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Agron. Sustain. Dev. 28 (2008) 439–447
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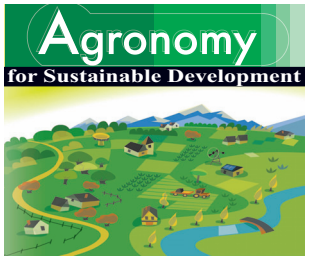
DOI: 10.1051/agro:2007041

Available online at:
www.agronomy-journal.org



Research article

Genotypic variation for drought tolerance in cotton

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(Accepted 5 September 2007)

Abstract – Increasing scarcity of irrigational water is a major threat to sustainable production of cotton (*Gossypium hirsutum* L.). It could be resolved by developing drought-tolerant cultivars. Osmotic adjustment and cellular membrane stability are well-documented traits that help to sustain yield under drought in cereals. However, their utility in cotton is not well established. Here, we studied genotypic variability and relationships among osmotic adjustment, cell membrane stability and productivity traits under field-induced water stress at the flowering stage. We evaluated a set of cotton germplasm comprising 32 cotton genotypes under contrasting water regimes for measurements of productivity including seedcotton yield, number of bolls per plant and boll weight, and physiological attributes such as osmotic adjustment and cell membrane stability in two field trials. The mean reduction in seedcotton yield due to water deficit was 20 and 43% in 2003 and 2004, respectively. Genotypes differed considerably for relative yield losses due to water stress ranging from 20 to 74%. Significant association between number of bolls and seedcotton yield under a water-limited regime suggests boll retention as the principal determinant of yield in a water-deficit-stress environment. Cell membrane stability varied significantly among the cotton genotypes; however, its association with productivity measurements was not significant in the water-limited regime. The significant positive correlation found between cell membrane stability and osmotic adjustment implicates the role of osmolytes in the protection of various cellular functions, including those associated with cellular membranes. Moderate but significant differences for osmotic adjustment were found among the genotypes in both years. Osmotic adjustment was positively associated with seedcotton yield under the water-limited regime and inversely correlated with the drought susceptibility index. These results demonstrated the contribution of osmotic adjustment in sustaining yield under water-deficit stress in cotton. Thus, like cereals, osmotic adjustment may be useful as a selection criterion in breeding programs with the objective of improving drought tolerance and yield in cotton under water-limited environments; however, the role of cell membrane stability as a drought-tolerant trait requires further investigation.

***Gossypium hirsutum* L. / drought tolerance / osmotic adjustment / cellular membrane stability**

1. INTRODUCTION

Cotton, the leading natural fiber crop both worldwide and in Pakistan, suffers from inadequate water supplies in many regions, resulting in low yield (Kramer, 1980; Boyer, 1982). Rapid climatic changes have further exacerbated this problem (Le Houerou, 1996). There is thus a need to improve cotton with respect to drought tolerance to sustain production in arid conditions (Blum, 1988).

Breeding to improve drought-tolerant genotypes requires identification of physiological mechanisms and morphological trait(s) conferring drought tolerance. The prerequisite for success requires determination of the extent of genotypic variation within a species for these traits, and their relative contribution to economic yield (Cooper, 1999).

A wide range of responses at molecular, cellular and whole-plant levels have been determined in plants that aid in tolerance for water-deficit stress (Bartels and Sunkar, 2005). A variety of adaptive mechanisms are considered important in conferring drought tolerance in different plant species; however, some basic cellular responses to drought appear to be conserved among all plants (Zhu et al., 1997). Accumulation of osmoprotectants (Ashraf and Harris, 2004; Ashraf and Iram, 2005) helps through osmotic adjustment to maintain metabolic activity, growth and productivity during drought (Morgan, 1984). A relationship between osmotic adjustment and plant productivity under water-limited environments has been found in a number of crops; for example, wheat (Moinuddin et al., 2005), sunflower (Chimenti et al., 2002) and canola and mustard (Niknam et al., 2003). Genotypic variation for osmotic adjustment and its association has also been reported in cotton (Nepomuceno et al., 1998; Saranga et al., 2001).

Cell membrane stability under water-limited conditions is another physiological criterion for selecting drought-tolerant plants. Sullivan (1972) described a drought- and heat-tolerance assay for sorghum based on electrolyte leakage that he designated the cell membrane stability test. This method measures increase in electrolyte diffusion resulting from increases in drought-induced cell membrane permeability.

Cell membrane stability has been widely exploited as an indicator of tolerance to different abiotic stresses, including

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high temperature (Ur-Rahman et al., 2004), salinity (Leopold and Willing, 1983) and drought (Ashraf et al., 1992). These studies revealed significant genetic variation for tolerance to different abiotic stresses using the cell membrane stability assay in a number of crops. Moreover, these reports also found a correlation between tolerance assessed by cell membrane stability and performance of the crops under field conditions. However, in most of the drought-tolerance studies the cell membrane stability assay was performed with osmotic stress induced in vitro with polyethylene glycol. Unlike field-induced water stress, which develops gradually, polyethylene glycol induces osmotic stress instantaneously. To our knowledge, the cell membrane stability assay has not previously been conducted on cotton where the water stress is imposed gradually under field conditions.

An objective of this study is to determine if genotypic differences exist among cotton genotypes for osmotic adjustment and cell membrane stability in response to field-induced water stress at the flowering period. A second objective is to determine if these physiological attributes can predict drought tolerance in cotton genotypes. The information obtained from this study will also supplement the existing database obtained from other crop plants which will be helpful in broadening the scope of osmotic adjustment and cell membrane stability in selecting drought-tolerant genotypes.

2. MATERIALS AND METHODS

2.1. Plant material

The experimental material consisted of 32 upland cotton, *Gossypium hirsutum* L., genotypes and cultivars, hereafter referred to as genotypes. Seeds of the genotypes were collected from their respective breeding stations located in different ecological regions of Pakistan (Tab. II).

2.2. Experimental design

Thirty-two cotton genotypes were evaluated under two irrigation regimes, well-watered and water-limited, in the field during 2003 and 2004 at the research area of the National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan (31°26'N, 73°06'E, elevation 185 m). The two water regimes:

- Well-watered. One irrigation at planting and 5 subsequent irrigations as required for normal crop growth and development. Total water applied including rainfall was 823 and 783 mm in 2003 and 2004, respectively.
- Water-limited. One irrigation at planting and one supplemental irrigation 40 days after planting. Total water applied including rainfall was 473 and 457 mm in 2003 and 2004, respectively.

Daily rainfall during each growing season was recorded at the experimental site.

A split-plot with four replications was used with water regimes as the main plot and genotypes as the sub-plots. Cottonseed was delinted with sulfuric acid and soaked in water for 12 h before planting. During both seasons, planting was completed during the 1st week of April. Four 6-m rows spaced 0.75 m apart were sown of each genotype with a hand drill. A commercial chemical fertilizer was applied at the rate of 100-50-50 kg N-P_2O_5-K_2O ha^{-1} at the time of seedbed preparation. Plant population was established at 4-plants m^{-2} by hand-thinning 25 days after germination. Throughout the season appropriate control measures were utilized as needed for insect and weed pests and applied evenly to all the plots.

2.3. Measurement of productivity traits

Seedcotton yield was measured as kg ha^{-1} on the two center rows of the four-row plots from both regimes each year. Seedcotton was hand-picked from all the plots 180 days after planting, and before weighing the cotton was sun-dried for one day and the trash and dry carpels removed.

Relative reduction in yield and yield components, hereafter referred to as reduction, of each genotype due to soil water deficit was calculated from the difference between the water-limited and well-watered regimes for the trait, i.e., reduction in yield for each genotype = 1 – (Yd/Yp), where Yd and Yp are mean yields of a given genotype in water-limited and well-watered regimes, respectively. This helped to eliminate genetic differences in yield potential among genotypes.

The formula proposed by Fisher and Maurer (1978) was used to calculate the drought susceptibility index for each genotype:

- Drought susceptibility index = Reduction in yield / Drought intensity
  Where drought intensity is determined as
- = 1 – {(Mean yield of all genotypes in well-watered regime)/(mean yield of all genotypes in drought)}.

Average seedcotton weight of 40 bolls picked from each plot was used to estimate boll weight. The total number of bolls per plant was calculated by dividing yield per plant by boll weight.

2.4. Measurement of physiological attributes

Physiological attributes were assessed on the youngest fully expanded main stem leaf (16–18 days old) 43 and 40 days after the 1st irrigation in 2003 and 2004, respectively, when all the genotypes were at least 50% flowering. There was no effective rainfall up to 92 and 81 days after planting in 2003 and 2004 at the experimental sites, respectively. Leaves were tagged on the day they unfolded (designated as day 1). Osmotic adjustment was measured on leaves sampled from both treatments by the rehydration method, which estimates osmotic adjustment as the difference in osmotic potential of fully rehydrated leaves sampled from water-stressed and control plants. For rehydration, petioles of detached leaves were inserted into water and incubated at 10 °C for 4 h in the dark. Upon rehydration a 5×5 mm^2 interveinal piece of leaf tissue was excised, placed immediately in a 5-mL disposable plastic syringe, and stored at −20 °C. After 2 weeks, samples were thawed and tissue sap was collected in 0.2-mL tubes. Following centrifugation

Table I. Analyses of variance for seedcotton yield per plot, number of bolls per plant and boll weight of 32 cotton genotypes under two water regimes during 2003 and 2004. (d.f: degree of freedom; * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$; n.s.: non-significant).

		Mean squares		
Source of variation	d.f	Seedcotton yield	Bolls per plant	Boll weight
2003				
Block	3	56451	4.3	0.008
Water regime	1	7508627*	413.4*	0.884*
Error A	3	135934	12.3	0.028
Genotype	31	945194***	80.3***	0.234***
Water regime × genotype	31	55267***	5.0***	0.025n.s.
Residual	186	23206	2.3	0.017
Coefficient of variability %		10	11	4
2004				
Block	3	71888	7.5	0.015
Water regime	1	55224218***	4037.8***	5.467***
Error A	3	91956	7.8	0.002
Genotype	31	1490521***	143.8***	0.315***
Water regime × genotype	31	432127***	53.1***	0.029**
Residual	186	52609	6.1	0.015
Coefficient of variability %		14	14	5

(13000 revolution per min) for 5 min, the sap was directly used to determine osmotic potential with a Wescor vapor pressure osmometer (model 5520, Wescor, Logan, Utah, USA).

For estimation of cell membrane stability, leaves were sampled at noon from water-stressed and well-watered plots on the same date samples for osmotic adjustment were taken. To minimize possible plant-to-plant variation, a bulked sample of 5 leaves was collected from the main stem apex of 5 random plants in each plot. Samples were rinsed with deionized water to remove surface contamination and carefully blotted dry. Twenty 1.0-cm^2 leaf discs were made from the bulked sample and submerged in 10 mL of deionized water in 20-mL screw-cap vials and kept at room temperature in the dark for 24 h. Subsequently, conductance of the sample solutions was measured with a conductivity meter (Model, 145 A+, Thermo Electron USA). The vials with samples were then autoclaved for 15 min and conductance of the sample solutions measured a second time to obtain an estimate of total electrolyte concentration. All measurements were recorded at 25 °C by keeping vials submerged in a water bath and vials were shaken vigorously to mix contents. Cell membrane stability (CMS) was calculated as reciprocal of relative cell injury (Blum and Ebercon, 1981) with the formula,

$$CMS\% = \{[1 - (T_1/T_2)]/[1 - (C_1/C_2)]\} \times 100$$

where T_1 = Stress sample conductance before autoclaving,

T_2 = Stress sample conductance after autoclaving,

C_1 = Control sample conductance before autoclaving,

C_2 = Control sample conductance after autoclaving.

2.5. Statistical analysis

Analysis of variance, appropriate for the specified experimental design, was performed with MSTAT-C software to evaluate the effects of water regime and genotypes on productivity and physiological attributes. Statistical significance was assumed at 5 and 1% levels of probability. Differences among means were tested by least significant difference (LSD) at a 5% probability level. Since analysis of variance for all the measured traits revealed a significant year by water regime and year by genotype interactions, the data for each year were analyzed separately. Simple linear regression and correlation analyses were performed to assess relationships among the variables of interest.

3. RESULTS AND DISCUSSION

3.1. Productivity traits

Analyses of variance of yield attributes for the growing seasons 2003 and 2004 revealed significant ($P < 0.001$) variation with respect to water regimes, genotypes and the interaction of these two parameters (Tab. I). However, the interaction of water regime by genotype was not significant for boll weight in 2003.

Mean values of seedcotton yield, number of bolls per plant and boll weight of genotypes in the well-watered and water-limited regimes for 2003 and 2004 are summarized in Tables II and III, respectively. In 2003, significant variation in seedcotton yield occurred among the 32 genotypes under a well-watered regime with values ranging from 1128 kg for FH-634 to 2635 kg for FH-901 (Tab. II). When the genotypes experienced water-deficit stress, the genotypes RH-510 and CIM-499 experienced only a 10% reduction in yield, and MNH-642,

Table II. Mean seedcotton yield, bolls per plant and boll weight of 32 cotton genotypes evaluated in well-watered (W_1) and water-limited (W_2) regimes during 2003.

Genotype	Origin[§]	Seedcotton yield				Bolls per plant			Boll weight		
		W_1	W_2	*RD*†	*DSI*‡	W_1	W_2	*RD*	W_1	W_2	*RD*
		kg ha^{-1}	kg ha^{-1}	%						g	%
BH-160	CRS, BWP	2486	1750	30	1.48	21.1	15.8	25	3.3	3.1	6
CIM-1100	CCRI, MN	2063	1743	16	0.78	17.4	15.7	9	3.3	3.1	7
CIM-443	CCRI, MN	1864	1261	32	1.62	17.2	11.8	31	3.0	3.0	2
CIM-473	CCRI, MN	2031	1749	14	0.69	17.9	15.9	11	3.2	3.1	3
CIM-497	CCRI, MN	1613	1361	16	0.78	14.8	12.3	17	3.0	3.1	–2
CIM-499	CCRI, MN	1464	1316	10	0.51	12.6	11.8	7	3.2	3.1	4
CIM-501	CCRI, MN	1318	1135	14	0.69	11.2	10.3	7	3.3	3.1	6
CIM-707	CCRI, MN	1797	1472	18	0.90	14.9	13.0	13	3.4	3.2	6
FH-1000	CRI, FSD	1998	1320	34	1.70	17.2	11.2	35	3.2	3.3	–2
FH-1200	CRI, FSD	1409	1227	13	0.65	12.0	11.2	7	3.3	3.1	6
FH-2000	CRI, FSD	2236	1612	28	1.39	20.4	14.8	27	3.1	3.0	1
FH-634	CRI, FSD	1128	987	12	0.62	9.9	9.4	6	3.2	2.9	7
FH-682	CRI, FSD	1253	965	23	1.15	12.1	10.1	17	2.9	2.7	8
FH-87	CRI, FSD	1424	1199	16	0.79	13.4	11.5	14	3.0	2.9	2
FH-900	CRI, FSD	1498	1194	20	1.01	12.5	9.8	21	3.3	3.4	–2
FH-901	CRI, FSD	2635	2020	23	1.17	24.0	19.5	19	3.1	2.9	6
FH-925	CRI, FSD	1530	1236	19	0.96	13.5	11.3	16	3.2	3.1	3
FH-930	CRI, FSD	1525	1333	13	0.63	12.7	11.0	14	3.3	3.4	–2
MNH-147	CRS, MN	2027	1508	26	1.28	18.6	14.7	21	3.0	2.9	6
MNH-552	CRS, MN	1594	1404	12	0.60	14.4	13.4	7	3.1	2.9	5
MNH-554	CRS, MN	2226	1785	20	0.99	19.4	16.1	17	3.2	3.1	5
MNH-642	CRS, MN	1202	1071	11	0.54	11.4	10.4	8	3.0	2.9	3
NIAB-111	NIAB, FSD	1480	1148	22	1.12	12.4	10.0	19	3.3	3.2	4
NIAB-78	NIAB, FSD	1922	1670	13	0.66	17.6	16.4	7	3.0	2.8	7
NIBGE-1	NIBGE, FSD	1375	1149	16	0.82	13.7	12.6	8	2.8	2.6	9
NIBGE-160	NIBGE, FSD	1284	888	31	1.54	11.3	8.0	29	3.2	3.1	2
NIBGE-2	NIBGE, FSD	1404	1046	26	1.28	12.8	10.6	17	3.1	2.8	10
NIBGE-4	NIBGE, FSD	1475	1077	27	1.35	13.4	9.2	31	3.1	3.3	–7
N-Karishma	NIAB, FSD	2082	1775	15	0.74	20.9	19.0	9	2.8	2.6	6
RH-510	CRS, RYK	1992	1801	10	0.48	16.7	16.1	3	3.3	3.1	7
SLH-257	CRS, SWL	2246	1904	15	0.76	19.5	16.6	15	3.2	3.2	0
VH-142	CRS, VR	1699	1210	29	1.44	15.3	11.4	25	3.1	3.0	4
Mean		1727	1385	20	1.0	15.4	12.8	16	3.1	3.0	4
LSD (0.05)		213				2.1			0.18		

[§] CRS: Cotton Research Station, CCRI: Central Cotton Research Institute, CRI: Cotton Research Institute, NIAB: Nuclear Institute for Agriculture & Biology, NIBGE: National Institute for Biotechnology and Genetic Engineering, BWP: Bahawalpure, MN: Multan, FSD: Faisalabad, RYK: Rahim Yar Khan, SWL: Sahiwal and VR: Vehari; LSD: least significant difference.
† *RD*: relative reduction yield due to water stress. ‡ *DSI*: drought susceptibility index.

Table III. Mean seedcotton yield, bolls per plant and boll weight of 32 cotton genotypes evaluated in well-watered (W_1) and water-limited (W_2) regimes during 2004. († *RD*: relative reduction yield due to water stress, ‡ *DSI*: drought susceptibility index, LSD: least significant difference).

Genotype	Seedcotton yield				Bolls per plant			Boll weight		
	W_1	W_2		‡ *DSI*	W_1	W_2	*RD*	W_1	W_2	*RD*
	kg ha^{-1}	kg ha^{-1}	%				%	g	g	%
BH-160	2928	1685	42	0.97	27.0	17.3	36	3.0	2.7	10
CIM-1100	2808	2043	27	0.62	27.2	21.9	20	2.9	2.6	10
CIM-443	1918	1070	44	1.01	20.4	13.1	36	2.6	2.3	14
CIM-473	2527	1532	39	0.90	25.1	16.1	36	2.8	2.7	5
CIM-497	1239	1059	15	0.34	13.8	13.6	2	2.5	2.2	13
CIM-499	2142	1162	46	1.06	21.1	12.5	41	2.8	2.6	9
CIM-501	2111	1258	40	0.92	18.8	12.5	34	3.1	2.8	11
CIM-707	2453	1661	32	0.74	23.2	16.9	27	3.0	2.7	7
FH-1000	1844	1206	35	0.80	17.4	13.0	25	3.0	2.6	13
FH-1200	1972	1496	24	0.55	17.2	14.5	16	3.2	2.9	10
FH-2000	2921	885	70	1.61	31.4	11.7	63	2.6	2.1	19
FH-634	2066	1224	41	0.94	22.6	16.4	27	2.6	2.1	19
FH-682	2497	797	68	1.56	29.3	9.7	67	2.4	2.3	3
FH-87	2664	1642	38	0.87	28.3	18.0	37	2.6	2.5	3
FH-900	2858	1775	38	0.87	28.2	19.1	32	2.8	2.6	8
FH-901	2534	662	74	1.70	26.4	7.6	71	2.7	2.4	9
FH-925	1770	1127	36	0.83	17.1	12.5	27	2.9	2.5	13
FH-930	1744	1298	26	0.60	15.7	12.3	22	3.1	2.9	6
MNH-147	2247	943	58	1.33	21.6	9.6	56	2.9	2.8	5
MNH-552	2261	749	67	1.54	22.0	7.9	64	2.9	2.6	8
MNH-554	3113	1190	62	1.43	29.8	12.6	58	2.9	2.6	10
MNH-642	1729	864	50	1.15	17.3	9.4	46	2.8	2.6	9
NIAB-111	2301	1748	24	0.55	22.7	19.9	12	2.8	2.5	13
NIAB-78	1908	1190	38	0.87	18.5	13.3	28	2.9	2.5	13
NIBGE-1	1402	764	46	1.06	14.8	8.7	41	2.7	2.5	8
NIBGE-160	1197	461	62	1.43	11.0	4.9	56	3.0	2.6	13
NIBGE-2	1230	880	28	0.64	13.0	10.0	23	2.7	2.5	8
NIBGE-4	1602	918	43	0.99	15.2	10.2	33	2.9	2.5	15
N-Karishma	2463	1521	38	0.87	24.3	16.9	31	2.8	2.5	11
RH-510	2673	2139	20	0.46	24.7	21.4	13	3.0	2.8	8
SLH-257	2111	987	53	1.22	21.2	10.8	49	2.8	2.5	9
VH-142	1104	677	39	0.90	11.7	9.4	19	2.6	2.0	24
Mean	2136	1207	43	1.0	21.2	13.2	36	2.8	2.5	11
LSD (0.05)	321				3.5			0.17		

MNH-552, and FH-634 also had less reduction in yield than the other genotypes (Tab. II). Average reduction in yield due to water-deficit stress was around 20%. Thirteen genotypes including FH-901, that produced high seedcotton yield under well-watered conditions, had a greater reduction in seedcotton yield than the average reduction due to water-deficit stress.

The effect of water deficit was more distinct on yield attributes in 2004 than in 2003. The average decrease in seedcotton yield due to water-deficit stress was 43% (Tab. III). Among the high-yielding genotypes under the well-watered regime, RH-510 and CIM-1100 maintained relatively high yield under the water-limited regime. Genotypes FH-901, FH-2000 and MNH-552 suffered substantial yield losses under water-deficit stress.

The drought susceptibility index was also calculated to provide an additional measurement of drought tolerance of the genotypes with respect to yield (Tabs. II, III). Among the high-yielding genotypes in the well-watered regime, RH-510,

Table IV. Phenotypic correlation among well-watered (W_1), water-limited (W_2), drought susceptibility index (*DSI*) and relative reduction (*RD*) for seedcotton yield (*SCY*), number of bolls per plant (*BN*) and boll weight (*BW*) for 32 cotton genotypes evaluated during the 2003 (upper diagonal) and 2004 (lower diagonal) seasons. (* $P < 0.05$ and ** $P < 0.01$).

		SCY			*BN*			*BW*		
		W_1	W_2	*DSI*	W_1	W_2	*RD*	W_1	W_2	*RD*
SCY	W_1	**1**	0.92**	0.24	0.97**	0.87**	0.23	0.08	0.06	0.02
	W_2	0.57**	**1**	–0.16	0.89**	0.96**	–0.15	0.10	0.01	0.12
	DSI	0.23	–0.64**	**1**	0.25	–0.17	0.94**	–0.07	0.11	–0.23
BN	W_1	0.96**	0.45*	0.32	**1**	0.90**	0.22	–0.14	–0.12	0.06
	W_2	0.55**	0.97**	–0.64**	0.48**	**1**	–0.23	–0.14	–0.27	0.29
	RD	0.31	–0.56**	0.98**	0.39*	–0.59**	**1**	–0.01	0.33	–0.54**
BW	W_1	0.07	0.33	–0.25	–0.18	0.13	–0.19	**1**	0.79**	–0.13
	W_2	0.21	0.42*	–0.23	–0.01	0.19	–0.08	0.84**	**1**	–0.68**
	RD	–0.34	–0.25	–0.04	–0.31	–0.10	–0.24	–0.15	–0.64**	**1**

CIM-473 and CIM-1100 were found to be relatively tolerant to drought in 2003. Whereas, BH-160, FH-2000, MNH-147 and FH-901 had relatively higher drought susceptibility indices (Tab. II), indicating their susceptibility to water-deficit stress. In 2004, cotton genotypes FH-2000 and FH-901 were the most susceptible to water-deficit stress, whereas RH-510, FH-1200, NIAB-111 and CIM-1100 were found to be the most tolerant to such stress.

Individual components of yield were also affected by drought in both years. In 2003, genotype FH-901, which had the highest seedcotton yield, also produced the highest number of bolls under both water regimes compared with the other genotypes. Low reduction in the number of bolls per plant due to water-deficit stress was observed in RH-510 and FH-634.

Cotton genotypes FH-901, MNH-554, FH-682, FH-87 and FH-900 produced a significantly higher number of bolls compared with the other genotypes under the well-watered regime, in 2004. However, a drastic reduction in the number of bolls of all genotypes occurred in the water-limited regime with an average decrease of 36% (Tab. III). Under water-deficit stress, 13 of the 32 genotypes, including FH-901 and FH-682, showed a reduction in bolls greater than the mean, whereas NIAB-111, RH-510, FH-1200 and CIM-1100 showed relatively lesser reductions (Tab. III). Considerable genotypic variation was recorded for boll weight in the two water regimes in both years; however, water-deficit stress had only a minor effect on boll weight in any genotype (Tabs. II, III).

These results for seedcotton yield and its components clearly indicate a significant magnitude of variation in the response of various cotton genotypes to water stress during the two years of experimentation. However, between the two years, the accessions had differential responses which resulted in a shift in their ranking regarding drought response. These differences are probably related to differences in the rainfall pattern between years (data not shown). During 2003 compared with 2004, seedcotton yields were higher in the water-limited regime due to well-distributed rainfall during the flowering and boll-setting periods.

Correlation coefficients between the well-watered and water-limited regimes were positive and highly significant ($P < 0.01$) for yield and yield components in both the years (Tab. IV). The seedcotton yield in the water-limited regime was negatively correlated with the drought susceptibility index for seedcotton yield and reduction in number of bolls per plant and boll weight. Seedcotton yield was also significantly associated with number of bolls per plant in both water regimes in both the years. Seedcotton yield was also associated ($P < 0.05$) with boll weight in the water-limited regime in 2003; however, the level of these associations was not significant in the 2003 trial.

Seedcotton yield per plant is determined by two constituent components, i.e. boll number and boll weight. When water-deficit stress occurs during the flowering stage, seedcotton yield reduction of cotton genotypes is mainly due to square and young boll shedding (Cook and El-Zik, 1992). The highly significant association of seedcotton yield with number of bolls per plant and reduction in boll number with the drought susceptibility index in this study confirmed this relationship.

Breeding strategies for drought tolerance depend strongly on the kind of target environment (s) to which a breeding effort is addressed. Significant positive correlations in seedcotton yield and its components under well-watered and water-limited regimes in 2003 and 2004 support the hypothesis that genotypic advantages selected under near-optimum growing conditions may be obtained under less favorable growing environments (Quisenberry et al., 1980).

3.2. Physiological attributes

Analysis of variance for osmotic adjustment and cell membrane stability revealed highly significant differences ($P < 0.001$) among the genotypes (Tab. V). All genotypes exhibited some degree of osmotic adjustment in response to water deficit (Tab. VI). In 2003, variation in osmotic adjustment ranged from 0.52 to 1.22 Mpa among the genotypes (Tab. VI). Thirteen out of the 32 genotypes showed above-average osmotic adjustment in response to water-deficit stress.

Table V. Analyses of variance for osmotic adjustment and cell membrane stability in cotton genotypes due to water-deficit stress during 2003 and 2004. (*** $P < 0.001$, d.f: degree of freedom).

		Mean squares			
		Osmotic adjustment		*Cell membrane stability*	
Source of variation	d.f	2003	2004	2003	2004
Block	31	0.008	0.022	4.41	11.7
Genotype	3	0.102***	0.084***	357.2***	399.5***
Error	93	0.009	0.022	35.9	60.2
Coefficient of variability %		**13.7**	**19.3**	**7.7**	**10.2**

Genotypes SLH-257, CIM-1100, CIM-707, FH-930 and RH-510 expressed relatively higher levels of osmotic adjustment, the values being 1.22, 1.09, 1.10 and 0.94 Mpa, respectively. The performance of genotypes for osmotic adjustment was consistent between both years with six exceptions. The cotton genotypes CIM-1100 and RH-510 showed repeatedly higher levels of osmotic adjustment in 2004 (Tab. VI).

The importance of osmotic adjustment as an effective mechanism for crop drought tolerance has received considerable recognition. All 32 cotton genotypes exhibited some degree of osmotic adjustment in response to water deficit. The maximum value of osmotic adjustment was comparatively greater than previous reports on osmotic adjustment in cotton leaves (Oosterhuis et al., 1987; Nepomuceno et al., 1998). The higher osmotic adjustment value is not unexpected, since osmotic adjustment is an inducible rather than inherent character. Active osmotic adjustment in response to water-deficit stress is more likely to occur if the drought is imposed slowly (Morgan, 1984). Previous studies reporting smaller values of osmotic adjustment in cotton were conducted in a controlled environment and instant drought was induced by withholding irrigation in sand pots (Oosterhuis et al., 1987) or addition of polyethylene glycol in hydroponically-grown plants (Nepomuceno et al., 1998).

The present study reveals moderate genotypic differences for osmotic adjustment, reflecting low diversity for the trait compared with previous reports on lentil (0.33–1.28 Mpa, Ashraf et al., 1992) and rice (0.1–1.7 Mpa, Babu et al., 1998). This low level of diversity might be related to the narrow genetic base of cotton that has resulted from intensive selection for productivity and fiber quality traits (Rahman et al., 2002, 2005).

Cell membrane stability has been widely exploited as an indicator of tolerance against water-deficit stress, and numerous reports of genotypic differences in cell membrane stability have established its association with economic yield under water stress in many crop species (Ashraf et al., 1992; Tripathy et al., 2000). However, in most of the drought-tolerance studies the cell membrane stability assay was performed with osmotic stress induced in vitro by polyethylene glycol. Unlike field-induced water stress, which develops gradually, polyethylene glycol induces osmotic stress instantaneously. To our knowledge, the cell membrane stability assay has not previously been conducted on cotton where the water stress is imposed gradually under field conditions.

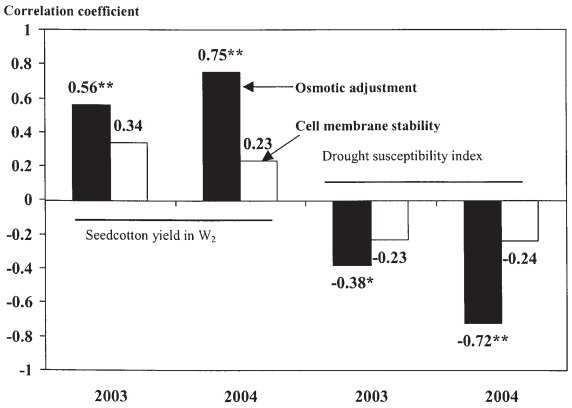

Figure 1. Association of osmotic adjustment and cell membrane stability with seedcotton yield in a water-limited regime (W_2) and the drought susceptibility index in cotton genotypes due to water-deficit stress during the 2003 and 2004 crop seasons. (* $P < 0.05$ and ** $P < 0.01$).

Considerable genotypic variation for cell membrane stability was present among the cotton genotypes in both years. It ranged from 92.3% for FH-1200 to 54.4% for NIBGE-2 in 2003 and varied from 94% for FH-1200 to 60% for NIBGE-1 in 2004 (Tab. VI). Sixteen and 15 genotypes exhibited above-average cell membrane stability in 2003 and 2004, respectively.

Correlations of physiological attributes with seedcotton yield under water-deficit stress and the drought susceptibility index for seedcotton yield were calculated to assess the association among the traits. Osmotic adjustment was significantly associated ($P < 0.01$) with yield under water deficit and negatively associated with the drought susceptibility index for seedcotton yield (Fig. 1). Cell membrane stability was neither correlated with yield under the water-limited regime nor with the drought susceptibility index for seedcotton yield (Fig. 1); however, it was associated significantly with osmotic adjustment in 2003 ($P < 0.01$) and 2004 ($P < 0.05$) (Figs. 2, 3, respectively).

Among physiological parameters, response to water deficit clearly implicates osmotic adjustment as an adaptation mechanism to a water-deficit environment. The positive association of osmotic adjustment with seedcotton yield in the water-limited regime and its negative correlation with the drought

Table VI. Mean osmotic adjustment and cell membrane stability in cotton genotypes due to water-deficit stress during 2003 and 2004. Bold figures represent genotypes showing osmotic adjustment and cell membrane stability above average, whereas non-bold figures represent those below average. (LSD: least significant difference).

	Osmotic adjustment (MPa)		*Cell membrane stability (%)*	
Genotype	**2003**	**2004**	**2003**	**2004**
BH-160	**0.83**	0.71	**79.4**	**85.4**
CIM-1100	**1.09**	**1.15**	**89**	**90.6**
CIM-443	0.73	0.68	62.6	**91.6**
CIM-473	0.69	0.7	69.1	67
CIM-497	**0.78**	**0.94**	75	73.6
CIM-499	0.67	0.69	64.5	68
CIM-501	0.76	0.76	66.8	63.6
CIM-707	**1.01**	**0.91**	**85.7**	60.1
FH-1000	0.68	**0.8**	**81.7**	**84.3**
FH-1200	0.77	**1.03**	**92.3**	**94.3**
FH-2000	0.65	0.65	63.9	61.4
FH-634	0.71	**0.8**	64.6	66.5
FH-682	0.61	0.69	74.6	71
FH-87	0.65	0.76	73.8	63
FH-900	**0.83**	**0.87**	**82.8**	70.6
FH-901	0.76	0.56	72.1	69.4
FH-925	0.77	0.74	**79**	71.7
FH-930	**0.98**	**0.86**	**85.5**	**85.5**
MNH-147	0.63	0.67	77.5	73.9
MNH-552	**0.81**	0.7	**79.6**	73
MNH-554	**0.79**	**0.79**	**89.9**	**85.1**
MNH-642	0.63	0.59	**84.3**	**79.4**
N-111	**0.92**	**1.04**	**83.4**	**79.3**
NIAB-78	**0.84**	**0.86**	73.7	**76.4**
NIBGE-1	0.56	0.66	67.1	**78.2**
NIBGE-160	0.59	0.64	70.8	72.3
NIBGE-2	0.67	0.7	57.4	60
NIBGE-4	0.52	0.55	77.7	72
N- Karishma	**0.87**	**0.82**	**83.4**	**79.1**
RH-510	**0.94**	**1.03**	**90.8**	**91.2**
SLH-257	**1.22**	0.74	**91.4**	**88**
VH-142	0.58	0.76	**83.3**	**86.7**
Mean	0.77	0.78	77.7	76
LSD (0.05)	0.14	0.21	8.4	10.9

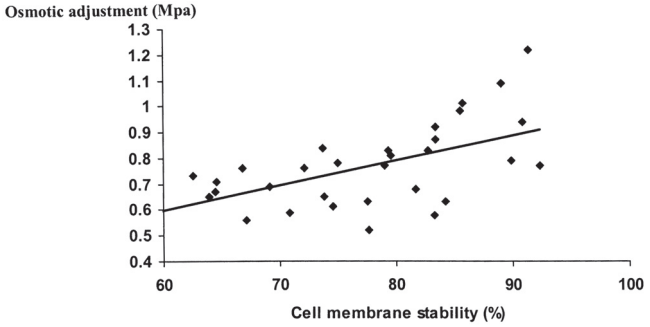

Figure 2. Association of osmotic adjustment with cell membrane stability in cotton genotypes due to water-deficit stress during the 2003 crop season ($y = 0.097x + 0.0203$ ($r^2 = 0.33$)).

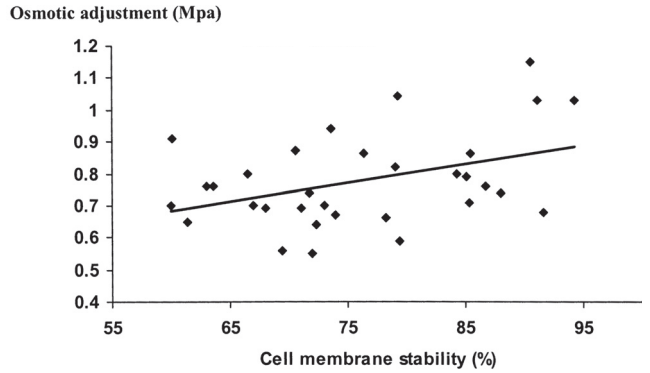

Figure 3. Association of osmotic adjustment with cell membrane stability in cotton genotypes due to water-deficit stress during the 2004 crop season ($y = 0.0058x + 0.3351$ ($r^2 = 0.16$)).

susceptibility index is in agreement with earlier reports in cotton (Saranga et al., 2001) and wheat (Moinuddin et al., 2005).

Osmotic adjustment results from accumulation of compatible solutes that can associate with lipid or protein and prevent membrane disintegration (Bohnert and Jensen, 1996). The significant positive correlation found in this study between cell membrane stability, as assessed by relative cell injury due to drought, and osmotic adjustment (Figs. 2, 3) indicates a role for osmolytes in the protection of various cellular functions, including those associated with cellular membranes (Blum and Pnuel, 1990).

Though appreciable genotypic variation was found for cell membrane stability in the present study, cell membrane stability was not associated with seedcotton yield or the drought susceptibility index. Perhaps the differences in cell membrane stability come from difference in leaf structure (MacRae et al., 1986), cell wall composition (Jarvis et al., 1988), degree of membrane lipid saturation (Tal and Shannon, 1983) or epicuticular wax coating (Sutter and Langhans, 1982). However, these associations may only be correlative and the mechanism involved has yet to be determined. Moreover, a number of mechanisms are involved in adaptation of crop plants to drought including drought escape, avoidance and tolerance. Cell membrane stability is only one of these mechanisms and, thus, an association between economic yield and cell membrane stability might not always be expressed.

4. CONCLUSION

The results reported here depict considerable genotypic variation for cell membrane stability and osmotic adjustment in cotton genotypes. Strong association of osmotic adjustment

with productivity measurements in a water-limited regime supports the hypothesis that osmotic adjustment helps to maintain seedcotton yield under water-deficit stress during the fruiting stage in cotton. Therefore, osmotic adjustment may be useful as a selection criterion in breeding programs with the objective of improving drought tolerance and seedcotton yield under water-limited environments. However, direct selection in the field for osmotic adjustment within a large population is difficult. Hence, its use in a breeding program will depend largely upon the development of associated molecular markers. Research is underway for mapping osmotic adjustment and other traits that may be associated with seedcotton yield under water deficit during the fruiting stage.

Acknowledgements: The funds for the present studies were provided by the Pakistan Science Foundation (PSF) through the Project No PSF/Biotech/P-NIBGE/Med (43) entitled "DNA Fingerprinting approaches to studying drought tolerance in cotton".

REFERENCES

Ashraf M., Iram A. (2005) Drought stress induced changes in some organic substances in nodules and other plant parts of two potential legumes differing in salt tolerance, Flora 200, 535–546.

Ashraf M., Bokhari M.H., Chishti S.N. (1992) Variation in osmotic adjustment of accessions of lentil (*Lens Culinaris* Medic.) in response to drought stress, Acta Bot. Neerl. 41, 51–62.

Ashraf M., Harris P.J.C. (2004) Potential biochemical indicators of salinity tolerance in plants, Plant Sci. 166, 3–16.

Babu R.C., Pathan M.S., Blum A., Nguyen T.N. (1998) Comparison of measurement methods of osmotic adjustment in different rice cultivars, Crop Sci. 39, 150–158.

Bartels D., Sunkar R. (2005) Drought and salt tolerance in plants, Crit. Rev. Plant Sci. 24, 23–58.

Blum A. (1988) Plant breeding for stress environment, CRC Press, Boca Raton, Florida.

Blum A., Ebercon A. (1981) Cell membrane stability as a measure of drought and heat tolerance in wheat, Crop Sci. 21, 43–47.

Blum A., Pnuel Y. (1990) Physiological attributes associated with drought resistance of wheat cultivars in a Mediterranean environment, Aust. J. Agric. Res. 41, 799–810.

Bohnert H.J., Jensen R.G. (1996) Strategies for engineering water-stress tolerance in plants, Trends Biotech. 14, 89–97.

Boyer J.S. (1982) Plant productivity and environment, Science 218, 443–448.

Chimenti C.A., Pearson J., Hall A.J. (2002) Osmotic adjustment and yield maintenance under drought in sunflower, Field Crops Res. 75, 235–246.

Cook C.G., El-Zik K.M. (1992) Cotton seedling and first bloom plant characteristics: Relationship with drought –influenced boll abscission and lint yield, Crop Sci. 32, 1464–1467.

Cooper M. (1999) Concept and strategies for plant adaptation research in rainfed lowland rice, Field Crops Res. 64, 13–34.

Fischer R.A., Maurer R. (1978) Drought resistance in spring wheat cultivars: I. Grain yield responses, Aust. J. Agric. Res. 29, 897–907.

Jarvis M.C., Forsyth W., Duncan H.J. (1988) A survey of the pectic content of nonlignified monocot cell walls, Plant Physiol. 88, 309–314.

Kramer P.J. (1980) Drought, stress, and the origin of adaptation, in: Turner N.C., Kramer P.J. (Eds.), In Adaptation of plants to water and high temperature stress, John Wiley, New York, pp. 7–20.

Le Houerou H.N. (1996) Climate changes, drought and desertification, J. Arid Environ. 34, 133–185.

Leopold A.C., Willing R.P. (1983) Evidence of toxicity effects of salt on membranes, in: Staples R.C., Toenniessen G.H. (Eds.), Plant improvement for irrigated crop production under increasing saline conditions, John Wiley & Sons, New York, pp. 70–91.

MacRae E.A., Hardacre A.K., Ferguson I.B. (1986) Comparison of chlorophyll fluorescence with several other techniques used to assess chilling sensitivity in plants, Physiol. Plant. 67, 659–665.

Moinuddin, Fischer R.A., Sayre K.D., Reynolds M.P. (2005) Osmotic adjustment in wheat in relation to grain yield under water deficit environments, Agron. J. 97, 1062–1071.

Morgan J.M. (1984) Osmoregulation and water stress in higher plants, Annu. Rev. Plant. Physiol. 35, 299–319.

Nepomuceno A.L., Oosterhuis D.M., Stewart J.M. (1998) Physiological responses of cotton leaves and roots to water deficit induced by polyethylene glycol, Environ. Exp. Bot. 40, 29–41.

Niknam S.R., Ma Q., Turner D.W. (2003) Osmotic adjustment and seed yield of *Brassica napus* and *B. Juncea* genotypes in water-limited environment in south western Australia, Aust. J. Exp. Agric. 43, 1127–1135.

Oosterhuis M.D., Stan D., Wullschleger S.T. (1987) Osmotic adjustment in cotton (*Gossypium hirsutum* L.) leaves and roots in response to water stress, Plant Physiol. 84, 1154–1157.

Quisenberry J.E., Roark B., Fryer D.W., Kohel R.J. (1980) Effectiveness of selection in upland cotton in stress environments, Crop Sci. 20, 450–453.

Rahman M., Hussain D., Zafar Y. (2002) Estimation of genetic divergence among elite cotton (*Gossypium hirsutum* L.) cultivars/genotypes by DNA fingerprinting technology, Crop Sci. 42, 2137–2144.

Rahman M., Hussain D., Malik T.A., Zafar Y. (2005) Genetics of resistance to cotton leaf curl disease in *Gossypium hirsutum*, Plant Pathol. 54, 764–772.

Saranga Y., Menz M., Jiang C., Wright R., Yakir D., Paterson A.H. (2001) Genomic dissection of genotype × environment adaptation conferring adaptation of cotton to arid conditions, Genome Res. 11, 1988–1995.

Sullivan C.Y. (1972) Mechanisms of heat and drought tolerance in sorghum and methods of measurements, in: Rao N.G.P., House L.R. (Eds.), Sorghum in the seventies, Oxford & IBH Publishing Co., New Delhi, India, pp. 247–264.

Sutter E., Langhans R.W. (1982) Formation of epicuticular wax and its effect on water loss in cabbage plants regenerated from shoot-tip culture, Can. J. Bot. 60, 2896–2902.

Tal M., Shannon M.C. (1983) Effect of dehydration and high temperature on the stability of leaf membrane of *Lycopersicon esculentum, L. peruvianum,* and *Solanum pennellii,* Z. Pflanzenphysiol. 112, 411–416.

Tripathy J.N., Zhang J., Robin S., Nguyen T.T., Nguyen H.T. (2000) QTLs for cell-membrane stability mapped in rice (*Oryza Sativa* L.) under drought stress, Theor. Appl. Genet. 100, 1197–1202.

Ur-Rahman H., Malik S.A., Saleem M. (2004) Heat tolerance of upland cotton during the fruiting stage evaluated using cellular membrane stability, Field Crops Res. 85, 149–158.

Zhu J.-K., Hasegawa P.M., Bressan R.A. (1997) Molecular aspects of osmotic stress in plants, Crit. Rev. Plant Sci. 16, 253–267.