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GENETIC LINKAGE STUDIES OF DROUGHT TOLERANT AND AGRONOMIC TRAITS IN COTTON

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Abstract

Genetic linkage among drought tolerant (relative water content, excised leaf water loss, stomatal size and stomatal frequency) and agronomic traits (fiber fineness, fibre strength, fiber length, number of bolls per plant, bolls weight, ginning out-turn, number of monopodial branches per plant and number of sympodial branches per plant) in upland cotton was studied by calculating the correlation matrix of a population of four parents and their F_1 hybrids. Relative water content (RWC) positively correlated with boll weight but negatively correlated with fiber length. Stomatal size had negative correlation with ginning out-turn (GOT). The stomatal frequency and excised leaf water loss revealed non-significant relationship with all the characters under study. The RWC and stomatal size had also non-significant relationship with number of bolls per plant, number of sympodial branches, number of monopodial branches, fiber fineness and fiber length. Negative correlation of RWC with fibre length showed linkage of genes for lower fibre length with the genes responsible for maintaining high RWC. However, negative correlation of stomatal size with GOT is favourable. The genotypes with lower stomatal size would help reduce transpiration rate and hence would show improved drought resistance as well as would yield high GOT.

Keywords: water stress, genetic studies, stomatal frequency, relative water content, cotton

Introduction

Cotton (*G. hirsutum*) is an important fibre, and food crop of Pakistan. The economy of Pakistan depends heavily on cotton and its products. It accounts for 10.5 percent of the value added in agriculture and about 2.4 percent to GDP. In Pakistan, it is grown over a vast area of about 3.19 million hectares with annual production of about 14.27 million bales (Anonymous, 2005). There are about 1035 ginning, more than 55 oil extraction and 450 textile units, in the country. About 30% of the country's manpower is engaged in cotton industry, the largest foreign exchange earner.

Drought is a major limiting factor in cotton production in many regions of the world including Pakistan. Drought stress significantly reduces cotton production in the country (Anonymous, 2005). The reduced size and number of bolls on plant due to drought stress affects yield in cotton. Drought stress also results in cotton motes, a condition in which cotton ovule fails to ripen into mature seed. This causes imperfection in ginned fibres and textile products from cotton. Therefore, there is need to breed cotton cultivars adapted to drought stress by incorporating traits which can confer drought resistance.

Cotton is normally not classified as a drought tolerant crop as are some other plants species such as sorghum (*Sorghum bicolor*) which is cultivated in areas normally too hot and dry to grow other crops (Poehlman, 1986). Nevertheless, cotton does have mechanisms that make it well adapted to semi-arid regions, such as its deep penetrating

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and extensive root systems, leaves, fruits that can be shed when plants are stressed, and a flexible fruiting period (Ray *et al*., 1974). Roark *et al*. (1973) reported differences in stomatal behavior and distribution among cotton cultivars grown in the high plains of Texas, USA. In plants, gaseous diffusion depends essentially on stomatal pore size and its distribution in the epidermis. Thus, it would seem that larger stomatal openings or greater number of stomata per unit of leaf could permit greater rates of photosynthesis by decreasing the diffusive resistance to CO_2 uptake (Teare *et al*., 1971). Miller (1938) pointed out that the number of stomata per unit of leaf surface was within certain limits, characteristic of a particular plant species or variety. Van de Roovaart & Fuller (1935) found that plants had a lower stomatal frequency under optimal moisture conditions than under stress conditions. However, they observed that the ratio of the number of stomata to that of the epidermal cells remained almost constant, as epidermal cells were larger under optimal conditions compared to the epidermal cells of plants grown under drought stress. Wood (1934) suggested that variations in stomatal frequency were connected more closely with genetic than with environmental conditions. Miskin *et al*., (1972) reported that a 25% decrease in frequency of stomata reduced transpiration rates by 24%. However, Stomatal frequency did not influence the rate of photosynthesis in barley. Hence, possibility exists in altering transpiration without affecting photosynthesis by selecting genotypes with lower stomata.

If a genotype can maintain optimum relative water content, or does not allow high rate of water loss from the leaf surface or by developing lower stomatal size and frequency without decreasing net photosynthesis, it would help plant producing good yield under drought stress. So lower excised leaf water loss, lower transpiration rate (lower stomatal size and frequency) and higher relative water content in leaf has been reported as selection criteria to breed plants against drought stress (Clarke & McCaig, 1982; Malik & Wright, 1997, 1999; Rahman *et al*., 2000). To breed a high yielding cultivar, breeder has to tailor a plant with combination of a number of desirable traits. It is important to study the linkage relationship of the traits related to yield and quality as well as those related to adaptability of the genotype to a certain environment. The present study investigated the linkage relationship of the traits, relative water content, excised leaf water loss, stomatal size, stomatal frequency, fiber fineness, fibre strength, fiber length, number of bolls per plant, bolls weight, ginning out-turn, plant height, number of monopodial branches per plant and number of sympodial, branches per plant. The information generated from this study would be useful for cotton breeder to tailor high yielding cotton cultivars under drought stress conditions.

Materials and Methods

The present study was conducted in the department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Four cotton genotypes were crossed to produce F_1 seeds in greenhouse during the winter season of the year 2003-04. The list of the plant genotypes and their crosses used in the study is given in the following:

a) MS-84
b) Cris-379
c) A-89/FM
d) FH-950
e) MS-84 × Cris-379
f) MS-84 × FH-950
g) MS-84 × A-89/FM
h) Cris-379 × FH-950
i) Cris-379 × A-89/FM
j) FH-950 × A-89/FM

The F_1 populations along with the parents were grown under field conditions on 17th May, 2004 in a randomized complete block design with three replications. In each replication, there was one row for each of the parents and F_1 hybrids. There were 10 plants in a row. Row to row and plant to plant distance was 75 and 30 cm respectively.

Five plants from each of the parents and the F_1 populations were selected at random from each replication for recording the data. Drought stress was imposed by terminating irrigation (106 days after sowing) during the month of September and October. According to the meteorological data from the Department of Crop Physiology, University of Agriculture Faisalabad, Pakistan, total rainfall at the university campus was 34 mm and temperature ranged from 35 to 41°C in September while in October there was only 8 mm rainfall on 2nd of the month and the temperature ranged from 30 to 39°C. During the last week of October, plants showed symptoms of severe drought. The data for the following physiological traits were recorded when symptoms of drought such as wilting of leaves appeared on the plants.

Relative water contents (RWC): Three fully developed leaf samples were taken from each of the selected plants at the middle of plant canopy when drought appeared. After excision each sample was covered with polythene bag. The samples were taken to laboratory and fresh weight was recorded using electronic balance. The leaf samples were kept in water for overnight to record turgid leaf weight. The samples were oven dried at 70°C for taking dry weight. The relative water content was calculated using the following formula:

$$\text{Relative water content} = \text{Fresh weight} - \text{Dry weight} / \text{Turgid weight} - \text{Dry weight}$$

Excised leaf water loss (ELWL): Three fully developed leaf samples were taken from each of the selected plants at the middle of plant canopy when drought appeared. After excision each sample was covered with polythene bag. The samples were taken to laboratory and fresh weight was recorded using electronic balance. The leaf samples were left on laboratory benches. After six hours the weight of wilted leaf samples was taken. The samples were then dried at 70°C in oven and dry weight was recorded. The ELWL was calculated using the following formula:

$$\text{Excised leaf water loss} = \text{Fresh weight} - \text{wilted weight} / \text{Dry weight}$$

Stomatal frequency and stomatal size: Strips of three fully mature leaves from each of the selected plants were fixed in acetic alcoholic fixative. The strips were kept in the fixative for overnight and then fixative solution was replaced by 70% alcohol. The stomatal size and frequency were measured under compound microscope. During the mid December 2004, when plants were fully mature, the data about number of bolls per plant, bolls weight, ginning out-turn, plant height, number of monopodial branches per plant and number of sympodial, branches per plant was recorded.

Fibre characteristics: Fibre length, fibre strength and fineness of the lint samples were measured using Spinlab HVI-900 from the Department of Fibre Technology, University of Agriculture Faisalabad. The computerized instrument records fibre length, fibre fineness and fibre strength according to international standards. The data were subjected to analysis of variance following the method given by Steel and Torrie (1980) and correlation coefficients were calculated by the formula as outlined by Dewey and Lu (1959) using Minitab programme of computer.

Results and Discussion

Analysis of variance revealed significant differences among the genotypes for all the traits (relative water contents, excised leaf water loss, stomatal size and frequency, fiber fineness, fiber strength, fiber length, boll weight, number of bolls per plant, Ginning out-turn, number of monopodial branches per plant and number of sympodial branches per plant) except for plant height. Mean values of the genotypes for the traits, the genotype effects and LSD values are given in Table-1. Correlation matrix among the traits is given in Table-2.

Breeding high yielding cultivars is a continuous process. This is generally achieved by crossing varieties/genotypes with desirable traits followed by selection. Correlation measures the degree of interdependence between a pair of characters. Knowledge of correlation is required to obtain the expected response of other characters when selection is applied to a particular character of interest in a breeding programme. Population with a variable combination of plant traits under study was used to estimate the correlation of the traits and the data of large population (150 plants) was used to calculate correlations. The correlation calculated in such a recombinant large population shows linkage behavior of the genes.

Lower stomatal size and frequency in leaves help reduce stomatal water loss. Negative correlation of stomatal size and frequency with relative water content (RWC) showed that lower stomatal size and frequency helped maintaing water balance, however, the correlation was not significant. The RWC was positively correlated with boll weight while it had negative association with fiber length. Boll weight was negatively associated with fiber length. Stomatal size had negative relationship with GOT. Excised leaf water loss and stomatal frequency did not show correlation with all the traits under study. Similarly, relative water contents and stomatal size as well as frequency did not show correlation with number of bolls per plant, number of sympodial branches per plant, number of monopodial branches per plant, fiber fineness and fiber strength. Relative water contents showed positive correlation with boll weight.

Under drought stress, plant maintains high relative water content through various morphological and physiological adaptations (Ray *et al*., 1974; Matin *et al*., 1989). In the present study cotton plants with genetic combinations to maintain high relative water content resulted in developing high boll weight. It may be suggested that selection for cotton plants for high relative water content would improve drought resistance. In wheat similar findings were reported by Clark and McCaig (1982) and Klar (1984).

However, the negative correlation of relative water contents with fiber length revealed that the genes which helped maintaining high relative water content might have linkage with alleles for producing poor fibre length. To breed cotton for drought resistance this negative correlation should be considered while selecting plants. Intensive crossing leading to crossovers may break this negative correlation. Hence a very large segregating population should be grown while selecting plants for high relative water content. Absence of correlation of relative water content with other traits (ELWL, stomatal frequency, stomatal size, fibre fineness, fibre strength, fibre length, number of bolls per plant, GOT, monopodial branches and sympodial branches) shows that the

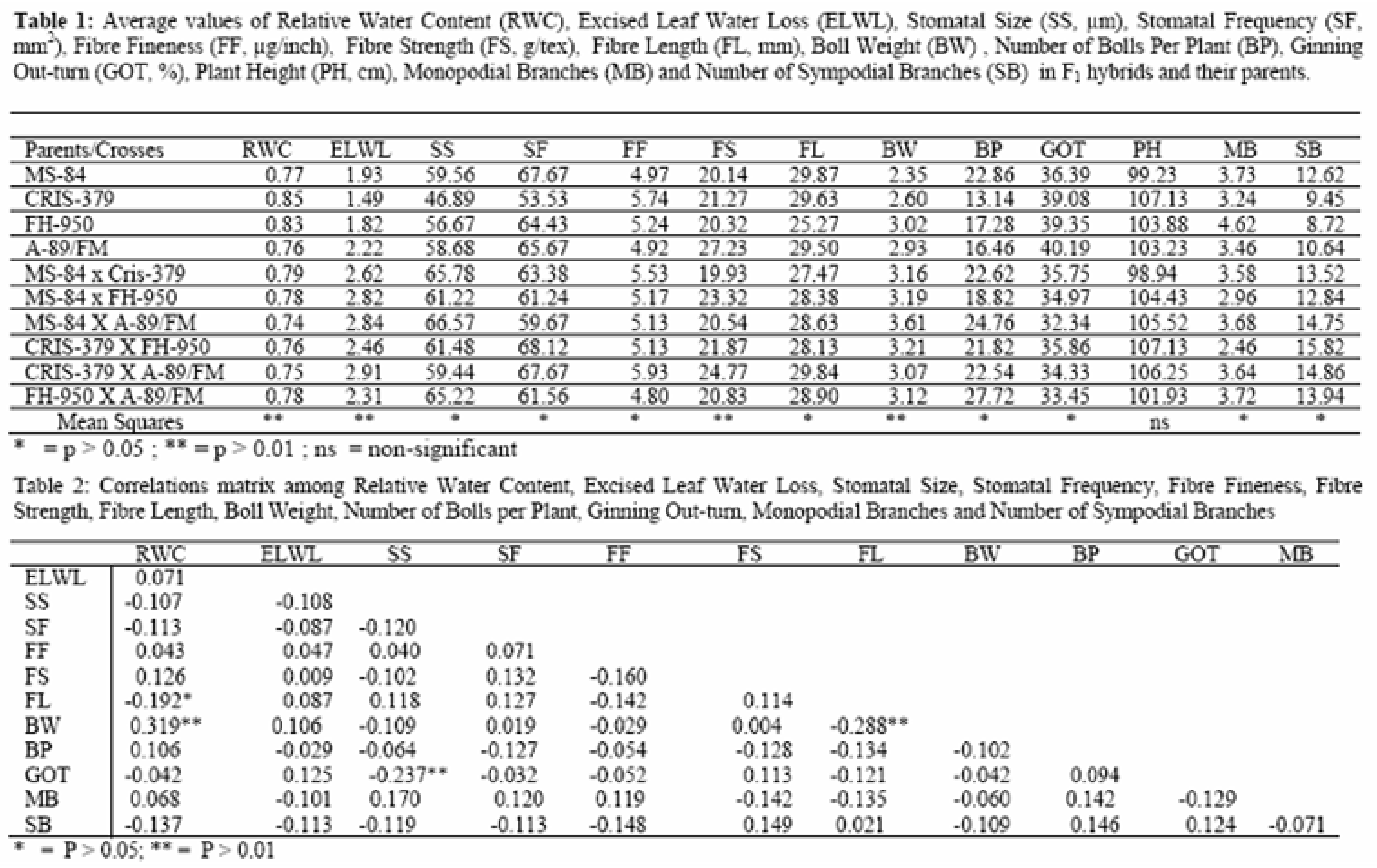

Table 1: Average values of Relative Water Content (RWC), Excised Leaf Water Loss (ELWL), Stomatal Size (SS, µm), Stomatal Frequency (SF, mm^2), Fibre Fineness (FF, µg/inch), Fibre Strength (FS, g/tex), Fibre Length (FL, mm), Boll Weight (BW) , Number of Bolls Per Plant (BP), Ginning Out-turn (GOT, %), Plant Height (PH, cm), Monopodial Branches (MB) and Number of Sympodial Branches (SB) in F_1 hybrids and their parents.

Parents/Crosses	RWC	ELWL	SS	SF	FF	FS	FL	BW	BP	GOT	PH	MB	SB
MS-84	0.77	1.93	59.56	67.67	4.97	20.14	29.87	2.35	22.86	36.39	99.23	3.73	12.62
CRIS-379	0.85	1.49	46.89	53.53	5.74	21.27	29.63	2.60	13.14	39.08	107.13	3.24	9.45
FH-950	0.83	1.82	56.67	64.43	5.24	20.32	25.27	3.02	17.28	39.35	103.88	4.62	8.72
A-89/FM	0.76	2.22	58.68	65.67	4.92	27.23	29.50	2.93	16.46	40.19	103.23	3.46	10.64
MS-84 x Cris-379	0.79	2.62	65.78	63.38	5.53	19.93	27.47	3.16	22.62	35.75	98.94	3.58	13.52
MS-84 x FH-950	0.78	2.82	61.22	61.24	5.17	23.32	28.38	3.19	18.82	34.97	104.43	2.96	12.84
MS-84 X A-89/FM	0.74	2.84	66.57	59.67	5.13	20.54	28.63	3.61	24.76	32.34	105.52	3.68	14.75
CRIS-379 X FH-950	0.76	2.46	61.48	68.12	5.13	21.87	28.13	3.21	21.82	35.86	107.13	2.46	15.82
CRIS-379 X A-89/FM	0.75	2.91	59.44	67.67	5.93	24.77	29.84	3.07	22.54	34.33	106.25	3.64	14.86
FH-950 X A-89/FM	0.78	2.31	65.22	61.56	4.80	20.83	28.90	3.12	27.72	33.45	101.93	3.72	13.94
Mean Squares	**	**	*	*	*	**	*	**	*	*	ns	*	*

* = p > 0.05 ; ** = p > 0.01 ; ns = non-significant

Table 2: Correlations matrix among Relative Water Content, Excised Leaf Water Loss, Stomatal Size, Stomatal Frequency, Fibre Fineness, Fibre Strength, Fibre Length, Boll Weight, Number of Bolls per Plant, Ginning Out-turn, Monopodial Branches and Number of Sympodial Branches

	RWC	ELWL	SS	SF	FF	FS	FL	BW	BP	GOT	MB
ELWL	0.071										
SS	-0.107	-0.108									
SF	-0.113	-0.087	-0.120								
FF	0.043	0.047	0.040	0.071							
FS	0.126	0.009	-0.102	0.132	-0.160						
FL	-0.192*	0.087	0.118	0.127	-0.142	0.114					
BW	0.319**	0.106	-0.109	0.019	-0.029	0.004	-0.288**				
BP	0.106	-0.029	-0.064	-0.127	-0.054	-0.128	-0.134	-0.102			
GOT	-0.042	0.125	-0.237**	-0.032	-0.052	0.113	-0.121	-0.042	0.094		
MB	0.068	-0.101	0.170	0.120	0.119	-0.142	-0.135	-0.060	0.142	-0.129	
SB	-0.137	-0.113	-0.119	-0.113	-0.148	0.149	0.021	-0.109	0.146	0.124	-0.071

* = P > 0.05; ** = P > 0.01

genes for this trait segregate independent of the other traits. Selection for higher relative water content to improve drought resistance would not affect the traits mentioned above.

Fiber length had negative linkage with boll weight which is in accordance with the results of Khan *et al*. (1980) who described that boll weight was negatively correlated with fiber length. Therefore, selection for higher boll weight would result in smaller fiber length. It may be suggested to raise larger segregating population to break the linkage to tailor plants having combination with both the useful traits.

Stomatal size showed negative relationship with GOT. This negative correlation is useful in a variety for cultivation under drought stress. As the lower stomatal size would help reduce transpiration rate and hence would help in maintaining high relative water content. So improvement in drought resistance would also have positive effect on GOT. It has been reported that generally there is correlation of lower cell size with lower stomatal size (Teare *et al*., 1971). Lower size of cells may result in smaller seed size in cotton. Cotton fibres are produced on epidermis. Hence, increase in surface area of seed by reducing cell size would have resulted in high GOT

Stomatal size and stomatal frequency, did not show correlation with ELWL, stomatal frequency, stomatal size, fibre fineness, fibre strength, fibre length, number of bolls per plant, monopodial branches per plant and sympodial branches per plant. This suggests that genes controlling stomatal size and stomatal frequency are not linked with genes for agronomic and quality traits. So selection for lower stomatal size and frequency to improve drought resistance through reduced transpiration rate would not affect yield potential and quality of cotton.

Absence of the correlation of ELWL with all the other traits studied shows that the genes for ELWL segregate independent of the other traits so plants with lower ELWL (having improved drought resistance) may be selected with combination of good quality and yield traits. ELWL is an indirect measure of cuticle thickness as stomata close about two minutes after the excision of leaf. So most of the water loss from the leaf after excision is from epidermis, hence differences in cuticular thickness would result in differences of ELWL. It has been reported that cuticular thickness and waxiness of leaf surface are genetically controlled and affect transpiration (Haque *et al*., 1992). Absence of correlation of ELWL with agronomic as well as the stomatal size, stomatal frequency and relative water content traits suggests that to engineer plants having combination of traits involved in improved drought resistance as well as with good yield potential and quality traits is possible.

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