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Water-deficit stress – Induced physio-biochemical changes in cotton (*Gossypium hirsutum* L.) Cultivars

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Water stress is a serious global issue regarding growth of agricultural crops and sustainable food production for the large population. In the present situation due to low rainfall and unavailability of advanced irrigation methods, water deficit stress is the most limiting factor decreasing crop production in many regions of the world. In this study, to assess the drought tolerance mechanism in cotton cultivars was monitored by drought– induced physio-biochemical changes. To assess the tolerance in cotton cultivars, a field experiment was conducted in split– plot design in which the main plot consists of irrigated and complete rainfed conditions as a stress and cotton cultivars arranged in the main plot as a subplot. The overall comparative analysis revealed that hybrid was superior over their parents under well-watered as well as in water deficit conditions in terms of chlorophyll content, wax content, accumulation of compatible solutes, photosynthesis rate, stomatal conductance, transpiration rate and yield parameters. The findings from the results indicate that under water deficit conditions plants having a different adaptive mechanisms for coping with the stress situation. So, some of the adaptive mechanisms such as accumulation of sugars, polyphenols, amino acids, non-enzymatic antioxidants, and wax deposition helps to maintain osmotic balance, to protect cellular macromolecules, to detoxify the cells, and to scavenge free radicals under water deficit condition.

Keywords: Compatible solutes, Photosynthesis rate, Transpiration rate, Wax content

Cotton is the valuable gift of nature to mankind, which contributed cloth to the population of the world. Out of the 50 species of cotton, four species are commercially cultivated in the world and India. India is the only country, where all the four species are cultivated on a commercial scale between 10° to 30° latitude and 70° to 80° longitude. India having a considerably larger diversity of cotton cultivars and cotton agro-climatic zones as compared to other major cotton–growing countries in the world. Among the different abiotic stresses, drought is a major limiting factor for plant productivity worldwide, especially in arid and semi-arid agro ecosystems. The severity of the drought is capricious as it depends on many factors such as the amount and distribution of rainfall, evaporative demands, and moisture storing capacity of soils[1]. Cotton is drought tolerant relative to susceptible crops, but severe water stress can slow plant development, cause less cotton bolls and squares to shed and thus reduce the seed cotton yield. Some physiological traits such as net photosynthesis and total chlorophyll content also decline due to plant water status decreases[2].

Water deficit stress– induced changes are characterized by reduction of water content, turgor changes and total water potential leads to closure of stomata, decrease in cell enlargement and growth. Water stress– induced stomatal closure limiting CO_2 uptake by leaves. In such events reduced CO_2 availability could lead to increased susceptibility to photo damage[3]. Water stress– induced changes in photosynthetic pigments and components[4], damaged photosynthetic apparatus[5], and reduced crop yield[6].

One of the negative effects of water stress involves damage to cell membranes and the release of ions into the intercellular space *i.e.* electrolyte leakage[7]. This disruption of cell membranes induces oxidative stress, which leads to lipid peroxidation, membrane permeabilization and cell death. At the biochemical level, soluble sugar accumulates in leaves, stems, and roots in response to drought stress in many plants[8]. Thus, soluble sugar accumulation leads to the lowering of osmotic potential in plant tissue, which helps to maintain the driving force for extracting soil water

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under drought conditions[9]. Similarly, other organic compounds such as total free amino acids that accumulate during water –stressed in plants also play a significant role in the osmotic adjustment of the cell sap[10]. Osmotic adjustment (OA) is an important mechanism, which alleviates some of the detrimental effects of water stress due to the accumulation of osmolyte like proline, total soluble sugar (TSS), and free amino acids (FAA).Therefore, the objective of this study was to evaluate the water deficit stress -induced responses on physiological and biochemical activities in cultivars to elucidate the possible tolerance mechanism of the stress during the growth of cotton.

Materials and Methods

Plant materials

In this study four cotton cultivars *viz*. G.Cot.16, H-1353/10 (tolerant genotypes), BS-30, and H-1452/10 (Susceptible genotypes) were obtained from Main Cotton Research Station, Navsari Agricultural University, Surat, Gujarat, India. These four cultivars were crossed in full diallel fashion during *Kharif* season 2011-12. Developed hybrids and their parents were grown in *Kharif* season 2012-13 for evaluation under irrigated (non-stress) and rainfed (stress) conditions.

Drought induction treatment

The experiment was carried out at Main Cotton Research Station (20°-12′ N, 72°-52′ E; altitude 11.34 M), Navsari Agricultural University, Surat, Gujarat during the *Kharif* season 2012-13. We used a split-plot design in which the main plot consists of irrigated and complete rainfed condition as a stress and cotton cultivars arranged in the main plot as a subplot under three replications for each treatment. For the vegetation period, the average maximum temperature ranged between 39.7 to 30.0°C, while the average minimum temperature ranged between 27.0°C to 14.7°C with a mean annual rainfall of 789 mM. the soil was drained clay soil, which represents the typical black cotton soil having predominant montmorillonite clay minerals by its origin and medium fertility. These soils crack vertically upon drying up to a depth of 80 to 120 cm. the clay content ranges from 56.4 to 64.9%. Plants were sown at the beginning of May and grown until the onset of flowering under well-watered conditions. to induce drought condition irrigation was completely stopped in a rainfed plot.

Photosynthetic gas-exchange parameters

Different leaf gas-exchange measurements such as net photosynthesis, stomatal conductance, and transpiration rate were measured from second fully expanded leaves from the apex of cotton using a portable gas analyzer-based photosynthesis system (LI-6400, Li-Cor, Inc., USA). Leaf gas exchange was measured between 09.00 and 11.00 AM. All measurements were carried out at a CO_2 concentration of 400 µmol/mol, a photosynthetic photon flux density of 1000 µmol/m^2/s, and leaf temperature of 30°C.

Growth analysis

Based on leaf area (LA) (cm^2) data and total dry matter DM or Biomass (g) per plant, using the equations proposed by Hunt[11], we calculated the NAR: rate of biomass increase per leaf area (g cm^{-2} day^{-1}) and RGR: the rate of biomass gain per biomass (g g^{-1} day^{-1}).

Photosynthetic pigments

0.5 g of fresh leaves were homogenized in chilled 80% acetone with mortar and pestle in dark at 4° and the homogenates were centrifuged at 10000 g for 10 min. The supernatant was collected and OD measured at 663, 646, and 470 nM using UV- visible spectrophotometer (Shimadzu UV 1800, Japan). The Chl *a*, Chl *b*, total chlorophyll, and carotenoid content were calculated by the equation of Lichtenthaler[12].

Chlorophyll stability index (CSI)

Chlorophyll stability index (%) was measured by exposing the leaf sample to a hot water bath at 56°C ± 1°C for 30 min, followed by grinding the sample in 100 mL of 80% acetone. The control sample was kept normal. The absorbance of the filtrate was recorded at 645 and 663 nM on UV-visible spectrophotometer. Then, the chlorophyll stability index was calculated by using the formula according to Sairam *et al.*[13].

Total Polyphenol

Total polyphenol was determined by the procedure of Chandler and Dodds[14]. In this method, 0.5 g leaves were homogenized in 5 mL of 80% ethanol using mortar and pestle with centrifugation at 10000 g for 20 min and the supernatant collected. The supernatant was pooled and evaporated upto the dryness. The residue was collected and dissolved in 5 mL of distilled water. 3 mL of aliquots were taken in to test tube and 0.5 mL Folin-Ciocalteau's reagent (1N) was added and mixed thoroughly. The solution was boiled in a water

bath for 1 min, cooled, and then OD was measured at 650 nM. Phenol concentration was determined using a calibration curve and expressed as mg g^{-1}FW.

Total soluble sugar and free amino acid

100 mg leaves were placed in a 10 mL centrifuge tube and mixed with 5 mL of 80% ethanol. The mixture was incubated in a water bath with shaking at 80°C for 30 min and centrifuged at 4000 rpm for 5 min to collect the supernatants. The pellets were extracted two more times with 80% ethanol and all supernatant were combined and diluted to 25 mL with 80% ethanol, mixed, and stored at −20°C for measuring the soluble sugar and amino acid content by using the anthrone method[15] and ninhydrin reagent method[16], respectively.

Epicuticular wax content

Epicuticular wax was determined by the method of Ebercon *et al*[17]. Ten leaf discs of area approximately 35.56 cm^2 from 3rd or 4th leaf from the upper part of plant were collected. Leaf wax was removed from leaf disc by stirring in 15 mL of chloroform in a test tube for 20 sec. Extracted wax was evaporated on a water bath maintained at 80°C for 30 min. The reagent was prepared by dissolving 20 g potassium dichromate in 40 mL of distill water and the resulting extract mixed with 1 L H_2SO_4 and heated boiling point until a clear solution was obtained. Samples were collected and added 12 mL of distill water, allowed to stand for 15 min, and the intensity of colour measured at 590 nM using UV-visible spectrophotometer. The wax content was measured on a leaf area basis (µg cm^{-2}) by using a standard curve.

Statistical analysis

The Analysis was performed to determine the water deficit stress –induced changes in physio-biochemical traits using SPSS software version 20.0 program for Windows (SPSS Inc., Chicago, IL, USA). Significant differences between treatments were evaluated using Duncan's multiple range test ($P< 0.05$).

Result and Discussion

Photosynthetic gas-exchange parameters

The photosynthesis rate significantly declines due to stress caused by the low availability of soil moisture with reduced relative water content (Fig. 1A). This

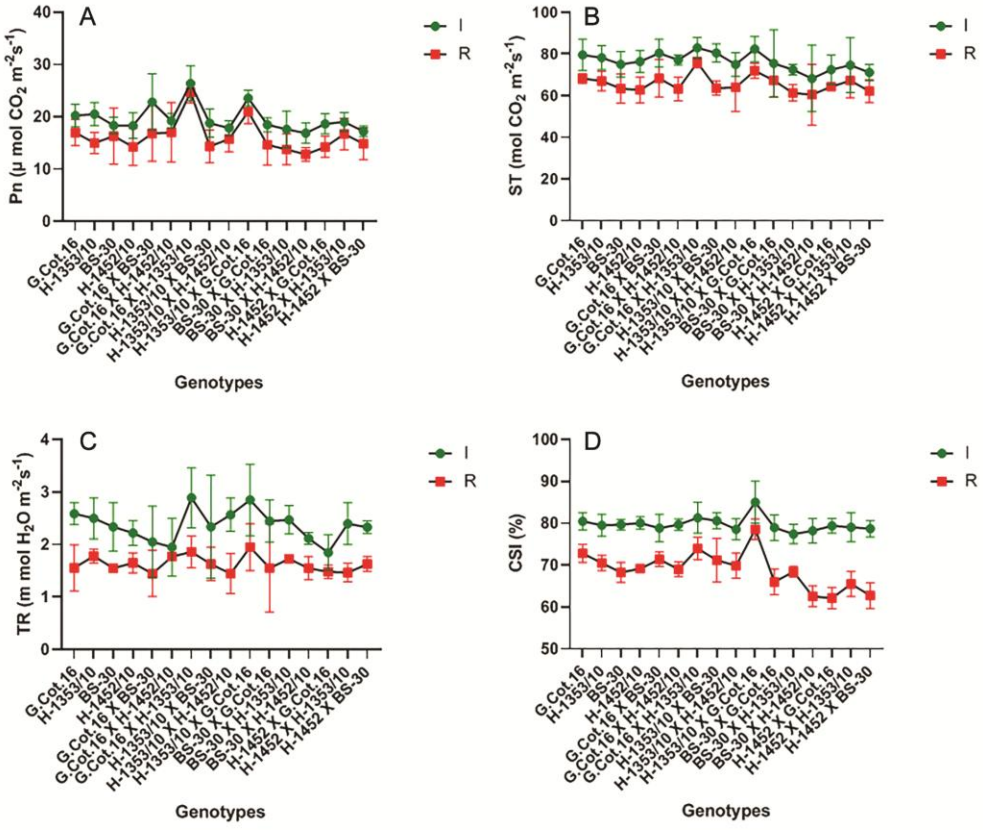

Fig. 1 — Impact of water stress on (A) Photosynthesis rate; (B) Stomatal conductance; (C) Transpiration rate; and (D) Chlorophyll stability index (CSI) of upland cotton cultivars under stress (R) and non-stress (I)

reflected on photosynthesis, which recorded a significant decrease under stress compared to non-stress. Nepomuceno *et al.*[18] also reported that photosynthesis significantly reduced under stress. Decreased photosynthesis under water stress was basically due to photoinhibition[19]. Ni and Pallardy[20] proposed that decrease in photosynthesis also responsible for the decrease in stomatal conductance under stress conditions. Stomatal and even non-stomatal limitations are responsible for reduction of photosynthesis under water deficit conditions[21]. The result of the present study indicates that the cultivars such as G.Cot.16 × H-1353/10 and H-1353/10 × G.Cot.16 showed higher photosynthesis rate in comparison of susceptible under stress condition. The differences in photosynthesis in cultivars under stress are correlated with RWC, osmotic potential, stomatal conductance, and transpiration rate. The tolerant cultivars show higher photosynthesis rate either they maintained photosynthesis near unstressed conditions or showed a minimum reduction in the same as also stated by Chaves[22].

Stomatal conductance is a measure of gaseous exchange from leaf lamina and greatly affected by water balance in the plant system. Pettigrew[23] stated that under stress conditions stomatal conductance substantially decreased as compared to non-stress conditions. The result showed variation amongst cultivars in terms of stomatal conductance (Fig. 1B). Variations among the stomatal conductance of cotton cultivars under stress also reported by Kumar and Bardhan[24], which is due to the genetic background of cultivars as well as parameters like RWC[25] and osmotic potential. Lv *et al.*[26] reported a reduction in RWC results in loss of turgidity, which leads to stomatal closure and reduced photosynthesis rate in plants.

Transpiration is important because stomatal conductance in the cotton leaf is correlated to transpiration rate because the mechanism of gas flux that drives the physiological processes is controlled by the stomata and stomatal sensitivity to relative water content[27]. In the present study, the transpiration rate significantly declined due to stress. It was interesting to observe that cultivars, G.Cot.16 × H-1353/10 and H-1353/10 × G.Cot.16 maintained a higher transpiration rate in comparison to other cultivars under stress conditions (Fig. 1C).

Photosynthetic pigments

Photosynthetic pigments such as chlorophyll content and carotenoids were decreases with increased water stress. The results indicated that chlorophyll 'a', 'b' and total chlorophyll were decreases significantly under drought stress over the non-stress conditions. Among the different cultivars G.Cot.16 × H-1353/10 and H-1353/10 × G.Cot.16 showed significantly higher chlorophyll 'a', 'b' and total chlorophyll in comparison of other cultivars (Table 1). Under drought stress

Table 1 — Changes in total chlorophyll, chlorophyll *a*, *b* and carotenoid content of upland cotton cultivars grown under water stress (R) and non-stress (I)

Cultivars	Total Chlorophyll (mg g^{-1} FW)		Chlorophyll *a* (mg g^{-1} FW)		Chlorophyll *b* (mg g^{-1} FW)		Carotenoid (mg g^{-1} FW)	
	Non-stress	Stress	Non-stress	Stress	Non-stress	Stress	Non-stress	Stress
G.Cot.16	2.37^{cde}	2.14^{e}	1.55^{fg}	1.40^{bc}	0.820^{h}	0.740^{def}	1.48^{cde}	1.36^{cd}
H-1353/10	2.33^{de}	2.10^{e}	1.47^{h}	1.37^{c}	0.860^{fg}	0.730^{ef}	1.53^{bc}	1.38^{c}
BS-30	2.42^{bcde}	2.12^{e}	1.52^{gh}	1.38^{c}	0.900^{de}	0.740^{def}	1.35^{fg}	1.24^{f}
H-1452/10	2.32^{de}	1.97^{g}	1.40^{i}	1.25^{fg}	0.920^{cd}	0.720^{f}	1.40^{efg}	1.20^{gh}
G.Cot.16 × BS-30	2.32^{de}	2.11^{e}	1.50^{gh}	1.38^{c}	0.820^{h}	0.725^{f}	1.42^{def}	1.30^{e}
G.Cot.16 × H-1452/10	2.30^{e}	2.02^{fg}	1.47^{h}	1.31^{de}	0.830^{gh}	0.714^{f}	1.54^{bc}	1.36^{cd}
G.Cot.16 × H-1353/10	2.59^{abc}	2.42^{b}	1.72^{b}	1.62^{a}	0.870^{ef}	0.803^{bcde}	1.57^{ab}	1.48^{b}
H-1353/10 × BS-30	2.43^{bcde}	2.11^{e}	1.53^{g}	1.35^{cd}	0.900^{de}	0.760^{cdef}	1.50^{bcd}	1.34^{d}
H-1353/10 × H-1452/10	2.35^{de}	2.13^{e}	1.47^{h}	1.36^{cd}	0.880^{ef}	0.770^{cdef}	1.52^{bc}	1.38^{c}
H-1353/10 × G.Cot.16	2.72^{a}	2.51^{a}	1.79^{a}	1.66^{a}	0.930^{bcd}	0.853^{ab}	1.64^{a}	1.57^{a}
BS-30 × G.Cot.16	2.46^{bcde}	2.13^{e}	1.51^{gh}	1.37^{c}	0.950^{bc}	0.760^{cdef}	1.34^{fg}	1.16^{i}
BS-30 × H-1353/10	2.51^{abcde}	2.20^{d}	1.59^{ef}	1.39^{c}	0.920^{cd}	0.810^{abcd}	1.38^{fg}	1.23^{fg}
BS-30 × H-1452/10	2.64^{ab}	2.30^{c}	1.65^{cd}	1.45^{b}	0.990^{a}	0.850^{ab}	1.35^{fg}	1.18^{hi}
H-1452/10 × G.Cot.16	2.60^{abc}	2.23^{d}	1.61^{de}	1.35^{cd}	0.990^{a}	0.884^{a}	1.39^{fg}	1.21^{fgh}
H-1452/10 × H-1353/10	2.62^{ab}	2.07^{ef}	1.67^{c}	1.22^{g}	0.950^{bc}	0.853^{ab}	1.38^{fg}	1.15^{ij}
H-1452/10 × BS-30	2.55^{abcd}	2.12^{e}	1.59^{ef}	1.29^{ef}	0.960^{ab}	0.830^{abc}	1.32^{g}	1.12^{j}

Values are the mean of three replications (n=3). Variants possessing same letters are not statistically significant at 5% probability level.

conditions slower synthesis or quicker breakdown of chlorophyll responsible for the reduction in chlorophyll content[28]. This decrease may be due to the formation of a proteolytic enzymes such as chlorophyllase, which is responsible for chlorophyll degradation[29].

Drought stress having the ability to decrease the concentration of carotenoids due to the production of reactive oxygen species in the thylakoids[30]. Carotenoid content is also reported higher in cultivars, G.Cot.16 × H-1353/10 and H-1353/10 × G.Cot.16 under stress in comparison to other cultivars because tolerant cultivars having the ability to maintained higher carotenoid content than sensitive cultivars under stress (Table 1).

Chlorophyll stability index (CSI)

The chlorophyll stability index decreases significantly in different cultivars under stress. The tolerant cultivars G.Cot.16 and H-1353/10 and their crosses showed significantly higher chlorophyll stability in comparison to susceptible cultivars because chlorophyll stability index decreases due to increasing chlorophyll degradation by heat (Fig. 1D). Chlorophyll stability affects the photosynthesis rate of the plant because to maintain better availability of chlorophyll to high chlorophyll stability index required by the plant to survive under stress condition. Due to this photosynthesis rate and total dry matter production increases[31]. High chlorophyll stability index (CSI) and relative water content (RWC) could be regarded as a selection index for the screening of drought tolerance and select tolerant cultivars from a large number of populations[32].

Growth analysis

Relative growth rate (RGR) is more associated with vegetative growth than with seed cotton yield, cotton lint, and earliness in cotton. In the present study relative growth rate was significant decreases in different cultivars under stress over non-stress conditions (Table 2). The tolerant cultivars such as G.Cot.16 × H-1353/10 and H-1353/10 × G.Cot.16 showed higher relative growth rate in comparison of susceptible cultivars under stress because more drought– tolerant genotypes maintained photosynthetic production under stress[33].

Greater leaf area index accompanied by higher photosynthesis in some of the cultivars or otherwise reflected on net assimilation, which is the product of the above two. The cultivars G.Cot.16 × H-1353/10 and H-1353/10 × G.Cot.16 indicated a higher net assimilation rate (Table 2). Distinct differences in NAR particularly the tolerant and susceptible cultivars are an indicator of the performance of hybrids. The NAR significantly decreased under stress is the reflection of physiological processes, which preceded it is LAI, RWC, Leaf osmotic potential, chlorophyll content, and photosynthesis rate. Hence again the tolerant cultivars registered a lesser decrease under stress vis-à-vis susceptible cultivars. Palomo and Godoy[34] opined that higher NAR is due to higher dry matter accumulation, which causes translocation of carbohydrates to reproductive organs.

Table 2 — Changes in Net assimilation rate (NAR) and Relative growth rate (RGR) of upland cotton cultivars grown under water stress (R) and non-stress (I)

Cultivars	Net Assimilation Rate (g cm^{-2} day^{-1})		Relative Growth Rate (g g^{-1} day^{-1})	
	Non-stress	Stress	Non-stress	Stress
G.Cot.16	0.430 ^{b}	0.496^{e}	27.15 ^{cd}	29.50 ^{g}
H-1353/10	0.419^{bc}	0.489 ^{e}	27.28 ^{cd}	29.33 ^{g}
BS-30	0.330^{f}	0.380 ^{k}	20.54 ^{f}	29.26 ^{g}
H-1452/10	0.358 ^{def}	0.393 ^{j}	20.62 ^{f}	27.58 ^{h}
G.Cot.16 × BS-30	0.393^{bcd}	0.460 ^{g}	27.07 ^{cd}	32.17 ^{f}
G.Cot.16 × H-1452/10	0.412^{bc}	0.480 ^{f}	24.00 ^{e}	28.33 ^{h}
G.Cot.16 × H-1353/10	0.493 ^{a}	0.596 ^{a}	31.27 ^{a}	33.42 ^{e}
H-1353/10 × BS-30	0.425 ^{b}	0.508 ^{d}	27.68 ^{cd}	32.47 ^{f}
H-1353/10 × H-1452/10	0.433 ^{b}	0.520 ^{c}	27.28 ^{cd}	29.74 ^{g}
H-1353/10 × G.Cot.16	0.439 ^{b}	0.539 ^{b}	30.24 ^{ab}	32.30 ^{f}
BS-30 × G.Cot.16	0.379^{cde}	0.459 ^{g}	29.06 ^{bc}	34.14 ^{de}
BS-30 × H-1353/10	0.346^{ef}	0.420 ^{h}	32.15 ^{a}	38.88 ^{a}
BS-30 × H-1452/10	0.326 ^{f}	0.389 ^{j}	27.84 ^{cd}	35.18 ^{c}
H-1452/10 × G.Cot.16	0.344^{ef}	0.402 ^{i}	28.12 ^{bcd}	34.91 ^{cd}
H-1452/10 × H-1353/10	0.355 ^{def}	0.405 ^{i}	27.13 ^{cd}	36.90 ^{b}
H-1452/10 × BS-30	0.332 ^{f}	0.390 ^{j}	26.28 ^{d}	35.42 ^{c}

Values are the mean of three replications (n=3). Variants possessing same letters are not statistically significant at 5% probability level.

Phenol, Total soluble sugar (TSS), and free amino acid (FAA)

Among the different classes of secondary metabolites, phenolics play variety of important roles in plants. In this study, phenol content was increased significantly under water stress comparison of non-stress condition (Table 3). Among different cultivars G.Cot.16 × H-1353/10 and H-1353/10 × G.Cot.16

Table 3 — Changes in Phenol, total soluble sugar (TSS), free amino acid (FAA) and epicuticular wax content of upland cotton cultivars under water stress (R) and non-stress (I)

Cultivars	Phenol (mg g^{-1} FW)		TSS (mg g^{-1} FW)		FAA (mg g^{-1} FW)		Wax content (µg cm^{-2})	
	Non-stress	Stress	Non-stress	Stress	Non-stress	Stress	Non-stress	Stress
G.Cot.16	0.430 ^{b}	0.496^{e}	27.15 ^{cd}	29.50 ^{g}	2.71^{abc}	3.58^{ef}	0.780 ^{a}	0.856^{c}
H-1353/10	0.419^{bc}	0.489 ^{e}	27.28 ^{cd}	29.33 ^{g}	2.60^{bcd}	3.50 ^{fg}	0.719^{abcd}	0.819^{d}
BS-30	0.330^{f}	0.380 ^{k}	20.54 ^{f}	29.26 ^{g}	2.65^{abcd}	3.84 ^{c}	0.646^{ef}	0.729^{h}
H-1452/10	0.358 ^{def}	0.393 ^{j}	20.62 ^{f}	27.58 ^{h}	2.74 ^{ab}	3.81 ^{cd}	0.661^{def}	0.714^{j}
G.Cot.16 × BS-30	0.393^{bcd}	0.460 ^{g}	27.07 ^{cd}	32.17 ^{f}	2.63^{abcd}	3.40 ^{ghi}	0.693 ^{cdef}	0.793 ^{e}
G.Cot.16 × H-1452/10	0.412^{bc}	0.480 ^{f}	24.00 ^{e}	28.33 ^{h}	2.60^{bcd}	3.33 ^{hi}	0.672^{def}	0.769 ^{g}
G.Cot.16 × H-1353/10	0.493 ^{a}	0.596 ^{a}	31.27 ^{a}	33.42 ^{e}	2.73 ^{ab}	3.30 ^{i}	0.771 ^{ab}	0.888 ^{b}
H-1353/10 × BS-30	0.425 ^{b}	0.508 ^{d}	27.68 ^{cd}	32.47 ^{f}	2.61^{bcd}	3.44^{gh}	0.709^{bcde}	0.816 ^{d}
H-1353/10 × H-1452/10	0.433 ^{b}	0.520 ^{c}	27.28 ^{cd}	29.74 ^{g}	2.50^{def}	3.49 ^{fg}	0.751^{abc}	0.857 ^{c}
H-1353/10 × G.Cot.16	0.439 ^{b}	0.539 ^{b}	30.24 ^{ab}	32.30 ^{f}	2.78 ^{a}	3.27 ^{i}	0.776 ^{a}	0.897 ^{a}
BS-30 × G.Cot.16	0.379^{cde}	0.459 ^{g}	29.06 ^{bc}	34.14 ^{de}	2.68 ^{abc}	3.70 ^{de}	0.684 ^{def}	0.770^{fg}
BS-30 × H-1353/10	0.346^{ef}	0.420 ^{h}	32.15 ^{a}	38.88 ^{a}	2.51^{def}	3.58 ^{ef}	0.689^{cdef}	0.776 ^{f}
BS-30 × H-1452/10	0.326 ^{f}	0.389 ^{j}	27.84 ^{cd}	35.18 ^{c}	2.57 ^{cde}	3.94^{abc}	0.636^{f}	0.720 ^{ij}
H-1452/10 × G.Cot.16	0.344^{ef}	0.402 ^{i}	28.12 ^{bcd}	34.91 ^{cd}	2.44 ^{ef}	4.04 ^{a}	0.648^{ef}	0.717 ^{j}
H-1452/10 × H-1353/10	0.355 ^{def}	0.405 ^{i}	27.13 ^{cd}	36.90 ^{b}	2.37 ^{f}	3.90^{bc}	0.629^{f}	0.704 ^{k}
H-1452/10 × BS-30	0.332 ^{f}	0.390 ^{j}	26.28 ^{d}	35.42 ^{c}	2.45^{ef}	4.01 ^{ab}	0.636^{f}	0.724 ^{hi}

Values are the mean of three replications (n=3). Variants possessing same letters are not statistically significant at 5% probability level.

showed significantly higher phenol content in comparison to other cultivars. An increase in polyphenol contents in different tissues under stress has been observed in a number of plants[35].

The results of the present study indicate that the total soluble sugar content of different cultivars was increased significantly under stress over the non-stress conditions to maintain the osmotic condition of the plant[10]. Among different cultivars BS-30 × H-1353/10 and H-1452 × H-1353/10 showed significantly higher total soluble sugar levels to maintain osmotic conditions to withstand under stress conditions (Table 3).

Free amino acid (FAA) content in the leaves was significantly increased intolerant cultivars under stress over non-stress condition because different protective/defensive mechanism like osmotic adjustment, protection of cellular macromolecules, maintaining cellular pH, storage of nitrogen, scavenging of free radicals, and detoxification of cells helped to cope drought stress due to higher accumulation of FAA in plants. Among different cultivars, G.Cot.16 × H-1353/10 and H-1353/10 × G.Cot.16 showed significantly higher FAA content because of increasing level of FAA indicating mode of adjustment to drought in cotton crop[28] (Table 3).

Epicuticular wax content

The epicuticular wax content of tolerant cultivar leaf was significantly higher in under stress over nonstress conditions. Water stress increases epicuticular was content in the leaf of water– stressed plant in comparison of well-watered plant due to increased number and levels of long– chain, higher molecular weight alkanes in the leaves[36]. The cultivars such as G.Cot.16 × H-1353/10 and H-1353/10 × G.Cot.16 showed higher accumulation of epicuticular wax in comparison to other cultivars (Table 3). Increased wax concentration in response to water stress contributed to functions such as conservation of water, minimization of leaching losses, and protection from injury due to various environmental factors which also seems to be true in the present study[37].

Yield attributes

The results indicated that due to water stress significant reduction occurred in yield in comparison to the irrigated conditions because of low biomass produced and reduced translocation efficiency (Fig. 2A). Genotypic differences amongst hybrids and parents in seed cotton yield was observed. The cross of tolerant parents such as G.Cot.16 × H-1353/10 and H-1353/10 × G.Cot.16 showed less reduction in seed cotton yield in comparison of susceptible parents and their hybrids.Therefore, these hybrids could be considered as stress– tolerant cultivars. Kar *et al*.[38] proposed that cultivars having the least reduction in yield in terms of a number of bolls and/or boll weight

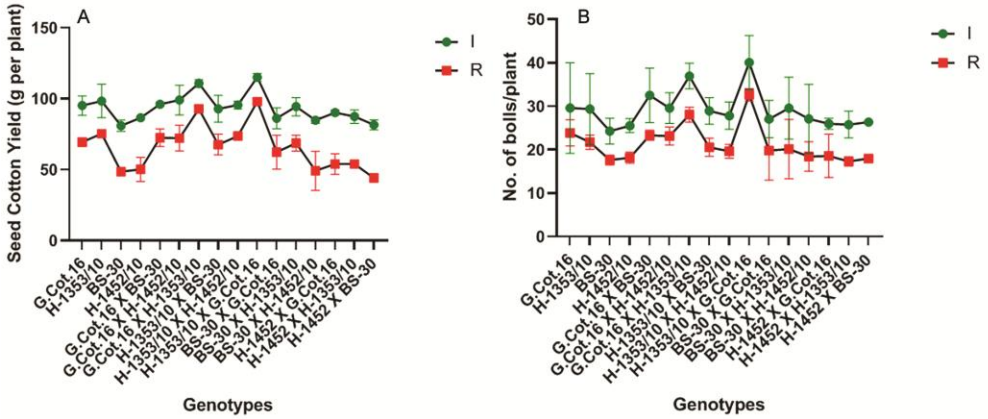

Fig. 2 — Impact of water stress on (A) Seed cotton yield (g/plant); and (B) Number of bolls/plants of upland cotton cultivars under stress (R) and non-stress (I)

or both under stress as compared to non-stress indicate their ability to tolerate stress condition.

In rainfed condition number of bolls was significantly reduced in comparison to irrigated condition (Fig. 2B). Variations in a number of bolls amongst different cultivars is widely reported earlier by Alishah and Ahmadikhah[39]. Amongst different cultivars in the present study G.Cot.16 × H-1353/10 and H-1353/10 × G.Cot.16 showed higher boll number with less reduction under stress over irrigated compared to susceptible cultivars, which showed lower bolls and greater reduction under stress[40].

Conclusion

The study concluded that water deficit stress strongly disrupted the normal metabolism of the plant such as a reduction in total chlorophyll, carotenoids, NAR, and RGR of both tolerant and susceptible cultivars. Butin tolerant cultivars less reduction was observed in the comparison of susceptible cultivars. Similarly, the level of a total free amino acid (FAA), wax content, TSS, and phenol content was increased under water stress. Increased accumulation of these metabolites in tolerant cultivars are basically responsible for osmotic adjustment, protection of cellular macromolecules, the storage form of nitrogen, maintaining cellular pH, detoxification of cells, and scavenging of free radicals. So, these adaptive traits could be exploited in breeding programs for the selection and development of drought–resistant genotypes.

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