INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
11–166/SBC/2011/13–6–902–908
http://www.fspublishers.org

Full Length Article

Alterations in Leaf Pigments in Cotton (*Gossypium hirsutum*) Genotypes Subjected to Drought Stress Conditions

AZWAR RAZA SHAH[1], TARIQ MANZOOR KHAN, HAFEEZ AHMAD SADAQAT AND ASHFAQ AHMAD CHATHA†
Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan
†*Department of Agronomy, University of Agriculture, Faisalabad, Pakistan*
[1]Corresponding author's e-mail: azwaruaf@yahoo.com

ABSTRACT

Oxidative stress is one of the most important consequences of drought stress in plants. Both enzymatic and non-enzymatic antioxidant systems are responsible to decrease oxidative injury in plant tissues. Fifteen different genotypes were sown in polythene bags (30 cm × 15 cm) following complete randomized design with three replications under normal and water stress conditions. After 45 days from sowing drought stress was induced by with holding irrigation for seven days. Normal regime was irrigated regularly. Simultaneous determination of chlorophylls, carotenoids and polyphenols were carried out to investigate the responses of cotton accessions to drought. As drought tolerant varieties have maximum amount of carotenoids and polyphenols and vice versa for susceptible varieties. We found that in cotton accessions, FH-113, PB-899 and MNH-789 had maximum amount of carotenoids (mg/100 mL) (0.79), (0.69) and (0.66) and polyphenols (µg/g of leaf) (0.014), (0.012) and (0.011) under normal conditions, respectively. CIM-506, FH-901 and CRIS-466 had minimum amount of carotenoids (0.29), (0.34) and (0.39) and polyphenols (0.005), (0.006) and (0.007) under normal conditions, respectively. Therefore, cotton accessions FH-113, PB-899 and MNH-789 were found drought tolerant and CIM-506, FH-901 and CRIS-466 were found drought susceptible. Chlorophyll *a* and *b* and carotenoids were decreased and polyphenols were increased under water stress conditions in all the accessions. Tolerant accessions have good antioxidant defense system, which protects from drought, should be selected for further breeding programme.

Key Words: Cotton; Drought; Phenolics; Carotenoids; Chlorophyll

INTRODUCTION

Cotton is one of the most vital crops in the world in terms of economic value. Cotton is the most essential textile fiber worldwide as it currently accounts for 90% of the commercially grown cotton. Cotton is the 2nd most important oil seed crop in the world averaging one fourth that of soybean (Zhang, 2001; Jones & Kersey, 2002).

Faced with lack of water resources, drought is the solitary crucial hazard to world food security. The severity of drought depends on many factors like distribution of rainfall, evaporative demands and moisture storing capability of soils (Wery *et al*., 1994). Investigations carried out in the past provide significant impending into the mechanisms of drought tolerance in plants at molecular level (Hasegawa *et al*., 2000). Three main mechanisms lessen crop yield by soil moisture scarcity (i) reduced canopy absorption of photosynthetically active radiation (ii) decreased radiation-use efficiency and (iii) reduced harvest index (Earl & Davis, 2003).

Development of crops for improved drought resistance entails the understanding of physiological mechanisms and genetic control of the contributing characteristics at different plant developmental stages. Significant work on drought tolerance has been done in plants (Ingram & Bartels, 1996). Drought stress results in photosynthetic reduction, which arises by a decrease in leaf expansion, impairs photosynthetic apparatus and associated decrease in food production (Wahid & Rasul, 2005). Drought stress influenced on photosynthetic pigments and components (Anjum *et al*., 2003), damaged photosynthetic apparatus (Fu & Huang, 2001).

Carotenoids are among large class of isoprenoid molecules, which are synthesized by all photosynthetic and numerous non-photosynthetic organisms (Andrew *et al*., 2008). They are separated into the hydrocarbon carotenes, such as lycopene and *β*-carotene or xanthophylls, typified by lutein (Jaleel *et al.,* 2007). Oxidative damage caused by drought stress in the plant tissue is eased by an intensive action of both enzymatic and non-enzymatic antioxidant systems. These include *β*- carotenes, ascorbate, *α*-tocopherol, reduced glutathione and enzymes including superoxide dismutase, peroxidase, ascorbate peroxidase, catalase, polyphenol oxidase and glutathione reductase (Prochazkova *et al.*, 2001). Carotenes form a major part of the plant antioxidant protection system, but they are very sensitive to oxidative damage. *β*-carotene, found in the chloroplasts of all green plants is completely bound to the core complexes of PSI and PSII (Havaux, 1998).

To cite this paper: Shah, A.R., T.M. Khan, H.A. Sadaqat and A.A. Chatha, 2011. Alterations in leaf pigments in cotton (*Gossypium hirsutum*) genotypes subjected to drought stress conditions. *Int. J. Agric. Biol*., 00: 902–908

Abiotic stress is the root cause of crop loss reducing average yield of most of the major crop plants by more than 50% worldwide (Boyer, 1982; Bray *et al*., 2000). In the present investigations, 15 cotton accessions were analyzed to study biochemical responses involved to combat drought stress. These studies will provide important information that can be used for genetic improvement of cotton to boost yield and fiber quality under normal and water stress conditions.

MATERIALS AND METHODS

Plant material: Fifteen different genotypes CIM-506 (Most susceptible), FH-901 (Susceptible), CRIS-466(Susceptible), FH-167 (Moderately susceptible), CIM-707 (Moderately susceptible), CIM-496 (Moderately susceptible), CIM-541 (Moderately susceptible), BH-160 (Moderately tolerant), FH-1000 (Moderately tolerant), N-111 (Moderately tolerant), MARVI (Moderately tolerant), CIM-554 (Moderately tolerant), MNH-789 (Tolerant), PB-899 (Tolerant), FH-113 (Most tolerant) were selected on the basis of survival rate. These cotton accessions were sown in polythene bags (30 cm × 15 cm), following complete randomized design with three replications under normal and water stress conditions. The irrigation was done on every alternate day with normal tap water. After 45 days from sowing, a drought cycle was induced by restricting irrigation for 7 days. Normal regime was irrigated regularly.

The leaf samples from both normal and drought stress conditions were collected for estimations of various biochemical traits. For each estimation, tissues from leaves of different plants possessing the same position were included.

Determination of chlorophyll and carotenoids: The contents of chlorophyll and carotenoids were analyzed. All pigments in sample were extracted with acetone at once, then absorbance of the extract was taken at 663 nm, 645 nm, 645 nm, 505 nm and 453 nm with spectrophotometer at the same time. From these values, the contents of chlorophyll *a, b* and carotenoids were calculated using following equations (Nippon, 1992).

Chlorophyll a (mg/100 mL) = $0.999A_{663}$-$0.0989A_{645}$
Chlorophyll *b* (mg/100 mL) = $-0.328A_{663}$+$1.77A_{645}$
β – Carotene (mg/100 mL) = $0.216A_{663}$-$1.22A_{645}$ -$0.304A_{505}$+$0.452A_{453}$

A663, A645, A505 & A45 are absorbance at 663 nm, 645 nm, 505 nm & 453 nm respectively.

Total phenolic contents (TPC): TPC in cotton leaves were quantified by using the method illustrated by Waterhouse (2001). Folin Ciocalteu Reagent (2N), Na_2CO_3 were used as reagents while gallic acid was used as standard (100, 150, 250 & 500 mg L^{-1} gallic acid) for making standard curve. The absorbance by gallic acid standards and moringa samples was noted at 760 nm by using UV-spectrophotometer (UV-4000, O.R.I. Germany).

Determination of morphological traits: Morphological traits viz. fresh root length (cm), fresh root weight (g), fresh shoot length (cm), fresh shoot weight (g), dry root weight (g), dry shoot weight (g) and root shoot ratio were measured. Fresh root and shoot length was measured by using a measuring tape while fresh root and shoot weights were taken by washing roots of each genotype carefully to free from sand and blotted dry fresh seedlings roots were weighed and fresh shoots were separated from the seedlings were weighed in grams by using an electronic balance. Dry root and shoot weights were taken by putting roots and shoots in Kraft paper bag and dried in an electric oven at 65±5°C for 72 h. After drying, dry root weights were recorded by using an electronic balance. Root shoot ratio was obtained by dividing the fresh root weight to shoot weight.

Statistical analysis: Analysis of variance was computed to compare the genotypes for each trait following complete randomized design. The difference among the treatments' means was compared using LSD test at 5% level of probability (Steel & Torrie, 1996).

RESULTS

Physiological basis of drought tolerance and susceptibility 15 cotton accessions was carried out in a pot study. It was found that genotypes FH-113, PB-899 and MNH-789 had maximum amount of chlorophyll *a* (mg/100 mL), (2.98), (2.89) and (2.81) under normal conditions, (2.89), (2.80) and (2.79) under water stress conditions, respectively (Fig. 1). CIM-506, FH-901 and CRIS-466 had minimum amount of chlorophyll (2.39), (2.42) and (2.44) under normal conditions, (1.97), (1.99) and (2.01) under water stress conditions, respectively. FH-113, PB-899 and MNH-789 had minimum amount of chlorophyll *b* under normal conditions and more amount of chlorophyll *b* under water stress conditions, respectively. CIM-506, FH-901 and CRIS-466 had maximum amount of chlorophyll *b*, (1.69), (1.67) and (1.65) under normal conditions and low value of chlorophyll *b* (0.99), (0.98) and (0.98) under water stress conditions, respectively.

FH-113, PB-899 and MNH-789 had maximum amount of carotenoids (0.79), (0.69) and (0.66) under normal conditions, (0.77), (0.67) and (0.64) under water stress conditions, respectively (Fig. 2). CIM-506, FH-901 and CRIS-466 had minimum amount of carotenoids, (0.29), (0.34) and (0.39) under normal conditions, (0.18), (0.24) and (0.26) under water stress conditions, respectively. It was noted that chlorophyll and carotenoids were reduced under water stress conditions. FH-113, PB-899 and MNH-789 had maximum *a/b* ratio (2.15), (2.10) and (2.05) under normal conditions, (2.44), (2.35) and (2.32) under water stress conditions, respectively. It was noted that *a/b* ratio was increased under water stress conditions. Same trend was noted for polyphenols. It was noted that polyphenols were increased under water stress conditions.

Analysis of variance for root length, shoot length, root weight, shoot weight, dry root weight, dry shoot weight and root shoot ratio under normal and water stress conditions showed significant results as depicted in Table I.

Table I: F-value and coefficient of variation (CV %) of 15 cotton genotypes for various seedling traits under normal and water stress conditions

Trait	Level	F-value	Error	C.V%
Root length (cm)	Normal	3.022**	0.5422	11.14
	Water stress	3.526**	0.5871	10.58
Shoot length (cm)	Normal	20.707**	1.0240	7.04
	Water stress	16.344**	0.8525	7.11
Root weight (g)	Normal	128.677**	0.0181	5.48
	Water stress	142.837**	0.0187	4.81
Shoot weight (g)	Normal	9.107**	0.0494	12.84
	Water stress	3.466**	0.0370	15.23
Dry root weight (g)	Normal	264.676**	0.0027	3.01
	Water stress	124.480**	0.0031	4.35
Dry shoot weight (g)	Normal	36.465**	0.0109	9.59
	Water stress	33.566**	0.0080	7.16
Root shoot ratio	Normal	20.166**	0.02758	3.88
	Stress	27.642**	0.0288	3.41

Fig. 1: Chlorophyll *a* and *b* determination under normal and water stress conditions

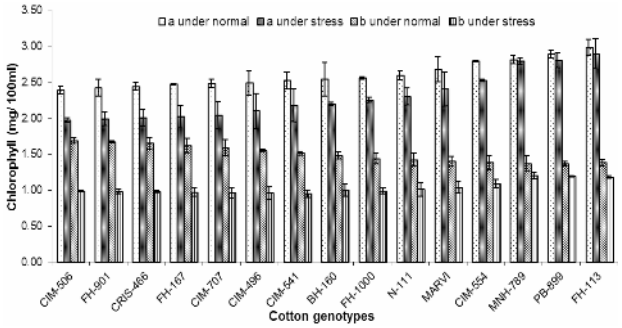
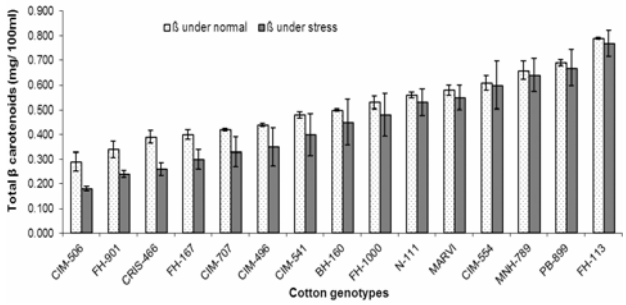


Fig. 2: Total *β*-carotenoids determination under normal and water stress conditions



Mean performance of cotton genotypes (Table II) revealed that FH-113 had maximum fresh root weight (0.145 g) and (0.155 g) under normal and water stress condition, respectively. While CIM-506 had minimum fresh root weight (0.034 g) and (0.049 g) under normal and water stress conditions, respectively which was followed by FH-113, PB-899 and MNH-789 showing maximum fresh root weight (0.140 g) and (0.120 g) under normal conditions, and (0.150 g) and (0.135 g) under water stress conditions, respectively followed by, CIM-506, FH-901 and CRIS- 466 had minimum fresh root weight (0.050 g) and (0.060 g) under normal conditions, (0.055 g) and (0.070 g) under water stress conditions respectively. There was increase in root weight under water stress conditions.

Fig. 3: Chlorophyll *a/b* ratio determination under normal and water stress conditions

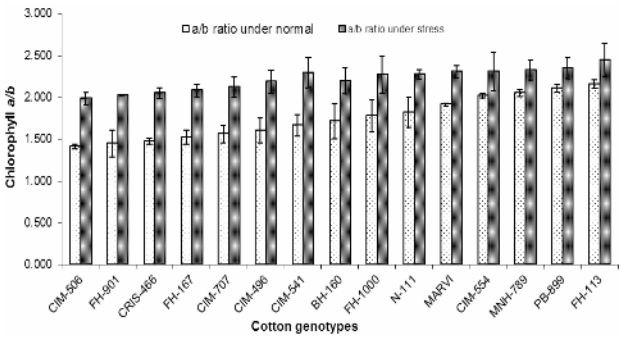
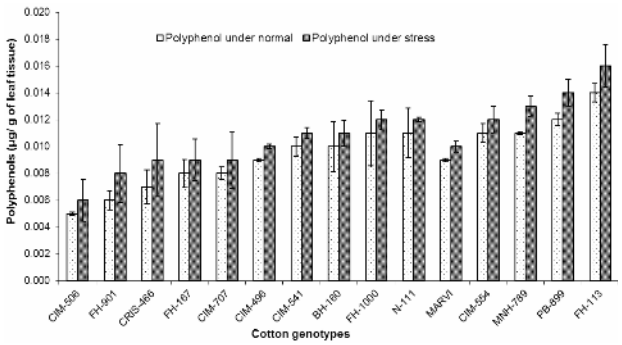


Fig. 4: Polyphenols determination under normal and water stress conditions



FH-113 had maximum root length (8.02 cm) and (8.51 cm) under normal and water stress conditions, respectively (Table II). CIM-506 had minimum root length (4.71 cm) and (5.01 cm) under normal and water stress conditions, respectively followed by FH-113, PB-899 and MNH-789 had maximum root length (7.02 cm) and (7.01 cm) under normal conditions, (7.52 cm) and (8.51 cm) under water stress conditions, respectively followed by CIM-506, FH-901 and CRIS-466 had minimum root length (5.75 cm) and (6.31 cm) under normal conditions, (6.51 cm) and (6.52 cm) under water stress conditions, respectively. It was noted that there was increase in root length under water stress conditions.

The cultivar FH-113 gained maximum shoot length (13.02 cm) and (12.01 cm) under normal and water stress conditions, respectively. CIM-506 had minimum shoot length (6.72 cm) and (6.01 cm) under normal and water stress conditions, respectively followed by FH-113, PB-899 and MNH-789 had maximum shoot length (13.01 cm) and (10.51 cm) under normal conditions, (11.02 cm) and (8.01 cm) under water stress conditions, respectively followed by CIM-506, FH-901 and CRIS-466 had minimum shoot length (8.53 cm) and (8.01 cm) under normal conditions, (7.22 cm) and (8.02 cm) under waters stress conditions, respectively. It was noted that there was decrease in shoot length under water stress conditions.

PB-899 achieved the maximum root dry weight, (0.026 g) and (0.030 g) under normal and water stress conditions (Table III), while CIM-506 showed minimum

Table II: Mean and statistical significance of 52 days old seedling of cotton genotypes under normal and water stress conditions

Genotypes	Root length (cm)		Shoot length (cm)		Root weight (g)		Shoot weight (g)	
	Normal	Water Stress	Normal	Water Stress	Normal	Water Stress	Normal	Water Stress
CIM-506	4.71 c	5.01 c	6.72 e	6.01 d	0.034 o	0.049 m	0.27 ef	0.26 c
FH-901	5.75 bc	6.51 b	8.53 cd	7.22 c	0.050 n	0.055 l	0.30 de	0.27 c
CRIS-466	6.31 b	6.52 b	8.01 d	8.02 bc	0.060 m	0.070 k	0.21 f	0.31 c
FH-167	5.92 bc	7.01 b	8.51 cd	8.11 bc	0.065 l	0.070 k	0.27 ef	0.27 c
CIM-707	6.41 b	7.21 ab	8.52 cd	8.01 bc	0.070 k	0.075 j	0.29 def	0.27 c
CIM-496	6.51 b	7.22 ab	8.71 cd	8.51 b	0.075 j	0.080 i	0.30 de	0.28 c
CIM-541	6.72 ab	7.31 ab	8.81 cd	8.41 b	0.080 i	0.090 h	0.35 cde	0.30 c
BH-160	6.91 ab	7.21 ab	8.62 cd	8.01 bc	0.085 h	0.100 g	0.36 cd	0.31 c
FH-1000	6.82 ab	7.01 b	8.51 cd	8.22 bc	0.090 g	0.115 e	0.36 cd	0.31 c
N-111	6.74 ab	7.11 ab	9.01 cd	8.81 b	0.10 f	0.110 f	0.37 cd	0.32 c
MARVI	6.83 ab	7.22 ab	9.51 bc	9.01 b	0.105 e	0.110 f	0.40 bc	0.35 bc
CIM-554	6.92 ab	7.51 ab	10.21 b	9.02 b	0.110 d	0.120d	0.40 bc	0.32 c
MNH-789	7.01 ab	8.51 a	10.51 b	8.01 bc	0.120 c	0.135 c	0.43 abc	0.30 c
PB-899	7.02 ab	7.52 ab	13.01 a	11.02 a	0.140 b	0.150 b	0.50 a	0.40 ab
FH-113	8.02 a	8.51 a	13.02 a	12.01 a	0.145 a	0.155 a	0.46 ab	0.45 a
LSD	**1.222**	**1.262**	**1.097**	**1.016**	**0.00288**	**0.00288**	**0.07457**	**0.0745**

Table III: Mean and statistical significance of 52 days old seedling of cotton genotypes under normal and water stress conditions

Genotypes	Dry root weight (g)		Dry shoot weight (g)		Root shoot ratio	
	Normal	Water Stress	Normal	Water Stress	Normal	Water Stress
CIM-506	0.007 f	0.012 f	0.030 h	0.030 h	0.700 de	0.833 cd
FH-901	0.010 e	0.015 e	0.080 a	0.080 a	0.673 e	0.900 b
CRIS-466	0.014 d	0.016 e	0.037 g	0.038 g	0.785 ab	0.814 cd
FH-167	0.014 d	0.016 e	0.040 f	0.038 g	0.694 e	0.862 bc
CIM-707	0.013 d	0.015 e	0.060 e	0.058 e	0.750 abcd	0.901 b
CIM-496	0.014 d	0.015 e	0.061 e	0.060 e	0.748 bcd	0.847 bcd
CIM-541	0.015 d	0.016 e	0.060 e	0.058 e	0.759 abc	0.865 bc
BH-160	0.018 c	0.020 d	0.062 de	0.060 e	0.801 a	0.899 b
FH-1000	0.019 bc	0.022 c	0.060 e	0.058 e	0.801 a	0.854 bcd
N-111	0.021 b	0.025 b	0.065 d	0.065 d	0.747 bcd	0.806 d
MARVI	0.020 bc	0.022 c	0.070 c	0.065 d	0.716 cde	0.799 d
CIM-554	0.018 c	0.020 d	0.065 d	0.060 e	0.677 e	0.831 cd
MNH-789	0.018 c	0.020 d	0.063 de	0.050 f	0.667 e	1.061 a
PB-899	0.026 a	0.030 a	0.010 i	0.070 c	0.540 g	0.682 e
FH-113	0.019 bc	0.030 a	0.076 b	0.075 b	0.615 f	0.708 e
LSD	**0.002358**	**0.00167**	**0.00288**	**0.00289**	**0.04613**	**0.0482**

Means sharing same letters are similar at P ≤0.05

dry root weight (0.007 g) and (0.012 g) under normal and water stress conditions, respectively followed by PB-899, FH-113 and MNH-789 had maximum dry root weight (0.019 g) and (0.018 g) under normal, (0.030 g) and (0.020 g) under water stress conditions, respectively. It was noted that there was increase in dry root weight under water stress conditions.

FH-113, PB-899 and MNH-789 have root shoot ratio of (0.615), (0.667) and (0.540) under normal, (0.708), (0.682) and (1.061) under water stress conditions, respectively (Table III). CIM-506, FH-901 and CRIS-466 have root shoot ratio of (0.700), (0.673) and (0.785) under normal, (0.833), (0.900) and (0.814) under water stress conditions, respectively. However, MNH-789 a drought tolerant variety has maximum root shoot ratio of (1.061) under water stress conditions. It was also observed that root shoot ratio was increased under water stress conditions.

Correlation between *β*-carotenoids and total chlorophyll contents were non-significant under normal conditions, while highly significant under water stress conditions. The relationship between *β*-carotenoids and polyphenols, and total chlorophyll and polyphenols were highly significant under normal and water stress conditions (Table IV). Accessions (drought susceptible) CIM-506 and FH-901 had negative correlation between *β*-carotenoids and polyphenols, and total chlorophyll and polyphenols, and also are non-significant (Table V). Drought tolerant accession MNH-789 showed positive correlation between *β*-carotenoids and total chlorophyll and drought tolerant accession FH-113 had positive correlation of *β*-carotenoids with total chlorophylls and polyphenols, and had also significant relationship (Table V).

DISCUSSION

Chlorophyll contents are reduced by water stress. Ashraf *et al.* (1994) reported decrease in chlorophyll (*a, b*) and an increase occurred in chlorophyll *a/b* ratio under water stress. A pronounced effect of water stress is reduction in photosynthesis, which arises by impaired

Table IV: Correlation among *β*-carotenoids, Chl (*a*+*b*) and polyphenols under normal and drought conditions

Varieties	*β*-carotenoids		Chl (*a*+*b*) vs polyphenols
	Chl (*a*+*b*)	polyphenols	
Control	0.2850	0.7970	0.4368
condition	0.0577 ns	0.0000 **	0.0027 **
Water	0.8965	0.7583	0.7730
stress	0.0000 **	0.0000 **	0.0000 **

Table V: Correlation of 15 cotton genotypes among β-carotenoids, Chl (a+b) and polyphenols

Varieties	*β*-carotenoids		Chl (*a*+*b*) vs polyphenols
	Chl (*a*+*b*)	polyphenols	
CIM-506	0.7080	-0.0209	-0.0978
	0.1154 ns	0.9685 ns	0.8537 ns
FH-901	0.7125	-0.0743	-0.0405
	0.1121 ns	0.8887 ns	0.9392 ns
CRIS-466	0.8163	0.0381	0.0491
	0.0475 *	0.9428 ns	0.9264 ns
FH-167	0.8533	0.5297	0.1212
	0.0307 *	0.2797 ns	0.8191 ns
CIM-707	0.8893	0.6855	0.2844
	0.0177 *	0.1327 ns	0.5848 ns
CIM-496	0.8307	0.5945	0.1729
	0.0405 *	0.2132 ns	0.7431 ns
CIM-541	0.7684	0.6681	0.3262
	0.0742 ns	0.1469 ns	0.5280 ns
BH-160	0.4473	0.7435	0.3557
	0.3737 ns	0.0902 ns	0.4889 ns
FH-1000	0.4878	0.4240	0.0277
	0.3263 ns	0.4020 ns	0.9583 ns
N-111	0.7387	0.6249	0.3406
	0.0935 ns	0.1846 ns	0.5088 ns
MARVI	0.8952	0.7792	0.4226
	0.0159 *	0.0677 ns	0.4038 ns
CIM-554	0.2675	0.9658	0.0092
	0.6083 ns	0.0017 **	0.9862 ns
MNH-789	0.8130	0.3218	-0.1726
	0.0492 *	0.5339 ns	0.7436 ns
PB-899	0.6673	0.7894	0.2726
	0.1476 ns	0.0618 ns	0.6011 ns
FH-113	0.8395	0.8665	0.6175
	0.0365 *	0.0255 *	0.1914 ns

Each cell contains double values in which upper value represents the correlation coefficient (r) and lower value represents probability at 5%
* = significant at P≤0.05
ns = non-significant

photosynthetic machinery. Changes were produced in photosynthetic pigments and components by drought stress (Anjum *et al.*, 2003) and damaged photosynthetic machinery (Fu & Huang, 2001). It is also confirmed in our study that the increase in *a/b* ratio was smaller in tolerant genotypes than susceptible ones under water stress. It has been reported by many workers that under saline conditions, chlorophyll *b* decreases more than chlorophyll *a*, thus shifting the ratio in favour of chlorophyll *a* (Ashraf & Mehmood, 1990). The contents of cholorophyll *a*, *b* and carotenoids and total chlorophyll (*a* + *b*), cholorophyll *a/b* and Carotenoid/cholorophyll *a* + *b* ratios decreased in the leaves of the more drought-sensitive cultivar under drought conditions (El-Tayeb, 2006).

β-carotene, not only function as an accessory pigment, but also as an effective antioxidant and plays an exclusive role in shielding photochemical processes and sustaining them (Havaux, 1998). A major defensive role of *β*-carotene in photosynthetic tissue may be through direct quenching of triplet chlorophyll, which restricts the production of singlet oxygen and protects from oxidative damage (Farooq *et al*., 2009). Drought stress has the ability to decrease the tissue concentrations of chlorophylls and carotenoids (Havaux, 1998; Kiani *et al.,* 2008), mainly with the production of reactive oxygen species in the thylakoids (Niyogi, 1999; Reddy *et al.*, 2004). Carotenoids of the xanthophyll family and some other terpenoids, such as isoprene or *α*-tocopherol, become stable and photo protect the lipid phase of the thylakoid membranes (Havaux, 1998; Sharkey, 2005; Velikova *et al*., 2005), in current study we found that chlorophyll *a, b* and total carotenoids were reduced under water stress. Chlorophyll *b* content increased whereas chlorophyll *a* remained unaffected resulting in a significant reduction in Chloropyhll *a:b* ratio in both cultivars under water limiting regimes (Ashraf *et al.*, 1994; Anjum *et al.,* 2003). Plants protect cells and sub-cellular systems from the cytotoxic effects of these active oxygen radicals with both non-enzymatic and enzymatic antioxidant system such as carotenoids, ascorbic acid, *α*-tochopherol, peroxidase and catalase (Fu & Huang, 2001). In the current study water stress reduced the chlorophyll contents in all the genotypes. Reduction in chlorophyll may be due to slower synthesis or its quicker breakdown under drought stress (Majumdar *et al*., 1991).

Phenolics play a variety of roles in plants as they are the most plentiful class of secondary metabolites. They act as powerful antioxidants in plant tissues under stress (Sgherri *et al*., 2004). They form the pigments in flowers and fruits, contribute to biotic and abiotic stress tolerance, provide UV shelter and have role in pollen fertility (Martens & Mithöfer, 2005). Reactive oxygen species scavenging ability of cotton genotypes differing in drought tolerance, cultivated in field conditions under water deficit conditions. Polyphenol species, carotenoids and proline act as powerful reactive oxygen species scavengers (Edreva 2005a & b; Leopoldini *et al*., 2006).

Abiotic stresses stimulate production of various secondary metabolites (Wahid & Ghazanfar, 2006). Qualitative patterns of two cultivars considerably differed in polyphenol amount grown at normal irrigation supply. The drought-tolerant cultivar was illustrious by higher content of all polyphenol types than the sensitive cultivar, the divergence being mainly expressed in the fraction of quercetin-containing flavonoids. At water deficit conditions, the polyphenol compounds reduced in both cultivars. However, the reduction was less significant in the tolerant as compared with the sensitive cultivar (Yildiz-Aktas *et al*., 2009).

Water stress increased the concentration of plant phenolics (Meyer *et al*., 2003; Morales *et al*., 2005; Hura *et al*., 2006). Water stress can bring defensive mechanisms against the lethal action of radiation, which could be immersed and transformed by phenolic compounds into

blue fluorescence that, in turn, could be exploited in the photosynthesis process as well as re-emitted as chlorophyll fluorescence (Bilger *et al*., 1997, 2001; Buschmann & Lichtenthaler, 1998), we found that polyphenols were increased in all cotton genotypes under water stress conditions. Increase in polyphenols contents in different tissues under stress has been observed in a number of plants (Muthukumarasamy *et al.,* 2000).

Fresh root weight was increased under water stress conditions. Roots are the chief plant organ for adaptation to water stress. Significant differences were found between the genotypes for all traits like shoot weight, root weight and root shoot ratios. There were no major interactions, however, between genotypes and experiments for all parameters except root-shoot ratios (McMichael & Quisenberry, 1999). The capability of a plant to alter significantly its root-shoot ratio may be only the sole part of the overall mechanism (Klepper *et al.,* 1973) that a plant exploits to tolerate environmental stress. Soil moisture status affects the growth, shape, structure, physiological function, and water uptake characteristics of crop root system as well as root shoot ratio (Li *et al*., 2000; Yang *et al*., 2007). The growth of cotton (*Gossypium hirsuturm* L.) root systems is under genetic control. Quisenberry *et al.* (1981) evaluated 35-days-old plants of exotic cotton germplasm in green-house conditions and showed significant variation for taproot length and number of lateral roots. McMichael and Quisenberry (1991) also evaluated exotic cotton genotypes as well as modern cultivars grown in containers for approximately 60-70 days and studied variability in root and shoot development.

Genotypic variation was found when plants were exposed to water stress but not in the absence of water stress in root development. The data indicated that tolerant genotypes responded to water stress by increasing root length density and root weight density significantly (Heggestad *et al*., 1988). Soil water content can have a direct impact on the growth rate and distribution of roots. Rooting depth and density may boost in a drying soil (Klepper *et al.,* 1973).

Some evidence suggests that genetic variation exist in the response of roots to changes in soil water. Quisenberry *et al.* (1981) concluded that there was significant variation in root growth of a number of cotton accessions that was positively related with shoot dry weights in dry_land conditions. They also proposed that root growth potentials appeared to be significant traits in the adaptation of cotton to water stress conditions. Dry matter production was greatly reduced in cultivars grown under water stress, which could be a result of stomatal and non-stomatal restriction of photosynthesis under water stress conditions (Kaiser, 1987).

In conclusion, accessions having more chlorophyll, carotenoids and better antioxidant defense system and polyphenols were drought tolerant. Chlorophyll, carotenoids are reduced and polyphenols are increased under water stress conditions. The information obtained from current study may be used to breed drought tolerant materials.

REFERENCES

Andrew, J.S., H. Moreau, M. Kuntz, G. Pagny, C. Lin, S. Tanksley and J. McCarthy, 2008. An investigation of carotenoid biosynthesis in *Coffea canephora* and *Coffea arabica*. *J. Plant Physiol.,* 165: 1087–1106

Anjum, F., M. Yaseen, E. Rasul, A. Wahid and S. Anjum, 2003. Water stress in barley (*Hordeum vulgare* L.). I. Effect on chemical composition and chlorophyll contents. *Pakistan J. Agric. Sci.,* 40: 45–49

Ashraf, M. and S. Mehmood, 1990. Response of four brassica species to drought stress. *Environ. Expt. Bot.,* 30: 93–100

Ashraf, M.Y., A.R. Azmi, A.H. Khan and S.S.M. Naqvi, 1994. Water relations in different wheat (*Triticum aestivum* L.) genotypes under soil water deficits. *Acta Physiol. Plant.,* 16: 231–240

Bilger, W., M. Veit, L. Schreiber and U. Schreiber, 1997. Measurement of leaf epidermal transmittance of UV radiation by chlorophyll fluorescence. *Physiol. Plant,* 101: 754–763

Bilger, W., T. Johnsen and U. Schreiber, 2001. UV-excited chlorophyll fluorescence as a tool for the assessment of UV-protection by the epidermis of plants. *J. Exp. Bot.,* 52: 2007–2014

Boyer, J.S., 1982. Plant productivity and environment. *Science*, 218: 443–448

Bray, E.A., J. Bailey-Serres and E. Weretilnyk, 2000. Responses to abiotic stresses. *In*: Gruissem, W., B. Buchannan and R. Jones (eds.), *Biochemistry and Molecular Biology of Plants*, pp: 1158–1249. American Society of Plant Physiologists, Rockville, M.D., USA

Buschmann, C. and H.K. Lichtenthaler, 1998. Principles and characteristics of multi-colour fluorescence imaging of plants. *J. Plant Physiol.,* 152: 297–314

Earl, H. and R.F. Davis, 2003. Effect of drought stress on leaf and whole canopy radiation use efficiency and yield of maize. *Agron. J.,* 95: 688–696

Edreva, A., 2005a. Generation and scavenging of reactive oxygen species in chloroplasts: a submolecular approach. *Agric. Ecosyst. Environ.,* 106: 119–133

Edreva, A., 2005b. The importance of non-photosynthetic pigments and cinnamic acid derivatives in photoprotection. *Agric. Ecosyst. Environ.*, 106: 135–146

El-Tayeb, M.A., 2006. Differential Responses of Pigments, Lipid Per-oxidation, Organic Solutes, Catalase and Per-oxidase Activity in the Leaves of Two Vicia faba L. Cultivars to Drought. *Int. J. Agric. Biol.,* 8: 116–122

Farooq, M., A. Wahid, N. Kobayashi, D. Fujita and S.M.A. Basra, 2009. Plant drought stress: effects, mechanisms and management. *Agron. Sustain. Dev.,* 29: 185–212

Fu, J. and B. Huang, 2001. Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environ. Exp. Bot.,* 45: 105–114

Hasegawa, P.M., R.A. Bressan, J.K. Zhu and H.J. Bohnert, 2000. Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Phys.*, 51: 463–499

Havaux, M., 1998. Carotenoids as membrane stabilizers in chloroplasts. *Trends Plant Sci.*, 3: 147–151

Heggestad, H.E., E.L. Anderson, T.J. Gish and E.H. Lee, 1988. Effects of Ozone and Soil Water Deficit on Roots and Shoots of Field-grown Soybeans. *Environ. Pollut.,* 50: 259–278

Hura, T., S. Grzesiak, K. Hura, M.T. Grzesiak and A. Rzepka, 2006. Differences in the physiological state between triticale and maize plants during drought stress and followed rehydration expressed by the leaf gas exchange and spectrofluorimetric methods. *Acta Physiol. Plant,* 28: 433–443

Ingram, J. and D. Bartels, 1996. The molecular basis of dehydration tolerance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 47: 377–403

Jaleel, C.A., P. Manivannan, A. Kishorekumar, B. Sankar, R. Gopi, R. Somasundaram and R. Panneerselvam, 2007. Alterations in osmoregulation, antioxidant enzymes and indole alkaloid levels in *Catharanthus roseus* exposed to water deficit. *Colloids Surf. B: Biointerfaces*, 59: 150–157

Jones, L.A. and J.H. Kersey, 2002. *Cottonseed Article*. National cottonseed products Association (NCPA) (USA)

Kaiser, W.M., 1987. Effects of water deficit on photosynthetic capacity. *Physiol. Plant,* 71: 142–149

Kiani, S.P., P. Maury, A. Sarrafi and P. Grieu, 2008. QTL analysis of chlorophyll fluorescence parameters in sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions. *Plant Sci.*, 175: 565–573

Klepper, B., H.M. Taylor, M.G. Hock and E.L. Fiscus, 1973. Water relations and growth of cotton in drying soil. *Agron. J.,* 65: 307–310

Leopoldini, M., N. Russo, S. Chiodo and M. Toscano, 2006. Iron chelation by the powerful antioxidant flavonoid quercetin. *J. Agric. Food. Chem.*, 54: 6343–6351

Li, S.K., C.T. Wang, W.F. Zhang and C.Y. Wang, 2000. Effects of cultivation on cotton root and shoot growth in north of Xinjiang. *China Cotton*, 27: 12–13

Majumdar, S., S. Ghosh, R. Bernord and E.B. Dumbroff, 1991. Activities of chlorophyllase, phosphoenolpyruvate carboxylase and ribulose-1, 5-bisphosphate carboxylase in the primary leaves of soybean during senescence and drought. *Pysiol. Plant,* 81: 473–480

Martens, S. and A. Mithöfer, 2005. Flavones and flavone synthases. *Phytochemistry,* 66: 2399–2407

McMichael, B.L. and J.E. Quisenberry, 1991. Genetic variation for root shoot relationships among cotton germplasm. *Environ. Exp. Bot.,* 31: 461–470

McMichael, B.L. and J.E. Quisenberry, 1999. Genetic variation for root-shoot relationships among cotton germplasm. *Environ. Exp. Bot.,* 31: 461–470

Meyer, S., A. Cartelat, I. Moya and Z.G. Cerovic, 2003. UV-induced blue-green and far-red fluorescence along wheat leaves: a potential signature of leaf ageing. *J. Exp. Bot.,* 54: 757–769

Morales, F., A. Cartelat, A. Alvarez-Fernandez, I. Moya and Z.G. Cerovic, 2005. Time-resolved spectral studies of blue-green fluorescence of artichoke (*Cynara cardunculus* L. *Var. Scolymus*) leaves: identification of chlorogenic acid as one of the major fluorophores and agemediated changes. *J. Agric. Food Chem.,* 53: 9668–9678

Muthukumarasamy, M., S.D. Gupta and R. Pannerselvam, 2000. Enhancement of peroxidase, polyphenol oxidase and super oxide dismutage activities by triadimefon in NaCl stressed *Raphanus sativus* L. *Biol. Plant*, 43: 317–320

Nagata, M. and I. Yamashita, 1992. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *J. Japanese Soc. Food Sci. Technol.*, 39: 925–928

Niyogi, K.K., 1999. Photoprotection revisited: genetic and molecular approaches. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* 50: 333–359

Prochazkova, D., R.K. Sairam, G.C. Srivastava and D.V. Singh, 2001. Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. *Plant Sci.*, 161: 765–771

Quisenberry, J.E., W.R. Jordan, B.A. Roark and D.W. Fryrear, 1981. Exotic cottons as genetic sources for drought resistance. *Crop Sci.,* 21: 889–895

Reddy, A.R., K.V. Chaitanya and M. Vivekanandan, 2004. Droughtinduced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol.*, 161: 1189–1202

Sgherri, C., B. Stevanovic and F. Navari-Izzo, 2004. Role of phenolic acid during dehydration and rehydration of Ramonda serbica. *Physiol. Plant*, 122: 478–485

Sharkey, T.D., 2005. Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species and thermotolerance provided by isoprene. *Plant Cell Environ*., 28: 269–277

Steel, R.G.D. and J.H. Torrie, 1996. *Principles and Procedures of Statistics: A biometrical approach,* 3rd edition. McGraw-Hill, New York

Velikova, V., A. Edreva and F. Loreto, 2005. Endogenous isoprene protects *Phragmites australis* leaves against singlet oxygen. *Plant Cell. Environ.*, 28: 318–327

Wahid, A. and A. Ghazanfar, 2006. Possible involvement of some secondary metabolites in salt tolerance of sugarcane. *J. Plant Physiol.* 163: 723–730

Wahid, A. and E. Rasul, 2005. Photosynthesis in leaf, stem, flower and fruit. *In*: Pessarakli, M. (ed.), *Handbook of Photosynthesis*, 2nd edition, pp: 479–497. CRC Press, Florida

Waterhouse, A.L., 2001. *Determination of Total Phenolics, in Current Protocol in Food Analytical Chemistry*. I1.1.1-I1.1.8, Wrolstad, R.E., Wiley

Wery, J., S.N. Silim, E.J. Knights, R.S. Malhotra and R. Cousin, 1994. Screening techniques and sources and tolerance to extremes of moisture and air temperature in cool season food legumes. *Euphytica*, 73: 73–83

Yang, S.Y., P. Yan, X.Y. Mei and X.B. Sun, 2007. Effects of different soilwater deficits on roots of winter wheat. *J. Triticeae Crops*, 27: 309–313

Yildiz-Aktas, L., S. Dagnon, A. Gurel, E. Gesheva and A. Edreva, 2009. Drought Tolerance in Cotton: Involvement of Non-enzymatic ROS-Scavenging Compounds. *J. Agron. Crop Sci.,* 195: 247–253

Zhang, T.G.W., 2001. *Present Status and Prospect on Cotton Genomic Studies*. Department of Agronomy, Nanjing Agricultural University, Nanjing, China

(Received 08 March 2011; Accepted 29 June 2011)