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STUDY OF BIOCHEMICAL CHANGES IN COTTON GENOTYPES AT SQUARING STAGE UNDER WATER STRESS CONDITIONS

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ABSTRACT

A study was conducted to find out the Cotton (*Gossypiumhirusutum*L.) varieties under pot culture technique (Gravimetric method) for drought tolerance as well as to study the effect of drought on fibre development and biochemical changes in Cotton. The research trials were carried out during the period from August 2012 to April 2013 with pot culture experiment in glasshouse, Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. Stress imposed at squaring seems to be much sensitive in terms of biochemical parameters studied. Result showed that, soluble protein, total sugar, reducing sugar and non-reducing sugar reduced as per increasing the water stress levels and also increased in proline content and peroxidase activity was observed.The variety Bunny Bt showed the increase in values of proline content in leaves at different moisture levels compared to Anjali and Pratima and also with respect to control. From the study it was concluded that, proline concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants the Bunny Bt found to be most tolerant among genotypes studied.

KEY WORDS
Gossypiumhirusutum L.
Total sugar
Soluble protein
Proline content and peroxidase activity

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INTRODUCTION

Water deficit is the major abiotic factor limiting plant growth and crop productivity around the world (Kramer, 1983). One third of the cultivated area of the world suffers from chronically inadequate supply of water (Massacci *et al*., 2008).In case of Cotton, where more than 65 per cent of Cotton cultivation is under rain fed conditions, available soil moisture is another key factor affecting growth and yield of Cotton. Approximately minimum of 50 cm of moisture is needed to mature the crop even at low acceptable yields of 0.75 bales ha^{-1} (Waddle, 1984). Cotton is sensitive to water deficit during both flowering and boll development (Constable and Hearn, 1981; Cull *et al*., 1981a, b; Turner *et al*., 1986).Water stress is commonly attribute to situations where water stress exceeds sufficient absorption intensity causing decrease in plant water content, turgor reduction and consequently, a decrease in cellular expansion and alternation of various essential biochemical processes that can affect growth and productivity. Eaton and Ergle (1948) showed that water stress significantly reduced starch concentrations and increased hexose sugars in Cotton leaves, with variable effects on sucrose accumulation. Parida *et al*. (2007) found that leaf starch contents decreased in both droughts tolerant and drought-sensitive cultivars. The reductions in reducing sugar, non-reducing sugar and total amino nitrogen were well established facts in Cotton under water deficit condition (Ahmed, 1989). Plant productivity is dependent upon the production, translocation, storage, and utilization of carbohydrates. Carbohydrate pools should, therefore, provide relevant information about the response of plants to stresses that reduce productivity. Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados and Savoure, 2009). Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants. It influences protein salvation and preserves the quaternary structure of complex proteins, maintains membrane integrity under dehydration stress and reduces oxidation of lipid membranes or photo inhibition (Demiral and Turkan, 2004). The proline content increase as the drought stress progressed and reached a peak as recorded after 10 days stress, and then decreased under severe water stress as observed after 15 days of stress (Anjum *et al*., 2011b). Diethelm and Shibles (1989) had opined that the Rubisco content per unit leaf area was positively correlated with that of the soluble protein content. During drought, quality of chloroplast protein decrease. Heat shock proteins (HSPs) and late embryogenesis abundant (LEA)-type proteins are two major types of stress induced proteins that are produced upon the induction of drought stress and are considered to play a role in cellular protection during the stress (Ingram and Bartels, 1996; Zhu *et al*., 1997). Heat shock proteins have been observed to be produced at any stage of crop development and under different environmental factors such as water-deficit stress.

The Peroxidase activity was studied to find out effect of drought on fibre elongation. Peroxidase activity was studied in 10 DPA bolls.

MATERIALS AND METHODS

Planting material (genotypes)

A total of three cotton varieties (Pratima, Anjali and Bunny Bt) were selected. Seeds of all varieties were collected from Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore.

Experimental set up

Three cotton varieties were used in experiment they are arranged in glass house. The experimental materials were subjected to four watering treatments, which induced T_1: Control, T_2: 75 per cent of Field Capacity, T_3: 50 per cent of Field Capacity and T_4: 25 per cent of Field Capacity with three replications.

Gravimetric method of drought imposition (pot preparation)

Each empty pot was weighed accurately and weight was noted as 'A'. The pots were filled with soil: sand: vermin compost mixture in the ratio of 2:1:1. The pots were weighed along with soil and weight was noted as 'B'. Above data was used to calculate the soil dry weight (C) as C = B - A. The pots were flooded with water and allowed overnight to drain the excess water and attain field capacity. Pot weight was taken after saturation and denoted as 'D'. Saturated soil weight (E) was calculated as E = D – A. The amount of water required to maintain 100 per cent field capacity was obtained by subtracting the dry soil weight from the saturated soil weight. Further pots were weighed once in two days and water was supplied to maintain the weight of pot at field capacity up to initiation of First Square. The water supply was with-held when square initiation was observed in plants and it was watered only when pot weighs below 75 per cent or 50 per cent or 25 per cent of field capacity as per the treatment.

Sowing/planting

Three seeds per pot were sown initially. After establishment the thinning was done and single healthy plant was maintained per pot. The treatments were imposed after thirty two days of sowing when first squaring observed. All the analyses were done only after 21 days of stress imposition.

Data collection of biochemical parameters

Total sugars content in leaves (mg g^{-1})

The total sugar content was determined using the anthrone method as described by Hedge and Hofreiter (1962).

Reducing sugars content in leaves (mg g^{-1})

The reducing sugar content in leaves was determined using Dinitrosalisylic acid method as described by Miller, (1972).

Non-reducing sugars content in leaves (mg g^{-1})

The non-reducing sugar content in leaves was calculated by using following formula;

Total sugars = Reducing sugars + Non reducing sugars

Soluble protein

Soluble protein was estimated from the leaf samples following the method of Lowry *et al*. (1951) and the content expressed in mg g^{-1} fresh weight.

Proline content

Proline content of leaves was estimated by the method described by Bates *et al*. (1973) and expressed in μmole g^{-1} fresh weight.

Peroxidase activity in bolls (Ä OD at 430 nm min^{-1} g^{-1})

The peroxidase activity was determined in bolls at 10 DPA as described by Maxwell and Bateman (1967) and was expressed in Ä OD at 430 nm min^{-1} g^{-1}.

RESULTS AND DISCUSSION

Total sugar content (mg g^{-1})

The total sugar content was highly significant among the treatments and their interactions. Highest sugar content was observed in Bunny Bt (45.28) in control, while at 75 per cent of field capacity Bunny Bt showed highest sugar content (37.94). At 50 per cent of field capacity, Anjali recorded the highest (38.39). Anjali recorded lowest values in two treatments *i.e.* 38.47 at control, 32.52 at 75 per cent of field capacity. At 25 per cent of field capacity Pratima recorded lowest values (20.72) and Bunny Bt recorded the highest value (29.68) (Table 1).

Reducing sugar content (mg g^{-1})

Data on reducing sugar content did not differ significantly in

Table 1: Effect of various moisture levels on total sugar (mg g^{-1}), reducing sugar (mg g^{-1}) and non reducing sugar (mg g^{-1}) content in leaves

	Total sugar					Reducing sugar					Non reducing sugar			
Mean	Control		75% FC	50% FC	25% FC	Mean	Control	75% FC	50% FC	25%	Mean	Control	75% FC	50% FC	25%
Pratima	44.92	37.11	26.56	20.72	32.33	16.43	16.95	16.19	15.85	16.36	28.49	20.16	10.37	4.87	15.97
Anjali	38.47	32.52	38.39	28.62	34.5	15.74	16.15	15.73	15.28	15.73	22.73	16.37	22.66	13.34	18.77
Bunny Bt	45.28	37.94	30.93	29.68	35.96	16.81	17.1	16.56	16.29	16.69	28.47	20.84	14.37	13.39	19.27
Mean	42.89	35.85	31.96	26.34	33.33	16.33	16.73	16.16	15.81	16.26	26.56	19.12	15.8	10.53	18

	V	T	V XT	V	T	V XT	V	T	V XT
SE(d)	1.281	1.47	2.562	0.252	0.291	0.504	1.029	1.179	2.058
CD (0.05)	2.657	3.069	5.318	0.52	0.601	0.041	2.137	2.468	5.277

Table 2: Effect of various moisture levels on soluble protein (mg g^{-1}), proline content in leaves(μ moles g^{-1}) and activity of peroxidase in bolls 10 DPA (activityg-1 min-1 enzyme)

	Soluble Protein					Proline Content					Peroxidase activity				
	Control	75% FC	50% FC	25% FC	Mean	Control	75% FC	50% FC	25% FC	Mean	Control	75% FC	50% FC	25% FC	Mean
Pratima	20.66	19.77	18.91	18.46	19.45	0.12	0.17	0.21	0.28	0.19	2.35	2.83	4.15	5.18	3.63
Anjali	28.83	26.43	26.15	25.76	26.79	0.11	0.16	0.19	0.24	0.17	3.76	4.88	5.18	5.87	4.92
Bunny Bt	29.4	28.73	27.96	27.3	28.37	0.13	0.2	0.22	0.29	0.21	4.16	4.26	5.48	6.27	5.04
Mean	26.3	24.98	24.34	23.84	24.86	0.12	0.17	0.2	0.27	0.19	3.42	3.99	4.94	5.77	4.46

	V	T	V XT	V	T	V XT	V	T	V XT
SE(d)	0.908	1.874	2.555	0.008	0.009	0.016	0.146	0.169	0.292
CD(0.05)	1.049	2.165	2.95	0.017	0.019	0.034	0.303	0.35	0.607

treatments and their interactions but showed significant difference in varieties. The highest reducing sugar content was found in Bunny Bt (16.81) and lowest in Anjali (15.74) in 100 per cent field capacity. When moisture level was reduced from 100 per cent to 75 per cent of field capacity, highest reducing sugar content (17.10) recorded was exhibits by Bunny Bt, the lowest was 16.15 found in Anjali. Bunny Bt also showed highest reducing sugar content (16.56) when moisture reduction was at 50 per cent of field capacity, and Anjali was the one to represent lowest (15.73). At 25 per cent field capacity, Bunny Bt showed highest reducing sugar content (16.29) and Anjali showed lowest (15.28) reducing sugar content (Table 1).

Non reducing sugar content (mg g^{-1})

The non reducing sugar content was significantly differ among the treatments and their interactions but did not differ by significant in varieties. Pratima registered highest content of non reducing sugars as 28.48 and lowest was in Anjali as (22.73) in control. Bunny Bt(20.84) and Pratima (20.16) were the varieties having highest non reducing sugar content at 75 per cent of field capacity. At 25 per cent field capacity, Bunny Bt recorded highest reducing sugar (13.39) and Anjali recorded lowest reducing sugar (4.87) (Table 1).

Soluble protein content (mg g^{-1}

Data on soluble protein content showed significant difference in the varieties, the treatments as well as their interactions was not significant (Table 2). The highest soluble protein content was found in Bunny Bt (29.40) and lowest in Pratima (20.66) in control. Bunny Bt recorded highest soluble protein at 75 per cent of field capacity as well as at 50 per cent and 25 percent of field capacity i.e. 28.73, 27.96 and 27.30 respectively. Pratima recorded lowest soluble protein at 75 per cent of field capacity as well as at 50 per cent and 25 percent of field capacity i.e. 19.77, 18.91 and 18.46 respectively.

Proline content (μ moles g^{-1})

There were significant differences in Proline content among the treatments and varieties but not significant in their interactions. At all watering treatments Bunny Bt showed increase proline content 0.13, 0.20, 0.22, 0.29 and Anjali registered lowest proline content 0.11, 0.16, 0.19, 0.25with respect to control, 75 per cent, 50 per cent and 25 percent of field capacity respectively (Table 2).

Peroxidase activity in bolls at 10 DPA (ÄOD at 430 nm min^{-1} g^{-1}

Peroxidase activity showed significantly increased activity in all Cotton varieties under water stress. There were significant differences in peroxidase activity among the treatments, the varieties and their interactions. Highest value was observed in Bunny Bt4.16, 4.26, 5.48 and 6.27 increasing as that of the water content decreases that of the Pratima which is having lowest activity 2.35, 2.83, 4.15, 5.18 increases with respect to drought stress in control, 75 percent, 50 percent and 25 percent respectively.

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