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STOMATAL REGULATION AND ABA CONCENTRATION IN COCOA PLANTS DUE TO DROUGHT

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Changes in abscisic acid (ABA) content, stomatal resistance and related gas exchange characteristics of container grown six month old cocoa lines with contrasting drought sensitivities were studied. Accumulation of ABA during stress period was maximum in NC 42/94 followed by I-21 x NC 42/94 which was associated with higher stomatal resistance while susceptible lines had lower ABA content. There was a direct relationship between stomatal resistance and ABA content indicating that ABA regulates stomatal closure. Susceptible lines showed higher photosynthesis during non-stress when compared to other accessions. Decrease in photosynthesis was to a lesser extent in tolerant lines as compared to susceptible ones during stress period. Water potential values also showed differences among the lines studied. The initial chlorophyll fluorescence (Fo) values showed an increase during drought period. The Fv/Fm showed significant difference and the tolerant accessions maintained higher values during stress as compared to susceptible ones.

INTRODUCTION

Environmental factors influence stomatal responses thereby affecting the net photosynthesis in higher plants. The regulation of water loss by stomatal movements is one of the important traits for drought tolerance in cocoa (Balasimha *et al.,* 1988; 1991). Several reports indicate that abscisic acid (ABA) controls water loss in plants under drought conditions by regulating stomatal behaviour (Shen and Ho, 1997). Selections for drought tolerance can be made on the basis of ABA levels and their response to stress (Jones, 1978). This paper describes the relationship between stomatal resistance and ABA levels and the associated photosynthetic characteristics in cocoa plants with varying degree of drought sensitivity.

MATERIALS AND METHODS

Based on the earlier studies (Balasimha et *al.*, 1988), five cocoa accessions were selected; they are II-67, drought susceptible parent; NC 42/94, drought tolerant parent; I-21 x NC 42/94 and I-29 x NC 23/43, tolerant hybrids and II-67 x NC 29/66, a susceptible hybrid. Six months old cocoa plants were grown in polybags. Stomatal resistance and photosynthetic parameters were measured using L1-6200 portable photosynthetic system as described by Balasimha *et al.* (1991). Fully expanded, healthy, third or fourth leaf from distal portion was used. Two observations were recorded for each leaf and atleast three leaves were measured from each plant. All measurements were made between 10.00 and 12.00 h. Chlorophyll fluorescence indices were measured with Hansatech Plant Efficiency Analyser (Balasimha, 1992) while leaf water potential was determined with Scholander pressure chamber. ABA was quantified by indirect enzyme linked immunoabsorbant assay (ELISA). Leaf tissue (50 mg) was extracted in 80 per cent methanol containing 10 mg/l of BHT at 40 oC for 48 h (Shashidhar *et al.*, 1996). Polyclonal antibodies were raised against ABA adopting the procedure of Quarrie and Galfre (1985). Each well received 20-µg/ml goat anti-rabbit IgG solution in 50 mM $NaHCO_3$ with a pH 9.6. After one-hour assay at 37°C, absorbance was measured in ELISA reader at 405 nm.

RESULTS AND DISCUSSION

Accumulation of ABA during stress period was maximum in NC 42/94 followed by I-21 x NC 42/94 (Fig. 1). There was about 52 per cent increase in ABA levels due to stress. However, the levels decreased by 64 per cent during recovery period as compared to stress. Susceptible lines showed lower concentrations of ABA as compared to tolerant ones. Stomatal resistance showed a similar trend among the accessions. Hybrid, I-21 x NC 42/94 and parent NC 42/94 showed highest stomatal resistance during stress period (Table 1). After rewatering, the plants recovered and original values of water potential were attained. There was a direct relationship between stomatal resistance and ABA content fitting the equation, Y = a+bx; where Y is stomatal resistance, a = regression constant (-5.35), b = 0.57 and r = 0.837, significant at one per cent probability (Fig. 2). Considering 50 per cent as threshold level for stomatal resistance, ABA levels increased beyond 18 ng/g dry weight in cocoa. Reduced leaf water potential resulted in considerable increase in stomatal resistance and higher ABA content in

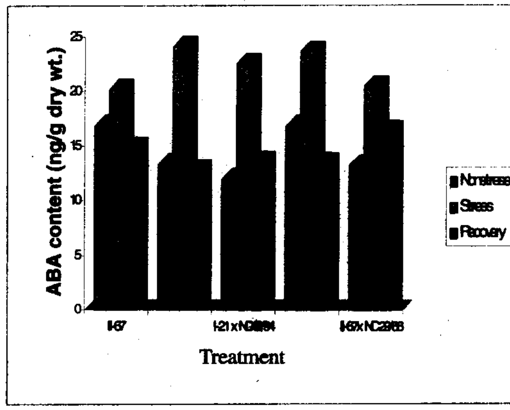

Fig. 1. ABA content in cocoa accessions

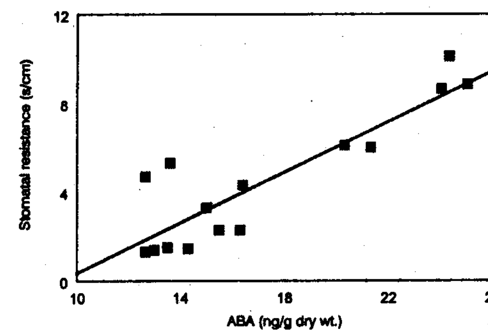

Fig. 2. Relationship between stomatal resistance and ABA concentration of cocoa leaves

Table 1. Physiological responses of cocoa accessions

Accessions	Stomatal resistance (S/cm)			Transpiration (M mole/m2/s)			Leaf water potential (-bars)		
	Non stress	Stress	Recovery	Non stress	Stress	Recovery	Non stress	Stress	Recovery
II - 67	2.207	6.073	3.327	5.78	1.59	4.49	7.60	16.50	11.33
NC 42/94	1.700	9.683	4.700	3.81	1.28	5.23	8.66	14.20	9.00
I - 21 X NC 42/94	1.507	10.003	4.954	5.12	1.60	5.04	8.36	14.70	10.05
I - 29 X NC 23/43	2.113	8.643	1.593	5.29	1.92	4.74	8.35	14.86	9.01
II - 67 X NC 29/66	1.527	6.140	4.523	5.54	1.35	4.47	9.00	16.13	10.93
C.D. at P = 0.05:									
Treatment		0.840			0.421			1.052	
Season		0.648			0.728			0.814	
Interaction		1.453			NS			NS	

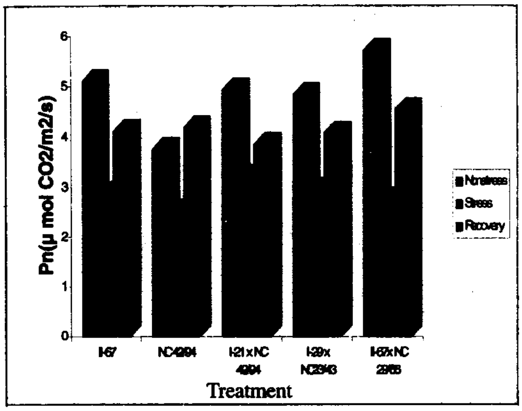

Fig. 3. Net photosynthesis in cocoa accessions

other plants (Ilahi and Dorffling, 1982). The high level of ABA was associated with drought tolerant cultivar of sorghum (Kannangara *et al.*, 1982).

Net photosynthesis (Pn) showed significant differences among the accessions (Fig. 3). Two susceptible accessions showed higher Pn during non-stress conditions as compared to others. Tolerant parent, NC 42/94 showed decrease in Pn during stress over pre-stress period as compared to susceptible accessions. Leaf water potential was higher in tolerant types during stress period as compared to susceptible ones (Table 1). Higher stomatal resistance resulted in lower transpiration rate in tolerant lines which is due to the higher leaf water potential. Similar results have been reported earlier (Jones, 1978; Balasimha *et al.*, 1988).

The initial values of chlorophyll fluorescence (Fo) showed an increase during stress period (Table 2). The Fv/Fm ratio is a quantitative measure of the potential photochemical efficiency of photosystem II and reliable indicator of quantum yield of photosynthetic oxygen evolution. The interaction between seasons and accessions were significant. During stress period the ratio was 0.555 to 0.608 in susceptible cocoa genotypes while it was higher (0.612-0.667) in tolerant accessions. It was evident from the results that tolerant accessions

Table 2. Chlorophyll fluorescence parameters of cocoa accession

Treatment	Non stress	Stress	Recovery	Non stress	Stress	Recovery
	Initial fluorescence			**Maximum fluorescence**		
II - 67	554	865	707	3130	1964	2386
NC 42/94	584	845	718	2650	2252	2291
I - 21 X NC 42/94	545	766	591	2480	2307	2514
I -. 29 X NC 23/43	646	770	541	2345	2109	2098
II - 67 X NC 29/66	604	790	593	2635	2129	2177
C.D. at P = 0.05:						
Treatment		NS			NS	
Season		52			289	
Interaction		104			NS	
	Variable fluorescence				**Fv/Fm ratio**	
II - 67	2609	1098	1714	0.823	0.55	0.665
NC 42/94	2093	1386	1573	0.786	0.612	0.678
I - 21 X NC 42/94	1934	1541	1946	0.774	0.667	0.766
I -. 29 X NC 23/43	1695	1338	1556	0.716	0.631	0.741
II - 67 X NC 29/66	2030	1364	1593	0.762	0.608	0.721
C.D. at P =0.05:						
Treatment		NS			NS	
Season		309			0.038	
Interaction		NS			0.086	

maintained better photochemical activity as compared to susceptible plants.

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