GENETIC VARIATION IN RELATIVE CELL INJURY FOR BREEDING UPLAND COTTON UNDER HIGH TEMPERATURE STRESS

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ABSTRACT

The objective of present study was to examine the presence of variation in relative cell injury percentage (RCI%), as a measure of heat tolerance in sample of *G. hirsutum* germplasm and also to investigate genetic basis, if any, of RCI% under heat stress. For this purpose, response of 70 cotton varieties/lines was studies to optimum and high temperature, and RCI% was measured at reproductive stage. Data showed differing responses of the germplasm to the two temperature regimes. On the basis of similarities MNH 552, FH 100 and NIAB 111 were screened out as tolerant whilst Cedix ST-362 (GL), LRA 5166 and 4F were identified as susceptible varieties. Three sets of crosses involving tolerant × susceptible varieties were made according to generation mean technique. Six generations of each cross was field planted under optimum and high temperature and at reproductive stage RCI% was again measured. Genetic analysis of the data showed the predominant effect of additive component in the inheritance of RCI% under both the temperature regimes and consequently estimates of h^2_{ns} were high in three crosses. These estimates were used to calculate response to selection in F_3 population that appear to be encouraging. The results of present investigation suggest that RCI% could be used as effective selection criteria for selecting the plants with lower value of RCI%.

Keywords: Generation mean analysis, *Gossypium hirsutum* L. heat tolerance, relative cell injury, variability

INTRODUCTION

For best growth and healthy development of cotton crop (*Gossypium hirsutum* L.), optimum day/night temperature is 30/20 ºC, with an optimum for photosynthesis being 28 ºC (Burke et al., 1988; Reddy et al., 1997a). Temperature higher than 35 ºC increase photorespiration and impaired reproductive phase seriously causing fruit shedding, pollen sterility and consequently decrease lint yield (Reddy et al., 1991a; Baloch et al., 2000 and Khan et al., 2008). In Pakistan, cotton crop is mostly grown in Sindh and Punjab provinces and in some part of these areas average daily temperature exceeds 35 ºC, and reproductive phase is highly sensitive to this temperature and in response to high temperature heavy loss occurs to young fruits i.e. 65-70% (Taha et al., 1981; Baloch et al., 2000). When temperature decrease during the month of September, 30-35% of bolls are retained which gives seed cotton yield to the farmers (Taha et al., 1981). It has been reported that high temperature during peak reproductive stage is the basic reason of low yield of cotton in Pakistan (Anonymous, 2005). There is evidence in the literature to how that for every degree of maximum daily temperature

over 30 ºC in July decreased lint yield to the extent of 50 Kg/hac (Ooesterhuis, 1999), and similarly Bradow and Davidonis (2000) found negative impact of high temperature on fiber quality. Thus there is ample scope for the development of cotton genotypes that are tolerant to high temperature beyond the documented effects on yield and fiber quality. However the use of selection criteria easy to measure heat tolerance of cotton is essential.

Assessment of germplasm at plan maturity particularly reproductive stage under heat stress is effective if relevant and repeatable screening environments are available (Ismail and Hall, 1999). Unfortunately significant, genotype × year and genotype × environment effects often hamper genetic advance in the field. Therefore, use of indirect selection criteria for the variation of heat tolerance is advantageous. Relative cell injury %age which is a measure of cell membrane thermostability (CMT) has been reported as a vital trait of heat tolerance (Blum and Abercon, 1981). This technique was used for the first time by Sullivan (1972) in sorghum for the identification of heat tolerant and sensitive genotypes. RCI had been used by various workers for the identification of temperature sensitive and temperature

tolerant cotton varieties (Ashraf et al., 1994; Rehman et al., 2004; Azhar et al., 2005, 2009 and Khan et al., 2008). Ashraf et al. (1994) evaluated five varieties of cotton for RCI % after three weeks of initial growth at 30 ºC, and found that MNH 93 with lower relative injury had greater dry mass production than the other three varieties evaluated. In another study, Khan et al. (2008) evaluated 45 varieties for RCI % which ranged from 44.8 to 79%, and reported NIAB 111 as tolerant variety. In the present study NIAB 111 with minimum injury to cell i.e., 27 % reflected it's greater tolerance to heat. Clearly the present data appeared to agree with that of previous work. Similarly high heat tolerance of MNH 552 has been studied by Rehman et al. (2004) and Azhar et al. (2009), and its superiority has been substantiated in the present studies, whilst 4F, an old and obsolete cultivar of this region has been found susceptible (Azhar et al., 2009). According to the suggestion of Bibi et al. (2003) that obsolete cultivar suffered more due to heat stress than new one has been substantiated by the data on 4F which revealed it's heat susceptibility. Such observations on heat tolerance had been reported in wheat (Galiba et al., 1997; Ibrahim and Quick et al., 2001a and 2001b; Ali et al., 2007), soybean (Martineau et al., 1979a and 1979b), rice (Agari et al., 1995), legumes (Srinavasan et al., 1996), cowpeas (Ismail and Hall, 1999), sorghum (Sullivan and Ross, 1979), tomato (Chen et al., 1982), and the existence of intraspecific variation for heat tolerance reported in various crop species is in agreement with the variation in *hirsutum* spp. examined here.

The development of heat tolerant plant material through breeding methods requires the existence of genetic variability for heat tolerance within the species. Previous studies revealed ample evidence on the existence of variation in heat tolerance in various crop species for example, wheat (Shanahan et al., 1990; Ibrahim and Quick, 2001a, 2001b), rice (Yoshida et al., 1981; Mackill et al., 1982), cowpeas (Mutters and Hall, 1992; Ahmed et al., l993ab; El-kholy et al., 1997), tomato (Dane et al., 1991; Abdul-Baki and Stommel, 1995), mung bean (Collins et al., 1995) and cotton (Rehman et al., 2004; Azhar et al., 2009). In the recent years a few studies showed a positive reasonably strong and significant association between CMT and yield (Blum et al., 2001; Rehman et al., 2004). However Rehman et al. (2004) cautioned against using CMT as an indirect selection measure under non-heat stressed environment, concluded that factors other than CMT may influence yield under high temperature, and that CMT alone is not suggested as a selection criteria. Therefore, better understanding of genetic and physiological basis of heat tolerance is essential to develop heat tolerant cotton cultivars. In this paper heat tolerance of *G. hirsutum* L. has been investigated by measuring RCI%, which is a measure of cellular membrane thermostability. Since no information to-date is available on genetic basis on RCI% in the literature, and therefore attempt has been made to explain genetic mechanism controlling RCI%.

In the present work heat tolerance has been studies in upland cotton measuring relative cell injury (RCI) which is a measure of cellular membrane stability (CMT) and has been identified as a vital trait of heat tolerance in crop. RCI level from leaf discs at high temperature has been suggested as a screening tool for heat tolerance in plants (Sullivan, 1972). Although it is dire need, no direct and useful attempts have been made towards understanding genetic, physiological and morphological basis of heat tolerance in Pakistan.

Keeping in view the importance of cotton crop in the buildup of the economy of Pakistan, development of tolerance against high temperature would be of a value both to the farmers and the country. Earlier information about the presence of variation for heat tolerance is very scanty, and what does exist in the literature revealed that variability within species is available (Trolinder and Shang, 1991; Baloch and Lakho, 2000; Rahman et al., 2004; Azhar et al., 2009). In addition, from the studies of intergeneric differences in heat tolerance (Srinavasan et al., 1996) it is clear that potential for selection and breeding for enhanced heat tolerance is existing.

MATERIALS AND METHODS

The plant material for the study of variation in relative cell injury (RCI%) in *G. hirsutum* L. and its genetic controlling mechanisms were studied in the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad (Pakistan) during 2012-2015. The cotton germplasm was sown under heat stress. Name of each genetic entry examined for RCI% is given in Table 1.

Evaluation of germplasm for RCI

The 70 varieties were grown in pots measuring 30 cm height and 30 cm diameter paced under optimum (to be called normal temperature, day/night) at 31-34/19-21 ± 2°C and higher temperature (heat stress), 42-44º C /24-27±2º C. The two temperatures were developed in two separate growth chambers in glass house. In one chamber optimal temperature was maintained and in other temperature was gradually increased to obtain desired degree of the temperature, till the start of the reproductive stage.

Table 1. RCI% of 70 varieties of *Gossypium hirsutum* L. grown under two temperature regimes

Code No	Varieties	Optimum	High
1	Greg 25V	45.0	53.3
2	Royal S. Okra	69.7	81.0
3	YEP5	63.3	72.3
4	Coker 310	61.0	69.7
5	Linea 100	57.0	67.3
6	Barb XL1	58.7	69.0
7	LRA5166	74.3	95.0
8	Acala1517C	55.0	62.3
9	Riba 50	52.0	59.3
10	TH 4183	50.7	58.0
11	Frego	50.3	57.3
12	DPL2775	50.0	57.3
13	DPL61	49.3	56.0
14	Cedix St 362 (GL)	74.0	94.7
15	GFS	36.0	44.0
16	CP/15/2	45.3	55.3
17	Tidewater	48.0	57.3
18	4F	73.0	93.7
19	SLH41	65.7	77.7
20	PB899	54.3	67.7
21	PB900	52.7	66.3
22	VH144	43.7	53.7
23	FH1000	27.0	29.3
24	FH900	70.7	83.0
25	AC134	52.0	61.3
26	CIM 443	64.3	77.0
27	MNH424	51.3	62.7
28	MNH129	78.0	88.0
29	MNH440	46.0	55.7
30	MNH394	43.7	54.3
31	NIBGE3701	53.3	62.0
32	MNH552	26.0	28.3
33	MNH786	43.0	50.3
34	MNH789	48.0	57.0
35	MNH633	59.7	66.3
36	MNH706	53.3	64.0
37	MNH765	40.7	50.3
38	MNH738	47.0	58.3
39	S12	71.3	84.0
40	FH 114	46.0	56.0
41	NIBGE1524	47.3	57.3
42	NIBGE601	44.7	53.7
43	CRISS403	56.7	66.0
44	CRISS134	47.3	58.0
45	CIM446	71.0	84.7
46	CIM448	58.7	71.3
47	CIM1100	41.3	47.7
48	CIM473	43.0	50.3
49	CIM240	70.7	83.7
50	CIM511	57.0	68.3
51	CIM557	39.7	49.3
52	CIM541	41.7	49.7
53	CIM554	53.0	66.7
54	BH118	68.7	79.0
55	BH126	41.0	51.3
56	BH121	45.7	55.7
57	BH160	54.7	65.3
58	BH162	32.3	37.7
59	NIAB 111	25.0	27.3
60	NIAB KARISHMA	47.7	59.0
61	NIAB 78	52.3	64.3
62	B557	35.0	44.0
63	NIAB 884	53.3	60.3
64	NIAB 999	44.0	52.7
65	FH 113	59.0	68.0
66	CIM 499	74.3	88.3
67	BH147	74.7	88.3
68	VH142	79.0	91.3
69	MNH 93	73.7	86.7
70	CIM707	72.3	87.0
	Cd 5%	2.54	2.08

These pots were filled with 9 kg pre-tested field soil. Components of soil testing were pH (8.1), EC (1.2 dS/m), organic matter (1.42 %), saturation percentage (31 %), phosphorous (28.9 ppm) and potassium (135 ppm). The seeds of 70 varieties were soaked for eight hours, and four seeds of each genetic entry were sown in each pot during October, 2012, and were thinned to one seedling at two true leaf stage. There were 5 pots of each genetic entry in one replication, thus having 15 plants in three replications. In total there were 1050 plants in each temperature regime, and these were arranged according to completely randomized design. In both the chambers day length was 14 hours, natural light (PAR ranged 1400-1600 μmol nf^2 s at noon) and 65-80 % humidity. The earthen ports were supplied with normal irrigation water at the rate of 1400 ml per pot daily during peak flowering period, and afterwards on alternate days. All other growing conditions were kept uniform, till maximum flush of flowers was noted on stress and non-stress plants.

For measuring RCI % of 70 varieties, leaves were tagged on the day they unfolded, and that was designated as day 1. A steel punch of 10 mm inner diameter was used for punching leaf discs. Samples were collected in paired sets from both sides of central rib of a leaf for heat stressed (high temperature) and non-heat stressed (normal). Leaf discs were excised between 1300 and 1500 hour, and were immediately placed in glass vials containing 2 ml deionized water. Vials were brought to the laboratory quickly. Leaf discs were thoroughly rinsed thrice with deionized water to wash out electrolyte adhered on the surface or those already released into water. After final washing, 2 ml deionized water was added to each vial and capped to avoid desiccation and evaporation during heat treatment. One set of vials was treated at 50°C for 1 hour in water bath, whilst other set was kept at 25°C for one hour. After heat treatment, 10 ml deionized water was added to each vial and held at 10 °C for 24 hours to allow diffusion of electrolytes. Vials were brought to 25°C and shaked to mix the contents. Initial reading was taken using EC meter (Model, 1-11-933300, Harina Instrument, USA). Vials were autoclaved for 10 minutes at 0.10 MPa pressure to kill the tissues and release of all the electrolytes. Vials were again brought to 25°C and final EC reading was measured. Relative cell injury percentage (RC1 %), an indicator of cell membrane thermostability (CMT), was calculated using following function (Sullivan, 1972).

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

Where, T and C denote EC of heat treated and controlled vials, and subscripts I and 2 denote initial and final EC readings, respectively.

Assessment of heat tolerance

The absolute of RCI% of 70 varieties were used to evaluate variation in the cotton germplasm. Many workers (Azhar et al., 2003; Akhtar et al., 2008; Iqbal et al., 2011) have found this method useful to distinguish tolerant and susceptible varieties of cotton under drought and heat stress.

Development of plant material for genetic studies

Comparison of RCI% of 70 varieties revealed the three varieties namely MNH 552, FH 1000 and NIAB 111 had low RCI%, and therefore were found to be heat tolerant, and in contrast Cedix ST-362, LRA 5166 and 4F with higher values of RCI% were identified as heat susceptible. The six varieties were sown in earthen pots placed in glasshouse during October 2013. Three sets of crosses i.e., MNH-552 × Cedix ST-362 (GL), FH-1000 × LRA 5166 and NIAB- 111× 4F, were attempted in glasshouse, these will be referred as to, Cross-1, Cross-2 and Cross-3 respectively.

Half quantity of the F_1 seed was stored and other half and their parents were planted during normal crop season, May-June 2014. When F_1 plants started to flower, these were crossed back with their respective parents to develop seeds of backcross 1 (BC_1), backcross 2 (BC_2) and backcross 3 (BC_3) whilst some of the F_1 plants were also selfed to obtain F_2 seed. At maturity seed cotton of these genotypes was collected and ginned.

Evaluation of plant material for RCI at plant maturity

Since development of these two temperature regimes in the field is very difficult and uncertain and therefore growing of plants under normal and high temperature in the field is not practicable, however this difficulty may be overcome by planting plant material at different sowing dates as had been done in Brassica (Morrison and Stewart, 2002), this method followed by many works and this method had been presently in cotton (Steiner and Jacobsen, 1992; Rahman et al., 2004; Azhar et al., 2009). For the assessment of different genotypes, seeds of six generations i.e. P_1, P_2, F_1, F_2, BC_1, BC_2 were sown in the field during 2015, during early April (for high temperature), and early June (normal temperature). A triplicated randomized complete block design was followed for the conductance of this experiment. Each genetic entry was planted in a single row plot of 450 cm long having 16 plants spaced 30 cm apart within the row and 75 cm between the rows. There was one row of each P_1, P_2 and F_1, three rows of BC_1 and BC_2 and six rows of F_2 of each genetic entry in one replication for two seeds of each entry were dibbled 5-6 cm deep per hill, and when seedling were 15 cm high, they thinned to one seedling. The plants were sprayed with suitable as and when needed to obtain clean plants. Both the experiments were conducted on the same piece of land to minimize possible soil heterogeneity. Adequate irrigation was applied when needed. All general production practices recommended for cotton crop were adopted identically for both the experiments. During the development and maturity of plants in six generations, data on the following characters were measured.

Statistical analysis

Absolute and relative data (indices of heat tolerance) on canopy temperature of 70 cotton varieties were analyzed following ordinary analysis of variance technique (Steel et al., 1997) to see whether the genotypic

differences were significant. For genetic analysis coefficients for partitioning of the six generations were made according to Little and Hills (1978). The genetic basis of variation in heat tolerance was investigated following the approach of generation means analysis (Mather and Jinks, 1982). Means and variances of two parents, F_1, F_2, BC_1 and BC_2 were calculated. A weighted least square analysis was performed on generation means commencing from the simplest model using parameter 'm' only. Further models of increasing complexity "md", "mdh", etc. were fitted if sum of squares were significant. The best fit model was the one which had significant estimates along with non-significant Chi square value.

RESULTS

Mean squares of RCI% of 70 varieties of *G. hirsutum* L. grown in optimum and high temperature were obtained from simple analysis of variance technique. Mean squares of 70 varieties were highly significant (P ≤ 0.01)(ms = 1234.36) and these varieties responded differently to two temperature regimes as the mean squares were highly significant (P ≤ 0.01)(ms= 17.99) and the two temperatures also differed significantly (P ≤ 0.01)(ms= 10934.40) (table is not given).

Comparison of RCI% of 70 varieties differed appreciably even under controlled temperature (Table 1), however, RCI measured under high temperature, minimum injury to cells i.e. 27%, 28%, and 29% was found in NIAB111 (No. 59), MNH552 (No.32) and FH 1000 (No.23) respectively, and were revealed to be more tolerant than any of the other variety assessed. By contrast, greater injury occurred in AC134 (No.25, 61%), NIAB 78 (No.61, 64%), BH126 (No.55, 51%), PB899 (No.20, 68%), DPL-2775 (No.12, 57%), CIM554 (No.53, 67%), CIM446 (No.45, 85%) and BH147 (No.67, 87%) and revealed essentially similar heat tolerance, and may be regarded as intermediate group under heat stress, cells of varieties of 4F (N. 18), Cedix ST. 362 (GL) (No. 4) and LRA 5166 (No. 7) were seriously injured to the extent of 93.77%, 94.7% and 95%, and these varieties may be called highly sensitive varieties.

Six generations i.e. P_1, P_2, F_1, F_2, BC_1 and BC_2 of each of the three crosses i.e. Cross -1, Cross -2 and Cross -3 were grown in the field during April (high temperature) and early June (normal temperature) as has been done in *Brassica* (Morrison and Stewart, 2002) and cotton (Akhter et al., 2008). At plant maturity, data on RCI% were collected, and assessment of the genotypic responses to high temperature was compared with that of control. Absolute data regarding RCI% in each of the three crosses under consideration measured in control and high temperature were subjected to ordinary analysis of variance technique (Steel et al., 1997). The study of the mean squares revealed highly significant differences (P ≤ 0.001) between the generations of three crosses (Table 2). Significant mean squares (P ≤ 0.001) due to high temperature indicated that temperature during April and June was also different, and the varieties responded differently to the two stress conditions. The data of means

of RCI% of these six generation and h^2_{ns} of three crosses is mentioned in also presented herein. The h^2_{ns} of three crossed grown in optimum conditions was found to be moderate to high (65 to 80%), while it was 93% existed for populations grown in high temperature (|Table 3).

Table 2. Mean squares due to absolute data for RCI% in three Crosses of *Gossypium hirsutum* L.

Sources of Variation	D.F.	Crosse-1	Cross-2	Cross-3
Replications	2	23.4	3175.6	95.6
Generations (G)	5	28581.30**	33211.30**	25633.80**
Temperature (T)	1	7280.20**	876.80*	6617.90**
G×T	5	31.60^{ns}	1119.90**	26.00^{ns}
Error	1336	131.3	165.5	129.9

* and ** denotes differences significant (P<0.05) and highly significant (P<0.01) respectively, whilst ns, reveals differences non-significant (P≥0.05)

Table 3. Means of six generations and heritability (h^2_{ns}) of RCI% in three crosses of *Gossypium hirsutum* L. grown under two temperature regimes

Generations	Cross- 1		Cross-2		Cross-3	
	Optimum	High	Optimum	High	Optimum	High
P_1	25.80	28.78	24.98	27.98	24.51	29.24
P_2	68.44	74.33	71.98	77.91	67.20	72.47
F_1	47.58	51.64	48.09	53.18	47.16	50.49
F_2	47.98	53.13	48.55	45.24	45.34	49.87
BC_1	37.31	41.79	37.41	41.88	36.48	41.59
BC_2	57.67	61.81	57.39	62.93	55.51	59.05
h^2_{ns}	0.65	0.91	0.73	0.90	0.80	0.93

Due to the presence of non-significant Chi square value, various genetic models are fitted on inheritance of RCI% in three populations. Three parameter models "mdh" was best fit for cross-1 and 2 while five parameter models "mdhij" was best fit for cross-3 grown in optimum conditions (Table 4). High values of "d" as compared to "h" revealed that inheritance of RCI% is governed by additive effects if three these crosses are grown in optimum conditions. The presence of "i and j" for cross-3 indicated that inheritance RCI% in cross-3 is also influenced by interaction of Additive × Additive and Additive × Dominant effects respectively. In contrast, two parameters (md) model was best fit for cross-1 grown in high temperature, but six parameter model (mdhijl) was fit for cross-2 and 3. The values of "d" and "h" indicated that additive effects were involved in the inheritance of RCI% in cross-1 and 3 while dominant genes were responsible in cross-2. In addition, epistatic effects were observed in inheritance of RCI% in Cross-2 and Cross-3. These effects can be seen due to the presence of opposite signs (+ and -) in "i" and "l" in generation mean analysis of certain traits. The collected data on RCI% was also analyzed for component of variance namely, additive (D), dominance (H), environments (E) and interaction (F) (Table 5). This analysis revealed the contribution of D in the inheritance of RCI under optimum and high temperature; however H also contributed in case of Cross-1 and Cross-2 under optimum conditions. Effects of interactions (F) were also noted in the inheritance of RCI in Cross-2 grown under optimum conditions.

Table 4. Components of generation means, m, [d], [h], [i], [j] and [l] for RCI % in three crosses of *Gossypium hirsutum* L. grown under two temperature regimes

Crosses	m	[d]	[h]	[i]	[j]	[l]	χ^2	DF	Probability
Optimum									
Cross -1	47.13 ± 0.067	21.31 ±0.067	0.45 ± 0.10				1.7641	3	0.90-0.100
Cross -2	48.45±0.14	23.47±0.14	-0.39±0.22				6.4889	3	0.100-0.050
Cross -3	43.61±0.75	21.35±0.14	3.54± 0.85	2.24±0.78	-4.64±1.29		0.0367	1	. 0.90-0.100
High									
Cross -1	51.58 ± 0.08	22.77±0.09					6.6625	4	0.90-0.100
Cross -2	24.32 ± 4.28	24.96 ±0.15	84.84 ± 8.61	28.62 ± 4.27	-2.95 ± 0.47	-25.99± 4.35	0.0000	0	Significant
Cross -3	59.59±3.81	21.61±0.12	-29.81±7.68	-8.74±3.81	-7.40±0.44	20.70±3.90	0.0000	0	Significant

Cross 2-Dominance, duplicate dominance
Cross 3-Additive, duplicate dominance

Table 5. Components of variance for RCI % in three crosses of *Gossypium hirsutum* L. grown under two temperature regimes

Crosses	D addit	H dominat	F interaction	E envi	χ^2	D.F	Probability
Optimum							
Cross -1	687.84±78.25	-489.92±83.69		0.33±0.04	1.6640	3	0.90-0.100
Cross -2	916.02±109.95	-620.24±123.84	-100.43±16.41	1.86±	0.23	2	0.90-0.100
Cross -3	96.76±6.26			1.84±0.22	0.7090	4	0.975-0.950
High							
Cross -1	512.64±31.35			0.83±0.10	8.078	4	0.100-0.050
Cross -2	626.58±38.42			1.54±0.19	5.4231	4	0.90-0.100
Cross -3	494.12±30.44			1.98±0.24	3.0864	4	0.90-0.100

DISCUSSION

In the present investigations, assessment of variation in heat tolerance based upon RCI was made at the commencement of reproductive stage. The data on RCI% suggest that there is considerable variability in heat tolerance in *G. hirsutum* L. The absolute measures of heat tolerance allowed the identification of highly tolerance, highly susceptible and medium tolerant groups. Similarly total number of varieties examined for RCI reveals general patterns of responses to optimum and high temperature showing a diverse range in responses to heat. Clearly the present data suggest that workable variability for heat tolerance is available in the material and may be exploited through selection and breeding.

The expression of heat tolerance in crop species is a complex phenomenon; both genetic and environmental factors are involved in the manifestation of heat tolerance (Bibi et al., 2003). In the current study data on RCI % was used to investigate genetic mechanism controlling RCI% in *Gossypium hirsutum* L. following generation mean analysis (Mather and Jinks, 1982). Generation means analysis revealed additive [d] and non-additive [h] gene effects in RCI under optimum and high temperature, except in Cross-1 under high temperature (Table 4). The negative value of [h] indicated that dominance is directional towards lower parent, and this information is useful for lowering RCI % in selected plants.

The presence of additive × additive [i] interaction was evidenced in the inheritance of RCI% in cross3 under optimum temperature, and Cross-2 and Cross-3 under high temperature and this epistatic component suggests that fixation of additive alleles is possible in later generation (Singh et al., 2000; Ali et al,. 2004). Genetic mechanism controlling RCI% appeared to be complicated by additive × dominance [j] in Cross-3 under optimum and Cross-2 and Cross-3 under high temperature and dominance × dominance [l] interactions in Cross-2 and Cross-3 and these epistatic components warrants the breeders to be careful while looking for plants having lower RCI% and for that matter heat tolerant plants in segregating generations

Genetic variance analysis has been widely adopted by the research workers to partition the total variance into additive (D), dominance (H), environments (E) and interaction (F). In the present study inheritance of RCI% was affected by D (additive) component under optimum and high temperature, however under optimum temperature dominance component (H) in Cross1 and

Cross-2 also appeared to be important (Table 5). Similarly, presence of F component complicated the inheritance of RCI% in Cross-2 under optimum temperature. These results indicated that both additive and non-additive variance was important for the genetic control of RCI%, however pronounced effect of "D" component indicated the dispersion of positive and negative alleles in the two parents. There is no information reported to support the present finding, however Randhawa et al. (1986), Kohel (1987) and Rahman and Malik (2008) observed additive component in the genetic variation of different plant traits of *G. hirsutum* L.. The presence of non-allelic interaction due to [i], [j], [l] observed in generation means analysis has not been detected through generation variance, and therefore suggestion of Malik et al. (1999), Shakoor et al. (2010) and Sarwar et al. (2012) was substantiated here.

Estimates of narrow sense heritability for RCI% appeared to be little inflated under high temperature (Table 3), whilst these were low under optimum temperature. The higher estimates of heritability might be due to better additive genetic dissimilarity due to appearance of genes related to stress tolerance or other environmental variation (Saranga et al., 1992). It has been suggested that hidden variation could be exposed due to moderate stress, and thus presenting the chance of increasing heritability estimates in plants (Bradshaw and Hardwick, 1989). Low and high estimates of h^2n in cotton had been reported for various plant characters (Ulloa, 2006; Shakoor et al, 2010; Saravanam et al., 2003; Ahuja 2004; Irshad et al., 2012). These estimates of h^2n appeared to be inspiring to cotton breeders, suggesting that selection of plants with lower degree of RCI%, and thereby enhanced heat tolerance in progenies is possible at the time of maturity. However, Falconer and Mackey (1996) had suggested that the estimates are subject to environmental variation, and therefore before making selection these estimates must be substantiated under differing temperature regimes.

The estimates of h^2ns may be used to predict response to selection (R) which is equal to the product of $h^2ns \times S\overline{d}$ (is difference between the mean of original F_2 population and the mean of the selected F_3 population). For example, the values of the response, R for the plant material under high temperature is 13.3, 12.17 and 11.47 in Corss-1, Cross-2 and Cross-3 and the mean of $\overline{X}F_3$ is 40.01, 33.07 and 38.40 respectively (Table 6). This revealed that progeny from F_2 population will have decrease amount of

RCI% in F$_3$ population, which will lead to the increase in heat tolerance. This genetic gain seems to be encouraging for the plant material. In previous studies significant increases over original population due to high estimates of h^{2}ns had been made in *Medicago sativa* L. (Dobrenz et al., 1981; Noble et.al., 1984). Due to the genetic basis of salt tolerance improvement in seven grass and four forage species (Ashraf et al., 1987) and wheat (Ali et al., 2004) had also been reported.

Table 6. Response to selection for RCI% in three crosses of *Gossypium hirsutum* L. grown under high temperature

	Cross 1	Cross 2	Cross 3
$\overline{X}_0 F_2$	53.3	45.24	49.87
$\overline{X}_S F_2$	38.81	31.72	37.53
$S\overline{d}$	14.32	13.52	12.34
Response= $S\overline{d} \times h^2_n$	13.3	12.17	11.47
$\overline{X}_{F_3}$	40.01	33.07	38.40

$\overline{X}_0$: mean of original segregating population; $\overline{X}_S$, mean of plants selected for the population; R, response to selection; $\overline{X}_{F_3}$, expected mean of next segregating population.

Comparison of the three crosses for genetic gains in physiological and agronomic characters, Cross-2 (FH1000 × LRA5166), exhibited its genetic potential for heat tolerance, and thus may be exploited through selection and breeding. The information reported herein cannot be generalized for whole of germplasm of upland cotton and all the cotton growing areas in Pakistan. Therefore another study involving different germplasm may be conducted to substantiate the information reported herein. However, the knowledge obtained from the present study may be of some value to the cotton breeders working in the cotton belt of Pakistan.

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