LEAF CHARACTERISTICS IN COCOA (*THEOBROMA CACAO* L.) ACCESSIONS

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Introduction

Among plantation crops cocoa is very sensitive to periods of drought stress. Several physiological processes are affected resulting in yield reductions over prolonged stress conditions. There are many leaf characteristics which could be related to drought tolerance (Parsons, 1979) and yield potentials (Barnes *et al.*, 1969). In order to obtain such information which could be valuable in further breeding programmes, we have determined the leaf morphology and some biochemical factors in forty cocoa accessions available in the germplasm collection.

Materials and methods

The Regional Station of the Central Plantation Crops Research Institute is located at Vittal in South Kanara District of Karnataka. The altitude of the station is 58 m above mean sea level; latitude 12.25° north and longitude 75.42° east. The soil is typically lateritic in nature. The nutrient status of soils (0-25 cm depth) is as follows : pH 5.3-5.6; organic carbon 0.7-1.1 %; total nitrogen 0.05-0.09 %; available P_2O_5 3.8-7.1 ppm; and available K_2O 35-85 ppm. The mean rainfall over last five years ranged from 3 500-4 000 mm annually distributed over one hundred and thirty days (April-October). There is usually a long dry period from December to April. Humidity ranges from *ca* 60 to 90 %, maximum temperature from 29° to 36 °C and minimum temperature from 20° to 25 °C during the year.

The cocoa accessions (*ca* 10-12 years old) used in the present study were cultivated at a spacing of 2.3 × 5.0 m under the shade of arecanut palm. Each tree was fertilized yearly with 100 g N : 40 g P_2O_5 : 140 g K_2O. The plots were irrigated once in a fortnight during dry periods of the year. Forty accessions of cocoa (*Theobroma cacao* L. var. *forastero*) were selected for the screening of leaf characteristics. The botanical and geographical origins of these accessions are detailed in table I, p. 96. Third to fifth leaves of the mature flush were sampled during July-August. Hand sections were taken with a sharp razor and examined under Olympus microscope and measurements recorded. Stomatal counts were taken from the epidermal peels. The specific leaf dry weight (SLW) was determined after oven drying to constant weight.

Epicuticular wax (EW) was extracted by dipping leaf in chloroform for 10 s, evaporated to dryness

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and determined gravimetrically. For qualitative analysis, the samples were plated on silica gel G plates (0.25 mm thickness) and developed in chloroform. The spots were visualised in iodine chamber. Cuticular transpiration was determined by the method described by Bengston *et al.* (1978) and nitrate reductase (NR) activity by *in vivo* procedures (Jaworski, 1971). The data were analysed statistically.

Results and discussion

There were significant differences in leaf characteristics among the forty accessions studied except the leaf area (tables I and II). One of the most sensitive processes to water stress is the leaf elongation rates. A reduction in leaf area might have an advantage in reducing transpirational rate but results in reduced yield through lowered photosynthetic area. There was no significant difference in leaf area in the present study which was done in well watered conditions. However, under water stress some of the accessions used in this study also showed variations in leaf expansion rates (Balasimha, 1982*a*).

The SLW was significantly different among the accessions. Some accessions having high SLW were also associated with high EW content. The accessions which are associated with high SLW and EW contents are reported to perform better under drought conditions, indicating the possible involvement of EW and SLW in the drought tolerance mechanisms. The high SLW has also been reported to be positively correlated with yield potential in annuals (Barnes *et al.*, 1969). The SLW can be a very good indicator of internal leaf anatomy and the results obtained clearly demonstrate its positive relation to leaf thickness, palisade layer thickness and wax contents. Although the stomatal frequency (table I) and size (191.6-271.4 μ^2) varied among the accessions, no positive relationship either with yield or drought tolerance was obtained.

The EW content (table I) and cuticular transpiration (table II) varied among the accessions. The EW content was highest in NC 23, NC 24, NC 29, NC 30 and NC 42 and was not necessarily associated with low cuticular transpiration as reported for oat cultivars (Bengston *et al.*, 1978). The first four « P » combinations are hybrids obtained between two Pound's collections which are known to perform better under drought conditions. In an earlier study it was also found that these hybrids of « P » combinations had high chlorophyll stability indicating heat tolerance (Ravindran and Menon, 1981). In CPCRI Vittal farm also we observed that leaves of these accessions remained turgid during severe drought in 1981 summer, while other accessions either showed wilting or wilting accompanied by yellowing symptoms. As the cuticular transpiration may account for less than 10 % of total water loss, the EW may play a rather indirect role by reflecting sunlight

TABLE I

Specific leaf dry weight, stomatal frequency, and epicuticular wax content in cocoa accessions

Accession	Stomatal frequency (n°/mm²)	SLW (mg/cm²)	EW (µg/cm²)
NC16 (P6xP6)*	92.3	7.6	..
NC20 (P4xP1)	95.3	7.4	98.2
NC21 (P176x719/5)	117.4	6.2	20.7
NC23 (P3xP)	93.9	7.8	383.5
NC24 (P6xP11A)	84.5	8.2	122.5
NC25 (T30/10xNa32)	114.6	8.5	12.1
NC29 (P6xP4)	94.9	7.4	369.6
NC30 (P3xP4)	122.0	7.3	642.3
NC31 (P12xP2)	91.7	7.0	57.5
NC32 (P9xP4)	92.2	6.9	42.8
NC34 (P9xP7)	135.5	7.2	92.8
NC36 (T85/5xNa32)	105.9	6.2	26.6
NC37 (P7xP6)	117.0	6.9	59.2
NC38 (P10xP1)	117.9	6.7	30.5
NC39 (T7/12)	99.4	7.4	54.8
NC40 (CW5/15/T63/884)	95.4	6.7	41.1
NC41 (T65/7)	95.8	7.0	32.9
NC42 (T86/2)	105.2	7.9	180.0
NC43 (W6/56/T63/9/10)	100.9	7.7	23.2
NC46 (W5/1/T63/884)	116.2	9.5	16.4
NC48 Unknown	112.8	6.7	22.6
NC51 (C44)	102.7	7.8	24.0
NC52 (C83)	101.7	6.6	27.8
NC53 (C76)	103.6	7.9	24.8
NC55 (T17/11)	116.2	7.3	41.3
NC56 (C42)	97.9	7.3	31.6
Na31	91.5	5.8	44.2
Na33	80.1	7.5	26.7
Na242	122.4	5.2	25.0
IMC10	97.6	6.9	23.4
IMC67	90.3	7.7	54.2
ICS1	90.2	5.9	50.8
ICS6	89.1	6.5	48.3
ICS89	94.7	7.9	58.3
ICS95	101.3	6.5	58.3
SCA6	112.2	5.2	37.5
SCA12	99.2	6.6	65.0
SIAL93	110.9	6.2	54.2
EET272	113.4	5.8	28.3
MOQ413	101.3	7.1	40.1
CD (P=0.05)	12.52	1.14	26.86

* CPCRI accession number (NC= Nigerian collection) showing parentage within parentheses,
"P" = Pound's collections.
Na - Nanay selections.
IMC - Iquitos Mixed Calabacillo.
ICS - Imperial College Selections.
SCA - Scavina populations.
SIAL - Seleção Instituto Agrônomico do Leste (Brazil).
EET - Estación Experimental Tropical (Pichilingue, Ecuador).
MOQ - Moquique selections (Ecuador).

TABLE II

Other leaf characteristics

Accession	Leaf area (cm^2)	Thickness (μ)	Palissade layer (μ)	Cuticular transpiration ($mg/cm^2/h$)	NR activity ($\mu M\ NO_2/g/h$)
NC20	292	135.3	35.6	0.51	15.6
NC23	299	142.5	38.1	0.61	9.6
NC24	332	144.4	28.5	0.31	12.2
NC29	318	156.8	42.3	0.59	12.0
NC30	349	133.0	34.1	0.64	13.3
NC31	292	142.0	36.4	0.28	9.2
NC32	319	130.6	35.6	0.29	4.8
NC34	382	122.5	33.9	0.43	9.8
NC37	358	123.6	34.3	0.28	9.4
NC38	335	130.7	34.6	0.51	4.0
NC39	317	118.6	36.3	0.45	4.2
NC42	308	161.8	45.4	0.29	10.5
ICS6	383	123.4	32.0	0.64	..
IMC67	332	128.1	33.2	0.31	..
Na31	382	121.8	33.3	0.32	..
CD(P=0.05)	NS	21.78	6.32	0.13	2.66

Correlation coefficients

Cuticular transpiration vs EW content = 0.53*
EW content vs leaf thickness = 0.59*
EW content vs palissade thickness = 0.65*
Palissade thickness vs leaf thickness = 0.94*

falling on leaf surface which could reduce leaf temperature and water loss.

The amount of wax was more or less equally distributed on both surfaces of cocoa leaf. However, there were no qualitative differences in the composition of waxes either among accessions or leaf surfaces (fig. 1).

There were wide significant variations in NR activity among accessions, highest being in NC 20, NC 24, NC 29 and NC 30. Genotypic differences in NR activity is reported in several other crops (Balasimha *et al.*, 1980; Reed and Hageman, 1980; Sinha *et al.*, 1974). However, NR activity is modulated by various internal and external factors like environmental stresses. It has been shown that cocoa NR activity shows a seasonal trend (Balasimha, 1982*b*) and is altered by water stress which is again a varietal character (Balasimha, 1982*a*). It is concluded that no single character can be employed in screening for drought tolerance in cocoa. Further, it is necessary to assess these characters under drought conditions and compare them with plants receiving adequate water. The study, however, reveals that using 2-3 morphological characteristics like SLW and EW content, it is possible to screen out over 80 % of the accessions in the preliminary screening programmes. The remaining can be evaluated in greater detail using biochemical parameters and field testing.

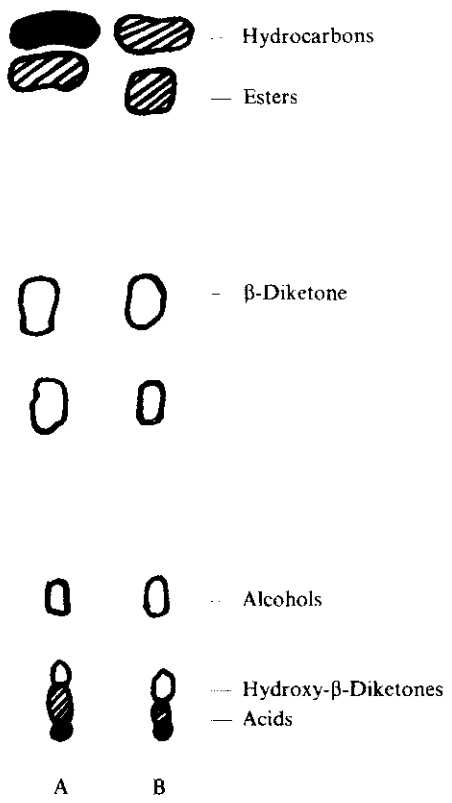

Fig. 1. - Qualitative distribution of wax components on lower (A) and upper (B) epidermis of cocoa leaf

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BALASIMHA (D.), SUBRAMONIAN (N.), CHENCHU SUBBAIAH (C.). — **Caractéristiques foliaires de cacaoyers (*Theobroma cacao* L.) acquis.** *Café Cacao Thé* (Paris), vol. XXIX, n° 2, avril-juin 1985, p. 95-98, 1 fig., 2 tabl., 10 réf.

Des cacaoyers (*Theobroma cacao* L.) acquis par la Station Régionale de l'Institut Central de Recherche sur les Plantes Cultivées (Inde) ont été sélectionnés pour leurs caractéristiques morphologiques et biochimiques. Des différences significatives, tout particulièrement en ce qui concerne le poids foliaire spécifique et la teneur en cire épicuticulaire, ont été notées parmi les caractéristiques foliaires des cacaoyers acquis. Il est possible de sélectionner 80 % du matériel mis à l'épreuve pour la tolérance à la sécheresse chez le cacaoyer en utilisant quelques-uns de ces caractères faciles à évaluer sur une grande échelle.

BALASIMHA (D.), SUBRAMONIAN (N.), CHENCHU SUBBAIAH (C.). **Leaf characteristics in cocoa (*Theobroma cacao* L.) accessions.** *Café Cacao Thé* (Paris), vol. XXIX, n° 2, avril-juin 1985, p. 95-98, 1 fig., 2 tabl., 10 réf.

Cocoa (*Theobroma cacao* L.) accessions of the Regional Station of the Central Plantation Crops Research Institute (India) were screened for leaf morphological and biochemical characteristics. There were significant differences in leaf characteristics among accessions ; noteworthy being specific leaf weight and epicuticular wax content. It is possible to screen out 80 % of test material for drought tolerance in cocoa by employing some of these characters which are easy to determine on a large scale.

BALASIMHA (D.), SUBRAMONIAN (N.), CHENCHU SUBBAIAH (C.). — **Blatteigenschaften erworbener Kakaobäume (*Theobroma cacao* L.)** *Café Cacao Thé* (Paris), vol. XXIX, n° 2, avril-juin 1985, p. 95-98, 1 fig., 2 tabl., 10 réf.

Von der regionalen Station des Zentralinstituts der Forschung der angebauten Pflanzen (Indien) erworbene Kakaobäume (*Theobroma cacao* L.) wurden nach ihren morphologischen und biochemischen Eigenschaften selektioniert. Signifikante Unterschiede wurden bei den erworbenen Kakaobäumen insbesondere betreffend das spez. Gewicht der Blätter und ihren Wachsgehalt festgestellt. Wenn einige dieser leicht feststellbaren Eigenschaften benützt werden, kann man 80 % des untersuchten Materials betreffend ihrer Toleranz der Trockenheit gegenüber selektionieren.

BALASIMHA (D.), SUBRAMONIAN (N.), CHENCHU SUBBAIAH (C.). — **Características foliares de árboles del cacao (*Theobroma cacao* L.) adquiridos.** *Café Cacao Thé* (Paris), vol. XXIX, n° 2, avril-juin 1985, p. 95-98, 1 fig., 2 tabl., 10 réf.

Se ha procedido a la selección de árboles del cacao (*Theobroma cacao* L.), adquiridos por la Estación Regional del Instituto Central de Investigaciones acerca de las Plantas Cultivadas (India), en consideración de sus características morfológicas y bioquímicas. Entre las características foliares de los ejemplares de cacao adquiridos se han señalado diferencias significativas, y concretamente por lo que respecta al peso foliar específico y el contenido de cera epicuticular. Existe la posibilidad de seleccionar un 80 % del material sometido a prueba para la tolerancia a la sequía en el caso del cacao, utilizando para ello algunos de estos caracteres de fácil evaluación cuando se procede a gran escala.