INT. J. BIOL. BIOTECH., 7 (1-2): 85-91, 2010.

HIGH DAY TEMPERATURE INDUCES CHANGES IN PROTEIN, SUGAR AND PHENOLIC CONTENTS IN COTTON GROWN UNDER ARID AND SEMIARID ENVIRONMENTS

Muhammad Naeem Akhtar[1], Shabab Ud-Din[1], Ghulam Abbas[2], Ilyas Raza[1], Ammarah Husnain[3] and Muqaddas Noreen[3]

[1]*Central Cotton Research Institute, Multan, Pakistan*
[2]*Department of Plant Breeding & Genetics, University of Agriculture., Faisalabad, Pakistan*
[3]*Department of Biochemistry, B.Z. University, Multan, Pakistan*

ABSTRACT

Thermal stress is a main limitation to achieve good and stable cotton yield in Pakistan. Cultivars differ in their adaptation to heat stress. Therefore, the screening of advanced strains is carried out by planting cotton in the month of April to coincide their fruiting phase with the hottest period of season (June-July). Fourteen genotypes were selected for screening against heat stress. Seed sowing was done during mid-April and the leaves were collected after 90 days of sowing while seed cotton yield was taken 125 days after sowing. Results revealed that genotypes CIM-496, Cyto-87, NIAB-V3, CIM-554, NIAB-V1 and CIM-541 resulted in higher seed cotton yield while Gomal 93 and Shahbaz, CRIS-342 and Cyto-89 exhibited lowest seed cotton yield. It was also observed that heat induced-stress caused certain quantitative and qualitative changes in phenolic, sugar and protein contents. The quantitative changes in the amounts of crude protein were also observed and such changes varied variety to variety but not in a regular fashion. Different types of sugar compounds were observed in all cultivars with no distinct relation with seed cotton yield. It was found that higher yielding cultivars maintain relatively higher nitrogen and protein contents and less sugar contents as compared to low yielding cultivars under high temperature environments. In qualitative analysis of phenolic contents, six standards phenolic compounds namely gallic acid, pyragallol, hydroquinone, phenol, 2-naphthol and o-nitro phenol were detected in different cultivars of cotton, indicating that high day temperature cause various changes in the metabolism of the plant helpful for their adaptation under arid climate.

Key words: Heat, Protein and sugar contents, Phenolics, Seed cotton yield, Arid environment

INTRODUCTION

Heat stress is a very common agricultural problem in many areas of the world (Porter, 2005). High temperature continuously for longer period of time or even for short interval cause morpho-anatomical, physiological and biochemical changes in plants, which affect plant growth and development leading to a drastic decline in economic yield. The adverse effects of heat stress can be counter-acted by developing crop plants with improved thermo-tolerance using plant breeding & genetic approaches (Wahid *et al*., 2007).

Being a native of the tropics, cotton thrives in warm weather with day-time temperature around 29-35 ^{0}C and cool night-time temperatures around 21 ^{0}C. Cotton is a summer crop but excessively high temperature impairs its growth and production. Excess heat triggers a series of physiological reactions in the plant which work to shut down non-critical growth functions for the sake of self-preservation (Ashraf *et al.* 1994). It had been examined that stress is a major factor limiting crop yield in many agricultural region. It has been found that under stress, different plant species may accumulate a variety of osmolytes such as sugars and sugar alcohols (polyols), proline, tertiary and quaternary ammonium compounds, and tertiary sulphonium compounds (Sairam and Tyagi, 2004). Phenolics, including flavonoids, anthocyanins, lignins, etc., is the most important class of secondary metabolites in plants and play a variety of roles including tolerance to abiotic stresses (Chalker-Scott, 2002 and Wahid *et al*., 2007). Studies suggest that accumulation of soluble phenolics under heat stress is accompanied with increased phenyl ammonia lyase (PAL) and decreased peroxidase and polyphenol lyase activities (Rivero *et al*., 2001).

Cotton plant is vulnerable to biotic and abiotic stresses. Thermal stress is a main problem faced in Pakistan to attain good and stable cotton yield. Generally, heat tolerance has been a primary selection criterion for higher lint yield in cotton breeding programs (Feaster and Turcotte, 1985; Percy and Turcotte, 1991). Cultivars differ in their adaptation to heat stress. The biochemical attributes are greatly influenced due to harsh climatic conditions prevailing during the growth period. The sustainability of crop productivity depends greatly on the stability of phenolic and protein contents during vegetative and reproductive development (Wahid et al., 2007). In cotton growing districts of the Punjab and Sindh provinces (Pakistan), summers are severe and maximum temperature often exceeds 45 °C. Such high temperatures cause considerable damage to cotton and are a major concern to physiologists and breeders working against stress environments. The temperature regime during the month of July-August, corresponding to peak flowering, is significantly associated with cotton yield. Temperature changes may act directly by modifying existing physiological processes, and indirectly by inducing an altered pattern of development after the imposition of temperature change (Rahman, 2004). According to Paulsen (1994), a large number of traits that have a direct relation with resistance to plants against high temperature indicate that thermo-tolerance is high complex trait.

A very little work in this regard has been carried out on local cotton germplasm. Therefore, field studies were carried out to evaluate the influence of heat induced stress on different cotton genotypes. The objectives of the present study were to determine the kind and extent of effects of heat induced stress on protein, sugar contents and phenolics in various cotton genotypes differing in their tolerance against heat induced stress.

MATERIALS AND METHODS

The field study was conducted at the Farm of the Central Cotton Research Institute, Multan in 2007 in randomized complete block design in triplicated fashion. Fourteen cotton genotypes viz., CIM- 496, CIM-541, CIM-554, NIAB-V1, Cyto-87, Cyto-89, Haridost, CRIS-129, NIAB-V2, Shahbaz, GH-102, Gomal-93, NIAB-V3 and CRIS-342 were sown on the ridges using seed @ 20 kg ha^{-1} during second week of April. An interplant distance of 30 cm was maintained by thinning out of the extra plants when crop grown up to 30 cm height. Nitrogen, Phosphorus and Potassium were applied @ 150, 50 and 50 kg ha^{-1}, respectively in form of urea, ammonium phosphate and sulphate of potash. Nitrogen was applied in three splits: sowing, squaring and flowering; while whole phosphorus and potassium was applied at the time of seed bed preparation. The leaf samples were collected from representative plants after 90 days of sowing. These leaves were washed, air dried, ground and preserved for further analysis. Seed cotton yield (kg/ha) was calculated by picking cotton at 125 days after sowing. The biochemical analysis was carried out in pant Physiology/Chemistry Section, Central Cotton Research Institute, Multan. Total nitrogen contents were determined by Kjeldahl method (Vogal, 1973). The percent crude protein was calculated by using the following formula:

% crude protein = % nitrogen × 6.25.

For the estimation of sugar contents (), One gram of finely ground leaves of 14 different genotypes of cotton was soaked separately in 75% ethanol (50 ml). After 24 hours, the samples were ground and filtered. The filtrates were collected into 14 different glass bottles while the residue was discarded. These extracts were used for the estimation of total sugars and reducing sugars (Travelson and Harrison, 1952; Hulme, and Narian, 1931b). Non-reducing sugars was calculated as follows:

Non-reducing sugars = Total sugars – reducing sugars

For qualitative analysis of sugars, the individual sugars were separated and identified by using paper chromatography. One-dimensional thin layer chromatography (T.L.C.) was employed for the separation of phenolic compounds.

The data was statistically analyzed using the procedure given by Steel & Torrie (1997), through ANOVA technique and ranking of genotypes was made by Tuckey's test at 0.05α level. Correlation coefficient was determined to find out the increasing or decreasing trend of protein and sugars with reference to yield of seed cotton. The yield of seed cotton was also compared with sugar compounds to find out some short-cut selection criteria. All analysis was done using Statistix® 8.1 statistical software.

RESULTS AND DISCUSSIONS

Data represented in Table 1 indicated that there were highly significant differences among all the 14 cotton cultivars with respect to Nitrogen and protein contents; total sugar, reducing and non-reducing sugar as well as for seed cotton yield. Maximum seed cotton yield (Kg/ha) was exhibited by cultivars CIM-496 (2009 Kg/ha), Cyto-89 (1965 Kg/ha), NIAB-V3 (1951 Kg/ha) and CIM-554 (1740 Kg/ha) hence these were declared to be the heat tolerant cultivars on the basis of seed cotton yield as it is the most widely used selection criteria for screening of cultivars under biotic and abiotic stress. Lower seed cotton yield was obtained in Cyto-89 (1377 Kg/ha), Gomal-93 (1263 Kg/ha) and Shahbaz (1062 Kg/ha). The nitrogen and protein contents differ greatly among the various genotypes. The nitrogen and crude protein contents were ranged from 3.92% to 4.76% and 24.5% to 29.75%, respectively. The higher contents of nitrogen and crude protein were found in the case of NIAB-V3 followed by NIAB-V2. In the case of NIAB-V3, nitrogen were 4.76% and crude protein 29.75%, while in case of NIAB-V2 nitrogen was 4.70% and crude protein 29.3%. Minimum nitrogen 3.92% and protein contents 24.5% were found in Gomal-93 (Table 1).

Fig. 1(a): Relationship between N (%) and Seed Cotton Yield

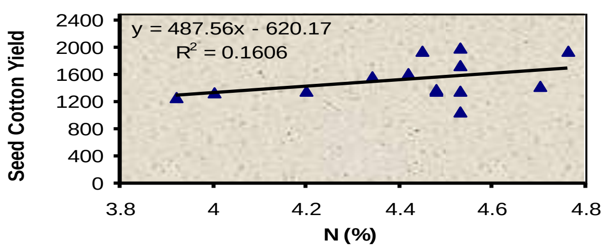


Fig. 1(b): Relationship between Protein contents (%) and Seed Cotton Yield

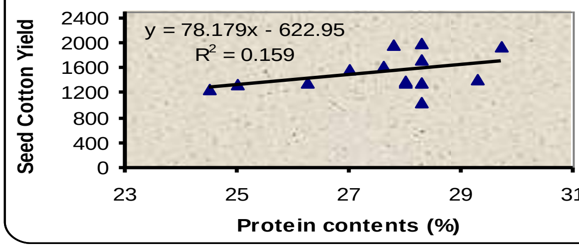


Fig. 1(c): Relationship between Total Sugar contents (%) and Seed Cotton Yield

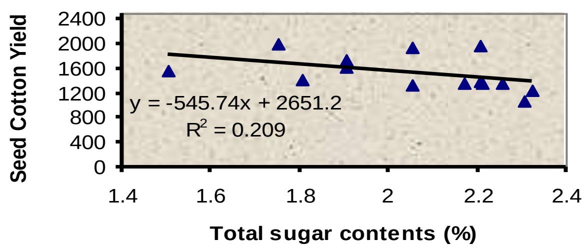


Fig. 1(d): Relationship between Reducing sugar contents (%) and Seed Cotton Yield

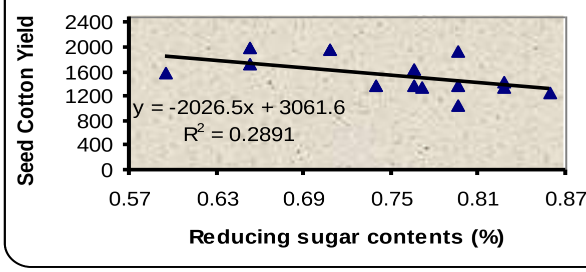
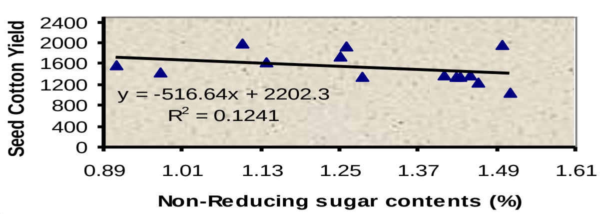


Fig. 1(e): Relationship between Non-Reducing sugar contents (%) and Seed Cotton Yield



Ashraf *et al.* (1994) reported similar findings that leaf soluble proteins were significantly higher in heat tolerant cultivars (B-557 and MNH-93) as compared with those in the other cultivars. Leaf soluble proteins are important adaptive components of heat tolerance of this crop through its impact on membrane thermo stability.

Data presented in Table 1 indicate that total sugars differ greatly among the various cotton genotypes. The higher contents of total sugars were found in genotype Gomal-93, whereas minimum in CIM-541. The values of total sugars ranged from 2.32% to 1.50%.The values of reducing and non-reducing sugars also differ with the genotypes, ranging from 0.859 to 0.595% and 1.5085 to 0.908%, respectively. Higher contents of reducing sugars were found in genotype Gomal-93, whereas minimum in CIM-541. Similarly, higher contents of non-reducing sugars were found in genotype Shahbaz, whereas minimum in CIM-541. Qualitative analysis of sugar compounds through paper chromatography of different genotypes has been shown in Table 3. Total nine sugars compounds viz., glucose, xylose, fructose, sucrose, arabinose, rhamnose, maltose, galactose and lactose were detected in all cultivars, though detection level varies among different genotypes. All the cultivars contained two types of sugar compounds among the earlier mentioned list except CRIS-342 which expressed three sugar types (Table 3). There was no clear cut relation between type of sugar compound and seed cotton yield.

Table 1. Mean performance of 14 genotypes of cotton for various traits under high day temp.

Genotypes	Nitrogen (%)		Protein (%)		Total Sugar (%)		Reducing Sugar (%)		Non-reducing Sugar (%)		Seed cotton Yield (kg/ha)	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Gomal-93	3.92 i	0.12	24.5 i	0.75	2.32 a	0.07	0.85 a	0.02	1.46 b	0.04	1263 h	44.36
Shahbaz	4.53 c	0.14	28.3 c	0.86	2.30 a	0.07	0.79 c	0.01	1.50 a	0.04	1062 i	37.30
CRIS-342	4.48 d	0.14	28.0 d	0.86	2.25 b	0.07	0.82 b	0.01	1.42 cd	0.04	1367 g	48.01
Cyto-89	4.20 g	0.13	26.3 g	0.80	2.21 bc	0.07	0.76 d	0.01	1.44 bc	0.04	1377 g	48.36
Cyto-87	4.45 de	0.14	27.8 de	0.85	2.20 c	0.07	0.70 f	0.01	1.49 a	0.04	1965 ab	69.01
Haridost	4.48 d	0.14	28.0 d	0.86	2.20 c	0.07	0.79 c	0.01	1.40 d	0.04	1382 fg	48.53
CRIS-129	4.53 c	0.14	28.3 c	0.86	2.17 c	0.07	0.73 e	0.01	1.43 cd	0.04	1370 g	48.11
NIAB-V3	4.76 a	0.15	29.7 a	0.91	2.05 d	0.07	0.79 c	0.01	1.25 ef	0.03	1951 b	68.52
GH-102	4.00 h	0.12	25.0 h	0.76	2.05 d	0.07	0.77 d	0.01	1.28 e	0.03	1349 g	47.38
NIAB-V1	4.42 e	0.14	27.6 e	0.84	1.90 e	0.06	0.76 d	0.01	1.13 g	0.03	1636 d	57.45
CIM-554	4.53 c	0.14	28.3 c	0.86	1.90 e	0.06	0.65 g	0.01	1.25 f	0.03	1740 c	61.11
NIAB-V2	4.70 b	0.14	29.3 b	0.90	1.80 f	0.06	0.82 b	0.01	0.97 i	0.02	1434 f	50.36
CIM-496	4.53 c	0.14	28.3 c	0.86	1.75 g	0.06	0.65 g	0.01	1.10 h	0.03	2009 a	70.55
CIM-541	4.34 f	0.13	27.0 F	0.82	1.50 h	0.05	0.59 h	0.01	0.90 j	0.02	1578 e	55.42
LSD (0.05%)	0.03		0.23		0.04		0.01		0.03		52.79	
df	26		26		26		26		26		26	
MS	0.168		6.151		0.174		0.174		0.116		249931	
CV	0.28		0.28		0.71		0.49		0.78		1.14	

Table 2. Correlation (r) among 14 genotypes of cotton for various traits under high day temperature.

Traits	Nitrogen (%)	Protein (%)	Total Sugar (%)	Reducing Sugar (%)	Non-reducing Sugar (%)	Seed cotton Yield (kg/ha)
Nitrogen (%)	1	1.00	-0.24	-0.12	-0.25	0.40
Crude protein (%)	1.00	1	-0.22	-0.11	-0.23	0.40
Total sugar (%)	-0.24	-0.22	1	0.69	0.96	-0.46
Reducing sugar (%)	-0.12	-0.11	0.69	1	0.46	-0.54
Non-reducing sugar (%)	-0.25	-0.23	0.96	0.46	1	-0.35
Seed cotton yield (Kg/ha)	0.40	0.40	-0.46	-0.54	-0.35	1

Table 3. Qualitative Analysis of Sugar contents by paper chromatography in 14 Genotypes of cotton under heat stress.

SName of Standard	Rf value of standards	CIM-496	CIM-541	CIM-554	NIAB-V1	Cyto-87	Cyto-89	Hari-dost	CRIS-129	NIAB-V2	Shah-baz	GH-102	Gomal-93	NIAB-V3	CRIS-342
Seed cotton yield kg/ha		2009	1578	1740	1636	1965	1377	1382	1370	1434	1062	1349	1263	1951	1367
Lactose	0.20	-	+	-	-	-	+	+	+	-	+	-	+	+	+
Maltose	0.23	+	-	+	+	-	-	-	-	+	-	+	-	-	-
Sucrose	0.28	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Glucose	0.32	-	+	-	-	-	-	+	-	-	-	-	+	+	-
Galactose	0.35	-	-	+	-	-	-	-	+	-	+	-	-	-	+
Rabinose	0.40	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fructose	0.41	+	-	-	-	-	+	-	-	+	-	+	-	-	-
Xylose	0.49	-	-	-	+	-	-	-	-	-	-	-	-	-	+
Rhaminose	0.68	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total		2	2	2	2	2	2	2	2	2	2	2	2	2	3

Table 4. Qualitative Analysis of Phenol compounds by paper chromatography in 14 Genotypes of cotton under Heat stress.

Name of Standard	CIM-496	CIM-541	CIM-554	NIAB-V1	Cyto-87	Cyto-89	Hari-dost	CRIS-129	NIAB-V2	Shah-baz	GH-102	Gomal-93	NIAB-V3	CRIS-342
Seed cotton yield kg/ha	2009	1578	1740	1636	1965	1377	1382	1370	1434	1062	1349	1263	1951	1367
Gallic acid	+	+	+	+	-	-	-	-	-	-	-	-	+	+
Pyrogallol	-	-	-	+	+	+	+	+	+	+	+	+	-	+
Hydro-quinone	+	+	+	+	-	-	-	+	-	-	-	-	+	+
Phenol	-	-	-	-	+	+	+	+	-	-	-	-	-	-
2-nephathanol	-	-	-	-	-	-	-	-	-	-	-	-	+	-
O-nitrophenol	+	+	+	+	+	+	+	-	+	+	+	+	-	-
Total	3	3	3	4	3	3	3	3	2	2	2	2	3	3

Due to thermal stress, biochemical changes are greatly affected by influencing the crop productivity. The maintenance of membrane thermo-stability attaches significant importance in adaptation to temperature stress. The maintenance of higher contents of total, reducing and non-reducing sugar have comparative advantage in maintaining integrity of cell membrane and continue the growth of plant (Sairam and Tyagi, 2004). The similar results are reported by Ashraf *et al.* (1994), who found that accumulation of higher concentration of sugars in various genotypes resulted in heat tolerance.

Correlation analysis was performed to check the relationship among protein, sugar contents and seed cotton yield. Positive correlation was observed between seed cotton yield and nitrogen as well as protein contents while negative correlation was found among total sugar, reducing and non-reducing contents and seed cotton yield (Table 2). Similar trend has been shown in Fig.1, indicating that cultivars with higher nitrogen and protein contents along with less sugar contents results in more seed cotton yield and vice-versa. This phenomenon may be used as selection criteria for screening of high temperature tolerant cultivars suitable for arid climate.

Genotypes of cotton (14) presented in Table 4 shows the phenolic compounds in various cotton cultivars under temperature stress. The phenolic contents were found higher in number in NAIB-V2 and Gomal-93, namely gallic acid, hydro-quinone and o-nitrophenol, while CIM-554, CRIS-129, GH-102, and CRIS-342 contained only phenolic substance of type hydroquinone and pyrogallol, respectively. It was found that lower yielding cultivars (e.g., Gomal-93 having seed cotton yield of 1263 Kg/ha) exhibited more phenolic compounds.

It apears that high temperature causes drastic metabolic changes in the plant internal system and cultivars which maintain higher contents of protein and lower contents of sugars and phenolic compounds may result in higher yield under high temperature stress.

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(*Accepted for publication December 2009*)