

Research Paper

**ALTERATIONS IN PHOTOSYNTHETIC, WATER RELATIONS AND
BIOCHEMICAL COMPONENTS IN COTTON SUBJECTED TO DROUGHT
STRESS**

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Abstract

The depiction of plant water relations, gas exchange parameters and biochemical components is essential for subsequent selection and genetic manipulation for drought tolerance in plants. In order to assess drought tolerance mechanism in cotton, short-term drought-induced water relations, gas exchange and biochemical responses were monitored in two cotton (*Gossypium arboreum* L.) genotypes contrasting their tolerance to water deficit. Significant differences were reported among both genotypes (FDH 786 and FDH 171) for the attributes measured. Gas exchange attributes (photosynthetic rate, stomatal conductance, transpiration rate) were found to be remarkably higher in cotton variety FDH 786 compared to FDH 171 during drought incantation. Drought-induced increases in water relations components (water, osmotic and turgor potential) were significantly higher in the cotton genotype FDH 786 than FDH 171 genotype. The total soluble sugars, total protein, proline and total free amino acids were found to be significantly reduced in FDH 171 stressed plants as compared to FDH 786 under drought stress. These results suggest that among these two cotton genotypes, cotton variety FDH 786 maintained prominently higher gas exchange attributes, water relations components and osmotic components under water deficit. The results demonstrated that the empirically determined differences in drought tolerance of these two cultivars can be related to measurable physiological parameters. These outcomes suggest that physiological, biochemical monitoring can be an effective tool in germplasm selection and improvement.

Key words: Gas Exchange, water relations, osmotic components, cotton, drought.

INTRODUCTION

Drought or water deficit stress is the major environmental factor that adversely impacts agricultural yield throughout the world, mainly when stress occurs during reproductive growth, affecting production whether it is for subsistence or economic gain [1]. The plant response to drought consists of numerous processes that must function in coordination to alleviate both cellular hyperosmolarity and ion disequilibrium. To cope with drought stress, plants respond

with physiological and biochemical changes. These changes aim at the retention of water in spite of the high external osmoticum and the maintenance of photosynthetic activity, while stomatal opening is reduced to counter water loss. Accumulation of low molecular compounds, such as sugars, proteins and proline, is a mechanism aimed at balancing water potential following drought [2]. Although an adaptive role for organic osmolytes in mediating osmotic adjustment and protecting subcellular structure has become a central dogma in stress physiology, the evidence in favor of this hypothesis is largely correlative [3]. Differences in the expression of specific genes between stress-sensitive and stress-tolerant plants indicate that tolerance is conferred by genetically encoded mechanisms in a network of biochemical pathways interacting to give a concerted response to stress [4]. Drought is one of the most important abiotic stress factors affecting plant growth and leaf photosynthesis [5] and altering biochemical properties of plants [6].

Cotton is one of the most important economy crops grown in rainfed and irrigated areas of the world. It is regarded highly by the governments not only in relation to people's lives, but also to the income of cotton farmers and the economic development of cotton planting zones, as well as to national textile supply and foreign exchange income. Many people consider cotton to be the purest fiber on earth, or the "fabric of our lives". Drought stress affects the cotton plants by limiting fiber yield and lint quality. Like other agricultural crops, then growth, development and performance of cotton is adversely affected by moisture stress. Cultivars are needed that can endure and recover from drought so as to minimize the losses in rainfed areas and to reduce the water needed in irrigated areas.

In Pakistan, cotton is an important agricultural commodity; being an exporting item it fetches a considerable amount of foreign exchange. In addition within the country cotton plant provides raw material to the expanding textile industry. Clearly the cotton crop is of immense importance in the economy of Pakistan. During summer season, the crop is extensively grown in the irrigated areas of southern parts of the Punjab province (so called "the cotton belt"), and Sindh province. Production of cotton in many areas of both Punjab and Sindh provinces is limited by inadequate amounts of water supply or small amount of rainfall during growth and development of cotton crop. Although there are many other reasons for low production levels in of cotton, decreasing ground water supplies and high energy costs are also emerging problems of cotton cultivation in the country [7].

No more significant studies available on the effect of water stress on gas exchange, water relations and biochemical behavior of *Gossypium arboreum*. The aim of this study was to find differences that may be implicated in conferring the ability to evaluate performance under drought condition in the controlled environment conditions by the two tolerant cotton varieties (FDH 786 and FDH 171) and determine any differences between them, and to examine the changes in gas exchange, physiological and biochemical responses between drought-stressed and control plants for these cultivars. The better responsive genotype under abiotic stress would be used in future molecular breeding program to develop abiotic stress tolerant cotton genotypes.

2. MATERIALS AND METHODS

2.1 Plant material (Genotypes)

A total of two cotton varieties (FDH 786 and FDH 171) were chosen. Seeds of both varieties were collected from local germplasm center Central Cotton Research Institute (CCRI) Multan.

2.2 Growth conditions and water stress treatment

The investigation was conducted at the National Centre of Excellence in Molecular Biology (CEMB) University of the Punjab, Lahore Pakistan. Seeds of cotton varieties were obtained from CCRI. This work was carried out in the green house of the Center of Excellence in Molecular Biology, University of the Punjab, Lahore. Seeds were germinated in plastic bags (size 16.25 × 21.25 cm) each containing 1kg soil, peat and sand (1:1:1) and grown under greenhouse environments. Temperature in green house was 30±2 °C at day and 25±2 °C at night with relative humidity approximately 45-50% and a photoperiod of 14h. Metal halide illumination lamps (400 W) were used to supplement natural radiation. Light radiation reached a maximum

of $1,500 \mu\text{mol m}^{-2}\text{s}^{-1}$ at the top of canopy at midday. The experiment was laid out in a completely randomized design (CRD) with three replications of each experimental unit (Treatments viz; control and stress plants). Seeds were sown in 60 plastic bags (10 bags per replication). Four seeds were sown per bag. After 2 weeks of emergence, seedlings were thinned to one plant per bag. The plants were irrigated every alternate day with normal tap water. After 45 days from sowing, a cycle of drought was induced by stopping irrigating the plants for 15 days. The volume of pure water added to the pots was calculated periodically to maintain the plastic bags of stressed treatments at 5% gravimetric humidity (GH) and non-stressed treatments at 15% GH [7]. Physiological parameters (photosynthetic rate (P_n), transpiration rate (E), stomatal conductance (C) and biochemical parameters (total proline, total soluble protein, total free amino acids and total soluble sugars) were determined 15 days after the imposition of water stress.

2.3 Gaseous exchange (Photosynthetic rate P_n , stomatal conductance C, transpiration E)

Photosynthetic rate (A), Stomatal conductance (C), and transpiration (E) from 3rd leaf from top of every plant were recorded by utilizing IRGA (infrared gas analyzer) (Model, LCA-4; Analytical Development Company, Hoddesdon, England). All these determinations were recorded at 13.00-14.00h. During data recording, leaf chamber molar gas flow rate $248 \mu\text{mol s}^{-1}$, ambient CO_2 conc was $352 \mu\text{mol mol}^{-1}$, temperature of leaf chamber ranged from $32.3\text{--}35.7^\circ\text{C}$, atmospheric pressure (P) 98.01 kPa , molar flow of air/leaf area $221.06 \text{ mol m}^{-2}\text{s}^{-1}$, photosynthetic active radiation (PAR) was maximum up to $890 \mu\text{mol m}^{-2}\text{s}^{-1}$. A caution was also made that the measurements of control plants were immediately followed by that of the both varieties under drought stress.

2.4 Water relations (water Ψ_w , Osmotic Ψ_s and Turgor Ψ_p potential)

2.3.1 Leaf water potential (Ψ_w)

The onset of responses to water deficit was observed by measuring the water potential and osmotic potential of leaf samples. In each replication per treatment, a disc of 1 cm diameter was sampled from 3rd leaf (a fully expanded youngest leaf) was removed with a sharp knife from each plant and leaf water potential measurement made from 6-8.00 a.m. using a pressure chamber (Plant Moisture Stress (PMS) Instrument Company, Model 670, Albany, USA).

2.3.2 Leaf osmotic potential (Ψ_s)

The leaves that used for Ψ_w measurements was subjected to -20°C for 72 hrs, after which time the frozen leaf tissue was extracted and the sap so extracted used for determining osmotic potential using Wescor Vapor Pressure Osmometer (Model VAPRO 5520, El Cajon, California, USA).

2.3.3 Turgor potential

Leaf turgor potential was estimated as the difference between the water potential and osmotic potential values [8-9].

$$\Psi_p = \Psi_w - \Psi_s$$

2.5 Biochemical Attributes

2.5.1 Extraction and estimation of total soluble sugars

Total soluble sugars were estimated in 20 ml of 80% (v/v) ethanol extract at 95°C for 1 h from 100 mg of leaf and root tissue powder frozen in liquid nitrogen. After centrifugation at 10,000 g for 10 min, starch was measured in the pellet [10]. Total soluble sugars were analyzed by reacting 0.25 ml of the supernatant with 3 ml freshly prepared anthrone reagent [0.06% (w/v) anthrone in 95% H_2SO_4] and placing in boiling water bath for 10 min. After cooling to room temperature (25°C), the absorbance at 625 nm was measured from a standard curve prepared against pure glucose ($0\text{--}50 \mu\text{g}$) using micro plate reader.

2.5.2 Extraction and estimation of total soluble protein

Total soluble proteins were determined followed by method of [11]. A sample of 0.5 g leaf and root tissue of control and stress plant was taken and chopped in 5 ml phosphate buffer 0.2 M (pH 7.0). Two tubes containing 0.5 ml and 1.0 ml of leaf and root tissue extract were prepared for protein estimation. Solution of 0.5, 0.1, 0.2, 0.4, 0.6 and 1.0 ml of standard Bovine Serum Albumin (BSA) were simultaneously used in the experiment. The volume of each tube was topped to 1.0 ml with distilled water. The blank contained only 1.0 ml distilled water. One ml of

solution (copper reagents) was added to each test tube. The reagents in the test tube were thoroughly mixed and allowed to stand for 10 minutes at room temperature. Then 0.5 ml of Folin-phenol reagent (1:1 diluted) was added, mixed well and kept for 30 minutes at room temperature. The optical density (O.D) was recorded at 620 nm on Micro plate reader (Molecular devices®, USA)

2.5.3 Extraction and estimation of proline

Proline was established according to the standardize method [12]. Roots and leaves weighing 0.5 g each from control and drought stressed plants were homogenized in 10 ml of 3 % sulfo-salicylic acid. The homogenate was filtered through Wattman filter paper (No. 2). Two ml of the filtrate was reacted with 2 ml acid ninhydrin solution ,1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml of 6 M orthophosphoric acid and 2 ml of glacial acetic acid in a test tube for 1 h at 100 °C. The reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed vigorously by passing a continuous stream of air for 1-2 minutes. The chromophore containing toluene was aspirated from the aqueous phase, warmed at room temperature and the absorbance was measured by Micro plate reader (Molecular devices®, USA) at 520 nm using toluene as a blank. The proline concentration was determined from a standard curve using 0-100 µg L-proline (sigma) and calculated on fresh weight bases as follows: $\mu\text{mol proline g}^{-1} \text{FW} = (\mu\text{g proline mL}^{-1} \times \text{mL of toluene}/115.5)/\text{sample wt (g)}$.

2.5.4 Extraction and estimation of total free amino acid

Total free amino acids were extracted and determined [13] with slight modifications. Roots and leaves of 0.5g each from control and drought stressed plants were weighed separately and homogenized with 5ml of 80% ethanol. The homogenate was centrifuged at 15000 rpm for 15min. the residues was re-extracted with 5ml of 80% ethanol and centrifuged. The supernatants were pooled and used for quantitative estimation of total free amino acid. 1ml of ninhydrin solution was added to 0.1ml of extracts in test tubes. The volume was made up to 2ml with distilled water. The tubes were heated in a boiling water bath for 20min. 5ml of diluents solvent was added and the contents were mixed well. After 15min, the absorbance of the purple color was documented at 570nm using Micro plate reader (Molecular devices®, USA).A standard curve was prepared against L-leucine 0-50µg. Using the standard curve the amount of free amino acids present in the leaf samples was calculated. The free amino acids content was expressed in terms of mg/g f.w (fresh weight).

2.6 Statistical Analysis

Statistical analysis of the results was performed with STATISTIX V 9.0 (Analytical software Tallahassee, USA) freely online available. Graphs were plotted using Microsoft Excel. The data was subjected to analysis of variance (ANOVA) procedure for a complete randomized design (CRD).The least significant difference (LSD) test (P=0.05) was done to compare the means [14] and determine whether there were any significant differences for the genotypes and treatments for measured parameters.

3. RESULTS

3.1 Statistical interpretation

Analysis of variance (ANOVA) for gas exchange and water relation components revealed that significant differences were found among genotypes and treatment (drought) for all parameters recorded except photosynthesis rate while no significant interaction was reported between genotype environment interaction (Table 3).The results of ANOVA values for, total soluble sugars, total soluble proteins and proline showed that except in proline root, between the genotypes studied (two genotypes studied for drought), there are significant differences. Values mentioned for all biochemical traits under drought condition significantly influenced the treatment (drought). The interaction of genotype and environment, except in total soluble sugars, total soluble protein and proline (root tissue) were significant (Table 4).

3.2 Gas exchange parameters

In the present study there was significant difference in photosynthetic rate (P_n) of drought stress plants between two cotton varieties; the photosynthetic rate of the FDH786 was meaningfully higher than that of the FDH171 (Table 1.).In case of drought stressed plants

FDH786 $7.25 \mu\text{molm}^{-2}\text{s}^{-1}$ photosynthetic rate was measured while it was found to be $5.88 \mu\text{molm}^{-2}\text{s}^{-1}$ in FDH 171 genotype (Table 1). A decrease P_n in under drought stress occurs through stomatal closure and reduction of protoplasm activity. A reduction in photosynthetic activity can be due to the reduction in stomatal conductance and uptake of water from roots but repetition of water stress cycles might cause photosynthetic adaptability [15]. Results showed that FDH786 has the potential to maintain a high photosynthetic rate under water stress.

Stomatal conductance (C) was significantly higher in FDH 786 i.e. $3.72 \text{ mmol m}^{-2}\text{s}^{-1}$ as compared to FDH171 which was reported to be $2.9 \text{ mmol m}^{-2}\text{s}^{-1}$ (Table 1). A significant difference in stomatal conductance (stomatal closure) was reported in FDH786 as that of FDH171 cotton variety. It reveals the more efficient stomatal closure attitude of FDH786 variety under drought stress period. Stomata close in response either to a decline in leaf turgor and/or water potential, or to a low-humidity atmosphere [16].

Based upon transpiration rate (E) (Table 1) plants appear to respond differently between drought stressed plants of both varieties. Data on absolute values of transpiration rate revealed that cotton variety FDH171 exhibited significantly lower transpiration rate of $0.90 \text{ mmolm}^{-2}\text{s}^{-1}$ that of $1.45 \text{ mmolm}^{-2}\text{s}^{-1}$ in FDH786 cotton variety (Table 1). Significant decrease of transpiration rate in FDH786 cotton variety may show its less adaptive behavior to drought stress (water deficit). For many crops, transpiration declines only after a third of the extractable soil water in the root zone has been left [17], but instances of crops showing a decline in transpiration at higher levels of extractable soil water have been reported [18].

3.3 Water relations

Plants under drought stress conditions markedly show an increase in water potential (Table 1) while those plants grown under control (irrigated) conditions had reduced water potential; similar results were to be reported in the current study. Significant difference was reported for water potential in drought stress plants of both cotton varieties was found. When soil water potential is high, plant water potential approaches soil water potential at night when stomata are closed. As soil dries under drought stress, hydraulic conductivity of soil decreases, and the rate of water movement toward root and absorption are slow to completely replace the water lost from the plant during the daytime because of transpiration so it might be reason for both cotton genotypes that could not lead to significant difference for water potential.

Significant difference was measured for osmotic potential (Table 1) between both cotton genotypes. In FDH786 osmotic potential of -3.52 Mpa as compared to -2.76 Mpa found in cotton genotype FDH 171 (Table 1). Regarding turgor potential (Table 1) a significant difference was measured between both cotton genotypes. Higher turgor potential was calculated in FDH786 drought stressed plants as compared to those of FDH171 stress plants. Turgor potential of 2.173 Mpa for stressed plants (Table 1) was calculated in FDH786 which is remarkably higher than that of turgor potential in FDH171 variety. Turgor loss point in the stressed leaves attained lower Ψ_w than in well-watered leaves.

3.4 Biochemical parameters

Both cotton genotypes under water stress conditions displayed a remarkable difference in total soluble sugars (Table 2). The total soluble sugar reported in stress leaf and root tissue was profoundly significantly lower than the FDH786 variety. Cotton variety FDH 171 under drought stress had total soluble sugar of $35.8 \mu\text{g/g}$ and $39.4 \mu\text{g/g}$ in leaf and root tissue accordingly (Table 2) while in case of FDH a higher significant concentration of $39.23 \mu\text{g/g}$ and $43.16 \mu\text{g/g}$ was reported in drought stressed leaf and root tissues respectively. This higher accumulation of organic molecule under drought stress condition shows significant attitude of cotton variety FDH786 towards drought as that of FDH171 variety. The concentration and components of carbohydrates differ according to the individual response of each plant species to drought.

As revealed from data for Proline (Table 2), cotton variety FDH786 drought stress leaf and root tissues generated significant difference in contrast to FDH176 variety. In FDH786 stress leaf and root; the amount of proline was found to be $44.8 \mu\text{g/g}$ and $199.06 \mu\text{g/g}$ (Table 2) respectively, similarly in FDH171 variety; $38.88 \mu\text{g/g}$ and $177.4 \mu\text{g/g}$ proline was found in stress leaf and root tissue respectively. It divulges more production of defensin stress organic molecules (proline) in FDH786 as compared to that of FDH171 variety under water deficit circumstances.

Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress [19].

The total soluble protein (Table 2) concentration was notably higher in cotton genotype FDH786 stress leaf and root tissues as compared to other cotton genotype. The concentration of protein estimated in FDH786 stress leaf and root tissues was 19.2 µg/g and 11.28 µg/g (Table 2) respectively while in FDH 171 in stress leaf and root tissue the total soluble protein was 15.92 µg/g and 7.95 µg/g respectively which is significantly lower as compared to FDH786 cotton genotype (Table 2). The more production of stress responsive biological molecules (protein) expresses the more tolerance behavior of cotton genotype FDH786 under water deficit conditions.

As far the matter of total free amino acid was related the cotton variety FDH786 drought stress leaf and root tissues possessed significantly higher amount of total free amino acids in comparison to cotton variety FDH171 (Table 2). In FDH786 stress leaf and root tissue amount of total free amino acid was estimated to be 1.29 µg/g and 0.896 µg/g (Table 2) respectively which was significantly more as compared to that of FDH171 variety. Total amino acid pool was markedly higher in FDH786 variety under drought stress which seems it to be more predominant to water deficit.

Table 1. Gas exchange and water relations parameters of FDH 786 and FDH 171 genotypes grown under control and drought stress

Gas Exchange and water relations parameters

Genotype	WR	P _n (µ mol CO ₂ m ⁻² S ⁻¹)	C (mmol m ⁻² S ⁻¹)	E (mmol H ₂ O m ⁻² S ⁻¹)	Ψ _w (-MPa)	Ψ _s (-MPa)	Ψ _p (-MPa)
FDH 786	C	10.02 ± 2.40 a	3.72 ±	2.17 ± 0.09 a	0.85 ±	2.62 ±	1.90 ±
	S	7.25 ± 2.40	0.34 a	1.45 ± 0.26 b	0.11b	0.21b	0.16 b
		ab	2.63 ±		1.34 ±	3.52 ±	2.17 ±
			0.48 b		0.04 a	0.39 a	0.43 a
FDH 171	C	9.30 ± 1.16	2.90 ±	1.60 ± 0.36 b	0.25 ±	2.22 ±	1.97 ±
	S	ab	0.20 b	0.90 ± 0.20 c	0.09 b	0.28 b	0.34 b
		5.88 ± 1.18 b	1.75 ±		1.14 ±	2.76 ±	1.62 ±
			0.17 c		0.20 b	0.41 b	0.34 b

Data are means of three replicates (9 observations) ± SE. Different letters in the same column indicate significant difference between treatments (P < 0.05). WR= water regime, C= Control, S= stress, P_n= photosynthetic rate, C= stomatal conductance, E= transpiration rate, Ψ_w= transpiration, Ψ_s= osmotic potential, Ψ_p= turgor potential

Table 2. Biochemical attributes of FDH 786 and FDH 171 genotypes grown under drought stress

Biochemical attributes

Genotype	WR	TSS (µg g ⁻¹)	TSP (µg g ⁻¹)	Proline (µg g ⁻¹)	TFA (µg g ⁻¹)
FDH 786 (leaves)	C	36.03 ± 0.60 b	8.44 ± 2.04 c	3.94 ± 1.30c	0.46 ± 0.06 c
	S	39.23 ± 0.50 a	19.20 ± 1.43 a	44.88 ± 1.23a	1.29 ± 0.06 a
FDH 786 (roots)	C	35.53 ± 0.76 c	3.72 ± 0.93 c	13.53 ± 2.89 b	0.33 ± 0.05 c
	S	43.16 ± 2.36 a	11.28 ± 2.14 a	199.06 ± 1.89 a	0.89 ± 0.10 a
FDH 171 (leaves)	C	32.33 ± 1.52 c	5.91 ± 0.52 d	4.27 ± 2.10 c	0.35 ± 0.05 c
	S	35.80 ± 0.72 b	15.92 ± 1.00 b	38.88 ± 0.90 b	0.80 ± 0.05 b
FDH 171 (roots)	C	33.53 ± 1.04 c	2.72 ± 1.03 c	11.16 ± 2.36 b	0.24 ± 0.04 c
	S	39.40 ± 1.27 b	7.95 ± 1.94 b	177.39 ± 29.07a	0.60 ± 0.02 b

Data are means of three replicates (9 observations) ± SE. Different letters in the same column indicate significant difference between treatments (P < 0.05). TSS= total soluble sugars, TSP= total protein, TFA= total free amino acids.

Table 3. Analysis of variance showing the mean squares of the genotypes, treatment factors and the interaction genotype × treatment for the gas exchange and water relations parameters.

Trait	Source of variation	SS	MS	F	P
Gas exchange and water relations parameters					
Photosynthesis rate (P _n)	Genotype	3.2552	3.2552	0.91 ^{NS}	0.3684
	treatment	28.7990	28.7990	8.04*	0.0220
	genotype× treatment	0.3169	0.3169	0.09 ^{NS}	0.7737
Stomatal Conductance (C)	Genotype	2.1675	2.1675	20.58*	0.0019
	treatment	3.7408	3.7408	35.51*	0.0003
	genotype× treatment	0.0027	0.0027	0.03 ^{NS}	0.8768
Transpiration rate (E)	Genotype	0.9520	0.9520	15.23*	0.0045
	treatment	1.5123	1.5123	24.20*	0.0012
	genotype× treatment	0.0003	0.0003	0.00 ^{NS}	0.9465
Osmotic Potential Ψ _s	Genotype	0.9861	0.9861	8.72*	0.0184
	treatment	1.5552	1.5552	13.75*	0.0060
	genotype× treatment	0.0972	0.0972	0.86 ^{NS}	0.3811
Turgor Potential Ψ _p	Genotype	0.1656	0.1656	1.50*	0.2562
	treatment	0.0044	0.0044	0.04*	0.8469
	genotype× treatment	0.2914	0.2914	2.63 ^{NS}	0.1435

NS, *, and **, non-significant, significant at P < 0.05 and P < 0.01, respectively MS= Mean square (estimate of variance between groups), SS= Sum of square, F= Significance probability (variance ratio between Treatment MS and Error MS) , P=Probability value

Table 4. Analysis of variance showing the mean squares of the genotypes, treatment factors and the interaction genotype × treatment for the biochemical attributes

Trait	Source of variation	SS	MS	F	P
Biochemical attributes					
Total soluble sugar (leaf)	Genotype	38.1633	38.1633	43.99*	0.0002
	treatment	38.1633	38.1633	43.99*	0.0003
	genotype× treatment	0.0533	0.0533	0.06 ^{NS}	0.8104
Total soluble sugar (root)	Genotype	24.941	24.941	11.23*	0.0101
	treatment	136.687	136.687	61.57*	0.0001
	genotype× treatment	2.341	2.341	1.05 ^{NS}	0.3345
Total soluble protein (Leaf)	Genotype	25.259	25.259	68.29*	0.0000
	treatment	323.752	323.752	875.28*	---
	genotype× treatment	0.414	0.414	1.12 ^{NS}	0.3207
Total soluble protein (root)	Genotype	14.083	14.083	5.47*	0.0475
	treatment	122.880	122.880	47.72*	0.0001
	genotype× treatment	4.083	4.083	1.59 ^{NS}	0.2434
Proline (leaf)	Genotype	24.08	24.08	7.55*	0.0252
	treatment	4279.72	4279.72	1340.97*	0.0000
	genotype× treatment	30.08	30.08	9.43*	0.0153
Proline (root)	Genotype	433.2	433.2	2.01 ^{NS}	0.1943
	treatment	92802	92802	430.05*	0.0000
	genotype× treatment	279.4	279.4	1.29 ^{NS}	0.2881

Total Free amino acid (leaf)	Genotype	0.2700	0.2700	26.28*	0.0009
	treatment	1.2288	1.2288	119.59*	0.0000
	genotype× treatment	1.2288	1.2288	119.59*	0.0118
Total Free amino acid (root)	Genotype	0.1102	0.1102	26.45*	0.0009
	treatment	0.6394	0.6394	153.46*	0.0000
	genotype× treatment	0.0330	0.0330	7.94*	0.0226

NS, *, and **, non-significant, significant at $P < 0.05$ and $P < 0.01$, respectively

4. DISCUSSIONS

4.1 Gas exchange components

The ability of FDH786 genotype to keep photosynthesis high during the stress could be related to their capacity to maintain tissue turgidity through higher water retention induced by osmotic adjustment; however, higher water absorption from roots cannot be ruled out. Reduction in photosynthesis rates in cotton plants due to impairment of electron flow and indirect inhibition due to lack of utilization of reducing power during water stress [20]. Similar results were reported in case of FDH171 and our results are supported by the previous studies. Consequently, FDH786 may also be able to keep photosynthesis high due the presence of a redox protection mechanism that helps maintain metabolic function. Net photosynthesis, transpiration rate and stomatal conductance was decreased in cotton genotypes as water stress was imposed. Our results suggested that the decrease of the P_n under stress conditions was mainly due to stomatal response and process which agree with previous findings [21]. It is a common observation that the photosynthetic rate in plants is reduced when they are subjected to drought. Water deficiency in plants may lead to physiological disorders, such as a reduction in stomatal, non stomatal [22] photosynthesis and transpiration [23], because in order to prevent transpiration, plants close their stomata [24]. This closure of stomata may result from direct evaporation of water from the guard cells (hydropassive closure).

In current studies decrease in Stomatal closure in FDH 171 genotype may be result of hormonal signaling from roots which probably involved in the decreased photosynthesis in FDH171 cotton variety [25-26]. Variability in stomatal conductance [27] and photosynthetic rate [28] have been suggested as tools for selecting genotypes with higher water deficit tolerance. Increases in atmospheric concentration of CO_2 have been shown to decrease stomatal conductance (g_s) for a wide range of species under numerous conditions [29-30]. Our findings in cotton variety FDH171 are reinforced by previous literature [31]. Stomatal responses are more closely linked to soil moisture content than to leaf water status. This suggests that stomata are responding to chemical signals (e.g. ABA) produced by dehydrating roots [32] which can be attributed in case of same results in cotton genotype FDH 171. This decline precedes changes in the water status of the plant, and is hence attributed to a non-hydraulic root signal produced by roots growing in a drying soil [33-34]. Increase in transpiration efficiency under drought has been reported in various crops [35] which is attributed to the fact that, partial stomatal closure under increasing water deficits leads to change in transpiration, as compared to dry matter production [36].

A decrease in leaf osmotic potential to maintain turgor, a process often called osmotic adjustment (OA), is also an important adaptive mechanism in plants subjected to drought [37]. The genotype FDH 171, exhibited significant reductions in osmotic potential under drought stress compared to the FDH 786. However, FDH 171 also exhibited lower water potential, meaning that the reduction in osmotic (solute) potential was caused by volume reduction rather than osmotic adjustment or by accumulation of apoplastic solutes. The reduction of water potential in FDH 171 is probably a reflection of changes in cell membranes, these changes being related to how the plants perceive the stress and initiate the signal transduction to the shoot. Changes in cell volume accompanying drought may trigger stretch-activated channels, alter the conformation or juxtaposition of critical sensory proteins or cause alterations in the cell wall-

plasma lemma continuum, thereby activating signal transduction pathways that elicit gene expression [38]. The similar results were analyzed in FDH171 cotton variety which reveals its less cell volume suspensions (intracellular osmotic adjustments) as reported [39] and decrease alterations in the plasma lemma range as that of FDH 786 which possess significantly higher adaptive trend to drought stress. Thus, drought results in lower plant water potential. The effects of drought on leaf water potential are progressive rather than immediate. The changes in the plant water potential can be attributed to change in osmotic pressure or osmotic component of the water potential. When leaf water potential is low, it causes the stomata to close, which causes decreased transpiration which in turn leads to increased water potentials.

4.2 Water relation aspects

The response that distinguishes two genotypes most clearly is the accumulation of solutes in stress tolerant species [40]. FDH171 leaves exhibited less significant change in water and turgor potential. These observations can be explained by solute accumulation in the cytosol, which causes water to be retained and keeps the water potential less negative [41]. Plants accumulate different types of organic and inorganic solutes in the cytosol to lower osmotic potential there by maintaining cell turgor [42]. Under drought, the maintenance of leaf turgor may also be achieved by the way of osmotic adjustment in response to the accumulation of proline, sucrose, soluble carbohydrates, glycine-betaine, and other solutes in cytoplasm improving water uptake from drying soil. Changes in turgor pressure in the cotton variety would translate into a signal that might lead to changes in guard cell osmotic pressure and consequently in stomatal aperture in response to changes in water supply and demand [43]. In our study the results are in concordance with the reported literature in the sense that FDH786 have more turgor potential than FDH171 variety which shows the wider performance of FDH786 cotton variety under drought stress.

4.3 Biochemical attributes

Like other cellular constituents, starch and sugar levels are also affected by stress [44-45]. In our studies both the genotypes of cotton, an increase in total soluble sugar, by drought was observed which suggests that drought induces starch sugar inter-conversion [46]. A drought-induced decrease in starch contents may also be associated with inhibition of starch synthesis [47]. Our results for FDH786 variety are supported by [45], who also reported an increase in sucrose and decrease in starch contents in safflower while decreased total soluble sugars in FDH171 as compared to FDH786 was supported by reported findings [47]. It shows that starch biosynthesis inhibition due to less starch contents ultimately lead to less biomass accumulation under drought stress which is not desirable in FDH171 variety. In general, a reduction in leaf starch concentration is common in water-stressed plants [48]. This change leads to an increase in the concentrations of soluble sugars that act as osmotic compounds and contribute to the stabilization of cell membranes. Like other cellular constituents, starch and sugar levels are also affected by stress [45].

It is well known that synthesis of proline in plants protects cell membrane and protein content in plants and enhanced by several stresses including drought stress [49]. Proline synthesis protects the plant against low water potential and causes osmotic regulation in plant organs. Also proline can act as an electron receptor preventing photosystems injuries in dealing with ROS function. Our results of dramatic increase in proline contents in different tissues of cotton agree with earlier reports of proline accumulation as a compatible osmolyte during drought exposure [45]. Increased accumulation of proline in cotton variety FDH786 might be due to the decreased activity of proline dehydrogenase, a catabolic enzyme of proline [50]. Thus, increase in proline contents during drought induction may confer adaptive mechanism in cotton. Accumulation of Proline under stress in many plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants. It influences protein solvation and preserves the quaternary structure of complex proteins, maintains membrane integrity under dehydration stress and reduces oxidation of lipid membranes or photo inhibition [51]. Furthermore, it also contributes to stabilizing sub-cellular structures, scavenging free radicals, and buffering cellular redox potential under stress conditions [52].

The marginal change in protein contents in cotton genotypes suggests that protein synthesis or proteolysis is affected minimally by short-term drought stress in this plant. Several reports concerning alteration of protein synthesis or degradation of protein in various plant species in response to drought [53] sustenance our results. Our results in cotton, contrasts with increasing evidences of drought-induced accumulation of proteins and physiological adaptations to water limitation [54]. Current studies speculated that proline increase in FDH 786 and decrease in FDH 171 genotypes speculated that the initial increase of proteins in drought stressed plants was related to stress proteins but the reduction occurred in next stage was due to the reduction in the amount of photosynthesis [55]. Changes in protein content suggest that protein synthesis or proteolysis is affected by drought stress. Several reports of alteration of protein synthesis or degradation of protein in various plant species in response to drought [53,56] support our results.

Significance of amino acids derived from their widely use for the biosynthesis of a large variety of non-proteinic nitrogenous materials, i.e. pigments, vitamins, coenzymes, purine and pyrimidine bases. Studies have proved that amino acids can directly or indirectly influence the physiological activities in plant growth and yield [57]. Total amino acid pool increased in present study under drought in both cotton genotypes is reflected by previous findings [58]. Thus, the drought induction in cotton showed an increase in total amino acid pool and marginal change in protein contents, which reflect the mode of adjustment to drought in this plan. The reduction in total amino acid as reported in cotton genotype FDH 171 could ascribed to water induced loss of solutes (mainly K⁺) from guard cells, which resulted in a selective reduction in guard cells turgor leading to stomatal closure. Our results in cotton variety FDH171 mirror the reported findings [59]. Increase in amino acid defines the FDH786 cotton variety more tolerable to drought as compared to FDH171 cotton variety because of its pivotal role in cytoplasmic osmotic adjustment in response to osmotic stress [60].

CONCLUSION

Taken as a whole, the present results show that photosynthetic activity, stomatal and water relations were significantly higher in cotton FDH 786 genotype. Significant changes were shown on total soluble carbohydrates, total soluble protein, proline and free amino acids with 15 days under water stress, indicating that the carbon metabolism is quickly modified and utilized as reserve source (defensing) during water deficit more efficiently in FDH 786 genotype ensuring efficient plant growth under drought stress. While less tolerance of FDH 171 genotype to drought stress may be due to decreased photosynthetic activity monitored by lower stomatal conductance, less osmotic adjustment and transpiration. Ultimately decreased osmotic adjustment in FDH 171 genotype led to decreased production of osmolytes and organic molecules which revealed less capability of this genotype to environment under drought stress. Knowledge of these findings should help to utilize FDH 786 cotton genotype by plant biologist and molecular breeders in cotton drought tolerance breeding program.

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REFERENCES

1. Selote D S, Chopra R K, Drought-induced spikelet sterility is associated with an inefficient antioxidant defence in rice plants. *Physiol Plantarum*, 2004, 121(3), 462-471.

2. Pilon-Smits E A, H, M. J. M. Ebskamp., Paul M J, Jeuken M J W, Weisbeek P J, Smeekens S C M, Improved performance of transgenic fructan-accumulating tobacco under drought stress. *Plant Physiol*, 1995, 107(1), 125-130.
3. Hare P D, Cress W A, Staden V J, S. Dissecting the roles of osmolyte accumulation during stress. *Afri. J. Sci*, 1996, 92, 431-439.
4. Ingram J, Bartels D, The molecular basis of dehydration tolerance in plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 1996, 47, 377-403.
5. Flexas J, Bota J, Loreto F, Cornic G, Sharkey T D, Diffusive and metabolic limitations to photosynthesis under drought and salinity in C₃ plants. *Plant Biol*, 2004, 6(3), 269-279.
6. Zobayed S M A, Afreen F, Kozai T, Phytochemical and physiological changes in the leaves of St. John's wort plants under a water stress condition. *Environ. Exp. Bot*, 2007, 59(2), 109-116.
7. Jamal A, Shahid M N, Aftab B, Rashid B, Sarwar M B, Mohamed B B, Hassan S, T Husnain T, Water stress mediated changes in morphology and physiology of *Gossypium arboreum* (var FDH-786). *J of Plant Sci*, 2014, 2(5), 179-186.
8. Nobel P S, Physiochemical and environmental plant physiology. (Academic Press: San Diego, CA). 1991.
9. Taiz L, Zeiger E, (2010) Plant physiology. Sinauer Associates, Inc.:Sunderland, MA
10. Jarvis C E, Walker J R L, Simultaneous, rapid, spectrophotometric determination of total starch, amylase and amylopectin. *J Sci Food Agri*, 1993, 63(1), 53-57.
11. Lowry O H, Rosebrough N J, Farr A L, Randall R J, Protein measurement with the Folin phenol reagent. *J Biol Chem*, 1951, 193(1), 265-75.
12. Bates L S, Waldren R P, Teare I D, Rapid determination of free proline for water stress studies. *Plant. Soil*, 1973, 39(1), 205-207.
13. Sugano N, Tanaka T, Yamamoto E, Nishi A, Phenylalanine ammonialyase in carrot cells in suspension cultures. *Phytochemistry*, 1975, 14, 2435-2440.
14. Sokal R R, Rohlf F J, Biometry, the principles and practice of statistics in biological research, 3rd ed. Freeman, New York, 1995, pp 321-356.
15. Matthews R.B, Azam-Alisn Peacock J M, Response of four sorghum lines to mid-season drought: II. Leaf characteristics. *Field Crops Res*, 1990, 25, 297-308.
16. Märoco J P, Pereirã J S, Chaves M, Stomatal responses to leaf-to-air vapour pressure deficit in Sahelian species. *Aus. J. Plant Physiol*, 1997, 24(3), 381-387.
17. Sadras V O, Milroy S P, Soil water thresholds for the responses of leaf expansion and gas exchange: *Field Crops Res*, 1996, 47, 253-266.
18. Améglio T, Roux X L, Mingeau M, Perrier C, Water relations of highbush blueberry under drought conditions. *Acta Hortic*, 2000, 537, 273-279.
19. Szabados L, Savoure' A, Proline: a multifunctional amino acid. *Trends Plant Sci*, 2009, 5(2), 89-97.
20. Genty B, Briantais J-M, Vieira da Silva J.B, Effects of drought on primary photosynthetic processes of cotton leaves. *Plant Physiol*, 1987, 83(2), 360-364.
21. Leidi E O, Lopez J M, Lopez M, Gutierrez J C, Searching for tolerance to water stress in cotton genotypes: photosynthesis, stomatal conductance and transpiration. *Photosynthetica*, 1993, 28, 383-390.
22. Samarah N H, Alqudah A M, Amayreh.s J A, McAndrews G M, The effect of late-terminal drought stress on yield components of four barley cultivars. *J. Agron. Crop Sci*, 2009, 195(6), 427-441.
23. Saccardy K, Pineau B, Roche O, Cornic G, Photo chemical efficiency of photosystem and xanthophyll cycle components in *Zea mays* L. leaves exposed to water stress and high light. *Photosynthesis. Res*, 1998, 56(1), 57-66.
24. Ashraf M, Iram A, Drought stress induced changes in some organic substances in nodules and other plant parts of two potential legumes differing in salt tolerance. *Flora*, 2005, 200(6), 535-54.
25. Khalil A A, J. Grace, Does xylem sap ABA control the stomatal behavior of water-stressed sycamore (*Acer pseudoplatanus* L.) seedlings? *J. Exp. Bot*, 1993, 44(7), 1127-113.

26. Kramer P J, Boyer J S, Water Relations of Plants and Soils. Academic Press, San Diego, 1995.
27. Laffray D, Louguet P, Stomatal responses and drought resistance. *Bull. Soc. Bot. Fr.-actual. Bot*, 1990, 137(1), 47 -60.
28. Leidi E O, Lopez J M, Lopez M, Gutierrez J C, Searching for tolerance to water stress in cotton genotypes: photosynthesis, stomatal conductance and transpiration. *Photosynthetica*, 1993, 28, 383-390.
29. Long S P, Ainsworth E A, Rogers A, Ort D R,) Rising atmospheric carbon dioxide: Plants Face the future. *Ann Rev Plant Biol*, 2004, 55, 591-628.
30. Ainsworth E A, Davey P A, Bernacchi C J, Dermody O C, Heaton E A, Moore D J, Morgan P B, Naidu S L, Ra H-S Y, Zhu X-g, Curtis P S, Long S P. A meta-analysis of elevated [CO₂] effects on soybean (*Glycine max*) physiology, growth and yield. *Glob Change Biol*, 2002, 8(8), 695-709.
31. Long S P, Ainsworth E A, Rogers A, D. R Ort, Rising atmospheric carbon dioxide: Plants Face the future. *Ann Rev Plant Biol*, 2004, 55, 591-628.
32. Davies W J, Zhang J, Root signals and the regulation of growth and development of plant in drying soil. *Ann. Rev. Plant Physiol. Plant Mol. Biol*, 1991, 42, 55-76.
33. Davies W J, Wilkinson S, Loveys B, Stomatal control by chemical signaling and exploitation of this mechanism to increase water use efficiency in agriculture. *New Phytologist*, 2002, 153(3), 449-460.
34. Chaves M M, Pereira J S, Maroco J, Rodrigues M L, Ricardo C P P, Osorio M L, Carvalho I, Faria T, Pinheiro C, How plants cope with water stress in the field? Photosynthesis and growth. *Ann. Bot*, 2002, 89, 907-916.
35. Jones H G, Plants and microclimate: A quantitative approach to environmental plant physiology. 2nd edition, Cambridge University Press, 1992.
36. Nobel P K, Physicochemical and Environmental Plant Physiology. 2nd edition, Academic Press, 1999, p. 341.
37. Hussain M, Malik M A, Farooq M, Khan M B, Akram M, Saleem M F, J. *Agron. Crop Sci*, 2009, 195, 98-109.
38. Hare P, Plessis S D, Cress W, Staden J V S, Stress-induced changes in plant gene expression. *Afri. J. Sci*, 1996, 92, 431-439.
39. Bohnert H J, Nelson D E, Jensen R.G, Adaptations to environmental stresses. *Plant Cell*, 1995, 7(7), 1099-1111.
40. Bohnert H J, Jensen R.G, Strategies for engineering water-stress tolerance in plants. *Trends in Biotech*, 1996, 14(3), 89-97.
41. Feng Y, Li X, Boersma L, Roles of osmotic adjustment and turgor potential in leaf area expansion. *Physiol. Plant*, 1994, 90(1), 1-8.
42. Rhodes D, Samaras Y, cellular and molecular physiology of cell volume regulation, (S. K. Strange, Ed). Boca Raton: CRC Press, 1994, 347-361.
43. Buckley T N, Mott K A, Stomatal water relations and the control of hydraulic supply and demand. *Progress in botany*, 2002, 63, 309-325.
44. Prado F E, Boero C, Gallardo M, Gonzalez J A, Effect of NaCl on germination, growth and soluble sugar content in *Chenopodium quinoa* Willd. *Bot Bull Acad Sinica*, 2000 41(1):27-34.
45. Laila E, Nasser A, A-Aal A E, Effect of elevated CO₂ and drought on proline metabolism and growth of safflower (*Carthamus mareoticus* L.) seedlings without improving water status. *Pak J Biol Sci*, 2002, 5, 523-528.
46. Chaves M M, Effects of water deficits on carbon assimilation. *J of Exp Bot*, 1991, 42,1-1.
47. Geigenberger P, Reimholz R, Geiger M, Merlo L, Canale V, Stitt M, Regulation of sucrose and starch metabolism in potato tubers in response to short-term water deficit. *Planta*, 1997, 201(4), 502-518.
48. Nemeskéri E, Sárdi E, Remenyik J, Kőszegi B, Nagy P, Study of the defensive mechanism against drought in French bean (*Phaseolus vulgaris* L.) varieties. *Acta Physiol. Plant*, 2010, 32(6), 1125-1134.

49. Behnamnia M, Kalantari Kh M, Rezanejad F, Exogenous Application of Brassinosteroid Alleviates Drought-Induced Oxidative Stress In *Lycopersicon esculentum* L. *Gen and Appl Plant Physiol*, 2009, 35, 22-34.
50. Sundaresan S, Sudhakara P R, Water stress-induced alterations in proline metabolism of drought-susceptible and tolerant cassava (*Manihot esculenta*) cultivars. *Physiol Plant*, 1995, 94(4), 635-642.
51. Demiral T, Turkan I, Does exogenous glycinebetaine affect antioxidative system of rice seedlings under NaCl treatment? *J. Plant Physiol*, 2004, 161(10), 1089-1110
52. Ashraf M, Foolad M.R, Roles of glycine-betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot*, 2007, 59(2), 206- 216.
53. Riccardi F, Gazeau P, Vienne D V, Zivy M, Protein changes in responses to progressive water deficit in maize. *Plant Physiol*, 1998, 117(4), 1253-1263.
54. Bray E A, Plant responses to water deficit. *Trends Plant Sci*, 1997, 2(2), 48-54.
55. Ghorbanli M, Gafarabad M, Amirkian T, Allahverdi M B, *Iranian J of Plant Physiol*, 2013, 3 (2), 651-658.
56. Chandler P M, Robertson M, Gene expression regulated by abscisic acid and its relation to stress tolerance. *Annu Rev Plant Physiol Plant Mol Biol*, 1994, 45, 113-141.
57. Mohamed A M, Effect of Some Bio-chemical Fertilization Regimes on Yield of Maize. *M.Sc. Thesis*, 2006, Faculty of Agric., Zagazig Univ., Egypt, pp. 70-177.
58. Mattioni C, Lacerenze N G, Troccoli A, De Leonardis A M, Fonzo N Di, Water and salt stress-induced alterations in proline metabolism of *Triticum durum* seedlings. *Physiol Plant*, 1997, 101(4), 787-792.
59. Hammad Salwa A R, Osama A M A, Physiological and biochemical studies on drought tolerance of wheat plants by application of amino acids and yeast extract. *Ann of Agri Sci*, 2014, 59(1), 133-145.
60. Alaei Y, Khanghah A M, Jafari M, Khaneghah A, Evaluation on leaf proline amount in three bread wheat cultivars in presence of two fertilizers containing amino acids in drought stress. *Environ. Exp. Bot*, 2007, 59(2), 109-116.