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Genetic studies of genotypic responses to water stress in upland cotton (*Gossypium hirsutum* L.)

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Abstract

The present study was carried out to examine the potential in cotton germplasm for breeding water stress tolerant plant material, and understand the genetic basis of different morphological traits related to water stress tolerance. Portioned analysis of variance was employed to obtain good parents for this purposes. The parental genotypes MNH-512, Arizona-6218, CIM-482, MS-39, and NIAB-78 were crossed in complete diallel fashion and F_0 seeds of 20 hybrids and five parents were planted in the field in randomized complete block design with three replications during 2010. Simple regression analysis of F_1 data revealed that additive-dominance model was quite adequate for all morphological traits. The unit slope of regression lines number of bolls (b = 1.07 ± 9.14), boll weight (b = 0.99 ± 0.11), yield per plant (b = 0.96 ± 0.31), plant height (b = 1.10 ± 0.34), leaf area index (b = 0.82 ± 0.27), and ginning percentage (b = 1.01 ± 0.12) suggested that the epistatic component was absent in the inheritance of all characters studied. The result of various plant characters including seed yield showed drastic effects of water stress as compared with those assessed in non-stressed condition. Leaf area index in the analysis of variance suggested that additive variation was more important for the character. Narrow leaf varieties NIAB-78 and CIM-482 were water stress tolerant while varieties Arizona-6218, MNH-512 and MS-39 were broader leaf showing less resistant to water stress. The information derived from these studies may be used to develop drought tolerant cotton material that could give economic yield in water stressed conditions of cotton belt.

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Introduction

Cotton is a major fiber crop grown in Pakistan. It is mainly grown in Sindh and Punjab provinces where climate is hot (Ahmad and Makhdum, 1992). Cotton is used as multipurpose crop; its fiber is used in textiles in around the world, and cotton seed oil is used for edible purpose (Khan, *et al.,* 2002).

Water stress is a serious problem that limits cotton production in many regions of the world. The climatic conditions of the cotton belt of Punjab are favorable for growth and development of cotton plant, but biotic and abiotic stresses badly affect crop productivity, and yield of seed cotton is affected adversely due to water stress at reproductive phase. Cotton plant has been adapted to semi-arid regions due to extensive root system and flexible fruiting period, but drought affects root distribution seriously. The effect of water stress on yield depends the time at which stress occurred, and its intensity (Malik *et al.*, 1979). High temperature and shortage of water adversely affect the cultivation of cotton crop and these two abiotic factors are positively correlated with each other (Vanschilfgaarde, 1990). It has been observed that prevalence of drought for longer time increase the canopy temperature which affects photosynthesis and transpiration processes. Under irrigation conditions use of water is necessary for major crop growth, and different physiological and metabolic functions in plant body are controlled by water contents (Redid and Reddi, 1995).

Surface and sub-surface water resources are limiting to meet the demand of water for irrigated areas, so there is need to develop high yielding and better performing drought resistant cotton varieties to overcome this problem. Yaseen and Rao (2002) indicated that cotton yield decreased considerably when irrigation was not applied at any critical growth stage. Krieg (1997) indicated that water stress reduced crop growth rate by reducing size and number of leaves and photosynthesis. The decrease in seed cotton yield is mainly due to the reduction in number of bolls under water stress (Pettigrew, 2004; Imran, 2012). Availability of variation in cotton germplasm for moisture stress condition and its genetic basis is essential for breeding cotton material having water stress tolerance following conventional breeding methods. In the previous studies, varied genotypic responses to water stress conditions, and the characters are under polygenic control, and these results indicated improvement in the potential of upland cotton to cope with less moisture supply may be possible.

Drought is yield limiting factor, its intensity is important because even moderate water stress decrease growth and yield of seed cotton (Heatherly *et al.*, 1977). Stress tolerance is genetically controlled and linked with different physiological and morphological characters of crop plants (Singh, 2004). The present study was planned to examine the genetic basis of water stress tolerance of twenty five genotypes using indices of water stress. The relative data were analyzed following simple genetic model (Hayman, 1954 a, b; Jinks, 1954).

Material and methods

The present investigations were carried out on the genetic basis of drought tolerance in upland cotton in the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad. The climatic data were obtained from Agromet Bullet in Agriculture Meterology Cell, Department of crop physiology, University of Agriculture, Faisalabad, Pakistan shown in Fig. 1.

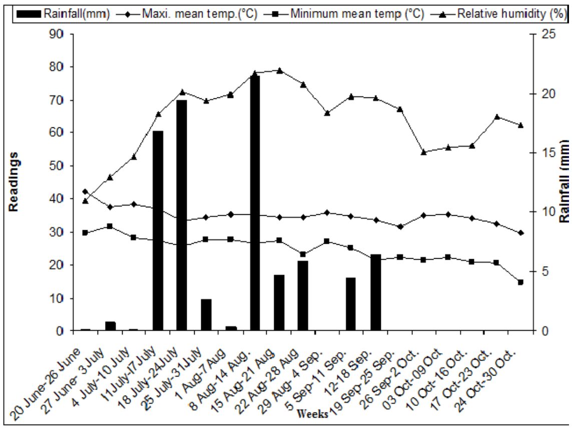

Fig. 1. Graph of meteorological data for cotton growing period in 2010.

Development of plant material in glasshouse

Five varieties of upland cotton namely MNH-512, Arizona-6218, CIM-482, MS-39, and NIAB-78, were grown in earthen pots with 30 cm height and 35 cm upper diameter. Nine kg soil was filled in these pots. Soil analyses were done before filling in the pots. The soil pH (8.4), EC (1.2 dS/m), organic matter (1.42%), saturation percentage (31%), phosphorous (28.9 ppm) and potassium (135 ppm) were noted. Seeds were soaked for eight hours before sowing. Four seeds were sown 2 cm deep in each pot and later on at two true leaf stage, the plants were thinned.

The parents were grown under day length of 14 hours, natural light (PAR raged 1400-1600 µmol m^{-2} s^{-1} at noon) and 65-80 % humidity, under optimum temperature throughout the growing period. Water was applied to the earthen pots at the rate of 1400 mL per pot daily during peak flowering period and on alternate days during off-peak flowering period. The period from 50 to 70 days after sowing was considered as peak flowering period. At peak flowering stage these varieties were crossed in diallel fashion. At maturity, seed cotton of ten direct, ten indirect hybrids and five parents were collected. This experiment was terminated after 120 days, and these crosses were ginned and seeds collected separately.

Assessment of the F_1 hybrids in the field

The seeds of 20 F_1 crosses and their parents were planted in two sets in water stressed conditions in the field during June 2010 in triplicate randomized complete block design. In each replication, there were 10 plant spaced 30 cm within row and 75 cm between the rows. No irrigation was applied throughout the growth period of entries tested under drought conditions. At the maturity eight middle plant were measured for plant height, number of bolls, boll weight, leaf area index, yield per plant and ginning percentage. Leaf area index (LAI)), ginning percentage and water stress tolerance was calculated by given below formula.

$$\text{LAI} = \frac{\text{Leaf area per plant (dm}^2\text{)}}{\text{Ground area covered by plant (dm}^2\text{)}}$$

$$\text{GOT\%} = \frac{\text{Lint weight}}{\text{Total seed cotton yield}} \times 100$$

$$\text{Relative water stress tolerance} = \frac{\text{Stressed}}{\text{Control}} \times 100$$

Statistical analysis

Portioned analysis of variance (Steel *et al.*, 1997) was performed on the data for all the characters in the order to see whether the genotypic differences are significant or non-significant. Only significant genotypic differences allowed use of Hayman-Jinks additive dominance model for genetic analysis of the data.

Results

Responses of 20 F_1 hybrids and their parents to water stress conditions were compared with that of controlled conditions, and this is called relative water stress tolerance. Partitioned analysis of variance was performed for the mean squares on different plant characters. The results showed significant differences among the 25 genotypes for all the characters measured under stressed and non-stressed conditions showing that genotypes performed differently under stress conditions.

Adequacy of additive-dominance model to the F_1 data sets

In order to know how precise the indices of water stress tolerance based upon various plant characters of *Gossypium hirsutum* L. Adequacy of the additive-dominance model and validity of some of the assumptions underlying the genetic model are usually tested by joint regression analysis (b), of the data. The results of the test are presented in Table 1. The regression coefficients (b) of all the characters, described above, deviated significantly from zero but not from unity. This property of the regression line indicated the presence of intra-allelic interaction (dominance), and independent distribution of the genes among the parents for the traits, and the genes were independent in action. Further unit slope of regression lines (b) for all the characters suggested that all the assumptions underlying the additive-dominance model were met as suggested by Hayman, (1954a).

Table 1. Component of variation in Upland cotton (*Gossypium hirsutum* L.).

Components	No of Bolls	Boll Weight	Yield per plant	Plant Height	Leaf Area Index	Ginning %
D = additive Variance	7.18±0.2	95.07±2.9	27.6±3.8	2.58±0.97	1.65±0.12	7.93±0.22
H_1= dominance Variance	-12.53±0.56	-3.73±7.84	-15.07±10.5	-13.9±2.64	-0.59±0.34	-4.73±0.59
H_2= proportion of positive and negative genes in the parents	-9.6±0.51	-3.98±7.11	-6.2±9.5	-9.42±2.39	-0.42±0.31	-3.3±0.54
F = relative frequency of dominant and recessive alleles in the parents	-4.93±0.52	0.66±7.25	-15.9±9.7	-7.91±2.44	-0.5±0.31	-1.6±0.55
E= environmental variance	6.6±0.34	10.78±1.18	16.4±1.5	8.09±0.39	0.53± 3.2	2.7±9.06
$\sqrt{H_1/D}$ = degree of dominance	0.98	0.19	0.74	2.32	0.61	0.77
$H_2 / 4H_1$ = proportion of genes with positive and negative effects in parents	0.19	0.26	0.1	0.16	0.18	0.17
$[\sqrt{4DH_1} + F] / [\sqrt{4DH_1} - F]$ = proportion of dominant and recessive genes in the parents	0.58	1.03	0.43	0.20	0.58	0.76
Heritability (ns)	0.57	0.82	0.54	0.34	0.68	0.67

Estimation of components of variation in various plant characters measured under water stress condition

The estimation of components of variation in the all components was made in the Table 1. The results showed that D was positive ($P \leq 0.05$) for all seed cotton yield components. Similarly H_1 and H_2 were non-significant ($P > 0.05$) for all studied characters. The extent of D was greater than those of H_1 and H_2 suggesting that additive gene affect appeared to be important for controlling number of bolls, boll weight, yield per plant, plant height, leaf area index and ginning percentage. The degree of dominance $\sqrt{H_1/D}$ was less than unity, thus showing partial dominance of genes and this was verified by the slope of regression line for boll weight, yield per plant, leaf area index and ginning percentage. While for number of boll and plant height, it was almost equal to one and more than one thus showed complete dominance and over dominance respectively. The genes were unequally distributed for number boll, plant height, leaf area index and ginning percentage as their magnitudes H1 is not equal to H2 with low ratio of $H_2/4H_1$ (0.19) for number of bolls, $H_2/4H_1$ (0.16) for plant height, $H_2/4H_1$ (18) for leaf area index and H_1 is greater H2 and the ratio of $H_2/4H_1$ is (0.17) for ginning percentage respectively. Whilst gene were equally distributed for boll weight and yield per plant with ratio of $H_2/4H_1$ (0.1) and $H_2/4H_1$ (0.26) respectively. The negative value of F supported by low ratio of $[\sqrt{4DH_1}+F] / [\sqrt{4DH_1}-F]$ for number of bolls (0.58), yield per plant(0.43), plant height (0.20), leaf area index (0.58) and ginning percentage (0.78) which indicated the presence of recessive genes in the parents controlling the characters. While the positive F value of boll weight (1.03) showed that the dominant genes were more significant in the parents. Due to the presence of additive gene in the genetic control the estimate of narrow sense heritability for number of bolls, boll weight, yield per plant, plant height, leaf area index and ginning percentage were 0.57, 0.82, 0.54, 0.34, 0.68 and 0.67, respectively.

The relative distribution of the array points along the regression line indicated the genetic diversity in the parental lines for number of bolls (Fig. 2). It is shown that NIAB-78 being closer to the origin had maximum number of dominant genes, whilst CIM-482 had maximum number of recessive genes. The varieties MS-39, MNH-512 and Arizona-6218 have formed an intermediate group, having both dominant and recessive genes in equal proportion. An examination of the distribution of variety points along the regression line depicted that there was greater diversity in the parents for boll weight (Fig. 3) it was shown that Arizona-6218 possessed the greatest number of dominant genes, whilst varieties MNH-512, MS-39 and CIM-482 have gained intermediate position, and in contrast NIAB-78 contained the maximum number of recessive genes for boll weight. The relative positions of array points along the regression line (Fig. 4) indicate that MS-39 carried more recessive genes for the character, whereas Arizona-6218 contained more dominant genes for yield per plant, and varieties MNH-512, CIM-482 and NIAB-78, appeared to carry both dominant and recessive genes. The scatter of varieties in fig. 5 revealed that parents differed widely from each other with respect to the presence of dominant and recessive genes. Variety MS-39 contained more number of dominant genes, and in contrast CIM-482 carried more number of recessive genes for the character. The remaining varieties *i.e.*, MNH-512, NIAB-78 and Arizona-6218 appeared to carry both dominant and recessive genes in the heritance of plant height. It is clear from fig. 6 that parents differed from each other with respect to the presence of dominant and recessive genes. Variety NIAB-78 contained the most dominant genes, and CIM-482 had more number of recessive genes for leaf area index, while the varieties like MS-39, MNH-482 and Arizona-6218 contained both dominant and recessive genes. The relative positions of array points along the regression line in fig. 7 indicate that MS-39 carried more number of dominant genes, whereas NIAB-78 contained most recessive genes for ginning percentage. Varieties MNH-512, CIM-482 and Arizona-6218 are in the mid-way of the two extremes.

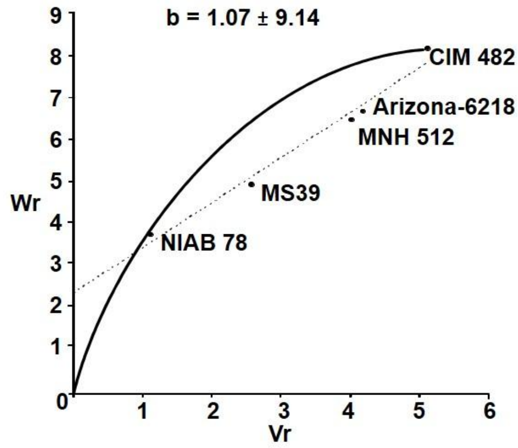

Fig. 2. Wr-Vr graph for number of bolls per plant.

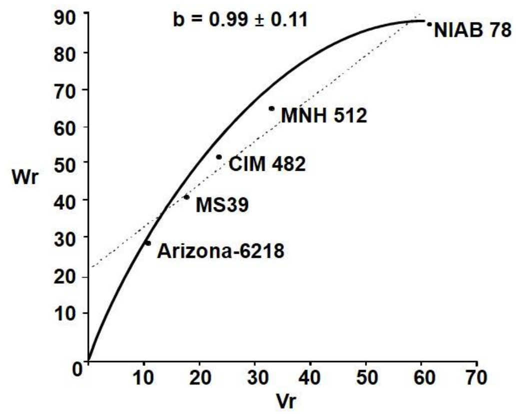

Fig. 3. Wr-Vr graph for boll weight.

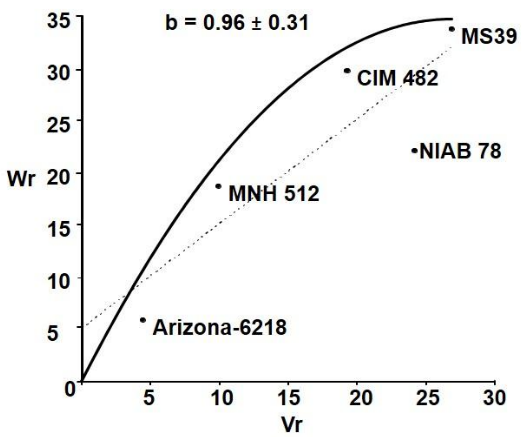

Fig. 4. Wr-Vr graph for yield per plant.

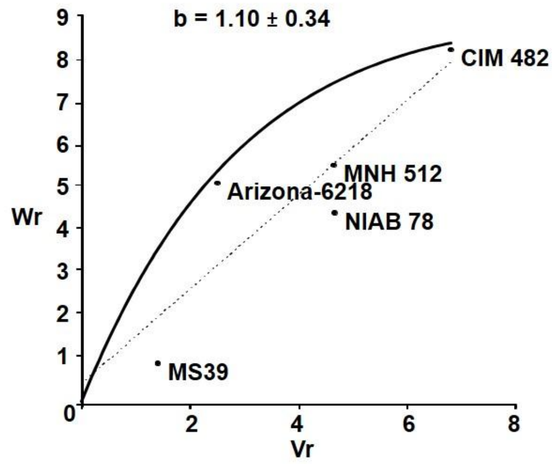

Fig. 5. Wr-Vr graph for plant height.

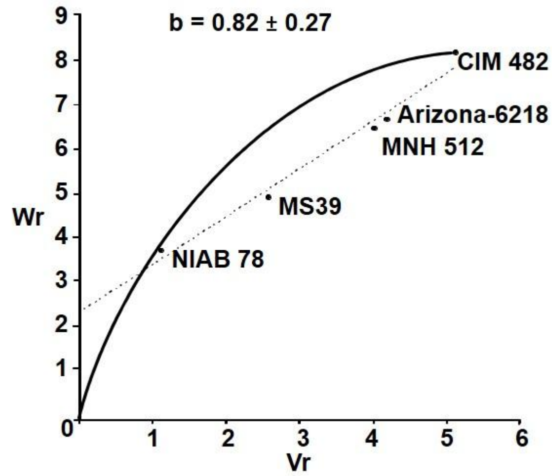

Fig. 6. Wr-Vr graph for leaf area index.

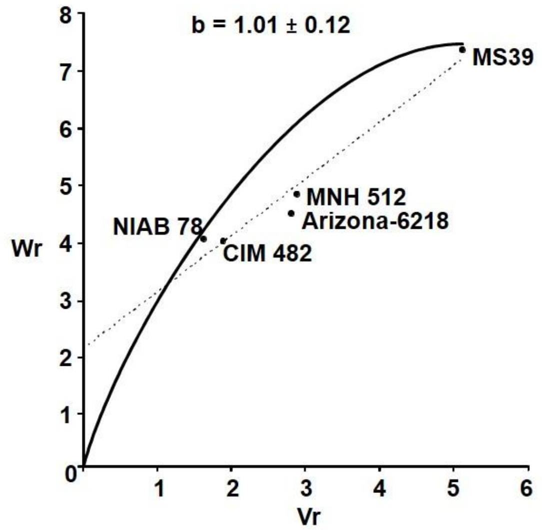

Fig. 7. Wr-Vr graph for ginning percentage.

Discussion

Irrigation water for the crops including *Gossypium hirsutum* L. is becoming a limiting factor for exploiting their yield potential throughout the world and for the same reason a research work was carried out here to study the genetic basis of variation for water stress tolerance. The expression of water stress tolerance in a crop species is a complex trait involving many plant characters, both physiological and morphological (Ingram and Bartles, 1996; Cushman and Bohnert, 2000). However, when a specific and readily quantifiable physiological mechanism conferring water stress tolerance is not available, it is suggested that plant material may be evaluated using other plant characters of agronomic importance (Turner, 1986). Data on various plant characters including yield of seed cotton showed drastic effects of water stress as compared with those assessed in non-stressed condition. It has been observed that 25 genotypes responded differently to water stress, and this indicated the existence of significant analysis of variation in all the plant characters examined in the present study. Indices of water stress tolerance (relative stress tolerance) based upon yield of seed cotton and its components and leaf area index were analyzed using partitioned analysis of variance which again provided a clue of the existence of significant genotypic differences. Indices of water stress tolerance has previously been used by Iqbal *et al.* (2011) from studying the genetic mechanism controlling water stress tolerance at seedling stage and at plant maturity. Therefore, the work reported has found sufficient instructions for the use of indices of water stress tolerance to study the genetic basis.

Simple additive-dominance model appeared to be adequate for analyzing all the plant characters measured in the present study, and in all these cases genes acted additively with varying degree of dominance. It is noted that trend of dominance in case of number of bolls, boll weight and yield of seed cotton, were towards the parents with decreasing the characters, whilst the negative sign of 'h' for plant height and leaf area index is good indication of the potential of plant material and provided hope for bringing further improvement under water stress conditions. Results regarding leaf area index in the analysis of variance suggested that additive variation was more important for the character. Narrow leaf varieties NIAB-78 and CIM-482 were water stress tolerant while varieties Arizona-6218, MNH-512 and MS-39 were broader leaf showing less resistant to water stress. Thus clearly, leaf area may be used as a tool for the selection of genetic material having tolerance for water stress. It is encouraging that inheritance of leaf area index was controlled by the genes affecting additively, and genetic components showed partial dominance in the inheritance of the character. The estimate of narrow sense heritability for leaf area index was 0.68. The previous studies showed that high estimates of h^2_{ns}, 0.82 and mode of gene action proposed that it was possible to improve drought tolerance in cotton (*Gossypium hirsutum* L) by single plant selection in later segregating generations (Iqbal *et al.* 2011).

When a lot of germplasm is available for screening against any stress condition, a rapid and efficient technique should be used for the identification of variation present in the material. The relative values of 20 F_1 hybrids and their five parents grown under water stressed and controlled conditions in the field were examined for six morphological characters. This method distinguished tolerant and susceptible genotypes. Previously, scientists had studied growth of cotton to moisture stress (Radin and Ackerson, 1981; Loffroy *et al.*, 1983; Ball *et al.*, 1994). In the present study, both additive and dominance properties of the genes appeared to be important for the variations in the characters related to water stress tolerance, the genes acting additively showed the predominance influence on the genetic control of all these traits. It was also studied that water stress tolerance cannot be credited to a single genotype due to its dominance for a single trait; therefore different parameters were required for evaluation (Al-Hamdani and Barger, 2003). It has been suggested that taller varieties with narrow leaves were found most suitable for water stress environment. The present results revealed that although plant height, leaf area index, seed cotton yield, and its components were reduced due to adverse effects of water stress, genotypes responded differently and some of the families like NIAB-78 × NIAB-78, CIM-482 × CIM-482, NIAB-78 × Arizona-6218, CIM-482 × Arizona-6218 and NIAB-78 × MS-39 showed have less indices for water stress tolerance while all the other families have greater values. It was also found that all the characters measured under water stress and controlled conditions, thereby meaning water stress tolerance, were largely influenced by additive genes. The previous work on water stress tolerance in cotton indicated significant variation in material tested under control and water stressed conditions (McMichael and Quisenberry, 1991; Ullah *et al.*, 2008).

In the present study it may be revealed that variation for water stress tolerance may be present in the cotton germplasm, and these variations were measured by genetic analysis of morphological characters finally, suggesting that all the characters studied were genetically controlled. These studies further concluded that morphological traits were controlled largely by additive genes. However, careful examination would be helpful in isolating suitable drought tolerant plants, based upon the heritability estimate.

Conclusion

Cotton production is mainly affected by several environmental constraints and water stress is the major constraint in Pakistan. From this experiment it may be concluded that drought has drastic effect on morphological traits and found to be genetically controlled. Further it was revealed that water stress tolerance was polygenic complex trait and governed by additive gene action. In case of number of bolls, boll weight, yield of seed cotton and ginning out turn, trend of dominance was towards the decreasing characters. Negative sign of ‘h’ for plant height, leaf area index and fiber fineness gave an indication of potential of plant material for further improvement. Narrow sense heritability for all the characters studied was very high suggesting single plant selection in later segregating generation. Assessment of genotypic responses to water stress in *G. hirsutum* is helpful to the breeders for comparing the potential of varieties/lines of cotton to drought tolerance. The information derived from these studies may be used to develop drought tolerant cotton material that could give economic yield in water stressed conditions of cotton belt.

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