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Generation mean analysis of some Physiological Traits Contributing to heat Tolerance in Upland Cotton (*Gossypium Hirsutum* L.)

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Abstract: Various physiological parameters help out the plant to overcome the adverse effect of thermal stress. To understand the genetic base of these traits, the current study is a step ahead. The objective of this research was to estimate the gene actions that are responsible for survival of plant under heat stress condition. Six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) of three crosses were developed by crossing among six cotton genotypes and evaluated at the Central Cotton Research Institute, Multan Farm. The experiment was done in randomized complete blocks besign in three replications under normal and heat stress conditions. The mean of data of all six generations of three crosses were recorded keeping in view the support from literature. Additive, dominance and epistatic genetic effects were operating in controlling the phenotypic expressions of most of the traits contributing to heat stress tolerance. Heterobeltosis for relative water content was observed only in on cross under heat stress condition with the value of 1.39 while the excised leaf water loss did not showed. heterobeltosis under both conditions.

Keywords: Cell Injury, Genetic Advance, Heritability, Heterosis, Thermal Stress

1. INTRODUCTION

Plant physiological functions greatly influenced by biotic and abiotic stresses. Every stress on cotton plant reduces biosynthetic ability of plants and led toward some critical damages on the plants (Lichtenthaler 1996). Heat is a significant restraining aspect for agricultural productivity and it generally inhibits plant growth through damage of leaves cells, and reduction in photosynthesis rate. Cotton belt of Pakistan in located in a hot and arid zone where temperature in summers may reach up to 50°C (Khan *et al*, 2008). It is estimated that due to long period rise in temperature, all part of world including Pakistan will face more frequent and intense thermal waves (Maida and Rasul, 2011). Other associated problems of high temperature such as drought and high light intensity which enhances the impact of heat and results in low number of plants per unit area which ultimately results in lower yield and quality (Rahman, 2006). Snider *et al* (2009) reported the adverse effects of temperature above 35°C on photosynthesis. Boyer (1982) reported about 25% less yield due to heat stress. Evaluation of heat in the field with estimation of irrigations required is a proper method to study heat stress (Hall, 2001). There is no obvious consent regarding the most favorable thermal conditions for cotton crop, as plant response fluctuate greatly with plant developmental phases and plant organ (Burke and Wanjura, 2009).. Whole plant life study in heat stress is a common and easier way to screen the stress (Burke, 2001). Yield losses can be minimized by developing heat resistant varieties. Stress tolerance is linked with different physiological traits like relative water content (RWC), excised leaf water loss (ELWL) and relative cell injury (RCI) which can be exploited by using advanced breeding tools such as DNA markers (Nguyen, 2000, Jenkins *et al*, 2001). The objective of present research work was to gain valuable information regarding gene action as well as the extent of hybrid vigour of some physiological traits contributing to heat tolerance in three cotton crosses

2. MATERIAL AND METHODS

On the bases of previous data of relative cell injury, six parents were selected for crossing. After developing the six basic generations of three crosses during 2012 and 2013 **(Table 1),** the same were sown in the experimental field area of Central Cotton Research Institute, Multan at two different sowing times during 2014. The first set of experiment was sown in last week of March while the second set was sown in 2nd week of May. The purpose of this sowing scheme was to expose the generations to different conditions of heat stress to identify the genetic response for various physiological traits. The experiment was planted by adopting RCB design with three replications. The plot size for parents and F_1's were 10 x 5 feet while for F_2s and backcrosses, the plot size was 40 x 7.5 and 10 x 10 respectively. Inter row distance was 75cm while spacing between the

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plants were kept 30 cm. Data were recorded on 30 random plants for each parent, F_1 and back cross generations, and 150 random plants were selected for each F_2 population.

The data of parameters under study was measured after the ninety days of planting. The relative water content (RWC) was calculated by following the formula described by Barrs and Weatherly (1962). The Net photosynthetic rate (NPR) was measured using portable photosynthesis system (CI-340, CID, USA). Relative cell injury (RCI) was calculated through the method described by Sullivan (1972) while excised leaf water loss (ELWL) was calculated by the method illustrated by Clarke and McCaig (1982a)

Table-1. Parental combination for different generations in Three crosses

Cross- 1		Cross- 2	Cross- 3
P_1	CIM-600 (Heat tolerant)	CYTO-178 (Heat tolerant)	FH-142 (Heat tolerant)
P_2	Allepo-1 (Heat susceptible)	AMSI-38 (Heat susceptible)	Marvi (Heat susceptible)
F_1	CIM-600 × Allepo-1	CYTO-178 × AMSI-38	FH-142 x Marvi
F_2	F_1× F_1(Self F_1)	F_1× F_1(Self F_1)	F_1× F_1(Self F_1)
BC1	(CIM-600×Allepo-1)×CIM-600	(CYTO-178 × AMSI-38) × Cyto-178	(FH-142 x Marvi) x FH-142
BC2	(CIM-600 × Allepo-1) × Allepo-1	(CYTO-178 × AMSI-38) × AMSI-38	(FH-142 x Marvi) x Marvi

Mather and Jinks, 1982 described the method for generation mean analysis. It was implemented by using computer software developed by Dr. JW Snape, Cambridge Laboratory, Norwich. All the characteristics of all the six generations were compared to investigation the legitimacy of additive-dominance model by means of Chi-square (χ^2) test. Firstly simplest model using m parameter only of weighted least square analysis was carried out on generation means. Then further models md, mdh etc. were applied. Best preferred model in use was that one which has significant values for the all parameters along with non-significant Chi- square.

3. RESULTS AND DISCUSSION

Outcomes of the analysis of variance specified significant differences at the 0.01 % probability for relative water content, leaf temperature, chlorophyll content, net photosynthesis rate, cell membrane Thermostability and excised leaf water loss among genotypes (Table 2). The diversity of parents was confirmed by significance of the 'F' for all the traits under study. These findings also proved that this genetic variability might be transferred to the progeny, consequently confirmed the genetic analysis of the traits. The means values and standard errors of all six generations with the three crosses for four physiological parameters are illustrated in **(Table 2).**

4. RELATIVE WATER CONTENT (RWC)

For RWC [m-d] is fit best Table 3 under normal environmental condition in cross no 1. The additive effects [d] significantly implicated in the genetic inheritance of the trait and selection from early generation could be beneficial. In the non-appearance of epistasis, it is possible to enhance the RWC by fixing the additive genetic effect as reported by Farshadfar *et al.* (2001), Golparavar *et al.* (2006) and Kumar and Sharma (2007). Under stress condition the trait was controlled by [m-d-h-i-l]. In cross 2, the [m-d-h-j-l] and [m-j] possibly the best fit for RWC **(Table 3)** under normal condition and heat stress conditions respectively. In cross no 3 three parameter model [m-i-l] at normal and [m-h-i] under stress condition, control the trait expression. The presence of epistasis, additive × dominant (j) and additive x additive (i) showed complex type of gene action and results in delaying of selection. Negative sign in the epistatic components represent the nonappearance of fixable genetic effect in the early segregates. The intense crossing of desired segregants could be the efficient breeding techniques by keeping adequate size of the generation in order to develop lines with instigation of early reproductive phase. These

Table-2. Generation means for RWC, ELWL, NPR and RCI in three crosses of cotton under normal (N) and heat stress (H) conditions.

Traits	Stress Levels	Generation Means P_1	P_2	F_1	F_2	BC_1	BC_2	Pop Effect
RWC	**N1**	**56.0**	**47.0**	**52.1**	**51.0**	**52.6**	**49.4**	**
	H1	**53.8**	**40.3**	**47.2**	**45.0**	**51.4**	**47.2**	**
	N2	**51.8**	**41.6**	**51.0**	**45.0**	**43.5**	**45.9**	**
	H2	**43.0**	**40.0**	**40.9**	**40.7**	**44.6**	**38.0**	**
	N3	**55.5**	**53.3**	**56.2**	**46.6**	**47.6**	**50.6**	**
	H3	**49.9**	**46.8**	**49.3**	**44.5**	**47.6**	**46.6**	**
ELWL (%)	**N1**	**0.7**	**1.2**	**0.9**	**1.2**	**1.3**	**0.9**	**
	H1	**0.8**	**1.2**	**1.1**	**1.0**	**1.3**	**0.9**	**
	N2	**0.8**	**1.1**	**1.0**	**0.7**	**0.8**	**1.0**	**
	H2	**0.7**	**1.0**	**0.7**	**0.8**	**0.7**	**0.8**	**
	N3	**0.9**	**1.1**	**1.0**	**0.9**	**0.8**	**0.7**	**
	H3	**0.8**	**1.0**	**0.9**	**0.8**	**0.8**	**0.6**	**
NPR (m mol $CO^2m^{-2}S^{-1}$	**N1**	**23.6**	**22.1**	**23.3**	**19.6**	**22.5**	**23.2**	**
	H1	**23.3**	**19.0**	**22.7**	**20.0**	**22.1**	**22.2**	**
	N2	**25.4**	**22.9**	**26.0**	**21.5**	**23.4**	**23.4**	**
	H2	**23.7**	**18.9**	**22.8**	**23.2**	**21.6**	**21.3**	**
	N3	**22.8**	**18.8**	**23.8**	**20.3**	**20.8**	**20.4**	**
	H3	**22.4**	**17.9**	**17.4**	**17.2**	**19.2**	**18.2**	**
RCI (%)	**N1**	**39.6**	**62.8**	**38.1**	**52.4**	**58.5**	**52.0**	**
	H1	**41.4**	**52.8**	**33.8**	**54.1**	**56.0**	**50.8**	**
	N2	**40.2**	**59.2**	**42.4**	**62.3**	**57.2**	**46.0**	**
	H2	**41.9**	**52.4**	**48.1**	**42.0**	**44.8**	**50.4**	**
	N3	**48.2**	**59.6**	**49.4**	**56.0**	**58.9**	**56.9**	**
	H3	**46.1**	**55.2**	**46.8**	**48.1**	**54.1**	**50.2**	**

RWC: relative water content, ELWL: excised leaf water loss, NPR: net photosynthesis rate, RCI: relative cell injury

kinds of existence of non-additive control of trait were already described by Farshadfar *et al.* (2011).

Narrow sense heritability found to be higher than broad sense heritability under normal temperature condition in all crosses concluded that environmental component was not very important in heritability. Increase in heritability under heat stress in cross 2 and cross 3 revealed that environmental component as well as genotypic × environmental interaction is not much influence on heritability of RWC (Table 4). Farshadfar *et al.*, (2001) also explained high narrow-sense heritability estimates for RWC. Positive heterosis for RWC was observed in all crosses under normal and heat stress conditions except cross 3 under normal condition. Relative score of heterosis for RWC was lower in non-stress condition than in stress conditions. Heterobeltosis was observed only in cross 3 under heat stress condition with the value of 1.39 (Table 4). Ganapathy and Ganesh (2008) calculated 51.9 percent heterosis for the trait of RWC which was relatively greater than the figures of heterosis revealed in current study.

5. EXCISED LEAF WATER LOSS (ELWL)

The trait was controlled by [m-d-h-i-j] in cross 1and 2 at normal condition while at stress condition it was controlled by [m-d] and [m-h]. The cross 3 was controlled by [m-d-h-l] and [m-d-h] at normal and heat stress condition respectively. **(Table 3)** while under normal condition epistatic was observed in all crosses. Existence of allelic interaction, the additive genetic control predominantly involved in inheritance this trait was reported before by Farshadfar *et al.* (2001) and Kumar and Sharma (2007). Genetic control of thickness of cuticular and leaf surfaces waxiness are already been proved and it affects the transpiration rate (Haque *et al.*, 1992). Diverse types of behavior of crosses for genetic interactions of ELWL showed a ample genetic diversity for the genes. The existence of interactions in the inheritance revealed the complexity of the trait. Reduction in heritability was observed in cross 2 and cross 3 at stress condition that is one more evidence regarding effect of environmental component as well as genotypic × environmental interaction (Table 4). Farshadfar *et al.*, (2001) also showed high narrow-sense heritability estimates for ELWL. Cross 1 and cross 3 showed positive heterosis for ELWL under heat stress condition while cross 1 showed positive heterosis under normal condition. All the crosses showed negative hetero-beltosis under both conditions of temperature **(Table-4)**. Same kinds of results were obtained by Lugojan *et al.* (2011).

Table- 3. Estimates of the best fit model for generation means parameters (±, standard error for RWC, NPR, RCI and ELWL in three crosses of cotton.

Trait	Stress level	Genetic Effects						X^2(DF)
		m±S.E.	[d]±S.E.	[h]±S.E.	[i]±S.E.	[j]±S.E.	[l]±S.E.	
RWC	N1	51.28±0.37	4.06±0.63	-	-	-	-	2.70(4)
	H1	30.08±4.44	5.84±1.04	42.36±12.26	16.42±4.19	-	-25.28±8.13	1.35(1)
	N2	46.71±0.67	5.11±0.67	-11.51±2.74	-	-15.28±3.43	15.83±2.81	0.087(1)
	H2	40.93±0.34	-	-	-	13.46±2.43	-	3.87(4)
	N3	43.35±0.92	-	-	11.06±1.21	-	12.89±2.16	4.36(3)
	H3	39.9±2.13	-	9.58±2.97	8.83±2.31	-	-	3.91(3)
ELWL	N1	1.53±0.09	0.23±0.01	-0.67±0.12	-0.56±0.09	0.32±0.08	-	1.30(1)
	H1	1.00±0.02	0.20±0.04	-				4.53(4)
	N2	0.49±0.07	0.15±0.04	0.49±0.09	0.466±0.080	-0.62±0.17	-	0.16(1)
	H2	0.82±0.04	-	-0.13±0.83	-	-	-	7.63(4)
	N3	0.99±0.03	0.13±0.03	-0.73±0.14	-	-	0.72±0.12	3.19(2)
	H3	0.91±0.03	0.14±0.03	-0.71±0.15	-	-	-	4.23(2)
NPR	N1	9.99±2.10		25.27±5.94	12.89±2.07	-	12.01±4.07	4.32(2)
	H1	21.93±0.45	3.19±0.39	-8.10±1.87		-	8.86±1.89	1.44(2)
	N2	17.09±0.43	0.89±0.33	8.87±0.59	7.25±0.58	-	-	2.88(2)
	H2	22.85±0.32	2.14±0.46	-	-1.64±0.62	-	-	5.569(3)
	N3	19.44±0.31	-	-	-	-	4.14±0.84	5.53(4)
	H3	17.43±0.24	2.10±0.32	-	2.78	0.45	-	4.03(3)
RCI	N1	40.83±0.92	11.59±0.29	23.44±1.03	10.37±0.99	-10.17±1.37	-	0.22(1)
	H1	38.50±0.17	1.23±0.18	-1.09±0.33	-	-	-	5.715(3)
	N2	91.89±4.10	9.82±0.77	-83.47±11.27	-42.10±3.99	-	48.58±7.60	0.87(1)
	H2	35.78±2.30	5.24±0.63	12.209±3.34	11.38±2.40	-	-9.62±3.217	0.068(1)
	N3	54.36±0.83	4.91±0.82	4.80±1.74	-	-	-	5.58(3)
	H3	**42.68±2.57**	**4.23±0.99**	**12.52±3.61**	**8.44±2.88**	**-**	**-**	**2.53(2)**

6. NET PHOTOSYNTHESIS RATE (NPR)

Generation mean analysis showed that this trait was under the effect of polygene. The trait was controlled by [m-h-i-l], [m-d-h-i] and [m-l] in cross 1, cross 2 and cross 3 respectively under normal condition while at stress condition it was controlled [m-d-h-l], [m-d-i] and [m-d-i-j] in stress condition. In the presence of epistasis, the selection in early generations for the improvement of trait will be destructive **(Table 3)** Epistasis was observed in all crosses under both stress situations.

A negative additive × additive interaction in cross 2 under stress condition confirmed the lack of accessibility of fixable additive components. Negative value of [i] revealed the dispersal of negative alleles in the parents involved in the cross. Schonfeld *et al.* (1988) have already described the additive genetic interactions along with the additive and dominant effects. Diverse types of behavior of crosses for genetic interactions of NPR showed a ample genetic diversity for the genes. Narrow sense heritability was higher than the broad sense heritability in cross 1 and cross 3 at normal condition and at thermal stress conditions. It is higher in cross 1 while all other crosses showed lower narrow sense heritability. It reflected that environmental component was very important in heritability of net photosynthesis rate increase in heritability was observed in all three crosses at stress condition. that is one more evidence regarding effect of environmental as well as genotypic × environmental interaction **(Table 4).**

Table 4 Narrow sense heritability (h^2_{ns}), broad sense heritability (h^2_{bs}), heterosis (Ht) and better parent heterosis (Hbt) for RWC, NPR, RCI and ELWL in three crosses of cotton

Trait	Cross	Stress Level	h^2_{nb}	h^2_{bs}	Ht	Hbt
RWC	1	N	0.90	0.72	1.17	-6.96
		H	0.10	0.45	0.24	-12.27
	2	N	0.24	0.15	9.25	-1.52
		H	0.67	0.51	-1.46	-4.97
	3	N	0.15	0.11	3.39	1.39
		H	0.61	0.68	2.02	-1.12
ELWL	1	N	0.73	0.93	-12.37	-29.17
		H	0.25	0.18	10.55	-7.56
	2	N	0.27	0.46	3.12	-10.81
		H	0.71	0.65	-17.71	-28.71
	3	N	0.44	0.44	-1.00	-13.16
		H	0.73	0.48	0.54	-10.58
NPR	1	N	0.23	0.12	1.77	-1.44
		H	0.64	0.53	7.33	-2.41
	2	N	0.12	0.28	7.58	2.20
		H	0.20	0.44	6.83	-3.96
	3	N	0.38	0.22	14.12	4.16
		H	0.51	0.60	-13.71	-22.29
RCI	1	N	0.88	0.92	-25.56	-39.30
		H	0.24	0.18	-28.30	-36.00
	2	N	0.14	0.10	-14.74	-28.38
		H	0.76	0.74	1.98	-8.22
	3	N	0.54	0.53	-8.40	-17.13
		H	0.71	0.60	-7.69	-15.26

Schonfeld *et al.* (1988) also reported higher narrow sense heritability for relative water content. All three crosses showed positive heterosis for NPR under normal and heat stress condition positive heterosis under normal condition and the range for positive heterosis was 1.77 to 14.12 while heterobeltosis was observed in cross 2 and cross 3 under heat stress condition (Table 4). Same kinds of results were obtained by Lugojan *et al.* (2011).

7. RELATIVE CELL INJURY (RCI)

Generation mean demonstrated that RCI was under the influence of multiple genes. This trait was controlled by [m-d-h-i-j] ,[m-d-h-i-l] and [m-d-h] in cross-1, cross 2 and cross 3 respectively in normal conditions while under heat stress condition it was controlled by [mdh], [mdhil] and [m-d-h-i] for cross 1, cross 2 and cross 3 respectively. It is concluded that the character was under the effect of duplicate epistasis both temperature conditions in cross 2 because [h] and [l] component have opposite signs (Table 3). Effect of negative allel in the parent line produces the negative value of additive x assistive component in cross 2 at normal condition. Same kind of outcome were recommended by Yildirim *et al* .(2009); Ullah *et al.*(2010), Salman *et al.* (2016), and divergent results was presented by Rahman (2006) and Farooq *et al.* (2011). Higher narrow sense heritability as compared to broad sense heritability was observed in all crosses at both conditions except cross 1 at normal condition (table 04). Ibrahim and Quick (2001); Rahman (2006) also produced results of same kind while Ullah *et al* .(2014) presented contradictory findings. Negative heterosis was observed in all the crosses under both temperature conditions except cross 2 under stress condition. Maximum negative heterosis (-28.3) was observed in cross1 under stress condition. Maximum negative heterobeltosis was obtained in F_1 of cross-1 in normal condition showed that F_1 could reduce cell injury than better parent in normal condition for cross-1(Table 4). Similar kinds of results were presented by Farooq *et al.*(2013). Contrasting findings were presented by Lal *et al.*(2013).

8. CONCLUSION

Breeding program is very crucial to successfully develop the heat tolerant plant material. Different type of genetic control like additive and non-additive type of gene action including epistasis in some of the traits were revealed. Additive into additive [i], additive into dominance [j] and dominance into dominance [l], *i.e.* three types of epistasis were imperative in nearly all the traits studied during normal condition and thermal stress conditions. Heterosis and heterobeltosis studies showed that F_1 hybrids could be most important for produce vigor while reduction in cell injury was observed in F_1

hybrids. Under severe condition of heat stress, use of hybrids for general cultivation may possibly be a good alternate for the survival of cotton.

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