MEASUREMENT OF CANOPY TEMPERATURE FOR HEAT TOLERANCE IN UPLAND COTTON: VARIABILITY AND ITS GENETIC BASIS

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The present study was conducted with the objective to assess germplasm of American cotton for heat tolerance using canopy temperature. For this purpose seventy varieties/lines of Upland cotton were screened out at flowering stage. Analysis of variance revealed significant differences among the germplasm lines. Three tolerant (MNH552, FH1000 and NIAB111) and three susceptible lines (Cedix ST-362 (GL), LRA5166 and 4F) were selected. The tolerant and susceptible genotypes were crossed to develop F₁ seed of the three crosses. Six generations i.e. F₁, F₂, BC₁ and BC₂ of each cross were grown along with their parents under normal and high temperature in the field following RCBD with three replications. At flowering stage, canopy temperature was measured in both the temperature regimes. Indices of heat tolerance were calculated for genetic investigation. Generation means analysis of the data revealed that additive, dominance and epistatic component were involved in the inheritance of canopy temperature. However genetic variance analysis showed that canopy temperature was predominantly controlled by additive genes, and thus estimates of h²ns were high. These results suggest that rapid improvement in reducing canopy temperature of the plant material is possible through breeding and selection in F₃ population.

Keywords: Heat stress, genetic components, indices of heat tolerance, heritability

INTRODUCTION

Although cotton plant of *hirsutum* spp. has originated in hot climates, it suffers greatly under extreme high day temperature (>36°C). In Pakistan, cotton crop is mostly grown in Sindh and Punjab provinces of the country (Ahmad and Makhdum, 1992), and sometimes in these areas daytemperature reaches up to 48-50°C, during planting and germination, resulting in low plant population (Rahman et al., 2004). Due to high temperature nearly 65-70% of fruiting points shed down due to heat-induced pollen sterility which causes the harvest of poor yield of seed cotton (Taha et al., 1981; Kittock et al., 1988; Baloch and Lakho, 2000; Liu et al., 2006). In the month of August and September. when temperature recedes, 30-35% of bolls are retained which contribute to acre-yield. The cotton research group of Agriculture Co-ordination Committee identified that high temperature during fruiting period was the basic reason of low yield of seed cotton in Pakistan (Anonymous, 2005). This situation suggests the local breeders to develop cotton cultivars, through breeding and selection, which may set maximum fruiting bodies under high temperature. Although commendable work had already been done by developing high yielding and heat-tolerant varieties in the region, the rising temperature, due to greenhouse effect and global warming, warrants the research workers to exploit the potential of cotton plant to make it able to grow under high temperature of the cotton belt of Pakistan.

For the development of heat tolerance in adapted cultivars, through conventional breeding approach, availability of a technique which could help screen a large number of varieties/lines quickly is important. The use of canopy temperature, a new physiological infra red technique, is gaining popularity these days to screen germplasm for heat tolerance (Singh et al., 2007). This technique had been used by various workers for screening varieties of cotton and corn for heat tolerance (Wanjura et al., 2004; Karademir et al., 2012), drought tolerance in cotton and wheat (Hatfield et al., 1987; Blum et al., 1989; Rashid et al., 1999). These studies revealed that workable variability in canopy temperature does exist in these species, but the work on the genetic basis had not been done vet. Earlier information about the presence of variation in canopy temperature in Gossypium hirsutum L. is not available. However variability in other characters related to heat tolerance within the species is available (Trolinder and Shang, 1991; Baloch and Lakho, 2000; Rahman et al., 2004; Azhar et al., 2009). The present work examines variability in canopy temperature of 70 varieties/lines of G. hirsutum L., and also reports information on its genetic control. This knowledge would be of some value to the local breeders for the development of heat tolerance in the plant material being grown in the cotton belt.

MATERIALS AND METHODS

Variation in canopy temperature, as a measure of heat tolerance, in Upland cotton and its genetic basis was studied in 70 varieties of *G. hirsutum* L. The experiments were conducted in the department of Plant Breeding and Genetics, University of Agriculture, Faisalabad during 2009-2012. Name of the germplasm examined in the present investigation is given in Table 1.

Table 1. Absolute and indices of heat tolerance using canopy temperature in 70 varieties of upland cotton

Code	**	Control	Heat	Indices of hear		
No.	Varieties /Lines		stress	tolerance		
1	Greg 25V	34.0	40.7	119.6		
2	Royal S. Okra	32.7	37.0	113.1		
2 3	YEP5	35.0	40.0	114.3		
4	Coker 310	33.7	40.7	120.8		
5	Linea 100	34.0	39.0	114.7		
6	Barb XL1	34.7	39.7	114.4		
7	LRA5166	33.7	44.0	130.6		
8	Acala1517C	32.0	39.0	121.9		
9	Riba 50	32.7	39.7	121.4		
10	TH 4183	31.0	38.7	124.8		
11	Frego	33.0	39.3	119.1		
12	DPL2775	33.0	38.7	117.3		
13	DPL61	32.7	37.7	115.3		
14	Cedix St 362 (GL)	34.0	44.0	129.4		
15	GFS	32.3	38.0	117.6		
16	CP/15/2	32.3	38.0	117.6		
17	Tidewater	33.3	38.3	115.0		
18	4F	33.0	43.3	131.2		
19	SLH41	32.3	38.7	119.8		
20	PB899	31.7	38.0	119.8		
21	PB900	32.0	38.0	118.8		
22	VH144	32.0	39.3	122.8		
23	FH1000	31.0	33.0	106.4		
24	FH900	33.0	39.3	119.1		
25	AC134	33.0	39.0	118.2		
26	CIM 443	32.7	39.0	119.3		
27	MNH424	33.7	40.0	118.7		
28	MNH129	33.7	39.0	115.7		
29	MNH440	33.7	39.7	117.8		
30	MNH394	33.0	39.7	120.3		
31	NIBGE3701	33.7	40.0	118.7		
32	MNH552	29.0	31.0	106.9		
33	MNH786	33.3	39.0	115.7		
34	MNH789	33.3	39.7	119.2		
35	MNH633	33.3	39.3	118.0		
36	MNH706	32.7	40.0	122.3		
37	MNH765	32.7	39.7	121.4		
38	MNH738	33.3	39.7	119.2		
39	S12	33.7	39.0	115.7		
40	FH 114	33.0	39.7	120.3		
41	NIBGE1524	32.7	39.3	120.2		
42	NIBGE601	33.0	39.7	120.4		
43	CRIS403	32.7	39.0	119.3		

44	CRIS134	32.7	39.0	119.3
45	CIM446	32.3	39.0	120.7
46	CIM448	32.7	39.3	120.1
47	CIM1100	33.3	39.7	119.2
48	CIM473	33.3	37.7	113.2
49	CIM240	32.7	38.7	118.3
50	CIM511	33.3	40.0	120.1
51	CIM557	33.3	39.7	119.2
52	CIM541	33.7	39.0	115.7
53	CIM554	32.7	37.7	115.3
54	BH118	33.3	39.3	118.0
55	BH126	33.0	39.3	119.1
56	BH121	32.7	39.3	120.2
57	BH160	32.7	40.0	122.3
58	BH162	32.7	39.7	121.4
59	NIAB 111	30.0	32.3	107.7
60	NIAB KARISHMA	33.7	40.0	118.7
61	NIAB 78	33.3	40.0	120.1
62	B557	33.0	39.7	120.3
63	NIAB 884	32.7	39.0	119.3
64	NIAB 999	32.3	39.7	122.9
65	FH 113	33.0	39.7	120.3
66	CIM 499	33.0	39.3	119.1
67	BH147	32.0	39.3	122.8
68	VH142	33.7	38.7	114.8
69	MNH 93	33.3	38.0	114.3
70	CIM707	33.3	40.7	122.2
	Cd 5%	-	2.26	10.52

Evaluation of germplasm for canopy temperature: This experiment was conducted using two temperature regimes The plants were grown under optimum temperature at 31-34°C/19-21±2°C (day/night) and higher temperature 42-44°C /24-27±2°C. These temperatures were maintained in two separate glasshouse chambers. In one chamber optimal temperature was maintained, whilst in other, temperature was gradually increased to the desired Celsius (°C) and maintained for rest of the period. The experiment was terminated when plants started to flowering and maximum flush of flowers was observed in both the chambers. In both the cases, plants were grown in earthen pots measuring 30 cm × 35 cm (high and upper diameter, respectively). These pots were filled with 9 kg soil which was tested before filling in the pots. The pH of soil was 8.1. EC 1.2 dS/m. organic matter 1.42%, saturation percentage 31%, phosphorous 28.9 ppm and potassium135 ppm.

The seeds of 70 varieties were soaked in tap water 8 hours before sowing. Four seeds of each variety were sown 2 cm deep in each pot, and at two true leaf stage, the young seedlings were thinned to one seedling. Each variety was grown in 5 pots, thus there were 15 plants in three replication and having 1050 plants in each temperature regime. The design of layout was randomized complete block. The plants were grown under day length of 14 hours, natural light (PAR ranged 1400-1600 µmol nf²s at noon) and 65-80% humidity in both the chambers. Water was supplied to earthen pots at the rate of 1400 ml per pot daily during

peak flowering period and on alternate days afterwards. All other growing conditions in the two chambers were kept uniform and identical. Canopy temperature of 70 varieties was recorded between 1300 to 1500 hours using infra red thermometer. The plants were measured at 2 days interval.

Assessment of germplasm for canopy temperature: For making comparison of different varieties, choice of a method which measures the varied response to temperature regimes is important to facilitate identification of tolerant and susceptible varieties within hirsutum spp. Assessment of varieties on the basis of absolute performance is a useful means, and this technique had been used previously by Azhar et al. (2005), Akhtar et al. (2008) and Igbal et al. (2011) to study drought and heat in G. hirsutum L. Therefore this method was also followed here. The response of different varieties to heat was also examined on the basis of indices of heat tolerance (relative heat tolerance). The technique had been extensively used in the study of salt tolerance (Ashraf et al., 1986a,b; Azhar and McNeilly, 1988), heat tolerance (Azhar et al., 2009), drought tolerance (Iqbal et al., 2011) of various plant species. In the present work, indices of heat tolerance were also calculated by the formula used by these workers.

Development of plant material for genetic studies: Data on canopy temperature measured on seventy cotton varieties were compared on absolute and relative basis and it showed that three varieties, MNH-552, FH-1000 and NIAB-111 had low canopy temperature, and therefore were found heat tolerant, whilst LRA-5166, Cedix ST-362 (GL) and 4F, had high canopy temperature, and were revealed heat susceptible genotypes. These six cotton varieties were sown in earthen pots in glasshouse during October 2009, and hybridized, to develop F₁ seed of three crosses i.e., MNH-552 × Cedix ST-362 (GL), FH-1000 × LRA 5166 and NIAB- 111× 4F. Half of the F₁ seed was kept in store, whilst other half and their parents were grown during May-June 2010. When F₁ plants started to flower, these were crossed back to their respective parents to develop seed of backcross-1 (BC₁) and backcross-2 (BC₂) and backcross-3 (BC₃), whilst some of the F_1 plants were selfed for F₂ seed. At maturity seed-cotton of these genotypes was collected and ginned to obtain seed.

Response of six generations to temperature regimes: Since growing of plants under normal and high temperature in the field is not practicable, therefore this difficulty may be overcome by planting plant material at different sowing dates as had been done in Brassica (Morrison and Stewart, 2002), and this method was followed in cotton by many workers (Steiner and Jacobsen, 1992; Rahman et al., 2004; Azhar et al., 2009). In the present investigation, seeds of six generations i.e. P₁, P₂, F₁, F₂, BC₁, BC₂ of each cross were sown in the field during early April (for high temperature), and early June (for normal temperature). A triplicate randomized complete block design was followed for the conduct of this experiment. Each genetic entry was planted

in a single row plot 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 in one replication. Two seeds of each entry were dibbled 5-6 cm deep per hill, and when seedlings were 15 cm tall, these were thinned to one seedling. The plants were sprayed with suitable insecticide, as and when, needed to obtain clean plants. Both the experiments were conducted on the same piece of land to minimize possible soil heterogeneity. All general production practices recommended for cotton crop were adopted identically for both the experiments. During flowering of six generations, canopy temperature was measured at 2 days interval.

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, F₁, F₂, BC₁ and BC₂ 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. For canopy temperature the higher parent was taken as P_1 in the model fitting.

RESULTS

Analysis of variance (mean squares) given in Table 2 reveals highly significant (P ≤ 0.01) differences among the 70 varieties (Var), and the two temperature regimes (T). Significant interaction (P \leq 0.01) Var \times T show that varieties responded differently to heat stress. Mean squares due to relative values (indices of heat tolerance) also revealed highly significant differences ($P \le 0.01$) in 70 varieties. Comparison of the means of absolute canopy temperature (Table 1) of 70 varieties revealed varied response to control temperature, but under high temperature the genotypic differences were found to be greater. Thus a comparison of the varieties/lines may be useful for the identification of highly tolerant and highly susceptible, and an intermediate group. The response of NIAB-111(No. 59), MNH552 (No.32) and FH 1000 (No.23) which showed minimum temperature i.e. 32°C, 31°C and 33°C appear to be more tolerant to heat than CIM554 (No.53), DPL-2775 (No.12) AC134 (No.25) BH126 (No.55), PB899 (No.20), NIAB78 (No. 61), CIM446 (No.45) and BH147 (No.67) which showed similar response to heat stress with temperature ranging from 38-40°C. By contrast, Cedix St 362(GL) (No. 14), LRA 5166 (No. 7) and 4F (No.18) displayed high leaf temperature ranging from 43-44°C, and thus seemed to be temperature-sensitive varieties. Indices of heat tolerance, given in Table 1, provided further estimate of tolerance of varieties, and thus LRA -5166 (No. 7), Cedix St 362 (GL) (No. 14) and 4F (No. 18) absorbed more heat (130°C, 129°C and 131°C, respectively) were found to be more sensitive to heat than FH1000 (No.23), MNH 552 (No.32) and NIAB 111 (No.59) which showed minimum temperature (100°C, 107°C and 108°C respectively). In other varieties, canopy temperature is essentially similar and formed an intermediate group for heat tolerance.

Table 2. Mean squares of canopy temperature of 70 varieties of upland cotton grown in control and high temperature

Sources of	DF	Absolute data	Indices of heat			
Variation			tolerance			
Varieties (Var)	69	10.47**	54.59**			
Temperature (T)	1	4042.40**	-			
Var x T	69	3.00*	-			
Residual	280	1.98	43.20			

^{**,} denotes highly significant difference (p< 0.01)

Canopy temperature of genetic material at plant maturity: At plant maturity, data on canopy temperature were collected and genotypic responses to high temperature were compared with that of control to calculate indices of heat tolerance in six generations and mean values over the replications is given in Table 3. These indices (relative values) were used to study genetic control of heat tolerance in the species under investigations. Mean squares obtained from analysis of variance reveal significant differences among six generations of the crosses for canopy temperature (mean squares are omitted from the text). Generation means analysis (Table 4) showed that four parameter genetic model m, [d], [h], [i] were found adequate for the data set in cross MNH552 × Cedix ST-362 (GL) and FH1000 × LRA5166,

whilst five parameter genetic model m, [d], [h], [i], [j] showed best fitness for the observed to the expected generation means of cross NIAB111 × 4F. Genetic variance analysis of Mather and Jinks (1982), given in Table 5 partitioned the total variances in to additive (D), dominance (H), environments (E) and interaction (F). In the present investigation three parameter genetic model, D, F and E were pronounced for data set on cross, MNH552 × Cedix ST-362 (GL) and NIAB111 \times 4F, whilst in FH1000 \times LRA5166 only D and E in the genetic model appear to be important for controlling canopy temperature. Estimates of h²ns given in Table 6 were appreciable in these three crosses, and are 0.93, 0.86 and 0.77 for MNH552 × Cedix ST-362 (GL), FH1000 \times LRA5166 and NIAB111 \times 4F respectively. These estimates were used to calculate response to selection (R) and the means of canopy temperature decreased to the extent of 2.49, 1.81 and 2.36 in the three crosses respectively (Table 6). It is important that lower values for canopy temperature are more desirable.

Table 3. Relative generation means of canopy temperature measured in three crosses of upland cotton

Crosses	P ₁	P ₂	$\mathbf{F_1}$	$\mathbf{F_2}$	BC_1	BC ₂
Cross-1	109.4	128.7	122.5	105.9	115.8	127.3
Cross-2	110.7	132.4	120.2	106.6	118.1	128.2
Cross-3	109.7	135.5	125.6	115.2	121.4	131.5

Cross-1=MNH552 × Cedix ST- 362 (GL), Cross-2=FH1000 × LRA5166 and Cross-3NIAB111 × F4

DISCUSSION

In the present investigations, assessment of variation for canopy temperature, as measure of heat tolerance, was made at early stage and at the commencement of reproductive stage. Therefore canopy temperature measured at midday was found to be useful to differentiate 70 cotton varieties for

Table 4. Best model for relative generation means of canopy temperature in three crosses of Gossypium hirsutum L.

Crosses	M	[d]	[h]	[i]	[j]	[1]	χ^2	DF	Prob.
Cross-1	107.91±2.21	3.52 ± 0.79	12.84 ± 3.18	6.51 ± 2.41			5.6550	2	0.10-0.050
Cross-2	107.83 ± 4.94	4.50 ± 0.78	33.91 ± 12.33	14.28 ± 4.85		-18.19±7.84	0.0333	1	0.90-0.100
Cross-3	114.07±1.60	2.34 ± 0.74	5.51 ± 2.44	6.17 ± 1.76	9.03 ± 2.31		0.8706	1	0.90-0.100

 $Cross-1 = MNH552 \times Cedix \ ST-\ 362 \ (GL), \ Cross-2 = FH1000 \times LRA5166 \ and \ Cross-3NIAB111 \times F4$

Table 5. Variance components, D (additive), H (dominance, F (interaction) and E (environment), h²_{ns} and response to selection for canopy temperature in three crosses of upland cotton

Crosses	D	H	\mathbf{F}	\mathbf{E}	χ^2	D.F	Prob.
Cross-1	367.98±44.12		-119.52±27.05	78.10 ± 9.23	6.2729	3	0.100-0.050
Cross-2	352.24±39.58			67.1±7.94	2.6883	4	0.90-0.100
Cross-3	82.72 ± 18.69		64.87 ± 10.0	46.72 ± 5.31	4.1145	3	0.90-0.100

Cross-1=MNH552 × Cedix ST- 362 (GL), Cross-2=FH1000 × LRA5166 and Cross-3NIAB111 × F4

heat susceptibility. In previous studies drought sensitive varieties grown under heat stress had warmer mid-days, and suffered relatively greater yield loss (Blum et al., 1989; Jawdad et al., 2012). Canopy temperature suggested that there was considerable variability for heat tolerance in G. hirsutum L. Both absolute and indices of heat tolerance allowed the identification of tolerant and sensitive cotton germplasm. Examination of the data for total number of varieties reveals general patterns of responses to optimum and high temperature showing a diverse range in responses to heat stress. Clearly the present data agree with the previous findings on canopy temperature in sorghum, soybeans, alfalfa and tomatoes (Hatfield et al., 1984), wheat (Winter et al., 1988; Rashid et al., 1999; Aveneh et al., 2002), and cotton and corn (Wanjura et al., 2004). The susceptibility of 4F, an old and obsolete cultivar of this region has been substantiated by Bibi et al. (2003), who emphasized that an obsolete cultivar is likely to suffer more than new ones.

Table 6. Estimates of heritability (h²ns) using mean components of various characters in three crosses of upland cotton

Temperature	Crosses	Canopy temperature
Normal temperature	Cross-1	0.36
	Cross-2	0.42
	Cross-3	0.4
High temperature	Cross-1	0.79
	Cross-2	0.64
	Cross-3	0.74
Index of heat	Cross-1	0.93
tolerance	Cross-2	0.86
	Cross-3	0.77

Cross-1=MNH552 × Cedix ST- 362 (GL), Cross-2=FH1000 × LRA5166 and Cross-3NIAB111 × F4

When such a potential plant material is available for exploitation through selection and breeding, the adoption of biometric method which could partition the genetic variation into different components is important for a breeder. Generation means analysis approach had been used previously for aluminum and salinity tolerance in wheat (Ahsan et al., 1996; Ali et al., 2007) and drought tolerance in cotton (Shakoor et al., 2010; Sarwar et al., 2012). Generation means analysis revealed that additive [d], nonadditive [h] and additive × additive [i] interaction were more pronounced in the inheritance of canopy temperature of three crosses. However for FH1000 × LRA5166 dominance × dominance interaction component was also evidenced. The presence of additive × additive [i] interaction in three crosses suggests that fixation of additive alleles is possible in later generation (Singh et al., 1980; Ali et al., 2007). The presence of genetic mechanism controlling heat tolerance appeared to be complicated by additive × dominance [j]

interaction in FH1000 \times LRA5166 and dominance \times dominance [1] interaction in NIAB111 \times 4F, and these epistatic components warrants the breeders to be careful while looking for 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 canopy temperature of the crosses was effected predominantly by D (additive) component. Similarly, presence of F interaction component complicated the inheritance of canopy temperature of MNH552 × Cedix-ST 362 (GL) and NIAB111 × 4F. The presence of significant and larger D component indicated the dispersion of positive and negative alleles in the two parents, used to develop the genetic material. In previous studies, Singh and Singh (1981), Randhawa *et al.* (1986), Yadava and Yadava (1987) and Rahman and Malik (2008) observed additive component in the genetic variation of different plant traits.

However, the presence of non-allelic interaction due to [i], [j], [l] observed in generation means analysis of canopy temperature has not been detected through generation variance as had been studied by Malik *et al.* (1999), Shakoor *et al.* (2010) and Sarwar *et al.* (2012).

High estimates of narrow sense hertabilities of canopy temperature at plant maturity appeared to be inspiring to cotton breeders, and suggest that selection of plants with enhanced heat tolerance in the progenies of these three crosses is possible. However, Falconer and Mackey (1996) had suggested that these estimates were subject to environmental variation, and therefore before subjecting plants to selection these estimates must be substantiated under differing temperature regimes. Further these estimates may be used to predict response to selection and possible genetic gain in subsequent segregating generations. The values of the response (R) in the three crosses are encouraging. The previous information on genetic progress in heat tolerance in cotton had not been reported in the literature. However due to high estimates of h²ns significant response to selection had been observed in lucerne, Medicago sativa L. (Dobrenz et al., 1981), and Noble et.al., (1984) working with the same spp. made significant improvement after two generation of selection. Due to the genetic basis of salt tolerance of seven grass and four forage species (Ashraf et al., 1986a and b; 1987) and wheat (Ali et al., 2007), increased salt tolerance in these spp. appears to be possible.

It is concluded that canopy temperature proved to be reliable indicator for assessment of heat tolerance in *G. hirsutum* L., and since the variation appears to be heritable, therefore the chances for improvement of heat tolerance in the spp. are there. Due to involvement of additive variance (D), estimates of h²ns were of larger magnitude, and therefore

appreciable amount of genetic progress was demonstrated. The knowledge about the genetic controlling system of heat tolerance may be advantageously used by the local cotton breeders of this area.

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