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Genetic Control of Thermo-Tolerance in Spring Wheat as Measured by Canopy Temperature Depression and Cell Membrane Thermostability

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Abstract: ***Terminal heat stress is an important production constraint for spring wheat and affects many plant biological activities. This comprehensive three years study was conducted to determine the genetic mechanism of heat tolerance through diallel analysis biometric technique in three environmental conditions i.e. stress free (Timely planting) and two heat stress regimes (Late planting and polyhouse-timely planting environments). Initially two hundred wheat strains of diverse origin were screened and identified four thermo-tolerant and three thermo-labile strains during year 2009-2010. Forty two F_1 hybrids were developed by hybridizing seven genotypes according to 7×7 full diallel fashion during 2010-2011. Response of forty nine genotypes to high temperature stress was measured by canopy temperature depression (CTD), cell membrane thermo stability (CMT) and grain yield per plant during next crop year 2011-2012. Data were subjected to diallel analysis of variance and estimation of variation of genetic parameters. Additive gene effects were highly significant for three traits in all test regimes. The overall dominance components were smaller but highly significant in stress free and heat stress indicating important role of dominance. Likewise highly significant b1 (Directional dominance) item indicated the directional dominance deviations of the genes. Symmetrical gene distribution and unimportant role of specific genes for canopy temperature depression and grain yield per plant were represented by non-significant b_2 (symmetry of gene distribution) and b_3(specific gene effects) items respectively. Regression and array variances analyses suggested the adequacy of model for canopy temperature depression and grain yield per plant. Estimation of genetic components of variation indicated the importance of additive gene effects in acquired thermotolerance for all the traits in three test regimes. Results indicated that heat tolerance based on CMT and CDT measurements can be enhanced by utilizing the genetic variability existing within genetic resources. In conclusion, diallel biometrical technique and integrated use of modified pedigree method of selection by involving parents like Ch-86, Bhakkar-2002, SH-02 and V00183 and use of specific crosses like Bhakkar-02 × SH-02, V00183 × Chakwal-86 and Chakwal-86 × SH-02 would be more effective for the evolution of post anthesis thermotolerant spring wheat varieties for hot irrigated dry climates.***

Keywords: Spring wheat, terminal heat, additive, genetic, polyhouse, gene action

1. Introduction

Wheat is cultivated in a diverse range of environments, from the arid plains of Africa to the humid valleys of Vietnam and from the cold of Nepal to the terminal heat of India/Pakistan. In hot irrigated dry climate, terminal heat stress has a strong influence on wheat growth and ultimately on yield. The stress intensity is more conspicuous in hot years on timely planting and as routine yield penalty in late planting. Wheat research endeavors have achieved significant genetic gains in yield potential without the aid of physiological selection tools.

Canopy temperatures depression (CTD) is an integrative, non-destructive and breeder friendly physiological trait with moderate heritability (Reynolds *et al.* 1997) and it has been reported upto 10°C in hot dry environments. CTD was strongly associated with yield (Fischer *et al.* 1998) performance in number of hot irrigated dry environments (Reynolds *et al.* 1994a). CTD is heritable and therefore amenable to early generation selection (Reynolds *et al.* 2001a). Heat tolerance is a polygenic trait (Puri *et al.* (1985) and selection in segregating populations is difficult and thereby it is necessary to develop novel approaches to enhance the selection efficiency. Balota *et al.* (2007) demonstrated consistent differences among the wheat lines for CTD.

Raison *et al.* (1980) reported that cell-membrane system of thermo-tolerant genotypes also remains functional in heat stress during grain filling stage of wheat crop. There are limited studies on the genetics of heat tolerance in cereals. Shanahan *et al.* (1990) obtained a significant increase in yield of spring wheat in hot locations by selection of membrane thermostable lines. Fokar *et al.* (1998) reported broad sense heritability for CMT 89% alongwith preponderance of additive genetic variance. Kumar *et al.* (2000) reported significant influence of genotypes on cell membrane thermostability, canopy temperature depression and grain yield per plant. Ibrahim and Quick (2001a) determined the genetic control of wheat heat tolerance through 6×6 complete diallel analysis and indicated the importance of additive gene effects.

Renu *et al.* (2004) concluded that wheat genotypes having MTS values in the range of 61.6 -72.5 appeared to be heat tolerant. Singh *et al.* (2005) reported positive association of grain yield with high CTD and lower RCI% values in post anthesis heat stress. Verma *et al.* (2006) screened 12 wheat genotypes for heat tolerance and reported significant genotypic differences in CTD and CMT, measured in terms

of relative cell injury percentage. In Pakistan, temperatures above the optimal range (28-30C^0) are very common even during the booting stage and short periods of chronic high temperatures (30-38^{o}C) are also encountered in some years. Heat shocks decrease more individual grain weight than progressively increasing temperatures. Anonymous (2007) suggested the incorporation of heat stress tolerance in future wheat varieties to sustain production. This study was design to establish the role of physiological traits in the evolution of high yielding and terminal heat stress tolerant wheat varieties for hot irrigated dry climates in present scenario of global warming. Main objective of this study was to explore the genetic makeup of forty two F_1 progenies along with seven parents through both field and laboratory testing in stress free and two heat stress environments.

2. Materials and Methods

2.1Location, Entries and Regimes

The research work was conducted in the wheat breeding section of Arid Zone Agriculture Research Institute, Bhakkar, Punjab, Pakistan during the three spring wheat crop seasons from 2009-10 to 2011-12. Experimental material was consisted of two hundred wheat genotypes of diverse origin, seven parental strains (Table 1) and 42 F_1 progenies of hexaploid spring wheat. The breeding material was evaluated for CTD and grain yield per plant in field conditions. Stress free environment was attained by timely planting while heat stress climates were provided by poly house and late planting along with cell membrane thermostability measurement in stress physiology laboratory.

2.2Preliminary Screening of wheat genotypes

A field experiment comprising 200 wheat genotypes was laid out according to RCBD having three replications in non stress and heat stress environments adjacently during crop season 2009-10. Stress free environment was attained by planting one set of experiment (control) on 10th November 2009 and thus allowing the earing and grain filling in optimal field environment. High temperature stress was provided by delaying the planting of second set of experiment up to the 20th December 2009, and thus exposing the crop to terminal heat stress during reproductive phase (Hussain, 2005). Each entry was sown in two rows of two meter length with plant to plant and row to row distance 0.15 m and 0.30 m respectively. Genotype to genotype spacing was also maintained 0.30 m to avoid border effect and to limit the gross area of experiment.

All the agronomic and cultural practices were carried out as and when required. Maximum and minimum mean temperatures remained above normal and crop remained almost disease free. Ten plants were harvested from each genotype per replication from both experimental units. Threshing was done with single plant thresher and average grain yield data per plant were recorded. Data were manipulated as per formulae for relative tolerance index % (Ali *et al.* 2007), relative performance ratio (Anonymous, 2006) and percent reduction in grain yield in heat stress in comparison with optimal situation (Rajaram,1998a) and identified four terminal heat tolerant (BKR-02, V00183, CH-86 and SH-02) and three thermo-labile wheat genotypes (3C001, 93T347 and Punjab-96) for further studies (Table-1).

$$\text{Relative tolerance index \%} = \frac{\text{Performance in normal} - \text{Performance in stress}}{\text{Performance in normal}} \times 100$$

$$\text{Relative tolerance ratio \%} = \frac{\text{Performance in stress}}{\text{Performance in normal}} \times 100$$

Table 1: Mean performance of seven selected wheat genotypes (parents for hybridization program) tested in stress free and heat stress

S. No	*Genotypes*	*Grain yield per plant (g)*		*RTI.%**	*RPR%***	*Yield Red.% ****	*Attribute*
		Stress free	*Heat stress*				
1	BKR- 02	30	19.67	34.43	65.66	34.34	Tolerant
2	V00183	31	20.63	33.32	66.54	33.45	Tolerant
3	Ch-86	30	19.67	34.33	65.66	34.43	Tolerant
4	SH-02	29	21	29.89	72.41	27.58	Tolerant
5	3C00l	22.33	8.67	61.12	38.82	61.17	Susceptible
6	93T347	25	11.33	54.66	45.32	54.68	Susceptible
7	Punjab-96	25	11.67	53.46	46.48	53.32	Susceptible

*=Relative tolerance index % **= Relative performance ratio %
*** = Yield reduction percentage in heat stress as compared with stress free environments

2.3 *Hybridization (Development of genetic material.*

Seven wheat genotypes, selected for the study of inheritance of high temperature stress tolerance were planted in field on 15th November 2010. These parental genotypes were hybridized in diallel fashion for forty two recombinants. Harvesting was done on physical maturity. F_0 and parental genotypes seed was reserved for further studies during next crop season (2011-12).

2.4 *Evaluation of genetic material in stress free and heat stress*

A) *Field experiments*

Three separate experiments were conducted during wheat crop seasons 2011-12. First experiment was laid-out on 10th November 2011 in stress free (Timely planting) and the second was also sown on the very date but poly-house was constructed over this plot after sixty days of sowing to mimic with heat stress environment. Maximum temperature in the poly house was maintained ±5°C above the ambient temperature. Third set of experiment was laid out on 20th December 2011 and thus exposed the crop to terminal heat stress during reproductive phase. Forty two F_1 hybrids along-with seven parents were laid out in each set according to randomized complete block design with three replications. Each entry consisted of six rows of 2.50 meter length with plant to plant and row to row distance of 0.15m and 0.30 m respectively. All the standard agronomic practices were adapted uniformly. Data were recorded for the two physiological traits, viz., canopy temperature depression and cell membrane thermostability along with grain yield per plant as per detail given below.

I Measurement of canopy temperature depression (CTD):

Canopy temperature depressions of all F_1 progenies and parents of 6-rows plot populations was recorded at grain filling stage (post anthesis) with a non-contact infra-red radiation thermometer. CTD was measured at high vapor pressure deficit and low relative humidity on warm, cloudless noon hours on three alternate days. Air temperatures were also noted before and after canopy temperature recording operation. Average CTD values were computed according to the formula (Balota *et al.* 2007) as given

$CTD = T_a - T_c$

Where

CTD= Canopy temperature depression

T_a = Air temperature

T_c = Canopy temperature

B) ***Laboratory experiments***

I Measurement of Cell membrane thermostability:

Cell membrane thermostability in terms of relative cell injury percentage (RCI %) of flag leaf tissue was measured at grain filling stage by adopting the procedure as proposed by Reynolds *et al.* (2001a). Plants were heat acclimated *in situ* as growing conditions were warm enough (>34/15°C) for more than 48 hours. Ten leaves (5 for treatment and 5 for control) per genotype of each replication in three planting regimes were sampled. Leaf samples were washed thoroughly with de-ionized water in stress physiology laboratory, and 1.0cm diameter disc from mid of each leaf was cut with a specially designed sharp steel puncture for both the control and heat shock treatments and put separately in vials containing 17ml de-ionized water. Five test tubes per entry were put in a water bath at 46.5°C for 60 minutes. The second set of vials was used as control and maintained at room temperature (25°C) for a period of an hour. After the treatment periods, the heat treated and controlled samples were held at 6°C over night. First reading (T_1) was recorded with electro conductivity meter at 25°C. Second EC meter reading (T_2) was also recorded at 25°C after autoclaving for 20 minutes at 120°C and 0.10 MPa pressure. Percentage relative cell injury (RCI %), an indicator of CMT, was calculated by using the following formula (Sullivan, 1972).

$$RCI\ \% = 1 - \frac{1 - (T_1/T_2)}{1 - (C_1/C_2)} \times 100$$

Where T and C refer to electro-conductivity values of heat treated and controlled vials, and subscripts 1 and 2 denote pre autoclaving and post autoclaving EC meter readings, respectively.

II Grain yield per plant (g):

Ten guarded plants of each genotype from all replications of three experiments were harvested at physiological maturity. Selected plants were threshed and grains were weighed with electronic digital balance and finally average value for yield per plant was computed accordingly.

3. Results and Discussion

3.1 Biometrical Analyses

Data collected of all F_1 hybrids and parental genotypes were subjected to standard analysis of variance (Steel *et al.*1997). Significant differences were observed for all traits in three test environments. The diallel analysis was carried out in two different phases. Firstly formal analysis of variance was conducted following Mather and Jinks (1982). Then genetic parameters of variation were computed in second step. Results obtained for the three traits (Canopy temperature depression, cell membrane thermostability and grain yield per plant) in three different test environments (Timely, late and poly-house cum timely plantings) are discussed as under.

3.2 Plant Response to High Temperature

a) *Canopy temperature depression (CTD):*

All the genotypes had higher canopy temperature depression in stress environments and displayed grand mean values of 5.97 and 8.73 °C in late planting and poly-house timely planting respectively as compared with stress free timely planting value of 5.10°C (Table 2). Maximum CTD values 11.00 and 11.33 °C were observed in parental genotypes SH-02 and V00183 in poly house heat stress environment. However, the highest CTD values 11.75°C, 11.33 and 11.00 were recorded in F_1 hybrids SH-02 × Ch-86, BKR-02 × SH-02 and SH-02 × BKR-02 respectively. These fore mentioned parental as well as F_1 progenies also performed better in late planted heat stress environment (Table 2).This situation resulted in establishment of cooler crop canopies and high grain yield were achieved in heat stress. These results are in accordance with those of Singh *et al*. (2005), Hirayama *et al.* (2006) and Balota *et al*. (2008).

b) *Cell membrane thermostability (RCI %):*

Cell membrane thermostability values for all the genotypes in three environments exhibited more or less the same trend for each particular genotype reflecting the stability irrespective of environmental conditions. RCI % displayed grand mean values of 48.85 and 49.73 in late planting and poly-house cum timely planting respectively as compared with stress free timely planting value of 45.80 (Table 2). However, the lowest cell membrane thermostability readings in terms of relative cell injury values 29.00, 29.33 and 32.00 were recorded for cross combinations V00183 × Ch-86, SH-02 × Ch-86 and BKR-02 × SH-02 respectively. These genotypes performed better in late planted heat stress environment as well (Table 2). F_1 hybrids characterized with low RCI % resulted in better grain yield in heat stress environments. These results are similar with those of Verma *et al*. (2006) and Anonymous (2007).

c) *Grain yield per plant (g):*

All the genotypes had lower grain yield per plant values in stress environments and displayed grand mean values 19.11 and 25.16g in late and poly-house timely planting respectively as compared with stress free timely planting value 30.34 g (Table 2). Among parental genotypes maximum grain yield per plant values 31.67 and 28.67 °C were observed in BKR-02 and SH-02 in poly house heat stress. However, the highest grain yield per plant viz. 33.67g, 32.33g and 32.00g were recorded in poly-house environment

for F_1 hybrids BKR-02 × SH-02, SH-02 × BKR-02 and Ch-86 × SH-02 respectively. These genotypes also performed better in stress free (Timely planting) and heat stress (Late planting) environments (Table-2). F_1 hybrid Bhakkar-02 × SH-02 had the highest grain yield per plant in three sowing conditions (39.67, 33.67 and 28.00g) with a reduction of 15.12% and 29.41%, in late and poly house cum timely planting heat stress environments respectively. Therefore polyhouse timely planting heat stress environment displayed net effect of heat stress on grain yield per plant. These results also get support from the findings of Akbar *et al*. (2008) and Prasad *et al*. (2008).

Table 2: Means, grand mean, coefficient of variability, LSD values of genotypes for canopy temperature depression, cell membrane thermostability and grain yield per plant in different heat stress regimes

Genotypes	*CTD (°C)*			*CMT* (RCI %)*			*Grain yield (g)*		
	Stress free	*Heat stress*	*Heat stress (P H)*	*Stress free*	*Heat stress*	*Heat stress (P H)*	*Stress free*	*Heat stress*	*Heat Stress (PH)*
BKR 02 (Parent)	07.00	09.33	10.33	30.00	38.67	37.00	39.00	25.00	31.67
BKR 02 ×V00183	06.00	08.67	10.33	30.33	38.00	39.00	33.00	23.33	29.00
BKR 02 × Ch-86	06.67	07.67	09.00	29.00	35.00	41.00	30.67	21.00	25.67
BKR 02 × SH 02	06.33	10.00	11.67	26.00	30.00	32.00	39.67	28.00	33.67
BKR 02 × 3C001	05.33	06.33	09.00	48.67	52.00	47.67	27.67	16.10	21.00
BKR02 × 93T347	04.67	05.00	08.33	43.33	42.33	56.00	34.00	21.67	27.67
BKR02 × Pb-96	05.00	05.33	09.00	47.00	52.00	52.00	31.00	17.67	24.67
V00183 × BKR02	06.00	07.33	10.33	31.00	37.00	38.00	33.00	21.00	25.33
V00183 (Parent)	07.00	08.00	11.00	28.00	35.00	34.00	34.33	22.33	28.00
V00183× Ch-86	07.00	09.33	11.33	27.67	31.00	32.00	34.00	24.00	29.33
V00183 × SH-02	08.67	08.00	09.00	23.00	33.00	29.00	29.67	20.67	25.33
V00183 ×3C001	05.00	05.67	07.67	56.00	58.00	53.00	27.67	17.00	23.33
V00183 × 93T347	04.00	04.67	07.33	60.00	67.67	57.00	32.67	20.00	26.33
V00183 × Pb-96	03.00	03.33	05.00	53.00	54.00	67.00	24.00	14.67	19.67
Ch-86 × BKR-02	06.67	07.67	11.00	28.00	37.00	39.33	34.67	24.67	30.67
Ch-86 ×V00183	06.33	07.33	10.67	26.33	32.00	34.00	35.67	25.33	30.00
Ch-86 (Parent)	07.67	09.33	10.33	28.00	34.00	36.00	33.00	22.67	28.33
Ch-86 × SH-02	08.67	08.33	10.33	26.00	30.00	28.67	38.00	24.67	32.00
Ch-86 × 3C001	06.00	06.67	08.33	48.00	46.67	50.00	27.00	17.00	22.00
Ch-86 × 93T347	04.33	05.00	07.67	54.00	47.67	53.00	27.00	17.00	23.33
Ch-86 × Pb-96	04.00	04.50	07.33	55.00	54.00	58.00	28.67	18.00	23.66
SH 02 × BKR-02	06.67	07.67	11.33	25.67	31.00	39.00	37.33	27.33	33.00
SH 02 × V00183	08.33	09.67	11.00	22.33	34.00	27.00	30.33	20.33	25.67
SH 02 × Ch-86	06.33	08.33	11.75	25.00	31.00	29.33	37.33	25.00	30.00
SH 02 (Parent)	06.67	09.67	11.33	26.00	28.33	35.33	35.00	22.67	28.67
SH 02 × 3C001	05.00	06.33	09.67	58.00	47.00	50.00	29.67	18.33	24.00
SH 02 × 93T 347	05.33	05.67	08.33	48.00	50.00	51.00	31.33	18.67	25.33
SH -02 × Pb-96	03.50	03.67	05.67	46.67	53.00	59.00	24.00	14.00	19.33
3C001 × BKR-02	04.67	05.15	09.00	48.33	52.00	49.00	30.00	16.67	24.67
3C001 × V00183	05.33	06.00	09.00	57.67	57.67	50.00	27.33	17.67	22.67
3C001 × Ch-86	05.33	06.00	08.67	46.67	45.00	48.33	29.33	19.00	24.00
3C001 × SH-02	04.67	05.33	09.00	57.67	46.00	51.00	29.33	18.00	24.67
3C001 (Parent)	02.33	02.67	06.00	69.67	74.00	72.00	24.00	13.67	19.33
3C001 × 93T347	05.00	05.50	08.00	61.00	66.00	58.00	32.33	18.33	26.33
3C001 × Pb-96	03.33	03.60	08.33	62.00	70.00	58.33	21.00	11.67	16.67
93T347 × BKR02	04.33	04.67	07.67	43.00	43.00	56.67	36.67	23.33	30.00
93T347 × V00183	04.00	05.50	08.33	60.33	68.33	56.00	28.33	17.00	22.33
93T34 7 × Ch-86	04.25	04.90	08.67	53.00	46.67	51.67	27.00	14.50	21.67
93T347 × SH-02	05.33	05.67	09.33	47.00	49.00	49.00	32.33	21.33	27.33
93T347 × 3C001	04.00	0 4.50	06.33	61.00	66.00	55.33	29.33	15.67	24.67
93T347 (Parent)	02.67	03.00	08.00	61.67	68.00	68.67	22.67	14.00	22.00
93T347Pb-96	02.65	03.00	07.00	63.00	65.33	70.00	28.33	16.00	23.67
Pb-96 × BKR-02	04.33	05.00	07.67	47.33	51.00	54.00	30.67	19.50	26.33
Pb-96 × V00183	03.00	03.67	06.33	52.00	52.00	65.00	24.67	12.75	20.33
Pb-96 × Ch-86	04.00	04.33	08.67	54.67	52.00	59.00	30.67	17.67	26.00
Pb-96 × SH-02	04.00	04.45	07.00	45.00	51.67	57.67	24.67	15.00	20.00
Pb-96 × 3C001	03.67	04.33	07.00	63.00	70.00	61.33	24.67	13.67	19.33
Pb-96 × 93T347	02.55	03.00	07.67	64.00	66.67	71.00	30.00	20.67	26.00
Pb-96 (Parent)	03.33	04.00	05.67	76.00	74.00	67.67	24.33	12.33	19.33
Grand mean	05.10	05.97	08.73	45.80	48.85	49.73	30.34	19.11	25.16
C.V.%	14.03	18.16	11.82	2.17	01.51	2.10	07.48	09.77	06.90
LSD	1.166	1.809	1.668	1.604	1.193	1.709	3.664	3.193	2.807

A) **Diallel analysis**

1) *Canopy temperature depression:*
Complete analysis of variance following Mather and Jinks (1982) for canopy temperature depression depicted that additive gene effects (Item *a*) were highly significant in all test regimes (Table 3).The overall dominance component *b* was smaller but highly significant in stress free and heat stress indicating important role of dominance. Likewise highly significant b_1 item indicated the directional dominance deviations of the genes. Symmetrical gene distribution and unimportant role of specific genes were represented by non-significant b_2 and b_3 items respectively. Absence of maternal and reciprocal effects was detected by non-significant *c* and *d* items in all test environments.

2) *Cell membrane thermostability (RCI %):*
Diallel analysis of variance following Mather and Jinks (1982) for cell membrane thermostability depicted that the item *a* (additive gene effects) was highly significant and accounted for high proportion of the total variation in three test regimes (Table 3). The overall dominance component *b* was smaller but highly significant indicating the important role of dominance. Similarly significant b_1 item indicated the directional dominance deviations of the genes in both stress free and heat stress conditions. Symmetry of gene distribution among the parents was represented by non-significant b_2 item. Significant b_3 item indicated the important effect of specific genes in three planting situations. Significant *c* and *d* required the retesting of *a* and *b* components. After retesting, significance of *a* component remained unchanged indicating that maternal effects did not influenced the additive gene effects. Highly significant *b* and b_1 items remained unchanged. Similarly, highlysignificantb_3 item was not changed indicating that specific gene effects were not invalidated by reciprocal effects in three test regimes.

3) *Grain yield per plant (g):*
Highly significant differences among the genotypes for grain yield per plant allowed to proceed for complete diallel analysis (Table 3). Additive gene effects were highly significant indicating the presence of greater amount of additive variation in all heat stress test regimes. Significant differences among F_1 generations were also reported by Budak, (2001). The overall dominance component *b* was smaller but highly significant in all planting conditions indicating the important role of dominance. Likewise significant b_1 item indicated the directional dominance deviations of the genes. Non-significant values of b_2 and b_3 items displayed the symmetrical distribution and unimportant role of specific genes respectively. Non-significant values of *c* items in timely and late plantings indicated the absence of maternal effects. Highly significant values of *c* and *d* items in poly-house stress environment required the retesting of *a* and *b* components. After retesting, significance of *a* component remained unchanged indicating that maternal effects did not influence the additive genetic effects.

Table 3: Diallel analysis of variance (Mean squares and F ratios) for CTD, CMT and grain yield per plant of hexaploid bread wheat (Mather and Jinks, 1982)

Sr. No.	Character	Condition		a	b	b1	b2	b3	c	d
1	Canopy temperature depression (°C)	Stress free (Timely Planting)	MS	73.15	5.08	136.15	0.68	1.80	1.06	1.55
			F.Ratio	75.11**	2.82**	96.51**	0.96	2.90	0.49	1.96
		Heat stress (Late Planting)	MS	59.04	2.51	67.21	0.009	1.01	0.20	0.48
			F. Ratio	87.01**	4.96**	129.15**	0.16	1.62	0.34	1.13
		Heat stress (Poly house)	MS	79.71	3.88	100.67	0.0074	1.36	1.13	2.17
			F. Ratio	98.81**	2.73*	77.49**	0.006	1.25	0.97	2.0
2	Cell membrane thermo-stability (%)	Stress free (Timely Planting)	MS	3978.37	95.60	2475.93	8.41	29.83	4.53	1.01
			F.Ratio	8805.34**	263.59**	3256.58**	2.10	25.97**	14.48**	2.71**
		Heat stress (Late Planting)	MS	4671.81	139.21	3380.98	0.12	32.62	0.63	1.52
			F.Ratio	3682.34**	98.54**	2681.33**	0.27	31.34**	1.08	2.37*
		Heat stress (Poly house)	MS	5300.64	73.14	2120.28	7.94	38.33	1.60	1.66
			F.Ratio	2693.07**	70.40**	1858.94**	2.68	35.67**	6.51**	2.16*
3	Grain yield per plant (g)	Stress free (Timely Planting)	MS	308.55	34.16	837.81	0.25	8.48	4.97	8.24
			F. Ratio	46.60**	4.52**	136.45**	0.15	2.35	0.90	2.7*
		Heat stress (Late Planting)	MS	232.04	24.08	568.47	0.25	4.36	8.13	6.44
			F. Ratio	52.37**	4.59**	134.50**	0.07	2.21	2.25	1.92
		Heat stress (Poly house)	MS	243.82	27.52	637.62	0.068	4.23	10.18	7.67
			F.Ratio	41.47**	7.11**	211.48**	0.41	2.84	5.30**	3.32**

* = $P \leq 0.05$. ** = $P \leq 0.01$ ● = each item tested against its own block interaction

B) Regression analyses and arrays analyses of variance

1) Canopy temperature depression:
Two scaling tests were employed for the validity of additive-dominance model following Mather and Jinks (1982). Analysis of array variances and regression coefficient test for canopy temperature depression depicted absence of non-allelic interaction and data were considered fully adequate for further analysis under both stress free and stress regimes (Table 4)

Table 4: Test of adequacy of additive-dominance model (regression analysis and arrays analysis of variance) of 7×7 diallel crosses for three traits studied in stress free and heat stress environments

S. No.	ChCharacters	*Regression analysis*		*Analysis of array Variances*		Re Remarks
		b=0	b=1	Wr+Vr	Wr-Vr	
I Stress free environment (Timely planting)						
1	Canopy temp. Depression (^oC)	**	NS	**	NS	Both tests suggested the adequacy of the model
2	Cell membrane Thermostability (RCI %)	*	NS	**	**	Regression analysis indicated the adequacy of the model but analysis of arrays invalidated the model, thus it was considered partially adequate
3	Grain yield per plant (g)	**	NS	*	NS	Both tests suggested the adequacy of the model
II Heat stress environment (Late planting)						
1	Canopy temp. depression(^oC)	**	NS	**	NS	Both tests suggested the adequacy of the model
2	Cell membrane thermostability	**	NS	**	**	Regression analysis indicated the adequacy of the model but analysis of arrays invalidated the model, thus it was considered partially adequate
3	Grain yield per plant(g)	**	NS	*	NS	Both tests suggested the adequacy of the model
III Heat stress environment (Timely cum polyhouse planting)						
1	Canopy temp. depression(^oC)	**	NS	**	NS	Both tests suggested the adequacy of the model
2	Cell membrane thermostability	**	NS	**	**	Regression analysis indicated the adequacy of the model but analysis of arrays invalidated the model, thus it was considered partially adequate
3	Grain yield per plant(g)	**	NS	*	NS	Both tests suggested the adequacy of the model.

* =Significant ** = Highly significant NS= Non-significant

2) Cell membrane thermostability (RCI %):
The joint regression coefficient test for cell membrane thermostability indicated that in all test regimes b differed significantly from zero but not from unity. Thus the data fulfilled assumptions of the model (Table 4). However, second test of analysis of array variances revealed significant differences, indicating the presence of both dominance and non-allelic interaction. Therefore data were considered partially adequate to explain the genetic information.

3) Grain yield per plant (g):
Test of adequacy of additive-dominance model for analysis of variance of arrays for grain yield per plant depicted absence of non-allelic interaction and data were considered fully adequate for further analysis under both stress free and stress regimes (Table 4).

iii Estimates of genetic components of variations

1) Canopy temperature depression:
Estimation of genetic components of variations revealed that both additive (D) and dominance (H) effects for CDT were significant. Unequal values of H_1 and H_2 and proportions of genes with positive and negative effects ($H_2/4H_1$ ratios) indicated the unequal distribution of positive and negative alleles among the parents in all planting environments (Table 5). 'Fr' mean over the arrays (F value) was found non-significant and positive. Dominant to recessive gene ratios indicated higher frequency of dominant gene in the parents. Dominance effects ($h^{\wedge 2}$values) were found positive in stress free climate, while negative in both heat stress environments but non-significant in all test conditions. Environmental component of variation (E value) depicted the influence of environment on the expression of this trait in stress free and poly house heat stress. Average degrees of dominance values viz. 0.727, 0.606 and 0.649 displayed the absence of complete dominance in timely, late and poly house-cum-timely plantings respectively. However, moderate heritability estimates were recorded for canopy temperature depression by Reynolds *et al*. (1997).

2) Cell membrane thermostability (RCI %):
Estimation of genetic components of variations revealed that additive portion (D value) was significant in all test climates, while dominance component exhibited non-significant value in stress free and late plantings. D values were higher as compared with dominance components in all test environments displaying predominance of additive effects. Variances due to dominance effects of gene (H_1 and H_2values) depicted unequal readings along with $H_2/4H_1$values viz. 0.206, 0.214& 0.192 in stress free, late and poly-house plantings respectively indicating the unequal distribution of positive and negative alleles among the parents. 'Fr' mean over the arrays was positive and non-significant in three temperature regimes. Dominant to recessive genes ratios i.e. 1.372, 1.348 & 1.383in normal, late and poly-house timely planting conditions respectively, also indicated higher frequency of dominant genes in the parents. Dominance effects ($h^{\wedge 2}$values) were found non-significant but positive in stress free and negative in both heat stress environments. Non-significant values of environmental component of variations ruled out the influence of environment. Mean degrees of dominance values (0.471, 0.498& 0.634) displayed the absence of complete dominance in three test environments. Additive gene action with partial dominance is reported by Singh *et al*. (2005).Likewise, high narrow sense heritability estimates

i.e. 0.905, 0.891& 0.850 in stress free, late and poly-house plantings respectively, also indicated considerably large additive proportion in the total heritable genetic variation (Table 5). Ibrahim and Quick (2001b) however reported low heritability estimates for membrane thermostability index. Contrary, Fokar *et al.* (1998) reported broad sense heritability 89%.

Grain yield per plant (g):

Estimation of genetic components of variations for grain yield per plant revealed that both additive (D) and dominance effects (H) were significant in all test climates. Components of variance due to dominance effects of genes (H_1 and H_2values) and proportion of genes with positive and negative effects ($H_2/4H_1$ratios) indicated the unequal distribution of positive and negative alleles among the parents in both stress free and heat stress environments. Dominant to recessive genes ratios indicated that dominant genes were more frequent than recessive genes in the parents in all situations. However significant values of environmental component of variations depicted the influence of environment on the expression of this trait in all regimes. Similar findings were also reported by Joshi *et al*. (2002). Mean degrees of dominance values viz. 0.847, 0.905 & 0.912 under timely, late and poly-house plantings respectively, displayed the absence of complete dominance (Table 5).

Additive gene action with partial dominance for this trait has been reported also by Joshi *et al*. (2003), Joshi *et al*. (2004) and Chandrashekhar and Kerketta (2004). High narrow sense heritability estimates indicated considerably large additive proportion in the total heritable genetic variation. Similar outcomes were also reported by Chandrashekhar and Kerketta (2004) and Khan *et al*. (2005).

Table 5: Estimates of genetic components

S. No	*Character*	*Condition*	*D*	*H_1*	*H_2*	*F*	*h^ 2*	*E*	*$(H_1/D)^{0.5}$*	*$H_2/4H_1$*	*$4DH_1^{0.5}+F/4DH_1^{0.5}-F$*	*$h^2_{(n.s)}$*
1	Cell membrane thermo-stability	Stress free (Timely Planting)	425.440*	94.421	78.043	63.005	8.104	0.278	0.471	0.206	1.372	0.905
		Heat stress (Late Planting)	501.044*	124.427	106.575	74.070	-0.060	0.378	0.498	0.214	1.348	0.891
		H Heat stress (Poly house)	333 334.771*	134.558*	103.179*	68.276*	-0.003	0.392	0.634	0.192	1.383	0.850
2	Canopy temperature depression	Stress free (Timely Planting)	7.903*	4.184*	3.483*	1.756	0.460	0.419*	0.727	0.208	1.360	0.726
		Heat stress (Late Planting)	6.078*	2.237*	1.793*	0.947	- 0.074	0.170	0.606	0.200	1.294	0.818
		H Heat stress (Poly house)	7.121*	3.005*	2.497*	0.137	-0.166	0.349*	0.649	0.208	1.030	0.793
3	Grain yield per plant	Stress free (Timely Planting)	36.781*	26.425*	22.996*	11.338	-0.628	1.800*	0.847	0.217	1.444	0.656
		Heat stress (Late Planting)	20.660*	16.950*	15.452*	0.429	-0.381	1.297*	0.905	0.227	1.023	0.677
		H Heat stress (Poly house)	23.713*	19.761*	18.155*	2.395*	-0.444	1.043*	0.912	0.229	1.117	0.672

* = Value is significant when it exceeds 1.96 after dividing with its standard error.

D = Variation due to additive effects. H = Component of variance due to dominance effect of the genes.

F = Mean of 'Fr' over the arrays E = Environmental component of variation

$(H1/D)^{0.5}$ = Mean degree of dominance$H_2/4H_1$ = Proportion of genes with positive and negative effects in the parents

$h^2_{(n.s)}$ = Heritability (Narrow sense) $4DH_1^{0.5}+F/4DH_1^{0.5}-F$ = Proportion of dominant and recessive genes in the parents

h^ 2 = Dominance effect (as the algebraic sum over all the loci in heterozygous phase in all loci)

4. Conclusions

1. Parental genotypes BKR-02 and SH-02 gave maximum grain yield per plant in heat stress environments. Similarly, the highest canopy temperature depression and grain yield per plant values were recorded for the F_1 hybrids BKR-02× SH-02, SH-02 × BKR-02 and Ch-86 ×SH-02 in poly-house environment. Likewise, the lowest cell membrane thermostability readings in terms of relative cell injury were recorded for cross combinations V00183× Ch-86, SH-02× Ch-86 and BKR-02× SH-02.
2. Biometrical analyses indicated preponderance of additive gene action with partial dominance for all the traits in three test environments. Therefore heat tolerance based on CMT and CTD measurements can be enhanced by utilizing the genetic variability available in plant resources.
3. Diallel biometrical technique and integrated use of modified pedigree selection method by involving parental genotypes and F_1 hybrids mentioned above would be more effective for the evolution of post anthesis thermotolerant spring wheat varieties for hot irrigated dry climates.

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