Full Length Article

Drought Induced Interactive Changes in Physiological and Biochemical Attributes of Cotton (*Gossypium hirsutum*)

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Abstract

Cotton is a drought tolerant plant which adapts the dehydrated conditions through significant ameliorations in its internal physiochemical activities. The objectives of present investigation were to examine its adaptive response to different intensities of dehydration stress under extreme arid climate of desert and quantify the extent of interactions between physiological and biochemical attributes in the course of drought adaptation. A micro scale field trial was conducted on southern oasis of Taklamakan desert, in which cotton was grown in soil buried 1 m^3 pots using four deficit irrigation levels. Results revealed that drought stress significantly enhanced the concentrations of abscisic acid (ABA), proline, total soluble sugars (TSS), and potassium ions (K$^+$) in cotton leaves, while, rates of photosynthesis (*A*), stomatal conductance (*gs.*), and transpiration (*E*) declined. On an average, the plants replenished with 80, 60, and 40% irrigation, accumulated relatively 37, 98 and 155% more quantities of stress combating biochemical substances than normal plants, respectively. The stomatal density on abaxial leaf surface increased, while, aperture and leaf water potential significantly reduced. Consequently, the average rate of photosynthetic attributes reduced by 7, 32, and 52% at 80, 60 and 40% irrigation replenishments, respectively. These results suggested that decline in leaf gas exchange activities was indeed a modulating effect of drought induced osmoprotectants enrichment in leaf tissues, thus cotton plants adapted the drought intensities of desert environment. However, further micro irrigation based large scale studies on water use optimization of cotton under desert ambiances are inevitable. © 2018 Friends Science Publishers

Keywords: Abscisic acid; Drought; Desert land; Osmoprotectants; Photosynthesis; Stomata

Introduction

Drought is one of the most damaging abiotic environmental factor, which is intensifying steadily and affecting the global agriculture and food security (Vurukonda *et al*., 2016). The knowledge of drought adaptive physiochemical behavior of a particular crop to adverse and water limited environments is equally important for complete description of its cultivable area range on globe. Cotton (*Gossipyum hirsutum* L.) is one of the most imperative cash crop of world's agricultural system, which not only provides raw material for textile and oil industry, but also feeds the animals through proteinaceous cottonseed cake (Eisa, 1994; Howell *et al*., 2004). It is widespread cultivated in most parts of the world but, Xinjiang Uyghur autonomous arid region in northwest China has a leading prestige in its total cotton production due to

compatible climate (Feng *et al*., 2017). Besides, due to higher yield and net profit per unit area, an increasing number of farmers are being engaged with cotton cultivation (Tang *et al*., 2010; Wang *et al*., 2014), that demands more arable land, which is limited. However, through intensive efforts, the cultivated area in the region in continuously being expanded by turning the deserts into oasis. Yet, the acute water shortage along with hyperaridity and mighty evaporative potential remained serious concerns in desert environment (Guo *et al*., 2008; Zeng *et al*., 2012; Zhang *et al*., 2017).

Since, cotton is a drought tolerant plant, therefore, it can be successfully grown under such adverse and extremely water scarce conditions using deficit irrigation approach (Howell *et al*., 2004; Fereres and Soriano, 2007). The deficit irrigation sets up partial drought situation in rhizosphere,

thus, the plants undergo various physiological and biochemical rearrangements, both at cellular and organismic levels (Timpa *et al.*, 1986; Farooq *et al.*, 2009; Deeba *et al.*, 2012; Chastain *et al.*, 2014). Concentration of abscisic acid (ABA) and compatible solutes enhances in leaves to combat desiccation stress (Alves and Setter, 2004) and, ABA solely acts inhibitory to complete stomatal opening (Krasensky and Jonak, 2012). In addition, the important osmolytes amass in leaf tissues, which make the cytosol more concentrated to reduce cell osmotic potential, minimize aerial water loss (transpiration), and maintain cell turgidity (Bray *et al.*, 2000; Mafakheri *et al.*, 2010). These osmoprotactants or compatible solutes mainly comprise of amino acids (proline, glycine betaine etc.), potassium ions (K^+), alcohols, carbohydrates (soluble sugars, sorbitol etc.), and some organic acids (Timpa *et al.*, 1986; Girousse *et al.*, 1996; Bray *et al.*, 2000). Besides, the rate of photosynthesis, stomatal conductance, and transpiration substantially decline (Deeba *et al.*, 2012; Pierantozzi *et al.*, 2013) due to partial or complete closure of stomata (Hsie *et al.*, 2015). Normally, the rate of assimilation and other gas exchange activities define growth and development of plants (Raines, 2011) but, drought and other environmental stresses affect them badly (Otieno *et al.*, 2005).

In numerous studies, such drought adaptive physiological, biochemical, and even proteomics level amendments in cotton plant have been reported under varying conditions (Ennahli and Earl, 2005; Chaves *et al.*, 2009; Chastain *et al.*, 2014). But, a little is known about the magnitude of interactions between these physiochemical aspects in the course of drought adaptation. Also, the literature explaining drought adaptive ameliorations in cotton plant under desert environment is also lacking. Thus, it was hypothesized that during acclimatizing drought stress under adverse ecological conditions of desert, the enhanced bioassay of stress combating biochemical substances rescues cotton plants interactively by alleviating gas exchange activities and, the plants acclimatize dehydration stress. The particular objective was to assess interactive effect of drought induced biochemical enrichment on physiological aspects of cotton through quantifying the relative concentrations of ABA, total soluble sugars (TSS), proline, and potassium ions (K^+) along with relative rates of gas exchange activities such as photosynthesis (*A*), stomatal conductance (*g_s*), and transpiration (*E*).The outcome of this biological inquiry would not only clarify drought adaptive mechanism of cotton but, also highlight the scope of cultivable desert ecosystems for cotton cultivation.

Materials and Methods

Experimental Site Description

The experiment was established at Cele Desert Research Station (80°43′45″E, 37°00′57″N) of Xinjiang Institute of Ecology and Geography, Chinese Academy of Science,

during cotton season of 2015. The station is located at south margin of Taklimakan desert in the northern piedmont of Kunlun Mountains, northwest China. The area is characterized as a typical continental desert climate. Long-term normal mean annual precipitation is only 35.1 mm that is mainly distributed between May and July, while, the average annual temperature and potential evaporation are 11.9°C and more than 2500 mm, respectively (Zeng *et al.*, 2012). The temperature extremes reach at -23.9°C in winter and 41.9 in summer, while data showing weather fluctuation during crop growth period are given in Fig. 1, that were obtained from the metrological station of Cele Desert Research Station. Soil at research site is mainly categorized as loamy sand/Aeolian sandy soil with bulk density and field capacity of 1.19 g cm^{-3} and 0.2 m^3 m^{-3}, respectively. Pre-sowing total N, P, and K concentrations were determined 0.28± 0.04, 0.79± 0.07, and 12.71± 0.89 g kg^{-1}, respectively.

Layout and Treatments Explanation

Cotton cultivar used in this trial was ''Zhongmian 24'' that was purchased from local market. The plantation was carried out on 20th April in 1 m^3 open bottom iron pots installed 1 m deep in soil for precise irrigation treatment in field conditions with minimum seepage loss. In each plot of 1 m^2 area, 54 seeds were dibbled in pairs, which were later thinned to 27 plants with plant to plant distance and row spacing of 10 cm and 30 cm, respectively. The recommended fertilizer dozes of NPK were 240, 120 and 60 kg ha^{-1}, respectively. Four deficit irrigation (DI) regimes were used to develop drought stress based on 100% (DI_0), 80% (DI_1), 60% (DI_2) and 40% (DI_3) replenishment of depleted soil moisture from field capacity. Drought treatments were initiated from early squaring stage after successful seedling establishment. For this purpose, moisture depletion from root zone was monitored by regular soil sampling from 30, 60 and 90 cm depths on weekly basis (Fig. 2), and the moisture content was determined by gravimetric method (105°C, 24 h). When moisture status in 100% irrigated pots reduced to 50% field capacity then the treatments DI_1, DI_2 and DI_3 were replenished with 80, 60 and 40% of the amount of water replenished to DI_0. Each plot received measured quantity of water through flow measuring water flow meters used to quantify the amount of water applied.

Gas Exchange and Stomatal Measurements

Stomatal conductance (g_s) (mmol m^{-2} s^{-1}), rate of photosynthesis (*A*) (μmol m^{-2} s^{-1}) and transpiration (*E*) (mol H_2O m^{-2} s^{-1}) were measured thrice starting from blooming at 15 days interval, between 11:00 am to 12:30 pm by using a Li-6400 portable photosynthetic system (LI-COR, Inc, Lincoln, Nebraska).

The quantum flux was customized according to clear sunny day whereas, reference CO_2 and block temperature were set at 400 μmols mol^{-1} and 30°C, respectively.
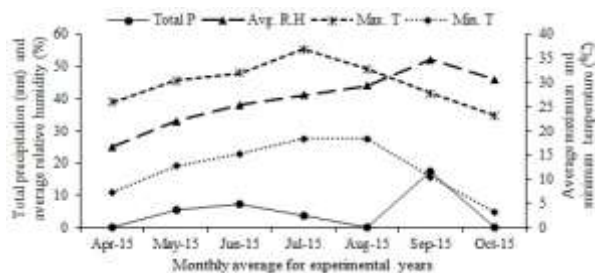
Fig. 1: Climatic data of experimental site for the entire crop season. The values represent monthly averages
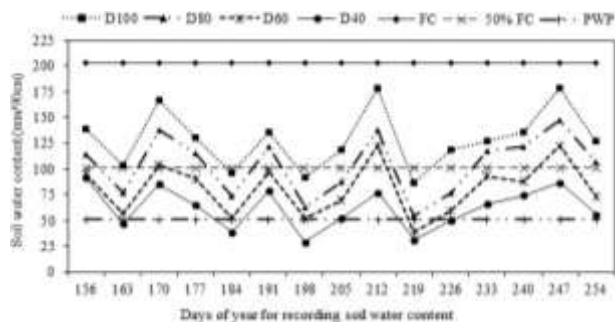

Fig. 2: Variation in soil moisture content under different irrigation treatments during experimental period

Measurements were made randomly, from young fully expanded photosynthetically active leaves of five plants in each treatment at each observation event by following the protocol of White and Raine (2009). Pre-down leaf water potential (Ψ_{pd}) MPa was measured through pressure chamber technique of Boyer (1967).

Stomatal traits including density (mm^{-2}) and aperture size (μm^2) on abaxial leaf surface, were measured using scanning electron microscopy (Waqas *et al.*, 2017). For this purpose the leaf discs (3 mm × 3 mm) were sampled at peak bloom on a sunny day just one day before stress relief. Samples were immediately preserved using FAA solution, which had percent concentrations of formalin, acetic acid, and alcohol as 38, 100 and 50 (5:5:90) respectively, and shifted to laboratory. After necessary processing, the leaf samples were observed under field emission scanning electron microscope (ZEISS SUPRA55 VP, Germany) to get the stomatal images. Measurements for stomatal density and aperture were made from microscopic images at 300x and 3.00kx magnifications, respectively. The aperture size was calculated by using the ellipse area equation, 1/4× (length × width ×π).

Biochemical Measurements

The quantities of ABA (ng g^{-1}), proline (μmol g^{-1} FW), sugars (mg g^{-1}), and K ions (g kg^{-1}) were determined from fresh leaf samples collected at boll maturation stage, just one day before irrigation. Five fully expanded photosynthetically active leaves were collected from each treatment. Instantly

they were wrapped in aluminum foil and preserved at ultra-freezing -80ºC temperature in liquid nitrogen. Later, the leaf samples were crushed and ground into fine dust. Concentration of ABA was determined using High-Pressure Liquid Chromatography (HPLC) by following procedure of Bousquet *et al.* (1986) with necessary modifications. Moreover, proline quantification was carried out according to standard procedures given by Bates *et al.* (1973). Total soluble sugars were measured by spectrophotometric method (Lo and Garceau, 1975) and, potassium ions concentration was quantified through flame photometer and digestion process using the protocols of Junsomboon and Jakmunee (2011).

Data Analysis

After all physiological and biochemical measurements, the net and relative concentration of stress combating molecules and the net and relative rates of gas exchange attributes were quantified on percentage (%) basis using simple percentage formulas. The treatments were arranged in a randomized complete block design with four repeats. All data were statistically analyzed using Fischer's analysis of variance (ANOVA) technique. The mean values were expressed as an average of four replications with the standard errors of means (S.E.M). The difference among means of different treatments were calculated by least significant difference (LSD) test at 5% probability. A statistical application for Windows (Statistix v.8.1) was used for all statistical calculations.

Results

Stomatal Response to Drought Stress

Electron microscopic images presented in Fig. 3a, b, c and d, are respectively showing the hierarchy of stomatal aperture response to drought stress regimes. Data analysis based on p ≤ 0.05 demonstrated that all treatments differed significantly from each other (Fig. 4a). The maximum aperture dimension of 17.24 μm^2 was observed from abaxial stomata of non-stressed cotton leaves that was followed by 15.35, 8.81 and 3.50 μm^2 of DI_1 DI_2 and DI_3 treatments, respectively. In addition, the results indicated a negative linear relationship between aperture and the concentrations of ABA ($R^2 = 0.86$) in response to degree of drought stress (Fig. 5a). Unlike aperture, stomatal density (mm^{-2}) on abaxial leaf surface increased with increasing drought stress. The highest density of 387 stomata mm^{-2} was observed on lower surface of highly stressed (DI_3) cotton leaves, while, it successively decreased with increasing irrigation amount in DI_2, DI_1 and DI_0 treatments (Fig. 4b).

Leaf Water Potential and Gas Exchange Attributes

Pre-down leaf water potential (Ψ_{pd}) (MPa) significantly (P≤0.05) reduced at respective level of drought stress (Table

1). Comparatively, the LWP (Ψ_{pd}) observed under DI_2 and DI_3 treatments was two and three fold, respectively lower than control (DI_0). The average of measurements made at five intervals, revealed the maximum LWP -0.98 MPa under no stress, and the minimum -2.76 MPa under 40% irrigation replenishment. Moreover, the detailed analysis indicated negative correlation between LWP and relative concentration of compatible solutes ($R^2 = 0.98$) in leaf tissues, with increasing intensity of drought stress Fig. 5b.

The photosynthetic rate of cotton significantly ($P \leq 0.05$) reduced under all drought stress treatments except in DI_1. Cotton plants replenished with 100% irrigation (control), assimilated CO_2 at the rate of 32.03 µmol m^{-2} s^{-1} that was recorded as the highest (Table 1), whereas, under 80% water replenishment, the rate was statistically similar with control. However, in DI_2 and DI_3 treatments, the assimilation rate decreased by 34 and 52%, respectively. Likewise, stomatal conductance (g_s) was also significantly ($P \leq 0.05$) affected by deficit irrigation supplies (Table 1). The lowest rate of conductance (306.73 mmol m^{-2} s^{-1}), recorded for DI_3 was about 50% less than control (616.53 mmol m^{-2} s^{-1}), while the 60% rehydrated (DI_2) plants depicted 30% decline. The conductance rate of 80% irrigated (DI_1) plants was also significantly affected, but its net decline from 100% treatment was not more than 07%. The Adverse effect of drought on rate of transpiration (E) is shown in Table 1. Based on 5% probability, the transpiration rate in 80% water supply was statistically at par with fully irrigated plants. However, with least significant difference of 2.66, the highest and lowest rate of transpiration was measured 20.5 and 9.3 mol H_2O m^{-2} s^{-1}, respectively.

Concentrations of Biochemical Substances

The concentration of ABA significantly ($p \leq 0.05$) increased in cotton leaves in response to increasing intensity of drought stress (Table 2). Its enrichment in 60 and 40% irrigated cotton leaves was quantified more than double the concentration 252.92 ng g^{-1} found in well-watered (DI_0) plants. On fresh weight (FW) basis, net ABA content in DI_1, DI_2, and DI_3 plant leaves increased by 54, 106, and 120% respectively, than in well water treatment. A dramatic change in proline accumulation was observed in cotton leaves against the degree of drought stress. Leaves chemical analysis indicated four folds more accumulation of proline in 40% water replenished (DI_3) plants as compared with 2.27 µmol g^{-1} FW determined from normal plants. However, in a comparison with control, the relative quantity of proline in leaves of DI_1, DI_2, and DI_3 plants was determined as 151, 268, and 422%, respectively (Table 2). The concentration of TSS and potassium K ions also showed significant ($p \leq 0.05$) elevation in cotton leaves under drought stress. The highest amount of sugars 12.17 mg g^{-1} accumulated in DI_3 cotton leaves was followed by 9.58 mg g^{-1} in DI_2 plants and vice versa (Table 2). Similar adaptive response was observed in potassium ions concentration that showed the maximum
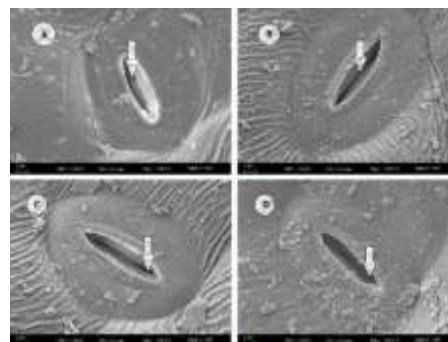

Fig. 3: Electron microscopic images of abaxial stomata showing aperture dynamics under 100% (A), 80% (B), 60% (C), and 40% (D) irrigation replenishment. The images were analyzed at 3.00 kx magnification
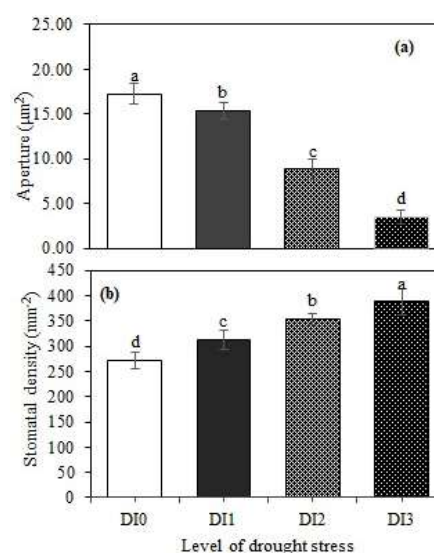

Fig. 4: Effect of drought stress regimes on stomatal aperture (a) and density (b). Values are means (± SE) of four replicates and, markers with different letters are differing significantly ($p \leq 0.05$) according to LSD test

accumulation of 22.4 g kg^{-1} in 40% irrigated (DI_3) leaves, and the least in normal plants. On percentage basis, the examined leaf K$^+$ content in DI_3, DI_2 and DI_1 was 38, 27, and 10% respectively, higher than the concentration found in control. On an average, the relative concentrations of ABA and all observed osmolytes showed that net quantity of all stress mitigating substances raised in cotton leaves by 37, 98, and 155% under 80, 60, and 40% water replenishment, respectively (Table 2 and Fig. 7).

Consequences of Biochemical Enrichment on Physiological Activities

The average relative rate of all gas exchange activities observed in 80% irrigated plants (DI_1) was recorded 93% that was negligibly apart from DI_0, whereas, this response

Table 1: Pre-down leaf water potential and the total and relative rates of leaf gas exchange attributes in response to respective levels of drought stress. The values are means (±SE) of four replications

| Level of stress	IR (mm)	LWP (MPa)	Photosynthesis (μmol $m^{-2}s^{-1}$)	RR (%)	Transpiration (mol $m^{-2}s^{-1}$)	RR (%)	Conductance (mmol $m^{-2}s^{-1}$)	RR (%)
DI_0	1040	-0.9±0.1a	32.0±1.0a	100	20.5±0.8a	100	616.5±13.7a	100
DI_1	832	-1.1±0.1a	29.5±0.6a	92	19.3±1.1a	94	575.3±6.6b	93
DI_2	624	-2.0±0.1b	21.2±0.7b	66	13.9±1.0b	68	433.9±13.9c	70
DI_3	416	-2.7±0.1c	15.3±1.1c	48	9.3±0.6c	45	306.7±11.1d	50
LSD		0.23	2.98		2.66		34.77	
CV		-8.49	7.61		10.56		4.50	

IR: applied irrigation equivalent to 1 ha, LWP: leaf water potential, RR: relative rate, LSD: least significant difference, CV: coefficient of variance
Values with different letters are significantly (*P*<0.05) apart from each other according to LSD test

Table 2: Total and the relative concentrations of ABA and major osmoprotectants in cotton leaves at respective levels of drought stress. The values are means (±SE) of four replications

Level of stress	IR (mm)	ABA (ng g^{-1})	RC (%)	TSS (mg g^{-1})	RC (%)	Prolin (μmol g^{-1})	RC (%)	K ions (g kg^{-1})	RC (%)
DI_0	1040	252.9±12.3d	100	5.1±0.2d	100	2.3±0.10d	100	16.2±0.9d	100
DI_1	832	388.8±8.8c	154	6.9±0.4c	136	3.4±0.03c	151	17.1±0.03c	105
DI_2	624	521.6±6.9b	206	9.6±0.2b	190	6.1±0.05b	268	20.6±0.1b	127
DI_3	416	555.6±6.3a	220	12.2±0.3a	241	9.6±0.06a	422	22.4±0.2a	138
LSD		19.66		0.68		0.15		0.45	
CV		2.86		5.06		1.77		1.48	

IR: applied irrigation, ABA: abscisic acid, TSS: total soluble sugars, K: potassium ions, RC: relative concentrations, LSD: least significant difference, CV: coefficient of variance
Values with different letters are significantly (*P*<0.05) apart from each other according to LSD test
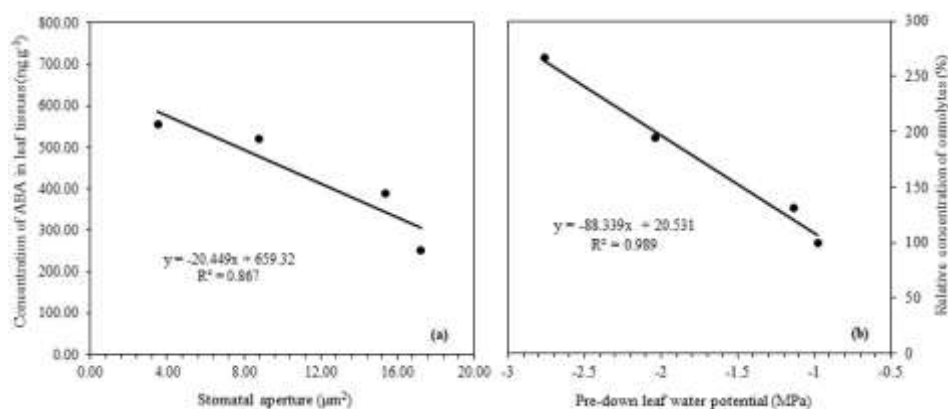
Fig. 5a: Negative linear correlation between stomatal aperture and concentration of ABA in leaf tissues. (5b) negative linear correlation between relative concentration of osmolytes and pre-dawn leaf water potential. The osmolytes indicate commulative average of the relative concentrations of prolin, TSS, and K ions

was more adversely affected by 60 and 40% replenishments (Table 1). In depth analysis exposed that it was the consequence of drought adaptive ameliorations in cytosol composition. At respective levels of drought stress, cotton leaf tissues accumulated relatively more concentration of stress hormone and compatible solutes (Table 2) to manage the excessive aerial water loss and ameliorate the physiological activities. Therefore, results reveal a linear negative correlation of leaf gas exchange activities with the relative concentrations of ABA ($R^2 = 0.86$) and osmolytes ($R^2 = 0.99$) (Fig. 6). Consequently, net performance of gas exchange responses under 80, 60, and 40% replenishment was 07, 32, and 52% respectively, lower than well-watered plants (Fig. 6). In another aspect, the quantity of stress mitigating biochemical substances showed positive relationship with intensity of drought ($R^2 = 0.98$) while, the

rate of gas exchange characteristics responded negatively to drought with regression (R^2) value of 0.95 (Fig. 7).

Discussion

Cotton plants brought out significant ameliorations in physiological and biochemical attributes during adaptation to drought under desert environment. The stressed plants substantially reduced stomatal aperture (SA) to reduce aerial water flow. It would have happened due to enhanced accumulation of ABA in leaves, which sets up ionic imbalance that compels K^+ to leak out from guard cells, thus, narrowing the aperture (Sarwat and Tuteja, 2017). These results are consistent with Krasensky and Jonak (2012) and Wijngaard *et al.* (2005) who reported that elevated leaf ABA content acts inhibitory to potassium channels, which retard
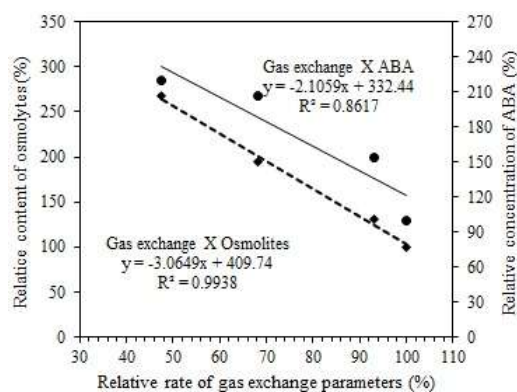
Fig. 6: Correlation indicating linear decline in photosynthetic gas exchange activities due to enrichment of osmolytes (dotted line) and abscisic acid (solid line) in leaf tissues under drought stress
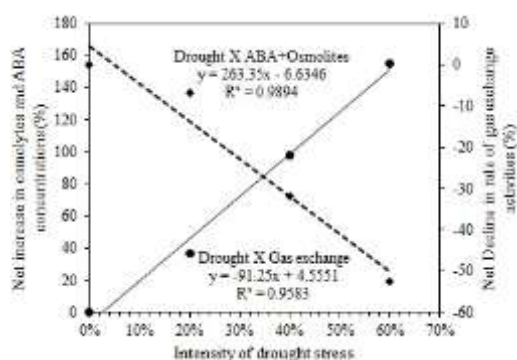

Fig. 7: Correlation indicates that net quantity of stress combating biochemical molecules linearly increased with increasing intensity of drought stress (solid line), while, net physiological activities showed negative response to intensities of drought (dotted line). Net values in both Y-axis were obtained as difference from control. Hence, the positive scale of osmoprotectants and negative scale of gas exchange activities depict an antagonistic relationship between the both in response to intensity of drought stress

the influx of K^+ ions. Contrarily, the stomatal density (SD) increased on abaxial surface under hyperosmotic conditions, which is in agreement with Wang *et al.* (2007). This enormous increase in SD would probably be a drought adaptive approach in the plants to attain higher membrane to surface area ratio for the ease of quicker response to stressed conditions and adjustment of gas exchange activities, accordingly (Drake *et al.*, 2013).

Results also indicated that highly desiccated plants decreased osmotic potential, which appeared as significant reduction in LWP ψ_{pd} up to -2.76 MPa. In accordance with these observations, Argyrokastritis *et al.* (2015) and Brito *et al.* (2011) reported substantial decline in LWP due to root zone dehydration stress. This decline in ψ_{pd} would be due to

increased solute potential (ψ_s) that triggered down the osmotic potential (ψ_{osm}). The phenomenon is characterized as osmotic adjustment in which dehydrated cells accumulate more compatible solutes and enhance the viscosity of cytosol to prevent water loss through ex-osmosis (Wang *et al.*, 2016). Furthermore, our results also depicted remarkable decline in stomatal conductance that would be due to reduced LWP and increased stomatal closure. Consistent with that, Hsie *et al.* (2015) also reported a connection between stomatal dynamics and conductance. In the same way, rates of transpiration (*E*) and photosynthesis (*A*) also showed substantial dropdown to drought stress regimes. This decline would be the consequence of stomatal pores reduction and enhanced accumulation of metabolites (Cornic and Massacci, 1996; Tezara *et al.*, 1999). These results are in line with Deeba *et al.* (2012) who reported similar declining trends in rates of assimilation (*A*), conductance (g$_s$) and transpiration (*E*) of cotton towards moisture deficit regimes. In addition, Ahmed *et al.* (2017) also reported substantial decline in net photosynthesis of camelina under similar patterns of drought stress. Such major adjustments in gas exchange attributes would probably be the strategic drought adaptive response of cotton. As, it is formerly reported that remarkable reduction in stomatal conductance, photosynthetic pigments, and net assimilation rate, are the key adaptive changes in plants during soil moisture deficit (Pierantozzi *et al.*, 2013).

Concentration of ABA triggered up in drought stressed cotton leaves and, its quantity in DI$_3$ plants was recorded more than double the amount observed in DI$_0$. In the line of these results, Ackerson (1980) reported enhanced concentrations of ABA in cotton and Alves and Setter (2004) in cassava leaves under dehydrated conditions. This enhancement would be due to increased bioassay of ABA in roots during soil moisture limitations, which is later transported to leaves, where it regulates stomata to limit water loss by transpiration (Gomes *et al.*, 2004). Proline is highly soluble in cytosol but, it does not interfere in cell's metabolic activities, while its build up in cytosol is a common drought prompted metabolic stress indicator of plants (Sen *et al.*, 2002). In present study, proline content in 80% watered cotton leaves increased by 51%, while in 40% irrigated plants, its increase was more than four folds higher than control. Consistently, Sekmen *et al.* (2014) also reported similar trends of proline accumulation in deficit irrigated cotton leaves. Moreover, Girousse *et al.* (1996) found higher proline and few other amino acids content in alfalfa leaves when ψ_{pd} decreased from -0.5 to -2.0 MPa. Most probably, this buildup of proline in cytosol could be the consequence of catabolism of stored inactive proteins during water stress. Because, significant movement of large inactive osmolites (proteins or starch) towards or retrieval from storage parts have been reported during the hyperosmotic stress (Pugnaire *et al.*, 1999).

The quantity of TSS significantly amassed in cotton leaves and its relative concentrations varied from 136% in

DI_1 stressed to 241% in DI_3 plants compared with control. These results are consistent with Timpa *et al.* (1986) and Burke (2007) who reported 2 to 3 fold higher accumulation of sucrose in desiccated cotton leaves compared with control. This elevated concentration of TSS could be due to conversion of insoluble starch into soluble sugars, because, the starches are sensitive to environmental stresses (Basu *et al.*, 2007). In the line of that, Burke (2007) also reported drought induced conversion of starch and, its accumulation as sucrose in cotton leaves. Similarly, K ions were also the major contributors in concentrating the cytosol. Their net concentration raised negligibly (5%) under 80% water supply while, in 40% irrigated plants their accumulation was approximately 38% higher than DI_0. These results are consistent with Wang *et al.* (2016) who reported that K^+ are the main osmolytes involved in osmoregulation of cotton during entire period of drought stress. Furthermore, Alves and Setter (2004) concluded that K-salt was a dominant osmolyte with its 60% role in osmotic adjustment of cassava leaves during water deficit stress. The enhanced accumulation of K in cytosol could be the result of substantial efflux of K^+ from guard cells that eventually resulted into stomatal closure at low leaf water potential (Mak *et al.*, 2014).

Overall, these results reveal an interactive role of stress combating biochemical substance in stomatal activity and gas exchange regulation of desert grown drought stressed cotton. ABA adjusted the stomatal aperture with respect to soil moisture availability. In the line of these results Wijngaard *et al.* (2005) reported that drought stress induced ABA enrichment in xylem sap retards K-ions influx in guard cells that limits stomatal apertures. Similarly, Krasensky and Jonak (2012) concluded that stomatal closure was the main consequence of ABA accumulation in leaves during soil moisture dryness. Subsequently, the compatible solutes including proline, TSS, and K ions, relatively enriched in cytosol to make it more viscous and, LWP reduced. In accordance with our findings, Burke (2007) and Alves and Setter (2004) found enhanced accumulation of osmoprotectants in cotton and cassava leaves, respectively. While, long before Timpa *et al.* (1986) explored a negative relationship between the quantities of osmolytes and LWP of cotton under water deficit stress. Consequently, the leaf gas exchange activities underwent decline at respective levels of deficit irrigation (Fig. 5). Our results are in agreement with (Alves and Setter, 2004) who reported a linear decline in conductance and transpiration rates of cassava leaves in relation to reducing stomatal pores due to ABA and K salts enrichment. In addition, Radin (1992) also found that C assimilation rate of cotton, both in laboratory and field conditions was directly related to rate of stomatal conductance. Finally, the results confirm that drought adaptation is a consequence of interactive variations in physiological and biochemical attributes of cotton (Fig. 7) and it would be more appropriate to interpret that ABA and osmoprotectants enrichment in leaves modulated the rates of photosynthetic gas exchange activities and, cotton plants adapted to drought under extreme conditions of Taklimakan desert.

Conclusion

It is concluded that cotton plants significantly amended their internal physiological and biochemical activities during adaptation to drought stress regimes. There was a linear relationship between the intensity of soil dryness and concentrations of ABA and osmoprotectants. On an average, the biochemical substances accumulated 155% more in 40% irrigated plants than normal ones. The rate of photosynthetic gas exchange activities decreased up to 50% at maximum level of stress due to reduced osmotic potential and narrowing of stomata. All physiochemical ameliorations occurred interactively, thus showing an inverse relationship between the quantities of stress combating biochemical substances and rate of leaf gas exchange activities under soil moisture dryness. The drought adaptive changes in 80% irrigated plants were not so vigorous, which suggested the use of mild stress for cotton cultivation under water limited environments. Following these results, further, cotton growth and productivity based water use optimization studies under desiccated desert conditions are inevitable.

Acknowledgements

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