Use of botanical descriptors for cocoa characterization: CRU experiences

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Efficient management of a genebank such as the International Cocoa Genebank, Trinidad (ICG,T) requires accurate identification of accessions and collation of appropriate characterization data along with passport and evaluation data. This is recognized in the Cocoa Research Unit (CRU), Trinidad and thus, in 1990, a programme was initiated to accumulate morphological data using the standardized, International Board for Plant Genetic Resources (IBPGR) descriptors. Nearly 3000 accessions are now housed in the ICG,T and the process of morphological characterization is prolonged by the use of an exhaustive list of 65 descriptors. Efforts are underway to develop a concise list for use in the ICG,T. It will consist of the most discriminative or taxonomically useful descriptors, which are easily measured and heritable. Agro-economically important descriptors such as butterfat content and quality and bean weight will also be included. Fifteen taxonomically useful quantitative descriptors have been identified using multivariate techniques and their heritabilities along with those of useful qualitative descriptors will be estimated subsequently. The concise list of descriptors should be adopted once this is done.

Keywords: *Theobroma cacao* L., morphological characterization, descriptor list

Introduction

To facilitate the efficient management of the International Cocoa Genebank, Trinidad (ICG,T) and the research of its scientists, the Cocoa Research Unit embarked on a project to collate morphological characterization data for the 2412 accessions. The accrued data are intended for international circulation and have therefore been incorporated into the International Cocoa Germplasm Database (ICGD).

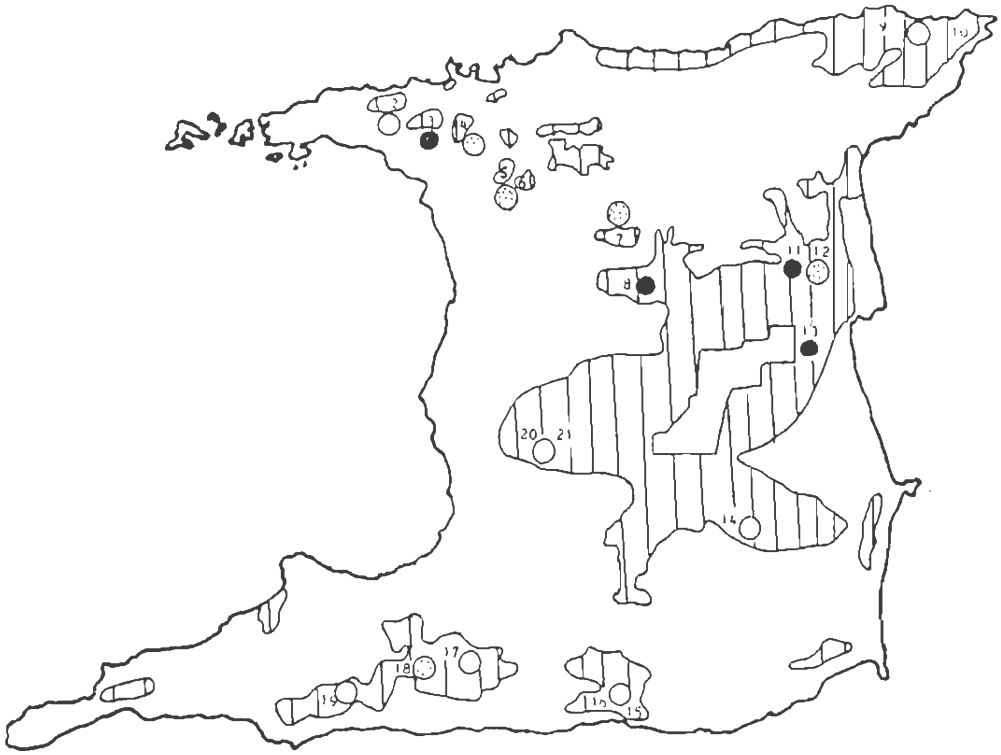
Botanical description of cocoa germplasm was conducted in the Cocoa Research Unit from as early as 1930, when the Unit was part of the Imperial College of Tropical Agriculture. Dr. F.J. Pound described the fruit morphology of the selected ICS accessions and this is documented in the 1933-1935 Annual Reports of the Cocoa Research Unit.

In the 1960s and 1970s, research scientists of the CRU recorded pod weight data along with other performance data for progenies of the River Estate (Diego Martin) and Las Hermanas (Figure 1) trials. The data were tabulated in the Annual Reports of the Cocoa Research Unit for those years.

In 1983, preliminary work was done to facilitate the collation of morphological data for the contents of the ICG,T. In a two year project, data were collated for 53 accessions representing six families (populations).

The process of data collection was continued after 1985 and involved mainly accessions situated at Marper Estate, St. Augustine Campus and San Juan Estate.

Figure 1 Cocoa growing areas in Trinidad



1. RIVER ESTATE ☒
2. BLUE BASIN
3. THE SADDLE
4. SANTA CRUZ
5. ST. JOSEPH ☒
6. ST. AUGUSTINE ☒
7. LA REUNION ☒
8. LAS HERMANAS ☒
9. SANS SOUCI
10. TOCO
11. EL REPOSO
12. NON PAREIL
13. MARPER ☒
14. RIO CLARO
15. MARAC
16. GRAND CHEMIN
17. PENAL
18. QUARRY VILLAGE
19. PALO SECO
20. GRAN COUVA
21. PEPPER VILLAGE

The morphological characterization project started in 1990 involves recently planted material from one site, viz., the University Cocoa Research Station (UCRS). Thus far, nearly 10% of the collection at the UCRS has been characterized using leaf and flower descriptors. The compilation of fruit descriptor data has lagged because of efforts to mechanize the process of mucilage removal, which has now been achieved. With augmented staff and facilities, it is hoped that the process of data accumulation will be markedly accelerated.

The fact that a lengthy descriptor list (65 standardized descriptors) recommended by the International Board for Plant Genetic Resources (IBPGR) (IBPGR, 1981 ACP: IBPGR/80/56) is employed in charaterization makes data recording rather tedious. The adoption of a concise descriptor list would reduce the time required to accumulate the data and concerted efforts are underway to develop such a list for use in the ICG,T. This list will consist of about 15 heritable, easily measured, discriminative characters, including some of interest to cocoa breeders internationally. The environmental stability of the descriptors will be taken into consideration. Descriptors of agro-economic importance such as pod and bean weight, bean number, butterfat content and quality will also be included.

The work is ongoing, but the results of the 1983-1985 project, which was designed to develop a concise descriptor list, will be useful and are outlined hereunder.

Materials and Methods

Several multivariate techniques were employed to determine the descriptive values of the IBPGR-recommended descriptors, using germplasm in the ICG,T. Data were collated between 1983 and 1985 at two sites in north Trinidad; the St. Augustine Campus and the St. Joseph Field Station (Figure 1). Thirty-one accessions were studied at St. Joseph and 53 at St. Augustine (20 being common to both sites). The populations represent the acknowledged centres of diversity of cocoa (Table 1).

Table 1 Populations included in the study

POPULATION	DESCRIPTION	NUMBER OF CLONES STUDIED
ICS	Trinidadian trinitario	16
IMC	Peruvian forastero	21
SPA	Colombian forastero	3
SCA	Amazonian forastero	6
E	Ecuadorian forastero	1
EET	Ecuadorian hybrids	6

Eleven leaf, 24 flower and 33 fruit characters were recorded from trees 21 to 25 years old. At both sites, the trees were distributed over a series of blocks or fields and each accession was replicated at least three times, but randomization only existed at St. Augustine.

To allow for the effects of season and time of collection on the expression of descriptors, data were collected during the same season for each group of descriptors. Leaf and flower data were collected throughout the rainy season and fruit data were collected during the main and light harvest periods, which occur during the dry season (January-June).

Data from three trees per accession were pooled to make up required sample sizes since Engels *et al.* (1980) found that for flower and fruit characters there are no significant differences between trees of the same clone. The characters recorded are listed in Table 2.

Table 2 Descriptors used in characterization

PLANT ORGAN	DESCRIPTORS		
Leaf	Total length	Basal length	Width
	Apical angle	Basal angle	Basal angle
	Basal: apical angle	Basal shape	Pulvinus size
	Flush colour	Apical shape	Total leaf length : width
Flower	Sepal width	Sepal length	Petal length
	Ligule length	Ligule width	Staminode length
	Ovary length	Ovary diameter	Ovule number
	Style length	Pedicel length	Bud colour
	Sepal colour	Sepal reflexion	Presence of glandular hairs
	Ligule colour	Ribbon colour	Theca colour
	Pigmentation at ovary base	Pigmentation of apex of ovary	Filament colour
	Colour at the base of the style	Anther disposition	Pedicel column colour
Fruit	Pod length	Pod width	Pod wall thickness
	Mesocarp thickness	Primary furrow depth	Secondary furrow depth
	Pod weight	Placenta weight	Wet bean weight
	Husk weight	Washed bean weight	Mucilage weight
	Bean number	Total dried bean weight	Total dried peeled bean weight
	Seed coat weight	Bean weight	Bean length
	Bean width	Bean thickness	Cotyledon colour
	Bean shape	Husk hardness	Pod apex form
	Pod basal constriction	Pod furrow pigmentation	Pod size
	Pod shape	Pod furrow disposition	Pod furrow separation
	Pod ridge colour	Pod surface texture	Pulp colour

Fifteen to twenty sound, fully expanded leaves free from insect damage and disease symptoms were randomly collected from two leaf nodes below the flush (from top to bottom) of mature plagiotropic branches, and were measured.

Disease-free flowers with pearl-coloured thecae were collected randomly from the trees and measured. The number of ovules per ovary was counted according to the method prescribed by Lucas (Lucas and Reffye, 1981). The ovaries were placed on a slide, covered with a drop of blue-black ink and the slide was then covered with another slide. In this way, the ovaries were squashed and the ovules could be detected under a binocular compound microscope as white globules on a bluish background.

The method for characterizing the fruit was developed by Toxopeus (pers. comm.). Four healthy, mature pods per accession were randomly selected, harvested, observed and measured.

An assessment of intraclonal versus interclonal variability of quantitative cocoa descriptors was achieved using the Analysis of Variance (ANOVA) Procedure of SAS Version 82.3. A normal distribution of the data was assumed. The model tested was that any variation in the expression of individual descriptors is due to genetic effects, viz. those of accession (clone) or family and is expressed by the following equation:

$$y_{ik} = \mu + d_i + \epsilon_{ik} \qquad 1.1$$

where y_{ik} is the observation corresponding to the k^{th} replicate

of the i^{th} genotype, μ is the general mean, d_i is the i^{th}

genotype effect and ϵ_{ik} is the random component associated with

the k^{th} replicate of the i^{th} genotype.

The variation in a given variable (descriptor) was attributed to genetic variation. Descriptors with high interclonal and low intraclonal variability were considered more discriminative.

Principal Component Analysis was used to identify a subset of descriptors that can effectively describe the accessions.

The discovery of less reliable descriptors correlated with more reliable ones could lead to a more concise, but still taxonomically valid descriptor list. Thus Correlation Analysis was performed using the mean values. The correlation coefficients were calculated using the correlation procedure of SAS Version 82.3. Separate analyses were performed using leaf, flower and fruit descriptors.

Hierarchical average-linkage Cluster Analysis was employed to test how well subsets of descriptors grouped or classified the accessions. The relationships among 53 accessions observed at the St. Augustine Campus were investigated using 68 quantitative descriptors. Then an attempt was made to determine a subset of descriptors that could also do this effectively. This subset would contain the most useful descriptors, which would be recommended for inclusion in a concise descriptor list. The investigation would reveal whether geographic origin was reflected by the genetic relationships between the accessions studied or whether the accessions from the Amazon Basin represented a single population of *Theobroma cacao* L.

The absence of distinct groupings in the germplasm under study would support the latter hypothesis. It has been suggested that the clones under investigation belong to six populations (Table 1). Five of these, represented by thirty-seven clones, originated in the Upper Amazon region, the reputed "centre of genetic diversity" of cacao (Cheesman, 1944).

The clustering procedure involved the calculation of a similarity measure between all the pairs of objects in the study. Individuals or groups were fused when their average similarity was greatest. The clusters became more and more inclusive as the similarity level dropped and the procedure stopped when all the objects formed a single cluster.

Results and conclusions

Interclonal versus intraclonal variability

Almost all the variables studied were found to show high interclonal and interfamilial variation. Intraclonal variation was not significant. This was inferred from the F values with significance probabilities which were less than or equal to 0.0001 in all but two cases. The latter involved the interfamilial variation for ovary diameter and washed bean weight, whose F values had significance probabilities of 0.02 and 0.001, respectively.

The ANOVA model accounted best for the variation in expression of flower characters, as compared to that of the fruit and leaf. The R^2 values for the flower and fruit characters were greater than 0.5, in all but one case (ovary diameter). The R^2 values for the leaf descriptors were less than 0.5 except in the case of apical and basal angles (Table 3).

The measure of variation within the populations for all the variables was relatively low (the coefficient of variation (c.v.) was less than 40 %). However, the c.v. was higher for leaf descriptors compared to flower descriptors as was found by Enriquez and Soria (1967a and b) and Asomaning and Lockard (1963) and significantly higher for the fruit descriptors. Thirteen out of 15 of the latter variables had coefficients of variation greater than 10.0. Placenta weight had the highest c.v. (37.7 %).

According to Enriquez and Soria (1967a), a c.v. of 10 is acceptable for a character to be used confidently in description. Leaf descriptors with their high R^2 values appear to be affected by factors other than genotype such as environment or genotype-environment interaction and their descriptive value is diminished. Fruit descriptors were very variable within the clones under study and this detracts from their descriptive value. Based on these analyses, bean and pod length and width are more reliable compared to the other fruit descriptors.

The flower descriptors appear to be of high taxonomic value. This was also found by Ostendorf (1957), Enriquez and Soria (1967a) and Engels (1981). Style, staminode and guideline lengths are particularly reliable, as is ovule number, which according to Lopez and Enriquez (1988) is a better indicator of yield and is not significantly affected by environment. Compared to fruit characterization, that of the flower is easier and less time-consuming.

Table 3 ANOVA Results

Descriptor (units)	Mean (s.e.)	Degrees of freedom	F	R^2	C.V.
Total leaf length (cm)	24.8 (2.5)	54	9.5	0.40	9.9
Basal leaf length (cm)	11.9 (2.3)	54	4.8	0.25	19.6
Leaf width (cm)	8.8 (1.0)	54	6.5	0.31	11.2
Leaf length : width ratio	2.9 (0.2)	54	9.7	0.40	8.4
Leaf apical angle (°)	62.8 (6.0)	54	16.1	0.53	9.5
Leaf basal angle (°)	105.2 (8.3)	54	70.7	0.83	7.9
Sepal width (mm)	2.2 (0.16)	52	51.9	0.64	7.2
Sepal length (mm)	7.7 (0.34)	52	97.8	0.76	4.5
Ligule length (mm)	3.0 (0.21)	52	43.9	0.75	7.2
Ligule width (mm)	2.3 (0.23)	52	29.9	0.68	9.9
Guideline length (mm)	2.4 (0.21)	52	54.8	0.79	8.8
Ribbon length (mm)	3.1 (0.23)	52	42.8	0.79	7.3
Staminode length (mm)	5.7 (0.28)	52	54.0	0.79	4.9
Ovary length (mm)	1.8 (0.14)	52	27.6	0.66	7.6
Ovary diameter (mm)	1.0 (0.08)	52	5.6	0.28	7.5
Ovule number	41.4 (3.9)	52	24.5	0.67	9.6
Style length (mm)	2.1 (0.14)	52	24.3	0.63	6.6
Pedicel length (mm)	14.5 (1.4)	52	35.2	0.54	9.5
Pod length (cm)	16.9 (1.7)	54	9.4	0.75	9.8
Pod width (cm)	8.2 (0.54)	54	10.7	0.78	6.5
Pod wall thickness (mm)	12.3 (1.14)	54	27.1	0.89	9.3
Primary furrow depth (mm)	6.3 (0.77)	54	42.0	0.94	12.2
Secondary furrow depth (mm)	4.3 (0.6)	54	53.0	0.95	13.9
Pod weight (g)	481.6 (113.0)	54	7.4	0.71	23.5
Placenta weight (g)	8.9 (3.3)	54	7.9	0.72	37.7
Wet bean weight (g)	118.6 (27.4)	54	7.1	0.69	23.1
Husk weight (g)	355.6 (85.2)	54	9.0	0.75	23.9
Washed bean weight (g)	91.9 (31.3)	54	3.0	0.49	34.1
Mucilage weight (g)	27.4 (9.9)	54	9.7	0.76	36.5
Bean number	42.4 (8.1)	54	3.6	0.54	19.0
Bean weight (g)	1.1 (0.18)	54	243.9	0.75	17.1
Bean length (cm)	2.2 (0.16)	54	156.5	0.66	7.4
Bean width (cm)	1.1 (0.13)	54	150.2	0.67	11.6
Bean thickness (cm)	0.6 (0.09)	54	100.3	0.59	15.3

Determining subsets of discriminative descriptors

There was a limitation in using Principal Component Analysis to reveal a small subset of reliable quantitative descriptors. The first principal components did not comprise more than 40% of the total variance expressed and at least 75% of the total variance should be represented by the first two components if the sample information is to be represented accurately in two dimensions.

However, a criterion for reducing the number of variables was applied and this involved finding the eigenvectors corresponding to the smallest eigenvalues and rejecting the variables with the largest coefficients (absolute values). The choice of retained variables was made realistic by selecting lambda$_0$ (λ_0), a threshold value, so that eigenvalues less than it could be regarded as contributing too little to the data. When a λ_0 of 1.2 was used, the following variables (Table 4) were discarded:

total dried bean weight
husk weight
leaf basal angle : leaf apical angle
pod wall thickness
pod width
total wet bean weight
secondary furrow depth
mucilage weight
mesocarp thickness

Table 4 Results of Principal Component Analysis

Descriptor (variable) discarded	Eigenvalue
Total dried bean weight	0.0001
Husk weight	0.003
Leaf basal angle : apical angle	0.004
Pod wall thickness	0.011
Pod width	0.029
Wet bean weight	0.033
Secondary furrow depth	0.054
Mucilage weight	0.061
Mesocarp thickness	0.085

Relationships within groups of descriptors

A subset of descriptors that may be representative of the whole group according to the results of correlation analysis would include total leaf length, leaf apical angle, petal length, staminode length, sepal length, ovule number, pod weight and length, total bean weight, individual bean length and width (Table 5).

The results of Cluster Analysis suggest that geographic origin partially accounts for the genetic distances among the accessions. Consequently, it may be inferred that the Amazon Basin does not represent one single population with a fair amount of genetic diversity.

To employ Cluster Analysis as a technique for discarding variables, one needs to consider how the groupings are affected when a subset of variables is used in clustering as compared to the full complement. The numbers of clusters formed at any given level of similarity, the contents of the clusters, the number of accessions in each cluster and the associations of accessions within clusters may be affected.

Table 5 Significant correlations between descriptors based on clonal means

DESCRIPTORS	Correlation Coefficient (PCC)	DESCRIPTORS	PCC
Leaf length:width	0.62***	Primary furrow depth:secondary furrow depth	0.86***
Leaf length:basal to apical angle ratio	0.35*	Pod weight : placenta weight	0.64***
Leaf length:basal angle	0.37*	Pod weight : washed bean weight	0.74***
Basal leaf length:width	0.66***	Pod weight: husk weight	0.96***
Sepal length:sepal width	0.30*	Pod weight: washed bean weight	0.50***
Sepal length:ligule length	0.60***	Placenta weight : husk weight	0.55***
Sepal length:ribbon length	0.48***	Wet bean weight : husk weight	0.58***
Sepal length:staminode length	0.47**	Wet bean weight: washed bean weight	0.79***
Sepal length: ligule width	0.45**	Pod weight: mucilage weight	0.66***
Staminode length:pedicel length	0.36*	Mucilage weight : wet bean weight	0.75***
Ligule length:ligule width	0.49**	Mucilage weight : husk weight	0.55***
Staminode length:ribbon length	0.45**	Bean length : pod width	0.60***
Sepal width:ovary length	0.45**	Bean length: pod weight	0.60***
Ovary length:style length	0.46**	Bean length: washed bean weight	0.58***
Pod length:pod width	0.50***	Dried (unpeeled) bean weight: peeled bean weight	0.90***
Pod length:pod weight	0.68***	Dried bean weight: bean thickness	0.60***
Pod length:husk weight	0.68***	Peeled bean weight : bean width	0.98***
pod length:washed bean weight	0.50***	Peeled bean weight : bean length	0.65***
Pod width:pod wall thickness	0.54***	Peeled bean weight : bean thickness	0.64***
Pod width:pod weight	0.90***	Husk weight: bean number	0.33*
Pod width:washed bean weight	0.67***	Husk weight: bean weight	0.30*
Pod width:husk weight	0.89***	Pod wall thickness : husk weight	0.66***
Pod wall thickness:mesocarp thickness	0.67***	Mesocarp thickness : husk weight	0.50***
Pod wall thickness:pod weight	0.56***		

LEGEND for Table 5

PCC - Pearson Correlation Coefficient
* 5% significance level ** 0.1% significance level ***0.01%significance level

In this study, a subset of desirable descriptors (Table 6) yielded information comparable to that provided by the full complement of descriptors (Figures 2 and 3). Since the most "useful" quantitative and qualitative descriptors were included in this data set, it is valid to recommend them for inclusion in a concise cocoa descriptor list.

Discussion

The analyses performed indicate that the quantitative reproductive descriptors, particularly those of the flower, are more useful taxonomically than the vegetative ones. This could be explained by tighter selection pressures and thus greater genetic control being exerted on the reproductive characters in the process of evolution. These results correspond with those of Ostendorf (1957), who proposed that the cocoa flower is the most effective character for distinguishing between clones.

The more useful quantitative descriptors indicated by the results of the analyses performed include total leaf length, leaf basal angle, staminode, petal, style and sepal lengths, sepal width, ovary length, ovule number, pod weight and length, individual dried bean weight, bean number and individual bean length and width.

The value of qualitative characters, which are highly heritable, has been demonstrated by Engels (1983a, 1983b, 1986). However, these descriptors are of little agro-economic value and thus not very important in breeding. However, assessment of the qualitative descriptors indicated that flush colour, pulvinus size, the intensity of anthocyanin in various parts of the flower and cotyledon colour are more useful than the other qualitative descriptors for differentiating between clones.

It is important to emphasize that detailed environmental information should accompany morphological data.

From a breeding perspective, the presence of a fair amount of diversity within this germplasm collection is indicative of its value in breeding programmes.

The results of this investigation suggest that as many quantitative and qualitative characteristics as possible be included in the description of populations of *T. cacao* with similar geographic origins. This will facilitate detailed inspections of inter-relationships. However, a subset of quantitative and qualitative descriptors (Table 7) should be adequate to identify distinct groups of accessions and will facilitate the process of characterization of accessions in large germplasm collections such as the ICG,T.

Table 6 Subset of 34 quantitative and qualitative descriptors found to be suitable for inclusion in a concise descriptor list for cocoa based on the results of Cluster Analysis

Quantitative descriptors	Qualitative descriptors
Total leaf length	Leaf apical shape
Leaf width	Leaf basal shape
Leaf apical angle	Pulvinus size
Leaf basal angle	Flush colour
Sepal length	Sepal colour
Sepal width	Filament colour
Staminode length	Pod apex form
Ovary length	Pedicel abscission zone colour
Ovule number	Pedicel column colour
Style length	Pod shape
Pod weight	Pod surface texture
Wet bean weight	Bean (cotyledon) colour
Washed bean weight	Bean shape
Mucilage weight	
Bean number	
Total dried bean weight	
Individual bean weight	
Bean length	
Bean width	
Pod length	
Pod width	

Table 7 List of descriptors recommended for a concise descriptor list for cocoa

QUANTITATIVE DESCRIPTORS	QUALITATIVE DESCRIPTORS
Total leaf length	Flush colour
Leaf basal angle	Pulvinus size
Sepal length	Leaf apical shape
Sepal width	Filament colour
Staminode length	Sepal colour
Petal length	Pedicel column colour
Ovary length	Cotyledon colour
Ovule number	Bean shape
Style length	Pod apex form
Pod length	Pod surface texture
Pod weight	Pod shape
Bean number	
Bean length	
Bean width	
Individual dried bean weight	

Quantitative descriptors = 15
Qualitative descriptors = 11

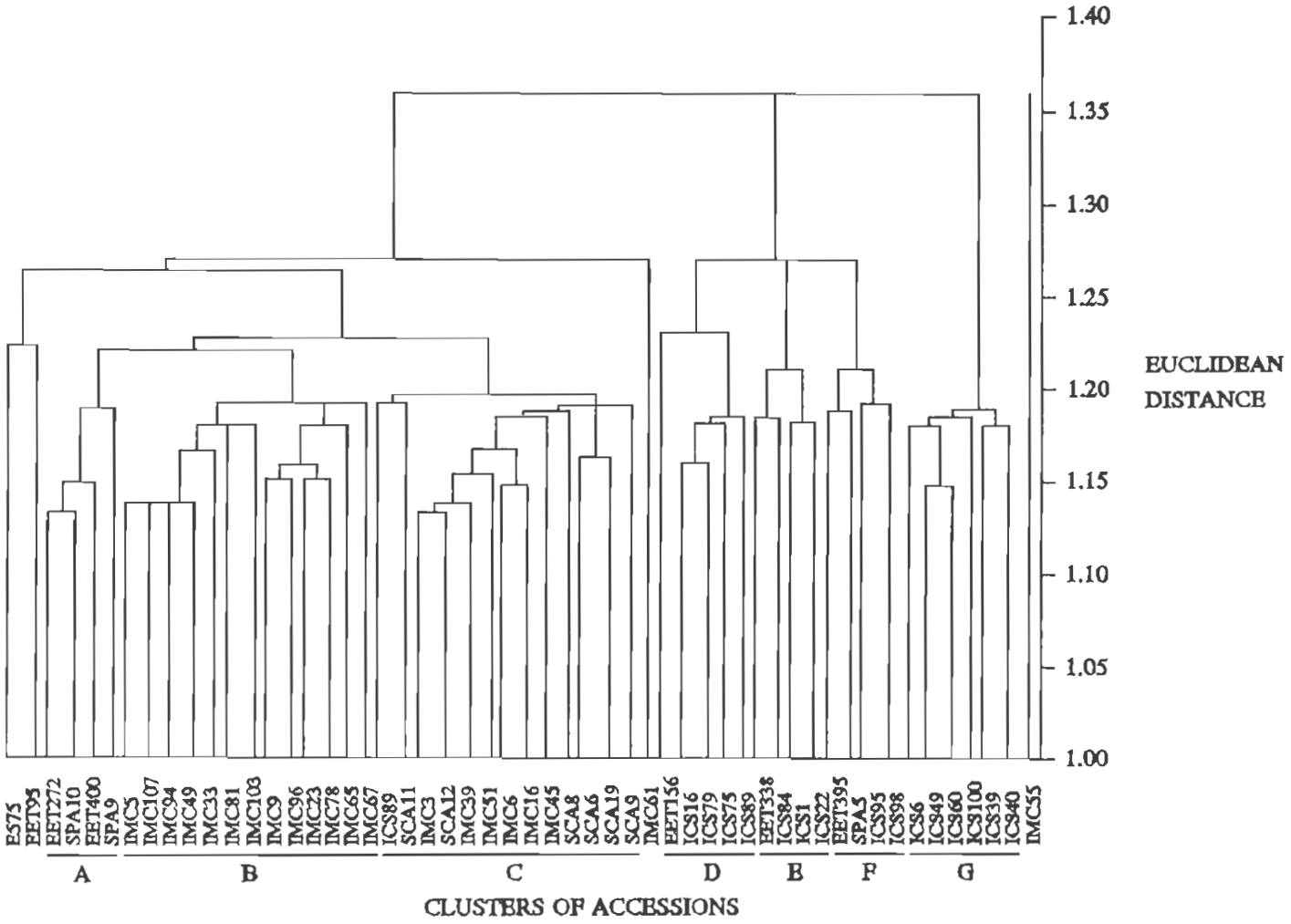


Figure 2 Dendrogram showing relationships between 53 cocoa clones after cluster analysis on the basis of the standardized variable-group method using 68 (quantitative and qualitative) variables.

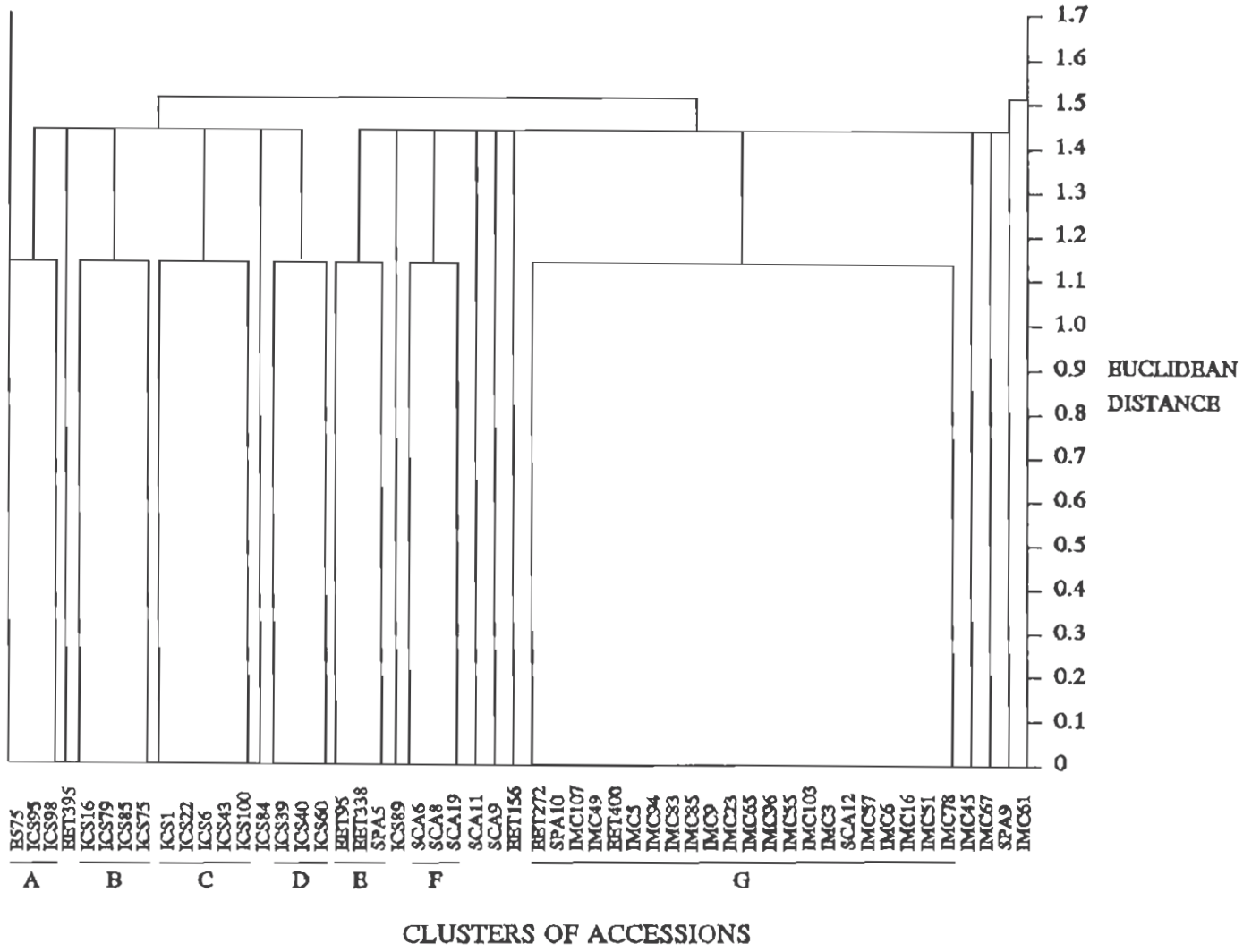


Figure 3 Dendrogram showing relationships between 53 cocoa clones after cluster analysis on the basis of the standardized variable-group method using 34 qualitative and quantitative descriptors.

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