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Inheritance Pattern of Physio-Morphological Traits of Cotton under Drought Stress

Muhammad Waqas Amjid[1*], Tanwir Ahmad Malik[2], Muhammad Kausar Nawaz Shah[1], Muhammad Asif Saleem[3], Yasar Sajjad[4], Rashid Mehmood[1]

[1]Department of Plant Breeding and Genetics, PMAS Arid Agriculture University, Rawalpindi, Pakistan
[2]Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan
[3]Department of Plant Breeding and Genetics, Bahauddin Zakariya University, Multan, Pakistan
[4]Department of Environmental Sciences, COMSATS Institute of Information Technology, Abbottabad, Pakistan

Abstract
Physiological traits play important role in breeding against drought stress. Intraspecific variability exists for drought tolerance in crop plants. Thirty seven upland cotton genotypes were screened in hydroponics on the basis of relative leaf water contents, excised leaf water loss and cell membrane stability. Drought stress was imposed by using 15% polyethylene-8000. On the basis of screening results, drought tolerant (FH-207) and drought susceptible (FH-901) genotypes were selected to develop F_1, F_2, BC_1 and BC_2 populations. The parents and all the populations were evaluated in the field under normal and drought stress conditions. The results of the genetic study revealed that all the traits were controlled by additive, dominance and epistatic type of gene actions. Correlation analysis revealed that relative leaf water content had a negative association with excised leaf water loss and positive association with cell membrane stability under drought stress conditions. Number of bolls/plant had positive correlation with relative leaf water contents and excised leaf water loss. These findings suggest that to develop a drought tolerant cultivar, selection of suitable plants should be delayed to the lateral generations so that genetic interactions could have been fixed. The positive association of number of bolls/plant with relative leaf water contents and cell membrane stability suggests that breeding for drought tolerance would improve yield in cotton under drought stress.
Keywords Upland cotton, hydroponic culture, relative leaf water contents, excised leaf water loss, cell membrane stability, drought.

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***Corresponding author** Muhammad Waqas Amjid **Email** waqasamjid@hotmail.com **Tel** +92-300-0300021

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Introduction

Cotton is the leading fiber crop grown in arid and semiarid regions of the world which often experience scarcity of water. Water deficit and global climate change are making conditions more adverse in the most agricultural domains of the world [1, 2]. The immediate response against drought stress is the reduction in leaf area to reduce transpiration, hence maintain the water status of plant and to protect membrane integrity. Leaf morphological traits such as stomatal size and density play a key role in plant response to drought stress. Drought stress leads to an increase in stomatal density [3] and a decrease in stomatal size, indicating this may enhance the adaptation of plant to drought. Stomatal size and frequency may affect the photosynthetic activity of leaves as plant exchanges CO_2 and water through stomata. So, the balance between CO_2 intake and water loss may affect water use efficiency.

Drought stress has adverse effects on yield [4]. Cotton has the genetic potential to cope with drought stress due to its semi-arid and sub-tropical origin which experience periodic drought stress [5], so there is a need to breed crop plants for drought stress.

Plants tolerate drought stress through osmoregulation by maintaining higher cell membrane stability and relative leaf water contents. There is substantial variation in the stomatal responses to environmental factors. Identification of genetic variability for stomatal properties would provide a new tool for plant breeders to improve crop adaptation to stressful environments. Plants can maintain optimum relative leaf water contents by developing lower stomatal size and frequency without decreasing net photosynthesis for producing good yield under drought stress. So lower stomatal size, stomatal frequency and excised leaf water loss while higher relative leaf water contents are important traits to breed plants against drought stress [6-9]. Screening of plants for drought tolerance could be more useful under controlled conditions. Polyethylene glycol (PEG) is used as an osmotic substrate to develop uniform and stable water stress in hydroponic culture [10, 11]. Plants tolerate water deficits stress through osmotic adjustment [12] by accumulation of organic

acids, sugars and ions in the cytosol to maintain leaf water potential near optimal levels [13, 14].

The linkage relationship of the traits related to yield and quality as well as those related to drought stress is very important. The breeder has to develop cultivars with the combination of yield related traits as well as physiological traits related to drought stress. The objective of the study was to assess genotypic variation for drought tolerance in cotton varieties under hydroponic conditions using physiological attributes as selection criteria, and to study the inheritance of physio-morphological attributes and their inter-relationship.

Materials and methods

Collection of cotton genotypes

The seed of thirty seven characterized cotton genotypes with known drought tolerance and susceptibility were collected from the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Ayub Agricultural Research Institute, Faisalabad and Central Cotton Research Institute, Multan (Table 1).

Screening of characterizing germplasm in hydroponics

The thirty seven genotypes were screened in hydroponics to select two contrasting genotypes for drought tolerance. The genotypes were sown in the polythene bags of 30×15 cm filled with sand as a medium. A plastic tank of 3×3×0.3 m^3 volume was filled with Hoagland solution to suspend seedlings in it. The seedlings were suspended in Hoagland solution by transferring on the Styrofoam sheet at the first true leaf stage. There were six seedlings for each genotype. Constant aeration was maintained in the root zone by installing a network of air-pipes connected to a motor. The Hoagland solution in the plastic tank was replaced with the freshly prepared Hoagland solution weekly. After two weeks when the seedling proved to be adapted in Hoagland solution, drought stress was imposed by dissolving 15% PEG-8000 in the Hoagland solution. The experiment was continued for 45 days from the date of emergence.

Relative leaf water content (RLWC)

A leaf sample was taken from each plant during early morning. Fresh weight of the leaf was recorded immediately after the excision. The samples were kept dipped in water over-night and turgid weight was measured. Then the samples were dried at high temperature (70^{o}C) and dry weight was recorded. The relative leaf water content of the leaf sample was calculated by using the following formula [15].

RLWC (%) = [(Fresh weight-dry weight) / (turgid weight-dry weight)] × 100

Excised leaf water loss (ELWL)

A leaf sample was 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 twenty four hours the weight of the wilted leaf samples was recorded. Then the leaf samples were oven dried at 70°C for recording dry weight. Excised leaf water loss was calculated using the following formula [16].

ELWL (g) = [(Fresh weight-wilted weight)/dry weight]

Cell membrane stability (CMS)

A leaf sample was 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 the dark for 24 hours. The conductance of the solution was measured with a conductivity meter (Jenway modal 4070). The vials were then autoclaved for 15 minutes at 121°C and conductance of the sample solution was measured again to estimate the 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 the reciprocal of relative cell injury using the following formula [17].

CMS (%) = [{1-(T1/T2)]/ [1-(C1/C2)}] ×100

Where, T1 is stress sample conductance before autoclaving, T2 is stress sample conductance after autoclaving, C1is control sample conductance before autoclaving and C2 is control sample conductance after autoclaving

Crossing work and field trial

FH-207 (tolerant) and FH-901 (susceptible) genotypes were grown in pots filled with loamy soil in glasshouse during winter season to produce F_1 hybrids. The selfed seeds of parents were produced by covering floral buds with butter paper bags. The seed of parents and half of the seed of F_1 was sown in the field in the normal growing season to raise plants for making backcrosses (BC_1 and BC_2). Some of the F_1 hybrids were selfed to produce F_2 seed. The parents,

F_1, F_2 and backcross populations were grown under normal and drought stress conditions in the field as separate trial using a randomized complete block design (RCBD) with three replications in normal cotton season. Seeds were sown keeping 75 cm row to row and 30 cm plant to plant distance. The trial under normal conditions received six supplemental irrigations at flowering, first boll opening and maturity stage while trial under drought conditions received two irrigations only at flowering and first boll opening stage. When symptoms of drought appeared on plants, 50 guarded plants per replication for each of the parents, F_1, BC_1 and BC_2, 500 plants per replication for the F_2 generation were selected to record the data on an individual plant basis for plant height, number of bolls per plant, boll weight, seed index, lint percentage, fiber length, fiber strength and fiber fineness, relative leaf water contents, excised leaf water loss, cell membrane stability, stomatal size and stomatal frequency. Strips of fully mature leaves were taken from each of the selected plants and were kept in Carnoy's solution for overnight to fix the material and the removal of chlorophyll from the leaf tissues. After 24 hours, the solution was replaced by 70% ethanol for preservation and further examination of strips. The stomatal size was measured under 40× objectives and stomatal frequency per microscopic field was counted under 10× objectives from upper (adaxial) surface of the leaf strips of each selected plant. The total cotton seed was collected from the selected plants for fiber analysis. Ginning was done on an individual plant basis by using Single Roller Electrical Gin available in the Department of Plant Breeding and Genetics. Before fiber testing, the ginned samples were re-conditioned by placing samples in blow room (65% humidity and 18-20°C temperature) using a humidifier. High Volume Instrument (HVI-900-SA; Zellweger Ltd., Switzerland) was used to analyze fiber length, fiber strength and fiber fineness. The data for yield traits was recorded for plant height, number of bolls per plant, boll weight, seed index and ginning-out-turn at maturity.

Statistical analysis

The data were subjected to analysis of variance following the method as outlined by Shakoor et al. [18] using MSTAT-C software package at 5% and 1% probability level. Generation means analysis and generation variance analysis was performed following the method described by Mather and Jinks [19]. Phenotypic correlation coefficients were calculated by the formula as outlined by Clarke and McCaig [20] using the Minitab programme of computer. Heritability in the narrow sense (h^2_{ns}) was calculated using the components of variance from the best fit model of weighted least squares analysis by the formula:

h^2_{ns}

(1) = 0.5D/(0.5D + E) (when a simple DE model was adequate without a significant dominance component)

(2) = 0.5D/(0.5D + 0.25H + E) (when a DHE model had to be fitted)

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

$h^2_\infty = D / (D + E)$

Results

Screening trial

The cotton genotypes differed significantly for relative leaf water content, excised leaf water loss and cell membrane stability. The means of all these traits are given in the Table 1. The genotype N-313/12 had the lowest mean value (0.97) for excised leaf water loss, while the genotype LRA-5166 had the highest (3.60) mean value for the excised leaf water loss. The maximum mean value of relative leaf water content was observed in genotype FH-207 (71.12) and the minimum in the genotype S-12 (54.22). For cell membrane stability, the genotype FH-207 (74.78) had the maximum mean value while the genotype CIM-496 (60.52) showed the minimum mean value. The genotype FH-207 was selected as most drought tolerant with high relative leaf water contents (71.12), low excised leaf water loss (1.07) and high cell membrane stability (74.78). The genotype FH-901was identified as most drought susceptible with low relative leaf water contents (55.21), high excised leaf water loss (1.88) and low cell membrane stability (60.69). Relative leaf water content, excised leaf water loss and cell membrane stability are directly related with the moisture percentage in the leaves. Tolerant genotype selected in this study had higher value for cell membrane stability. Relative leaf water contents always decreases under drought stress [20] but drought tolerant genotypes maintains higher relative leaf water contents compared to drought susceptible genotypes under drought stress [21]. The genotype selected as drought tolerant in this study maintained higher relative leaf water content under drought stress while selected as susceptible had the lowest value. The parameters, cell membrane stability, relative leaf water content and excised leaf water loss are directly related to drought tolerance and have been used to select drought tolerant genotypes [12].

One most drought tolerant genotype (FH-207) and one most drought susceptible genotype (FH-901) was selected for the field evaluation.

Table 1 The relative leaf water content (RLWC), cell membrane stability (CMS) and excised leaf water loss (ELWL) of 37 cotton genotypes at seedling stage in hydroponics experiment.

Sr. No.	Genotypes	ELWL (g)	RLWC (%)	CMS (%)
1	FH-207	1.07	71.12	74.78
2	FH-930	1.46	68.36	70.13
3	FH-634	2.82	56.13	61.63
4	B-557	1.26	62.51	66.11
5	MNH-552	1.68	66.04	70.06
6	BH-118	1.08	66.10	73.03
7	NIAB-111	2.05	64.18	72.05
8	N-313/12	0.97	61.14	70.14
9	RH-510	1.89	62.07	71.10
10	CP-15/2	1.80	54.88	70.36
11	MNH-554	1.56	57.11	65.96
12	CIM-1100	3.28	69.67	65.52
13	N-Karishma	2.83	56.77	61.48
14	CIM-496	1.88	60.40	60.52
15	BH-160	1.69	54.74	65.87
16	CIM-707	1.88	56.18	63.78
17	FH-1200	2.85	55.81	71.72
18	VH-144	2.82	55.77	70.39
19	MNH-642	2.51	66.77	69.18
20	BH-124	2.72	69.14	63.83
21	FH-901 (S)	1.88	55.21	60.69
22	MNH-147	2.96	59.07	71.64
23	N-801/2	3.21	58.14	66.21
24	Acala-1517-C	3.11	67.22	75.81
25	4-F	2.43	56.74	70.77
26	CedixS-362-T-362	2.74	55.14	70.18
27	H-493-3	1.94	55.51	60.65
28	MNH-129	2.04	54.48	57.77
29	S-12	3.13	54.22	75.71
30	VH-142	1.48	60.52	72.18
31	CIM-446	2.71	67.29	53.88
32	CIM-240	2.67	70.48	60.59
33	FH-1000	1.55	61.96	60.85
34	LRA-5166	3.60	70.92	66.96
35	NF-801-2	2.66	60.59	75.88
36	CIM-70	2.00	62.34	65.85
37	MNH-93	3.09	70.76	65.32
Genotypes/cultivars effects		**	**	**

* = P < 0.05; ** = P < 0.01

Field trial

The coefficients of means are given in Table 2 which were used to calculate genetic effects and coefficients of variance are given in Table 3 which were used to calculate variance components. The coefficients are given as reference for statistical analysis used to calculate the components of genetic means and variance. The analysis of variance revealed significant differences for all the traits under drought stress conditions. Under well-watered conditions, all the traits differed significantly except relative leaf water content, cell membrane stability, plant height, ginning-out-turn and fiber length (Table 4).

Generation means analysis

Genetic effects for all the traits are given in Table 5 while narrow sense heritability estimates are given in Table 6. For relative leaf water content, five parameters genetic model [m, d, i, j, l] under drought stress conditions was proved satisfactory to data. The relative leaf water content had high narrow sense heritability. In excised leaf water loss, model with four parameters [m, d, j, l] was proved fit to data under well-watered and drought stress conditions. Narrow sense heritability estimates were from lower to higher for excised leaf water loss. Model with five parameters [m, d, h, i, j] under drought stress conditions was proved fit to data for cell membrane stability with higher narrow sense heritability. For stomatal size, the model with five parameters [m, d, h, i, j] under well-watered conditions and five parameter model [m, d, h, i, l] under drought stress conditions was proved fit to data with higher narrow sense heritability estimates. For stomatal frequency, four parameters model [m, d, h, j] was found fit to data under well-watered conditions while five parameter model [m, d, h, i, j] was proved satisfactory to data under drought stress conditions. Narrow sense heritability estimates of stomatal frequency were higher in well-watered and drought stress conditions. For plant height, four parameters model [m, h, i, l] under drought stress conditions was proved fit to data. Narrow sense heritability estimates were higher for plant height. For number of bolls per plant, four parameter model [m, d, h, l] was fit to data under well-watered and drought stress conditions and heritability estimates (narrow sense) were medium under well-watered and drought stress conditions. For boll weight, five parameter model [m, d, h, j, l] under well-watered conditions and four parameter model [m, d, j, l] under drought stress conditions proved satisfactory to data and narrow sense heritability estimates were higher under well-watered and drought stress conditions. For seed index, five parameter model [m, d, h, i, l] was proved fit to data under both conditions with medium narrow sense heritability estimates. For ginning-out-turn, four parameter model [m, d, j, l] was found fit to data under drought stress conditions. Narrow sense heritability estimates for ginning-out-turn were lower to medium. For fiber length, three parameter model [m, h, j] under drought stress conditions was proved satisfactory to data. Fiber length exhibited lower to higher in narrow sense heritability estimates. The genetics of fiber strength showed four parameter model [m, h, i, j] fitting under well-watered and drought stress conditions and narrow sense

heritability estimates were medium to high. For fiber fineness, a model with two parameters [m, j] under well-watered conditions and three parameters model [m, d, h] under drought stress conditions was satisfactory to data. Heritability estimates (narrow sense) were medium for fiber fineness.

Table 2 Coefficients of the mean (m), additive [d], dominance [h], additive × additive [i], additive × dominance [j] and dominance × dominance [l] parameters for the weighted least squares analysis of generation means [19].

Generations	Components of genetic effects					
	m	[d]	[h]	[i]	[j]	[l]
P_1	1	1.0	0.0	1.00	0.00	0.00
P_2	1	-1.0	0.0	1.00	0.00	0.00
F_1	1	0.0	1.0	0.00	0.00	1.00
F_2	1	0.0	0.5	0.00	0.00	0.25
BC_1	1	0.5	0.5	0.25	0.25	0.25
BC_2	1	-0.5	0.5	0.25	-0.25	0.25

Table 3 Coefficients of generation variances analysis [19].

Generations	Components of variation			
	D	H	F	E
P_1	0.00	0.00	0.00	1
P_2	0.00	0.00	0.00	1
F_1	0.00	0.00	0.00	1
F_2	0.50	0.25	0.00	1
BC_1	0.25	0.25	-0.5	1
BC_2	0.25	0.25	0.50	1

Generation variance analysis

Existence of variation is due to genetic and environmental effects. Generation variance analysis revealed that additive, dominance and environmental components of variance were generally found suitable to explain the variation under well-watered and drought stress conditions except stomatal size and stomatal frequency under drought stress (Table 6). The variance model uses the difference of variance in the parental and segregating populations, whereas the generation means model uses the data of the parents and the segregating populations. Generation variances analysis calculates only cumulative interactions while generation mean analysis calculates all the components of interaction so generation means analysis is more robust than generation variance analysis. Infinity generation heritability was consistently higher than the narrow sense heritability of all the traits given in Table 6.

Phenotypic and genotypic correlations

Phenotypic (Lower diagonal) and genetic correlation (Upper diagonal) matrix are given in Table 7. Relative leaf water contents showed positive correlation with cell membrane stability while correlated negatively with excised leaf water loss under drought stress conditions. Cell membrane stability showed negative correlation with excised leaf water loss under drought stress condition. Stomatal size showed negative correlation with stomatal frequency under well-watered and drought stress conditions. Stomatal frequency correlated positively with cell membrane stability. Plant height had positive correlation with the number of bolls per plant under well-watered as well as drought stress. Number of bolls per plant showed positive correlation with boll weight, seed index and relative leaf water content under well-watered and drought stress conditions. Number of bolls per plant correlated positively with cell membrane stability under drought stress conditions. Boll weight revealed a positive correlation with seed index under well-watered and drought stress conditions. Fiber length correlated positively with fiber strength and relative leaf water contents while correlated negatively with fiber fineness, excised leaf water loss and stomatal frequency under drought stress conditions. Fiber strength showed positive correlation with cell membrane stability and stomata size while correlated negatively with stomatal frequency. Fiber fineness correlated negatively with fiber length under drought stress conditions.

Discussion

Generation means analysis and generation variance analysis showed that the inheritance of physiological and morphological traits were complex. Generation means analysis revealed the existence of interaction for the traits; however, in variance analysis differences occurred due to the difference of technique used to identify interactions [22]. Similar results were reported in cotton by other researchers [18, 23, 24]. There was a general difference in gene action of relative leaf water contents, excised leaf water loss, cell membrane stability, stomatal size, stomatal frequency, plant height, number of bolls per plant, boll weight, seed index, ginning-out-turn, fiber length, fiber strength and fiber fineness under well-watered and drought conditions. A combination of physiological and morphological traits related to drought tolerance may enhance the efficient use of moisture to produce better yield.

The positive correlation of relative leaf water contents with cell membrane stability showed that the genes which maintain higher relative leaf water content may also contribute towards cell membrane stability. Cell membrane stability has been considered as a reliable parameter for screening against drought tolerance [17, 25]. Relative cell injury has been used to screen *Gossypium hirsutum* [26]. Moisture contents are required for the integrity

Table 4 Generation means for a cross FH-207 × FH-901 under normal [N] and drought (D) conditions in the field.

Traits	Field conditions	Generations						Pop. Effects
		P_1	P_2	F_1	F_2	BC_1	BC_2	
RLWC	N	78.78	80.17	81.14	78.08	79.42	80.58	N.S
	D	74.36	62.98	70.69	65.29	60.81	62.88	**
ELWL	N	1.36	2.40	1.32	1.88	1.82	1.95	**
	D	1.11	1.98	1.16	1.56	1.26	1.42	**
CMS	N	79.85	76.21	79.11	71.84	68.56	69.03	N.S
	D	75.92	62.32	70.79	67.11	64.00	61.92	**
SS	N	292.15	358.97	365.42	335.40	313.14	362.08	**
	D	240.09	296.82	303.81	247.26	273.62	301.02	**
SF	N	134.87	91.23	90.47	106.83	104.60	93.33	**
	D	169.37	117.83	114.83	155.15	137.73	110.40	**
PH	N	107.37	106.57	110.97	99.01	105.30	101.90	N.S
	D	104.83	95.69	108.20	94.55	101.70	95.50	**
NB	N	15.17	10.87	13.10	9.54	11.77	11.37	*
	D	10.93	8.63	11.93	7.97	9.87	8.97	**
BW	N	3.80	2.84	3.82	3.27	3.15	2.87	*
	D	3.39	3.10	3.48	3.26	3.28	3.11	**
SI	N	7.32	7.09	7.86	7.20	7.60	7.44	*
	D	7.07	6.81	7.79	7.14	7.53	7.31	**
GOT	N	39.16	39.24	39.66	37.96	38.24	38.00	N.S
	D	39.04	36.82	39.46	38.49	37.98	37.36	**
FL	N	26.99	27.54	28.03	26.91	27.00	27.21	N.S
	D	25.08	24.75	24.72	23.59	24.04	23.75	**
FS	N	24.91	24.69	24.81	23.20	24.30	25.11	*
	D	24.59	24.10	23.59	22.90	23.47	23.08	**
FF	N	4.29	4.20	4.29	4.48	4.60	4.12	*

* = significant (p<0.05), ** = highly significant (p<0.01); RWLC = relative leaf water contents; ELWL = excised leaf water loss; CMS = cell membrane stability; SS = stomatal size; SF = stomatal frequency, PH = Plant height; NB = number of bolls/plant; BW = boll weight; SI = Seed index; GOT = ginning out turn; FL = fiber length; FS = fiber strength; FF = fiber fineness.

Table 5 Estimates of the best fit model for generation means parameters (±standard error) by weighted least squares analysis in a cross FH-207 × FH-901 under normal (N) and drought (D) conditions in the field.

Traits		Genetic Effects						X^2(df)
		[m]	[d]	[h]	[i]	[j]	[l]	
RLWC	D	56.78±0.93	5.69±0.21	-	11.89±0.97	16.28±2.49	16.91±0.96	0.07 (1)
ELWL	N	1.93±0.05	0.52±0.07	-	-	0.84±0.32	0.55±0.14	2.58 (2)
	D	1.54±0.04	0.44±0.05	-	-	1.31±0.24	0.48±0.06	3.36 (2)
CMS	D	54.73±3.03	5.82±0.70	24.16±3.51	19.17±3.17	14.20±3.88	-	2.30 (1)
SS	N	250.16±7.63	28.36±1.38	53.67±7.80	18.36±7.93	83.92±8.74	-	0.28 (1)
	D	190.41±30.86	32.76±1.44	316.31±30.892	135.16±30.82	-	141.30±34.05	1.92 (1)
SF	N	112.97±0.32	21.79±0.32	22.64±0.54	-	76.04±2.20	-	3.89 (2)
	D	158.43±4.37	25.77±0.77	43.60±4.49	14.84±4.54	141.43±4.76	-	0.07 (1)
PH	D	123.04±4.95	-	58.91±12.49	16.07±4.92	-	46.83±7.84	2.53 (2)
NB	N	13.02±0.42	2.14±0.35	9.20±1.73	-	-	9.28±1.80	3.70 (2)
	D	9.79±0.42	1.23±0.35	6.45±1.63	-	-	8.60±1.46	3.66 (2)
BW	N	3.32±0.05	0.48±0.05	1.42±0.24	-	1.73±0.23	1.93±0.25	0.53 (1)
	D	3.20±0.04	0.15±0.05	-	-	0.47±0.21	0.25±0.09	3.45 (2)
SI	N	8.38±0.37	0.15±0.04	2.60±0.84	1.18±0.36	-	2.07±0.51	1.39 (1)
	D	8.16±0.43	0.14±0.50	2.18±0.96	1.22±0.42	-	1.81±0.56	0.06 (1)
GOT	D	37.69±0.21	1.15±0.29	-	-	4.06±1.06	1.72±0.35	1.46 (2)
FL	D	24.96±0.21	-	4.95±0.88	-	4.70±0.87	-	1.45 (3)
FS	N	26.55±1.70	-	18.34±3.81	6.20±1.69	12.38±2.19	-	2.22 (2)
	D	22.93±0.57	-	1.86±0.61	1.81±0.59	4.06±0.69	-	2.95 (2)
FF	N	4.30±0.014	-	-	-	0.74±0.21	-	4.83 (4)
	D	4.65±0.032	0.11±0.032	0.16±0.39	-	-	-	2.09 (3)

df = degree of freedom; RWLC = relative leaf water contents; ELWL = excised leaf water loss; CMS = cell membrane stability; SS = stomatal size; SF = stomatal frequency, PH = plant height; NB = number of bolls/plant; BW = boll weight; SI = seed index; GOT = ginning out turn; FL = fiber length; FS = fiber strength; FF = fiber fineness.

Table 6 Variance components following weighted analysis of components of variance, and heritability in a cross FH-207 × FH-901 under normal (N) and drought (D) conditions in the field

Traits	Field conditions	Variance Components				x^2 (df)	Heritability	
		D	H	F	E		NS	F∞
RLWC	D	163.81±16.40	-	-	1.61±0.24	3.56 (4)	0.57	0.97
ELWL	N	0.62±0.73	2.81±1.36	-	0.34±0.05	0.01 (3)	0.33	0.65
	D	0.95±0.12	-	-	0.09±0.01	2.49 (4)	0.57	0.87
CMS	D	171.37±21.61	-	-	16.62±2.47	5.38 (4)	0.56	0.95
	D	240.16±27.32	-	-	13.85±2.06	6.94 (4)	0.58	0.73
SS	N	4450.92±499.19	-	2206.74±257.92	133.09±19.83	1.58 (3)	0.77	0.97
	D	3922.01±425.55	-	1820.34±221.96	65.97±9.83	0.40 (3)	0.65	0.96
SF	N	897.69±95.86	`-	446.87±48.03	5.90±0.88	2.44 (3)	0.67	0.96
	D	1372.17±149.20	-	677.50±75.53	21.39±3.19	0.08 (3)	0.61	0.94
PH	D	2618.05±342.70	2355.92±356.44	-	11.78±1.76	0.01 (3)	0.69	0.97
NB	N	7.36±4.43	-	-	10.23±1.45	1.26 (4)	0.41	0.58
	D	7.90±3.48	-	-	7.58±1.08	4.71 (4)	0.45	0.62
BW	N	0.65±0.10	-	-	0.11±0.02	0.12 (4)	0.77	0.87
	D	0.47±0.10	-	-	0.15±0.02	4.62 (4)	0.76	0.86
SI	N	0.68±0.21	0.67±0.33	-	0.18±0.03	0.11(3)	0.49	0.79
	D	0.45±0.09	-	-	0.14±0.02	0.82 (4)	0.49	0.65
GOT	D	6.84±1.90	-	-	4.56±0.51	1.12 (4)	0.35	0.67
FL	D	2.66±1.22	-	-	2.68±0.38	5.06 (4)	0.29	0.60
FS	N	11.20±1.25	-	-	0.56±0.084	4.42 (4)	0.74	0.94
	D	0.46±5.25	17.96±9.31	-	1.43±0.21	2.57 (3)	0.34	0.74
FF	N	1.60±0.62	4.27±1.19	-	0.14±0.02	1.48 (3)	0.40	0.92
	D	1.20±0.35	2.90±0.68	-	0.041±0.006	3.48 (3)	0.44	0.97

D = additive; H = dominance; F = additive × dominance; E = environmental; RWLC = relative leaf water contents; ELWL = excised leaf water loss; CMS = cell membrane stability; SS = stomatal size; SF = stomatal frequency, PH = plant height; NB = number of bolls/plant; BW = boll weight; SI = seed index; GOT = ginning out turn; FL = fiber length; FS = fiber strength; FF = fiber fineness; NS = narrow sense heritability

of membrane. Plants tend to maintain their moisture percentage under water storage conditions to maintain their membrane integrity due to higher relative leaf water content. The negative correlation of excised leaf water loss with relative leaf water content under draught stress showed that plants which maintained their higher relative leaf water contents may be linked to genes for cutical thickness. Stomatal size correlated negatively with stomatal frequency in cotton [10, 27, 28]. Water deficit stressed plants exhibit higher stomatal frequency due to the smaller leaf area than non-stressed plants [29]. The genotypes with lower stomatal size show reduced transpiration and hence are comparatively more resistant against drought stress [25]. Positive correlation of plant height with bolls per plant suggested that taller plants would bear more bolls [11, 30]. Number of boll per plant directly contributes to yield. The positive correlation of number of bolls per plant with relative leaf water content shows that genotype, which maintain higher relative leaf water contents would bear a higher number of bolls. The results of correlation studies revealed that selection for increased boll weight will also increase seed index. Both of these traits contribute to high lint yield. Negative correlation of fiber fineness with fiber length observed in the present study is also reported in earlier studies [1, 2, 31].

The quantitative traits stomatal size, stomatal frequency, relative leaf water contents, excised leaf water loss and cell membrane stability have a complex inheritance due to their polygenic nature and interactions [32]. Additive and dominance with the interaction type of genetic effects control the inheritance of relative leaf water content and excised leaf water loss in upland cotton [24]. Drought causes closure of stomata after two minutes of leaf excision so differences in excised leaf water loss arise due to cuticle thickness [33]. The drought tolerant genotypes exhibit low rate of excised leaf water loss. Therefore, it has been used to select drought tolerant genotypes [16]. Higher cell membrane stability is required for sustaining normal cellular processes under drought stress [10, 34]. The genotypes with lower stomatal size showed reduced transpiration and hence are comparatively more resistant against drought stress [22]. Moisture deficiency decreases leaf area due to lower leaf water potential [35], hence increasing stomatal frequency. Water stressed plants exhibited higher stomatal frequency and smaller leaf area than non-stressed plants [29].

Genetic effects for plant height and number of bolls per plant were additive and dominance with interactions in nature [24]. Drought stress affects yield negatively by decreasing number of bolls [4, 36]. The presence of interactions in the inheritance of number of bolls per plant showed that the trait is not simply inherited. Additive and dominance with epistasis genetic effects control the inheritance of boll

Table 7 Phenotypic (Lower diagonal) and genetic correlation (Upper diagonal) matrix for a cross FH-207 × FH-901under normal (N) and drought (D) conditions in the field.

Traits		PH	NB	BW	SI	GOT	FL	FS	FF	RLWC	ELWL	CMS	SS	SF
	N		0.110	-0.050	0.034	0.066	-0.022	-0.116	0.047	-0.209	-0.151	-0.187	0.064	-0.223
PH	D		0.033	0.021	0.156	-0.065	-0.158	-0.163	0.028	0.192	-0.079	0.007	-0.049	0.027
	N	0.104*		-0.236	0.348	0.192	0.127	-0.102	0.194	-0.058	-0.251	0.107	0.152	-0.044
NB	D	0.113**		0.241	-0.351	-0.370	-0.263	-0.116	-0.249	0.222	-0.147	0.286	-0.139	0.158
	N	0.001	0.210**		0.297	-0.059	-0.196	-0.208	-0.088	-0.122	-0.277	0.278	-0.160	0.244
BW	D	0.014	0.120*		0.266	-0.101	-0.197	0.398	-0.158	0.091	0.257	0.260	.0.087	0.092
	N	0.020	0.167**	0.103*		0.522	0.099	0.288	-0.130	0.152	0.277	-0.209	-0.156	0.095
SI	D	0.132**	0.149**	0.110*		-0.255	0.106	-0.066	-0.289	0.060	-0.276	-0.101	-0.111	0.084
	N	0.037	0.112	0.040	0.094		0.284	-0.042	-0.136	-0.360	0.156	0.232	-0.056	-0.120
GOT	D	0.039	0.048	-0.056	0.073		0.340	-0.135	0.075	-0.108	-0.327	-0.153	0.158	-0.207
	N	0.020	0.092	0.085	0.075	0.149		-0.092	-0.139	0.102	-0.250	-0.172	0.088	-0.141
FL	D	-0.052	.-0.012	-0.003	-0.067	-0.053		-0.220	0.180	0.196	-0.275	-0.086	-0.133	-0.209
	N	-0.037	0.186**	-0.030	0.224**	0.033	0.016		-0.213	-0.053	0.151	0.113	0.090	-0.108
FS	D	-0.013	0.106	0.072	-0.016	-0.002	0.189**		-0.130	-0.084	0.105	-0.149	0.140	-0.143
FF	N	0.027	0.012	0.049	-0.036	-0.014	-0.038	-0.079		-0.169	0.177	0.113	-0.162	0.142
	D	0.006	0.103	-0.110	0.003	-0.035	-0.146*	-0.011		-0.140	-0.137	0.023	0.190	-0.132
RLWC	N	0.041	0.124*	0.105	0.114*	0.083	0.036	0.043	-0.136*		0.098	-0.096	-0.021	0.078
	D	0.185**	0.190**	0.062	0.036	0.093	0.180**	0.078	-0.082		-0.290	0.349	-0.169	0.098
ELWL	N	-0.064	-0.059	-0.274**	-0.105	-0.062	-0.004	0.048	0.014	-0.036		-0.151	0.119	0.045
	D	-0.065	-0.032	0.046	-0.117*	-0.064	-0.200**	-0.069	0.118*	0.215**		-0.236	-0.074	0.067
CMS	N	0.018	0.050	-0.219**	-0.007	0.182*	-0.012	0.100	0.021	0.081	-0.065		-0.122	0.173
	D	0.002	0.133*		0.083	0.073	0.019	0.117*	0.047	0.312**	0.198**		-0.130	0.212
SS	N	0.060	-0.054	-0.141	0.101	0.028	0.078	0.036	-0.128*	0.007	0.089	-0.073		-0.727
	D	0.009	0.014	-0.060	0.019	-0.038	0.111	0.119*	0.033	-0.049	-0.067	-0.101		-0.763
SF	N	-0.139*	0.094	0.174*	-0.087	-0.095	-0.139	-0.063	0.105	-0.004	-0.096	0.133*	-0.527**	
	D	-0.018	0.005	0.074	.-0.068	0.065	0.154**	-0.122*	-0.008	0.093	0.039	0.141*	-0.618**	

* = significant (p<0.05), ** = highly significant (p<0.01); RWLC = relative leaf water contents; ELWL = excised leaf water loss; CMS = cell membrane stability; SS = stomatal size; SF = stomatal frequency, PH = plant height; NB = number of bolls/plant; BW = Boll weight; SI = seed index; GOT = ginning out turn; FL = Fiber length; FS = fiber strength; FF = fiber fineness

weight [37] so breeding for this trait is fruitful in advanced generations when interaction would have been fixed. Drought stress affects the fiber length and strength positively while the micronaire value negatively [9]. The fiber traits are controlled by polygenes without involvement of any major genes, so the genetic behavior of these traits changes due to the interaction of genotype with the environment [21]. Additive and dominance genetic effects control inheritance of fiber length with high narrow sense heritability (>0.78) while fiber strength was controlled by additive and additive × dominance type of genetic effects [38]. High heritability indicated that a higher proportion of the genetic variance was additive so improvement is possible by selection in early generation. In the present study, micronaire value increased under drought stress compared to well water conditions. Micronaire is inversely proportional to fiber fineness. Under low water availability conditions, photosynthetic accumulation also decreases as a result accumulation of cellulose layers decreases in the fiber development to decrease fiber fineness. The genetic control of fiber fineness is complex with polygenic inheritance [39] and most genes involved are recessive in nature with less additive affect [40]. The presence of different patterns of genetic variance such as additive, dominance and epistatic interactions in the breeding populations is useful to adopt a comprehensive breeding strategy in crop species. If the major portion of the genetic variance for a trait is of additive nature, selection of individual plants in the early segregating population would be effective. In case of dominance and interactions, individual plant selection in early segregating population would result in extra work without any progress so carrying the bulk population until advanced segregating population would be economically useful.

Conclusions

The results of this study revealed that the relative leaf water content, excised leaf water loss, cell membrane stability, stomatal size and frequency are important traits which may be exploited to develop drought tolerance in upland cotton. Positive correlation of relative leaf water content and cell membrane stability reveals that the genes which help to maintain higher relative leaf water contents may also be involved for cell membrane stability.

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