Pak. J. Agri. Sci., Vol. 57(6), 1491-1501; 2020
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI:10.21162/PAKJAS/20.9910.
http://www.pakjas.com.pk

GENETIC BASIS OF VARIATION FOR PHYSIOLOGICAL AND YIELD CONTRIBUTING TRAITS UNDER NORMAL AND HIGH TEMPERATURE STRESS IN *Gossypium hirsutum* L.

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Cotton is severely affected by heat stress in most parts of Pakistan which results in poor quality and low cotton yield. Sixty cotton strains were screened against heat stress on the basis of cellular membrane thermostability, chlorophyll contents, canopy temperature, node number of first fruiting branch, boll weight and seed cotton yield to explore the genetics of variation among genotypes for heat tolerance. Five tolerant and five sensitive parents were sorted out and crossed in Line × Tester mating design during 2018.The F_1 crosses along with parents were grown in field under normal and heat stress conditions by using two sowing dates to create heat stress in the field. Sowing during 1st week of April was considered as heat stressed because its peak flowering period was supposed to be coincided with the maximum temperature of May-June; while, sowing during 1st week of June was taken as normal because its peak flowering period coincided with normal temperature of August. Data regarding cell membrane themostability (CMT%), chlorophyll contents, canopy temperature, node number to first fruiting branch, bolls/plant, boll weight, ginning out turn and seed cotton yield under normal as well as heat stressed conditions were collected which showed significant differences among genotypes for all the traits under investigation. Most of the traits showed greater mean square for female × male interaction than female and male individual mean squares and higher dominance variance than additive variance. Broad sense heritability for investigated traits was observed in the order of boll weight (94.56), seed cotton yield (94.51), CMT% (91.88), canopy temperature (90.48), node number to first fruiting branch (75.68) and chlorophyll contents (51.93). FH-458 and PB-76 indicated high general combining ability for most of the traits whereas the cross combination FH-458× FH-326 was described as the best cross showing 56.85% better parent heterosis for seed cotton yield under heat stress conditions. The present study was planned to investigate the genetics of some important physiological and agronomic traits of cotton which is a pre-requisite for the development of high yielding cotton varieties under high temperature stress.

Keywords: CMT%, chlorophyll contents, canopy temperature, sowing dates, heat stress tolerance, cotton.

INTRODUCTION

Cotton has major share in agricultural economy of Pakistan and any decline in cotton production causes serious threat to the country's GDP. There are many factors that limit cotton production but one main factor in the recent times is increasing temperature in the cotton season which negatively affects cotton yield and quality (Abro *et al*., 2015). A temperature increase of about 1°C during first decade of 21st century has surpassed the temperature increment of 0.7°C during 20th century (Rasul *et al*., 2011; Kamran *et al.,* 2017). A continuous increase in worldwide air temperature has been recorded during last few decades which have resulted in low yield in various cotton growing areas of the world (Hatfield *et al*., 2011). By the end of this century, an increase of 1.1-6.4°C in air temperature is expected (IPCC, 2018) which could result in reduced water potential of plants due to elevated general warming process by affecting leaf cooling process and transpiration rate (Carmo-Silva and Salvucci, 2011). Although cotton is a crop of hot and semi-arid regions of the world, the plant grows well in a temperature range of 15-36°C and too high temperature affects its growth and development very badly (Baloch *et al*., 2000). Oosterhuis (2002) correlated year to year variation in cotton yield with high day and night temperature which produced heat and drought stress (Brown *et al*., 2003). The cotton plants show the maximum photosynthetic rate at 33°C and start to decrease at 36°C or above mainly due to thylakoid membrane leakage (Bibi *et al*., 2008). Cotton yield and fiber quality are negatively affected by increase in daily mean temperature (Roussopoulos *et al*., 1998). Fiber is more than 90% cellulose and its development depends upon the deposition of cellulose in the primary and secondary cell walls, while the rate of cellulose synthesis was significantly affected by temperature variations. The rate of cellulose synthesis tends to increase above 18ºCand remained high between 28-37ºC and then decrease after 40ºC (Roberts *et al*., 1992). It was determined by Kakani *et al.* (2005) that pollination process in cotton was negatively affected above 30°C. Pollen germination tends to decrease when temperature increased from 37°C, while, growth of pollen tube was affected above 32°C (Burke *et al*., 2004). The thermal kinetic window for most of the enzymatic activities of cotton plant ranged from 23.5-32°C (Burke *et al*., 1995). Pettigrew (2008) claimed that with an increase of 1°C from optimum (35-38°C) cotton yield may decrease up to 10%. A maximum decrease of 110 kg/ha in cotton yield was noticed for 1°C rise in temperature above maximum day limit of temperature for cotton plant (Singh *et al*., 2007). Although all growth stages are vulnerable to heat stress however, reproductive phase in general is more affected by high temperature. For example, a short spell of heat stress during reproductive stage may result in shedding of floral buds and flowers (Xu *et al*., 2017). Different morphological indicators, biochemical and physiological processes get significantly changed in cotton plant under heat stress which affect plant growth and development and ultimately result in low economic yield. These negative impacts of heat stress can be reduced upto maximum level by developing high temperature stress tolerant cotton strains through molecular and conventional breeding (Wahid *et al*., 2007). Plant spp. show great variety in response to heat stress (Rodríguez *et al*., 2005) and have evolved different mechanisms to combat with heat stress but genetic improvement for heat stress tolerance is the main focus of plant breeders. Genetic structure for heat resistance in cotton plant is however quite complicated with extended level of epistatic relationships (Khan *et al*., 2014). However, some physiological and morphological traits can be used as indicators of heat stress tolerance. For example, high CMT% indicates more tolerance and stability in cotton yield under heat stress environment (Azhar *et al*., 2009; Khan *et al*., 2014; Arfan *et al*., 2018). Lower canopy temperature indicates thermotolerance in cotton (Khan *et al*., 2014), lower node numbers to first fruiting branch is an indication of tolerance to heat stress (Baloch and Baloch, 2004; Hajazi *et al*., 2014; Arfan *et al*., 2018). Similarly, Boggs *et al*. (2003) described that cotton leaf chlorophyll was significantly correlated with soil nitrate-nitrogen and seed cotton yield. Availability of genetic variations among cotton strains against heat stress and knowledge of genetic effects, combining ability and heterotic impacts are essential tools of a cotton breeder for developing high temperature tolerant genotypes trough conventional breeding (Rauf *et al*., 2005). A researcher can better understand the general and specific trend of combination among different strains to produce desirable results from the study of combining ability effects (Braden *et al.*, 2003). Heterosis shows superiority of progeny over parents and relies upon genetic differences, extent of dominance and hereditary separation among selected parental genotypes (Kearsey and Pooni, 1996). Therefore, present study was planned to investigate the genetics of some important physiological and agronomic traits of cotton which is a pre-requisite for the development of high yielding cotton varieties under high temperature stress.

MATERIALS AND METHODS

The current study plan was executed during the years 2017 and 2018 at post Agricultural Research Station (PARS), University of Agriculture of Faisalabad under semi-arid climatic condition.

***Screening of cotton genotypes against heat stress*:** Sixty upland cotton genotypes were collected from different research organizations in public sector including Cotton Research Station (CRS) Ayub Agriculture Research Institute (AARI) Faisalabad, Cotton Research Institute (CRI) Multan, Cotton Research Station (CRS) Vehari, Nuclear Institute for Agriculture and Biotechnology (NIAB) Faisalabad, National Institute for Biotechnology and Genetic Engineering (NIBGE) Faisalabad and Central Cotton Research Institute (CCRI) Multan and also from private sectors to explore genetic variation against heat stress. The experiment was conducted in field by sowing sixty genotypes at two different sowing dates with three replications under Randomized Compete Block Design (RCBD) in split plot arrangement. Hand sowing (chopa method) was adopted for bed sowing of three rows of each genotype consisted of ten plants with inter rows and intra rows distance of 75 cm and 30 cm respectively. First irrigation was applied at the time of sowing; 2nd irrigation after 4-5 days; 3rd and 4th irrigations with the interval of 7-10 days and subsequent irrigations were applied at 15 days interval or according the need of the crop. Fertilizer @140 kg/ha N and 60 kg/ha $P_2O_5$was applied. Half of the N and whole P_2O_5 were applied at sowing and the remaining N was given at the square stage in the form of Urea. All recommended cultural practices of crop production and protection were adopted from sowing to harvesting of the cotton crop. Two different sowing dates with an interval of about sixty days were taken as treatments to induce heat stress under field conditions. The April sowing was considered as heat stressed as its peak flowering period (70-80 days after sowing) coincided with maximum field temperature of June where as the June sowing was taken as normal. Available germplasm was screened against heat stress on the basis of cellular membrane thermostability (CMT), chlorophyll content (SPAD value), canopy temperature, node number to first fruiting branch, boll weight and yield per plant.

***Cell membrane thermostability*:** After 70-80 days of sowing, at peak flowering period in both sowing dates (heat stressed and normal) young fully expended leaves from five plants of each genotype were picked and cut into circular shapes of equal size and were put in distilled water in falcon tubes at 25°C after giving 2-3 washing with distilled water. Samples were collected in paired sets from both sides of leaf midrib. One set was used as control and the second one was used for heat treatment. One set of falcon tubes was placed in water bath at 50°Cfor 1 hr; while, control set was kept at 25°Cfor the same period. After heat treatment, the both sets were held at 25°Cfor 24 hr to allow diffusion of electrolytes. After 24 hr, the vials were shaken well to mix the electrolytes and electrical conductivity (EC) was measured with EC meter (Model: HI-933300, Hanna Instruments, USA). EC of control was designated as C_1 and that of heat treated samples was designated as T_1. Vials were then autoclaved for 10 minutes at 0.01 MPa to kill the tissues completely and release the electrolytes. C_2 (EC of control) and T_2 (EC of treatment) was measured after cooling the falcon tubes at room temperature. CMT% was calculated by using formula as used earlier by Blum and Ebercon (1981).

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

Where, T_1= EC of sap (treatment) before autoclaving, T_2= EC of sap (treatment) after autoclaving, C_1= EC of sap (control) before autoclaving, C_2= EC of sap (control) after autoclaving

***Canopy temperature*:** Canopy temperature is another important parameter for the assessment of heat stress tolerance in *Gossypium hirsutum* L. Canopy temperature of five selected plants of each genotype from both treatments was recorded with the help of Infra Red Thermometer (IRT, model: DT-8811H) in between 11.00 am and 04.00 pm. The data from all entries were recorded on the same day to minimize experimental error.

***Chlorophyll content*:** At peak flowering period, chlorophyll content of fifth fully expanded leaf below the terminal of plant was measured with the help of Minolta SPAD-502 chlorophyll meter as proposed by Johnson and Saunders (2003).

***Node number to first fruiting branch*:** Node number to first fruiting branch for five selected plant was calculated taking the cotyledon node as zero node.

***Boll weight*:** Twenty-five well developed matured bolls were picked at random and average boll weight was calculated by dividing the total yield with number of bolls.

***Seed cotton yield*:** At maturity, five plants from each genotype were selected and picked twice after the dew was off. Average seed cotton yield per plant was computed by dividing the total yield with number of plants.

***Development and evaluation of genetic material*:** Five high temperature tolerant genotypes (PB-76, MNH-992, FH-LALAZAR, MNH1016 and FH-458) and five sensitive genotypes (SLH-337, A-555, FH-326, CIM-511 and VH-282) were grown in glass house during 2017.All the conditions necessary for optimum cotton growth and development (temperature (35/21°C±2 day/night), humidity (60-70%), day length (14 hr) and natural light (1400-1600 μmol $m^{-2}s^{-1}$) were maintained artificially. At flowering stage, each female parent was crossed to five male parents in line×tester fashion taking the tolerant genotypes as female parent and sensitive as male parents to develop F_0 seed. The crossed seed along with parents was grown in field at two different sowing dates viz. April (heat stressed) and June (normal) following split plot arrangement under randomized complete block design (RCBD) with three replications in 2018 at research area of Post Agricultural Research Station (PARS), University of Agriculture, Faisalabad. All the standard practices of cotton crop production and protection were adopted to conduct the experiment.

***Data collection*:** At maturity, five plants from each genotype were selected at random for recording data of physiological parameters (Cell membrane thermostability, Chlorophyll content, Canopy temperature) and agronomic parameters (node number to first fruiting branch, number of bolls per plant, boll weight, ginning out turn, yield per plant).

***Better Parent heterosis (Heterobeltiosis)*:** Heterosis of crosses over better parent was calculated by the formula given below

$$\text{Heterobeltiosis} = \frac{F_1 - \text{Better parent value}}{\text{Better parent value}} \times 100$$

T-test proposed by Wyne *et al*. (1970) was applied to test the significance of heterotic effects

$$x = \frac{F_1 - \text{Better parent value}}{\sqrt{\frac{1}{2}\text{MSE}}}$$

***Statistical analysis*:** Analysis of variance technique given by Steel *et al*. (1997) was used to analyze the data and the traits showing significant results were further analyzed for combining ability effects by adopting procedure given by Kempthorne (1957). Methodology developed by Mather and Jinks (1971) was followed for the calculation of heterosis for various characteristics.

RESULTS

The genotypes exhibited highly significant differences for female, male and female × male interaction mean squares under normal as well as heat stressed environment among themselves for all the traits under investigation except node number to first fruiting branch which showed non-significant male × female mean square under control and non-significant female mean square under heat stressed environment (Table 1&2). Cell membrane thermostability, chlorophyll contents, canopy temperature, number of bolls/plant and GOT% displayed higher and significant female mean square than male and female × male interaction mean squares, node number to first fruiting branch, boll weight and seed cotton yield showed significant higher male mean square than female mean square and female × male interaction mean square under control environment. Under heat stress condition, chlorophyll contents, canopy temperature and boll weight displayed higher female mean square; while, cell membrane thermostability, node number

Table 1. Mean squares and genetic components for various physiological and agronomic traits of cotton under normal condition.

SOV	d.f	CMT%	C.C.	C.T	NNFFB	B/plant	B.W(g)	GOT%	SCY(g)
Replication	2	3.120	2.510	0.0070	0.4090	0.3520	0.0370	0.8800	63.720
Genotypes	34	250.150**	64.610**	8.7700**	1.6800**	72.9600**	0.3360**	14.7500**	1691.960**
Cross	24	175.065**	60.949**	9.4128**	0.1200**	76.2644**	0.3487**	16.0834**	1315.737**
Female	4	411.937**	109.945**	43.8627**	0.1200**	244.3533**	0.8389**	50.3598**	1308.653**
Male	4	206.686**	38.335**	3.4277**	2.8533**	57.3867**	1.0742**	21.3811**	1603.520**
M x F	16	107.942**	54.354**	2.2966**	0.8033$^{N.S}$	38.9617**	0.0448 $^{N.S}$	6.1898**	1245.562**
Error	48	1.149	1.148	0.7207	0.6061	1.5878	0.0312	0.3585	22.898
σ^2gca		1.678	0.165	0.1779	0.0178	0.9326	0.0076	0.2473	1.754
σ^2sca		35.598	17.735	0.5253	0.0657	12.4580	0.0045	1.9438	407.554
σ^2A		6.712	0.660	0.7116	0.0711	3.7303	0.0304	0.9894	7.018
σ^2D		142.391	70.940	2.1012	0.2630	49.8319	0.0181	7.7751	1630.218

*Significant, **Highly significant whereas cell membrane thermostability (CMT%), Chlorophyll contents (C.C), Canopy temperature (C.T), Node number to first fruiting branch (NNFFB), Bolls per plant (B/Plant), Boll weight (B.W), Ginning out turn (GOT) and Seed cotton yield (SCY)

Table 2. Mean squares and genetic components for various physiological and agronomic traits of cotton under heat stress.

SOV	d.f	CMT	C.C.	C.T	NNFFB	B/plant	B.W	GOT	SCY
Replication	2	22.413	8.300	0.5470	3.8380	2.1230	0.0740	0.0980	9.266
Genotypes	34	392.810**	174.660**	43.0800**	5.2440**	87.1800**	0.4220**	14.7900**	2968.590**
Cross	24	434.889**	203.827**	48.3242**	4.4478**	88.4644**	0.4349**	19.2304**	3032.139**
Female	4	658.467**	543.112**	103.0450**	0.7533$^{N.S}$	57.6867**	1.2655**	15.7678**	2569.367**
Male	4	922.033**	330.659**	63.4430**	12.4533**	189.1530**	0.9678 **	48.7211**	9451.833**
M x F	16	257.208**	87.298**	30.8643**	3.3700**	70.9867**	0.0940**	12.7234**	1542.908**
Error	48	4.502	3.305	0.9764	0.8911	4.5094	0.0314	0.2870	48.111
σ^2gca		4.442	2.913	0.4365	0.0269	0.4369	0.0085	0.1627	37.231
σ^2sca		84.235	27.998	9.9626	0.8263	22.1591	0.0209	4.1455	498.266
σ^2A		17.768	11.652	1.7460	0.1078	1.7478	0.0341	0.6507	148.923
σ^2D		336.941	111.991	39.8505	3.3052	88.6363	0.0834	16.5819	1993.064

**Highly significant whereas cell membrane thermostability (CMT%), Chlorophyll contents (C.C), Canopy temperature (C.T), Node number to first fruiting branch (NNFFB), Bolls per plant (B/Plant), Boll weight (B.W), Ginning out turn (GOT) and Seed cotton yield (SCY)

Table 3. General combining ability effects for various physiological and agronomic traits of cotton under normal condition.

Genotype	CMT	C.C	C.T	NNFFB	B/plant	B.W	GOT	SCY
Lines								
PB-76	1.54**	-0.22^{NS}	2.33**	0.03^{NS}	-1.36**	-0.01^{NS}	1.75**	4.49**
MNH-992	-7.03**	-4.10**	1.30**	0.23^{NS}	-5.56**	-0.38**	-2.99**	-12.31**
FH Lalazar	-3.05**	-0.15^{NS}	-1.21**	-0.77**	-0.56^{NS}	0.13**	-0.21^{NS}	-0.97^{NS}
MNH-1016	1.77**	1.15**	-0.89**	0.16^{NS}	2.31**	0.02^{NS}	0.35*	-3.91**
FH-458	6.76**	3.33**	-1.53**	0.36^{NS}	5.17**	0.24**	1.11**	12.69**
Tester								
SLH-337	2.83**	-0.65*	-0.48*	-0.57**	1.44**	0.25**	-0.91**	3.16*
A-555	-6.21**	-1.80**	0.79**	-0.04^{NS}	-1.63**	-0.25**	-1.51**	-9.91**
FH-326	2.48**	1.99**	0.05^{NS}	-0.24^{NS}	2.11**	-0.02^{NS}	1.03**	10.69**
CIM-511	1.45**	1.35**	-0.19^{NS}	0.36^{NS}	0.51^{NS}	0.30**	1.22**	7.96**
VH-282	-0.56^{NS}	-0.88**	-0.17^{NS}	0.49*	-2.43**	-0.27**	0.19^{NS}	-11.91**
S.E	0.2768	0.2767	0.2192	0.2010	0.3253	0.0456	0.1546	1.2355

*Significant, **Highly significant, NS = Non significant whereas cell membrane thermostability (CMT%), Chlorophyll contents (C.C), Canopy temperature (C.T), Node number to first fruiting branch (NNFFB), Bolls per plant (B/Plant), Boll weight (B.W), Ginning out turn (GOT) and Seed cotton yield (SCY)

to first fruiting branch, number of bolls/plant, GOT% and seed cotton yield exhibited higher male mean square than female and female × male interaction mean squares. All the traits under study produced higher SCA variance than GCA variance under normal conditions except boll weight which showed higher GCA than SCA variance under control.

Table 4. General combining ability effects for various physiological and agronomic traits of cotton under heat stress.

Genotype	CMT	C.C	C.T	NNFFB	B/plant	B.W	GOT	SCY
Lines								
PB-76	-6.60**	-4.39**	0.94**	-0.11^{NS}	0.51^{NS}	0.01^{NS}	-0.80**	0.53^{NS}
MNH-992	1.27*	5.25**	-1.30**	0.03^{NS}	-0.76^{NS}	-0.07^{NS}	0.44**	-9.87**
FH Lalazar	10.73**	7.43**	-3.91**	-0.31^{NS}	1.71**	0.44**	1.61**	19.47**
MNH-1016	-2.13**	-2.07**	1.61**	0.29^{NS}	-3.03**	-0.37**	-0.67**	-13.93**
FH-458	-3.27**	-6.21**	2.65**	0.09^{NS}	1.57**	-0.00^{NS}	-0.58**	3.80*
Tester								
SLH-337	2.80**	1.34**	-0.60*	0.16^{NS}	-0.16^{NS}	0.09^{NS}	-0.71**	-8.40**
A-555	-11.53**	-6.60**	3.17**	0.83**	-3.63**	-0.15**	-1.69**	-17.27**
FH-326	9.40**	4.41**	0.22^{NS}	-1.44**	4.04**	0.05^{NS}	1.31**	26.80**
CIM-511	2.53**	3.81**	-2.53**	-0.24^{NS}	3.11**	0.35**	2.45**	26.20**
VH-282	-3.20**	-2.97**	-0.25^{NS}	0.69**	-3.36**	-0.33**	-1.38**	-27.33**
S.E	0.5479	0.4694	0.2551	0.2437	0.5483	0.0458	0.1383	1.7909

*Significant, **Highly significant, NS = non-significant whereas cell membrane thermostability (CMT%), Chlorophyll contents (C.C), Canopy temperature (C.T), Node number to first fruiting branch (NNFFB), Bolls per plant (B/Plant), Boll weight (B.W), Ginning out turn (GOT) and Seed cotton yield (SCY)

Table 5. Specific combining ability effects of crosses under normal conditions.

Cross	CMT%	C.C	C.T	NNFFB	B/plant	B.W	GOT	SCY
PB-76 * SLH-337	-5.39**	-3.01**	-0.52^{NS}	0.64^{NS}	-2.51**	0.09^{NS}	0.25^{NS}	-21.23**
PB-76 * A-555	2.81**	5.67**	0.15^{NS}	0.44^{NS}	0.56^{NS}	-0.24*	1.75**	-0.83^{NS}
PB-76 * FH-326	-0.77^{NS}	-3.92**	0.39^{NS}	-0.03^{NS}	0.49^{NS}	0.03^{NS}	0.07^{NS}	7.57**
PB-76 * CIM-511	4.72**	5.09**	0.42^{NS}	-0.63^{NS}	1.09^{NS}	0.14^{NS}	-0.79*	7.97**
PB-76 * VH-282	-1.37*	-3.82**	-0.43^{NS}	-0.43^{NS}	0.36^{NS}	-0.02^{NS}	-1.29**	6.51*
MNH-992 * SLH-337	2.78**	1.64*	0.27^{NS}	-0.56^{NS}	-0.64^{NS}	0.10^{NS}	0.43^{NS}	-1.09^{NS}
MNH-992 * A-555	4.15**	2.18**	-0.46^{NS}	-0.09^{NS}	0.43^{NS}	0.03^{NS}	0.49^{NS}	7.31*
MNH-992 * FH-326	-6.91**	-2.28**	0.38^{NS}	0.11^{NS}	-2.64**	-0.06^{NS}	0.42^{NS}	-12.63**
MNH-992 * CIM-511	0.65^{NS}	2.30**	-0.19^{NS}	0.51^{NS}	1.63*	0.05^{NS}	-1.07**	-0.56^{NS}
MNH-992 * VH-282	-0.67^{NS}	0.76^{NS}	-0.01^{NS}	0.04^{NS}	1.23^{NS}	-0.12^{NS}	-0.27^{NS}	6.97*
FH Lalazar * SLH-337	-3.26**	-1.65**	-0.15^{NS}	-0.23^{NS}	-4.64**	-0.08^{NS}	-1.12**	-12.76**
FH Lalazar * A-555	-0.34^{NS}	-7.77**	-0.79^{NS}	-0.09^{NS}	-3.91**	0.12^{NS}	-1.39**	-25.69**
FH Lalazar * FH-326	0.39^{NS}	3.18**	0.19^{NS}	0.11^{NS}	1.36^{NS}	0.02^{NS}	-0.66^{NS}	2.71^{NS}
FH Lalazar * CIM-511	-5.51**	0.39^{NS}	0.82^{NS}	0.17^{NS}	0.63^{NS}	-0.16^{NS}	0.58^{NS}	2.77^{NS}
FH Lalazar * VH-282	8.72**	5.85**	-0.07^{NS}	0.04^{NS}	6.56**	0.10^{NS}	2.58**	32.97**
MNH-1016 * SLH-337	3.73**	3.35**	-0.04^{NS}	0.51^{NS}	6.49**	-0.08^{NS}	1.58**	39.51**
MNH-1016 * A-555	-5.85**	2.30**	2.03**	0.31^{NS}	0.23^{NS}	0.02^{NS}	-1.29**	-9.76**
MNH-1016 * FH-326	6.46**	2.54**	-0.60^{NS}	-0.83^{NS}	0.49^{NS}	0.06^{NS}	1.31**	-6.69*
MNH-1016 * CIM-511	6.67**	-3.55**	-1.80**	-0.43^{NS}	-0.57^{NS}	0.08^{NS}	0.51^{NS}	0.71^{NS}
MNH-1016 * VH-282	-11.02**	-4.65**	0.41^{NS}	0.44^{NS}	-6.64**	-0.09^{NS}	-2.12**	-23.76**
FH-458 * SLH-337	2.15**	-0.33^{NS}	0.44^{NS}	-0.36^{NS}	1.29^{NS}	-0.03^{NS}	-1.14**	-4.43^{NS}
FH-458 * A-555	-0.78^{NS}	-2.39**	-0.93^{NS}	-0.56^{NS}	2.69**	0.07^{NS}	0.43^{NS}	28.97**
FH-458 * FH-326	0.83^{NS}	0.49^{NS}	-0.35^{NS}	0.64^{NS}	0.29^{NS}	-0.06^{NS}	-1.15**	9.04**
FH-458 * CIM-511	-6.54**	0.37^{NS}	0.75^{NS}	0.37^{NS}	-2.77**	-0.11^{NS}	0.76*	-10.89**
FH-458 * VH-282	4.34**	1.86**	0.09^{NS}	0.09^{NS}	-1.51*	0.12^{NS}	1.09**	-22.69**

Cell membrane thermostability (CMT%), Chlorophyll contents (C.C), Canopy temperature (C.T), Node number to first fruiting branch (NNFFB), Bolls per plant (B/Plant), Boll weight (B.W), Ginning out turn (GOT) and Seed cotton yield (SCY)

Similarly, all the traits except boll weight showed higher dominance variance than additive variance under normal environment. Under heat stress, all the traits produced higher SCA variance than GCA variance and higher dominance variance than additive variance.

***Combining ability effects*:** Results for general combining ability effects under control and heat stressed environments are presented in Table 3&4. The results revealed that the female parent FH-458 produced the highest positive values of general combining ability effects for most of the traits including cell membrane thermostability, chlorophyll contents, node number to first fruiting branch which was non-significant, number of bolls/plant, boll weight and seed cotton yield while PB-76 produced the highest positive value for canopy temperature and GOT% under normal environment. The tester/male parent SLH-337 showed the highest negative values for canopy temperature and node number to first fruiting branch, tester A-555 exhibited the highest negative values for cell membrane thermostability, chlorophyll contents and GOT%; whereas, the tester VH-

282 showed the highest negative values for number of bolls/plant, boll weight and seed cotton yield for general combining ability effects under control condition. The line FH Lalazar produced the highest positive values for general combining ability estimates under heat stress for most of the traits including cell membrane thermostability, chlorophyll contents, number of bolls/plant, boll weight and seed cotton yield, the line MNH-1016 showed the highest positive but

Table 6. Specific combining ability effects of crosses under heat stress.

Cross	CMT%	C.C	C.T	NNFFB	B/plant	B.W(g)	GOT%	SCY(g)
PB-76 * SLH-337	-5.47**	-3.77**	5.95**	0.44NS	-4.57**	-0.03NS	-1.83**	1.20NS
PB-76 * A-555	2.53*	-6.57**	0.61NS	0.77NS	-6.77**	-0.03NS	0.05NS	-29.93**
PB-76 * FH-326	-5.07**	5.85**	-3.11**	0.37NS	-0.77NS	0.08NS	-0.71*	-7.67NS
PB-76 * CIM-511	-2.87*	-0.25NS	-2.02**	-0.16NS	2.83*	-0.09NS	-0.29NS	-1.07NS
PB-76 * VH-282	10.87**	4.73**	-1.43*	-1.43*	9.29**	0.05NS	2.78**	37.47**
MNH-992 * SLH-337	1.00NS	2.89**	-1.85**	-0.69NS	5.03**	0.05NS	2.37**	28.27**
MNH-992 * A-555	-1.33NS	2.13*	3.72**	0.64NS	-0.51NS	-0.09NS	-1.15**	-3.53NS
MNH-992 * FH-326	1.40NS	2.05NS	-1.30*	0.24NS	2.83*	-0.15NS	0.51NS	-9.27*
MNH-992 * CIM-511	3.93**	-4.28**	-0.51NS	-1.29*	-2.57*	0.29**	0.47NS	-4.67NS
MNH-992 * VH-282	-5.00**	-2.80*	-0.06NS	1.11*	-4.77**	-0.11NS	-2.19**	-10.8**
FH Lalazar * SLH-337	0.20NS	0.41NS	1.09NS	-1.36*	3.89**	0.11NS	-2.03**	0.60NS
FH Lalazar * A-555	-1.13NS	1.69NS	-3.81**	-0.36NS	3.36**	-0.03NS	1.65**	35.8**
FH Lalazar * FH-326	-2.07NS	-3.39**	0.17NS	-0.09NS	1.69NS	-0.22*	-0.55NS	2.7NS
FH Lalazar * CIM-511	-7.87**	-1.66NS	2.06**	2.37**	-5.71**	-0.22*	-1.06**	-27.67**
FH Lalazar * VH-282	10.87**	2.95**	0.48NS	-0.56NS	-3.24*	0.35**	2.01**	-11.47**
MNH-1016 * SLH-337	15.73**	9.31**	0.64NS	0.71NS	-1.71NS	-0.05NS	3.91**	-16.00**
MNH-1016 * A-555	5.07**	0.69NS	0.64NS	-0.63NS	-0.57NS	0.05NS	-0.44NS	-6.80NS
MNH-1016 * FH-326	-5.87**	-7.73**	1.72**	-0.36NS	-3.91**	0.16NS	-0.87**	-11.53**
MNH-1016 * CIM-511	-6.33**	2.47*	0.21NS	-0.56NS	5.69**	0.06NS	-0.61NS	27.07**
MNH-1016 * VH-282	-8.60**	-4.75**	-3.21**	0.84NS	0.49NS	-0.23*	-1.98**	7.27NS
FH-458 * SLH-337	-11.47**	-8.85**	-5.83**	0.91NS	-2.64*	-0.09NS	-2.41**	-14.07**
FH-458 * A-555	-5.13**	2.06NS	-1.17*	-0.43NS	4.49**	0.08NS	-0.10NS	4.47NS
FH-458 * FH-326	11.60**	3.21**	2.51**	-0.16NS	0.16NS	0.12NS	1.63**	25.73**
FH-458 * CIM-511	13.13**	3.71**	0.27NS	-0.36NS	-0.24NS	-0.05NS	1.49**	6.33NS
FH-458 * VH-282	-8.13**	-0.14NS	4.22**	0.04NS	-1.77NS	-0.07NS	-0.61NS	-22.47**

Cell membrane thermostability (CMT%), Chlorophyll contents (C.C), Canopy temperature (C.T), Node number to first fruiting branch (NNFFB), Bolls per plant (B/Plant), Boll weight (B.W), Ginning out turn (GOT) and Seed cotton yield (SCY)

Table 7. Better parent heterosis of crosses under normal conditions

Cross	CMT%	C.C	C.T	NNFFB	B/plant	B.W(g)	GOT%	SCY(g)
PB-76 * SLH-337	-19.40**	-12.76**	2.34NS	-17.39*	-18.56**	13.86**	1.66NS	7.22NS
PB-76 * A-555	-20.50**	2.58NS	8.51**	-9.09NS	-15.96**	-10.89**	1.79NS	6.33NS
PB-76 * FH-326	-13.73**	-11.40**	1.62NS	-18.18*	-18.92**	-3.67NS	0.24NS	-12.88**
PB-76 * CIM-511	-7.80**	6.65**	6.28*	-19.17*	-17.92**	8.26*	0.64NS	-8.06**
PB-76 * VH-282	-18.56**	-14.87**	-2.50NS	-13.64NS	-11.63**	-4.95NS	0.83NS	29.34**
MNH-992 * SLH-337	17.44**	3.32NS	-1.34NS	-30.43**	-25.77**	0.97NS	-15.09**	10.83*
MNH-992 * A-555	2.51NS	1.90NS	0.31NS	-17.39*	-29.79**	-15.53**	-16.34**	-2.33NS
MNH-992 * FH-326	-30.29**	-15.85**	-1.52NS	-17.51*	-38.74**	-16.51**	-10.56**	-36.70**
MNH-992 * CIM-511	-19.71**	-16.05**	-1.86NS	-4.35NS	-28.30**	-4.59NS	-13.60**	-25.58**
MNH-992 * VH-282	4.13*	0.71NS	-4.30NS	-8.70NS	-10.81*	-20.39**	-14.15**	31.19**
FH Lalazar * SLH-337	-2.10NS	8.23**	-12.07**	-39.13**	-22.68**	6.54NS	-8.18**	1.32NS
FH Lalazar * A-555	-12.37**	-10.77**	-10.14**	-27.27**	-27.66**	-1.87NS	-10.67**	-24.5**
FH Lalazar * FH-326	-14.66**	2.85NS	-9.71**	-15.79NS	-14.41**	4.11NS	-6.13**	-19.53**
FH Lalazar * CIM-511	-22.80**	-2.69NS	-8.22**	-10.10NS	-16.98**	3.67NS	-0.80NS	-15.44**
FH Lalazar * VH-282	12.34**	22.70**	-12.01**	-14.29NS	10.23*	-2.80NS	3.60**	31.79**
MNH-1016 * SLH-337	38.45**	-3.38NS	-0.23NS	-17.39*	13.59**	0.91NS	-4.39**	19.47**
MNH-1016 * A-555	5.51**	-7.58**	8.05**	-9.09NS	-13.59**	-10.00**	-12.53**	-29.74**
MNH-1016 * FH-326	0.44NS	0.13NS	-11.12**	-15.79NS	-9.01**	-2.73NS	-0.47NS	-27.47**
MNH-1016 * CIM-511	1.24NS	-12.74**	-9.23**	-5.23NS	-12.26**	6.36NS	-1.88NS	-18.89**
MNH-1016 * VH-282	6.50**	-19.11**	-9.61**	4.76NS	-35.92**	-13.64**	-10.49**	-42.37**
FH-458 * SLH-337	45.18**	17.38**	-0.79NS	-26.09**	13.40**	30.77**	-3.41*	34.3**
FH-458 * A-555	35.54**	9.73**	-3.86NS	-18.18*	11.70**	11.46**	-3.01*	44.33**
FH-458 * FH-326	-0.46NS	4.44*	-12.34**	-4.55NS	-1.80NS	0.92NS	-4.16**	-6.65*
FH-458 * CIM-511	-10.38**	4.30*	-3.04NS	4.81NS	-10.38**	8.26*	2.80*	-15.44**
FH-458 * VH-282	48.84**	21.51**	-12.51**	-4.55NS	11.11*	20.00**	11.98**	15.74**
S.E	0.863	0.925	0.776	0.640	1.196	0.135	0.543	3.996

Cell membrane thermostability (CMT%), Chlorophyll contents (C.C), Canopy temperature (C.T), Node number to first fruiting branch (NNFFB), Bolls per plant (B/Plant), Boll weight (B.W), Ginning out turn (GOT) and Seed cotton yield (SCY)

non-significant value for node number to first fruiting branch whereas the line FH-458 produced the highest general combining ability effects under heat stress for canopy temperature. FH Lalazar×VH-282 proved to be the best specific cross for CMT% under normal condition; whereas, MNH-1016×SLH-337 was proved to be the best cross under heat stress as it produced the highest value. FH lalazar×VH-282 produced the highest positive value of

Table 8. Better parent heterosis of crosses under heat stress

Cross	CMT%	C.C	C.T	NNFFB	B/plant	B.W(g)	GOT%	SCY(g)
PB-76 * SLH-337	-11.37**	-18.35**	3.43NS	28.57*	-7.35NS	-3.88NS	-8.85**	-5.94NS
PB-76 * A-555	-24.63**	-44.15**	7.01**	42.86**	-32.35**	-10.68*	-6.41**	-47.9**
PB-76 * FH-326	3.28NS	12.18**	-16.88**	4.76NS	27.94**	-1.94NS	-0.36NS	21.68**
PB-76 * CIM-511	-6.49NS	-3.93NS	-21.08**	14.29NS	39.71**	1.94NS	3.88**	27.97**
PB-76 * VH-282	10.26*	-8.25*	-15.23**	3.92NS	39.71**	-13.59**	1.81NS	12.24*
MNH-992 * SLH-337	8.42*	13.13**	-22.37**	9.09NS	27.54**	4.21NS	4.18**	9.25NS
MNH-992 * A-555	-23.47**	-6.45*	9.41**	36.36**	-11.59NS	3.12NS	-7.82**	-32.53**
MNH-992 * FH-326	21.81**	18.15**	-18.01**	4.15NS	36.23**	4.55NS	4.62**	6.85NS
MNH-992 * CIM-511	13.52**	2.55NS	-22.98**	-4.55NS	8.70NS	23.91**	7.56**	10.96NS
MNH-992 * VH-282	-14.54**	-9.38**	-17.45**	34.78	-28.99**	-6.82NS	-9.78**	-50.34**
FH Lalazar * SLH-337	28.02**	14.17**	-21.51**	5.10NS	31.43**	19.59**	-7.57**	10.20NS
FH Lalazar * A-555	-2.68NS	-1.07NS	-18.63**	30.23*	14.29*	8.25NS	-0.60NS	37.07**
FH Lalazar * FH-326	36.51**	12.49**	-20.95**	2.87NS	40.00**	8.25NS	1.46NS	48.30**
FH Lalazar * CIM-511	11.69**	15.08**	-23.07**	55.12**	4.29NS	17.53**	3.10*	16.67**
FH Lalazar * VH-282	37.17**	10.13**	-22.72**	8.70NS	-12.86NS	14.43**	1.12NS	-21.43**
MNH-1016 * SLH-337	26.08**	5.79NS	-8.48**	52.63**	-21.79**	-16.35**	7.73**	-45.11**
MNH-1016 * A-555	-20.27**	-29.71**	8.95**	35.00*	-30.77**	-20.19**	-6.82**	-44.79**
MNH-1016 * FH-326	-1.73NS	-24.14**	-2.60NS	10.53NS	-14.10*	-11.54**	0.18NS	-7.57NS
MNH-1016 * CIM-511	-15.33**	-3.57NS	-13.53**	20.00NS	19.23**	-5.77NS	4.00**	28.39**
MNH-1016 * VH-282	-30.16**	-33.57**	-18.04**	34.78**	-25.64**	-33.65**	-10.18**	-41.01**
FH-458 * SLH-337	-21.81**	-32.78**	-22.45**	38.1**	1.41NS	2.11NS	-10.33**	-20.21**
FH-458 * A-555	-37.58**	-25.41**	6.83**	28.57*	16.90*	4.40NS	-6.74**	-10.27NS
FH-458 * FH-326	36.66**	4.80NS	2.16NS	5.64NS	30.99**	12.09*	6.02**	56.85**
FH-458 * CIM-511	26.15**	4.55NS	-10.67**	14.29NS	25.35**	15.22**	8.72**	36.30**
FH-458 * VH-282	-27.07**	-21.85**	3.57**	21.74NS	-8.45NS	-6.59NS	-7.28**	-48.29**
S.E	1.907	1.365	0.830	0.891	1.640	0.145	0.496	5.548

Cell membrane thermostability (CMT%), Chlorophyll contents (C.C), Canopy temperature (C.T), Node number to first fruiting branch (NNFFB), Bolls per plant (B/Plant), Boll weight (B.W), Ginning out turn (GOT) and Seed cotton yield (SCY)

specific combining ability effects for chlorophyll contents under control condition; while, MNH-1016×SLH-337 showed best specific combining ability effect under heat stress.

The cross MNH-1016×A-555 exhibited highest specific combining ability for canopy temperature under normal environment and the cross PB-76×SLH-337 produced highest specific combining ability for the trait under heat stress. PB-76×SLH-337 and FH-458×FH-326 produced the highest positive but non-significant value of specific combining ability effects for node number to first fruiting branch under normal environment whereas FH lalazar×CIM-511 exhibited the highest positive value for this trait under heat stressed environment. FH lalazar×VH-282 proved to be the best cross for number of bolls/plant by producing the highest positive value for specific combining ability effects under normal condition while the cross PB-76×VH-282 showed the highest specific combining ability estimates under heat stress. The cross PB-76×CIM-511 produced the highest specific combining ability for boll weight under normal condition while the cross FH Lalazar×VH-282 exhibited the highest specific combining ability for boll weight under heat stress.

***Heterobeltiosis*:** Results for better parent heterosis revealed that the cross FH-458×VH-282 showed the highest increase over better parent for cell membrane thermostability and GOT% and the highest decrease for canopy temperature under normal conditions (Table 7&8). FH Lalzar×VH-282 produced the highest heterobeltiosis for chlorophyll contents, FH Lalazar×SLH-337 gave better performance for node number to first fruiting branch and showed the highest decline over better parent, MNH-1016×SLH-337 produced better for bolls/plant, FH-458×SLH-337 performed better for boll weight and the cross FH-458×A-555 produced the highest heterobeltiosis for seed cotton yield under control condition. FH Lalzar×VH-282 showed highest better parent heterosis for cell membrane thermostability, MNH-992×FH-326 performed better for chlorophyll contents, FH lalazar×CIM-511 and MNH-992×CIM-511 showed highest decline over better parent for canopy temperature and node number to first fruiting branch respectively, FH lalazar×FH-326 gave better performance for bolls/plant, MNH-992×CIM-511 produced better results for boll weight, FH-458×CIM-511 performed better GOT% and FH-458×FH-326 showed highest better parent heterosis for seed cotton yield under heat stress.

DISCUSSION

Plant yield in the field is often affected by various types of stresses like high temperature, water deficit, insect and disease stress and final economic yield of any plant/crop reflects its ability to fight against these stresses. Bita and Gerats (2013) recommended continuous and rapid assessment of plant germplasm to include high yielding and thermo tolerant material in a breeding program. Results of current study revealed that tolerant genotypes exhibited higher cell membrane thermostability whereas, susceptible genotypes produced lower value for the trait. Thus, it could be established that cell membranes of tolerant genotypes resist high temperature while those of sensitive strains become leaky under heat stress. CMT% is a fast screening tool which can assess genetic material at any stage of life cycle and thus it could be used reliably for screening and selecting the cotton germplasm against heat stress (Azhar *et al*., 2009; Arfan *et al*., 2018). Earlier researchers used chlorophyll content measurements for the identification of the best parent for stress breeding and concluded that high chlorophyll contents under stressed environment is an indication of thermotolerance and vice versa (Rana *et al.*, 2011). It was concluded from this study that genotypes with higher canopy temperature, due to more closed stomata, produced the highest yields. However, some researchers like Mason *et al.* (2014) and Bennani *et al.* (2016) also showed negative association between canopy temperature and seed cotton yield. This might be due to different crops under study or may become from differences of irrigation as some studies were conducted under well watered conditions while others were conducted under water deficit conditions. Node number to first fruiting branch has been found as an efficient morphological indicator of earliness and thermo-tolerance in cotton (Shakeel *et al*., 2008; Baloch *et al*., 2014; Hajazi *et al*., 2014; Arfan *et al.*, 2018). Yield is the final outcome of any plant after facing all negative forces in the field and increase in yield is the ultimate objective of any breeding program. Under heat stress, plant's first priority is its survival and so it tends to shed extra fruit and even leaves under stress. Similarly, the seed cotton yield is considerably decreased by fruit (flowers and bolls) shedding due to heat stress. Brown *et al*. (2003) considered drought and heat stress as two major constraints for seed cotton yield. Combining ability estimate is very useful tool to evaluate the potential of parents to combine with each other and with other genotypes under study (Olfati *et al*., 2012; Shankar, 2013). Estimation of general combining ability effects and genetic components is helpful to identify the desirable general combiner for the improvement of traits of interest (Wu *et al*., 2010). Heterobeltiosis describes the performance superiority of F1 hybrids over better parent. Cotton yield and quality could be improved by exploiting heterosis breeding (Meredith and Brown, 1998). Certain characters like seed cotton yield, CMT%, boll weight and number of bolls/plant require positive heterosis for their improvement while negative heterosis is desirable for some other characters e.g., negative heterosis is required for canopy temperature and node number to first fruiting branch (Singh *et al.*, 2012). Results of present experiment showed that the cross FH-458 × FH-326 produced the highest positive heterotic effects for seed cotton yield under heat stress. The present findings are in accordance with those of Rauf *et al*. (2005) and Arfan *et al.* (2018) who claimed considerable amount of heterosis for seed cotton yield and its contributing traits. During current study, dominance was found to be involved in the inheritance of most of the characters under normal as well as heat stressed environments which described that hybrid breeding could be fruitful for developing thermo-tolerant genetic material in cotton for sustainable cotton yield and lint quality. The findings of present investigations intensify the role of both additive and non-additive types of gene action for maximum improvement of yield and yield related traits. Similar results about heritability, combining ability effects and gene action were obtained by Arfan *et al*. (2018). It was concluded from current experiments that superior cross combinations involved at least one best general combiner, which got support from the previous findings of Rauf *et al*. (2005) and Arfan *et al.*(2018) who observed non-additive gene action for the inheritance of seed cotton yield and other yield contributing traits. It was also observed during current study that the cross combinations showing better specific combining ability involved at least one parent with high positive general combining ability effects which suggested heterosis breeding for heat stress in cotton.

***Conclusion*:** It can be concluded from current findings that cell membrane thermostability, chlorophyll contents and canopy temperature could be utilized reliably for rapid assessment and screening of thermo-tolerant cotton germplasm and because of heritable variations, heat tolerant strains could be developed in cotton. Presence of non-additive gene action revealed the importance of hybrid breeding for yield and heat stress tolerance improvement. The information gathered from present experiments may advantageously be used by cotton breeders in future.

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[Received 03 Dec. 2019; Accepted 27 July 2020 Published (Online) 25 Oct. 2020]