	2.72	18.53	7.69	18.51	2.16	5.68	0.22	0.54	0.29	3.43	5.40	4.55	0.39
	Heat stress	3.16	0.84	1.25	2.22	3.69	4.00	0.81	0.85	0.69	5.51	2.55	2.93	0.17
H	Normal	1.43	5.15	1.54	11.45	27.42	7.48	0.13	1.88	0.19	0.75	0.44	4.57	0.07
	Heat stress	2.50	0.16	0.35	0.29	35.94	7.37	0.02	2.60	0.12	4.59	0.32	1.98	0.11
E	Normal	0.69	5.67	0.48	6.07	2.21	1.64	0.28	0.00	0.35	0.14	1.83	0.61	0.26
	Heat stress	0.18	1.31	0.64	1.48	0.15	0.29	0.15	0.01	0.49	0.19	1.14	0.52	0.26
$h^2_{(n.s)}$	Normal	56	63	79	51	7	38	35	22	45	79	75	47	60
	Heat stress	54	39	56	56	10	34	82	25	59	54	69	54	40

Table. 4. Genetic estimates for physiological traits under normal and heat stress.

	Conditions	ProS	PNS	ES	ProR	PNR	ER	CMIS	CMIR	CTDS	CTDR	SG
D	Normal	0.0001534	1.84	0.0022	0.000178	1.61	0.00211	12.30	0.21	9.88	1.41	168.47
	Heat stress	0.0001889	1.84	0.0012	0.000143	0.84	0.00036					
H	Normal	0.0000630	0.10	0.0981	0.000098	0.30	0.00136	0.31	0.13	0.23	0.39	236.81
	Heat stress	0.0001077	0.11	0.0003	0.000089	0.09	0.00125					
E	Normal	0.0000002	0.59	0.0003	0.000000	0.25	0.00027	25.66	3.07	27.41	0.37	2.48
	Heat stress	0.0000027	0.39	0.0004	0.000003	0.31	0.00037					
$h^2_{(n.s)}$	Normal	71	76	84	65	74	56	32	6	26	65	41
	Heat stress	64	83	62	62	73	50					

Nature of gene action: Additive genetic effects were exhibited by all physio-morphic studied traits except plant height, flag leaf area, tillers per plant, spike length and stay green those were controlled by non-additive genetic effects under both normal and heat stress conditions. Transpiration rate at seedling stage was exhibited by non-additive genetic effects that were changed to additive effects whereas transpiration rate at reproductive stage was controlled by additive genetic effects that were altered to non-additive effects under heat stress. Biomass per plant was governed by non-additive genetic effects that were altered to additive genetic effects under heat stress conditions as shown in Tables 3 & 4.

Heritability

Narrow sense heritability ($h^2_{n.s}$) ranged from 7%-84% under normal and 6%-83% under heat stress conditions (Table 2 & 3). Higher heritability estimates were observed for anthesis period (63%), maturity (79%), grains per spike (79%), grain yield (60%), thousand grain weight (75%), proline content at both seedling and reproductive stages (71% and 65%), photosynthetic rate at both stages (76% and 74%) and transpiration rate at seedling stage (84%) under normal conditions. Heritability estimates were higher for tillers per plant (82%), thousand grain weight (69%), proline content at both stages (64% and 62%), photosynthetic rate at both stages (83% and 73%), transpiration rate at seedling stage (62%) and canopy temperature depression at reproductive stage (65%) under heat stress condition.

Discussion

Heat stress adversely affects the metabolic activities in wheat that reduces the growth and development (Semenov & Halford, 2009). Delayed planting completes the growing degree days earlier, vulnerable to pre-flowering and reduction in grain filling duration due to enhanced temperature. Cardinal temperature of wheat crop are 16-22ºC at heading, 21-28ºC at anthesis and 26-32ºC at grain filling duration (Acevedo *et al.*, 2002; Asseng *et al.*, 2015; Tack *et al.*, 2015). However, 1ºC rise in temperature during these three critical stages can reduce 3-15% wheat yield (Lobell *et al.*, 2008). Asian countries are facing severe threats of heat stress where 4-5ºC rise in temperature at grain filling duration causes 20-40% reduction in grain yield (Ortiz *et al.*, 2008).

In current study, normal planting faced 25-28ºC temperature at anthesis and 28-32ºC at grain filling duration that was below the threshold level. Delayed planting faced the wheat plant high temperature (28-31ºC) at anthesis and (34-36ºC) grain filling duration as shown in Fig. 1. Delayed planting genotypes compensate high temperature by completing their growing days earlier with reduction in the efficiency of physiological process and metabolic activities. Similarly, targeted terminal heat stress, 23-29ºC at anthesis and 30-36ºC at grain filling stage was also observed during 2017-18 that was above the optimum temperature for wheat (Fig. 2).

Screening of genotypes is pre-requisite to develop heat tolerance in wheat. Proline content and cell membrane injury are useful indicators for selecting heat tolerant lines that maintains the osmoregulation and membrane activity for different metabolic activities (Ahmed & Hasan, 2011; Bala & Sikder, 2017; Prasad *et al.*, 2016; Rehman *et al.*, 2016; Zhang *et al.*, 2005). Cooler canopy temperature also stabilizes the transpiration rate and photosynthetic rate which sustain the metabolism under heat stress conditions (Abdipur *et al.*, 2013; Kumari *et al.*, 2013; Ray & Ahmed, 2015; Reynolds *et al.*, 2009; Saxena *et al.*, 2016).

General combining ability (GCA) estimates of testers and lines helps to select parents for productive hybrids development against heat stress (Jyoti *et al.*, 2017). Our study elucidated the parents with high combining ability for cell membrane injury, proline content, photosynthetic rate, stay green, canopy temperature depression, transpiration rate, yield and yield related components under heat stress conditions. Similarly, Punia *et al.*, (2011a) also selected parents based on physiological traits with high GCA estimates for heat stress. Ram *et al.*, (2014) suggested that parents retaining high GCA estimates along with canopy temperature depression and cell membrane injury should be utilized in breeding program against heat stress.

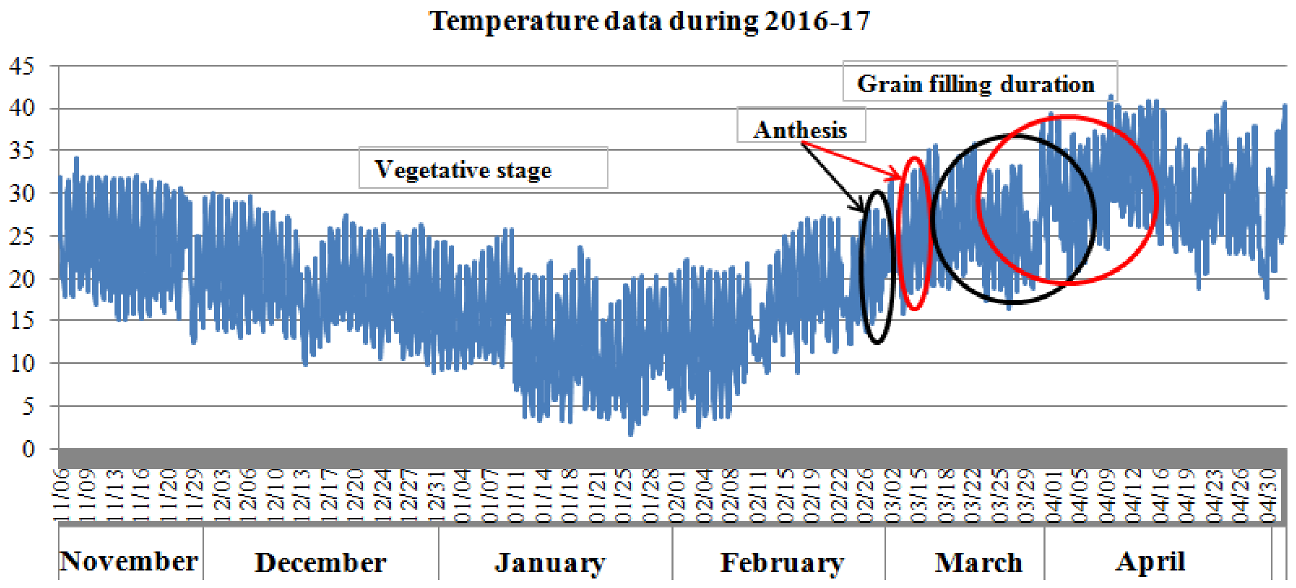

Fig. 1. Temperature data collected at interval of ten minutes during life cycle of wheat crop 2016-17.
Source: Department of Environmental Science, PMAS-Arid Agriculture University Rawalpindi.

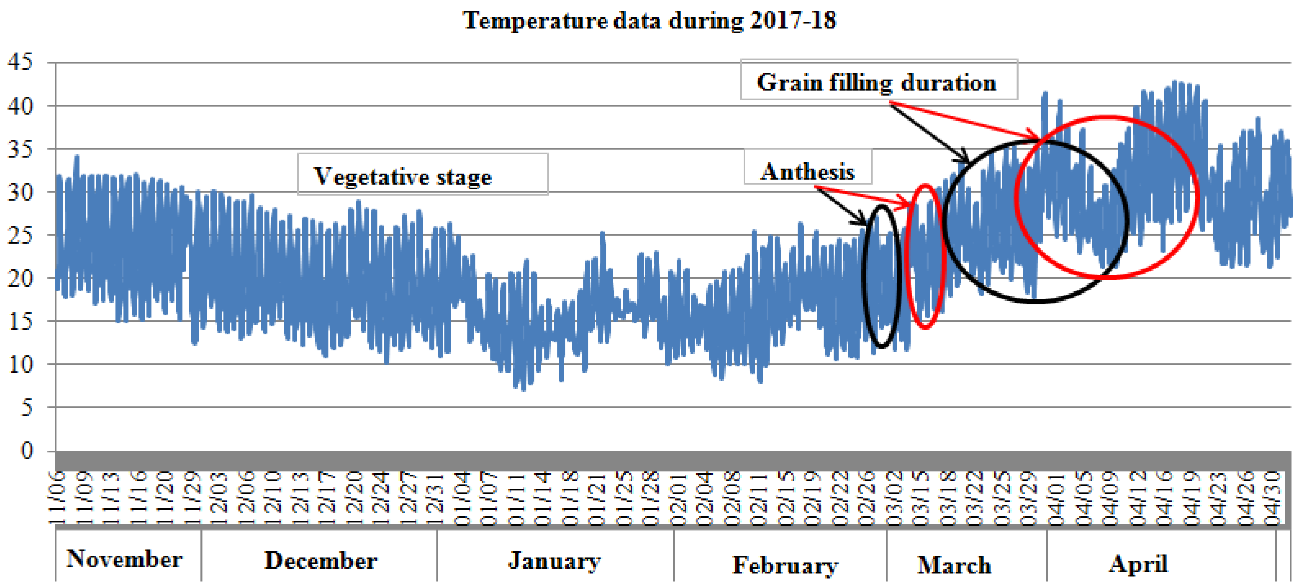

Fig. 2. Temperature data collected at interval of ten minutes during life cycle of wheat crop 2017-18.
Source: Department of Environmental Science, PMAS-Arid Agriculture University Rawalpindi.

GCA and SCA variances indicate the nature of gene action. Higher GCA variances designated the preponderance of additive genetic effects whereas higher SCA variances depicted the non-additive genetic effects. Preponderance of non-additive genetic effects suggested that selection of superior plants should be delayed in later segregating generations or heterosis breeding for improvement against heat stress. Additive genetic effects suggested that selection should be achieved in early generations or pedigree selection in segregating generations to improve traits against heat stress.

Additive genetic effects for heading, plant height, seeds per spike, thousand grain weight tillers and grain yield per plant were also observed against heat stress conditions (Farooq *et al.*, 2011; Irshad *et al.*, 2014b; Irshad *et al.*, 2012; Kaukab *et al.*, 2013). Non-additive genetic effects for yield and yield related components in wheat were detected by Istiplier *et al.*, 2015 and Titan *et al.*, 2012 those were contrary to our results. It might be due to different environmental conditions or combining ability of parents due to their different genetic makeup. Cell membrane injury and canopy temperature depression exhibited by additive genetic effects was also noticed by Irshad *et al.*, (2014a) whereas Dhanda & Munjal (2009) observed non-additive genetic effects for cell membrane injury.

Heritability estimates determines the genetic improvement and it may differ among different populations and environments (Naveed *et al.*, 2016). In our study the heritability ranged from 6-84% under both conditions and it differed under heat stress as compared to normal conditions. Abd-Allah *et al.*, (2013) also noticed 31-78% heritability for yield components in wheat under heat stress conditions. Similarly, higher estimates of heritability were also observed for seed yield, canopy temperature depression and cell membrane injury (Kumar *et al.*, 2018). High estimates of heritability coupled with additive genetic effects for photosynthetic rate, cell membrane injury, proline content, transpiration at seedling stage and canopy temperature depression at reproductive stage suggested that selection of these traits would be effective in early transgressive segregating generations against heat stress.

Conclusion

Combining ability estimates were high for lines AUR-09, AUR-10, Pirsabak-15 and tester Miraj-08, 307 for cell membrane injury, canopy temperature depression, photosynthetic rate while line Dharabi-11 and tester Pakistan-13 for grain yield under heat stress conditions. Crosses Pirsabak-15 × Pakistan-13 and AUR-10 × Miraj-08 were best for canopy temperature depression, grain filling duration, cell membrane injury and photosynthetic rate whereas Chakwal-50 × Pakistan-13 for grain yield. Non-additive gene action was observed for plant height, transpiration rate at reproductive stage, spike length and stay green. High narrow sense heritability coupled with additive genetic effects suggested the selection of plants for these traits would be effective in early segregating generations utilizing pedigree method for thermotolerance.

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