GENETIC ANALYSIS AND EXPRESSION PROFILING OF *TAHSP90A* TRANSCRIPTS CONFER HEAT TOLERANCE IN WHEAT

A. AMMAR[1], Z. ALI[1, 2,*], M.A.B. SADDIQUE[1], M. HABIB-UR-RAHMAN[3], and I. ALI[4]

[1]Institute of Plant Breeding and Biotechnology, MNS University of Agriculture Multan, Pakistan
[2]Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan
[3]Department of Agronomy, MNS University of Agriculture Multan, Pakistan
[4]Regional Agricultural Research Institute, Bahawalpur, Pakistan
* Corresponding author's email: zulfiqarpbg@hotmail.com
Email addresses of co-authors: abubakar.siddique@mnsuam.edu.pk, habib.rahman@mnsuam.edu.pk,
imtiaz.malghani@gmail.com, ammarali79@gmail.com

SUMMARY

Heat stress has emerged as a chief problem impeding wheat crop productivity. In several crops, specific HSP90A genes have intensively managed induced fluctuations in temperature. A wheat plant with *TaHSP90A* transcripts had the potential to cope with temperature stress. It enables plants to survive in transient extremes of temperature and under heat stress. The presented study design enhanced temperature tolerance plasticity with high yield in wheat through a line × tester mating design containing lines (12) and testers (4) having a differential expression of *TaHSP90A* transcripts (TraesCS2A02G033700.1, TraesCS5B02G258900.3, and TraesCS5D02G268000.2), then hybridized to get the F$_1$ (48) wheat hybrids. For heat treatment, temperature raising was only in the daytime, through the tunnel at anthesis (for two weeks). Data recording for several morphological and physiological parameters went along with the relative expression of *TaHSP90A* transcripts for hybrid evaluation. After one hour of heat treatment, the relative expression of *TaHSP90A* transcripts' determination in the flag leaf followed. The manifestation of *TaHSP90A* transcripts' upregulation was two folds in several hybrids after heat treatment. Best lines, testers, and selected crosses having *TaHSP90A* transcripts with high yield and heat tolerance compared with parents can further benefit breeding programs aiming toward tolerance against heat stress in changing climate scenarios.

Keywords: *Triticum aestivum* L., climate change, expression, *TaHSP90A,* food security

Key findings: This study mainly focuses on the upregulation of *TaHSP90A* transcripts showing a contribution to heat tolerance in wheat. Heat-tolerant wheat genotypes with *TaHSP90A* transcripts can produce significant yields under changing climate scenarios. The importance of this work lies in the potential for these genes by breeders to improve the plant's natural defenses against heat stress.

INTRODUCTION

About 35% of the world's population (Ogbonnaya *et al*., 2013) depends on wheat, one of the first domesticated food crops. It is essential to many lives, with contributions, such as, food, feed, medicines, cosmetics, and other industrial products. Although Pakistan is self-sufficient in wheat, there is a need to produce more due to food security threats from sudden climatic conditions and to feed an increasing population. Environmental changes, such as, stress from water, heat, cold, and salt, affect wheat yield (Irshad *et al.,* 2022). Several climate constraints, especially high temperatures, are responsible for declining wheat production. Occurring high temperatures (Stratonovitch and Semenov*,* 2015) around anthesis threatens a projected increase. Short spells of stirring increase in temperature (Prasad and Djanaguiraman, 2014) and the main impact of heat stress reduce crop yield (Zhao *et al.,* 2017). An estimated 8% reduction in wheat production is due to a 1 °C rise in temperature (Lesk *et al.,* 2016; Zhao *et al.,* 2017). As the temperature rises, all organisms undergo a stress response, which causes a global change in gene expression. The discovery in 1962 of heat shock proteins (Vierling, 1991) led to describing a variety of proteins whose influence of heat shock and other stresses have triggered (Wang *et al.,* 2016). Heat shock proteins (HSPs) conferred improving physiological traits like membrane stability, photosynthesis, water usability, and assimilate partitioning as a tolerance mechanism.

At high temperatures, the fast synthesis of several new proteins simultaneously maintained the physiological equilibrium. HSPs are the most significant of these proteins. Previous use of HSPs worldwide created thermo-tolerant plants responding to increasing temperatures (Kumar *et al.,* 2020). HSPs prevent irreversible protein denaturing and maintain their biological functions under heat stress. Activation and accumulation of these proteins occur as a reaction to retain cell stability (Richter *et al.,* 2010; Papsdorf and Richter, 2014). The five classes of HSPS are typically according to molecular weight: HSP 60, HSP 70, HSP 90, HSP 100, and small HSPs. In this regard, employing HSP90s is a proposed strategy for plant improvement and environmental stress response (Xu *et al.,* 2012). The molecular chaperone HSP90, which performs housekeeping duties on crucial biological processes, is a highly conserved protein required for eukaryotic viability (Zhang *et al.,* 2021). A 30%–40% increase in HSP90 appeared under high-temperature stress. The *HSP90As* showed sensitivity to high-temperature stress (Ogiso *et al.,* 2004). These genes play a vital role in protein folding and transportation (Banilas *et al.,* 2012) and help maintains conformation. In addition to folding, degrading, and transporting proteins, HSP90A acts as chaperones that regulate protein kinase activity and signal transduction using ATP (Schopf *et al.,* 2017). HSP90A is mainly found in the cytoplasm, mitochondria, chloroplasts, and endoplasmic reticulum (Prassinos *et al.,* 2008). Molecular chaperones are abundant and much conserved in eukaryotes. They also serve as housekeeping genes for crucial biological processes and are essential for viability. The evolution of HSP90s in the wheat functional conversation, polyploidization, and among *TaHSP90* homologs divergence needs exploration (Lu *et al.,* 2020).

Temperature changes showed a strong correlation with the expression of *HSP90A* (Xu *et al.,* 2012), and these fluctuations induced the *HSP90A* manifestation. Therefore, this mechanism for improving heat tolerance can also be factual in wheat. Hence, there is a need to replace or enhance the existing cultivars with newly developed improved varieties and hybrids possessing attractive traits, heat resistance, and high-yield potential. Plant breeding aims to identify new genetic recombinations to create improved genotypes that provide high yields for changing climatic conditions. It may result in combining the ideal parental genotypes for general combiners and choosing the hybrids for desirable traits. Combining ability analysis plays a significant role in parent and cross selection about the method of breeding used for trait improvement (Salgotra *et al.,* 2009).

This study mainly focused on the wheat genetic variability for tolerance against heat stress and identified physiological trait associations in wheat showing adaptability against heat stress with no impact on morphological traits. The most efficient breeding plan to progress the desired features' determination used the line × tester analysis. The research performed a *TaHSP90A* gene profiling assessment to gain a more comprehensive understanding of the spatial and temporal gene expression patterns of the wheat F_1 population. Using flag leaf tissue under heat stress and control conditions helps profile their gene expression levels. Breeding of wheat genotypes with abundant *TaHSP90A* transcripts may be helpful for the development of high-yielding and heat-tolerant germplasm.

MATERIALS AND METHODS

Study site and experimental material

The experiments ran in the fields at the MNS-University of Agriculture, Multan, located at 30.08° latitude, 71.26° longitude, and 189 m elevation above sea level. This study comprised three experiments, and the details of each follow.

Screening of germplasm for heat tolerance

A great set of wheat genotypes, including 103 entries sown in the field under randomized complete block design (RCBD), had three replications and three sowing dates (i.e., normal and late after 20 days of intervals). Row-to-row and plant-to-plant spacing between the wheat genotypes were 30 cm and 15 cm, respectively, in a 1 m^2 plot. The genotypes sown in three sets had the following dates: 15 November, 05 December, and 25 December. The first set was standard, while the second and third faced heat stress at grain filling and flowering, respectively. All the recommended crop management practices performed in the experiment were the same. The data measurement came from five guarded plants for morphological traits and relative cell injury.

Development and evaluation of breeding material for heat tolerance

For the hybridization, selecting a set of 16 parental lines (Table 1) and then mating in a line × tester pattern created the hybrids, and finally, 48 cross combinations produced F_0 seed at the flowering stage. The parents and their respective crosses sown in the field under normal and heat stress (5 ± 1 °C above ambient) comprised three replications under RCBD. Row-to-row and plant-to-plant spacing for the various wheat genotypes in a 1 m^2 plot were 30 and 15 cm, respectively. For heat treatment, raising the temperature happened only in the daytime, through a tunnel at the anthesis stage (for two weeks). Row-to-row space remained at 30 cm, with seeds placed 15 cm apart. The data collection came from five guarded plants for the morphological, physiological, and yield-controlling traits; further statistical analysis followed to find the combining ability and heterotic effects.

Measurement of morpho-physiological traits

Relative cell injury (RCI)

Determination of RCI (%) followed the procedure by Blum and Ebercon (1981) for measuring RCI. Cut a disc from the flag leaf with a diameter of 10 mm using a steel punch, with the samples transferred to an Eppendorf tube containing 2 ml of deionized water to prevent desiccation in the field. Then washing three times with deionized water cleansed the material of electrolytes from tissues in the laboratory. Vials placed in a water bath received heat up to 44 °C for 1 h. Control vials remained at 25 °C over the same time. After adding 10 ml of deionized water, holding the vials at 10 °C for 18–24 h allowed diffusion. Afterward, bringing the vials to 25 °C, they gained shaking to mix the contents. The initial conductance of the vial contents identification used an electrical conductivity meter. Vials

Table 1. List of wheat genotypes used for hybridization in line × tester mating design.

Line No.	Genotype	Expression	Tester No.	Genotype	Expression
1	E-01	(T)	1	38ESWYT E-145	(T)
2	25SAWYT E-328	(T)	2	25SAWYT E-349	(T)
3	38ESWYT E-135	(T)	3	25SAWYT E-302	(S)
4	SHP-44	(T)	4	25SAWYT E-329	(S)
5	25SAWYT E-332	(T)			
6	Suntop	(T)			
7	38ESWYT E-134	(S)			
8	AAS-11	(S)			
9	Jauhaur-16	(S)			
10	25SAWYT E-304	(S)			
11	SHP-52	(S)			
12	38ESWYT E-118	(S)			

T: Heat tolerant, S: Heat susceptible

received autoclaving for 10 min at 0.10 MPa pressure, then cooled to 25 °C, followed by a final conductance measurement. Distinguishing the level of injury used the following formula:

$$RI\ (\%) = 1 - ([1 - \{T_1/T_2\}] / [1 - \{C_1/C_2\}]) \ 100$$

Where, numbers 1 and 2 denote the start and final conductance measurements, and T and C stand for the conductance values for treatment and control, respectively.

Stay green

Differences in leaf and spike greenness served to measure the stay-green trait. The scale used for scoring was a 0–9 scale (a modified version of the 1–10 scale) at the late dough stage (Silva *et al.,* 2000).

Canopy temperature (°C)

A canopy temperature assessment operated by a handheld infrared thermometer (Fluke 572-2, Fluke, Everett, Washington, USA). Data recordings occurred on sunny days between 12:00 noon to 2:00 p.m. The canopy temperature estimation used the following equation:

$$CT = Air\ temperature - Canopy\ temperature$$

Physiological traits

The Portable Photosynthesis System (CIRAS-3, PP Systems, Amesbury, MA, USA) served to measure stomatal conductance (mmol H_2O m^{-2} s^{-1}), net photosynthetic rate (µmol CO_2 m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), and water usage efficiency (WUE) (mmol CO_2 mol^{-1} H_2O), following the procedure mentioned by Merrium *et al.* (2022). Measurements started from the mid-portion of flag leaves between 12:00 noon to 2:00 p.m. (full sunlight). The use of ambient CO_2 and H_2O maintained the concentration of CO_2 constant level. All measured physiological traits were from each random wheat genotype with three replications, with the mean values of the obtained data sourced for statistical analysis.

Morphological traits

Morphological traits of wheat genotypes observed in the presented study consisted of plant height, days to heading and maturity, flag leaf area, spike length, spike/plant, number of grains/spike, 1000-grain weight, and grain yield per plot. The data had recordings for three plants per plot per genotype, calculating the average by the procedure mentioned by Merrium *et al.* (2022).

Table 2. Primer sequences of three *TAHSP90A* transcripts in wheat.

Serial. No.	Gene Locus	Temp	Amplicon size (bp)	5′F	5′R
T1	*TraesCS2A02G033700.1*	58.3	2448	AAGCTTCGGGACAAGGCTC	TCAGTCGACCTCCTCCATCT
T2	*TraesCS5B02G258900.3*	54.3	2063	ATGTATACAGATAAAACTTCATCG	CTACCTTTCAGTGGAGATCGA
T3	*TraesCS5D02G268000.2*	61.6	1276	ATGAGGAAGCCAGAGGAGAT	CCACAAACAGCTAACCGCG

Expression profiling of *TaHSP90A* genes

For expression analysis, 48 wheat hybrids, including 16 parents, served for spatial and temporal *TaHSP90A* transcripts profiling. The protein sequences' retrieval of all the transcripts came from the plant genomic resource v13 Phytozome (https://phytozome-next.jgi.doe.gov/info/Taestivum_v2_2), and designing the primer used AmplifX version 1.7.0 (Table 2). RNA samples taken from all wheat hybrids came from flag leaf tissue after 1 h of heat treatment, and total RNA extraction employed the TRIzol protocol (Zhang *et al.,* 2016). RNA quantification checking on a spectrophotometer (nanodrop 2000c, Thermo Fisher Scientific, Washington, USA) continued. Carrying out cDNA synthesis used 5 µl RNA (500 ng) (Liu *et al.,* 2018). The cDNA synthesis followed the manufacturer's guidelines (Thermo Fisher Scientific, 2000). Performing Semi-q PCR on synthesized cDNA helped analyze the expression profiling of *TaHSP90A* transcripts by Ali *et al.* (2012).

The cDNA synthesis and Semi-q PCR

Add 1 µl of oligo dt and 6.5 µl of nuclease-free water to 5 µl of RNA (500 ng) to produce a total volume of 12.5 µl. After centrifugation at 5000 rpm for 2 min, samples attained incubation at 65 °C temperature for 5 min in a thermo cycler and kept on ice to lower the temperature to 4 °C. Then adding 0.5 µl Ribo lock RNase inhibitor, 4 µl 5× reaction buffer, 2 µl 10mM dNTP's Mix, and 1 µl of Revert aid Reverse Transcriptase helped make a volume of 20 µl. A thermocycler ran samples for 60 min at 42 °C, 10 min at 70 °C, 15 min at 10 °C, and 1 min at 72 °C for the final termination.

An optimization for semi-qPCR cycles ran at 30, 32, 34, 35, 36, 39, and 40 cycles up

to the plateau stage of the project. Ethidium bromide served for staining of PCR products, with the product running on 1% agarose gel, and the bands' visualization under UV radiance used a gel documentation apparatus (Omega Fluor™ Plus Gel Documentation System). The analysis had three repeated independent experiments. The gel band intensity quantification employed the Image J software (Saddique *et al.,* 2020). Determining the change in transcript expression of *TaHSP90A* used numerical values that represented band strength.

$$\% \text{ change in expression} = \frac{\text{Gel band strength under heat stress} - \text{gel band strength under control}}{\text{gel band strength under control}} \times 100$$

Statistical analysis

The mean values of RCI and yield traits aided in constructing clusters and heat maps. The R software agricolae package version 4.1.2 helped the calculation of the analysis of variance. Biplots developed used the mean data of the recorded traits by using the R software ggbiplot package. Employing L × T (line × tester) and combining ability analysis provided genetic and variance components estimates. All data presented for spatial and temporal expression of *TaHSP90A* transcripts were mean values of relative expression ± SE. The estimated difference among the mean values operated one-way ANOVA and Tukey's HSD (Tukey's honestly significant difference) test in R software.

RESULTS

Screening of wheat germplasm for heat tolerance

Screening of wheat genotypes (parents for hybridization) based on morphological traits (grains/spike, 1000-grain weight, and grain

yield) and RCI % from a diverse 103 entries achieved visuals through a heat map and clustering (Figure 1C). The heat map showed several clusters based on tolerance and susceptibility index. From the screening of 103 genotypes, selection led to 16 genotypes (Table 1) for hybridization. The biplot analysis revealed that the PC1 variance was 30.9%, and the PC2 variation was 28.7% (Figure 1B). Among 16 selected wheat genotypes, for yield, genotypes 74 and 86 had the highest value, while for 1000-grain weight (TGW), genotypes 78 and 55 showed maximum scores. On relative cell injury (RCI), genotypes 16 and 84 gave the minimum values. Grain yield had a strong positive correlation with TGW and grains/spike (GPS), but a negative correlation emerged with RCI (Figure 1A).

Development and evaluation of wheat breeding materials for heat tolerance

Analysis of variance (lines, testers, and hybrids)

A comparison of variances for the different morphological traits observed in lines, testers, and hybrids grown under normal and heat experimental conditions appear in Table 3, and for physiological features, in Table 4.

Several traits provided a considerable variation between the lines, except for spike length, flag leaf area, photosynthetic rate, stomatal conductance, and water use efficiency. All the studied traits' results indicated to be insignificant in the case of testers except plant height, transpiration rate, and stay green. Analysis of variance of both crosses and lines × tester interaction resulted in significant differences for all the studied traits, and parents vs. crosses, all the observed features differed significantly, except for 1000-grain weight and water use efficiency attributes.

Analysis of variance of treatments, parents, parents vs. crosses, and lines × tester interaction resulted in significant differences for all the observed traits. Substantial differences emerged for some studied qualities observed in lines, excluding spike length, photosynthetic rate, stomatal conductance,

and water use efficiency. Regardless of testers, among all parameters observed in the presented study, plant height, transpiration rate, and stay green showed significant variation. On the other hand, the crosses expressed notable variability for all observed traits. In the case of the treatment of parents versus crosses (P vs. C), a significant difference in variability appeared for all characteristics except for 1000-grain weight and water use efficiency.

Estimates of GCA and SCA effects

The GCA (general combining ability) effects of 16 selected parental wheat genotypes for all the studied traits (morphological and physiological) are in Tables 5 and 6, respectively. Under normal conditions, significant GCA effects for some morphological features (grains/spike, 1000-grain weight, flag leaf area, days to heading) emerged. For instance, line 11 showed positive GCA effects on the days to heading, peduncle length, and days to maturity traits; line 2 showed positive GCA effects for flag leaf area, and line 4 indicated positive reactions for plant height. Among yield-contributing traits, line 1 revealed positive GCA for SPS, GPS, SPP, TGW, and yield characters. Wheat lines with positive and significant GCA effects are good combinations of their respective traits. Regarding the GCA effects on physiological qualities, line 3 showed remarkable outcomes on water use efficiency, stomatal conductance, and photosynthetic rate traits, and line 1 is a good combiner with significant GCA effects for canopy temperature, transpiration rate, stay green, and relative cell injury. Wheat genotypes with good general combining ability (lines 1, 3, and 4) may further benefit breeding programs to develop high-yielding varieties.

Estimation of GCA effects for agro-physiological traits observed from 16 parental lines revealed that two lines, such as lines 7 and 12, showed better combining ability for two vital agronomic traits: days to heading and maturity. Wheat genotypes of lines 2, 4, and 7 also showed the maximum GCA effects for flag leaf area, plant height, and peduncle length, respectively. Before the yield-contributing
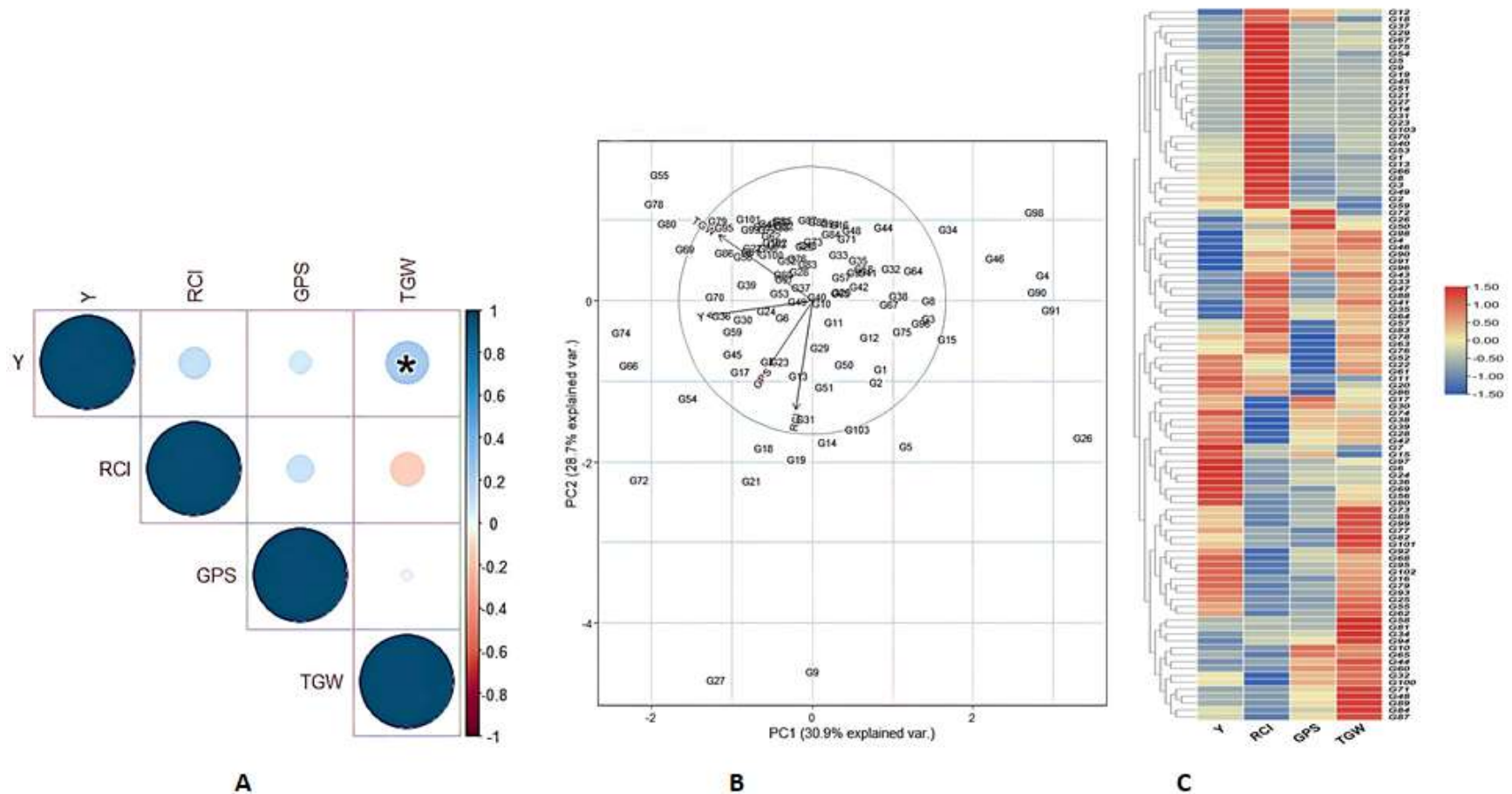
Figure 1. Correlation analysis, biplot analysis, and heat map of 103 wheat genotypes for relative cell injury (RCI), grains/spike (GPS), 1000-grain weight (TGW), and grain yield (Y).

Table 3. Mean squares values for morphological and yield-contributing traits of parents and F$_1$ crosses under normal and heat conditions.

Source	Df	DHE		DM		PH		SL		PL		SPS		FLA		GPS		SPP		TGW		Y	
		N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H
Replications	2	40.94*	16.34*	258.40*	6.94	342.13*	12.89*	10.12*	20.32*	430.48*	4.41	97.77*	49.64*	236.13*	307.61*	82.62*	169.86*	133.34*	209.01*	81.04*	23.56*	2.19 NS	15.99*
Treatments	63	16.05*	9.73*	27.93*	27.44*	49.29*	53.74*	2.93*	2.77*	20.98*	37.54*	8.18*	7.07*	167.05*	167.05*	48.91*	50.20*	47.95*	47.95*	27.69*	24.99*	235.60*	235.60*
Parents	15	4.31*	7.39*	9.64*	30.51*	54.79*	45.97*	2.01*	2.07*	25.97*	38.88*	7.06*	5.84*	431.46*	431.46*	16.33*	23.03*	38.70*	38.70*	28.86*	22.41*	181.96*	181.96*
Parents vs. Crosses	1	190.21*	90.25*	665.21*	484.00*	45.98*	261.36*	19.78*	26.47*	39.76*	210.25*	73.67*	90.25*	199.58*	199.58*	390.47*	443.62*	21.39*	21.39*	2.77*	0.39	852.34*	852.34*
Crosses	47	16.09*	8.76*	20.21*	16.75*	47.61*	51.80*	2.86*	2.49*	18.99*	33.44*	7.14*	5.70*	857.93*	857.94*	52.04*	50.51*	51.46*	51.46*	27.85*	26.33*	239.60*	239.60*
Lines	11	46.89*	23.25*	54.97*	32.04*	98.66*	108.32*	3.11 NS	3.95*	27.24*	92.41*	14.41*	12.90*	978.87 NS	978.87	161.52*	159.60*	166.45*	166.45*	96.03*	91.89*	1002.99*	1002.99*
Testers	3	2.39 NS	4.58 NS	15.48 NS	9.90 NS	110.34*	106.35*	3.38 NS	1.34 NS	28.77 NS	11.19 NS	6.62 NS	4.81 NS	176.50 NS	176.50 NS	33.01 NS	28.71 NS	9.12 NS	9.12 NS	2.72 NS	0.60 NS	7.12 NS	7.12 NS
Lines × Testers	33	7.07*	4.30*	9.05*	12.28*	24.89*	28.00*	2.74*	2.11*	15.35*	15.79*	4.77*	3.38*	879.56*	879.56*	17.27*	16.12*	16.98*	16.98*	7.41*	6.83*	6.28*	6.28*
Error	126	1.66	1.76	3.40	2.67	1.82	2.22	0.86	1.04	1.80	2.14	1.68	1.75	0.00	0.00	4.14	3.74	2.11	2.11	2.68	2.62	0.81	0.81

*, indicates significant differences at p ≤ 0.05 and NS sign indicates non-significant. Morphological and yield traits, days to heading (DHE), days to maturity (DM), plant height (PH), spike length (SL), peduncle length (PL), spikelets/spike (SPS), flag leaf area (FLA), no. of grains/spike (GPS), spike per meter row (SPP), 1000-grain weight (TGW), and yield (Y) under normal and heat conditions, where, N indicates normal and H for heat.

Table 4. Mean squares values for physiological traits of parents and F$_1$ crosses under normal and heat conditions.

Source	Df	P		SC		CT		T		WUE		SG		RCIA		RCIL	
		N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H
Replications	2	31.42*	34.05*	144.31*	118.61*	149.51*	256.00*	161.53*	47.91*	28.34*	11.09*	0.01	0.01	133.16*	153.75*	124.49*	97.74*
Treatments	63	11.01*	11.07*	910.44*	910.67*	45.90*	45.93*	4.93*	4.93*	1.91*	6.05*	4.79*	4.79*	313.29*	313.30*	305.41*	305.80*
Parents	15	140.27*	14.07*	135.37*	1353.45*	55.54*	55.56*	1.72*	1.72*	1.09*	1.29 NS	6.70*	6.70*	655.50*	655.17*	737.73*	737.68*
Parents vs Crosses	1	4.37*	43.75*	568.29*	568.32*	553.37*	553.48*	0.00*	0.00*	0.00*	3.42 NS	10.56*	10.56*	289.59*	289.64*	321.67*	321.18*
Crosses	47	10.23*	10.26*	776.21*	776.65*	32.00*	32.06*	6.05*	6.05*	2.19 NS	7.63*	4.05*	4.05*	204.12*	204.70*	167.50*	167.64*
Lines	11	7.24 NS	72.42 NS	510.94 NS	510.73 NS	105.86*	105.42*	13.64*	13.68*	3.41 NS	5.68 NS	9.98*	9.98*	629.01*	629.04*	603.12*	603.56*
Testers	3	2.45 NS	24.55 NS	994.23 NS	994.37 NS	4.13 NS	4.13 NS	102.19*	10.26*	2.08 NS	10.42 NS	6.64*	6.64*	105.32 NS	105.48 NS	46.53 NS	46.54 NS
Lines × Testers	33	11.09*	11.98*	845.14*	845.49*	10.15*	10.14*	3.12*	3.12*	1.79*	8.02*	1.84*	1.84*	72.23*	72.27*	33.31*	33.35*
Error	126	0.00	0.00	6.05×10^{-20}	0.00	0.00	0.00	5.99×10^{-29}	0.00	0.00	1.56	0.01	0.01	1.74×10^{-29}	0.00	0.00	0.00

*, indicates significant differences at p ≤ 0.05 and NS sign indicates non-significant. Physiological parameters, viz., photosynthesis (P), transpiration (T), stomatal conductance (SC), canopy temperature (CT), water use efficiency (WUE), stay green (SG), relative cell injury anthers (RCIA), and relative cell injury leaf (RCIL) under normal and heat conditions, where N indicates normal and H for heat.

Table 5. Estimates of general combining ability effects of lines and testers for morphological and physiological traits under normal conditions.

GCA Effects	Morphological traits											Physiological traits							
	DHE	DM	PH	SL	PL	SPS	FLA	GPS	SPP	TGW	Y	P	SC	CT	T	WUE	SG	RCIA	RCIL
Lines																			
L1	-2.18	-1.85	0.65	1.02	-0.99	1.52	-1.60	6.88	8.58	6.04	14.37	1.28	1.18	-6.90	2.13	0.72	2.50	-12.76	-13/09
L2	1.56	1.31	-3.37	-0.13	-1.47	0.86	5.52	4.13	5.16	1.21	10.59	-0.54	-6.81	-5.15	1.48	0.15	0.00	-4.43	-4.03
L3	-1.84	-2.18	-5.03	0.11	-2.39	1.36	-0.93	1.55	1.16	2.96	9.51	1.30	-0.56	-0.15	0.10	1.04	0.00	3.86	4.72
L4	-1.51	-1.68	4.63	0.19	0.52	0.52	0.02	1,13	0.08	1.54	4.65	-0.74	3.00	0.60	0.79	-0.72	0.50	-6.86	-5.42
L5	-0.01	-2.77	-0.03	0.11	-2.22	1.02	2.59	3.38	-0.33	-1.03	6.53	-0.88	-5.68	1.84	-0.98	0.11	-0.16	-3.05	-3.42
L6	-0.76	-0.52	-0.28	-0.63	1.02	-1.30	0.37	-2.44	-0.08	0.04	3.38	-0.25	-9.50	1.09	0.30	-0.34	-0.16	0.45	2.19
L7	-1.01	-1.10	4.29	-0.22	0.60	-1.13	-0.73	0.52	1.58	0.29	-9.02	0.40	-1.50	1.72	-0.43	-0.05	-0.83	11.12	11.58
L8	-0.93	-0.02	1.29	0.86	0.18	0.02	4.07	0.63	-2.25	-1.45	-10.33	0.55	9.21	2.59	-1.57	-0.16	-1.00	6.98	3.59
L9	-1.59	-0.35	0.45	-0.22	-0.47	-1.30	-1.85	4.27	0.08	0.46	-11.70	0.03	-6.16	2.34	-1.01	-0.26	-0.58	-3.55	-3.70
L10	2.48	3.22	2.04	-0.47	1.60	-0.63	-3.43	-4.19	-2.75	-2.95	-6.41	0.08	5.68	0.59	-0.59	-0.81	-0.50	9.02	10.29
L11	3.90	3.22	-1.53	-0.13	1.77	0.36	0.06	-1.44	-5.25	-4.28	-6.48	-0.24	11.43	1.34	0.08	0.12	-0.25	4.88	2.30
L12	1.90	2.72	-2.20	-0.47	1.85	-1.30	-4.07	-4.86	-2.83	-2.86	-5.09	-1.01	-0.31	0.09	-0.29	0.20	0.50	-5.65	-4.99
S.E.	0.37	0.53	0.38	0.26	0.38	0.37	0.00	0.58	0.41	0.47	0.26	$3.68×10^{-15}$	$2.24×10^{-14}$	$6.04×10^{-15}$	0.00	$1.07×10^{-15}$	0.02	$1.20×10^{-8}$	$1.19×10^{-14}$
Tester																			
T1	-0.18	-0.71	1.93	0.27	-0.50	0.58	-0.73	1.22	-0.25	-0.03	0.65	-0.29	-2.53	-0.03	0.09	-0.20	0.55	0.20	-0.62
T2	0.09	0.86	0.35	-0.22	0.93	0.02	-0.24	-0.91	-0.05	0.13	-0.17	-0.10	6.74	-0.40	0.68	-0.12	0.05	-1.89	-0.33
T3	-0,23	0.00	0.01	-0.30	0.54	-0.41	0.05	0.27	-0.41	0.27	-0.16	0.09	-5.42	0.00	-0.21	0.34	-0.47	2.20	1.68
T4	0.31	-0.16	-2.30	0.25	-0.98	-0.19	0.92	-0.58	0.72	-0.36	-0.31	0.30	1.21	0.42	-0.56	-0.00	-0.13	-0.51	-0.73
S.E.	0.21	0.30	0.22	0.15	0.22	0.21	0.00	0.33	0.24	2.47	0.15	$2.13×10^{-15}$	$1.29×10^{-14}$	0.00	$1.29×10^{-15}$	$6.20×10^{-16}$	0.01	0.00	$6.88×10^{-15}$

Where, days to heading (DHE), days to maturity (DM), plant height (PH), spike length (SL), peduncle length (PL), spikelets/spike (SPS), flag leaf area (FLA), no. of grains/spike (GPS), spike per meter row (SPP), 1000-grain weight (TGW), and yield (Y); and physiological parameters, viz., photosynthesis (P), transpiration (T), stomatal conductance (SC), canopy temperature (CT), water use efficiency (WUE), stay green (SG), relative cell injury anthers (RCIA), and relative cell injury leaf (RCIL) under normal conditions.

Table 6. Estimates of general combining ability effects of lines and testers for morphological and physiological traits under heat conditions.

GCA Effects	Morphological traits											Physiological traits							
	DHE	DM	PH	SL	PL	SPS	FLA	GPS	SPP	TGW	Y	P	SC	CT	T	WUE	SG	RCIA	RCIL
Lines
L1	-1.14	-1.66	0.88	1.01	-2.79	1.33	-1.60	6.73	8.58	5.70	14.37	1.28	1.18	-6.90	2.13	-0.52	2.50	-12.76	-13.09
L2	1.93	1.33	-3.52	0.59	-3.12	1.16	5.52	4.09	5.16	1.45	10.59	-0.54	-6.81	-5.15	1.48	-0.86	0.00	-4.43	-4.03
L3	-0.22	-0.75	-3.69	0.51	-4.12	1.50	-0.93	1.48	1.16	2.62	9.51	1.30	-0.56	-0.15	0.10	-0.03	0.00	3.86	4.72
L4	-1.06	-0.41	5.72	0.01	-0.87	0.66	0.02	1.48	0.08	1.70	4.65	-0.74	3.00	0.60	0.79	-0.84	0.50	-6.86	-5.42
L5	-0.14	-1.33	2.55	-0.15	-2.54	0.66	2.59	3.48	-0.33	-0.70	6.53	-0.88	-5.68	1.84	-0.98	0.48	-0.16	-3.05	-3.42
L6	-0.81	-2.58	-1.61	-0.23	2.62	-1.00	0.37	-2.51	-0.08	0.70	3.38	-0.25	-9.50	1.09	0.30	-0.44	-0.16	0.45	2.19
L7	2.43	-1.33	3.38	-0.65	4.70	-0.83	-0.73	-0.34	-1.58	0.12	-9.02	0.40	-1.50	1.72	-0.43	-0.05	-0.83	11.12	11.58
L8	0.93	1.75	1.63	0.76	1.04	-0.16	4.07	0.40	-2.25	-1.37	-10.33	0.55	9.21	2.59	-1.57	0.96	-1.00	6.98	3.59
L9	1.18	-0.08	-2.77	-0.48	-0.04	-1.33	-1.85	-4.34	0.08	0.12	-11.70	0.03	-6.16	2.34	-1.01	0.54	-0.58	-3.55	-3.70
L10	0.35	2.16	0.88	-0.40	3.20	-0.83	-3.43	-4.43	-2.75	-2.95	-6.41	0.08	5.68	0.59	-0.59	1.24	-0.50	9.02	10.29
L11	-1.89	0.50	-2.69	-0.40	1.54	0.00	0.06	-1.51	-5.25	-4.20	-6.48	-0.24	11.43	1.34	0.08	-0.50	-0.25	4.88	2.30
L12	-1.56	2.41	-0.77	-0.56	0.37	-1.16	-4.07	-4.51	-2.83	-3.20	-5.09	-1.01	-0.31	0.09	-0.29	0.03	0.50	-5.65	-4.99
S.E.	0.38	0.47	0.43	0.29	0.42	0.38	0.00	0.55	0.41	0.46	0.26	0.00	0.00	0.00	0.00	0.36	0.02	0.00	0.00
Tester
T1	-0.09	-0.61	1.91	0.23	-0.59	0.44	-0.73	1.12	-0.25	0.18	0.65	-0.29	-2.53	-0.03	0.09	-0.43	0.55	0.20	-0.62
T2	0.46	-0.25	0.69	-0.01	0.40	0.05	-0.24	-0.84	-0.05	-0.04	-0.17	-0.10	6.74	-0.40	0.68	-0.49	0.05	-1.89	-0.33
T3	-0.39	0.33	-0.50	-0.23	0.54	-0.44	0.05	0.29	-0.41	-0.01	-0.16	0.09	-5.42	0.00	-0.21	0.43	-0.47	2.20	1.68
T4	0.02	0.52	-2.11	0.01	-0.34	-0.05	0.92	-0.56	0.72	-0.12	-0.31	0.30	1.21	0.42	-0.56	0.49	-0.13	-0.51	-0.73
S.E.	0.22	0.27	0.24	0.17	0.24	0.22	0.00	0.32	0.24	0.27	0.15	0.00	0.00	0.00	0.00	0.20	0.01	0.00	0.00

Where, days to heading (DHE), days to maturity (DM), plant height (PH), spike length (SL), peduncle length (PL), spikelets/spike (SPS), flag leaf area (FLA), no. of grains/spike (GPS), spike per meter row (SPP), 1000-grain weight (TGW), and yield (Y); and physiological parameters, viz., photosynthesis (P), transpiration (T), stomatal conductance (SC), canopy temperature (CT), water use efficiency (WUE), stay green (SG), relative cell injury anthers (RCIA), and relative cell injury leaf (RCIL) under heat conditions.

traits, one wheat genotype coded by line 1 indicated significant and positive GCA effects for SL, SPS, GPS, SPP, TGW, and grain yield. For physiological traits, three wheat genotypes, such as lines 3, 11, and 8, showed a substantial effect on photosynthetic rate and stomatal conductance traits.

Estimates of specific combining ability effects for morphological and physiological traits of crosses under normal and heat conditions are in Supplementary Tables 1 and 2. Six out of 48 wheat cross combinations used for the combining ability test, such as, L7×T1, L6×T4, L2×T1, L6×T1, L5×T3, and L4×T4, showed the best values for days to heading and maturity, flag leaf area, plant height, spike length, and grain yield. On the other hand, among yield-contributing qualities observed in the presented study, the F_1 population derived from cross L10×T3 emerged as best performing for spikelets/spike and grains/spike with maximum SCA effects. For the yield and 1000-grain weight features, the L5×T3 population exhibited the highest SCA effects, though the cross of L6×T1 showed the best SCA effects for the SPP trait. Concerning the SCA effect on physiological characteristics, the F_1 crosses of L9×T3, L3×T2, and L1×T1 performed best with maximum SCA results in photosynthetic rate, canopy temperature, and transpiration rate, respectively. On the other hand, three wheat cross combinations, L3×T4, L6×T3, and L3×T3, performed best for stay green and relative cell injury features, respectively. Similarly, the F_1 cross of L5×T3 showed maximum SCA effects for SC and WUE attributes.

Among 48 cross combinations of F_1 plants grown under heat stress in the field, the following cross combinations, i.e., L5×T1, L4×T3, L3×T1, L6×T1, L8×T3, L1×T3, L10×T4, L3×T4, L6×T3, and L2×T3 showed the best SCA performances with maximum SCA effects observed for DM, PL, SPS, SPP, P, T, WUE, SG, and RCI traits. The cross of L3×T2 showed maximum SCA effects for plant height and canopy temperature features. In addition, the cross of L12×T4 exuded the best SCA effects for days to heading and flag leaf area characters, and the cross of L10×T3 is best for

spike length and grains/spike. Regardless of the stomatal conductance, 1000-grain weight, and yield qualities, the cross of L5×T3 exhibited the highest value for SCA effects.

Biplot analysis of genotypes (lines, testers, and hybrids) for yield and physiological traits

Biplot analysis depicted the response of all lines, testers, and their hybrids for the morphological and physiological parameters and yield's influential traits under normal and heat conditions (Figure 2). Under normal conditions, traits, such as, yield (Y), grains per spike (GPS), 1000-grain weight (TGW), stay green (SG), transpiration rate (T), and the number of spikes per meter row (SPP), exhibited a positive correlation with each other. However, relative cell injury (RCI), canopy temperature (CT), and plant height (PH) showed negative correlation with the previously stated traits. For yield, line 1 and cross 1 had the highest values. Similarly, for TGW, crosses 2 and 3 provided the topmost values. In the case of GPS, cross 6 and line 4 achieved the maximum scores.

In heat-stress conditions, based on the results, line 1, cross 2, and cross 3 showed maximum values for the yield trait. For physiological traits, (WUE, SC, and T), notably, tester 4, cross 16, and cross 3 showed the highest values. Under normal conditions, the grain yield had a strong positive correlation with T, WUE, SG, GPS, TGW, SPP, and SPS. Regardless, a negative correlation existed for features like PH, PL, CT, and RCI. However, a positive correlation also emerged between GPS and SPP traits. In heat stress conditions, the correlation of all studied parameters appears in Figure 3. Results depicted that grain yield had a strong positive correlation with attributes GPS, TGW, SPP, SPS, T, and SG, but showed a negative correlation with PH, PL, DHE, DM, CT, and RCI. PH and PL showed a negative correlation with yield, yet their correlation indicated positive for RCI and CT. Photosynthetic rate (P) correlated positively with FLA, GPS, SPP, TGW, and Y but negatively correlated with PH and SL.
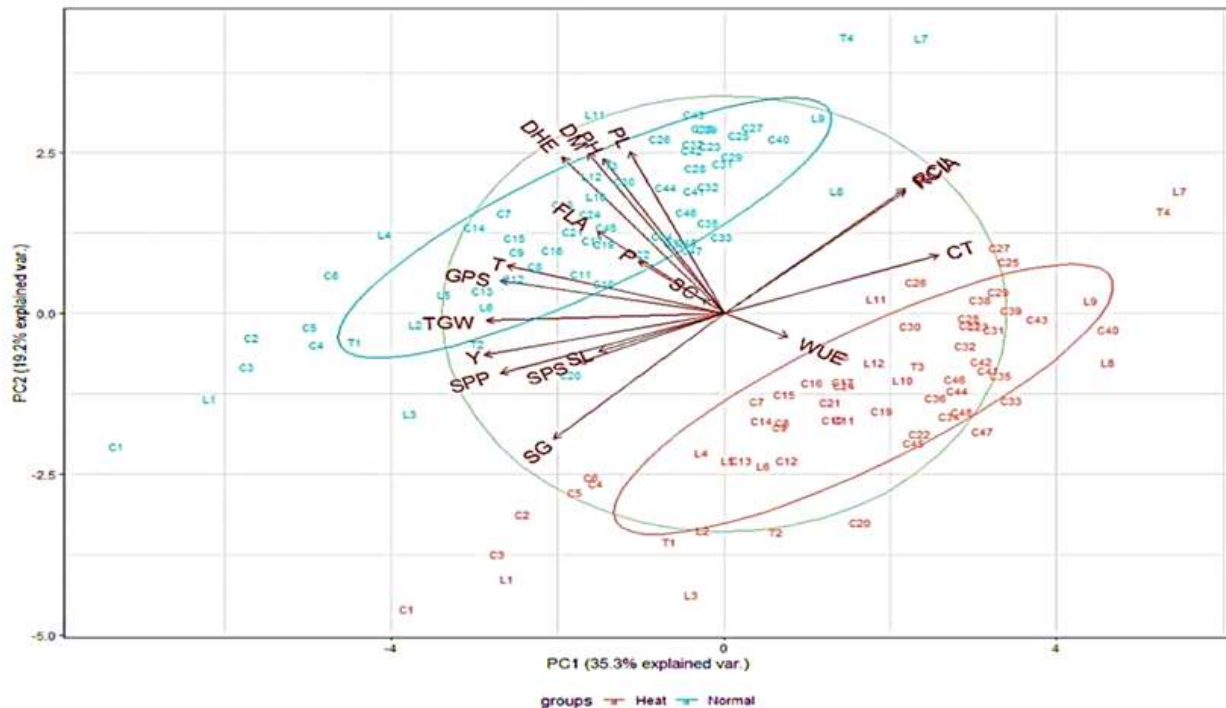
Figure 2. Biplot analysis of lines, testers, and crosses for days to heading (DHE), days to maturity (DM), plant height (PH), spike length (SL), peduncle length (PL), spikelets/spike (SPS), flag leaf area (FLA), no. of grains/spike (GPS), spike per meter row (SPP), 1000-grain weight (TGW), and yield (Y). Physiological parameters, viz., photosynthesis (P), transpiration (T), stomatal conductance (SC), canopy temperature (CT), water use efficiency (WUE), stay green (SG), relative cell injury anthers (RCIA), and relative cell injury leaf (RCIL). Arrows show the correlation among the traits for their respective environment, where blue color represents normal and red shows heat.

Expression profiling of hybrids

Under normal conditions, transcript 1 showed differential expression in wheat hybrids 5 and 19, while transcript 2 showed expression in hybrids 2 and 14. No significant expression occurred for transcript 3 in any of the hybrids. Under high temperatures, transcript 1 showed differential expression in hybrids 1, 10, 17, and 41. For transcript 1, hybrids 1 and 17 showed upregulation of 2-folds. Transcript 2 appeared to be 2-fold upregulated in hybrids 9 and 45. Transcript 3 exhibited a 2-fold upregulation in hybrids 2 and 30, but its appearance did not show in normal conditions (Figure 4).

DISCUSSION

Increasing plant genetic variability can proceed via hybridization, one of the most crucial sources for crop improvement. In addition to identifying new traits, hybridization is central to exploiting genetic diversity in crop plants and the evolution of new phenotypes. Hybridization application on contrasting wheat genotypes boosts yield improvement and temperature tolerance. Evaluation of the F_1 populations showed that significant variation in hybrids inheritance transpired. An observation is that the hybridization of different parents containing heat-sensitive and insensitive genes in different combinations produces distinct phenotypes that result in grain yield improvement.
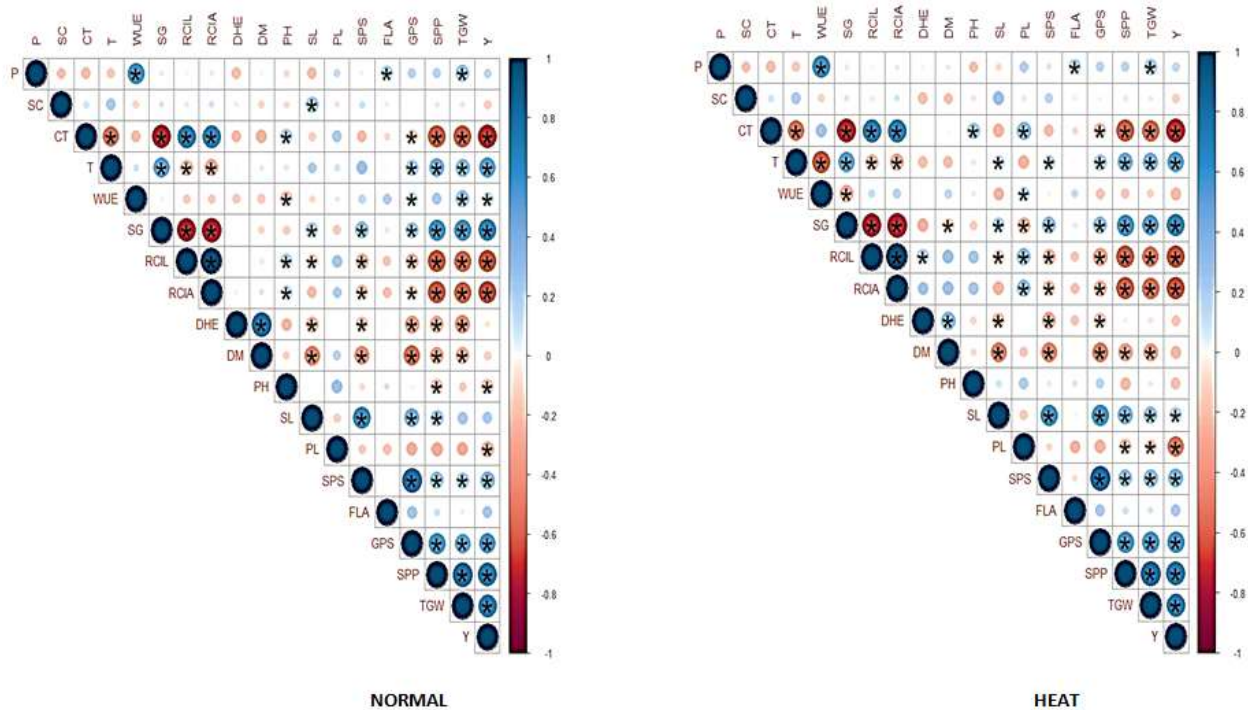
Figure 3. Correlation plot of yield-contributing traits, relative cell injury leaf (RCIL), relative cell injury anther (RCIA), days to heading (DHE), days to maturity (DM), plant height (PH), spike length (SL), peduncle length (PL), spikelets/spike (SPS), flag leaf area (FLA), spikes per meter row (SPP), no. of grains/spike (GPS), 1000-grain weight (TGW), and grain yield/plot (Y). Physiological parameters, viz., photosynthesis (P), transpiration (T), stomatal conductance (SC), canopy temperature (CT), water use efficiency (WUE), and stay green (SG) under normal and heat conditions. Blue shade shows the positive correlation and pink shade shows negative correlation. Size of the circle shows how traits are associated with each other. More size means strong association. * indicates significant (p < 0.05) and without sign * indicates non-significant (p ≥ 0.05).
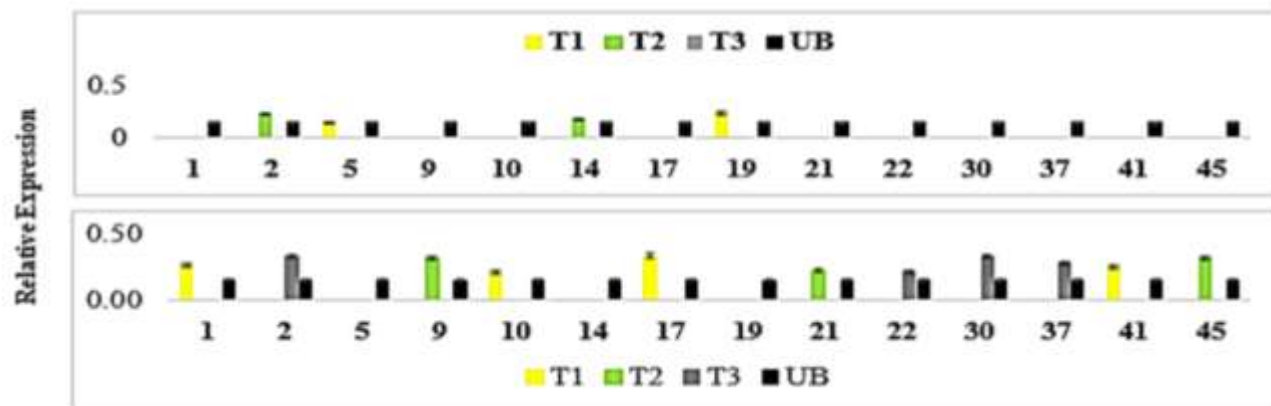

Figure 4. Relative expression of wheat hybrids under normal (N) and high temperature (HT) from flag leaf tissue after 1 h of heat treatment, where, transcript 1 (T1), transcript 2 (T2), transcript 3 (T3), and Ubiquitin (UB) was used as a housekeeping gene. Values represent data from three biological replicates and three technical replicates. Error bars indicate values ± SD.

When selecting parents for hybridization programs, understanding gene action is essential. Identifying best-performing parental lines is critical in crop breeding to get the best hybrid combinations for creating genetic variability. It is necessary to identify suitable parents and promising hybrids in developing high-yielding varieties (Longin *et al.,* 2013).

In this study, significant variations occurred for all studied traits in wheat genotypes under normal and heat stress, indicating the presence of sufficient variability for the estimation of combining ability. For all traits, additive genetic variation was less than the non-additive genetic variance signifying its importance in inheritance. The results revealed that the GCA variance was lower than the SCA, suggesting the predominance of non-additive gene action. Thus, the selection may be fruitful for desired trait improvement. Similar results also came from Jatav *et al.* (2014) but in contrast with the results of Titan *et al.* (2012). Most often, non-additive genetic factors are in charge of regulating the inheritance of the traits under study. Similarly, Alhossary (2020) reported finding the same gene action outcomes. With the predominance of non-additive gene actions, it is better to postpone the selection of superior plants to later generations. Verma *et al.* (2007) described similar results for wheat concerning the predominance of non-additive gene action.

Finding the ratios of GCA/SCA to be less than one for several studied traits represented a prevalence of non-additive gene effects in the inheritance traits. Similarly, Jatav *et al.* (2014) and Potla *et al.* (2013) reported the difference between SCA and GCA variances that indicate the non-additive gene action predominance. Combining ability effects from different parents might result in negative or positive results. Among yield-contributing traits, genotype line 1 exhibited significant and positive GCA effects for SPS, GPS, SPP, and TGW, as well as, for yield in normal and heat conditions. The lines with significant GCA impacts were good combiners of their particular traits. Likewise, for physiological features, line 3 showed good cross combination for photosynthetic rate, stomatal conductance, and water use efficiency traits, while line 1, for canopy temperature, transpiration rate, stay green, and relative cell injury, showed good combiners with significant GCA effects. The study recognized the wheat cross of L2×T1 as the best cross for relative cell injury trait, with highly remarkable SCA effects in the negative direction. A previous report stated that changing canopy temperatures affected grain yield traits in stress conditions (Reynolds *et al.,* 2007), and a low canopy temperature is desirable.

The contributions of lines were better than the tester and line × tester to the total variance. However, the hybrids' involvement in total variation was higher than testers, which showed the superiority of hybrids over parents. Line 1 had positive GCA effects for grain yield, grains per spike, and 1000-grain weight, indicating that it is a good combiner for these critical characteristics, with similar findings also reported by Kumar *et al.* (2015).

Contrasting parents with sensitive and insensitive genes in various combinations produce contrasting phenotypes, and hybrids showed more heat tolerance than their parents. New phenotypes also acquired significant SCA effects for several traits, signifying these hybrids had the potential to acclimatize to heat conditions without affecting their performance. Moreover, the association with cell injury also improves the performance of hybrids and stabilizes them under heat stress. Utilization of *TaHSP90A* transcripts in the breeding program resulted in significant tolerance in heat stress conditions, with an increase in the number of grains/spike, less cell injury, low canopy temperature, and more grain yield. Wheat hybrids with *TaHSP90A* transcripts with less cell injury are concomitant with high grain number and harvest. Upregulation of *TaHSP90A* transcripts contributed to temperature tolerance in hybrids compared with parents at high temperatures. The expression of *TaHSP90A* transcripts indicated an upregulation (2 folds) in several wheat hybrids (1, 17, 30, 37, and 41) after heat treatment. These findings shed fresh light on the function of *TaHSP90A* transcripts in

phenotypic plasticity for temperature tolerance to develop heat-tolerant wheat cultivars under the current changing climate scenario.

CONCLUSIONS

The study concluded that the wheat genotypes showing upregulation of *TaHSP90A* transcripts showed increased grain yield and more heat tolerance. The hybridization of wheat genotypes containing *TaHSP90A* transcripts results in significant heterosis for increased yield and heat tolerance. The high SCA effects of selected hybrids (L1×T1, L5×T3, L2×T1, L3×T3, and L4×T4) have proven to be beneficial for traits contributing to yield and heat tolerance. New phenotypes also acquired significant SCA effects for several features (RCI, GPS, TGW, CT, and Y), indicating these hybrids can potentially acclimatize to heat conditions without affecting their performances. In wheat breeding, extending germplasm variation using different *TaHSP90A* transcripts will facilitate temperature tolerance plasticity under the current changing climate scenario. Line 1 and tester 1 need promotion for developing temperature-tolerant wheat genotypes with high yield for the farming community, and hybrids (L1×T1, L5×T3, L2×T1, L3×T3, and L4×T4) having *TaHSP90A* transcripts with high harvests can benefit breeding programs targeting heat tolerance.

ACKNOWLEDGMENTS

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Supp. Table 1. Estimates of specific combining ability effects for morphological and physiological traits of crosses under normal conditions.

Crosses	DHE	DM	PH	SL	PL	SPS	FLA	GPS	SPP	TGW	Y	P	SC	CT	T	WUE	SG	RCIA	RCIL
L1×T1	-2.56	-2.61	-2.70	0.63	-2.73	1.58	1.31	3.36	2.08	1.36	0.88	2.62	27.78	-1.96	0.10	0.08	0.11	-3.44	-3.00
L2×T1	-0.98	-2.45	5.64	0.47	0.08	1.58	6.68	1.11	1.50	1.53	0.73	-0.25	-5.21	-0.71	-1.84	-0.37	0.94	-7.55	-5.31
L2×T3	1.06	1.16	0.23	-0.61	-1.29	-0.75	1.29	-0.61	-4.66	1.56	0.79	0.65	-6.32	0.24	-0.43	0.28	-0.02	4.44	5.07
L3×T1	-0.90	1.04	-0.35	-1.11	2.33	1.08	4.43	2.36	-1.16	0.11	0.62	0.39	-3.46	1.28	0.72	0.71	-0.05	0.24	1.22
L3×T2	-0.84	-1.53	-0.10	-0.61	-1.77	-0.36	-1.85	-0.50	1.63	-1.04	1.66	-0.39	0.25	3.65	-1.76	-0.89	-0.55	-2.34	-2.16
L3×T3	1.15	-0.00	-3.10	1.47	-2.04	-1.25	-4.05	-2.36	2.00	0.47	-1.14	0.80	-12.57	-4.75	-0.05	0.22	-1.02	3.34	-0.28
L3×T4	0.59	0.49	3.55	0.25	1.48	0.52	1.47	0.50	-2.47	0.45	-1.14	-0.80	15.78	-0.17	1.09	-0.03	1.63	-1.24	1.23
L4×T3	-0.51	-2.50	1.56	0.72	1.70	-0.41	0.69	0.38	-0.58	-0.10	-1.71	-0.80	10.79	-0.52	0.31	-0.49	0.47	-2.02	-0.04
L4×T4	-1.40	-0.67	1.22	1.50	-1.10	0.69	1.82	1.91	-1.05	-1.79	0.57	-1.75	15.75	2.06	-0.03	-0.21	-0.86	4.72	4.27
L5×T1	-1.40	2.63	4.31	0.55	2.17	-1.25	-4.88	-3.80	-2.00	-0.54	-1.57	-1.40	3.18	0.28	0.71	-0.91	0.11	2.46	2.47
L5×T3	-1.01	-1.75	-1.76	-0.86	0.45	-0.91	8.81	0.13	-0.16	2.81	3.53	2.60	33.07	1.24	-1.00	2.08	-0.52	-5.23	-4.13
L6×T1	-0.98	-3.28	-0.10	0.30	2.92	-0.91	5.83	-1.97	5.41	-0.96	-0.45	-2.10	11.00	0.03	0.99	-0.62	0.11	-2.75	1.65
L6×T3	0.06	1.32	-0.11	-0.18	-0.13	-0.58	2.74	-1.69	-0.75	1.06	-1.07	1.76	0.89	0.99	-0.85	0.54	-0.52	8.62	3.12
L6×T4	-0.81	3.16	-2.52	-1.00	-0.27	1.19	5.57	5.16	-2.22	0.03	1.02	3.09	-23.01	-1.42	0.36	0.80	-0.19	-3.82	-2.93
L7×T1	2.26	2.29	-2.01	-1.11	-0.66	-1.75	0.43	-1.88	-0.41	0.11	0.26	-0.30	-15.59	-0.60	-0.54	0.18	-0.88	-2.11	-1.34
L7×T3	-2.01	-0.42	-0.10	0.47	-1.38	-0.08	-1.85	-1.61	2.41	-0.85	-0.78	0.30	-5.11	1.36	0.98	-0.77	0.13	4.89	3.36
L8×T3	-0.09	1.49	2.89	-0.94	-0.63	-0.58	0.54	1.22	0.08	-1.77	-1.51	-3.24	-8.82	-0.50	0.21	-1.18	0.97	-2.87	-1.83
L9×T3	-1.43	-0.50	-2.68	-1.19	2.36	0.75	0.86	0.47	1.08	0.64	0.21	3.28	-19.37	0.74	-0.63	0.45	-0.11	-5.64	-2.65
L10×T3	-0.18	-0.75	-1.18	1.38	0.61	2.08	-0.40	5.38	1.25	-1.93	1.46	-2.37	18.17	1.49	0.24	-0.70	0.47	4.89	3.36
L10×T4	-1.40	0.74	3.47	-0.16	-0.18	0.52	-3.48	-3.08	-0.88	-0.63	0.95	1.21	-4.46	0.07	-1.20	-0.13	-0.86	2.32	-3.78
L12×T4	0.51	-0.09	2.39	-1.50	-1.43	-1.47	-2.07	0.58	2.86	1.61	1.51	0.41	-2.46	0.57	-0.50	-0.27	0.13	-2.52	1.36
S.E.	0.74	1.06	0.77	0.53	0.77	0.74	0.00	1.17	0.83	0.94	0.52	0.00	4.49 x10-14	1.20×10-14	4.47	2.15×10-15	0.05	2.41×10-14	2.38×10-14

Where, days to heading (DHE), days to maturity (DM), plant height (PH), spike length (SL), peduncle length (PL), spikelets/spike (SPS), flag leaf area (FLA), no. of grains/spike (GPS), spike per meter row (SPP), 1000-grain weight (TGW), and yield (Y); and physiological parameters, viz., photosynthesis (P), transpiration (T), stomatal conductance (SC), canopy temperature (CT), water use efficiency (WUE), stay green (SG), relative cell injury anthers (RCIA), and relative cell injury leaf (RCIL) under normal conditions.

Supp. Table 2. Estimates of specific combining ability effects for morphological and physiological traits of crosses under heat conditions.

Crosses	DHE	DM	PH	SL	PL	SPS	FLA	GPS	SPP	TGW	Y	P	SC	CT	T	WUE	SG	RCIA	RCIL
L1×T1	-2.82	-2.47	-4.25	0.34	-1.48	1.05	1.31	2.87	2.08	1.23	0.88	2.62	27.78	-1.96	0.10	0.68	0.11	-3.44	-3.00
L2×T1	0.42	-0.47	4.83	0.09	0.18	1.22	6.68	1.20	1.50	1.15	0.73	-0.25	-5.21	-0.71	-1.84	0.60	0.94	-7.55	-5.31
L2×T3	0.39	-0.08	-0.41	-0.09	-0.62	-0.55	1.29	-0.62	-4.66	1.34	0.79	0.65	-6.32	0.24	-0.43	-0.19	-0.2	4.44	5.07
L3×T1	-1.07	-0.72	-4.00	-0.81	3.51	1.55	4.43	2.12	-1.16	-1.01	0.62	0.39	-3.46	1.28	0.72	0.10	-0.05	0.24	1.22
L3×T2	0.70	-0.08	4.88	-0.23	-0.48	-0.05	-1.85	-0.23	1.63	-0.45	1.66	-0.39	0.25	3.65	-1.76	0.88	-0.55	-2.34	-2.16
L3×T3	0.56	0.66	-1.58	1.31	-1.73	-2.22	-4.05	-2.30	2.00	0.84	-1.14	0.80	-12.57	-4.75	-0.05	-0.11	-1.02	3.34	-0.28
L3×T4	-0.18	0.13	0.69	-0.26	-1.73	0.72	1.47	0.48	-2.47	0.62	-1.14	-0.80	15.78	-0.17	1.09	-0.88	1.63	-1.24	1.23
L4×T3	-0.27	-4.33	2.66	0.48	3.79	-0.05	0.69	-0.04	-0.58	-0.23	-1.71	-0.80	10.79	-0.52	0.31	-0.60	0.47	-2.02	-0.04
L4×T4	-0.02	1.80	1.61	0.90	-0.65	0.22	1.82	2.15	-1.05	-0.79	0.57	-1.75	15.75	2.06	-0.03	-0.75	-0.86	4.72	4.27
L5×T1	-0.49	4.19	4.08	0.18	2.59	-0.27	-4.88	-3.87	-2.00	0.65	-1.57	-1.40	3.18	0.28	0.71	-1.23	0.11	2.46	2.47
L5×T3	-0.18	-1.41	-2.83	-0.68	-0.20	-0.05	8.81	-0.04	-0.16	2.51	3.53	2.60	33.07	1.24	-1.00	3.62	-0.52	-5.23	-4.13
L6×T1	0.84	-1.22	0.58	0.26	0.09	-1.27	5.83	-1.87	5.41	-0.09	-0.45	-2.10	11.00	0.03	0.99	-0.44	0.11	-2.75	1.65
L6×T3	-0.18	1.50	-1.00	-0.26	-0.04	-0.38	2.74	-1.37	-0.75	1.09	-1.07	1.76	0.89	0.99	-0.85	0.51	-0.52	8.62	3.12
L6×T4	-1.93	1.97	-2.72	-0.18	2.18	1.22	5.57	4.81	-2.22	-0.12	1.02	3.09	-23.01	-1.42	0.36	0.36	-0.19	-3.82	-2.93
L7×T1	1.59	3.52	-1.41	-1.31	-0.65	-2.11	0.43	-2.04	-0.41	-0.18	0.26	-0.30	-15.59	-0.60	-0.54	0.36	-0.88	-2.11	-1.34
L7×T3	-0.43	-2.08	1.66	0.81	1.20	0.11	-1.85	-1.87	2.41	-0.65	-0.78	0.30	-5.11	1.36	0.98	-0.71	0.13	4.89	3.36
L8×T3	0.06	1.16	2.41	-0.59	-3.12	-0.55	0.54	1.37	0.08	-1.81	-1.51	-3,24	-8.82	-0.50	0.21	-2.30	0.97	-2.87	=1.83
L9×T3	-0.52	0.66	-1.83	-1.34	3.62	0.61	0.86	0.45	1.08	0.68	0.21	3.28	-19.37	0.74	-0.63	2.71	-0.11	-5.64	-2.65
L10×T3	-0.35	-0.58	-1.16	1.56	-0.95	0.77	-0.40	4.87	1.25	-1.90	1.46	-2.37	18.17	1.49	0.24	-2.67	0.47	4.89	3.36
L10×T4	-0.43	0.22	2.11	-0.34	0.93	1.05	-3.48	-2.93	-0.88	-1.12	0.95	1.21	-4.46	0.07	-1.20	5.04	-0.86	2.32	-3.78
L12×T4	1.81	1.30	-0.22	-1.18	0.76	-1.27	-2.07	0.15	2.86	1.45	1.51	0.41	-2.46	0.57	-0.50	-0.31	0.13	-2.52	1.36
S.E.	0.76	0.94	0.86	0.59	0.76	0.76	0.00	1.11	0.83	0.93	0.73	0.52	0.00	0.00	0.00	0.00	0.72	0.05	0.00

Where, days to heading (DHE), days to maturity (DM), plant height (PH), spike length (SL), peduncle length (PL), spikelets/spike (SPS), flag leaf area (FLA), no. of grains/spike (GPS), spike per meter row (SPP), 1000-grain weight (TGW), and yield (Y); and physiological parameters, viz., photosynthesis (P), transpiration (T), stomatal conductance (SC), canopy temperature (CT), water use efficiency (WUE), stay green (SG), relative cell injury anthers (RCIA), and relative cell injury leaf (RCIL) under heat conditions.