

Epicuticular waxes, lipids and membrane stability of cocoa trees in relation to drought tolerance*

Prakash G Bhatt, E V Daniel & D Balasimhat

Central Plantation Crops Research Institute, Regional Station, Vittal 574 243, India

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Higher leaf turgor was maintained in tolerant accession types of cocoa during dry periods which was accompanied by higher wax deposition. There were however no qualitative changes in wax composition between accession types. The lipid content decreased due to stress. Degree of unsaturation was lower, and total sterol/phospholipid ratio was higher with lower electrolytic leaching in tolerant accessions as compared to susceptible ones, especially under stress. All these changes in wax and lipid fractions help cocoa in better adaptation to drought by improving membrane stability.

Epicuticular wax (EW) increases due to stress and helps the plants to withstand drought^{1,2}. Water deficit decreases the polar lipids and increases neutral lipid fractions³. Cocoa, which is mostly cultivated in Southern India, experiences long period of drought from November to May. Work so far carried out has resulted in better understanding of drought tolerance in cocoa and an identification of few drought tolerant accessions⁴ which are characterised by thicker leaves, higher EW and efficient stomatal regulation reducing transpirational water loss⁵. This paper presents EW and lipid composition, membrane stability and fatty acid profiles with reference to drought tolerance in cocoa, (*Theobroma cacao* Linn.).

Materials and Methods

Cocoa accessions used in the present study were planted in 1975 at a spacing of 2.3 × 5.0 m under the shade of coconut palms. Five trees each belonging to tolerant (NC 23, NC 29, NC 31, NC 39, NC 42) and susceptible (NC 24, NC 30, NC 52, NC 55) groups were selected⁵. The stress in April was induced by withholding irrigations for 20 days which resulted in soil drought. Methods for collecting plant material, leaf samplings and separation of wax on TLC are described elsewhere⁴. Leaf water potential (Ψ) was measured using Scholander's pressure chamber (Soil Moisture Equipment Corp., USA).

Lipids were extracted from leaf samples initially with hot isopropanol and later with chloroform-methanol (2:1 v/v) mixture⁶. After concentration under vacuum (at 45-60°C), lipids were fractionated by sili-

cic acid chromatography⁷ and fractions again concentrated. Neutral lipid and wax were estimated by chromic acid method of organic carbon determination using stearic acid as standard⁸, phospholipids (PL) by estimating for inorganic phosphorus⁹ (Pi) and glycolipids (GL) by sugars content¹⁰. Using the relationship $PL = P_i \times 25$ and $GL = \text{sugars} \times 4.6$, the polar lipid contents were calculated. Total sterols were extracted after saponification and estimated by the Liebermann-Burchard reaction¹¹.

Lipid fractions were saponified with alcoholic potassium hydroxide to liberate fatty acids. Fatty acid methyl esters were analysed by gas chromatography (Shimadzu GC-6AM) fitted with flame ionization detector. Separation was achieved on steel column (1.5 m length and 3 mm internal diam.) packed with chromosorb W coated with 15% diethyleneglycol succinate. The column and injector temperatures were 190° and 230°C respectively. Flow of air, N₂ and H₂ were 11, 35 and 50 ml/min respectively. Retention time of the peaks were compared with authentic fatty acid methyl esters and quantified by calculating area under each peak.

For electrolytic leaching, 1 g leaf discs (1.5 cm) were taken in 20 ml distilled water after surface washing and kept at room temperature in dark. After 24 hr electrolytic conductivity was determined¹². Later, the samples were boiled for 15 min, cooled and conductivity determined again. Using these two readings percentage leaching was calculated¹².

Results and Discussion

The cocoa trees maintained higher Ψ during monsoon (June) as compared to summer period (Table 1). Further, the tolerant accessions maintained higher Ψ than susceptible ones under stressed condition. Wax

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† Present address: National Research Centre for Citrus Seminary Hills, Nagpur 440 006, India

‡ Correspondent author

accounted for 0.4-0.8% of total leaf dry weight. The contents increased due to stress and tolerant accessions showed 22 to 27% higher levels (Table 1). In rice, EW varied in drought tolerant upland cultivars¹³. Cocoa which is usually cultivated under partial shade of palms is not directly or completely exposed to sun. Nevertheless, because of its extreme sensitivity to atmospheric and soil droughts, the wax deposition increased. The higher levels of wax content in tolerant accessions may help cocoa plants to adapt to drought conditions.

Waxes contained hydrocarbons, esters, ketones, alcohols, free fatty acids and two unidentified components (Table 2). No qualitative differences were observed

between the accession types. Due to stress, however, hydrocarbons and free fatty acids increased whereas an unidentified component (R_f , 0.15), esters and alcohols decreased. Increased amount of hydrocarbons under stress has been reported in several annual crops^{1,14}. Glaucous plants of *Eucalyptus* and *Poa* have a high β -diketone content¹⁵.

Lipids constituted 0.62 to 1.04% of total leaf fresh weight. Water stress in April decreased total lipid content to an extent of 30% in tolerant and 38% in susceptible accessions (Table 3). While the polar lipids fractions decreased neutral lipid increased due to stress. The main fatty acids of the lipid fractions were myristic, palmitolic, oleic, linoleic and linolenic acids. The

Table 1—Seasonal changes in leaf water potential and EW content

[Values are means \pm SE of 5 trees]

Accession type	Seasons			
	June	December	April (unstressed)	April (stressed)
Water potential (- MPa)				
Tolerant	0.42 \pm 0.17	0.59 \pm 0.02	0.55 \pm 0.02	0.93 \pm 0.07
Susceptible	0.55 \pm 0.04	0.72 \pm 0.01	0.61 \pm 0.07	1.26 \pm 0.08
EW content (μ g/cm ²)				
Tolerant	17.63 \pm 2.03	24.78 \pm 1.32	33.79 \pm 1.53	38.66 \pm 0.54
Susceptible	12.65 \pm 0.79	18.01 \pm 1.1	24.88 \pm 1.49	31.66 \pm 0.84

Table 2—EW components as percentage of total wax

Wax composition	Tolerant		Susceptible		R_f value in benzene
	Unstressed	Stressed	Unstressed	Stressed	
Hydrocarbons	28.54	34.32	25.75	38.43	0.82
Esters	9.41	5.62	11.45	11.13	0.77
Ketones	2.33	6.60	9.70	2.01	0.61
Alcohols	41.42	26.38	25.55	23.33	0.27
Unidentified	12.95	7.95	8.80	4.01	0.15
Unidentified	0.64	1.00	2.93	3.01	0.08
Fatty acids	4.71	21.13	15.85	18.18	0

Table 3—Changes in lipids and membrane stability

Parameter	Tolerant		Susceptible	
	Unstressed	Stressed	Unstressed	Stressed
Total lipid (mg/g fresh wt)	10.46 \pm 2.38	7.39 \pm 0.97	9.97 \pm 1.41	6.27 \pm 0.92
Neutral lipid (%)	69.4	77.2	66.4	78.0
Glycolipid (%)	20.9	19.1	22.8	17.0
Phospholipid (%)	9.7	3.7	10.7	4.9
Total sterol (%)	3.5	4.4	3.6	4.8
Total sterol/phospholipid ratio	0.36	1.19	0.34	0.98
Electrolytic leaching (%)	15.56 \pm 1.20	20.19 \pm 1.69	17.96 \pm 1.87	27.66 \pm 2.36

other fatty acids present in smaller quantities were palmitic and stearic acids. Lauric acid was present only in neutral and glycolipid fractions in small quantity. Unsaturated fatty acids (USFA, mainly oleic, linolenic and palmitic acids) constituted 70-95% of total fatty acid of GL fraction. In response to water stress the USFA percentage decreased in all lipid fractions. The USFA/SFA ratio was 1.15 and 1.72 in tolerant and susceptible accessions respectively. It is interesting to note that this ratio is lower in case of the tolerant accessions offering better membrane stability against peroxidation. Ratio of total sterol to PL is known to be an indicator of membrane stability. This increased with water stress, being higher in tolerant accessions (Table 3). Susceptible accessions which showed higher degree of unsaturation also showed higher membrane electrolyte leaching (Table 3). Increased unsaturation affects the membrane packing by lowering the hydrophobic bonding which results in a more fluid lipid bilayer¹⁶. Thus, these changes in wax and lipid fractions increase membrane stability in cocoa leaves.

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