



Full Length Article

Variability for Drought Tolerance in Cotton (*Gossypium hirsutum*) and its Genetic Basis

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ABSTRACT

Responses of 80 genotypes/lines of *Gossypium hirsutum* were examined at seedling stage under water stressed and non-stressed conditions in glasshouse. Plant growth was measured as longest root and shoot after 45 days. Genotypic differences for indices of drought tolerance were statistically significant. Based upon the differences and similarities, four tolerant i.e., 149F, B-557, DPL-26, BOU 1724-3 and 4 susceptible namely FH-1000, NF-801-2, CIM-446 and H-499 genotypes/lines were crossed in all possible combinations. The responses of 64 families were examined under water stress and non-stressed (control) conditions at seedling stage. Seedlings were allowed to grow for 45 days. Root lengths of all the families were measured under the two conditions. Indices of drought tolerance (relative root length) were analyzed following Hayman-Jinks approach. Regression coefficient ($b=1.0\pm 0.15$) and analysis of variance of (W_r+V_r) and (W_r-V_r) provided no evidence of the presence of epistasis and the genetic model was found fully adequate for analyzing the data. The results revealed that both additive and non-additive genes affected variation for drought tolerance, but the influence of additive gene was more pronounced. High estimates of h^2_{ns} , 0.82 and mode of gene action suggest that it is possible to improve drought tolerance in *G. hirsutum* by single plant selection in later segregating generations. © 2011 Friends Science Publishers

Key Words: Cotton; Relative root length; Variance; Selection; Breeding

INTRODUCTION

Amongst the abiotic stresses reducing crop productivity, shortage of irrigation water is a primary limiting factor in many regions of the world (Turner, 1997; Sinclair, 2005). As a result of increasing demand and competition from environmental, industrial and domestic sectors, supply of fresh water to agriculture sector is likely to be reduced in the coming years. With the increase in world's population, production of food and cash crops has become a top endeavor and the major challenge for agriculture sector in Pakistan will be to raise crops under water stress. It has been reported that most of the crops are sensitive to water stress, particularly during flowering to seed development stage (Salter & Goode, 1967). Thus keeping in view the current situation of water supply in rivers and canals, it has become indispensable to breed drought tolerant varieties of various crops through selection and breeding.

Cotton (*Gossypium hirsutum* L.) is the most important textile fiber crop and the world's 2nd important oil seed crop after soybean. Although cotton is considered to be a drought tolerant crop, its sensitivity varies greatly among genotypes (Gorham, 1996; Naidu *et al.*, 1998). Water stress affects the cotton plant by limiting fiber yield and lint quality,

suggesting the development of drought tolerant cultivars to get economic yield in water deficit areas.

For successful breeding of cotton cultivars tolerant to drought through conventional approach, basic information about the breeding material must be available to the breeders. Firstly, there must be significant variability in genotypic responses to water stress and secondly, this variation must be genetically controlled. Thus, an understanding of the knowledge of these two components about the breeding material under consideration is necessary (Mitra, 2001).

Previous work on drought tolerance provide sufficient evidence on the occurrence of variation within the *G. hirsutum* (Quisenberry *et al.*, 1982; Pereira *et al.*, 1998; McCarty *et al.*, 2004; Pettigrew, 2004; Basal *et al.*, 2005; Kar *et al.*, 2005). However, because of a general lack of the genetic studies on drought tolerance, very little is known about the genetic mechanism controlling variation in drought tolerance in *G. hirsutum*. Only few studies revealed that water stress tolerance in cotton is under genetic control (Liu *et al.*, 1998; McCarty *et al.*, 2004, Singh & Singh, 2004). In rice, root characters such as long root and greater root number are indicators of drought tolerance and affected by multiple genes (Ekanayake *et al.*, 1985).

This paper examines the variation in 80

lines/genotypes of cotton for water stress tolerance and genetic mechanism controlling that variation at seedling stage. Indices of stress tolerance based upon shoot length and root length were analyzed following simple additive dominance model of Hayman (1954 a, b) and Jinks (1954).

MATERIALS AND METHODS

Assessment of variation in germplasm: Response of 80 genotypes/lines of *G. hirsutum* (Table I) was assessed in glasshouse. The optimum temperature in glasshouse was maintained at 35/21°C (day/night). Seeds of 80 genotypes were planted during October, 2005 in polythene bags measuring 25 cm × 15 cm, filled with about 1.5 kg of silt mixed with 100 g farm yard manure. The pH of soil was of 8.4, EC 1.2 dS/m, saturation 31%. Soil in the bags was saturated to field capacity before planting overnight soaked seeds. Four holes, each 2.5 cm deep, were made in each bag and two seeds were sown in one hole. After germination, seedlings were thinned to one seedling per hole and thus, there were four seedlings per bag. There were two sets of experiment, each having 80 genotypes/lines. In each treatment, the bags were arranged following completely randomized design in triplicate. Initially, seedlings in both the sets were watered and fertilized till the development of first true leaf. Thereafter, in one set, supply of water was withheld to develop water stressed conditions, whilst in other set; seedlings were grown under normal moisture conditions to designate as control.

The seedlings were grown under 14 h day length. The effect of water stress was monitored visually and with soil moisture meter (HH₂ Theta Probe Type, Delta-T device, Cambridge, England). At initial wilting (observed visually), when soil had 14 to 16 % moisture contents, the stressed plants were watered to relieve from wilting. Seedlings were allowed to grow for 45 days, from the date of emergence, till the 3rd main stem leaf fully expanded. Young plants under both the moisture conditions were measured for longest shoots and roots. Indices of water stress tolerance were calculated as the mean, shoot and root length in water stressed relative to that in non-stressed condition following Azhar and McNeilly (1988). The responses of 80 accessions to water stress were subjected to ordinary analysis of variance in order to see the genotypic differences in water stress tolerance (Steel *et al.*, 1997).

Development of plant materials for genetic studies: Based upon the similarities and differences in water stress tolerance, eight cotton varieties/lines namely 149F, B-557, DPL-26, BOU 1724-3, FH-1000, NF 801-2, CIM-446 and H-499 were selected from the lot. The parents were crossed according to diallel mating system in the glasshouse. In order to produce sufficient quantity of F₁ seeds, maximum number of pollinations was attempted. During emasculation and pollination, all necessary precautionary measures were adopted to avoid alien pollen contamination. Some of the

buds of the parents were covered with glycine bags to obtain selfed seed. At maturity, crossed and selfed bolls were collected and seeds of 56 hybrids and eight parents were collected by ginning seed cotton from bolls.

Assessment of F₁ hybrids for water stress tolerance: The response of 56 hybrids and eight parents to water stress was assessed in glasshouse during October, 2006. The plant material was grown and treated in water stressed and non-stressed and conditions following the same procedure described previously for assessment of variation in the germplasm. Seedlings under both the conditions were measured for longest root. Relative root lengths (indices of water stress tolerance) were calculated as described previously. Before analyzing the data for genetic interpretation following simple genetic model of Hayman (1954a, b) and Jinks (1954), mean relative root lengths of 56 hybrids and eight parents were subjected to ordinary analysis of variance technique in order to see whether the genotypic differences for root length are significant. Genetic analysis of data using simple genetic model is valid only when such genotypic differences are significant.

To determine the adequacy of the additive-dominance model to account for the data and to assess the validity of some of the assumptions underlying the model, preliminary analysis of data was under taken following Hayman (1954a). From the diallel data, variances of the components of each array (V_r) and covariances of all the offsprings included in each parental array with non-recurrent parent (W_r), variance of parental mean (V_0L_0), variance of array means (V_0L_1), means of array variances (V_1L_1) and mean array covariances (W_0L_0) were calculated (Table IV). These statistics were involved in the estimation of four genetic components i.e., D, an estimate of the additive genetic effects; H₁, H₂, measure of variation due to dominance effects and F, provide an estimate of relative frequency of dominant to recessive alleles in the parental lines.

RESULTS

Assessment of variability for water stress tolerance: Mean squares showed that indices of water stress tolerance based upon root and shoot lengths of 80 genotypes/lines differed significantly ($P \leq 0.01$) in water stress condition. In order to simplify the data presentation and examine the responses of the genotypes/lines to water stress conditions, a sub-sample of 34 genotypes from the 80 genotypes assessed, has been taken (Table II).

The comparison of indices of water stress tolerance based upon shoot length shows that some of the genotypes/lines were more tolerant than the others (Table II). Varieties 149 F, DPL-26, B-557 and BOU-1724 with indices ranging from 83 to 89% appeared to be less affected by moisture stress. In contrast, CIM-446 with 49% shoot length showed susceptibility to water stress. Similarly, FH-1000, 1118, H499-3, FH-679 and NF801-2 with indices ranging from 51 to 56%, appeared to be affected more than

Table I: Description of cotton germplasm assessed for drought tolerance

Genotypes/lines	Source
CIM448, CIM511, CIM482, CIM473, CIM109, CIM1100, CIM70, CIM240, CIM-496, CIM-446, CIM497, CIM-707, S12, S14	Cotton Research Station, Multan
VH28, VH57, VH54, VH37, VH55, VH141, VH144, VH-53	Cotton Research Station, Vehari
FH945, FH679, FH-634, FH-1000, FH87, FH950, FH925, FH938, FH901, FH900, FH682, B-557, LSS, 149F, 4F, 199F, 268F	Cotton Research Institute, Faisalabad
MNH-147, MNH93, MNH513, MNH-129, MNH3570, MNH552, MNH554, MNH700	Cotton Research Station, Multan
NIAB999, NIAB KRISHMA, NIAB228	Nuclear Institute Of Agriculture & Biology, Faisalabad
BH121, BH89, BH126, BH-124, BH123, BH95, BH36, BH147, BH162, BH118, BH-116, BH160, BH-125	Cotton Research Station, Bahawalpur
Rehmani	Cotton Research Station, Tandojam
SLH257	Cotton Research Station, Sahiwal
S362T362(GL), LINE A-100, H499-3, OKRA659, 1118, OKRA3101, NF801-2, BOU-1724, COKER 4601, Paymaster, Dixi-King, DPL-26, MR 73, LRA5166, LB391	Exotics

the others.

Indices of water stress tolerance based upon root length data provided further estimates of tolerance of the germplasm. The root length of some genotypes/lines was markedly reduced due to water stress than others. B-557 and DPL-26 had the greatest tolerance index (98.4%) and appeared to be tolerant to water stress. In addition, BOU-1724, 149F, VH-57, CIM-497 and BH-124 with indices of tolerance ranging from 95 to 97%, also seem to be the tolerant genotypes/lines. The genotypes/lines NF 801-2, FH-1000, Dixi-king and H499-3 were susceptible showing 40 to 48% root length of the control. The data presented in Table II clearly showed the differences in genotypic responses to water stress. Likewise, there were considerable differences among the remaining 46 genotypes/lines.

Genetics of water stress tolerance: Indices of water stress tolerance of 64 genotypes are given in diall table (Table III). Analysis of variance of these indices revealed highly significant ($P \leq 0.01$) differences for root length in 64 families. To test the fitness of the data to the genetic model, and the validity of some of the assumptions underlying the simple model, two scaling tests i.e., joint regression analysis and analysis of variance of ($V_r + W_r$) and ($V_r + W_r$) were carried out. The unit regression co efficient ($b = 1.0 \pm 0.15$) and significant differences between the arrays ($V_r + W_r$) and non-significant differences within the arrays ($V_r - W_r$) provided no evidence of the presence of epistasis. Thus the additive-dominance model was found to be fully adequate for analyzing the data.

Estimates of genetic components of variation in water stress tolerance: The estimates of five components of variation, D , H_1 , H_2 , F and h and their standard errors are presented in Table IV. Although magnitude of both D and H_1 were significant, a greater magnitude of D item revealed the importance of additive genes in the inheritance of water stress tolerance. The ratio of $\sqrt{H_1/D}$ (0.65) indicated partial dominance of the genes controlling the character, which was verified by the slope of the regression line on W_r axis (Fig. 1). Equal magnitude of H_1 and H_2 items indicated equal distribution of the genes in the parents. The ratio of $H_2/4H_1$ (0.24) is almost equal to the maximum value i.e., 0.25,

Table II: Relative water stress tolerance of 34 *Gossypium hirsutum L.* varieties based upon relative shoot and root length

Varieties/lines	Relative shoot length	Relative root length
CIM473	75.2	67.1
CIM1100	74.5	69.8
CIM70	77.1	74.5
CIM-446	48.7	48.7
BOU-1724	83.2	97.1
CIM497	75.3	96.0
NF801-2	55.6	39.5
VH57	69.6	96.3
VH37	77.8	64.5
FH679	55.5	52.4
FH-1000	51.2	38.2
FH950	72.0	54.8
FH925	72.4	58.7
MNH-147	63.8	75.2
MNH93	71.0	69.7
MNH-129	58.8	74.2
MNH554	68.5	79.5
NIAB228	77.4	72.8
BH121	69.3	56.2
149F	89.0	96.9
COKER 4601	62.5	72.5
BH-124	79.2	95.3
BH36	59.3	53.8
BH162	59.0	51.8
H499-3	54.7	47.8
199F	64.8	85.7
268F	67.1	90.0
BH-125	65.3	88.5
B557	84.7	98.4
DPL 26	85.4	98.4
SLH257	66.8	89.0
1118	53.1	79.1
DIXI-KING	65.8	47.6
VH-53	73.0	88.2
Cd_1	4.72	5.38
Cd_2	6.36	7.24

which provided further evidence for the equal distribution of genes in the parents. The positive value of F indicate the presence of more dominant alleles for water stress tolerance, and the higher ratio of $\sqrt{4DH_1+F}/\sqrt{4DH_1-F}$ (1.10) further strengthened this claim. Positive sign of h revealed the trend of dominance being towards the parents showing greater water stress tolerance. Because of the presence of additive

Table III: Indices of water stress tolerance (over replication and reciprocals) of 64 families of *G. hirsutum* L. and variances (V_r) and covariances (W_r)

Parents	FH-1000	NF801-2	H499-3	CIM-446	DPL-26	B-557	BOU-1724	149F	V_r	W_r
FH-1000	0.46									
NF801-2		0.42								
H499-3			0.48							
CIM-446				0.51						
DPL-26					0.55					
B-557						0.75				
BOU1724							0.66			
149F								0.60		
Means	0.56	0.54	0.60	0.78	0.80	0.80	0.75	0.79	0.020	0.027

Table IV: Statistics calculated from the diallel table and components of genetic variation in root length of 64 families of *G. hirsutum* L

Components of variation	Estimates
Statistics	
V_0L_0 =Variance of parents	6×10^{-2}
V_0L_1 =Variance of mean arrays	1×10^{-2}
V_1L_1 =Mean variance of arrays	0.02
W_0L_0 =Mean co-variance between the parents and the arrays	0.03
Component of variance	
D=additive variance	$6 \times 10^{-2} \pm 2 \times 10^{-3} *$
H_1 =dominance variance	$2 \times 10^{-2} \pm 5 \times 10^{-3} *$
H_2 =proportion of positive and negative genes in the parents	$2 \times 10^{-2} \pm 4 \times 10^{-3} *$
F=relative frequency of dominant and recessive alleles in the parents	$4 \times 10^{-3} \pm 5 \times 10^{-3}$ ns
h=dominance effect(overall loci in heterozygous phase)	$2 \times 10^{-3} \pm 3 \times 10^{-3}$ ns
E=enviournmental variance	$3 \times 10^{-4} \pm 3 \times 10^{-4}$ ns
$\sqrt{H_1/D}$ =mean degree of dominance	0.65
$H_2/4H_1$ =proportion of genes with positive and negative effects in the parents	0.24
$\sqrt{4DH_1+F}/\sqrt{4DH_1-F}$ =proportion of dominant and recessive genes in the parents	1.10
Heritability in narrow sense	0.82

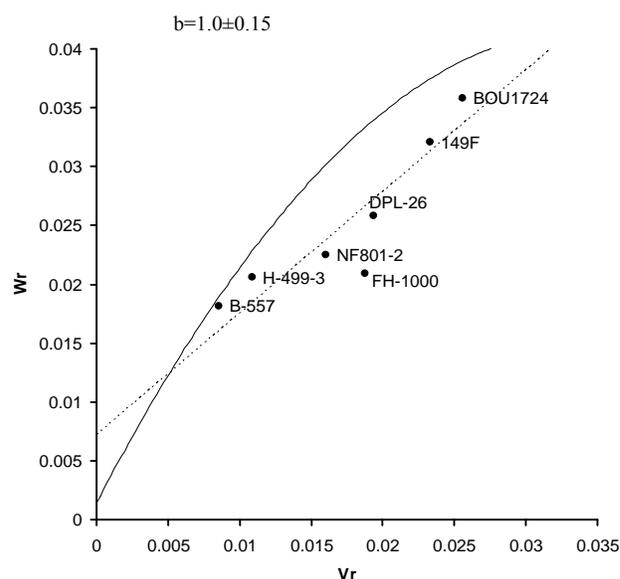
genes effects, the estimate of narrow sense heritability was high (0.82).

Relative distribution of array points along with the regression line indicated that BOU 1724 and 149F were away from the origin and thus, carried the maximum number of recessive genes for root length, whilst the remaining six lines/varieties carried varying number of dominant and recessive genes (Fig. 1).

DISCUSSION

The availability of genetically based variation in water stress tolerance is essential for breeding cultivars with enhanced tolerance to moisture stress through selection and breeding. In order to obtain such information in *G. hirsutum*, 80 genotypes/lines were assessed at seedling stage. In the present investigations, 45-days old seedlings of 80 accessions, grown under water stress and watered conditions in glasshouse, were examined for shoot and root length. This method distinguished tolerant and non-tolerant genotypes. Assessment of genotypic responses to water stress was carried out using indices of water stress tolerance

Fig 1: W_r/V_r regression for relative root length of *G. hirsutum* L. under water stress



(relative tolerance) following the method extensively used in the study of heavy metal tolerance (Bradshaw & McNeilly, 1981). The differing measurement of root and shoot lengths provided clear indication of varieties/lines to the adverse effect of water stress as shown previously in cotton (Radin & Ackerson, 1981; Loffroy *et al.*, 1983; Ball *et al.*, 1994). Water stress tolerance cannot be attributed to a genotype, because of its superiority for a single trait, therefore many different parameters required to be evaluated (Al-Hamdani & Barger, 2003). Root growth is a reliable indicator of the response of drought tolerance (Basal *et al.*, 2005) and therefore this character was examined at the seedling stage.

Comparison of 34 varieties/lines revealed useful information about potential of the material to withstand water stress and allowed the identification of some tolerant and non-tolerant genotypes. Comparison of the relative water tolerance suggests that they may be useful source of genes for enhancing the tolerance of more vigorous lines through breeding. In previous work on water stress tolerance of cotton, McMichael and Quisenberry (1991) and Ullah *et al.* (2008) indicated great variation in material tested under

control and water stress conditions and thus agreed with the present work.

In the genetic study, the additive-dominance model of Hayman (1954a, b) and Jinks (1954) was used to study the genetic mechanism controlling variation in genotypic responses to water stress at seedling stage. The data on relative root length deviated significantly from zero but not from unity, suggesting that all the assumptions underlying the genetic model were fulfilled (Hayman, 1954a). The distribution of variety points along with the regression line (Fig. 1) provided another evidence of the absence of epistasis in the inheritance of root length (Hayman, 1954a).

Genetic analysis revealed that the genes having both additive and non-additive properties appeared to be significant in controlling water stress tolerance in *G. hirsutum*, but effect of cumulative genes was more pronounced. The trend of dominance in the parents was towards the parents having higher water stress tolerance, and this was clearly advantageous in a breeding programme aimed to breed varieties for moisture stress conditions. Based upon the inheritance pattern of water stress tolerant and high estimate of narrow sense heritability, it seems that the trait is less complex in nature as suggested by Gamble (1962a, b) and Azhar and McNeilly (1988). Although estimate of narrow sense heritability appeared to be generally inflated, this is encouraging to a cotton breeder for making straightforward selection of stress tolerant plants in segregating populations.

The results of all these studies indicated that both additive and dominance gene effects are important in controlling root length in the stress conditions applied. Thus, considering root length as better indicator of response to water-stress-tolerance (Basal *et al.*, 2005), plants having longest roots may be selected from segregating population for increased, drought tolerance as had been done previously (Ashraf *et al.*, 1986a, b, 1987). Lawrence (1984) had argued that populations subjected to strong directional selection pressure showed reduced additive component for the character under selection. As there is no evidence that cotton had previously been subjected to directional selection pressure, either in the wild or cultivated material for enhanced drought tolerance, it seems likely that additive variation in water stress tolerance may be available to cotton breeders for exploitation through selection. The availability of additive component suggests that the chances of improving water stress tolerance in *G. hirsutum* are considerable subjecting the breeding material to strong selection pressure.

The estimate of heritability of water stress tolerance is inflated. Increased estimates of heritability had previously been reported in tomato (Saranga *et al.*, 1992) and maize (Khan *et al.*, 2003). In the present study, higher estimate of heritability might be the result of greater genetic variation due to the expression of genes associated with water stress tolerance or a smaller environmental variation as speculated

by Saranga *et al.* (1992). It had also been argued that hidden variation, previously unselected, could be uncovered when moderate stress is applied thus possibly increasing heritability (Bradshaw & Hardwick, 1989). As has been stated previously, the estimates of heritability are affected by numerous environmental factors, and therefore, must be used/interpreted with great care (Falconer & Mackay, 1997).

In conclusion, the estimate of heritability of water stress tolerance seem to be encouraging for breeding and making direct selection for plants in subsequent generations, based upon root length measurements at seedling stage and other characters of agronomic importance at maturity. However, further studies are needed to substantiate the present data and for developing a well conceived breeding program for water scarce areas. Such a breeding program would help to utilize the limiting water resources of the country more efficiently.

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(Received 09 November 2009; Accepted 31 July 2010)