LEAF GROWTH AND ASSOCIATED PHYSIOLOGICAL CHANGES IN SIX CACAO ACCESSIONS UNDER WATER STRESS*

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ABSTRACT

Leaf growth rate was compared in seedlings of six accessions of cacao (*Theobroma cacao* L.) differing in their drought tolerant characteristics subjected to water stress. The leaf elongation rate was severely inhibited under drought but failed to indicate any positive relationship with their drought tolerance. Under stress, relative water content of leaf decreased, accompanied by an accumulation of free proline and reduction in nitrate reductase activity. This was more pronounced in drought-susceptible accessions. Epicuticular wax content was generally higher in drought tolerant accessions and did not change appreciably due to stress, while in susceptible accessions the wax content increased considerably. The specific leaf dry weight did not show significant changes.

INTRODUCTION

Cell growth and elongation are adversely affected due to water deficits. One of the first physiological responses to water stress to be affected is leaf elongation rate (Acevedo *et al.*, 1971; Hsiao *et al.*, 1970). Cacao is sensitive to water stress and various physiological parameters are affected (Balasimha, 1981). However, most of the metabolic changes occur only after the leaves are desiccated considerably (below –15 bars or 70 per cent RWC). This study examined the leaf growth rates under increasing stress conditions and associated physiological changes in six cacao accessions.

MATERIALS AND METHODS

Seedlings of six cacao (*Theobroma cacao* L.) accessions were raised in polyethylene bags containing 1:1 mixture of garden soil

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and farmyard manure, and 4-month old plants were used for the experiments. Leaf elongation rate was measured in first three young leaves in 6 replicates. All other determinations were done in 3 replicates. Water stress was induced by withholding irrigation.

For analyses, fully expanded leaves were sampled. Relative water content (RWC) was estimated by floating 1 cm^2 leaf discs in distilled water for 6 hr (Weatherely, 1950). Epicuticular wax was extracted in chloroform, evaporated to dryness at room temperature and determined gravimetrically. The *in vivo* assay procedures were employed for nitrate reductase (NR) activity with 0.1M KNO_2, 20 mM K-phosphate buffer and 0.25 per cent n-propanol (Jaworski, 1971). Proline was extracted in 3 per cent sulfosalicylic acid, centrifuged and supernatant used for estimation (Bates *et al.*, 1973).

Results and Discussion

There were differences in leaf elongation rates among the six cacao accessions under irrigated and stress environments (Table 1). The elongation rates did not show significant variations among 1st, 2nd and 3rd leaves. However, due to water stress the elongation was inhibited significantly. There was, however, no difference in elongation rates among accessions (NC 29, 31, 32 and 43) which possess some drought tolerant characteristics (Balasimha *et al.* 1982) as compared to susceptible ones (ICS 6 and IMC 67) under stress. Similar varietal differences in growth of leaf among annual crops have been reported (Acevedo *et al.* 1971; Boyer, 1970;, Parameshwara & Krishna Sastry, 1982). After rewatering, the leaf growth recovered after 4 days to prestress levels. The metabolic parameters like proline accumulation and reduction in NR activity changed only after irrigations were stopped for some time and when RWC reached below 70 per cent (Tables 2, 3; Fig. 1). It is apparent that leaf growth is much more sensitive to drought as compared to other parameters. There is increasing evidence for this contention (Hsiao *et al.*, 1970; Acevedo *et al.*, 1971; Boyer, 1970).

After 7 days of drought, the RWC decreased to a great extent in susceptible accessions (Table 2). This was associated with retention of high NR activity (Table 2) and low proline accumulation in drought tolerant accessions (Fig. 1). It is interesting to note that proline accumulated only when the plants were severely wilted and was negatively correlated with the relative water content ($r = -0.68$). As RWC was low in ICS 6 and IMC 67, proline content also was high. It is pertinent to view their relationship in the light of better utilization of metabolites like proline as leaf turgidity was maintained. Similarly, nitrate reduction could occur more efficiently because of higher energy pools available.

Table 1. Leaf elongation rates (cm/day)

Accession No.	Prestress	Stress (days)			Recovery*
		2	3	4	
			1st leaf		
ICS 6	2.92	1.92	1.56	0.80	1.00
IMC 67	2.02	2.15	0.78	1.00	1.50
NC 29	1.18	1.88	1.37	0.85	ND**
NC 31	1.33	1.45	1.18	0.75	1.70
NC 32	1.57	2.10	1.30	0.44	1.25
NC 42	1.89	1.70	1.35	0.63	1.15
		CD (P=0.05)=0.4295			
			2nd leaf		
ICS 6	2.44±0.91	2.00±0.86	1.32±1.27	1.05±1.84	1.40
IMC 67	2.32±0.28	2.87±2.04	1.63±2.09	1.12±1.13	2.10
NC 29	1.94±0.64	1.05±0.89	0.77±0.87	0.33±0.53	ND
NC 31	1.83±0.84	2.10±0.95	1.05±1.12	0.78±1.22	2.50
NC 32	2.61±0.83	1.48±1.12	1.35±1.35	0.95±0.88	2.70
NC 42	3.15±1.90	2.18±1.16	2.12±1.48	1.44±0.46	2.50
			3rd leaf		
ICS 6	3.35±1.10	0.35±0.21	0.25±0.35	0.23±0.40	4.40
IMC 67	2.54±0.63	1.85±1.77	0.55±0.47	0.30±0.38	2.50
NC 29	1.66±0.56	1.30±1.56	0.62±1.02	0.58±1.30	ND
NC 31	2.20±0.30	1.70±1.73	1.26±0.97	0.28±0.49	ND
NC 32	2.54±0.83	1.55±1.20	0.07±0.12	0.00	ND
NC 42	2.91±0.95	1.23±1.24	1.28±1.65	0.18±0.35	3.00

*4 days after rewatering

**ND, New flushing leaf not present for measurement

Table 2. Changes in relative water content (%) after 7 days' stress

Accession No.	Relative water content	
	Control	Stress
ICS 6	82.7	50.3
IMC 67	84.7	57.6
NC 29	84.7	69.5
NC 31	83.3	75.0
NC 32	82.7	71.3
NC 42	83.7	69.3

CD (P=0.05): Accessions 8.8; Treatments=3.6; Interactions=5.13

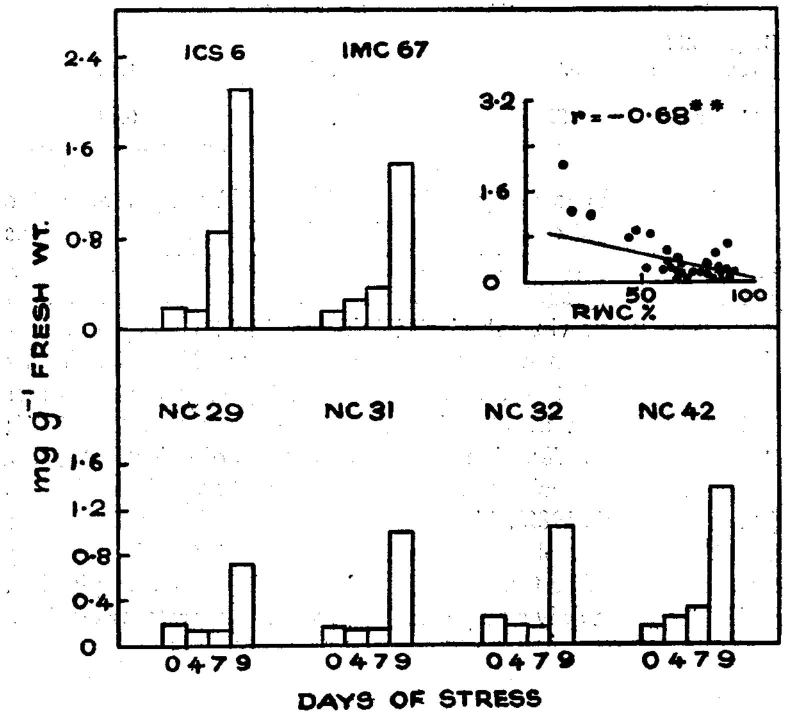

Fig. 1. Changes in proline content during stress; CD (P=0.05): Accessions =0.464 mg g^{-1} Treatments=0.184 mg g^{-1}. Inset shows the regression curve for proline content *vs* relative water content.

Table 3. Specific leaf weight, epicuticular wax content, and NR activity in leaves after 7 days' stress

Accession No.	Specific leaf weight (mg/cm^2)		Epicuticular wax ($\mu g/cm^2$)		NR activity $NO_2\ g^{-1}\ hr^{-1}$)	
	Control	Stress	Control	Stress	Control	Stress
ICS 6	3.37	3.70	149.5	197.5	8.27	3.27
IMC 67	4.25	4.20	56.7	198.7	7.60	3.33
NC 29	4.55	3.63	174.2	188.5	8.53	6.07
NC 31	3.48	3.52	203.3	164.6	8.00	5.93
NC 32	4.20	3.77	33.1	229.8	8.80	3.47
NC 42	3.48	3.77	153.9	151.6	6.93	6.27
CD(P=0.05)						
Accessions	0.24		38.04		1.18	
Treatments	NS		21.96		0.68	

The specific leaf dry weight varied significantly among the accessions but not due to stress. The epicuticular wax content was significantly higher in NC-29, 31, and 42 which did not show any changes on being subjected to water stress (Table 3). However, in other accessions, viz. ICS-6, IMC-67 and NC-32 there was considerable increase in wax contents during stress. Epicuticular wax plays an important role in the plant's ability to withstand water deficits, and is known to increase due to stress (Baker, 1974; Bengston *et al.*, 1978). In fact, higher contents of epicuticular wax content may be associated with drought tolerant accessions of cacao (Balasimha *et al.*, 1982). This increased wax deposition may provide an ability to the plants for reflecting sunlight, thereby reducing transpiration rather than a direct effect on cuticular transpiration, which may form only a very small portion of total water transpired.

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DISCUSSION

P. S. SREENIVASAN (Palghat): This study, I think, refers to soil drought. How far the findings will hold good for aerial drought? What is the type of proline accumulation, water content of leaf, wax content etc.?

D. BALASIMHA: This study was done with increasing soil drought conditions at atmospheric humidity. The crop response would change at varying humidity levels.

V. S. SHARMA (UPASI): While calculating any physiological character like drought, no single factor should be judged in isolation but in conjunction with several other factors responsible for the particular physiological function under study.

D. BALASIMHA: Yes, in fact I have made this point very clear in my paper that no single parameter should be evaluated while dealing with drought tolerance.

N. VASUDEVA (CCRI, Chickmagalur): Apart from proline, is there any other biochemical character responsible for drought tolerance/ resistance in coffee?

D. BALASIMHA: No single growth or metabolic parameter can be considered in isolation, but studied *in toto*. To my knowledge, RWC and NR activity have been shown to be influenced by drought in coffee.