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Research Article

RAPID DETERMINATION OF SOLUBLE PROTEIN CONTENT, NITRATE REDUCTASE ACTIVITY AND YIELD STUDIES IN COTTON GENOTYPES UNDER WATER STRESS

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ABSTRACT
Moisture deficits can depress cotton (*Gossypium spp* L.) in all cotton production regions. Water scarcity limits crop production and further expansion of agriculture in the world. In general, stress imposed at squaring seems to be much sensitive in terms of biochemical parameters studied. Soluble protein reduced under drought The Nitrate Reductase activity observed to increase when there was a stress. Soluble protein constitutes 40 percent of RUBP carboxylase (RUBP case), an enzyme responsible for CO_2 fixation in leaves of higher plants. RUBPCO activity reduced under drought. A study was conducted to determine the biochemical and yield responses to water stress in cotton. The experiment was conducted by adopting Factorial Randomized Block Design with three replications. The treatments comprised of water stress imposed at vegetative, squaring and boll development stages of crop growth. The plants submitted to stress suffered an decrease in the amount of soluble protein and yield content. The aim of this study was to evaluate and compare water stress effects on soluble protein, NRase and yield of cotton genotypes, as well as reveal which genotypes better adopts to water stress conditions using these parameters.

Key Words: *Nitrate Reductase Activity, Soluble Protein, Yield*

INTRODUCTION
Water stress is commonly attributed to situations where the water loss exceeds sufficient absorption intensity causing a decrease in plant water content, turgor reduction and, consequently,a decrease in cellular expansion and alterations of various essential biochemical processes that can effect growth or productivity. Diethelm and Shibles (1989) had opined that the Rubisco content per unit leaf area was positivity correlated with that of the soluble protein content. Drought causes reduction in ribulose-1, 5-biphosphate carboxylase / oxygenase (RUBPco) activity (Berkowitz and Wahlen, 1985; Pandy *et al*., 2000). During drought, quality of chloroplast protein decreased and electrophoretic spectrum of proteins changed in the tree plants. Many researchers have reported alterations in the functioning and speed of enzymatic activity, like amino acid synthesis (Andrews *et al*., 2004) and decrease in protein levels (Zhu and Xiong, 2002), as metabolically responses to water restrictions (Pimental, 2004). The nitrate reductase (NRase) is the rate limiting enzyme in nitrogen assimilation and is a key point of metabolic regulation (Eilrich and Hageman, 1973) in crops. Thus, NRase is intimately associated with the plant growth and development (Sinha and Nicholas, 1981). The decrease in NRase was accompanied by an increase in free amino acids and a decline in protein synthesis. The plants submitted to water stress suffered and decrease in the amounts of total protein casued by the decrease in their synthesis and a fall in nitrate reduction activity caused by the low nitrate flux were reported (Costa *et al*., 2008). Kaur and Singh (1992) found that flower number and percentage of boll abscission were decreased by water stress at flowering stage of cotton. Seed cotton yield decreased as the allowable water deficit increased (Cudrak and Reddel, 1988). Seed yield and yield components are severely affected by water deficit.

MATERIALS AND METHODS
The aim of this experiment was to investigate the responses caused by progressive water stress and the necessary time for have biochemical and physiological changes of *Gossipium spp*. during the vegetative,

squaring and boll development stages. For present investigation, twenty one genotypes including eight parents, four F_1 hybrids, five F_2's and four back crosses along with parents were subjected for genetic diversity analysis using physiological features. Field trials were conducted at Kharif 2008-2009 in the Department of Cotton, Centre for Plant Breeding and Genetics, TNAU, Coimbatore.

Treatments

1. T_1- Control
2. T_2- Stress at vegetative
3. T_3- Stress at squaring
4. T_4- Stress at boll development

Varietal Details:

Parents:

1. JKC 770
2. AS1
3. AS2
4. KC2
5. KC3
6. MCU 13
7. Suvin
8. Surabhi

F1 Hybrids:

1. AS1XSuvin
2. KC2XMCU 13
3. AS2XMCU13
4. KC2X JKC 770

F2's:

1. KC2XMCU 13
2. AS3XJKC 770
3. AS2XMCU 13
4. KC2XJKC 770
5. AS1XSuvin

Back Crosses:

1. (AS2XMCU13) XMCU13
2. (AS2XMCU13) X AS2
3. (KC2XMCU 13) X MCU 13
4. (KC2XMCU13) XKC2

Enzyme assay:

Soluble protein content: Soluble protein content of the leaf sample is a measure of indirect assessment of the photosynthetic efficiency of crop plants. The content of soluble protein was estimated from the leaf samples following the method of Lowry *et al.* (1951) and expressed as mg g^{-1} fresh weight.

Nitrate reductase activity: Nitrate reductase activity in the leaves was determined by adopting the method of Nicholas *et al.* (1976) and the enzyme activity was expressed as µg of NO_2^- g^{-1} hr^{-1}.

Yield parameters: At final harvest flower number, boll number and seed cotton yield per plant were determined.

Statistics: The data of three replications were statistically analyzed by Factorial completely randomized design.

RESULTS AND DISCUSSION

Drought stress adversely affects multiple physiological and biochemical pathways contributing to the growth and development, and ultimately yield of cotton. Although breeding programs have generally focused on yield as a cultivar selection tool, there exists potential for the development of stress specific screening tools for rapid identification of superior cotton cultivars. Water stress caused a steep decline in soluble protein content irrespective of stages and genotypes. The mean soluble protein content was found to be higher in KC 2 X MCU 13 at boll development stage. Among the F_1, F_2 and F_4 generations, KC 2 X MCU 13 has shown higher values irrespective of the treatment indicating that KC 2 X MCU 13 is fairly tolerant to drought situation than others. Drought induced decrease in RUBPCO activity should be attributed not only to proteolitic decomposition of enzyme protein but also to the partial inhibition of its catalytic activity, because decrease in RUBPCO activity was more than that in RUBPCO content (Chernyad'ev and Monakhova, 1998). Higher value of NRase activity was observed at the boll development stage for all the genotypes including control. Among the genotypes AS 2, KC 2, KC 2 X MCU 13 and KC 2 X JKC 770 (F_1, F_2 and F_4, respectively) have recorded the highest NRase activity at boll development stage (24.47, 23.33, 25.37, 24.08 and 22.97). NRase activity was more in the control than in stressed plants. The NRase, a substrate inducible enzyme, mediates conversion of nitrate to nitrite. The reduction in the activity might be either due to reduction in enzyme level (Bardzik *et al*., 1971) or due to the inactivation of the enzyme (Nicholas *et al*., 1976) caused by stress condition. Sivaramakirishnan *et al*. (1988) studied the midseason drought indicating that there is a sharp decline in NRase activity under water stress situation. NRase activity was found to be more in KC 2 and AS 2 which may be tolerant irrespective of the treatments. The stress imposed at squaring stage has shown a marked reduction in seed cotton yield when compared to the control. The seed cotton yield recorded as 128.99 in KC 2 X MCU 13 (F_2) irrespective of treatments. Significant differences were also observed between the genotypes, treatments and their interactions. The genotypes KC 2 and AS 2 have the highest value of seed cotton yield (120.28 and 110.22) than other genotypes at all stages irrespective of the treatmental effects. Yield was remarkably reduced when stress was imposed at squaring stage. Earlier report also indicated that the most critical phenophase for water stress in cotton is flowering (Singh and Sahay, 1992).

Table 1: Effect of drought on Soluble protein content (mg g^{-1}) and Nitrate Reductase activity (µg of $NO_2^- g^{-1} hr^{-1}$) at squaring stage of cotton in F_1, F_2, back crosses along with parents

Stages										
Genotypes	**Squaring**									
Parents	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
MCU 13	5.78	5.77	5.34	5.99	5.72	21.64	21.34	20.38	21.53	21.22
AS 2	6.46	6.32	6.33	6.23	6.34	21.62	22.43	21.45	20.54	21.51
JKC 770	6.53	6.78	5.98	6.81	6.53	19.44	20.21	19.35	18.42	19.36
KC 2	6.85	6.88	6.22	6.72	6.67	21.12	21.04	19.98	21.23	20.84
AS 1	5.44	5.64	5.12	5.58	5.45	17.71	17.84	17.46	15.67	17.17
Surabhi	5.26	5.32	5.01	5.34	5.23	17.73	18.43	17.82	16.44	17.61
KC 3	7.23	7.3	7.24	6.99	6.99	22.45	23.22	22.3	20.76	22.18
Suvin	7.68	6.88	6.58	7.44	7.15	16.29	16.15	15.27	15.52	15.81
F_1 Hybrids										
AS1 X Suvin	6.68	6.73	5.04	5.55	6.00	15.98	14.82	14.25	15.39	15.11
KC 2 X MCU 13	7.42	7.54	6.43	6.55	7.19	22.67	22.2	21.89	21.54	22.08
AS 2 X MCU 13	6.55	6.65	5.24	5.63	6.02	20.65	19.10	19.53	19.35	19.66
KC 2 X JKC 770	6.31	6.33	6.1	5.44	6.05	22.34	21.38	20.15	20.18	21.01
F_2'S										
KC 2 X MCU 13	6.76	6.34	5.54	5.57	6.05	22.1	22.33	21.89	22.34	22.17
AS 3 X JKC 770	6.45	6.52	5.35	5.39	5.93	22.99	21.76	20.16	21.28	21.55
AS 2 X MCU 13	6.11	7.02	4.98	5.42	5.88	19.20	19.56	18.21	19.20	19.04
KC 2 X JKC 770	5.87	5.98	4.29	5.49	5.54	19.92	18.2	18.82	18.76	18.93

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AS 1 X Suvin	5.95	5.51	4.63	5.04	5.28	18.29	17.66	17.01	17.17	17.53
Back Crosses										
(AS2XMCU13) X MCU13	6.34	6.44	6.23	6.32	6.33	17.89	19.10	18.80	17.39	18.30
(KC2XMCU13) X KC2	7.03	7.23	6.56	6.54	6.84	22.78	24.88	23.92	22.65	23.56
(AS2XMCU13) X AS2	6.45	6.66	6.34	6.21	6.42	21.10	22.98	21.76	22.10	21.99
(KC2XMCU13) X MCU13	6.54	6.69	6.22	6.05	6.38	21.36	20.72	20.65	21.39	21.54
Mean	6.46	6.50	5.75	6.18	7.47	20.25	20.25	19.57	19.47	19.91
	T		G		TXG	T	G		TXG	
SEd	0.137		0.060		0.275	0.849	1.947		3.894	
CD(P=0.05)	0.272		0.118		0.544	1.677	3.844		7.689	

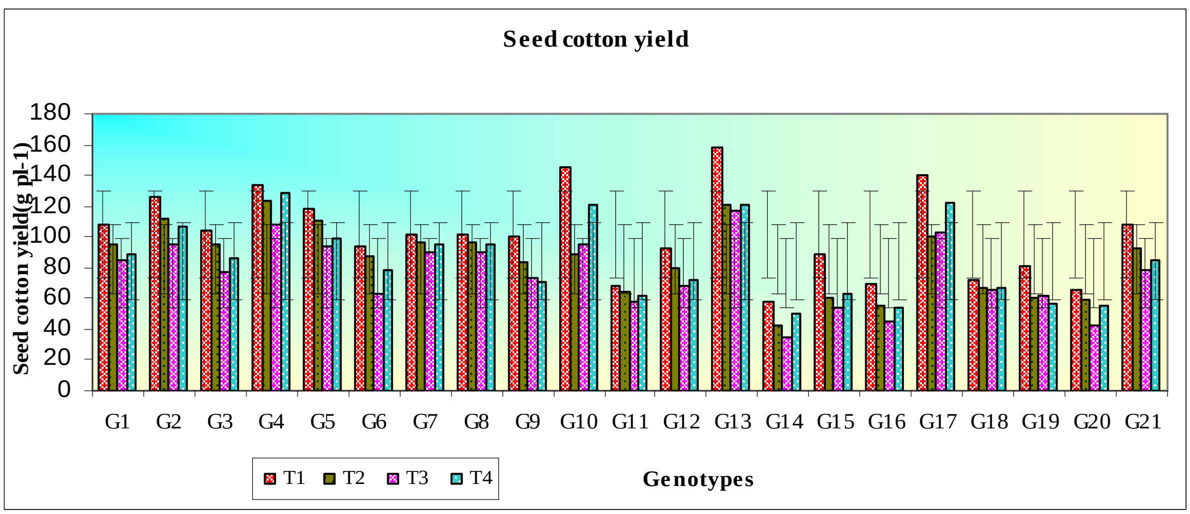

Figure 1: Effect of drought on yield components of cotton in F_1, F_2, back crosses along with parents

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Table 2. Effect of drought on yield components of cotton in F_1, F_2, back crosses along with parents

Stages											**Seed cotton yield (g pl^{-1})**				
Genotypes	**No of flowers per plant**					**No of bolls**									
Parents	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean	T_1	T_2	T_3	T_4	Mean
MCU 13	52.8	49.5	46.9	48.6	49.5	37.5	35.2	31.6	33.3	34.4	108.23	94.59	85.45	88.27	96.09
AS 2	45.7	44.4	41.2	42.5	43.5	33.4	21.1	25.9	27.2	26.9	126.43	112.34	94.82	107.29	110.22
JKC 770	38.3	26.1	22.6	24.1	27.8	21.0	20.8	17.3	18.8	19.5	103.68	95.48	77.29	86.3	90.69
KC 2	**73.2**	62.1	51.5	56.2	60.3	46.3	44.2	40.7	42.9	43.5	133.16	123.16	108.17	128.32	120.28
AS 1	57.6	51.3	46.6	49.4	51.2	40.3	35.7	33.4	37.5	36.7	118.63	110.35	93.76	99.59	105.58
Surabhi	44.2	42.6	41.3	43.4	42.6	38.7	32.4	28.7	30.5	32.6	93.6	86.9	62.9	78.34	80.44
KC 3	48.9	46.5	43.2	44.8	45.9	36.0	33.6	30.3	31.9	33.0	102	96.4	89.76	95.2	95.5
Suvin	59.6	55.5	52.5	54.2	54.8	45.6	42.2	36.6	37.3	42.9	102	96.4	89.76	95.2	95.5
F_1 Hybrids															
AS1 X Suvin	37.5	35.5	33.6	35	35.40	30.7	28.7	26.8	28.2	28.60	99.96	83.27	73.1	70.38	81.68
KC 2 X MCU 13	63.3	52.7	45.7	48.9	52.65	56.5	45.9	38.9	42.1	45.85	144.824	88.39	95.33	120.29	112.21
AS 2 X MCU 13	26.8	26	22.7	25	25.13	20.0	19.2	15.9	18.2	18.33	68.432	64.29	57.33	61.21	62.82
KC 2 X JKC 770	42.8	41.7	40.7	41.5	41.68	35.3	34.2	33.2	34.0	34.18	92.778	79.39	68.34	72.29	78.2
F_2'S															
KC 2 X MCU 13	55.3	49.6	46.1	48.2	49.8	47.8	42.1	38.6	40.7	42.30	157.973	121.23	116.38	120.38	128.99
AS 3 X JKC 770	33.8	32.3	28.9	31.2	31.55	26.3	24.8	21.4	23.7	24.05	58.045	42.66	35.34	49.72	46.44
AS 2 X MCU 13	34.5	33.1	29.3	31.2	32.03	27.0	25.6	21.8	23,7	24.80	89.11	60.72	54.22	63.54	68.01
KC 2 X JKC 770	36.1	35.2	31.7	32.9	33.98	26.7	25.8	22.3	23.5	24.58	69.09	55.28	45.23	54.46	56.25
AS 1 X Suvin	54.7	54.2	49.5	52.6	52.75	45.3	44.8	40.1	43.2	43.35	140.008	99.82	103.29	122.26	116.28
Back Crosses															
(AS2XMCU13) X MCU13	37.1	35.2	33.2	34.1	34.90	27.7	25.8	23.8	24.7	25.50	71.968	66.23	65.29	66.39	67.47
(KC2XMCU13) X KC2	24.4	23	21.2	21.6	22.55	15.0	13.6	11.8	12.2	13.15	81.263	60.38	62.28	57.19	65.28
(AS2XMCU13) X AS2	39.6	37.4	36.2	26.9	35.03	30.2	28	26.8	17.5	25.63	65.8	58.92	42.1	55.1	55.48
(KC2XMCU13) X MCU13	57.9	56.4	53.2	53.8	55.33	49.3	47.8	44.6	45.2	46.73	108	92.88	78.36	85.12	92.59
Mean	45.91	42.4	38.94	40.29	41.83	36.01	32.83	29.45	31.13	32.29	101.67	85.19	76.12	84.61	86.95
	T		G		TXG	T	G			TXG	T	G		TXG	
SEd	1.584		0.691		3.198	1.602	0.699			3.204	3.632	1.585		7.264	
CD(P=0.05)	3.127		1.365		6.255	3.163	1.380			6.327	7.171	3.129		14.342	

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