

## EVALUATION OF SEED PHYSICAL TRAITS IN RELATION TO HEAT TOLERANCE IN UPLAND COTTON

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### Abstract

Studies was carried out to study the effect of high temperature regimes on the phenotypic expression of physical seed traits in relation to heat tolerance in upland cotton and to determine their relationship with seed cotton yield in the presence and absence of heat stress. There was differential response of cultivars in the expression of these traits across temperature regimes which suggested that the expression of these traits in the presence of heat-stress could be useful in differentiating relatively heat tolerant and sensitive cultivars in upland cotton.

### Introduction

Cotton is primarily known and grown for its fiber, nevertheless, it's second product, seed, also enjoys significant economic value. Cottonseed, on an average, represents two-third of the total seed cotton production, and about 38-40 million tones of cottonseed is produced in the world every year, of which only 2.3% is used for planting.

Physical seed traits like seed weight, volume, and density are related to seed vigour (Heydecker, 1960, 1972; Tupper *et al.*, 1970), that in turn determines seedling vigour, crop stand, seedcotton yield, and quality in upland cotton (Wanjura *et al.*, 1969; Kreig & Carroll, 1978; Leffler & Williams, 1983). Seed size and density are therefore, important determinants of seed quality and vigour (Ferguson & Turner, 1971; Krieg & Barte, 1975; Minton & Supak, 1980; Leffler & Williams, 1983). Planting of high-density seed results in increased lint yield and vice versa (Minton & Supak, 1980; Hofmann *et al.*, 1988). Seed traits have been related to seed cotton yield (Rehman *et al.*, 1991), and therefore are important yield components in cotton (Coyle & Smith, 1997). Worley *et al.*, (1974) have reported number of seeds per boll as the largest contributor to lint yield after number of bolls per unit area. The desirability of higher number of seeds per boll is also important as ground for greater surface area for lint production with in the boll (Culp & Harrell, 1975; Harrell & Culp, 1976).

Seed development under diverse environments strongly affects both morphological, physiological (Daliens, 1982) and compositional seed traits (Thomas *et al.*, 2003). Seeds, for example, are smaller in cotton bolls emerging in the later season (Kohel & Cherry, 1983; Leffler, 1986). Variations in these traits, as a result of physical stresses like high temperature may, therefore, be helpful in determining the level of whole plant heat tolerance in crop plants, because evidence exists for availability of substantial genetic variability among cotton germplasm for physical seed traits (Rehman *et al.*, 1991; 1993a; Eissa *et al.*, 1983). In the present study, the objectives were to evaluate the effect of high temperature regimes on the phenotypic expression of physical seed traits in relation to heat tolerance in upland cotton and to determine their relationship with seed cotton yield in the presence and absence of heat stress.

## Materials and Methods

The experimental material in the present study comprised of 8 upland cotton (*Gossypium hirsutum* L.) cultivars having diverse leaf and plant morphology (Rehman, 2004) and their 15 F<sub>1</sub> hybrids obtained by crossing each of the 5 female parents (serial # 1-5 in Table 2) with 3 male parents (serial # 6-8 in Table 2). These 23 genotypes were then evaluated under optimum and heat stressed regimes in the controlled greenhouse and natural field conditions.

**Greenhouse experiments:** In greenhouse, two temperature regimes were maintained in two separate chambers, designated as optimum and supra-optimum regimes. Optimum (non-stressed) regime was maintained at (day/night) 35/21<sup>0</sup>C and supra-optimum (heat-stressed) regime at 46/30<sup>0</sup>C +/-2<sup>0</sup>C. Plants were allowed to go under optimum temperature regime in both chambers from sowing to 30 days after sowing (DAS). After 30 DAS, temperatures in the supra-optimum chamber were increased at an average rate of 2<sup>0</sup>C per day till desired level of supra-optimum regimes was reached. Layout in the greenhouse experiments was completely randomized design with three repeats, while cultivars and temperature regimes were treated as factors. Each entry was represented, per replication, by three pots each harbouring two plants. Pot size measured 30cm height, 35cm upper diameter and contained 9-kg of soil (mixture of silt and peat in 3:1 ratio). Soil analysis carried out before filling in the pots showed EC, 0.59 dSm<sup>-1</sup>; soil pH, 8.1; organic matter, 3.1%; saturation percentage 29; available phosphorous 30.1 ppm and potassium 130 ppm. Urea (46% nitrogen) was applied to the pots in solution form (10grams urea/litre of water) 30, 60, and 90 days after sowing as irrigation water. Ambient CO<sub>2</sub> concentration remained between 320 to 324 μmol mol<sup>-1</sup>, relative humidity from 60-65%, and PAR (photosynthetically active radiation) varied between 1300-1400 μmol m<sup>-2</sup>s<sup>-1</sup> in both the chambers of greenhouse. Pots were watered in the afternoon (400ml/pot) on the alternative days before and after maximum flowering and daily during maximum flowering period to eliminate confounding effects of drought on seed traits. Greenhouse experiment was terminated 120 days after sowing (DAS).

**Field experiments:** Field experiments were sown on 7<sup>th</sup> April and 29<sup>th</sup> May and 14<sup>th</sup> April and 4<sup>th</sup> June during 2000 and 2001 crop sessions, respectively. Early sowing helped to coincide maximum flowering period with the hottest days of the years (May & June) while maximum flowering in the late sown experiments appeared during optimum temperatures of August and September. Minimum and maximum temperatures in the April regime were significantly higher than those in June regime (Rehman, 2004; Rehman *et al.*, 2004). Early sowing (April regime) was, therefore, regarded as heat-stressed and later (June) sowing as non-stressed field regime. PAR (photosynthetically active radiation) at noon during maximum flowering period ranged between 1800-2000 μmol m<sup>-2</sup>s<sup>-1</sup> in the April regime and between 1600-2000 μmol m<sup>-2</sup>s<sup>-1</sup> in the June regime. Ambient CO<sub>2</sub> concentration remained between 340 and 351 μmol mol<sup>-1</sup> in the field during both the years. Experiments in the field were terminated 180 DAS in both regimes and years. Both experiments (regimes) in a year received identical agronomic treatments. The crops were fertilized at 150:50:00; N:P:K, per hectare. Potash (K) was not added as the pre-experiment soil test revealed 145 ppm potassium in the experimental field. Nitrogen was split in three equal doses and applied at sowing, first irrigation (33-35 DAS) and at maximum flowering. Experiments were sprayed for proper insect control when required. Adequate irrigation was applied by flooding when necessary to minimize the confounding effect of drought, especially during reproductive stage.

**Determination of seed traits:** At harvest, 50 bolls were randomly picked by hand from upper, middle and lower portions of the plants from each genotype per replication, weighed and ginned to separate seed from the seed cotton. Three subsamples each of one hundred seeds were drawn per replication, and weighed to obtain linked seed index. Linted seed weight was used in determining number of seeds per bolls (SN) as:

$$SN = (100 \times \text{seed weight per boll}) / \text{linted seed index}$$

where,

Seed weight/boll = weight of seed from 50 bolls/50, and linted seed index was the weight of 100 seeds prior to delinting with Sulphuric acid.

Hundred-seed samples were delinted with commercial Sulphuric acid (approximately 5ml of H<sub>2</sub>SO<sub>4</sub> for each gram of seed) and later washed thoroughly with tap water. After surface drying on filter paper, seed samples were placed in oven for 48 hours at 30°C in the oven and weighed to get seed weight (SW). Seed volume (SV) was determined from the displacement in the volume of 30ml ethanol by the same seed samples in the graduated jars. Seed density (SD) was calculated as the ratio of seed weight to seed volume. Seed surface area (SA) was determined in relation to seed volume from the table generated following Hodson (1920).

**Statistical procedures:** Data was analyzed in a factorial arrangement after running test of homogeneity of variances. Genotypes (parents and hybrids), temperature regimes and years were treated as factors, assumed to be having fixed effect. Variations among genotypes were partitioned into that due to parents, crosses and parent verses crosses. Variations due to genotype x years and genotype x temperature regimes interaction was also partitioned on the same pattern. Statistical significant was sought at 5 and 1% levels of probability. Genotypic and phenotypic correlation coefficients were determined following analysis of covariance (Kwon & Torrie, 1964). Since the magnitude of years interaction for the seed traits was either non-significant ( $p < 0.05$ ) or smaller than the main effects, data were pooled over years under each temperature regime for subsequent correlation analysis (Gomez & Gomez, 1984). Significance of phenotypic correlation was obtained from the statistical table, and that of genotypic correlation following Lothrop *et al.*, (1985).

## Results

**Phenotypic expression of seed traits under greenhouse regimes:** Significant effect of temperature regimes in the greenhouse ( $p < 0.01$ ) was evident in the phenotypic expression of all the seeds traits (Table 1). Presence of significant interaction of temperature regimes with the genotypes (parents and hybrids) masked phenotypic differences among cultivars and those among hybrids. In the greenhouse experiment, parents x temperature regimes interaction was significant for SV and SA ( $p < 0.01$ ) while hybrids x temperature regime interaction was significant ( $p < 0.01$ ) for SN, SV and SA. This indicated that temperature regimes in the greenhouse substantially modified the relative expression of SV and SA in the parental cultivars and that of SN, SV and SA in hybrids. Parents versus hybrid x temperature regime interaction was also significant for all the seed traits except SW, indicating that deviation of hybrids from their parental performance (heterotic expression) for seed traits was also subject to modification across temperature regimes. Mean phenotypic expression of seed traits under greenhouse regimes is presented in

Table 2. Substantial decrease in SN, SW, SD, and SA was observed under supra-optimum regime as compared to optimum regime in the greenhouse. Cultivars FH-900, MNH-552, NIAB-Karishma and CRIS-19 had relatively higher number of seeds per boll under supra-optimum temperature regimes (Table 2). Interestingly, cultivars FH-634, CIM-443 and HR109-RT showed lowest (1%) reduction in SN and MNH-52, FH-900 and Karishma, higher reduction (14, 13 and 11%, respectively) under supra-optimum temperature regime.

**Table 1. Analysis of variance among upland cotton genotypes for number of seeds per boll, seed weight (g), seed volume (cc), seed density (g/cc), and seed surface area (cm<sup>2</sup>) across temperature regimes in greenhouse.**

Source	df	Mean Squares				
		SN	SW	SV	SD	SA
Temp Regime (R) (G)	1	1659.56**	30.57**	27.12**	0.58**	0.54**
Genotypes (G)	22	10.15**	1.58**	3.15**	0.03**	0.06**
Parents (P)	7	3.29 <sup>ns</sup>	0.60 <sup>ns</sup>	2.60**	0.01 <sup>ns</sup>	0.05**
P versus H	1	9.25**	22.38**	6.10**	0.43**	0.12**
Hybrids (H)	14	13.65**	0.58 <sup>ns</sup>	3.21**	0.01 <sup>ns</sup>	0.06**
G x R	22	8.32**	1.10**	4.89**	0.02**	0.10**
P x R	7	3.36 <sup>ns</sup>	0.34 <sup>ns</sup>	4.51**	0.01 <sup>ns</sup>	0.09**
P vs. C x R	1	25.47**	16.46 <sup>ns</sup>	31.17**	0.31**	0.62**
C x R	14	9.57**	0.38 <sup>ns</sup>	3.19**	0.01 <sup>ns</sup>	0.06**
Residuals (G)	92	2.65	0.39	0.83	0.01	0.02
Residuals (P)	32	2.60	0.51	0.72	0.01	0.01
Residuals (C)	60	2.68	0.34	0.89	0.01	0.02
<b>Total</b>	<b>137</b>					

\*,\*\*= Significant at 5 & 1% levels of probability, respectively. ns = Non-significant (p>0.05)

**Table 2. Mean phenotypic expression of upland cotton cultivars of number of seeds per boll, seed weight (g), seed volume (cc), seed density (g/cc) and seed surface area (cm<sup>2</sup>) in parental cultivars in non-stressed (optimum) and heat-stressed (supra-optimum) regimes in greenhouse.**

Cultivar	SN		SW		SV		SD		SA	
	Opt	Supra	Opt	Supra	Opt	Supra	Opt	Supra	Opt	Supra
1. FH-634	18.45	18.24	7.40	5.05	7.28	7.32	1.02	0.70	1.03	1.03
2. FH-900	24.45	21.25	7.32	5.10	6.18	6.92	1.01	0.70	0.87	0.98
3. MNH-552	24.00	20.57	6.66	5.07	7.60	3.56	0.92	0.70	1.07	0.50
4. CIM-448	18.91	17.56	5.72	4.76	5.05	5.84	0.79	0.66	0.71	0.82
5. CIM-443	18.61	18.37	6.52	4.90	7.39	6.56	0.90	0.68	1.04	0.93
6. Karishma	22.68	20.25	6.84	4.65	6.26	7.65	0.94	0.64	0.88	1.08
7. CRIS-19	22.64	21.56	7.17	4.86	7.21	5.66	0.99	0.67	1.02	0.80
8. HR109RT	19.80	19.54	6.89	5.02	6.41	6.56	0.95	0.69	0.90	0.93
Mean	21.19	19.67	6.81	4.93	6.67	6.26	0.94	0.68	0.94	0.88
CD 5%	1.75	1.39	1.96	1.98	2.43	2.26	0.28	0.28	0.34	0.32

**Table 3. Analysis of variance among upland cotton genotypes for number of seed per boll, seed weight (g), seed volume (cc), seed density (g/cc) and seed surface area (cm<sup>2</sup>) across years and temperature regimes in the field.**

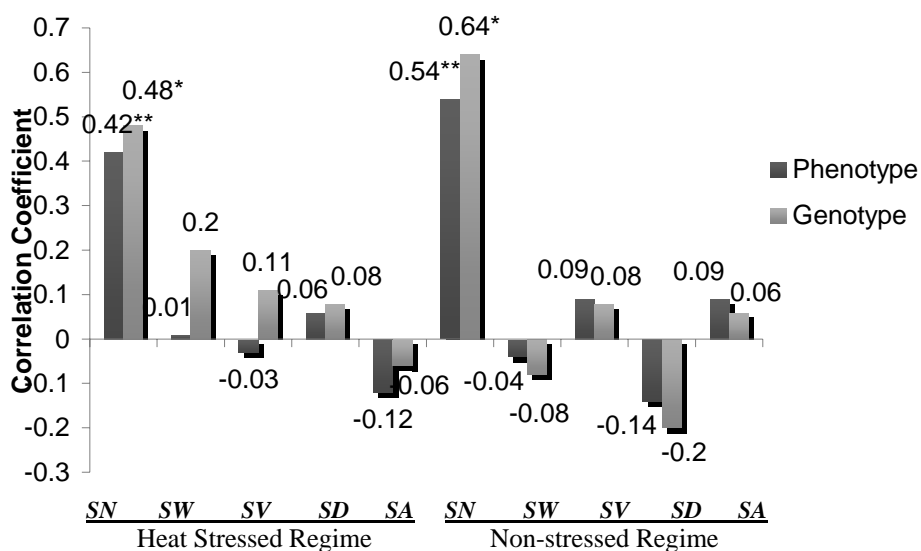
Source	df	Mean Square				
		SN	SW	SV	SD	SA
Replications for (G)	2	0.02 <sup>ns</sup>	0.57**	0.39 <sup>ns</sup>	0.33 <sup>ns</sup>	0.002 <sup>ns</sup>
Years (Y) for (G)	1	518.33**	147.83**	129.71**	0.31 <sup>ns</sup>	0.207**
Field Regimes (R) (G)	1	65.01**	0.89**	38.58**	0.01 <sup>ns</sup>	0.155**
Y x R	1	184.56**	1.03**	1.34 <sup>ns</sup>	0.28 <sup>ns</sup>	0.002 <sup>ns</sup>
Genotypes (G)	22	57.55**	2.55**	5.49**	0.33 <sup>ns</sup>	0.012**
Parents (P)	7	43.10**	3.01**	4.79**	0.05**	0.006**
P versus C	1	386.45**	9.83**	0.77 <sup>ns</sup>	0.01 <sup>ns</sup>	0.010**
Hybrids (C)	14	41.28**	1.80**	6.17**	0.49 <sup>ns</sup>	0.015**
G x Y	22	35.47**	1.03**	1.27 <sup>ns</sup>	0.29 <sup>ns</sup>	0.004 <sup>ns</sup>
P x Y	7	14.19**	0.28 <sup>ns</sup>	0.17 <sup>ns</sup>	0.01 <sup>ns</sup>	0.002 <sup>ns</sup>
P versus C x Y	1	301.58**	9.34**	6.84**	0.16 <sup>ns</sup>	0.013 <sup>ns</sup>
C x Y	14	27.11**	0.81**	1.42 <sup>ns</sup>	0.44 <sup>ns</sup>	0.005 <sup>ns</sup>
G x R	22	49.11**	1.03**	7.46**	0.47**	0.017**
P x R	7	41.52**	0.62**	9.35**	0.21**	0.026**
P versus C x R	1	297.96**	2.85**	11.27**	0.02 <sup>ns</sup>	0.009 <sup>ns</sup>
C x R	14	35.13**	1.09**	6.24**	0.63 <sup>ns</sup>	0.013**
G x Y x R	22	23.41**	1.62**	1.95**	0.29 <sup>ns</sup>	0.004 <sup>ns</sup>
P x Y x R	7	5.97 <sup>ns</sup>	0.46**	0.37 <sup>ns</sup>	0.01 <sup>ns</sup>	0.003 <sup>ns</sup>
P versus C x Y x R	1	147.13**	20.66**	20.62**	0.14 <sup>ns</sup>	0.021 <sup>ns</sup>
C x Y x R	14	23.29**	0.84**	1.41 <sup>ns</sup>	0.44 <sup>ns</sup>	0.003 <sup>ns</sup>
Residuals (G)	182	5.60	0.14	0.86	0.26	0.003
Residuals (P)	62	5.35	0.16	0.53	0.01	0.002
Residuals (C)	118	5.75	0.12	0.97	0.37	0.003
<b>Total</b>	<b>275</b>					

\*,\*\* = Significant at 5 & 1 % levels of probability, respectively. ns = Non-significant (p>0.05)

**Phenotypic expression of seed traits under field regimes:** Analysis of variance revealed significant (p<0.01) effect of years and field temperature regimes on all the seed traits except SD (Table 3). Genotypes x years interaction was significant for SN and SW (p<0.01). parents x years interaction was significant for SN only, and hybrids x years interaction for SN and SW. parents x temperature regimes and hybrids x temperature regimes interactions were significant for all the seed traits except hybrids x regimes interaction for SD, indicating that temperature regimes in the field substantially modified relative ranking among parents and among hybrids for most of the seed traits. The expressions of average heterosis for SN, SW, and SV were also substantially modified across years and temperature regimes. The magnitude of year interaction was smaller than of main effects and temperature regimes interactions. Mean performance of parental cultivars for various seed traits under April and June temperatures regimes (Table 4) indicated that on over all bases, heat-stressed field regime caused relatively lesser depression in seed traits than that observed in the heat-stressed regime of the greenhouse. It was obviously due to continuous and severer heat-stress in the greenhouse. Cultivars FH-900, MNH-552 and N-Karishma showed relatively higher depression in SN and FH-900, CIM-448 and N-Karishma in SV under heat-stressed regime in the field. On the contrary, MNH-552, CIM-443 and CRIS-19 showed higher mean SV and SA in the heat-stressed regime in the field. This could be due to the higher adaptability of these cultivars to heat stresses (April) regime showing their heat tolerance based on these traits.

**Table 4. Mean phenotypic expression of number of seeds per boll, seed weight (g), seed volume (cc), seed density (g/cc) and seed surface area (cm<sup>2</sup>) in parental cultivars under heat-stressed (April) and non-stressed (June) regime in the field.**

Cultivar	SN		SW		SV		SD		SA	
	April	June	April	June	April	June	April	June	April	June
1. FH-634	24.05	22.97	8.14	8.09	7.94	8.59	1.03	0.94	0.98	1.02
2. FH-900	22.85	28.13	8.06	7.55	6.82	8.26	1.18	0.93	0.91	1.06
3. MNH-552	19.87	25.94	7.19	6.93	8.04	5.15	0.91	1.41	1.00	0.90
4. CIM-448	24.43	25.41	6.42	6.97	5.66	7.29	1.14	0.96	0.90	0.95
5. CIM-443	25.41	20.46	7.47	7.69	8.29	7.53	0.91	1.02	1.00	0.95
6. Karishma	25.21	30.37	7.41	6.73	6.75	8.67	1.10	0.78	0.91	1.03
7. CRIS-19	20.69	23.08	8.37	7.65	8.33	6.90	1.01	1.11	1.00	0.93
8. HR109RT	22.58	23.73	7.45	7.58	6.89	8.03	1.08	0.96	0.92	1.01
Mean	23.14	25.01	7.56	7.40	7.34	7.55	1.04	1.01	0.95	0.98
CD 5%	2.99	4.19	1.22	0.92	1.87	2.26	0.20	0.36	0.09	0.11



Note: \*,\*\*, Significant at 5 and 1% levels of probability, respectively.

Fig. 1. Genotypic and phenotypic association of seed cotton yield with number of seeds per boll, seed weight (g), seed volume (cc), seed density (g/cc), and seed surface area (cm<sup>2</sup>) under heat stressed regime in the field.

**Relationship of seed traits with seed cotton yield:** Correlation analysis (Fig. 1) indicated that among physical seed traits, SN was significantly associated with seed cotton yield (SCY) at genotypic and phenotypic levels ( $p < 0.01$ ) under both heat-stressed and non-stressed field regimes. The strength of correlation was stronger under non-stressed regime as compared to the under heat-stressed regime. SW was significantly associated with SCY at genotypic level under heat-stressed and SD under non-stressed regime. The result of correlation analysis indicated that the simultaneous selection of SCY with SN, SW or SD could be effective.

The relationship between SCY and seed traits was further analyzed through regression analysis under the two temperature regimes and years separately. Regression analysis did not reveal significant and worth mentioning relationship, except that between SN and SCY. The relationship between the two traits was more linear and stronger under non-stressed regime, suggesting that SCY had relatively stronger dependency on SN in the absence of heat stress. It was also obvious that regression points were relatively more scattered under heat-stressed regimes than under non-stressed regimes in both the years, indicating that heat stressed regime caused more variation in the relationship between the two traits. This also implies that SN under heat-stress could be a dependable indicator of whole plant heat tolerance based on seed cotton yield under heat-stress.

### Discussion

The result of the present study revealed that high temperature both in field and greenhouse considerably modified the phenotypic expression of physical seed traits. Rehman, *et al.*, (1993b), have reported environmental effects on seed weight in upland cotton. Moreover, breeding and agronomic practices affecting both physical and chemical properties of cottonseed have also been reported (Cherry & Leffler, 1984). Interaction of temperature regimes modified the expression of seed traits in cultivars and hybrids more than did the years interaction, suggesting that repeating experiments over temperature regimes (sowing dates) provided more variable environment for seed traits than repeating experiments over years. The differential response of cultivars for depression in seed traits under heat-stress suggested that these traits could be useful in assessing heat tolerance in upland cotton and relatively tolerant or sensitive cultivars and hybrids could be differentiated based on higher number of seeds per boll and seed weight. Number of seeds per boll and seed weight appeared to be relatively more important seed traits *vis-à-vis* their relatively high sensitivity to heat-stress and significant and positive relationship with seed cotton yield under heat-stressed regimes. These traits, therefore, provided a link between seed vigour and higher seed cotton yield under heat-stressed regimes. Worley *et al.*, (1974, 1976) have reported number of seeds per boll as the largest contributor to lint yield after number of bolls per unit area. The desirability of higher number of seeds per boll has also been emphasized (Culp & Harrell, 1975) as a ground for greater surface area for lint production with in the boll (Harrell & Culp, 1976; Coyle & Smith, 1997). SN in the presence of heat-stressed conditions reflects the ability of a genotype to produce higher number of variable pollens, develop pollen tube, fertilize and mature ovules in the presence of heat-stress. Since number of seeds per boll depends upon the number of ovules fertilized by the variable pollen, it should be considered a good indication of the heat tolerance.

It is also suggested that seed traits in upland cotton are modified across temperature regimes in the greenhouse as well as in the fields and these traits, especially, number of seeds per boll and seed weight are important indicators of heat tolerance in upland cotton. The differential response of cultivars in the expression of these traits across temperature regimes suggested that the expression of these traits in the presence of heat-stress could be useful in differentiating relatively heat tolerant and sensitive cultivars in upland cotton.

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