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Effect of Physio-biochemical Factors Influencing Moisture Stress Tolerance in Cotton (*Gossypium hirsutum* L.)

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A B S T R A C T

Physio-biochemical parameters were recorded under moisture stress and normal condition of tolerant and susceptible cotton *G. hirsutum* genotypes, when plants experiencing moisture stress at 65-67 DAS (15-17 days of water withholding and 80-82 DAS (30 days water withholding). The genotypes, Khandwa-2, F-2226, RAJ-2, Bikaneri nerma, PH1009, CCH1831 and 5433A2A03N83 were found to exhibit one or more than one physiological parameters towards tolerance to higher RWC, less reduction in photosynthetic rate, stomatal conductance and transpiration rate with higher canopy temperature in drought tolerant genotypes than susceptible MCU-5. Similarly biochemical traits like higher proline and peroxidase enzyme activity play important role in exhibiting drought tolerance under moisture stress condition. This change in physio-biochemical process indirectly helps for increased yield potential in cotton genotypes, Khandwa-2, F-2226, 5433A2A03N83, RAJ-2, and RHC0811.

Introduction

Moisture stress incited by soil water deficit (Chaves *et al.*, 2009) at reproductive stage of cotton (Michael *et al.*, 1973; Quisenberry *et al.*, 1985; Turner *et al.*, 1986; Loka *et al.*, 2012) is one of the reasons for reducing cotton productivity. India's cotton yield 568 kg per hectare continues to be lower than the global average of 800 kg per hectare (*Anon*., 2016). In India, maximum area of cotton cultivation, particularly hot and dry region of central and south zone under rainfed condition limits productivity, due to moisture stress. Irrigated cotton partially solves the problem in north India, where productivity is higher than rainfed condition (*Anon*., 2016). Therefore it is not just sustainability but need of elevated production of cotton, it is the major challenge to meet the need of increasing world population under deteriorating arable land and depletion of water resources creating moisture stress. The identification of moisture stress tolerant cotton genotype based on physio-biochemical parameters with yield contributing traits to increase yield has been the major focus of researchers worldwide as a direct way of selection for breeding purpose (Rahman *et al.*, 2008; Aktas *et al.*, 2009; Brito *et al.*, 2011; Ullah *et al.*, 2017). Cotton genotypes tolerating moisture stress with low yielding ability were identified in several studies (Blum, 1988; Imran *et al.*, 2012; Pettigrew, 2004; Kamaran *et al.*, 2016).

However, high yielding genotypes under water stress could likely to be low yielding under well-watered environments (Rosielle and Hamblin, 1981). Moreover the too dry on post-germination stage (Ananthi and Vijayaraghavan 2012; Karademir *et al.,* 2009) probably do not reflect the conditions of natural drought. Even though drought avoidance (Kramer, 1983) of drought tolerance is almost impossible for increase productivity. Thus, identifying this traits for tolerance response, that can be assessed under both watered and water-stress conditions can characterize genotypes and may support cotton breeding programs in finding cultivars that are more tolerant to water stress or that may be used in the preliminary stages of a breeding program (Hassan *et al.,* 2015; Kamaran *et al.,* 2016; Zhang *et al.,* 2010).

It has been known that plants started experiencing drought when soil water potential is less by more than 50 per cent in stressed plot than normal (Santos *et al.,* 2011). Therefore in the present study observations were recorded only when the soil moisture of stressed plot was less by 50 per cent than normal. The physiological parameters included were canopy temperature, relative water content, transpiration rate, stomatal conductance and photosynthetic rate and chlorophyll content as an important attributes for moisture stress tolerance in cotton (Isoda *et al.,* 2002; Massacci *et al.,* 2008; Lahong *et al.,* 2000). In the present study, RWC reflects the balance between water supply to the leaf tissue and transpiration rate through stomatal closure (Lugojan and Ciulca, 2011) and it results in reduction of transpiration rate which activated cooling system of leaf water potential and maintain higher canopy temperature leads to greater biomass production in dry land (Conaty *et al.,* 2015). Lower transpiration rate along with higher relative water content (RWC) has been reported as selection criteria for plants against moisture stress (Malik *et al.,* 1999; Rahman *et al.,* 2000). When plants expose to water stress, produce abscisic acid (ABA), which can promote stomatal closure by causing the efflux of solutes and water from the guard cells (Radin *et al.,* 1988; Schroeder *et al.,* 2001) and reduced stomatal conductance is positively associated for higher photosynthetic rate (Cornic and Massacci 1996; Tezara *et al.,* 1999).

Moisture stress induces oxidative stress leads to increase production of reactive oxygen species (ROS), such as superoxide radicals (O2-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH), which can attack lipid, proteins, carbohydrates and nucleic acid of plant system (Khatun *et al.,* 2008). In order to eliminate ROS, plants increase activity of antioxidant enzymes peroxidase (Hosseini *et al.,* 2015) that minimize cellular damage like oxidation of photosynthetic pigments and destruction of lipids, proteins and nucleic acids (Reddy *et al.,* 2004). In addition to the production of antioxidants, osmotic adjustment occurs in plant cells through accumulation of compatible solutes like proline (Bray *et al.,* 2000), regulate water loss by reducing the cell water potential (Fumis *et al.,* 2002). Proline acts as an osmoregulator and cellular protectant under moisture stress (Hanower and Brzozowska, 1975) and it is variable in species according to factors genotypes (Patil *et al.,* 2011). The increased osmoprotectant proline content and antioxidant peroxidase enzyme activity has been studied in different crops (Parida *et al.,* 2007; Aktas *et al.,* 2009; Amudha *et al.,* 2014; Borgo *et al.,* 2015; Marechaux *et al.,* 2015; Jamal *et al.,* 2015).

In cotton, the sensitivity to drought stress during flowering and boll development has been well established (Constable and Hearn, 1981; Cull *et al.,* 1981; Turner *et al.,* 1986; Loka *et al.,* 2012) and insufficient soil water at

this stage leads to a reduced plant height, number of fruiting branches, boll shedding, developed bolls and seed cotton yield (Pettigrew, 2004 and Ahuja *et al.,* 2001; Karademir *et al.,* 2011; Ananthi and Vijayaraghavan 2012; Loka *et al.,* 2012). The amount of water utilized by cotton plants is related to the efficacy of physiological (Deeba *et al.,* 2012) and biochemical processes (Hatfield *et al.,* 1987) responsible for crop growth and yield. With all these viewpoints, the present investigation was planned to identify moisture stress tolerant cotton genotypes based on physio-biochemical and yield contributed traits towards moisture stress.

Materials and Methods

Plant materials, experimental site, location and design

Fourteen drought tolerant and one susceptible cotton genotype selected based on All India Co-ordinated Crop Improvement Project (AICCIP) report from the year of 1999-2014 were used. The seed of the 15 cotton (*G. hirsutum* L.) genotypes were collected from their respective breeding stations located in different ecological regions of India. List of the genotypes their source is given in Table 1.

During *kharif* season of the year 2015-16, field experiment conducted at IABT Garden, UAS, Dharwad is situated in northern transitional zone of Karnataka altitude of 678 m above mean sea level with latitude $15^{0}26^{1}$ N and longitude $76^{0}7^{1}$ East. In the year of 2016-17 *kharif*, the same experimental taken in ARS, Dharwad Farm, Dharwad is situated in the northern transitional zone (Zone No. 8) of Karnataka with latitude of 15° 46^{1} north and longitude of 75° 0^{1} east altitude of 724 m above mean sea level (MSL), having similar agro climatic and rainfall as that of first location.

There were six blocks, in each block all 15 genotypes were sown randomly. Three blocks namely R4, R5 and R6 were used as control (maintain moisture at field capacity level) and another three blocks (R1, R2 and R3) later used to induce moisture stress. Each genotype was raised in a single row of 4.0 m length with a spacing 90×20 cm in rain out shelter.

Imposition of moisture stress

Water was applied to entire field plot at field capacity up to 50 DAS (Days after sowing). To treated plot (considered to expose to moisture stress), after 50 DAS, watering was withheld to one plot (treated) which was separated by two layers of polythene sheets inserted up to 1-2 m in the soil to avoid lateral movement of water from one plot to another. The soil moisture content was measured in 10 random spots of soil depth 20 cm of entire plot using soil moisture meter at different days of plants leaf drooping response to moisture stress. One access tubes for Delta-T PR1 Profile probes were inserted into 1m depth of soil bin. They were placed equidistant from the edge and 100 cm apart randomly. The mean of the 15 measurements was used to indicate the water content of the soil in the entire drought and control plot presented in Table 2. The plant response to moisture stress (50 per cent field capacity) was observed by various parameters at 65-67 DAS and 80-82 DAS. Rewatering was done to plot from 90 DAS onwards and continued to water till the end of the experiment and recorded yield contributed traits at harvesting.

Physio-biochemical and productivity traits

After induction of soil moisture stress (per cent reduction of soil moisture), treated plants were subjected to show leaf drooping and wilting symptoms due to decreasing leaf water deficiency (RWC) recorded by Barrs and Weatherly (1962) formula content [(RWC =

[(FW-DW)/ (TW– DW)] × 100 Where, FW- fresh weight; DW- dry weight; TW- turgid weight (weight after the leaf was kept immersed in distilled water for 12 hr)]. Other non-destructive physiological parameters such as chlorophyll content observed by SPAD meter (502 Plus, Spectrum Technologies, Plainfield, IL, USA) and stomatal conductance, transpiration rate and photosynthetic rate were recorded through IRGA (Infrared Red Gas Analyzers) system LI- 6400 (L1COR 6400, Lincoln Nebraska, USA).

The biochemical non-enzymatic and enzymatic process, like proline content and peroxidase enzyme activity were estimated by standard procedure (Bates *et al.,* 1973 and Costa *et al.,* (2002). During harvesting, productivity traits like number of sympodia, plant height, number of fruiting bodies/plant, number of harvested bolls/plant, per cent boll shedding and yield (kg/ha) were recorded. The data were analyzed statistically using standard protocols (Panse and Sukhatme 1967) and used Windows Stat 9.1 software for analyzing the data

Results and Discussion

Induction of moisture stress at square formation stage

In the world good crop of cotton can be raised with an annual rainfall of 800 mm distributed uniformly from March to November. There is sufficient moisture in soil to support normal germination, while delaying irrigation on this stage was found by several investigators as an effect on decreased yield (Singh *et al.,* 1975; Grimes *et al.,* 1970; Loka *et al.,* 2012). This is occurred mainly due to soil water scarcity in central and southern region of India. In cotton the induction of reproductive parts (square) starts at 50-55 days after sowing depending on varieties within and between the species. Flowering followed by squaring and finally boll setting continue to goes up to 120-180 days after sowing depending on species. Post germination moisture stress hinders the initial good growth, recovery may be expected but it depends on duration of moisture stress. In case of continued stage of moisture stress, crop may not recover at all then farmers will not continue to spend. In other situation, where crop experience moisture stress after normal establishment of crop leading to cause adverse effect on development of reproductive parts which might be enhance abscission of flowers and bolls and subsequently resulting in yield or quality loss (Ananthi and Vijayaraghavan 2012; Loka *et al.,* 2012). In cotton, it is suggested that cotton plants experiencing moisture stress 15-30 days withholding water, considering this cotton plants experiencing moisture stress during 60-80 DAS is said to be one of the most important critical stages (Michael *et al.,* 1973; Quisenberry *et al.,* 1980).

It has also been known that plants started experiencing drought when soil water potential is less by more than 50 per cent in stressed plot than normal (Santos *et al.,* 2011). In this study, after 15-17 days of water withholding (65-67 DAS), soil moisture content in stressed plots was 17.72 per cent reduced by 26.47 per cent over control condition and after 80-82 DAS (30-32 days water withholding), 8.9 per cent soil moisture content was recorded in stress induced plots. In comparison to control plot, 63.26 per cent reduction of moisture was recorded in stress induced plot (Table 2). Observations on different physio-biochemical traits were recorded when leaf relative water content at 65-67 DAS and 80-82 DAS in stressed plot was respectively less by 10.87 and 19.03 per cent than normal watered plots. Therefore these observations were helpful in identification of the plants experiencing moisture stress.

Physio-biochemical parameters

After 15-17 days of water withholding (65-67 DAS), moisture stress effect in diverse cotton genotypes on physio-biochemical traits was studied. The significant difference was observed irrespective of genotypes between the conditions for several traits (Table 3). Although at 65-67 DAS, significant difference was not observed for transpiration rate and chlorophyll content (Table 3), after 30-32 days water withholding (80-82 DAS), ANOVA showed significant difference between conditions for all traits irrespective of genotypes (Table 4). To identify genetic variability under moisture stress, data was analyzed individually (condition wise separately), the significant difference was observed between the genotypes for physio-biochemical traits in moisture stress condition (Table 5).

Berger *et al.,* (2010) reviewed that canopy temperature is one such integrative trait that reflects the plant water status or the resultant equilibrium between root water uptakes and shoot transpiration. Under stress condition canopy temperature is changing in cotton due to closure of stomata, reducing leaf activity, leaf area and increase leaf senescence (Marani *et al.*, 1985) and it is affected by both the water status of plant (Meyer and walker, 1981) and the water status of soil (Wang *et al.,* 2007). In this study, leaf senescence symptoms observed due to changes in plant and soil water status that leads to significant difference between the conditions for physio-biochemical traits. Under moisture stress condition (80-82 DAS), significant difference between genotypes observed for canopy temperature and the mean canopy temperature in moisture stress condition (29.17) was higher than control condition (27.94). Reddy *et al.*, in 1996 reported that most advantageous canopy temperature ranged between 20 to 30˚C in cotton. Lugojan and Ciulca (2011) reported the balance between water supply to the leaf tissue and transpiration rate is maintained through higher relative water content. During moisture stress, reduced transpiration rate activated cooling system of leaf water potential and maintain higher canopy temperature (Conaty *et al.,* 2015). Although RWC was reduced after 30 days of water withholding, but their reduction rate was less in drought tolerant genotypes than susceptible MCU-5 in this study (Table 6). Therefore maintenance of higher RWC in drought tolerant genotypes (Sahana, RS-810, Khandwa-2, L-761, Bikaneri nerma and 5433A2A03N83) recorded higher canopy temperature than susceptible MCU-5. The susceptible MCU-5 recorded 31.42 per cent reduction of RWC at 80-82 DAS of moisture stress condition over control condition (Table 6). It indicates that higher RWC plays an important role for moisture stress resistance in drought tolerant genotypes. There are some studies reported, that the higher RWC in drought tolerant genotypes, had warmer canopy temperature than the sensitive genotypes in chickpea (Zaman-Allah *et al.,* 2011), cowpea (Belko *et al.,* 2012) and wheat (Rebetzke *et al.,* 2013). Ananthi *et al.,* (2012) observed lowest RWC (61.4) per cent in susceptible cotton genotype, "Surabhi" and highest in "KC2" (77.2), drought tolerant genotype. In this experiment also all drought tolerant cotton genotypes recorded higher RWC in stress condition (30 days of water withholding) than susceptible, MCU-5 (58.66).

Lower transpiration rate along with higher relative water content (RWC) has been reported as a selection criterion for plants against moisture stress (Malik *et al.,* 1999; Rahman *et al.,* 2000). Passioura (1982) and Zhang *et al.,* (2010) implies a water conservation strategy by reducing transpiration rate, that preventing water loss from plant system leads to water saving for

plant growth and helps to withstand in moisture stress condition. Isoda *et al.,* (2002) reported, that reduction in transpiration rate under moisture stressed plants by 5-15 per cent than in well-watered plants, considered as typical for the field-grown cotton plants. Farquhar and Richards (1984) concluded, that under drought condition, low water use efficiency leads to decreased transpiration and at cellular level, abscisic acid was increased in shoots. Roberts and Dumbroff, (1986) reported, that the increase in levels of ABA was closely associated with a decrease in rate of transpiration. In this study decrease in transpiration rate was recorded in stress condition at 80-82 DAS than control condition (Table 6). But, per cent reduction rate of transpiration rate was less in drought tolerant genotypes PH1009 (2.74), F-2226 (2.32), Bikaneri nerma (4.03), JK-4 (4.53) and Khandwa-2 (7.97) than susceptible MCU-5 (49.21). Previous studies in rose (Williams *et al.,* 1999, 2000; Jenks *et al.,* 2001) and tree tobacco (Cameron *et al.,* 2006) reported, that plant adaptation in water deficit limit transpiration rate and delay the onset of cellular dehydration during prolonged drought (Kosma and Jenks, 2007).

When plants expose to water stress, produce abscisic acid (ABA), which can promote stomatal closure by causing the efflux of solutes and water from the guard cells (Radin *et al.,* 1988; Schroeder *et al.,* 2001). Gorham *et al.,* (1998) reported that stomatal conductance was reduced by water deficit with consequent reductions in gas exchange parameters like net photosynthesis, transpiration and water use efficiency and an increase in leaf temperature of cotton.

Table.1 List of genotypes and their source of locations

Sl. No.	Genotype	Source
P1	Sahana	UAS, Dharwad, Karnataka
P2	RS-810	RAU, Sriganganagar, Rajasthan
P3	Khandwa 2	Khandwa, Madhya-Pradesh
P4	L-761	LAM, Guntur, Andhra-Pradesh
P5	GJHV-358	Surat, Gujarat
P6	F-2226	LAM, Guntur, Andhra-Pradesh
P7	JK-4	JAU, Junagadh, Gujarat
P8	RAJ-2	MPUAT, Udaipur, Rajasthan
P9	AK-23	PDKV, Akola, Maharashtra
P10	Bikaneri nerma	PAU, Punjab
P11	PH 1009	MAU, Nanded, Maharashtra
P12	CCH 1831	NAU, Surat, Gujarat
P13	5433A2A03N83	CICR, Nagpur, Maharashtra
P14	MCU-5 (susceptible)	TNAU, Coimbatore, Tamilnadu
P15	RHC-0811	MPKV, Rahuri, Maharashtra

Table.2 Percent of soil moisture content

Conditions	Days of interval		
	50 DAS	**65 DAS**	**80 DAS**
Control plots	23.23	24.10	24.23
Moisture stress plots	23.02	17.72	8.9
Percent reduction	0.9	26.47	63.26

Table.3 Analysis of variance for physio-biochemical traits in moisture stress and control condition at 65-67 DAS

Source of variance	d.f.	CT (˚C)	RWC (%)	TR (m mole of H_2O /m^2/s)	SC (µ mole CO_2/m^2/s)	PR (µ mole CO_2 /m^2/s)	Chl (%)	Proline (µg/g fresh wt.)	GPOX (nM/min/g protein)
Replication	1	1.11	30.88	0.31	0.004	0.84	31.45	2765.31*	301.17
Genotypes (G)	14	0.56	34.35**	0.39	0.009**	2.58	5.6	311.93	7354.29**
Treatments (T)	1	32.07**	1308.75**	0.63	0.33**	24.58**	2.11	22502.06**	630857.00**
T*G	14	1.03	15.36	0.23	0.002	1.72	5.05	204.34	5831.92**
Error	30	0.7	8.57	0.33	0.003	2.47	5.53	157.13	1174.51
Total	59	1.28	38.33	0.32	0.014	2.69	5.37	583.79	21872.24

*, ** significant at 5 % and 1 % respectively

Table.4 Analysis of variance for physio-biochemical traits in moisture stress and control condition at 80-82 DAS (Analyzed condition wise)

Source of variance	d.f.	CT (˚C)	RWC (%)	TR (m mole of H_2O /m^2/s)	SC (µ mole CO_2/m^2/s)	PR (µ mole CO_2 /m^2/s)	Chl (%)	Proline (µg/g fresh wt.)	GPOX (nM/min/g protein)
Replication	1	96.35	26.45	3.32	0.025	4.25	1.1	8.06	74309.12
Genotypes (G)	14	1.45*	19.18*	2.21*	0.006*	7.06*	6.435*	199.78**	35327.36*
Conditions	1	22.51**	4063.58**	26.41**	0.23**	141.87**	150.21**	35869.30**	2144364.37**
T*G	14	1.77	14.01	1.35	0.028**	10.89**	4.31	192.02**	47353.48**
Error	30	4.34	7.78	1.04	0.011	3.08	1.74	51.04	16637.25
Total	59	3.35	80.71	1.82	0.02	8.23	5.98	726.88	64423.96

*, ** significant at 5 % and 1 % respectively

CT- Canopy temperature (˚C); RWC- Relative water content (percent gm of leaf sample); Chl- Chlorophyll content (% leaf area); SC- Stomatal conductance (µ mole CO_2/m^2/s); TR- Transpiration rate (m mole of H_2O /m^2/s**)**; PR- Photosynthetic rate (µ mole CO_2 /m^2/s); Proline (µg/g fresh wt.); GPOX- Peroxidase activity (nM/min/g protein)

Table.5 Analysis of variance for physio-biochemical traits in moisture stress condition at 80-82 DAS (Analyzed condition wise)

Source of variance	d.f.	CT (˚C)	RWC (%)	TR (m mole of H_2O /m^2/s)	SC (µ mole CO_2/m^2/s)	PR (µ mole CO_2 /m^2/s)	Chl (%)	Proline (µg/g fresh wt.)	GPOX (nM/min/g protein)
Replication MSS	1	49	7.41	0.06	0.034	0.02	0.4	303.15	51706.01
Genotype MSS	14	2.70*	28.66**	2.02*	0.019**	15.66**	9.04**	366.22**	71078.33**
Error MSS	14	1.49	11.09	1	0.008	4.28	2.16	34.57	23122.67
C. V.		4.18	4.76	19.39	19.75	11.52	3.56	5.17	13.54
C. D. 5 %		2.61	7.14	2.14	0.2	4.44	3.15	12.61	326.14
S.Em.±		0.86	2.36	0.71	0.07	1.46	1.04	4.16	107.52

*, ** significant at 5 % and 1 % respectively

CT- Canopy temperature (˚C); RWC- Relative water content (percent gm of leaf sample); Chl- Chlorophyll content (% leaf area); SC- Stomatal conductance (µ mole CO_2/m^2/s); TR- Transpiration rate (m mole of H_2O /m^2/s); PR- Photosynthetic rate (µ mole CO_2 /m^2/s); Proline (µg/g fresh wt.); GPOX- Peroxidase activity (nM/min/g protein)

Table.6 Physiological traits in cotton genotypes after 30-32 days of water withholding (80-82 DAS)

Genotype	CT (˚C)			RWC (%)			TR (m mole of H2O /m2/s)			SC (µ mole CO2/m2/s)			PR (µ mole CO2 /m2/s)			Chl (%)		
	C	D	% C	C	D	% C	C	D	% C	C	D	% C	C	D	% C	C	D	% C
Sahana (P1)	27.25	30.63	12.42	86.90	70.38	-19.00	8.08	4.80	-40.59	0.67	0.22	-66.89	22.30	17.00	-23.77	45.30	39.18	-13.52
RS-810 (P2)	27.66	31.51	13.94	86.77	69.54	-19.86	6.96	6.55	-5.90	0.63	0.36	-41.93	21.10	18.83	-10.78	45.88	42.95	-6.38
Khandwa 2 (P3)	28.58	30.32	6.06	87.42	71.78	-17.89	6.40	5.89	-7.97	0.54	0.45	-16.46	21.50	20.45	-4.88	44.23	44.08	-0.34
L-761 (P4)	28.25	30.35	7.43	86.99	67.05	-22.92	6.66	4.56	-31.61	0.44	0.49	11.72	19.70	18.75	-4.82	43.38	41.03	-5.43
GJHV-358 (P5)	28.54	28.74	0.70	85.88	69.58	-18.99	5.71	3.75	-34.33	0.47	0.42	-10.99	19.85	16.30	-17.88	45.80	43.00	-6.11
F-2226 (P6)	27.78	27.23	-1.96	88.46	72.68	-17.83	6.25	6.10	-2.32	0.43	0.56	29.82	22.10	19.10	-13.57	44.78	40.80	-8.88
JK-4 (P7)	28.94	27.64	-4.47	87.90	66.97	-23.82	5.30	5.06	-4.53	0.46	0.31	-31.62	19.40	19.35	-0.26	44.98	39.73	-11.68
RAJ-2 (P8)	28.22	28.80	2.06	85.15	70.43	-17.29	5.17	3.86	-25.44	0.45	0.40	-11.06	21.45	19.96	-6.95	43.70	41.48	-5.10
AK-23 (P9)	27.80	28.76	3.43	89.84	75.01	-16.50	5.24	4.71	-10.12	0.45	0.38	-15.41	21.45	18.80	-12.35	44.26	39.35	-11.10
Bikaneri nerma (P10)	27.84	29.59	6.32	85.57	72.86	-14.85	6.83	6.55	-4.03	0.42	0.55	30.62	21.00	18.70	-10.95	45.20	42.03	-7.03
PH 1009 (P11)	27.04	28.29	4.61	86.43	70.82	-18.05	6.02	5.85	-2.74	0.59	0.43	-27.95	21.40	20.20	-5.61	43.70	43.93	0.51
CCH 1831 (P12)	27.51	29.08	5.68	84.27	70.92	-15.84	6.63	5.34	-19.47	0.40	0.38	-4.68	19.05	18.90	-0.79	43.36	42.58	-1.81
5433A2A03N83 (P13)	28.16	29.38	4.32	84.77	72.73	-14.21	7.05	4.37	-38.09	0.58	0.31	-47.25	21.35	19.53	-8.55	45.48	42.53	-6.49
MCU-5 (P14)	27.80	29.04	4.45	85.53	58.66	-31.42	7.27	3.69	-49.21	0.61	0.24	-60.63	22.65	10.11	-55.36	43.10	36.00	-16.47
RHC-0811 (P15)	27.79	28.18	1.41	85.28	70.85	-16.93	7.66	6.24	-18.54	0.57	0.33	-41.59	21.30	13.50	-36.62	44.76	41.80	-6.60
Mean	27.94	29.17	4.43	86.48	70.02	-19.03	6.48	5.15	-19.66	0.51	0.39	-20.29	21.04	17.96	-14.21	44.53	41.36	-7.09
Range	27.0-28.9	27.2-31.5		84.2-89.8	58.6-75.0		5.1-8.0	3.6-6.5		0.40-0.67	0.22-0.56		19.0-22.6	10.1-20.4		43.1-45.8	36.0-44.0	
	Genotypes	Conditions	Interaction	Genotypes	Conditions	Interaction	Genotypes	Conditions	Interaction	Genotypes	Conditions	Interaction	Genotypes	Conditions	Interaction	Genotypes	Conditions	Interaction
CD 5%	1.11	1.1	NS	4.03	1.47	NS	1.47	0.54	NS	NS	0.05	0.18	2.54	0.93	3.59	1.9	0.69	2.69

C-Normal condition, D-moisture stress condition, % C- per cent change

Table.7 Biochemical traits in cotton genotypes after 30-32 days of water withholding (80-82 DAS)

Genotype	Proline content (µg/g fresh wt)			GPOX (nM/min/g protein)		
	C	D	% C	C	D	% C
Sahana (P1)	61.7	102	65.22	834.6	1000.5	19.88
RS-810 (P2)	68.1	94	38.03	880.2	927.7	5.4
Khandwa 2 (P3)	62.4	119.7	91.72	774.8	1201.1	55.02
L-761 (P4)	57.9	106.2	83.6	640.3	1046.1	63.37
GJHV-358 (P5)	65.1	142.7	119.25	647.7	1150.3	77.6
F-2226 (P6)	65.9	121.7	84.7	719.9	1184	64.46
JK-4 (P7)	64.5	107.3	66.36	749.5	887.1	18.36
RAJ-2 (P8)	60.1	116.2	93.37	664.4	1448.6	118.03
AK-23 (P9)	62.1	119.1	91.83	809.5	1275.5	57.57
Bikaneri nerma (P10)	66.8	110.2	64.88	634.6	1215.7	91.58
PH 1009 (P11)	69.4	122.3	76.37	821.8	1247.7	51.82
CCH 1831 (P12)	66.5	131	97.02	798.9	1041.6	30.39
5433A2A03N83 (P13)	71.5	109.2	52.6	736.7	1366	85.42
MCU-5 (P14)	65.1	90.2	38.55	710.2	731.6	3.01
RHC-0811 (P15)	64	112.7	76.15	747.8	1118.8	49.61
Mean	64.74	113.65	75.98	744.72	1122.82	52.77
Range	57.8-71.5	90.1-142.7		634.5-880.2	731.5-1448.5	
	Genotypes	**Conditions**	**Interaction**	**Genotypes**	**Conditions**	**Interaction**
CD 5%	13.89	5.07	19.65	250.82	91.59	354.71

Table.8 Analysis of variance for productivity traits in moisture stress and control condition at harvesting stage

Source of variance	d.f.	No. of sympodia/ plant	Plant height	No. of fruit bodies/plant	No. of bolls/plant	Boll shedding (%)	Yield (kg/ha)
Replication	1	81.95*	22.2	12.8	3.70*	0.017	561129.44*
Genotypes (G)	14	7.87	165.74*	12.38**	4.61*	39.85*	178430.98**
Treatments (T)	1	765.94**	3788.18	1126.41**	144.43**	2702.31**	2791162.63**
T*G	14	4.92	63.24	15.26**	5.67	61.57**	47513.07
Error	30	8.79	78.1	4.21	1.51	10.83	25911.59
Total	59	20.49	158.25	27.8	5.66	75.37	114097.1

*, ** significant at 5 % and 1 % respectively

Table.9 Analysis of variance for productivity traits in moisture stress condition at harvesting stage (Analyzed condition wise)

Source of variance	d.f.	No. of sympodia/ plant	Plant height	No. of fruit bodies/plant	No. of bolls/plant	Boll shedding (%)	Yield (kg/ha)
Replication MSS	1	0.41	14.18	7.5	0.97	67.71	153184.4
Genotype MSS	14	3.22	93.24*	18.31**	5.46**	91.82**	75849.24**
Error MSS	14	1.92	85.31	2.21	0.41	11.6	1012.25
C. V.		8.82	8	10.33	6.12	7.75	3.19
C. D. 5 %		2.97	19.81	3.19	1.37	7.3	68.24
S.Em.±		0.98	6.53	1.05	0.45	2.41	22.5

Table.10 Yield and yield contributing traits in cotton genotypes at harvesting stage

Genotype	No. of sympoida/ plant			Plant height (cm)			No. of fruiting bodies/plant			No. of bolls harvested/plant			% boll shedding			Yield (kg/ha)		
	C	D	% C	C	D	% C	C	D	% C	C	D	% C	C	D	% C	C	D	% C
Sahana (P1)	26.69	16.44	-38.41	136.19	119.19	-12.48	24.28	15.63	-35.63	13.90	10.90	-21.58	31.1	43.4	39.53	1955.7	1034	-47.13
RS-810 (P2)	19.5	16.13	-17.31	130.44	111.25	-14.71	22.98	19.7	-14.25	12.20	13.80	13.11	27.7	43.4	56.65	1031.3	883.6	-14.32
Khandwa 2 (P3)	19.44	15.06	-22.51	135.81	112.31	-17.3	20.25	20.15	-0.49	12.30	13.80	12.2	29.2	44.1	51.12	1482.7	1213.9	-18.13
L-761 (P4)	21.38	15.44	-27.78	138.81	124.31	-10.45	20.27	13.59	-32.98	11.65	9.60	-17.56	28.6	50.3	76.01	1309.6	1135.3	-13.31
GJHV-358 (P5)	22.31	17.06	-23.53	143.25	125.25	-12.57	21.25	14.1	-33.65	12.80	9.00	-29.69	29	42.6	46.95	1126.1	967.6	-14.08
F-2226 (P6)	20.25	16.13	-20.37	113.44	103.25	-8.98	21.1	12.08	-42.77	12.20	9.80	-19.67	30.4	51.3	68.96	1361.2	959.4	-29.52
JK-4 (P7)	21.88	14.38	-34.29	132.56	118.38	-10.7	24.29	10.98	-54.81	13.90	9.20	-33.81	27.6	50.1	81.57	1403.3	784.5	-44.09
RAJ-2 (P8)	23.75	15.88	-33.16	132	125.69	-4.78	23.83	12.44	-47.8	12.80	9.50	-25.78	27.7	49.3	77.98	1969.8	1410.5	-28.39
AK-23 (P9)	24.81	16.25	-34.51	139.13	112.88	-18.87	24.25	12.49	-48.51	13.80	9.30	-32.61	31.5	36.2	14.77	1286.1	878.1	-31.73
Bikaneri nerma (P10)	22.5	14.75	-34.44	120.44	110	-8.67	20.33	13.61	-33.03	12.10	9.60	-20.66	32.2	38.1	18.49	1289.4	921.3	-28.55
PH 1009 (P11)	21.81	13.19	-39.54	130.63	109.25	-16.36	25.19	13.95	-44.62	16.10	9.00	-44.1	33.5	31.2	-7.06	1455.4	1002.4	-31.13
CCH 1831 (P12)	24.44	15.19	-37.85	140.88	108.94	-22.67	21.35	13.86	-35.07	12.40	9.80	-20.97	32.7	35.2	7.61	1400.1	1119.9	-20.01
5433A2A03N83 (P13)	23.75	17.38	-26.84	125.25	118.81	-5.14	25.04	18.84	-24.76	14.80	12.30	-16.89	31.7	41.6	31.1	1423.5	1009.9	-29.06
MCU-5 (P14)	25.06	14.31	-42.89	127.19	111.06	-12.68	24.43	10.01	-59.01	15.00	9.30	-38	34.4	55.2	60.17	1169.6	557.7	-52.32
RHC-0811 (P15)	25.13	17.94	-28.61	123.56	120.63	-2.38	27.18	14.59	-46.32	16.70	11.20	-32.93	30.9	47.6	54.25	1744.9	1060.2	-39.24
Mean	22.85	15.7	-30.8	131.3	115.41	-11.92	23.07	14.4	-36.91	13.51	10.41	-21.93	30.54	43.96	45.21	1427.25	995.89	-29.4
Range	19.4-26.6	13.1-17.9		113.4-143.2	103.2-125.6		20.2-27.1	10.0-20.1		11.6-16.7	9.0-13.8		27.6-34.4	31.2-55.2		1031.3-1969.7	557.7-1410.5	
	Geno types	**Conditions**	**Interaction**	**Geno types**	**Conditions**	**Interaction**	**Geno types**	**Conditions**	**Interaction**	**Geno types**	**Conditions**	**Interaction**	**Geno types**	**Conditions**	**Interaction**	**Geno types**	**Conditions**	**Interaction**
CD 5%	NS	1.56	NS	12.76	4.66	NS	2.96	1.08	4.19	1.78	0.65	2.51	4.75	1.74	6.72	232.46	84.88	NS

C-Normal condition, D-moisture stress condition, % C- per cent change

Massacci *et al.*, (2008) found irrigated plants had greater stomatal conductance than dryland cotton and 30 per cent reduction of stomatal conductance observed in stressed plants as compared to non-stressed (Khan *et al.,* 2015). It shows the blocking of the stomata to reduce water loss and increase adaptability of plants to stress condition. Therefore in this study, all genotypes showed decreased stomatal conductance at 80-82 DAS of moisture stress condition than control condition (Table 6).

However in moisture stress condition, per cent reduction of stomatal conductance was less in drought tolerant genotypes RAJ-2 (11.06), AK-23 (15.41), CCH-1831 (4.68), GJHV-358 (10.99), Khandwa-2 (16.46) and RAJ-2 (31.62) than susceptible MCU-5 (60.63). Contrastingly, in some genotypes, L-761 (11.72), F-2226 (29.82) and Bikaneri nerma (30.62) higher per cent of stomatal conductance recorded in water deficit condition than watered condition, probably as a result of a higher capacity for water uptake in this genotypes. In cotton many studies reported, as soil water availability diminished, stomatal conductance decrease (Lopez *et al.*, 1995; Laffray and Louguet, 1990; Costa and Cothren 2011; Cornish *et al.*, 2016).

Photosynthesis is closely related to water and carbon (C) metabolism in plants, moisture stress is known to reduce photosynthetic rate by decreased RWC and is mainly through stomatal closure or metabolic impairment (Cornic and Massicci, 1996). Many studies observed significant inhibition of photosynthetic rate in cotton under a water-limited environment (Turner *et al.,* 1986; Leidi *et al.,* 1993). In this study, all cotton genotypes recorded lower photosynthetic rate at 30 days of water withholding (80-82 DAS) than control condition (Table 6). Under severe water stress when relative water contents drops below 70 per cent, inhibition of photosynthesis is attributed to non-stomata1 effects (Kaiser, 1987; Ennahli and Earl, 2005), therefore higher reduction of photosynthetic rate observed, might be due to reduction of chlorophyll content (Table 6). Nepomuceno *et al.,* (1998) demonstrated that the tolerant genotypes were able to maintain higher photosynthesis, stomatal conductance and relative water content near unstressed control levels. Similarly in this study drought tolerant genotypes CCH1831 (0.79), JK-4 (0.26), L-761 (4.82), Khandwa-2 (4.88), PH1009 (5.61) and 5433A2A03N83 (8.55) recorded smaller reduction of photosynthetic rate than susceptible MCU-5 (55.36) in moisture stress condition. Reduction in stomatal conductance in susceptible MCU-5 is to save water for survival, but due to reduced photosynthesis, susceptible genotype MCU-5 recorded very low yield. Therefore less reduction of photosynthetic rate in drought tolerant genotype under moisture stress condition (80-82 DAS) is considered a decisive factor for higher cotton production (Lopez *et al.,* 1995; Ullah *et al.,* 2008).

Plant chlorophyll content is generally controlled and there was wide genetic variability existed among all crop species. However, irrespective of genotypes, moisture stress reduces the chlorophyll content in crop plants. The extent of chlorophyll content is again depend on genotypes and their level of moisture stress tolerance (Yi *et al.,* 2000; Li *et al.,* 2012; Li *et al.,* 2014).

In the present study after 30 days of water withholding (80-82 DAS), all cotton genotypes showed reduced chlorophyll content than control condition and in drought tolerant genotypes Khandwa-2 (0.34), RAJ-2 (5.10), L-761 (5.43), GJHV-358 (6.11) rate of reduction was less than moisture stress susceptible MCU-5 (16.47). Genotypes with higher chlorophyll recorded higher yield under moisture stress, Cetin *et al.,* (2009)

revealed that leaf chlorophyll content may be one of the major factors influencing seed cotton yield of cotton under drought stress.

As normal phenomenon, proline is acting as an osmoprotectant, in the moisture stress condition (Bates *et al.,* 1973). Singh *et al.,* (1992) reported that under water stress condition, reduction RWC and increased leaf proline content was observed in drought tolerant cotton variety (SRT 1). Similarly, in this study also, under moisture stress situation reduced RWC rate was observed at 80-82 DAS with higher proline rate (Table 7) in genotypes, GJHV-358 (119.25), PH1009 (76.37), CCH1831 (97.02), Khandwa-2 (119.7) and RAJ-2 (93.37) than susceptible MCU-5 (38.55). The increased proline content under moisture stress condition was found in many studies (Parida *et al.,* 2007; Aktas *et al.,* 2009; Amudha *et al.,* 2014). This is due to the fact that drought tolerant plants can regulate their solute potential to compensate for transient or extended periods of water stress by the process called osmotic adjustment and proline acts as an osmoregulator and cellular protectant under moisture stress (Hanower and Brzozowska, 1975; Patil *et al.,* 2011). Moisture stress induces oxidative stress leads to increased production of reactive oxygen species (ROS) like superoxide (O_2^-), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2), which can attack lipids, proteins, carbohydrates, and nucleic acids of plant system (Smirnoff, 1993). However there were several enzymes that detoxify ROS resulting from stress. Superoxide dismutase (SOD) is the first defense enzyme that converts superoxide to H_2O_2, which can be scavenged by catalase (CAT) (Hosseini *et al.,* 2015). Hydrogen peroxide is produced in the process of stress and is detoxified in living organisms by catalase and glutathione peroxidase (Jin *et al.,* 2006). In the present study, increase in peroxidase activity in cotton genotypes (Table 7) under moisture stress was recorded than normal condition and the rate of increase in peroxidase enzyme in moisture stress tolerant genotype RAJ-2 (118.03), Bikaneri nerma (91.58), 5433A2A03N83 (85.42) was greater than susceptible MCU-5 (3.01).

Yield and yield contributing traits

The ANOVA showed significant variation in irrespective of genotypes between conditions (Table 8) for yield contributed traits. When data was analyzed in individually condition wise, under moisture stress condition significant difference among genotypes was observed for all studied productivity traits except sympodia (Table 9). In many studies, yield and yield contributed traits have been found to reduce under moisture stress condition (Luz *et al.,* 1997; Osborne and Banks, 2006; Karademir *et al.,* 2011; Sahito *et al.,* 2015).

However the rate of reduction in moisture stress condition than control condition is vary among genotypes and Karademir *et al.,* (2011) reported, that in drought tolerant varieties less yield reduction observed than susceptible varieties. In the present study (Table 10). The genotypes, L-761 (13.31), GJHV-358 (14.08), RS-810 (14.32), Khandwa-2 (18.31), CCH1831 (20.01) recorded lower reduction of yield than susceptible MCU-5 (52.32). Yield is a complex trait and is the end product of various developmental and physiological processes. Therefore in the present study, as compared to control condition, under moisture stress condition, cotton yield was reduced due to changes in physio-biochemical process. At 80-82 DAS (30 days water withholding), drought tolerant genotypes recorded higher relative water content, stomatal conductance, photosynthetic rate and transpiration rate with increasing canopy temperature than susceptible MCU-5, resulted

in higher yield in moisture stress tolerant genotypes (Khandwa-2, RAJ-2, CCH1831, PH1009, and RHC0811) than susceptible MCU-5. Ephrath (1990); Genty (1987); Marani (1985); Turner *et al.,* (1986) reviewed that adequate water availability in plant system is the main factor maintained photosynthesis and stomatal conductance and, consequently positively correlated with increased yields in drought tolerant genotypes than susceptible (Fischer *et al.,* 1998). Although several physiological traits studied in relation to yield potential, but no single parameter has been identified as the sole trait of improving yield (Barbour *et al.,* 2000; Bray, 1993; Radin *et al.,* 1994; Ullah *et al.,* 2008) in moisture stress induction.

Therefore, in this study, the maximum yield of desirable drought tolerant genotypes showed variation for possible traits like sympodial number, number of fruiting points, number of bolls and plant height and these productivity traits were ultimately affected by physio-biochemical plant response. Higher chlorophyll content and photosynthetic rate recorded in Khandwa-2 and higher stomatal conductance, RWC recorded in genotypes F-2226 and Bikaneri nerma than susceptible MCU-5 in same level of moisture stress condition and it leads to maximum number of bolls in moisture stress condition than susceptible MCU-5. It indicates that significant difference between genotypes for moisture stress tolerance is regulated by different metabolic processes under stress condition.

The tolerance of genotypes to moisture stress also result in increasing antioxidants, proline and peroxidase enzyme. All their activities were higher in drought tolerant genotypes as compared to susceptible, MCU-5. Khatun *et al.,* (2008) reported that antioxidant peroxidase minimize cellular damage which caused by reactive oxygen species (ROS) such as superoxide, perhydroxy radicals, hydrogen peroxide, hydroxy radicals and results in to higher yield in tolerant genotypes grown in water deficit condition. Meanwhile, Ullah *et al.,* (2017); Zhang *et al.,* (2014) revealed that antioxidant enzyme activities, decreased in drought susceptible genotypes and increased in drought tolerant genotypes which is responsible for higher yield potential in stress condition.

When water-deficit stress occurs during the square formation stage, reduction in seed cotton yield of cotton genotypes is mainly due to square and young boll shedding (Cook and El-Zik, 1992). Other study reports Alishah and Ahmadikhah (2009); Khorgade and Ekbote (1980); Karademi *et al.,* (2011); Zare *et al.,* (2014) evaluated cotton genotypes in moisture stress condition and recorded reported higher seed cotton yield in stress condition due to maintenance of higher water use efficiency and other physiological parameter. The higher productivity traits were related to plant physio-biochemical metabolic process. Therefore in this study, under moisture stress condition significant variation between genotypes for physio-biochemical traits associated with variation in number of fruiting bodies, boll number, plant height and sympodial number was observed.

Substantial genotypic variation found for physio-biochemical attributes among the cotton genotypes and as expected higher rate of RWC, lower transpiration rate, higher photosynthetic rate, proline content and peroxidase enzyme activity would record higher yield in genotypes Khandwa-2, F-2226, 5433A2A03N83, RAJ-2, and RHC0811 under moisture stress condition. The moisture stress tolerance due to regulation of physio-biochemical metabolic process results in to less reduction of yield in genotypes, GJHV-358 (14.08), RS-810 (14.32), Khandwa-2 (18.31) and CCH 1831 (20.01) than susceptible MCU-5.

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