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GENETICS OF PHYSIOLOGICAL AND AGRONOMIC TRAITS IN UPLAND COTTON UNDER DROUGHT STRESS

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Development of drought tolerant cultivars requires genetic information of physiological and agronomic traits of a crop under drought stress condition. A drought tolerant (B-557) and a drought susceptible (FH-1000) cultivar of upland cotton (*G. hirsutum* L.) were crossed to develop F_1, F_2 and backcross generations (BC_1& BC_2). All the generations along with parents were grown under drought stress in the field. Genetic analysis was conducted for relative water content, excised leaf water loss, cell membrane stability, plant height, number of monopodial branches, number of sympodial branches, number of bolls per plant, boll weight, ginning out-turn, fibre length, strength and fineness. The results revealed that all the traits were quantitatively inherited. Additive, dominance as well as genetic interactions were found in the inheritance of the traits. Medium to high narrow sense heritability was observed for all the traits. Correlation analysis showed that the genes involved in maintaining high relative water content and cell membrane stability had genetic linkage with those controlling bolls per plant, fibre length and fibre strength. The genetic analysis suggested that while breeding cotton for drought tolerance, selection of promising plants in advanced segregation populations would be appropriate when the genetic interactions are fixed and the selected plants reproduce for the desirable traits.
Keywords: Cell membrane stability, excised leaf water loss and relative water content.

INTRODUCTION

A plant is said to be under drought stress when soil water supply is not adequate to meet the transpirational demands (Krieg, 2000). Drought stress, among all types of abiotic stresses, is the most serious threat to the production of field crops (Sinclair, 1985; Almeselmani *et al.,* 2011). Cotton is relatively more sensitive against drought (Ray *et al.,* 1974). Drought stress affects cellular growth (Turner *et al*., 1986) hence retards leaf and stem elongation (Jordan, 1970), plant height (Ahmad *et al*., 2013; Yagmur *et al*., 2014) number of floral buds (Ahmad *et al*., 2013; Zare *et al*., 2014) boll development (Radin *et al*., 1992; Plaut *et al*., 1992) and eventually reduces yield of cotton (Yagmur *et al*., 2014; Zare *et al*., 2014). Boll development starts after pollination and is considered as the most sensitive stage to drought stress (Constable and Hearn, 1981). Cotton yield is directly affected by number of bolls per plant (Stockton *et al*., 1961; Grimes *et al.,* 1969; Gerik *et al*., 1996). Fibre quality is also affected by drought stress. The turgor pressure in the fibre cell is badly affected under drought stress conditions (Dhindsa *et al.,* 1975), which affects fibre quality traits (Yagmur *et al*., 2014).
Breeding cultivars for drought tolerance requires genetic knowledge of physiological and agronomic traits under drought stress condition. The traits like relative water content (Tahara *et al.,* 1990; Kumar and Singh, 1998), excised leaf water loss (Basal and Ünay, 2006) and cell membrane stability (Kakani *et al*., 2005; Azhar *et al*., 2009) are considered as key parameters in screening crops for drought tolerance. Generation means analysis is commonly used to study genetic basis of variation by analyzing segregating populations. The analysis detects additive, dominance as well as additive × additive, additive × dominance and dominance × dominance interactions. Correlation analysis using an F_2 population indicated linkage relationship of traits (Chen and Lubberstedt, 2010). The objective of present study was to generate genetic information about physiological and agronomic traits in upland cotton under drought stress.

MATERIALS AND METHODS

A highly drought tolerant B-557 (Iqbal *et al*., 2010) and susceptible FH-1000 (Ullah *et al*., 2008; Iqbal *et al*., 2010) cultivars were crossed in glasshouse to develop F_1 generation. During the normal cropping season, F_1 generation and parents were grown in the field for developing BC_1, BC_2 and F_2 generations. The parents, F_1, F_2, BC_1 and BC_2 populations were evaluated under field conditions using randomized complete block design with three replications. Each replication was comprised of two rows for each of the parent/ F1/ backcross and six rows for F_2 generation. Ten plants in each row of 3 m in length were planted with plant to plant distance of 30 cm and row to row distance of 75 cm. Single irrigation was applied after 40 days of planting. On rainfall, water was drained from experimental plot because of its higher elevation compared to other experimental area. Data on different physiological traits like relative water content, excised leaf water loss and cell membrane stability were recorded when plants showed signs of drought stress. Data were recorded from 30 plants of each parents/ F_1/, 100 plants of each BC_1& BC_2 and 150 plants of F_2 population.

***Relative water content (RWC)*:** Three fully developed leaf samples were taken from each plant around 7 a.m. Their fresh weight was recorded immediately after the excision. The samples were kept dipped in water over-night and turgid weight was measured. Afterwards the samples were kept under high temperature (70ºC) to record dry weight. The RWC of the leaf samples was calculated by using the following formula Clarke and Townley-Smith, 1986).

***Excised leaf water loss (ELWL)*:** Three fully developed leaf samples were taken from each plant. The samples were covered with polythene bags soon after excision and fresh weight was recorded using electronic balance. The leaf samples were left on laboratory bench at room temperature. After 24 hours the weight of the wilted leaf samples was recorded. After that the leaf samples were oven dried at 70°C for recording dry weight. Excised leaf water loss was calculated using the following formula as by Clarke and McCaig (1982).

***cell membrane Stability (CMS)*:** Three fully developed leaf samples were taken from each plant. The samples were rinsed with deionized water to remove surface contamination. Leaf discs of 1.0 cm^2 were sliced from samples and were submerged in 10 ml deionized water in 20 ml screw-cap vials which were kept at room temperature in dark for 24 hours. Conductance of the solution was measured with a conductivity meter (Jenway modal 4070). The vials were then autoclaved for 15 minutes and conductance of the sample solutions was measured again to estimate electrolyte concentration. All measurements were recorded at 25°C by keeping vials submerged in a water bath. The cell membrane stability of the leaf discs was calculated as reciprocal of relative cell injury (Blum and Ebercon, 1981) using the following formula:

Where, T1= Stress sample conductance before autoclaving.
T2= Stress sample conductance after autoclaving.
C1= Control sample conductance before autoclaving.
C2= Control sample conductance after autoclaving

***Agronomic traits*:** At crop maturity, data on different agronomic traits such as plant height, number of monopodial branches and number of sympodial branches. All the bolls which were picked during all pickings from each individual plant were recorded and summed up as bolls per plant. Average boll weight was calculated by dividing the total weight of seed cotton obtained from a plant by its total number of bolls.Samples of seed cotton obtained from individual plants were weighed and ginned separately with a single roller electrical gin in the laboratory. Lint was weighed and GOT was calculated as percentage of lint in seed cotton. For fibre analysis total seed cotton was collected from the selected plants. Ginning was done on individual plant basis using Single Roller Electrical Gin available in the department of Plant Breeding and Genetics. Before fibre testing, the ginned samples were re-conditioned by placing samples in blow room (65% humidity and 18-20°C temperature) using humidifier. High Volume Instrument (HVI-900-SA; Zellweger Ltd., Switzerland), available in the Department of Fibre Technology, was used to analyze fibre length, strength and fineness.

***Statistical analysis*:** Analysis of variance among the generations was conducted as in Steel *et al*. (1997). The traits showing significant differences were further subjected to generation means analysis following the method described by Mather and Jinks (1982). A weighted least squares analysis of variance based on the method as described in Mather and Jinks (1982) was performed on the data of the experiment containing six generations (Parents, F_1, F_2, BC_1 and BC_2). Heritability in narrow sense (h^2_{ns}) was calculated using the components of variance from the best fit model of weighted least squares analysis of generation variances (Mather and Jinks, 1982) by the formula.

h^2_{ns} = (When a simple DE model was adequate without a significant dominance component)

(When a DHE model had to be fitted)

Heritability in the F_∞ generation was also calculated by using the formula:

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***Correlations*:** The phenotypic and genotypic correlation coefficients between pairs of plant traits were calculated using the individual plants data of the F_2 population (Singh and Chaudhary, 1985)

RESULTS AND DISCUSSION

Significant differences were observed among the generations for all the traits (Table 1). Normal distribution of the traits in F_2 populations showed that the traits were quantitative in nature. The genetic segregation of traits in F_2 population showed continuous variation for relative water content, excised leaf water loss, cell membrane stability as well for yield/quality traits (Fig. 1 and 2. The small classes with smaller differences in histogram showed that the traits were multi-genic with equal additive effect (Falconer and Mackay, 1996), suggesting that the selection for the traits would be fruitful (Brown *et al*., 2014). Additive, dominance

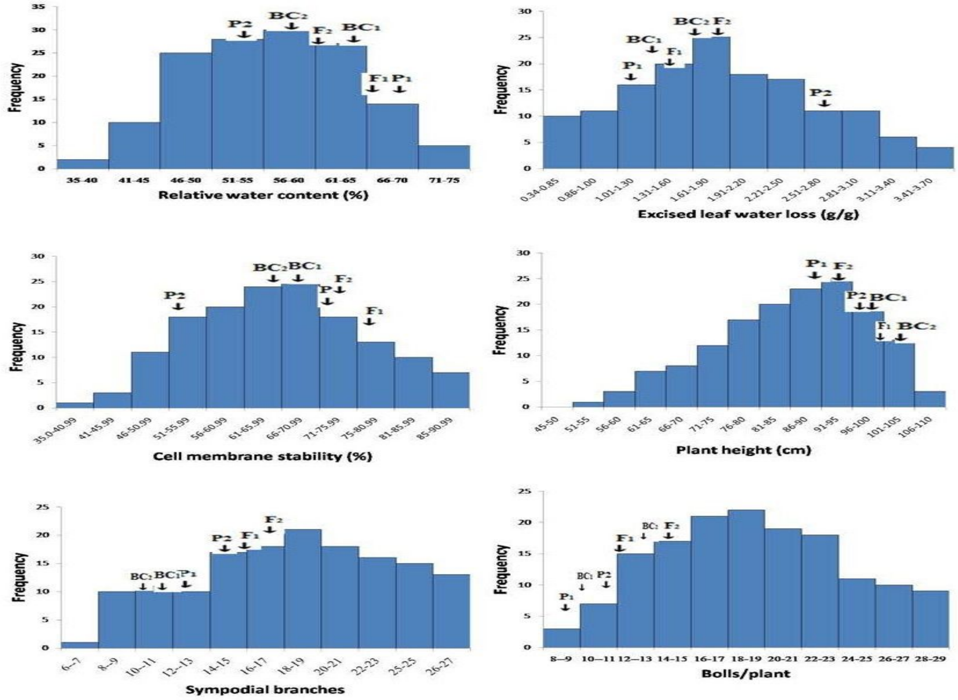

Figure 1. Frequency distribution for relative water content, excised leaf water content, cell membrane stability, plant height, sympodial branches and bolls per plant in F_2 population of the cross B-557 × FH-1000 of cotton under drought conditions.

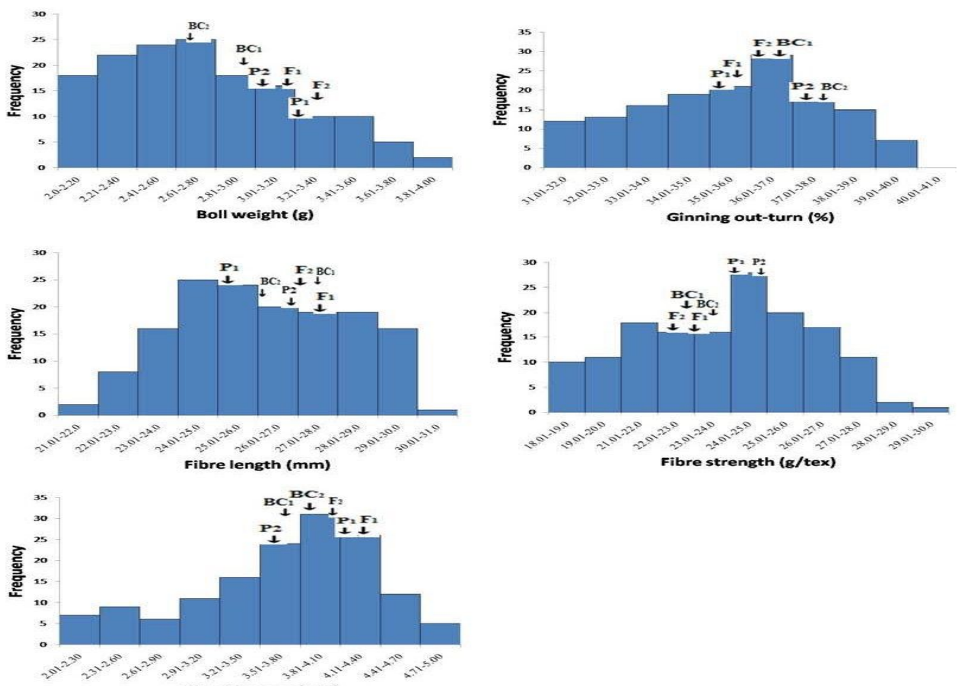

Table 1. Generation means for different morpho-physiological traits in the cross B-557×FH-1000 under drought conditions.

Traits	Generations						Pop. Effects
	P_1	P_2	F_1	F_2	BC_1	BC_2	
RWC	68.84	53.26	70.91	62.41	64.31	59.77	**
ELWL	1.35	2.90	1.54	1.84	1.34	1.90	**
CMS	72.42	53.93	78.31	74.43	68.83	69.45	**
PH	95.32	92.53	101.7	91.05	103.16	101.45	**
MB	3.10	3.40	3.10	3.17	3.10	2.90	**
SB	12.81	14.40	15.02	16.65	11.07	11.00	**
BP	7.75	11.70	11.93	14.70	10.56	12.40	*
BW	3.32	3.15	3.14	3.23	3.04	2.72	**
GOT	36.14	37.21	36.85	36.66	36.57	37.59	*
FL	25.81	27.07	27.51	27.47	27.40	26.11	**
FS	24.18	25.09	23.39	23.34	23.27	23.75	**
FF	4.27	3.72	4.19	4.09	3.88	3.99	*

Table 2. Estimates of the best fit model for generation means parameters (±, standard error) by weighted least squares analysis in the cross B-557 × FH-1000 under drought conditions.

Traits	Genetic Effects						χ^2(df)
	[m]	[d]	[h]	[i]	[j]	[l]	
RWC	52.04±1.03	12.20±1.03	16.79±4.22	-	21.58±3.83	-	1.41(2)
ELWL	1.32±0.03	0.57±0.04	0.51±0.08	-	2.25±0.29	-	0.48(2)
CMS	54.11±0.97	15.52±0.44	3.45±1.23	3.30±1.10	-	-	1.63(2)
PH	62.47±5.46	3.41±0.89	53.58±4.88	26.01±5.37	-	33.05±9.84	0.04(1)
MB	3.34±0.10	0.24±0.09	1.37±0.41	-	-	1.62±0.41	0.73(2)
SB	13.65±0.28	0.66±0.26	10.91±1.26	-	-	10.26±1.44	1.74(2)
B/P	11.65±0.15	1.93±0.25	-	7.72±1.38	-	-	0.69(3)
BW	3.18±0.02	0.07±0.06	-	0.40±0.25	-	-	5.91(3)
GOT	36.76±0.13	0.003±0.26	-	-	-	-	2.57(4)
FL	27.41±0.07	0.36±0.14	-	-	-	-	1.46(4)
FS	18.59±1.47	0.87±0.23	13.14±3.88	6.51±1.44	-	7.34±2.59	0.52(1)
FF	3.12±1.02	-	4.54±1.65	0.92±0.33	-	-	0.78(3)

***, P < (0.05); **, P < (0.01)**

Figure 2. Frequency distribution for boll weight, ginning out-turn, fibre length, fibre strength and fibre fineness in F_2 population of the cross B-557 × FH-1000 of cotton under drought conditions.

and epistatic type of gene action predominated in the inheritance of relative water content, excised leaf water loss and cell membrane stability (Table 2). In quantitative traits, gene action is determined as additive, dominance and epistatic effects (additive × additive, additive × dominance and dominance × dominance). Additive effect is normally the average effect of genes from both parents, dominance is the interaction of allelic genes and epistasis is the interaction of non-allelic genes affecting a particular trait. The inheritance of physiological traits related to drought tolerance was complex because of the involvement of additive and non-additive interactions. The epistatic effect in the gene action of the traits could be fixed in later generations (Singh and Narayanan, 2000). So to breed cotton genotypes which may maintain higher relative water content and cell membrane stability under drought, selection of the plants may be delayed or performed in later segregating generations.

Additive, dominance and epistatic type of gene action was observed for plant height, number of monopodial branches and number of sympodial branches (Table 2). These traits constitute the plant architecture and very important from yield point of view. The presence of additive and dominance along with epistasis type of gene action in the traits showed that the selection for these traits would be fruitful only in later segregation generations (Ahmad *et al*., 2009; Shakoor *et al.,* 2010). Additive along with additive × additive gene action was seen for number of bolls per plant and boll weight while for ginning outturn only additive type of gene action was observed. Boll development is the most sensitive phase of cotton plant life cycle to drought stress (Radin *et al.*, 1992; Plaut *et al.*, 1992). These traits are most important for breeding high yielding cotton genotypes. Plants which may bear higher number of bolls under drought conditions would help in increasing seed cotton yield. Similarly, higher boll weight positively affect yield of seed cotton (Mohan, 2011). Present study suggests that breeding for these traits would be fruitful. Additive and non-additive gene action has been reported in cotton under drought for number of bolls per plant (Abd-El-Haleem *et al*., 2010; Mahalingam *et al*., 2011) boll weight and ginning out-turn (Shakoor *et al.,* 2010; Sarwar *et al*., 2012). Additive type of gene action was observed for fibre length whereas, additive, dominance and epistatic type of gene action was observed for fibre strength and fineness. Higher narrow sense heritability for fibre length depicts that breeding for increased fibre length would be possible. Narrow sense heritability for the traits indicated that most of the traits were highly heritable (Table 3) and breeding could be fruitful for these traits.

In the present study simple model with D and E parameters explained the additive variance for the traits (Table 3). Similar findings were reported earlier by Mukhtar *et al.* (2000) and Bertini *et al.* (2001). In generation means analysis, genetic interactions were also observed for the traits however in the variance analysis the interactions were not revealed. The difference is mainly due to the techniques applied. The generation means analysis is comparatively more robust.

Correlation analysis indicates linkage among genes responsible to maintain leaf water content and cell membrane stability (Table 4), it may be due to pleiotropic effects (Chen and Lubberstedt, 2010). Correlation of relative water content with excised leaf water loss also indicates association of genes responsible for maintaining higher relative water content with the genes for low excised leaf

Table 3. Variance components D (additive), H (Dominance), F (Additive × Dominance) and E (environmental) following weighted analysis of components of variance, and heritability (ns, narrow sense and F∞ generation) in the cross B-557 × FH-1000 under drought conditions.

Traits	Variance Components				χ^2 (df)	Heritability
	D	H	F	E		ns
RWC	382.84± 68.88	-	-	64.98±9.68	0.66(4)	0.58
ELWL	3.16±0.54	-	-	0.17±0.02	0.30(4)	0.61
CMS	71.98±11.80	-	-	7.09±1.05	2.46(4)	0.76
PH	74.74±26.36	-	-	54.02±7.75	0.03(4)	0.74
MB	9.28±2.70	-	-	5.17±0.74	2.27(4)	0.59
SB	2.52±0.38	-	-	0.11±0.01	0.11(4)	0.57
B/P	1.20 ±0.64	-	-	1.46±0.20	0.72(4)	0.72
BW	6.24±2.32	-	-	4.83±0.69	3.87(4)	0.70
GOT	2.58±1.87	-	-	4.45±0.63	1.56(4)	0.77
FL	0.13±0.08	-	-	0.11±0.01	0.83(4)	0.71
FS	3.30±2.73	-	-	6.58±0.92	2.22(4)	0.55
FF	0.13±0.08	-	-	0.19±0.02	2.38(4)	0.61

Table 4. Phenotypic (Lower diagonal) and genetic correlation (Upper diagonal) matrix for traits under study in the cross B-557 × FH-1000 under drought conditions.

Trait	RWC	ELWL	CMS	PH	MB	SB	BP	BW	GOT	FL	FS	FF
RWC		-0.51	0.57	-0.13	0.28	0.23	0.23	0.20	0.21	0.36	0.26	-0.24
ELWL	-0.59**		-0.47	0.14	-0.27	0.31	0.16	-0.26	-0.10	-0.45	0.10	0.21
CMS	0.68**	-0.58**		-0.11	-0.28	0.21	0.45	0.33	-0.17	0.41	-0.19	-0.22
PH	-0.17	0.26	-0.16		0.22	0.11	-0.21	-0.23	0.26	-0.08	-0.23	0.29
MB	0.39**	-0.40	-0.29	0.27*		0.39	-0.17	-0.24	0.09	-0.18	0.16	-0.21
SB	0.38**	0.36	0.14	0.13	0.45**		0.32	0.22	0.51	0.27	0.17	-0.17
BP	0.29**	0.17	0.48*	-0.26	-0.24	0.39*		-0.47	0.15	0.18	-0.16	0.55
BW	0.23	-0.27	0.35*	-0.26	-0.39**	0.28	-0.57**		0.53	-0.14	-0.17	0.15
GOT	0.22	-0.19	-0.11	0.13	0.13	0.59*	0.22	0.59**		0.11	0.25	0.26
FL	0.42**	-0.47**	0.38*	-0.17	-0.11	0.17	0.23	-0.23	0.17		-0.20	0.19
FS	0.31	0.12	-0.12	0.27**	0.19	0.19	-0.19	-0.25	0.23	-0.23		-0.35
FF	-0.29**	0.27	-0.29*	-0.32**	-0.29	-0.24	0.57*	0.17	0.28	0.20	-0.41**	

*, P < (0.05); **, P < (0.01)

water loss in cotton. Association of relative water content with number of monopodial branches, sympodial branches, bolls/plant, fibre length and fibre fineness (Table 4) depicts the importance of maintaining higher water content for growth as well as for fibre quality. Excised leaf water loss had negative correlation with cell membrane stability. Cell membrane stability had positive correlation with number of bolls per plant, boll weight, fibre length and fineness which indicated that the genotypes with good cell membrane stability under drought stress could be better in performance under drought stress (Bibi *et al*., 2003; Rahman *et al*., 2004) Correlation analysis revealed that plant height had positive association with monopodial branches, fibre strength and fineness. Monopodial branches had negative correlation with boll weight and sympodial branches. The negative correlation of monopodial branches with boll weight indicated that plant with lower number of monopodial branches would be associated with heavier bolls. Positive association between number of sympodial branches with number of bolls and ginning outturn was observed in this study. Correlation analysis indicates that number of bolls per plant had strong negative correlation with boll weight and positive with micronaire value. This correlation depicted that the alleles for higher number of bolls per plant in cotton have linkage with smaller bolls with low quality fibre. So selection for higher number of bolls may result in smaller boll weight. Boll weight had strong positive correlation with ginning outturn. The results suggest that selection for increased boll weight would give rise to higher lint yield. Correlation analysis revealed that fibre strength had strong negative correlation with micronaire which indicates that fibre strength and fibre fineness could be improved simultaneously. Similar correlation between fibre strength and fibre fineness was observed by Desalegn *et al.* (2009).

Conclusion:Additive, dominance and epistatic type of gene action for the plant yield and drought tolerance traits revealed that the selection of plants may be carried out at later segregation population in cotton. Positive correlation of relative water content and cell membrane stability with plant yield and fibre quality related traits revealed that the traits may be used as screening criterion to develop drought tolerant cotton cultivar

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