

## Soluble Protein, Nitrate Reductase Activity and Yield Responses in Cotton Genotypes under Water Stress

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**Abstract:** In general, stress imposed at squaring seems to be much sensitive in terms of biochemical parameters studied. **Background:** Soluble protein reduced under drought The NRase observed to increase when there was a stress. **Results:** Cotton genotypes possessing drought tolerance requires thorough understanding of physiological and yield component traits which directly or indirectly plays an important role in influencing the yield. If, traits for drought tolerance could be identified and contributing significantly to yield, they could prove as alternative selection criteria in yield improvement program. Soluble protein constitutes 40% of RuBP carboxylase (RuBP case), an enzyme responsible for CO<sub>2</sub> fixation in leaves of higher plants. RuBisCO 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. **Conclusion:** Evaluated and compared 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:** Soluble protein, nitrate reductase activity, 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 (RuBisCO) activity (Berkowitz and Wahlen, 1985; Pandey *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 (Xiong and Zhu, 2002), as metabolically responses to water restrictions (Pimentel, 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 Nrased 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 Reddell, 1988). Seed yield and yield components are severely affected by water deficit.

The objective of the study was to evaluate and compare water stress effects as well as to reveal that which genotypes better edopts water stress conditions using parameters.

### 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<sub>1</sub> hybrids, five F<sub>2</sub>'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:**

- T<sub>1</sub>: Control
- T<sub>2</sub>: Stress at vegetative
- T<sub>3</sub>: Stress at squaring
- T<sub>4</sub>: Stress at boll development

**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<sup>-1</sup> 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<sub>2</sub><sup>-</sup> g<sup>-1</sup> h<sup>-1</sup>.

**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×MCU 13 at boll development stage (Table 1). Among the F<sub>1</sub>, F<sub>2</sub> and F<sub>4</sub> generations, KC 2×MCU 13 has shown higher values irrespective of the treatment indicating that KC 2×MCU 13 is fairly tolerant to drought situation than others. Drought induced decrease in RuBisCO activity

Table 1: Effect of drought on Soluble protein content (mg g<sup>-1</sup>) and Nitrate Reductase activity (µg of NO<sub>2</sub><sup>-</sup> g<sup>-1</sup> hr<sup>-1</sup>) at squaring stage of cotton in F<sub>1</sub>, F<sub>2</sub>, back crosses along with parents

Genotypes	Stages									
	Squaring					Vegetative				
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	Mean	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	Mean
<b>Parents</b>										
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	22.30	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
<b>F<sub>1</sub> hybrids</b>										
AS1×Suvin	6.68	6.73	5.04	5.55	6.00	15.98	14.82	14.25	15.39	15.11
KC 2×MCU 13	7.42	7.54	6.43	6.55	7.19	22.67	22.20	21.89	21.54	22.08
AS 2×MCU 13	6.55	6.65	5.24	5.63	6.02	20.65	19.10	19.53	19.35	19.66
KC 2×JKC 770	6.31	6.33	6.1	5.44	6.05	22.34	21.38	20.15	20.18	21.01
<b>F<sub>2</sub> hybrids</b>										
KC 2×MCU 13	6.76	6.34	5.54	5.57	6.05	22.10	22.33	21.89	22.34	22.17
AS 3×JKC 770	6.45	6.52	5.35	5.39	5.93	22.99	21.76	20.16	21.28	21.55
AS 2×MCU 13	6.11	7.02	4.98	5.42	5.88	19.20	19.56	18.21	19.20	19.04
KC 2×JKC 770	5.87	5.98	4.29	5.49	5.54	19.92	18.20	18.82	18.76	18.93
AS 1×Suvin	5.95	5.51	4.63	5.04	5.28	18.29	17.66	17.01	17.17	17.53
<b>Back crosses</b>										
(AS2×MCU13)×MCU13	6.34	6.44	6.23	6.32	6.33	17.89	19.10	18.80	17.39	18.30
(KC2×MCU13)×KC2	7.03	7.23	6.56	6.54	6.84	22.78	24.88	23.92	22.65	23.56
(AS2×MCU13)×AS2	6.45	6.66	6.34	6.21	6.42	21.10	22.98	21.76	22.10	21.99
(KC2×MCU13)×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
Source of variation					Source of variation					
	T		G		T×G	T		G		T×G
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

Table 2: Effect of drought on yield components of cotton in F<sub>1</sub>, F<sub>2</sub>, back crosses along with parents

Genotypes	Yield parameters																	
	No. of flowers per plant					No. of bolls					Seed cotton yield (g plant <sup>-1</sup> )							
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	Mean	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	Mean	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	Mean			
<b>Parents</b>																		
MCU 13	52.8	49.5	46.9	48.6	49.50	37.5	35.2	31.6	33.3	34.40	108.23	94.59	85.45	88.27	96.09			
AS 2	45.7	44.4	41.2	42.5	43.50	33.4	21.1	25.9	27.2	26.90	126.43	112.34	94.82	107.29	110.22			
JKC 770	38.3	26.1	22.6	24.1	27.80	21.0	20.8	17.3	18.8	19.50	103.68	95.48	77.29	86.30	90.69			
KC 2	73.2	62.1	51.5	56.2	60.30	46.3	44.2	40.7	42.9	43.50	133.16	123.16	108.17	128.32	120.28			
AS 1	57.6	51.3	46.6	49.4	51.20	40.3	35.7	33.4	37.5	36.70	118.63	110.35	93.76	99.59	105.58			
Surabhi	44.2	42.6	41.3	43.4	42.60	38.7	32.4	28.7	30.5	32.60	93.6	86.90	62.90	78.34	80.44			
KC 3	48.9	46.5	43.2	44.8	45.90	36.0	33.6	30.3	31.9	33.00	102	96.40	89.76	95.20	95.50			
Suvin	59.6	55.5	52.5	54.2	54.80	45.6	42.2	36.6	37.3	42.90	102	96.40	89.76	95.20	95.50			
<b>F<sub>1</sub> hybrids</b>																		
AS1×Suvin	37.5	35.5	33.6	35.0	35.40	30.7	28.7	26.8	28.2	28.60	99.96	83.27	73.10	70.38	81.68			
KC 2×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×MCU 13	26.8	26.0	22.7	25.0	25.13	20.0	19.2	15.9	18.2	18.33	68.432	64.29	57.33	61.21	62.82			
KC 2×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	78.20			
<b>F<sub>2</sub> hybrids</b>																		
KC 2×MCU 13	55.3	49.6	46.1	48.2	49.80	47.8	42.1	38.6	40.7	42.30	157.973	121.23	116.38	120.38	128.99			
AS 3×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×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×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×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			
<b>Back crosses</b>																		
(AS2×MCU13)×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			
(KC2×MCU13)×KC2	24.4	23.0	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			
(AS2×MCU13)×AS2	39.6	37.4	36.2	26.9	35.03	30.2	28.0	26.8	17.5	25.63	65.8	58.92	42.10	55.10	55.48			
(KC2×MCU13)×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			
<b>Source of variation</b>																		
	T			G			T×G			T			G			T×G		
SED	1.584			0.691			3.198			1.602			0.699			3.204		
CD (p = 0.05)	3.127			1.365			6.255			3.163			1.380			6.327		
	7.171			3.129			14.342											

should be attributed not only to proteolytic decomposition of enzyme protein but also to the partial inhibition of its catalytic activity, because decrease in RuBisCO activity was more than that in RuBPisCO 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×MCU 13 and KC 2×JKC 770 (F<sub>1</sub>, F<sub>2</sub> and F<sub>4</sub>, 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. Sivaramakrishnan *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 (Table 2). The seed cotton yield recorded as 128.99 in KC 2×MCU 13 (F<sub>2</sub>)

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).

## REFERENCES

- Andrews, M., P.J. Lea, J.A. Raven and K. Lindsey, 2004. Can genetic manipulation of plant nitrogen assimilation enzymes result in increased crop yield and greater N-use efficiency? An assessment. *Ann. Applied Biol.*, 145: 25-40.
- Bardzik, J.M., H.V. Marsh Jr. and J.R. Havis, 1971. Effects of water stress on the activities of three enzymes in mize seedlings. *Plant Physiol.*, 47: 828-831.
- Berkowitz, G.A. and C. Wahlen, 1985. Leaf K<sup>+</sup> interaction with water stress inhibition of nonstomatal-controlled photosynthesis. *Plant Physiol.*, 79: 189-193.

- Chernyad'ev, I.I. and O.F. Monakhova, 1998. The activity and content of ribulose-1,5-bisphosphate carboxylase/oxygenase in wheat plants as affected by water stress and kartralin-4. *Photosynthetica*, 35: 603-610.
- Costa, R.C.L., A.K.S. Lobato, C.F.O. Neto, P.S.P. Maia, G.A.R. Alves and H.D. Laughinghouse, 2008. Biochemical and physiological responses in two *Vigna unguiculata* (L.) wulp cultivars under water stress. *J. Agron.*, 7: 98-101.
- Cudrak, A.J. and D.L. Reddell, 1988. A stimulus response model to predict crop yields due to water deficits. ASAE Paper No. 88-2514, American Society of Agricultural Engineers, St. Joseph, MI., USA., pp: 1-17.
- Diethelm, R. and R. Shibles, 1989. Relationship of enhanced sink demand with photosynthesis and amount and activity of ribulose 1,5-bisphosphate carboxylase in soybean leaves. *J. Plant Physiol.*, 134: 70-74.
- Eilrich, L.G. and R.H. Hageman, 1973. Nitrate reductase activity and its relationship to accumulation of vegetative and grain nitrogen in wheat (*Triticum aestivum* L.). *Crop Sci. J.*, 13: 59-66.
- Kaur, R. and O.S. Singh, 1992. Response of growth stages of cotton varieties to moisture stress. *Indian J. Plant Physiol.*, 35: 182-185.
- Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the *Folin phenol* reagent. *J. Biol. Chem.*, 193: 265-275.
- Nicholas, J.C., J.E. Harper and R.H. Hageman, 1976. Nitrate reductase activity in soybeans (*Glycine max* L. Merr.) I. Effects of light and temperature. *Plant Physiol.*, 58: 731-735.
- Pandey, D.M., C.L. Goswami, B. Kumar and S. Jain, 2000. Hormonal regulation of photosynthetic enzymes in cotton under water stress. *Photosynthetica*, 38: 403-407.
- Pimentel, C., 2004. The relationship of the plant with the water. EDUR, Seropedica.
- Singh, D. and R.K. Sahay, 1992. Drought stress in cotton: Identification of critical growth stages. *Plant Physiol. Biochem.*, 19: 55-57.
- Sinha, S.K. and J.D. Nicholas, 1981. Nitrate Reductase in Relation to Drought. In: *Physiology and Biochemistry of Drought Resistance*, Palag, L.G. and L.D. Aspinall (Eds.). Academic Press, Sydney, Australia.
- Sivaramakrishnan, S., V.Z. Parell, D.J. Flower and J.M. Peacock, 1988. Proline accumulation and nitrate reductase activity in contrasting sorghum lines during mid-season drought stress. *Physiol. Plant.*, 74: 418-426.
- Xiong, L. and J.K. Zhu, 2002. Molecular and genetic aspects of plant responses to osmotic stress. *Plant Cell Environ.*, 25: 131-139.