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Full Length Article

Characterization of Cotton (*Gossypium hirsutum*) Germplasm for Drought Tolerance using Seedling Traits and Molecular Markers

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Abstract

Drought tolerance is the major area of thrust nowadays, due to the water scarcity and climate change scenario, around the globe. Looking for the novel cotton germplasm tolerant to drought is an important breeding objective of the major breeding programs. On the basis of these grounds, 42 cotton varieties were evaluated for their genetic potential to perform under limited water conditions (60% field capacity). The germplasm was subjected to the seedling stage drought stress, to observe genetic variation within germplasm for various traits related with drought stress viz. shoot length (cm), root length (cm), excise leaf water loss (ELWL) and relative water contents (RWC). These traits were used as stress indicators and significant variation was observed for these estimates. Among the seven tolerant accessions, DPL-26 and 149F were identified as the highly tolerant due to their least SSI estimates for most of the characters studied. Likewise among the susceptible lines, FH-1000 and NF-801 were identified as highly susceptible. Our studies showed the presence of genetic variability for the trait of interest and it exhibits its potential for exploitation in the future breeding for drought tolerance.

Keywords: Drought; Seedling characters; Stress susceptibility index (SSI); Variability; SSR similarity matrix

Introduction

The production potential of major agricultural crops is hampered seriously due to non-availability of irrigation water in arid and semiarid regions of the world (Nawaz *et al*., 2013). Various soil surveys indicate that major land area of the world is either completely desert (64%) or suffering from severe water shortage and is categorized as dryland (57%) drought (FAO, 2000).

Low yield, in all arable regions, is due to the adverse effects of water stress (Henry and Lehouerou, 1996). It signifies the need for the development and characterization of new cotton germplasm for subsequent exploitation in the cotton breeding programs targeting this economically important trait. Pakistan is also not an exception to this situation; therefore, breeding for drought tolerance is also an important breeding objective in the country. Upland cotton (*Gossypium hirsutum* L.) is an important cash crop of Pakistan which bring substantial amount of foreign exchane to home. Although various factors related to management policies are involved in the low productivity but drought is the major future concern due to the diminishing water resource of the country.

Existence of genetic variability in morpho-physiological characters associated with stress tolerance can be exploited successfully to cope with the stress. Various additive and non additive genetic interactions are involved to drive this trait (Ullah *et al*., 2008; Iqbal *et al*., 2010). Quantitative and complex genetics of this trait, in *G. hirsutum* L., demands more consistent and focused efforts to exploit it successfully in the breeding programs (Ullah *et al*., 2008; Iqbal *et al*., 2010).

Selection for morpho-physiological components of drought, at the seedling stage, is of great importance because tolerance, at this critical growth stage, contributes to high yield of seed cotton (Longenberger *et al*., 2006; Iqbal *et al*., 2010). It is particularly true when the breeding program is targeting the water stress tolerance (Khan *et al*., 2008; Jajarmi, 2009; Qayyum *et al*., 2011). Among different seedling traits, root characteristics play an important role for carrying out different physiological processes for plant growth such as uptake and assimilation of nutrients, stress signals about water deficit stress and movement of water from soil to plant (Rauf and Sadaqat, 2008; Bibi *et al*., 2012). Therefore, root length could be used for rapid and early screening of large amount of germplasm against water stress.

To screen large amount of germplasm, Fisher and Maurer (1978) suggested the use of stress susceptibility index (DSI/SSI) as an efficient and high through put tool to

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assess the genetic potential for drought tolerance. This tool was used by different people, with slight modification, in cotton and successfully identified the variation for this trait (Cook, 1989).

During current study efforts were directed to use the SSI as a high through put measure to assess the genetic potential of 42 cotton cultivars for exploitation in the drought tolerance program. Technique was used due to its simplicity and high efficiency and consistency.

Materials and Methods

In the present studies, response of 42 cotton accessions including varieties and breeding lines to water stress and non-stress conditions was examined at seedling stage in glasshouse (Table 1). Seeds of all the varieties were planted during November, 2009 in polythene bags measuring 30 × 15 cm, filled with about 1.7 kg silt mixed with 100 g farm yard manure(FYM). The pH of soil was 8.4, EC 1.2 dS m^{-1}, saturation of the mixture (Soil + FYM) 38%. Thus, field capacity of the soil-FYM mixture was 19%, being half of the saturation percentage. All the bags were saturated to field capacity before planting seeds. Seeds were soaked overnight before sowing in the bags. Two seeds were sown in each bag, and, after germination, were thinned to one seedling. Recommended dose of nitrogen@0.25g urea was applied to each bag after every 14 days (Murtaza, 2004). Temperature in the glasshouse was maintained at 35°C/30°C (day/ night) using hot water circulation system. Day light intensity was maintained at 2500 lux for 14 hours with the help of electric lamps. Triplicate completely randomized design was used in the glasshouse. Thirty polythene bags of each accession were divided into two sets i.e. non-stressed (T_0) and stressed (T_1) treatments accounting for 5 bags per replicate. Growing seedlings in each water regime were watered to 100% field capacity daily till the development of first true leaf, and at this stage water stress was imposed to plants (T_1). When water content in soil reached to maximum allowable deficit (MAD; that is 50% of the field capacity), non-stressed (T_0) seedling plants were watered to 100% field capacity following Samarah (2005), however, the seedlings, treated under water stress, were irrigated to 60% of the field capacity. Polythene bags were weighed daily, and seedlings were watered accordingly when control plants reached to MAD. The experiment was continued till full expansion of 3rd main stem leaf, and at this stage three fully expanded leaves of all the varieties were evaluated for relative water content (RWC) and excise leaf water loss (ELWL) in three replicates. Soon after excision, fresh weight of leaves was evaluated using digital electronic balance (KEISO KKI 71482, USA). The leaf samples were left on the bench in the laboratory for six hours, and thereafter wilting weight of the samples were taken. These leaf samples were dipped overnight in water tank for recording turgid leaf mass. The samples were oven dried weighted. RWC was calculated using the formula used by Ali *et al.* (2011).

$$RWC = [(\text{Fresh mass} - \text{dry mass})/ (\text{Turgid mass} - \text{dry mass})] \times 100$$

ELWL was calculated as:
Fresh mass – wilted mass/dry mass (Ali *et al.*, 2011).

Further five seedling of each accession grown under non-stressed (T_0) and stressed (T_1) conditions were gently uprooted to avoid breakage of roots, and were separated by cutting at the junction of root and shoot. Measuring tape was used for the measurement of shoot and root lengths (cm), and means of each variety assessed under both the water regimes.

Stress Susceptibility Index (SSI)

SSI is a measure of stress resistance based on minimization of yield loss under stress compared with that under optimum conditions. It was used to characterize relative stress tolerance of all genotypes according to the formula given by Fisher and Maurer (1978):

$$SSI= [1-(Ys/Yp)]/SI$$

Where, Ys = reading of character in water stress, Yp = reading of character in control condition, while SI = stress intensity was calculated as:

$$SI= 1-(Ys/Yp).$$

Where, $\bar{Y}s$ = mean of all genotypes in susceptible condition, $\bar{Y}p$ = mean of all genotypes in control condition.

Molecular Marker Studies

Five most tolerant and five most susceptible cotton lines were identified from a sample of 42 genotypes and they were further used for genetic characterization to find out the most diverge genotypes that will be exploited in further breeding programs. For this purpose the PCR-based co-dominant microsatellite markers (SSRs) were used. Few young leaves of each genotype were collected at seedling stage. DNA extraction was performed by CTAB method (Murray and Thompson, 1980). DNA samples giving smear in the gel were rejected. DNA dilutions were prepared from stock samples in a concentration of 30 ng/µL with ddH_2O for SSR analysis.

For microsatellite analysis ten, out of twenty five, primers of JESPR series were used to select five tolerant and five susceptible cotton parents. PCR amplifications were performed in Peqlab (Germany) master cycler gradient. Polyacrylamide gel electrophoresis was used to resolve the SSR (PCR) fragments for further analysis. The preparation of PAGE is as follows.

SSR Data Analysis

After gel electrophoresis good quality gel photographs were used to score the all visible and unambiguously scorable fragments amplified by SSR primers. The primers that

produced polymorphic fragments were used in this study. The PIC value of each SSR locus was also calculated using the equation developed by Anderson *et al*. (1993).

$$PIC_i = 1 - \sum_{j=1}^{n} P^2ij$$ (*pij* is the frequency of the *j*th allele for locus *i*)

Mean band frequency was computed by the following equation:

$$MBF = n/N$$

Where n = number of plants carrying particular band
N = total number of varieties.

The genetic similarity between 10 genotypes of cotton was estimated according to the method developed by Nei (1973). Based on similarity data, an un-weighted pair group method of arithmetic averages (UPGMA) cluster analysis was used to assess genetic diversity among the germplasm under observation.

Results

Mean squares obtained from analysis of variance of all the characters (Table 1) showed highly significant ($P \leq 0.01$) differences among the accessions and between the two water regimes, and varieties responded differently to water regimes as the interaction component (Var × W) was also highly significant ($P \leq 0.01$) except root length which is significant ($P \leq 0.05$).

Forty two accessions of cotton were assessed using SSI estimates for four traits examined at seedling stage (Table. 2). It had been suggested that cultivars with smaller values had better tolerance to water stress than those having bigger values (Fisher and Manurer, 1978). The SSI of 14 accessions based upon four seedling traits is given in Table 3.

The SSI estimates based upon shoot length ranged from 0.51 to 1.57. The lowest estimate was found in cultivar DPL-26 (No.40) followed by BH-124 (No.3), 149F (No.48), NIAB-26 (No.45), BH-118 (No.43) and BH-160 (No.17) with 0.59, 0.74, 0.77, 0.70 and 0.84 SSI, respectively, and thus appeared to be more tolerant to water stress, whilst genotypes showing higher SSI values (1.57, 1.51, 1.34,1.32,1.27 and 1.14) of FH-1000 (No.5), NF-801-2 (No.23), SLH-257 (No.7), BOU-1724 (No.29) ,CIM-446 (No.41) and S-12 (No.49), respectively were found to be susceptible to water deficit condition.

For assessment of water stress tolerant and non-tolerant accessions based upon root length data, cultivar 149F (No.48) had the lowest SSI value i.e. 0.20, whilst the most suceptible variety was FH-1000 (No.5) with SSI value of 2.42. The accession BH-160 (No.17), DPL-26 (No.40), BH-124 (No.3) and VH-55 (No.26) showed moderate response to water stress, whilst the remaining cultivars showing SSI >1 have shown greater susceptibility to water stress.

Based upon relative water content, the lowest SSI estimates were 0.45 0.56, 0.72, 0.77 and 0.85 for BH-118 (No.43), NIAB-26 (No.45), DPL-26 (No.40), BH-124 (No.3), VH-55 (No.26) and 149F (No.48) respectively, thus appeared to show their good response to water stress. The highest SSI estimates of BOU-17-24 (No.29), FH-1000 (No.5), S-12 (No.49), CIM-446 (No.41), NF-801(No.23) and SLH-257 (No.7) were 1.51, 1.45, 1.33, 1.32, 1.30 and 1.23 respectively, which indicated poor retention of water, and thus may be categorized as the sensitive varieties to water stress. When excised leaf water losses of 42 accessions were compared for assessing stress tolerance, it was revealed that BH-118 (No.43) and VH-55 (No.26) with SSI values 0.77 and 0.83 were better tolerant than BH-160 (No.17) and NIAB-26 (No.45) which measured 0.85 and 0.90 respectively.

Genetic Similarities using Molecular Data

Genetic similarities using microsatellite data with selected polymorphic primers were used (Unpublished data) to assess the genetic relatedness and divergence among the selected sample of ten cotton accessions. Genetic similarity matrix of the within cotton accessions has been shown in Table 4. Maximum genetic relatedness was observed between genotypes BH-124 and BH-118 which showed highest similarity coefficient i.e. 88.39%. The accessions BH-124 and NF-801 were found least similar due to smaller similarity coefficient (52.76%). The overall narrow genetic base was observed among cotton genotypes used in this study.

Discussion

Existence of genetic variability is prerequisite for the improvement of any crop species. DNA based screening techniques such as RAPD, SSR markers, micro arrays and various biochemical markers have been used to access the overall genetic diversity within crop species (Rauf *et al*., 2010;). These markers depicted polymorphism in neutral and functional sites within the genome. The information has been exploited for the selection of Parent for hetrotic or transgressive breeding. Our studies showed narrow genetic base in the selected (drought resistant and susceptible) 10 cotton accessions. Exploitation of similar types of the parentages in establishment of transgressive segregation, high selection pressure in establishment of progenies has been found to be the major cause for the depletion of genetic diversity within elite germplasm (Khan *et al.*, 2009; Rauf *et al.*, 2012). Rauf *et al.* (2010) noted that breeder selection for similar types of breeding goals resulted in the substantial loss of alleles within breeding population. Our results also showed that selection for drought resistance resulted in the reduced nucleotide polymorphism. However, within the selected group, few accessions exhibited some genetic distance, which may be

Table 1: Analyses of variance of four characters of *G. hirsutum* under two water regimes

Sources of variation	Degree of freedom	Root length	Shoot length	Relative water content	Excise leaf water loss
Varieties (Var)	41	135.35**	86.49**	64.09**	0.24**
Water regimes (W)	1	19.32*	628.51**	564.70**	5.71**
Var × W	41	19.66*	10.35**	66.70**	0.18**
Error	168	3.61	3.39	33.90	0.06

*, **: Denote differences, significant at 5% and 1% probability respectively

Table 2: Shoot and root lengths (cm), relative water loss and excise leaf water loss of 42 cotton varieties/lines measured under two water regimes

Genotypes	SL-NDR(cm)	SL-DR(cm	Decrease %	SSI	RL-N (cm)	RL-D (cm)	Decrease %	SSI	RWC-NDR	RWC-DR	Decrease %	SSI	ELWL-NDR	ELWL-DR	Decrease %	SSI	
CIM-240	16.25	12.82	21.11	0.59	21.28	19.25	9.54	0.38	73.86	52.00	29.60	1.18	2.02	1.36	32.67	1.32	
BH-124	16.25	12.82	21.11	0.59	21.28	19.25	9.54	0.38	75.76	62.03	18.12	0.72	1.15	0.87	24.35	1.00	
MNH-129	18.50	11.20	39.46	1.10	22.13	13.75	37.87	1.51	77.31	57.94	25.05	1.00	1.60	1.06	33.75	1.37	
FH-1000	18.88	8.20	56.57	1.57	20.63	8.15	60.49	2.42	72.55	46.18	36.35	1.45	2.37	1.46	38.40	1.55	
SLH-257	22.25	11.50	48.31	1.34	20.25	12.10	40.25	1.61	73.70	51.06	30.72	1.23	1.85	1.24	32.97	1.35	
LSS	12.63	7.52	40.46	1.12	22.88	14.75	35.53	1.42	78.62	64.29	18.23	0.73	1.71	1.21	29.24	1.18	
VH-57	25.00	12.42	50.32	1.40	20.00	15.75	21.25	0.85	69.64	55.47	20.35	0.81	2.44	1.65	32.38	1.31	
BH-147	20.25	15.25	24.69	0.69	22.17	20.15	9.11	0.36	81.82	67.95	16.95	0.68	0.97	0.70	27.84	1.12	
MNH-93	20.75	14.17	31.71	0.88	16.75	12.03	28.18	1.13	69.54	55.59	20.06	0.80	1.85	1.29	30.27	1.23	
FH-925	21.88	14.33	34.51	0.96	20.50	14.67	28.44	1.14	63.72	48.95	23.18	0.93	2.35	1.59	32.34	1.31	
MNH-513	19.75	12.78	35.29	0.98	17.88	13.14	26.51	1.06	68.27	49.26	27.85	1.11	1.18	0.81	31.36	1.26	
BH-160	18.00	12.58	30.11	0.84	18.25	16.80	7.95	0.32	80.51	56.25	30.13	1.21	1.18	0.93	21.19	0.85	
S-14	20.75	12.23	41.06	1.14	17.50	12.55	28.29	1.13	72.86	53.23	26.94	1.08	0.93	0.59	36.56	1.47	
FH-682	16.25	11.14	31.45	0.87	27.00	17.38	35.63	1.43	69.91	51.60	26.19	1.05	1.42	1.02	28.17	1.13	
CIM-448	19.50	11.52	40.92	1.14	24.25	20.46	15.63	0.63	72.81	56.71	22.11	0.88	1.56	1.03	33.97	1.39	
NF- 801	24.50	14.57	40.53	1.13	16.50	7.17	56.55	2.26	81.06	54.76	32.45	1.30	1.30	0.79	39.23	1.59	
4F	19.25	14.75	23.38	0.65	17.75	9.58	46.03	1.84	72.19	50.38	30.21	1.21	1.43	1.05	26.57	1.07	
H-499-3	18.50	11.33	38.76	1.08	14.75	10.00	32.20	1.29	69.65	53.47	23.23	0.93	1.12	0.74	33.93	1.37	
VH-55	16.25	12.75	21.54	0.60	20.12	17.92	10.93	0.44	86.43	69.89	19.14	0.77	0.72	0.57	20.83	0.83	
B-557	18.63	10.92	41.38	1.15	20.75	15.50	25.30	1.01	68.55	48.93	28.62	1.14	1.53	1.06	30.72	1.24	
BH-126	19.75	11.05	44.05	1.22	21.75	16.83	22.62	0.90	75.89	50.58	33.35	1.33	1.38	0.86	37.68	1.51	
BOU-1724	21.00	11.00	47.62	1.32	19.75	9.00	54.43	2.18	66.92	41.67	37.73	1.51	2.54	1.63	35.83	1.46	
OKRA-3101	27.25	16.33	40.07	1.11	18.00	12.16	32.44	1.30	73.14	56.50	22.75	0.91	1.04	0.79	24.04	0.96	
FH-87	21.25	13.42	36.85	1.02	16.50	12.90	21.82	0.87	76.61	53.38	30.32	1.21	1.14	0.77	32.46	1.32	
MNH-554	19.00	12.50	34.21	0.95	22.50	17.08	24.09	0.96	73.80	45.80	37.94	1.52	1.52	1.09	28.29	1.13	
VH-54	16.50	13.50	18.18	0.51	18.75	16.50	12.00	0.48	83.88	63.18	24.68	0.99	0.93	0.68	26.88	1.08	
CIM-707	22.00	14.25	35.23	0.98	20.00	13.25	33.75	1.35	72.17	48.72	32.49	1.30	1.36	0.98	27.94	1.12	
FH-634	17.25	10.90	36.81	1.02	19.75	17.00	13.92	0.56	73.20	60.58	17.24	0.69	1.39	0.90	35.25	1.44	
FH-679	21.75	12.75	41.38	1.15	21.25	16.67	21.55	0.86	74.43	54.76	26.43	1.06	1.02	0.75	26.47	1.09	
FH-938	21.00	13.17	37.29	1.04	23.00	18.25	20.65	0.83	68.13	53.24	21.86	0.87	2.25	1.53	32.00	1.30	
REHMANI	22.25	14.67	34.07	0.95	18.50	16.00	13.51	0.54	73.55	61.42	16.49	0.66	1.67	1.12	32.93	1.33	
DPL -26	19.75	16.10	18.48	0.51	21.25	19.50	8.24	0.33	80.78	69.08	14.48	0.58	1.07	0.78	27.10	1.10	
CIM-446	23.00	12.50	45.65	1.27	21.75	11.25	48.28	1.93	67.38	45.17	32.96	1.32	2.10	1.26	40.00	1.62	
LRA-5166	18.75	14.25	24.00	0.67	17.75	16.10	9.30	0.37	79.02	61.15	22.61	0.90	1.28	0.99	22.66	0.93	
BH-118	24.00	17.92	25.33	0.70	19.45	17.00	12.60	0.50	72.51	65.24	10.03	0.40	1.34	1.08	19.40	0.77	
VH-53	18.75	11.42	39.09	1.09	15.75	13.25	15.87	0.63	76.69	62.70	18.24	0.73	1.85	1.18	36.22	1.47	
NIAB-26	22.75	16.42	27.82	0.77	16.00	13.85	13.44	0.54	71.95	61.91	13.95	0.56	0.93	0.72	22.58	0.90	
BH-116	17.00	11.40	32.94	0.92	26.25	16.17	38.40	1.54	80.01	60.26	24.68	0.99	0.91	0.57	37.36	1.50	
LINE-A-100	18.50	11.17	39.62	1.10	20.50	14.34	30.05	1.20	71.56	55.89	21.90	0.88	1.25	0.89	28.80	1.18	
149-F	19.13	14.00	26.82	0.74	23.25	22.10	4.95	0.20	74.15	58.46	21.16	0.85	0.84	0.63	25.00	1.03	
S-12	17.88	10.53	41.11	1.14	16.85	10.64	36.85	1.47	74.74	49.93	33.20	1.33	2.04	1.51	25.98	1.06	
FH-901	16.75	13.17	21.37	0.59	22.00	20.33	7.59	0.30	78.15	65.04	16.78	0.67	1.21	0.91	24.79	1.02	
Mean ± SD	19.69	12.79	12.79	0.97	20.08	15.01	20.08	1.01	74.22	55.71		0.99	1.01	1.02	74.22	1.22	55.97
	±2.88	±2.07		±0.26	±2.73	±3.58		±0.58	±4.88	±6.86		±0.27	±0.48	±0.30		±0.22	

Where ±SD= Standard deviation, RL=Root length, SL=shoot length, RWC=Relative water content, ELWL=Excise Leaf Water Loss, SSI=Stress susceptibility index N=Normal water supply and D= Water deficit condition

utilized for the establishment of segregating population. It has been suggested that the accessions with similarity coefficient less than 65 can be highly informative for future breeding crosses (Meredith, 2000).

Phenotypic evaluation of the germplasm offers an easy, cheaper and rapid method to identify the target source. The phenotypic evaluation is an important resource in the hands of breeders because of their high throughput and cost economical nature. It has been identified earlier that seedling stage is the most sensitive stage of plant growth, and thus

Table 3: Stress susceptibility indices of 14 *Gossypium hirsutum* genotypes for four seedling traits measured in water stress condition

Entry No.	Varieties/lines	SL(cm)	RL (cm)	RWC	ELWL
3	BH-124	0.59	0.40	0.72	1.00
5	FH-1000	1.58	2.40	1.45	1.55
7	SLH-257	1.34	1.61	1.23	1.35
17	BH-160	0.84	0.32	1.21	0.85
23	NF- 801	1.51	2.26	1.30	1.59
24	4F	1.13	1.84	1.21	1.07
26	VH-55	0.65	0.44	0.77	0.83
29	BOU-17-24	1.32	2.18	1.51	1.46
40	DPL -26	0.51	0.31	0.58	1.10
41	CIM-446	1.27	1.93	1.32	1.62
43	BH-118	0.70	0.50	0.40	0.77
45	NIAB-26	0.77	0.54	0.56	0.90
48	149-F	0.74	0.20	0.85	1.03
49	S-12	1.14	1.47	1.33	1.06

Where RL= Root length, SL= Shoot length, RWC= Relative water content and ELWL= Excise leaf water loss

Table 4: Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Pop ID	1	2	3	4	5	6	7	8	9	10
1	****	0.8839	0.5276	0.7882	0.8018	0.7267	0.5833	0.7882	0.6670	0.7826
2	0.1234	****	0.7107	0.9147	0.8189	0.7906	0.6285	0.8575	0.6860	0.7906
3	0.6393	0.3415	****	0.8044	0.7252	0.6742	0.8040	0.8044	0.6581	0.6068
4	0.2380	0.0892	0.2177	****	0.7130	0.8135	0.7276	0.8235	0.7059	0.7593
5	0.2209	0.1998	0.3213	0.3382	****	0.6574	0.5345	0.9075	0.7130	0.7171
6	0.3192	0.2350	0.3942	0.2064	0.4195	****	0.6708	0.7593	0.8135	0.8500
7	0.5390	0.4644	0.2181	0.3180	0.6264	0.3993	****	0.7276	0.7276	0.5963
8	0.2380	0.1537	0.2177	0.1942	0.0971	0.2754	0.3180	****	0.8235	0.8135
9	0.4050	0.3769	0.4183	0.3483	0.3382	0.2064	0.3180	0.1942	****	0.8677
10	0.2451	0.2350	0.4996	0.2754	0.3325	0.1625	0.5170	0.2064	0.1419	****

Where 1=BH-124, 2=BH-118, 3=NF-801, 4=149F, 5=SLH-257, 6=NIAB-26, 7=FH-1000, 8=DPL-26, 9=S-12, 10=CIM-446

screening for stress tolerance at this stage has been targeted in various crops like wheat (Gesimba *et al*., 2004; Farshadfar *et al*., 2012), sorghum (Ali *et al*., 2011; Bibi *et al*., 2012) and cotton (Cook, 1989; Longenberger *et al*., 2006; Ahmad *et al*., 2009; Iqbal *et al*., 2011). Present studies were also based on the model used by these researchers.

During the present work, four drought related traits, two morphological like root length and shoot length and two physiological like ELWL and RWC, were used to study the variation for this trait. SSI indicated the significant variation among all the 42 cultivars under study. However seven highly susceptible and seven highly drought tolerant, based on SSI, were used for detailed elaboration. Under low moisture stress shoot lengths of 42 varieties appeared to differ greatly, and the decrease ranged from 18.48% to 56.93% in DPL-26 and FH-1000, respectively. On the basis of stress susceptibility indices based upon shoot length data, FH-1000 and NF-801 were found to be susceptible, whilst DPL-26, VH-124, VH-118 and 149F were tolerant varieties.

Drought tolerance, based on the reduction in different characters of cotton plant under water deficit conditions (Pettigrew, 2004; Longenberger *et al*., 2006; Iqbal *et al*., 2010) have used absolute values, for the performance of drought related traits, under stress conditions, whereas during present study both SSI and absolute values were used to explain the drought tolerance and susceptibility. Such approaches are more reliable estimates for any germplasm characterization program through conventional breeding.

Among the drought related morphological traits, root length has been reported relatively more important measure of the trait and thus may be used to measure the level of tolerance of cotton varieties at seedling phase (Basal *et al.*, 2005). In the present investigation water stress sensitive genotypes showed more reduction in root growth e.g. FH-1000, NF-801 and BOU-17-24 whereas, drought tolerant genotypes appeared to be relatively less affected under moisture stress conditions. The accessions 149F, DPL-26 and BH-124 showed 4.9%, 7.9% and 7.9% reduction, under drought stress, in root length respectively and were marked as tolerant genotypes. The results of stress susceptibility indices agreed with the absolute data of tolerant (149F, DPL-26 and BH-124) and susceptible varieties FH-1000, NF-801 and BOU-17-24.

Previously relative water content had been used by Malik *et al.* (2006), Parida *et al.* (2008) and Ahmad *et al.* (2009) and excise leaf water loss had been used by Basel *et al*. (2005) to identify tolerant and non-tolerant crops for moisture stress. In the present studies the stress susceptibility index for relative water content and water loss in excised leaf showed that varieties/lines differed from each other and some of them were distinctly more tolerant than the others. It was revealed that varieties with greater relative water content had shown more losses of water in excised

leaf, and in susceptible varieties RWC decreased due to water stress as reduction in RWC, in tolerant varieties, had been reported by Farooq and Azam (2002). Varieties belonging to tolerant group maintained their superiority over the susceptible ones for moisture stress tolerance as BH-118, NIAB-26, DPL-26 and 149F showed the minimum decrease in relative water content. However, contrary to this, varieties BOU-1724, FH-1000, NF-801 and 4F were found susceptible. Rauf *et al*. (2009) viewed that osmotic adjustment is increased with the increase in root length, and thus plants showed more tolerance under water deficit conditions therefore, during the present investigation longer root length of tolerant varieties might be due to higher osmotic adjustment and retention of more water content in plants, suggesting that root growth is a reliable indicator for moisture stress tolerance in crops (Gesimba *et al*., 2004). The data generated by some previous workers substantiate our results (Malik *et al*., 2006; Ullah *et al*., 2008; Ahmad *et al*., 2009; Iqbal *et al*., 2011), which make the present technique reliable and efficient for any drought screening program.

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