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BIOMETRICAL ANALYSIS OF VARIABILITY IN PLANT HEIGHT AND SEED COTTON YIELD IN *GOSSYPIUM HIRSUTUM* UNDER WATER STRESS CONDITIONS

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Seedlings of 50 varieties/lines of *Gossypium hirsutum* were screened for water stress tolerance during 2010-2013. Indices of water stress tolerance were calculated on the basis of shoot and root lengths. Based on similarities and differences, CIM-496, 149F, DPL-26, BOU-1724 and B-557 were found tolerant to moisture stress, whilst FH-1000, NF-801-2-37, MNH-129 and H-499 exhibited susceptibility to water stress. Results of screening revealed that variability among germplasm was present which may be exploited through hybridization. Therefore, F_1 crosses were developed in the field keeping stress tolerant genotypes as lines and susceptible as testers using line × tester technique. The genetic material developed was field-planted and 50% moisture stress was applied to examine responses in plant height and seed cotton yield. The analysis of F_1 and parental data revealed the preponderance of non-additive genetic effects for both traits. Testers and interaction between parents contributed more to expression of plant height and seed cotton yield respectively. CIM-496 with significant and positive general combining ability estimates, i.e. 1.83 for plant height and 3.65 for seed cotton yield was found to be good general combiner for both the traits. The cross B-557 × H-499 was the best varietal combination for plant height and seed cotton yield. Non-additive variation for both traits suggests possibility of using this material for hybrid development in drought hit areas. Plant height did not significantly affect seed cotton yield as shown by non-significant correlation coefficient (0.3).
Keywords: Water stress tolerance, Line × Tester analysis, Additive variation, Cotton germplasm

INTRODUCTION

Cotton (*Gossypium hirsutum*), being originated in hot climates of Asia, Africa and America, is adapted to limited moisture conditions, and thus has been categorized as drought tolerant (Wendel and Cronn, 2003). In Pakistan, *hirsutum* species regardless of whether it is irrigated or not, is often exposed to water deficit conditions, and it had been reported that shortage of fresh irrigation water at flowering and fruiting stages significantly affected lint production and quality, besides inhibition of new flowering sites (Pettigrew, 2004; Karl *et al.,* 2009). Thus owing to the growing problem of water shortage, breeding of cotton suitable for water deficit areas has emerged as an area of special interest for plant breeders. This alternative approach constitutes genetic modification of conventional crop plants, to make them suitable for water stressed areas. In order to be able to produce such material, two basic requirements fundamental to all plant breeding programmes, must be available. Firstly, there must be variability for water stress tolerance in a crop as a whole, and secondly this variation must involve significant genetic component. For effective exploitation of genetic resources through selection and breeding, information about these two components must be obtained. Previous studies on drought tolerance are few, but these provide evidence on the occurrence of variation in various plant species, for example, sunflower (Chimenti *et al*., 2002, Rauf *et al.*, 2009), soybean (Daneshian *et al.*, 2010) and corn (Khodarahmpour, 2011; Meeks *et al.,* 2013).

Previous information on variability for drought tolerance in upland cotton is not extensive, however only few studies reported presence of variation for water stress tolerance at seedling stage (Pace *et al.,* 1999; Longenberger *et al.,* 2006; Irum *et al.,* 2011). Such information at adult stage is also necessary and workable variation had been reported in characters like plant height, root length, shoot length, stem diameter, number of bolls and boll weight (Mahmood *et al.*, 2006; Ahmad *et al.*, 2009; Iqbal *et al.*, 2010). Early researchers reported that selection among segregating generation under moisture stress conditions had been rewarding in wheat (Kirigwi *et al.,* 2004; Golabadi *et al.,* 2006). In different studies conducted to investigate genetic basis of water stress tolerance of mature cotton plants, Karademir *et al.* (2007), Ahmad *et al.* (2009) and Iqbal *et al.* (2011) reported preponderance of additive gene action whilst Shakoor *et al.* (2010) and Basal *et al.* (2011), found non-additive genes controlling the inheritance of different agronomic traits.

MATERIALS AND METHODS

The present research work was conducted in glasshouse and research area of Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan during 2010-2013. As a first step, 50 varieties/lines were examined at seedling stage under water stress conditions to identify water stress tolerant and susceptible genotypes. The selected drought tolerant and susceptible parents were hybridized in field following line × tester mating design (Kempthorne, 1957) to develop plant material for genetic studies. Information derived from these investigations may be of potential use for continued improvement in drought tolerance in *Gossypium hirsutum* in this area.

Assessment of germplasm in glasshouse: Responses of 50 cotton varieties/lines (listed in Table 1) to water stress were examined at seedling stage using polythene bags (25×15 cm) filled with 1.15 kg soil. The experiment was conducted in two different sets. Presoaked seeds of each varieties/lines were sown in holes made in polythene bags at 2.5 cm depth. During germination and growth, temperature and humidity in glasshouse was maintained at 35°C and 65-80% respectively during day and night using hot water circulating in pipes and electric heaters. The plants were exposed to natural sunlight and supplemented with artificial lighting to attain a photoperiod of 16 hours. Seedlings were thinned to one plant per bag after two weeks of planting, and after every 14 days, 0.25 g Urea (46% Nitrogen) was added to each bag (Murtaza, 2006). Plants were watered daily and sprayed (when required) to avoid the attack of sucking and chewing pests. At the appearance of first true leaf, two different water levels were used according to the calculated field capacity of soil in bags, first set was watered daily up to 100% field capacity (T_o); and in second set stress was developed by withholding moisture upto 80% of field capacity (T_1). Stress treatment was continued for four weeks and thereafter seedlings were measured for longest shoot and root lengths. Data on plants of each variety grown under

Table 1. Values of relative shoot and root lengths of 50 cotton genotypes used for water stress tolerance study with their origin

Sr.#	Cultivars	Source/Origin	RSL	RRL	Sr.#	Cultivars	Source/Origin	RSL	RRL
1	NIAB-78	NIAB, FSD	68	78	**26**	Paymaster-54	Exotic	65	71
2	NIAB-111	NIAB, FSD	80	82	**27**	NF-801-2-37	Exotic	61	60
3	CIM-70	CCRI, Multan	71	69	**28**	NIAB-313/86	NIAB, FSD	67	75
4	CIM-109	CCRI, Multan	60	65	**29**	NIAB-313/36	NIAB, FSD	63	76
5	CIM-240	CCRI, Multan	60	65	**30**	NIAB-766	NIAB, FSD	68	76
6	CIM-496	CCRI, Multan	80	83	**31**	CIM-443	CCRI, Multan	63	74
7	CIM-446	CCRI, Multan	65	78	**32**	CIM-435	CCRI, Multan	66	73
8	CIM-448	CCRI, Multan	80	84	**33**	CIM-90	CCRI, Multan	66	79
9	CIM-473	CCRI, Multan	65	74	**34**	MNH-552	CRS, BWP	79	83
10	CIM-707	CCRI, Multan	65	78	**35**	MNH-129	CRS, Multan	58	58
11	BH-36	CRS, BWP	80	83	**36**	CRIS-164	CRIS, Sakrand	64	69
12	BH-118	CRS, BWP	79	83	**37**	CRIS-209	CRIS, Sakrand	67	74
13	FH-900	CRI, FSD	63	74	**38**	CRIS-403	CRIS, Sakrand	65	71
14	FH-901	CRI, FSD	66	74	**39**	CRIS-90	CRIS, Sakrand	64	71
15	MNH-93	CRS, Multan	65	72	**40**	Babdale	Exotic	65	72
16	MNH-554	CRS, Multan	69	79	**41**	FH-925	CRI, FSD	64	79
17	DPL-26	Exotic	81	84	**42**	FH-87	CRI, FSD	61	63
18	149F	CRS, Multan	83	85	**43**	124F	CRI, FSD	65	77
19	B557	CRI, FSD	84	84	**44**	4F	CRI, FSD	61	67
20	BOU-1724	Exotic	83	86	**45**	FH-1000	CRI, FSD	61	63
21	S-12	CRS, Multan	62	64	**46**	Acalasj.4	Exotic	64	67
22	HR-1	Exotic	67	72	**47**	PB-899	PBG,UAF, FSD	65	79
23	H-499	Exotic	57	60	**48**	PB-900	PBG,UAF, FSD	64	79
24	Acala 1517C	Exotic	68	73	**49**	PB-38	PBG,UAF, FSD	79	83
25	NIAB Karishma	NIAB, FSD	70	69	**50**	PB-39	PBG,UAF, FSD	64	71

RSL: Relative shoot length, RRL: Relative root length, NIAB: Nuclear Institute for Agriculture and Biology, Faisalabad, CIM:Cotton Institute, Multan (for CCRI, Multan), CRS: Cotton Research Station, CRI:Cotton Research Institute, CCRI: Central Cotton Research Institute, FSD: Faisalabad, BWP: Bahawalpur, PBG: Plant Breeding and Genetics, UAF: University of Agriculture.

T_o and T_1 conditions, were used to calculate indices of water stress tolerance (Baloch *et al.* 2011; Iqbal *et al.,* 2011). These indices were then subjected to ordinary analysis of variance in order to see significant genotypic differences among 50 cotton varieties/lines (Steel *et al*., 1997).

Development of plant material for genetic studies: Based upon longest shoot and root lengths, five genotypes namely B-557, BOU-1724, DPL-26, CIM-496 and 149F were identified as water stress tolerant, whilst FH-1000, NF-801-2-37, MNH-129 and H-499 turned out to be susceptible due to showing drastic reduction in shoot and root lengths under water stress. The genetic material was developed in field during year 2011 by hybridizing these nine genotypes following line × tester mating design (Kempthorne, 1957). Water stress tolerant genotypes were used as females (lines) and susceptible genotypes as males (testers). At maturity, seed cotton from crossed and selfed bolls were collected, and ginned with single roller electric gin machine. The number of cotton seed obtained per cross was approximately 20-25.

Assessment of F_1 hybrids for water stress tolerance: Whole-plant-response of the genetic material to water stress was studied in field. Seeds of 20 F_1 hybrids and their nine parents (five lines and four testers) were field-planted during May 2012 in three replications following randomized complete block design. Seeds of each of the 29 entries in each replication were planted in single row plot having six plants, spaced at the distance of 75cm between the rows and 30cm within the rows. All agronomic practices i.e. hoeing, irrigation and fertilization from sowing to maturity were followed. The seeds of 29 entries were soaked in tap water for 8 hours before sowing, and four seeds of each entry were sown at 2cm depth. This experiment was conducted using normal water supply i.e. 100% field capacity (T_o), and giving stress i.e. 50% of field capacity (T_1). On the emergence of first square i.e. 30 days after sowing, water stress was imposed. At maturity, data on four guarded plants per entry, in each replicate were recorded for plant height and seed cotton yield to evaluate the genotypic responses to water stress.

Plant height (cm) was measured from first cotyledonary node to the apical bud, and for seed cotton yield, two pickings of the genetic material were done from the bolls showing maximum opening. The harvest of a plant was weighed (g) on electronic balance. Relative plant height and seed cotton yield (indices of water stress tolerance) of each family were computed for analysis of variance technique (Steel *et al.,* 1996) in order to estimate the significance of genotypic differences. Genetic analysis of data was done according to Line × Tester technique (Kempthorne, 1957) to calculate general combining ability (GCA) and specific combining ability (SCA) effects. Genetic correlation was also worked out to study the relationship between plant height and seed cotton yield.

RESULTS

Variability for water stress tolerance**:** Complete results of analysis of variance have been omitted from the text, however mean square values are given here. These mean square values revealed significant differences ($P \leq 0.01$) among 50 cultivars/lines for shoot (MS value: 162.03^*) and root lengths (MS value: 170.65^*). The comparison of indices of water stress tolerance based on shoot lengths indicated that NIAB-111, DPL-26, 149F, B-557, BOU1724 exhibited better shoot growth under water deficit conditions with indices ranging between 80 to 84%, and therefore were found to be water stress tolerant. In contrast, varieties S-12, CIM-109, CIM-240, H-499, MNH-129, FH-87 and FH-1000 with low indices, i.e. 57-60% revealed susceptibility to water stress, and thus were characterized as sensitive genotypes. Varying responses against water stress were also manifested by other cotton genotypes studied under the current investigations.

The comparison of indices values based on root lengths revealed BOU1724 (18.8cm) to be a tolerant variety with highest tolerance index 88%, while DPL-26 (19.7cm), 149F (18.9cm), B-557 (18.9cm), CIM-448 (16.2cm), BH-118 (17.8cm) and PB-38 (16.4cm) appeared to be less affected under water deficit conditions with indices ranging from 83 to 88%. Varieties which showed significant decrease in root lengths were NF-801-2-37 (12.1cm), H-499 (13.7cm), MNH-129 (12.1cm), FH-87 (11.5cm), FH-1000 (11.2cm) with indices ranging from 54 to 63%, and therefore identified as sensitive to water stress.

Genetic analysis of plant height and seed cotton yield: Mean square values from analysis of variance indicated significant differences among nine parents and their 20 F_1 hybrids ($P \leq 0.01$) for plant height and seed cotton yield under water stress condition (Table 2). Biometrical analysis showed that mean squares due to GCA effects (parents) and interaction due to parents vs crosses revealed significant differences for plant height and seed cotton yield. Mean squares due to testers differed highly significantly for plant height.

Comparison of general combining abilities (GCA) of five lines and four testers revealed CIM-496 to be good general combiner due to significant and positive GCA coefficients for plant height (1.83) and seed cotton yield (3.65). Among testers, H-499 and FH-1000 having significant and positive GCA estimates appeared to be best general combiners for plant height (3.33) and seed cotton yield (3.36) respectively (Table 3).

Table 2. Mean squares based on indices of water stress tolerance for plant height and seed cotton yield using Lines × Testers analysis

Source of variation	Df	Plant height	Seed cotton yield
Replications	2	50.76^{ns}	303.96*
Genotypes	28	161.78**	123.02**
Parents	8	257.27**	132.81**
Crosses	19	1862.93**	1075.52**
Parents vs Crosses	1	32.04^{ns}	68.76^{ns}
Lines	4	18.12^{ns}	76.25^{ns}
Testers	3	2.63*	79.75^{ns}
Line x Tester	12	19.87^{ns}	63.53^{ns}
Error	56	37.68	66.32^{ns}

* denotes differences significant at 5% probability level, ** denotes differences significant at 1% probability level, ^{ns} denotes non significant

Table 3. General combining ability estimates of lines and testers for plant height and seed cotton yield

Parents	Plant height	Seed cotton yield
Lines		
CIM-496	1.83*	3.65*
149F	-0.87	-2.14
DPL-26	-1.68	-2.53
BOU-1724	0.53	1.04
B-557	0.79	-0.02
Standard Error	1.77	2.35
Testers		
FH-1000	0.29	3.36*
NF-801-2-37	-0.78	-0.37
MNH-129	-2.84	-1.36
H-4993	3.33*	-1.63
Standard Error	1.58	2.10

Specific combining abilities (SCA) of different crosses for plant height and seed cotton yield are given in Table 4. The cross BOU-1724 × FH-1000 was identified as best varietal combination for plant height due to significant and positive SCA (3.73). For seed cotton yield, crosses DPL-26 × MNH-129 and B-557 × H-499, with significant SCA coefficients 4.72 and 7.43 respectively were identified as superior combinations.

Table 4. Specific combining ability estimates of various crosses obtained from relative values for plant height and seed cotton yield

Name of crosses	Plant height	Seed cotton yield
CIM-496 × FH-1000	0.14	1.36
CIM-496 × NF-801-2-37	0.63	1.68
CIM-496 × MNH-129	0.92	4.50
CIM496 × H-499	-1.69	-7.55
149F × FH-1000	-2.57	-0.51
149F × NF-801-2-37	-0.28	1.76
149F × MNH-129	0.54	-1.10
149F × H-499	2.32	-0.16
DPL-26 × FH-1000	0.93	-1.02
DPL-26 × NF-801-2-37	1.09	-1.95
DPL-26 × MNH-129	-3.02	4.72*
DPL-26 × H-499	1.00	-1.75
BOU-1724 × FH-1000	3.73*	0.99
BOU-1724 × NF-801-2-37	-1.67	3.28
BOU-1724 × MNH-129	2.14	-6.29
BOU-1724 × H-499	-4.20	2.03
B-557 × FH-1000	-2.23	-0.82
B-557 × NF-801-2-37	0.23	-4.78
B-557 × MNH-129	-0.58	-1.83
B-557 × H-499	2.58	7.43*
Standard Error	3.54	4.70

Genetic components computed for plant height and seed cotton yield using indices of water stress tolerance revealed that variance due to specific combining ability ($\sigma 2$sca) is greater than that due to general combining ability ($\sigma 2$gca) for plant height and seed cotton yield, indicating the pre-dominant role of non-additive genes and the negative sign suggested the direction of dominance towards dwarf parent (Table 5). The ratio of variance resulting from general and specific combining

Table 5. Genetic components of variation based on indices of water stress tolerance for plant height and seed cotton yield using Lines × Testers analysis

Plant Characters	Genetic components of variation				
	σ^2_{gca}	σ^2_{sca}	σ^2A	σ^2D	$\sigma^2_{gca}/\sigma^2_{sca}$
Plant height	0.36	-5.94	0.71	-5.94	-0.06
Seed cotton yield	0.15	-0.93	0.31	-0.93	-0.16

σ^2_{gca} = Estimate of GCA variance, σ^2_{sca} = Estimate of SCA variance, σ^2A = Additive variance, σ^2D = Dominance variance, $\sigma^2_{gca}/\sigma^2_{sca}$ = Variance ratio

Table 6. Contribution of parents and their interaction to total variance for plant height and seed cotton yield and their correlation (r)

Plant Characters	Lines	Testers	Line × Tester	r
Plant height	12	49	39	0.3
Seed cotton yield	23	18	59	

ability ($\sigma 2_{gca}/\sigma 2_{sca}$) was below unity, thus confirmed the presence of non-additive genes in the inheritance of both the traits.

The phenotypic expression of plant height was largely determined by the testers (49%) followed by interaction between lines × testers (39%). However, interaction term (59%) significantly influenced the final expression of seed cotton yield as compared to contribution of lines (23%) and testers (18%) (Table 6).

Genetic correlation (r) between plant height and seed cotton yield: Simple correlation analysis using indices of plant height and seed cotton yield revealed positive but non-significant correlation between plant height and seed cotton yield (r = 0.30) under water stress conditions (Table 6).

DISCUSSION

In the present research work, shoot and root length data at seedling stage were used to study variation for these two characters for water stress tolerance. It was observed in the current investigations that effect of water stress on shoot growth was greater than that on root growth. For shoot length, H-499 showed least tolerance against water stress i.e. 57% whilst the variety B557 revealed maximum tolerance (84%) against water stress for the same trait. In previous studies, similar responses of shoot lengths to water stress were observed in bajra (*Pennisetum glaucum*) and cotton (*Gossypium hirsutum*) (Govindaraj *et al.,* 2010; Iqbal *et al.,* 2011), whilst Huang and Gao (2000) reported extreme effect of water shortage on root growth in tall fescue (*Festuca arundinacea*). Root is an important organ for the study of water relations to plants. Examination of the data for total number of varieties revealed general pattern of responses to water stress, showing a diverse range in responses to moisture stress. It is clear from these data that workable variability is available in the plant material examined here, and it is possible to exploit it through hybridization. Development of roots in cotton was genetically controlled (Basal *et al.,* 2003; Iqbal *et al.,* 2010; Klueva *et al.,* 2000; McMichael and Quisenberry, 1991; Pace *et al.,* 1999; Riaz *et al.,* 2013), but change in expression of root traits due to environmental influence had also been reported (Cooper *et al.,* 2009; Dorlodot *et al.,* 2007). Malik *et al.* (2011) had suggested that seedling data must be substantiated with those of mature plants. In the present studies, it was not possible to measure root length of plants at adult stage due to destructive, laborious and tedious methods for extraction of roots from soil profile (Herder *et al.,* 2010; Comas *et al.,* 2013).

When such a potential variability is available in the germplasm, it is important to examine the components of variation i.e. additive and non-additive using a biometric method. Line × tester analysis (Kempthorne, 1957) partitioned additive and non-additive variation at maturity. The variance due to general combining ability (σ^2_{gca}) was lower than that due to specific combining ability (σ^2_{sca}) for plant height and seed cotton yield, indicating the predominant role of non-additive genes under water stress conditions (Griffing, 1956), and consequently may exhibit low heritability (Falconer and Mackay, 1996). Thus based on this information, direct selection of desirable plants is not recommended in segregating generations as had been suggested by Basal *et al.* (2011) and Fellahi *et al.* (2013). Previous studies on cotton reporting significant control of non-additive genetic effects for plant height and seed cotton yield supported the findings of the present investigation (Sarwar *et al.,* 2012; Simon *et al.,* 2013).

Comparison of general combining abilities of nine parents (five lines and four testers) revealed that CIM-496 was a good general combiner for plant height and seed cotton yield. Among testers, H-499 and FH-1000 showed good GCA for plant height and seed cotton yield respectively. Varietal combination B-557 × H-499 was best for plant height and seed cotton yield, involving B-557 with good GCA and H-499 with poor GCA.

In the present study, plant height did not appear to affect seed cotton yield significantly due to weak correlation between two traits (r = 0.3). Taller plants have more fruiting branches and fruiting initiation points which contribute directly to increased yield. However, tallness was undesirable during water deficit conditions because taller varieties had higher water requirement due to rapid growth rate, therefore, medium heighted varieties have been considered as suitable for areas facing water scarcity. Therefore, parents having negative GCA for plant height may be considered better as had been observed in cotton by Rauf *et al.* (2006), Ahmad *et al.* (2009), Baloch *et al.* (2011) and Soomro *et al.* (2012). Variation in performance of parents and hybrids could be justified on the basis of differences in genetic makeup and environmental conditions prevailing during experiments (Pettersen *et al.*, 2006; Imran *et al.,* 2012).

The information generated from the present investigations is limited to the plant material observed under local conditions and therefore, may not be generalized for whole of the areas facing shortage of irrigation water in the cotton belt of Pakistan. Therefore, it is suggested that this information must be substantiated by another genetic experiment which may involve a reasonable number of cotton genotypes, evaluated under diverse environments in order to enhance stress adaptation of existing commercial cultivars and to develop new cultivars with improved drought tolerance for the cotton growing areas.

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