THE COMPILATION AND ANALYSIS OF A DESCRIPTOR LIST FOR COCOA (*THEOBROMA CACAO* L.)

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ABSTRACT

THE COMPILATION AND ANALYSIS OF A DESCRIPTOR LIST FOR COCOA (Theobroma cacao L.)

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The usefulness of several cocoa descriptors was assessed in this study. Thirty-five quantitative descriptors were analyzed to determine whether, and to what extent, they differentiate between fifty-three accessions. The latter represent a range of diversity. All the descriptors were discriminative. However, the leaf descriptors were more influenced by environment than the reproductive ones and the fruit descriptors displayed the most variability within the populations studied.

There were several correlations between these descriptors. For the purpose of quick identification of accessions, the most discriminative were retained and those correlated with them discarded. The resulting subset of descriptors includes pod weight and length, total bean weight, bean length and width;

staminode, petal and sepal lengths, ovule number; total leaf length and leaf apical angle.

Information on the diversity of the accessions was important for assessing the usefulness of these descriptors. Seven homogeneous groups of accessions were identified by clustering with sixty-eight mixed variables. The presence of diversity was demonstrated and the accessions could be differentiated according to their geographic origin.

No subsets of descriptors provided a classification identical to that of the full complement. However, a subset of thirty-four mixed descriptors furnished a similar classification. It may be appropriate for quick classification in the International Cocoa Genebank, Trinidad. Subsets of descriptors may be useful for differentiating between distinct accessions. However, as large a group of descriptors, as is practicable, is recommended for the reliable determination of the relationships between the accessions.

In an attempt to reduce the subjectivity involved in measuring qualitative descriptors, the level of anthocyanin in the sepals of several accessions was determined spectrophotometrically. This proved to be a very useful and fairly rapid method and this approach is recommended for improving the existing cocoa descriptor list.

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GLOSSARY

GENERAL

cm	= centimetre
^{o}C	= degrees Celsius
g	= gramme
λ max.	= lambda maximum
m.e.	= molar equivalent
ml	= millilitre
mm	= millimetre
nm	= nanometre
ns	= not significant
p	= probability
S.D.	= standard deviation
S.E.	= standard error
uv	= ultraviolet
vis	= visible
v/v	= volume by volume ratio
vs	= versus

DESCRIPTOR	ACRONYM
LEAF	
Leaf apical angle	AA
Apical : basal angle ratio	ABR
Apical shape	AS
Leaf basal angle	BA

Basal leaf length	BL
Basal shape	BS
Flush colour	FL
Total leaf length : width ratio	LWR
Pulvinus size	PS
Total leaf length	TL
Leaf width	W
FLOWER	
Anther disposition	AD
Average petal (ligule) length	APLL
Bud colour	BC
Filament colour	FLC
Guideline length	GL
Presence of glandular hairs	GR
Ligule colour	LC
Ligule length	LIL
Ligule width	LW
Ovary apex colour	OAC
Ovary base colour	OBC
Ovary diameter	OD
Ovary length	OL
Ovule number	ON
Pedicel abscission colour	PAC
Pedicel apex colour	PAPC

Pedicel colour	PEC
Pedicel length	PL
Ribbon colour	RC
Ribbon length	RL
Sepal colour	SC
Sepal length	SL
Sepal length : width ratio	SLWR
Sepal position	SP
Style colour	STC
Staminode length	STL
Style length	STYL
Sepal width	SW
Thecae colour	TC
FRUIT	
Bean colour	BEC
Bean number	BEN
Bean shape	BES
Bean thickness	BET
Bean width	BEW
Bean length	BL
Bean weight	BW
Dry bean weight	DBWT
Primary furrow depth	FD1
Secondary furrow depth	FD2

Husk hardness	HH
Husk weight	HW
Mesocarp thickness	MESOTH
Mucilage weight	MUW
Pod apex form	PAF
Pod basal constriction	PBC
Peeled bean weight	PBWT
Pod furrow colour (immature)	PFCI
Placenta weight	PLW
Pod length : width ratio	PLWR
Pod size	POS
Pod shape	POSH
Pod length	POL
Pod width	POW
Pod weight	POWT
Pod furrow colour (mature)	PFCM
Pod furrow disposition	PFD
Pod furrow separation	PFS
Pod ridge colour (immature fruit)	PRCI
Pod ridge colour (mature)	PRCM
Pod surface texture	PST
Pulp colour	PUC
Pod wall thickness	PWT
Seed coat percentage	SCPE
Seed coat weight	SCWT

Washed bean weight	WABW
Wet bean weight	WBW

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 IMPETUS FOR THIS STUDY

This project entails the compilation and analysis of a descriptor list for cocoa, Theobroma cacao L.. It was initiated, in 1983, as part of a scheme to reorganize the cocoa genetic resources in Trinidad. In 1981, the Cocoa Research Unit (CRU), in St. Augustine, acknowledged the need to develop a genebank (germplasm collection centre) to conserve ex-situ valuable local and exotic germplasm. Consequently, arrangements were made to develop the International Cocoa Genebank, Trinidad (ICG,T); a collection plantation. Further information on the ICG,T is given in Chapter Two.

Efficient management of a genebank requires accurate identification of accessions and collation of appropriate documentation. Such documentation may include genealogical, morphological, agronomic, breeding, pathological and economic information. Data collection can be facilitated by the adoption of a list of standardized descriptors, which may also serve several other purposes, as outlined in Chapter Two.

Fifty-three accessions representing six populations (ICS, IMC, SPA, SCA, E and EET) were

Table 1.1 Description of the populations studied at the St. Augustine site

POPULATION	DESCRIPTION	NUMBER OF CLONES.
ICS	Trinidadian Trinitario[1]	16
IMC	Peruvian (Upper Amazon)[2] Forastero	21
SPA	Colombian Forastero[3]	3
SCA	Amazonian Forastero[4]	6
E	Ecuadorian Forastero[5]	1
EET	Ecuadorian Hybrids[6]	6

1 - described by Pound (1934-36). (ICS 39, 40, 45, 47, 60, 100 = Nicaraguan Criollo (Lockwood and Gyamfi (1979))

2 - collected by Pound (in 1937)

3 - collected by Pound

4 - collected by Pound (in 1938?)

5 - selected by Jolly on the San Juan Estate (Trinidad)

6 - collected by Pound (in 1942/43)

characterized on the St. Augustine Campus (refer to Appendix 2a) between November, 1983 and July, 1985 (refer to Table 1.1). Thirty-one accessions were studied at St. Joseph (refer to Appendix 2b) and twenty of these were common to the studies at the two sites. These populations represent the major geographical distribution of Theobroma cacao L., but exclude germplasm from Brazil and Central America (Criollo) and in particular represent the acknowledged centre of diversity for T. cacao (refer to Appendix 3). They are also of interest for agronomic and economic reasons. Some of the clones, such as ICS 95, are notable for the superior flavour of their beans. Others, like IMC 67, display desirable characteristics such as large pod (fruit) size, vigor, high yields and resistance to some disease(s) like Witches' Broom disease. (IMC 67, is highly susceptible to Black Pod disease, however).

The main objective of the study was to determine the usefulness of the descriptor list for cocoa, (refer to Appendix 9) proposed by the International Board For Plant Genetic Resources (IBPGR (1981)), to discriminate among accessions. It was hoped that this study could result in the development of a shorter list of effective descriptors.

To achieve this objective several analyses were

performed. Section 3.2 (Experimental) contains an assessment of the intraclonal and interclonal variability of individual descriptors. Those descriptors with high interclonal and low intraclonal variability would be considered most diagnostic or discriminative.

In Section 3.3 (Experimental), correlations within groups of descriptors were examined. The discovery of less reliable descriptors, which bear a close association with other more reliable ones, could lead to a more concise, but still taxonomically valid descriptor list. Principal Component Analysis (PCA) was also done in an attempt to identify a subset of descriptors that can effectively describe cocoa accessions (refer to Section 3.4 (Experimental)).

In Section 3.5 (Experimental) an effort was made to assess the role of environment in the expression of the descriptors. Descriptors, whose expression is not significantly affected by the environment, are considered most useful in the descriptor list.

Hierarchical average-linkage cluster analysis was performed to determine relationships among the clones studied and to test how well subsets of descriptors group or classify the clones. The results are given in Section 3.6.

A preliminary investigation was also undertaken

to determine the feasibility of using a biochemical descriptor i.e. pigment intensity in cocoa flowers. This type of descriptor has not yet been included in the IBPGR descriptor list for cocoa. (In Chapter Two, another example of the use of biochemical parameters to characterize cocoa germplasm is given.) Such descriptors may prove to be better than morphological ones and may be studied further in the future. The findings of this investigation are furnished in Section 3.7 (Experimental).

1.2 SOME BACKGROUND ON THE COMPILATION OF A DESCRIPTOR LIST FOR COCOA

The need for descriptors of cocoa has been recognized in the course of selection, testing and propagation ((Ostendorf (1956) and Soria and Enriquez (1967b)) and the collection of data in accordance with the specifications of a standard descriptor list will facilitate future research in cocoa (Williams (1984)).

Work on descriptors was begun as early as 1935 in Java (Ostendorf (1956)). In 1964, Enriquez and Soria recommended that a catalogue of cocoa cultivars be established and it was decided in 1967 (in Bahia, Brazil) that a short, descriptive format, including main distinctive characteristics be used in cocoa descriptor lists (Soria and Enriquez (1967b)).

In 1972, a format and instructions for the list were presented at the Fourth International Cocoa Research Conference (Trinidad) by a subcommittee under B.G.D. Bartley and it was decided only to include the most easily observed descriptors, which can be estimated with relatively small samples (Soria and Enriquez (1967b)). A standard descriptor list for cocoa was subsequently published (Engels et al (1980); IBPGR (1981)). Further work may still be necessary to improve the existing IBPGR descriptor list (Allen (1984)).

1.3 RECORDING DESCRIPTOR DATA

1.3.1 LEAF DESCRIPTORS

Fifteen leaf descriptors were included in this study. Several workers have made observations on recording these characters. For the purpose of cultivar comparison, Asomaning and Lockard (1963) recommended that the leaves be taken from a standardized position, such as the second mature leaf counted from the tip to the base of the flush. This suggestion was followed in this investigation.

The laminae of most cocoa varieties were described as similar in shape; ovate, elliptical to somewhat acuminate with an entire margin (Asomaning

and Lockard (1963) and Soria (1977)). Murray (1952) reported that the leaf shape of normal leaves is practically constant whatever the size.

Asomaning and Lockard (1963) also found that leaf width is not readily determined and that measurement is subject to bias since one has to first find the widest point of the leaf. Thus, care is necessary when recording it.

Although petiole lengths were not measured in this study, Brook (1950), cited by Enriquez and Soria (1967c) found clear differences within genotypes between the petiole lengths of trunk leaves and those of lateral branches at Turrialba.

1.3.2 FLOWER DESCRIPTORS

Twenty-four flower descriptors were employed in this research. Since their measurement is influenced by the age of the flower (Enriquez and Soria (1967a)), care was taken to select only freshly opened flowers for observation.

Within any given flower, the dimensions of its organs were shown by Enriquez and Soria (1967a) to be very similar and thus only one organ per flower was measured during this study.

Random selection should be used to offset the

effect of position of the flower on the tree on the size of individual organs. Sepal width was one descriptor found to be affected by position (Enriquez and Soria (1967a)). The widest sepals were found on the trunk flowers and the narrowest on those of fine branches.

Toxopeus and Jacob (1970) and Enriquez and Soria (1967a) remarked on the usefulness of reference to external factors, when recording such flower features as the number of ovules per ovary. This advice was followed to some degree and the reasons and implications are stated in Section 3 (Experimental).

Engels et al (1980) showed that for flower, as well as fruit characters, there were no differences of statistical significance between trees of the same clone, grown at the same site. Data from an average of three trees were therefore pooled to make up the required sample sizes, while disregarding the possible effect of tree on any variability found between clones.

1.3.3 POD (FRUIT) AND BEAN (SEED) DESCRIPTORS

Thirty-six fruit and seed descriptors were used in this study. There have been many studies to develop appropriate methodology for the collection of pod (fruit) and bean (seed) data.

Pound (1931) observed that pods borne in the canopy were smaller in size than those on the trunk. Glendinning (1963) and Amponsah (1973) noted differences in the sizes of pods within the same variety and in the same tree. Thus a random selection of pods was attempted in this study, but this was dependent on the availability of disease-free, mature or ripe pods at the time of collection.

Toxopeus (1981) described pod shape as one of the most variable characters of cocoa. Several descriptions of shape have been proposed by workers, including Van Hall (1932); Pound (1933); Cheesman (1944); and Ostendorf (1956). Van Hall's classification was adopted in the IBPGR'S (1981) descriptor list for cocoa and in this study.

Most inherent variability in pod length and diameter within trees and from tree to tree within plots was observed by Pound (1932). Pod weight was found to vary considerably over a tree, over seasons and from year to year (Glendinning (1963)). Pound (1932) found it to be affected by the degree of ripeness of the pod. Pod shell thickness and furrow depths are also affected by season and environment (Pound (1932); Enriquez and Soria (1966)).

Pound (1932) also found bean number per pod to vary markedly from pod to pod and to decrease from the

main trunk to the main branches and the finer branches.

All the above observations make it necessary to sample pods randomly over trees and to note the nature of the environment. The inherent variability of pod characters (refer to plate 1 for a representation of the diversity of fruit types in the ICG,T) necessitates the use of large sample sizes.

Pound (1932) recommended a random sample of thirty pods to be sufficient to characterize a tree, giving means for pod length and diameter accurate within 5%. Toxopeus and Jacob (1969) suggested that a sample of no less than six pods be used from a harvest. In 1967, Enriquez and Soria stipulated that eight randomly harvested pods per variety be used. In 1968, they suggested twenty pods, from which five beans each are weighed. Bartley (1964) used a sample size of ten. Engels (1981) recommended between ten and thirty-five to forty pods be used for various pod and bean measurements.

In 1981, Toxopeus recommended selective random sampling so that a sample size of four could be used. In this study, only four, uniformly well-developed pods were collected. To offset the effects of season observed by Pound (1932), Toxopeus and Jacob (1969) and Engels (1981), among others, pods were harvested

Plate 1 A representation of the diversity of fruit types in the ICG,T.

at the same time of year (same months) when collection spanned more than one year. An observation, made by Bartley (1964), that the validity of individual tree estimates are not altered if harvesting is done within three weeks of pod maturity, was taken into consideration.

1.4 ENVIRONMENTAL FACTORS AFFECTING THE PHENOTYPE OF COCOA

The importance of recording information on the physical environment of cocoa trees has been noted by workers such as Allen (1984); Bartley (1971); McDonald (1932) and Murray (1967). Such information may be a valuable addition to cocoa descriptor lists.

Temperature and rainfall are described as the most critical climatic factors for growth of cocoa trees (Alvim (1977)). Annual rainfall between 1500 and 2000 mm with a dry season of no more than three months with less than 100 mm per month is required. The mean maximum temperature should be between 30 and 32°C and the mean minimum between 18 and 21°C (Lass and Wood (1985)). Wind, solar radiation and relative humidity also affect the physiological processes of the plant (Cunningham and Lamb (1959)).

Cocoa may be grown on many different soils, provided they have some physical and chemical

properties of particular importance for the crop (Hardy (1960) and Smyth (1966)). The best cocoa soils are usually formed by aggregated clay or loamy sand. The proportions of the different particles are usually 30-40% clay, 50% sand and 10-20% silt-sized particles (Smyth (1966)). Pearce (1953) partially attributed "the wide variability in the performance of cacao from tree to tree" to soil effects. Fennah (1957) noted the effects of soil heterogeneity on the wide variations among clonal trees.

Cunningham and Lamb (1959) cited nutrient supply as a factor limiting the vegetative growth of cocoa. A close correlation between response to nitrogen (N) and exposure of trees to light or shade has been discovered (Evans and Fennah (1953)). Trees growing under full light were generally found to give a response to N. Younger leaves tended to be affected by N deficiency. They are undersized and harden into a pale, yellow colour.

The effects of magnesium were described as pronounced on sandy soils, towards the end of the wet season. Copper deficiency is said to have a similar effect to that of magnesium in the cocoa plant (Evans and Fennah (1953)).

Goodall and Posnette (1947) reported that morphological change is induced in cocoa leaves by

mineral deficiency eg. of iron and manganese.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.0 INTRODUCTION

The global importance of crop genetic resources is now widely accepted (Altieri and Merrick (1987); Budin (1973) and the management of these resources has been of concern for a number of years (Hondelmann (1974); Ng (1982); Williams (1984)). Since programmes of collection and conservation are usually distinct from breeding programmes that utilise genetic resources, efficient documentation and dissemination of information is essential (Frankel (1970); Seidewitz (1978); Suzuki and Kamagai (1981)). Characterization and evaluation of germplasm therefore become significant aspects of the management of genetic resources (Iltis et al (1986); Creech and Reitz (1971)).

Until 1980, the documentation of genetic resources was inadequate for a large number of crops (Williams (1984)), not internationally standardized and seldom readily accessible (Konzak and Dietz (1969); Rogers et al (1975); Jain (1979)). Important descriptive information necessary for identification is still seldom collated (Budin (1973); Brown (1983)). It has been argued that pedigrees of valuable stocks

may be confused by numerous designation forms and abbreviations (Budin (1973); Enriquez and Soria (1967b)). The development of a standard descriptor list allows a systematic accumulation of data, that becomes internationally accessible (Dambroth (1978); Burt et al (1971); Estabrook and Rogers (1966); IBPGR Secretariat (1981); Konzak and Dietz (1969)).

In recognition of the above, an international Working Group on the Standardization in Crop Research and Data Recording was initiated in 1966 under the joint sponsorship of the Food and Agriculture Organization (FAO) and the Institute of East Asian Agriculture (IAEA) (Konzak and Dietz (1969)). Much of their work is now being effected by the International Board of Plant Genetic Resources (IBPGR) established in 1974 by the Consultative Group on International Agricultural Research (CGIAR) (Annual Report of IBPGR (1984)). The IBPGR co-operates with one hundred and ten countries and six hundred key agricultural institutes. Over one hundred crop species are under study (IBPGR Annual Report (1989)).

To date, several descriptor lists for commercially important plant species have been compiled and recognized within a worldwide network of over one hundred genebanks (Hondelmann (1974); Jain (1979); IBPGR Annual Report (1989)). Information

included relate to the identity, genealogy, morphology and agronomy of the plants (Konzak and Dietz (1969)). The first two types of information are described as accession and collection data, respectively. The others are characterization and preliminary evaluation data.

2.1.1. GUIDELINES FOR DESCRIPTOR LIST COMPILATION

Certain guidelines are necessary in descriptor list compilation to ensure the maximum value of the data in agronomic and taxonomic work. These are outlined hereafter.

Characterization data should consist of characters, which are highly heritable, can be easily seen by the eye and are expressed in all environments (Konzak and Dietz (1969); Jain (1979); Williams (1984)). Individual accessions should be evaluated in a systematic manner for a number of characters thought desirable by a consensus of users of the crop (Miri (1980)). These may include ethnobotanical, food and industrial information (Rogers et al (1975)).

It is important that descriptors are well-defined for all users of a crop, taking into consideration the different uses of the crop, the various breeding objectives and the methods of measuring the same character (Konzak and Dietz (1969); Sneath and Sokal

(1973)). The diversity of the crop must be taken into account, along with the growth stage and growth environment where observations are made (Erskine and Williams (1980)).

The mass of data, that is accumulated in the course of descriptor list compilation, is easily handled using a computer-based, Communication, Information and Documentation System (CIDS), especially for global networks (Sokal and Rohlf (1969)).

Modern information-processing technology has been widely used to date to provide an efficient, flexible, rapid and continuous mechanism through which information can be stored, retrieved and exchanged (Konzak (1972)).

2.2.0 THE COMPILATION OF A DESCRIPTOR LIST FOR COCOA

2.2.1 INTRODUCTION

In 1990, the world production of cocoa was estimated at 2.43 million metric tonnes and the world consumption was 2.2 million metric tonnes for 1988/89 (Wakeling (1990)). Over 70% of this cocoa came from seven developing countries; Indonesia, Malaysia, Brazil, Ivory Coast, Ghana, Nigeria, and Cameroon with Africa producing about 60%. The rest of the world's

cocoa is derived from South and Central America, the West Indies, Asia and the Pacific region. Malaysia's production has increased steadily over the past five years. Trinidad produced 3,000 metric tonnes of raw cocoa in 1980, but as much as 34,000 tons in 1921 (Anon. (1959)).

2.2.2 BOTANY

Cocoa (cacao), of the family Sterculiaceae, is a tropical crop. Linnaeus designated this name in the first edition of Species Plantarum in 1753 (Cope (1976)).

Theobroma cacao L. is the only economically important species of the twenty-two species, which comprise the genus Theobroma (Cuatrecasas (1964)) Two of its subspecies are T. cacao subsp. cacao and T. cacao subsp. sphaerocarpum (Cuatrecasas (1964)).

The former is divided into four forms and the latter consists of calabacillo types (Soria (1977)). This classification is based mainly on the morphology of the fruit, but is not fully justified from the genetic point of view (Soria (1977)).

Cope (1976) reported that all the subspecies and forms of cocoa interbreed to give fully fertile F1 hybrids. The chromosome number of cocoa is 2n=20 (Munoz (1948) cited by Soria (1977)).

Of the four forms of T. cacao subsp. cacao, three are recognized by the "cocoa trade" (Cope (1976)). These are the "criollos", the "amelonados" and the "trinitarios" (Soria (1977)). Cheesman (1944) classifies the latter two forms as "forastero". The fourth form, "pentagona" is not commercially important (Wood (1975); Soria (1977)). However, it may be the oldest known form of cultivated cocoa, which gave rise by mutations and later hybridizations among the mutants to the presently cultivated hybrids (Mora Urpi (1958)). Commercial cocoa is largely forastero (Cheesman (1944)).

"Criollo" cocoa is typified by large, plump beans, round in cross-section, with white or very pale violet cotyledons in the fresh bean (Cheesman (1944)). The latter is described as a recessive character. The chocolate yielded lacks astringency and is of a high quality (Wood (1975)). The fruit wall is five-angled, soft and warty. This form of cocoa suffers from two major drawbacks in that it has low yields and lacks hardiness (Cope (1976)). With respect to the latter feature, it shows low resistance to Witches' Broom disease (Crinipellis pernicious) and Ceratocystis wilt (Gregory (1974), (1977)).

Two criollos of interest are the Venezuelan and the Nicaraguan criollos (Cheesman (1944)). The former

is perhaps the one that existed in Trinidad before the "blast" (hurricane or disease epidemic) of 1727. The latter criollo was brought to Trinidad by Hart in 1893. It is noted for its large beans; a favourable characteristic in the Cocoa Trade.

There is little "criollo" available today (Cope (1976)). Only two clones of pure origin are found in Ghana (Lockwood and Gyamfi (1979)). In Trinidad, the "blast" destroyed much of the Criollo planting (Wood (1975)).

The Amazonian forastero type of cocoa is believed by Pittier to have Theobroma leiocarpa in its ancestry (Cheesman (1944)). It is characterized by flattened beans with violet cotyledons and a very astringent, roasted product (Cheesman (1944)). (T. leiocarpa also has violet cotyledons.) The hard pericarp is not usually conspicuously angled or warty. Forasteros are hardy and often high-yielding (Soria and Esquivel (1970)). Thus they supply the bulk of the world's cocoa today (Cope (1976)). They were introduced into Trinidad in 1757 (after the blast) to replace the "criollo" (Soria (1975)).

The "trinitario" group is widely regarded as a variable hybrid population of "criollo" and "forastero" ancestry. Hybridization is said to have occurred in Trinidad, after the introduction of the

"forastero" (Soria (1975)). The cotyledon colour, bean size and shape, pod wall characters and astringency are highly variable (Cuatrecasas (1964)). This type of cocoa is described as "fine cocoa" (Wood (1975)). Trinitario was transported to Venezuela in 1825. It also exists in Grenada and other West Indian islands, as well as in Costa Rica, Ceylon and Java.

Van Hall (1932) stated that the previously mentioned types are not sharply separated from one other. He recognizes the main groups of cocoa as follows:

Criollo: Central and South America.

Forastero: Amazon and Trinitario.

These most widely cultivated varieties of cocoa are believed to represent only a part of the total genetic variation in the original wild species (Allen and Lass (1983)). All current breeding programmes are at least partly based on crosses with wild cocoa, which are expected to yield improved varieties (Allen and Lass (1983)).

2.2.3 LIFE HISTORY AND MORPHOLOGY

The cocoa tree is generally described as small; attaining a height of about six metres. The mode of branching is unusual. Growing from the seed, it first forms a straight main orthotropic stem and at a height

of roughly one to two metres from the ground, divides into up to five plagiotropic lateral branches. Growth of the fan branches is by a series of flushes, each consisting of three to six pairs of leaves (Lass and Wood (1985)). The terminal bud itself is divided in the process and no vertical leader remains (Cheesman (1944); Baker in Urquhart (1961)).

The lateral or "fan" branches comprise the "jorquette". A subsequent vertical shoot or "chupon" arises from the main stem just below the jorquette and repeats the behaviour of the original stem. This method of growth is repeated during the life history of the tree.

The habit of the budded tree depends on the type of bud used (Cheesman (1944)). Clonal trees may be erect or reclining in habit, depending on whether the rooted cutting is taken from a chupon or a jorquette.

Leaves generally persist through two flushes and drop at the time of the third.

The cauliflorous inflorescences are produced in the leaf-axils of shoots of old wood. Flowers are abundant at the start of the wet season. The individual flower is hermaphrodite and regular with all parts in fives. The floral formula is as follows:

$$K_5\ C_5\ A_5 + {}_5\ G\ (\underline{5})$$ (Are and Gwyne-Jones (1974)).

The sepals are the most conspicuous organs (white

or pinkish). The petals are narrow at the base and expand above into a cup-shaped pouch, beyond which they end in a spathulate tip. Five long, sterile staminodes and five fertile stamens in an inner whorl comprise the androecium. All ten members are joined at the base into a short tube.

The staminodes stand erect and form a ring around the style, protecting the stamens, whose anthers lie concealed in the pouch of the petals. This makes pollination difficult to accomplish.

Cocoa is both self and cross-pollinated. Ceratopogonid midges (Forcipomyia spp) (Young et al (1987) and crawling insects such as ants (Cheesman (1944) and Urquhart (1961)) are said to be agents of pollination. The flowers fall off the day after opening if they are not pollinated.

The fruit or pod is a berry with a thick husk and contains about twenty to forty beans (seeds), surrounded by pulp. The latter develops from the outer integument of the ovule (Baker, in Urquhart (1961)). The pod does not open or fall off when ripe and dissemination of the beans depends on the pod being opened by some animal, which discards the beans after sucking off the pulp (Lass and Wood (1985)).

2.2.4 THE HISTORY OF COCOA: ITS ORIGIN AND COLLECTION

Theobroma cacao L. originated in Tropical America (Central and South) and is native to extensive areas of the humid lowlands (Chatt (1953); Cheesman (1944); Cuatrecasas (1964); Lockwood (1985); Wood (1975)). According to Cheesman (1944), its chief centre of genetic diversity was the Amazon Basin, between the headwaters of the Caqueta, Putomayo and Napo rivers. This area appears to embrace the widest range of variability, as recorded by Pound (1938). There is a secondary centre in Mexico and Central America, where it was domesticated by the Mayas, thousands of years before the arrival of Columbus (Cuatrecasas (1964)).

Patino (1963), cited by Soria (1975) stated that cocoa cultivation was started in the valleys of the Cauca, Caldas, Antioquia and Huila rivers in Colombia; the extreme south of Lake Maracaibo, the northern coastal valleys of Aragua, the Charua valley in Merida State and the Sucre state in Venezuela, in Trinidad and on the west coast of Ecuador. Cope (1976) gives the centre of cultivation of cocoa as Central America. Mora Urpi (1958) named Mexico as the specific centre.

2.2.5 COCOA COLLECTING EXPEDITIONS

Several expeditions by collectors have been made

in the past to obtain "wild" cocoa. In the 1930's, scientists from the Imperial College of Tropical Agriculture (ICTA), Trinidad, made collections in Mexico, Central America and in the northern part of South America (Soria (1975)).

Dr J. Pound went to the Amazon to collect varieties resistant to Witches' Broom disease (Bartley and Chalmers (1971)). In 1937, he collected material from Peru (refer to Table 2.1). This material is held at Marper Farm, Trinidad. In 1942/43, he collected refactarios in Ecuador.

In 1945, G.E.L. Spencer made introductions from Mexico and Central America into Trinidad (Bartley and Chalmers (1971)). Becker, Bartley, Cope, Holliday and Taylor went to the Caqueta, Orteguaza and Vaupes rivers of Columbia to collect material in 1952/54. Soria, Vello, Murca Pines and Medeiros selected and collected material in Brazil, in 1965.

In 1968/73, the Experimental Station, Pichilingue, Ecuador (INIAP) and the ICTA carried out four expeditions to the headwaters of the Putumayo, Napo, Coca and Pastaza rivers (Soria (1975)). Chalmers collected material from the Oriente region, Ecuador in 1968/1970. Material was again collected from the Amazon, near the Napo river, in 1973 by the INIAP/UWI collecting group (Bartley and Chalmers (1971)).

Table 2.1 The contents of the ICG,T

(After Kennedy (1985))

Population	Origin	Number of Genotypes
1) Pound Upper Amazon	Peru	521
Nanay(Na)	Peru	301
Parinari(Pa)	Peru	129
Morona(Mo)	Peru	23
Iquitos(IMC)	Peru	53
Scavina(SCA)	Peru	15
2) Pound Refactarios	Ecuador	969
3) Imperial College clones (ICS)	Trinidad	90
4) Chalmers Oriente	Ecuador	85
5) Grenada Selections (GS)	Grenada	15
6) Anglo-Colombian (Spec)	Colombia	43
7) EET	Ecuador	8
8) Pound	Peru	21
9) Brazilian clones	Brazil	6

In 1979, John Allen began a collection on behalf of the London Cocoa Trade Amazon Project in Ecuador (Allen (1983)). It was completed in 1985. By the end of 1981, he had made three hundred and ten collections from the Amazon and these were held at Napo, Ecuador. In 1983, three hundred and sixty-four plants had been collected (Allen and Lass (1983)).

In 1981, the chief cocoa germplasm collections were distributed as follows :

(1) At Marper Farm and the Imperial College of Tropical Agriculture (ICTA) or The University of the West Indies (UWI), Trinidad, as it is called now.

(2) At Turrialba, Costa Rica.

(3) At the Estacion Experimental Tropical, Pichilingue, Ecuador.

(4) At Ceplac, Brazil.

(5) At Mayaguez, Puerto Rico (IBPGR Secretariat (1981)).

2.2.6 COCOA COLLECTIONS IN TRINIDAD

Organized cocoa research in Trinidad may date from the inception of The Cocoa Research Scheme in 1930 (Bartley and Chalmers (1971)). Marper Farm in Manzanilla was then one of the major collection sites. Most of Pound's collections were kept there.

The St. Joseph Nursery is another collection site and contained roughly six hundred genotypes (Bartley and Chalmers (1971); Chalmers and Murray (1973)). However, a part of this collection was destroyed by fires in May, 1984 and March, 1985 (refer to Appendices 1 and 2 b).

There is a fairly large, integrated clonal and hybrid collection on the St. Augustine Campus of the University of The West Indies (refer to Appendix 2a). It was established in 1961.

All the material, including two thousand clones, in Trinidad, is being consolidated into one site with a rationalized planting design (Kennedy (1985)) (refer to Table 2.1 for a list of the accessions being included). The site was selected at La Chaguaramas and here the International Cocoa Genebank, Trinidad (ICG,T) is being established on about forty hectares of land. Over four hundred and ninety-eight accessions were planted by the end of 1986 (Muttscheller, Gonzales and Toyer (1987)). These were distributed over one hundred and thirty-one accession plots (with spacings of 2.5 by 2.5 metres.)

2.3 USE OF A DESCRIPTOR LIST FOR COCOA

Data has been recorded for the Turrialba collection according to a descriptor list for cocoa

and it is computerized (IBPGR Secretariat (1984)). Comprehensive documentation, involving the use of a descriptor list, has also been initiated in Trinidad (Kennedy (1985)).

The IBPGR Secretariat also reported that generally all cocoa accessions are well identified in the field, but catalogues are available for only two collections; those at the Caucagua Station, Venezuela (by Reyes (1973)) and at the Turrialba site.

The IBPGR has embarked on a project to promote the use of standard descriptor lists in the preparation of inventories of all cocoa genebanks. This is being undertaken as an initial step in the effort to improve the collection, evaluation and maintenance of new cocoa germplasm. The latter is a major task, only now being addressed internationally (Lass and Wood (1985)).

2.4 ASSESSMENT OF THE USEFULNESS OF COCOA DESCRIPTORS

Ostendorf (1956) indicated that a distinction should be made between descriptive characters, used in assessment of the agricultural value of a given variety, and descriptors studied for their value in identifying varieties. Cheesman (1944) reported that all the diagnostic descriptors of cocoa are

descriptive and concern the part of the plant useful to man i.e the pod (fruit). Among the pod characters, Cheesman (1944) described those of the bean (seed) as being more constant than the external fruit characters. Differences in flower characters were found to occur between clones, but could not necessarily distinguish between "criollo" and "forastero". Leaves and general plant habit were said to provide no definite distinguishing characters.

Fruit descriptors have been studied in detail by several workers from as far back as 1931. Their usefulness as identifying characters, however, have been described as restricted by Ostendorf (1956). He based this on the fact that these characters necessitate a fairly large number of measurements per tree and cannot be used until parents have reached the productive stage. Enriquez and Soria (1967c) and Engels (1981) supported this observation.

Descriptors based on the general habit of the leaf storeys and the morphology and general appearance of leaves were developed after investigations on identifying characters for young Hevea buddings (Ostendorf (1928), cited by Ostendorf (1956)). These characters proved to be of limited value for discrimination between cocoa clones since they do not seem to differ significantly from clone to clone and

are extremely variable within clones (Enriquez and Soria (1967c) and Engels et al (1980)). The high degree of variability may be partly attributed to environmental factors such as the density of the shade canopy.

Ostendorf (1956) conceded that fairly reliable identifications, based on the general appearance of trees may be made for a limited number of clones. Balasimha et al (1985) also found significant differences between accessions in the expression of eight leaf characters. Their study involved forty accessions.

The IBPGR has included leaf and plant habit descriptors in the recommended descriptor list of 1981. These characters were also studied in Turrialba (1964) to compile the International Cacao Cultivar Catalogue (Enriquez and Soria (1967b)) and some are being used in the description of cocoa germplasm in Ecuador (Allen, personal communication).

Atkinson et al (1986) attempted to characterize cocoa germplasm using isoenzyme markers in leaf tissue. Eight enzyme systems exhibited repeatable variation. An investigation was also undertaken by Yidana et al (1988) to determine the potential use of isoenzyme analysis as a tool in description. Three main iso-peroxidase zymogram patterns were obtained

from the bark extracts of clonal material. This research is being continued by other workers at the Cocoa Research Unit, Trinidad.

If proved a useful descriptive tool, the isoenzyme technique may become a valuable supplement in descriptor list compilation. The resulting descriptors may reflect genotype more closely than other morphological data.

CHAPTER THREE

3.0 EXPERIMENTAL

3.1.0 GENERAL MATERIALS AND METHODS

3.1.1 INTRODUCTION

The stipulations recommended for the proper collection of characterisation data for descriptor lists were outlined in Chapter 2. A detailed account of the methodology employed in this study will be given hereafter.

Between 1983 and 1985, data were collected from two clonal collections in Trinidad; those in St. Augustine and St. Joseph (refer to Appendices 1 and 2a-2d). At the start of data collection, the trees in St. Augustine were about twenty-five years old and those at St. Joseph were twenty-one years old.

At both sites, the material studied is distributed over a series of blocks or fields and each accession is replicated at least three times (refer to Appendices 2c and 2d). Although, the accessions are not planted in a completely randomised block design, there is randomisation at the St. Augustine site. Over thirty of the fifty-three accessions studied there are randomly distributed between at least two fields

(blocks). Samples from the thirty-one accessions observed at St. Joseph were taken from replicate trees, which are all in the same row and therefore it was not valid to analyse this dataset using Analysis of Variance.

There were several important considerations that were relevant to this study. Two of these were the effects of season and time of collection on the expression of descriptors (refer to Section 3.4). Engels (1981) commented on the effect of these factors on two fruit descriptors; pod weight and pod length. Data were collected during the same season for each group of descriptors; leaf, flower and fruit (between November, 1983 and July 1985). Leaf and flower data were collected throughout the rainy season; mainly between August and November. The pod (fruit) data were collected during the main harvest periods, which occur during the dry season. More pods were available in late December to February. (A higher incidence of diseased pods is common during the rainy season (June-December).)

The majority of colour-related characteristics observed in the laboratory were assessed under fairly uniform daylight conditions. However, no "artificial daylight lamp" or other rigidly standardized light source was used, as was done by Engels et al (1980).

The distinctness of the categories, in most cases, made this unnecessary. Reference was made to the colour charts of The Royal Horticultural Society (RHS Enterprises Limited, Surrey, England).

In order to facilitate statistical analysis, the qualitative data collected during this study were converted into a quantitative form.

3.1.2 SAMPLING

A survey of the existing germplasm collection i.e. of the types and numbers of accessions and their locations, is a prerequisite for descriptor list compilation (refer to appendix 2a, b, c and d for the plans of the fields where sampling was done). For each accession observed in this project, data were collected randomly from the available trees.

Different workers such as Enriquez (1969); Enriquez and Soria (1967b); and Engels et al (1980) have suggested adequate sample sizes for the various cocoa descriptors and have supported these with statistical analyses. In this study, the sample sizes stipulated by the IBPGR were used with few exceptions. The latter involved pod (fruit) and bean (seed) descriptors (refer to section 3.1.5). All sampling was done during the morning period (from 7.00 to 10:45 am).

3.1.3.0 RECORDING LEAF DESCRIPTORS

3.1.3.1 METHOD

In the field, an average of three trees with flushes were selected for sampling. The plant habit of the tree was noted and fifteen leaves, which were sound and free from insect damage or disease symptoms, were randomly collected from the second leaf positions below the flushes (from top to bottom) of the mature plagiotropic (fan) branches. When fifteen leaves could not be found in the second position, additional leaves were taken from the third position. The colour of the new terminal flushes was noted (refer to plate 2).

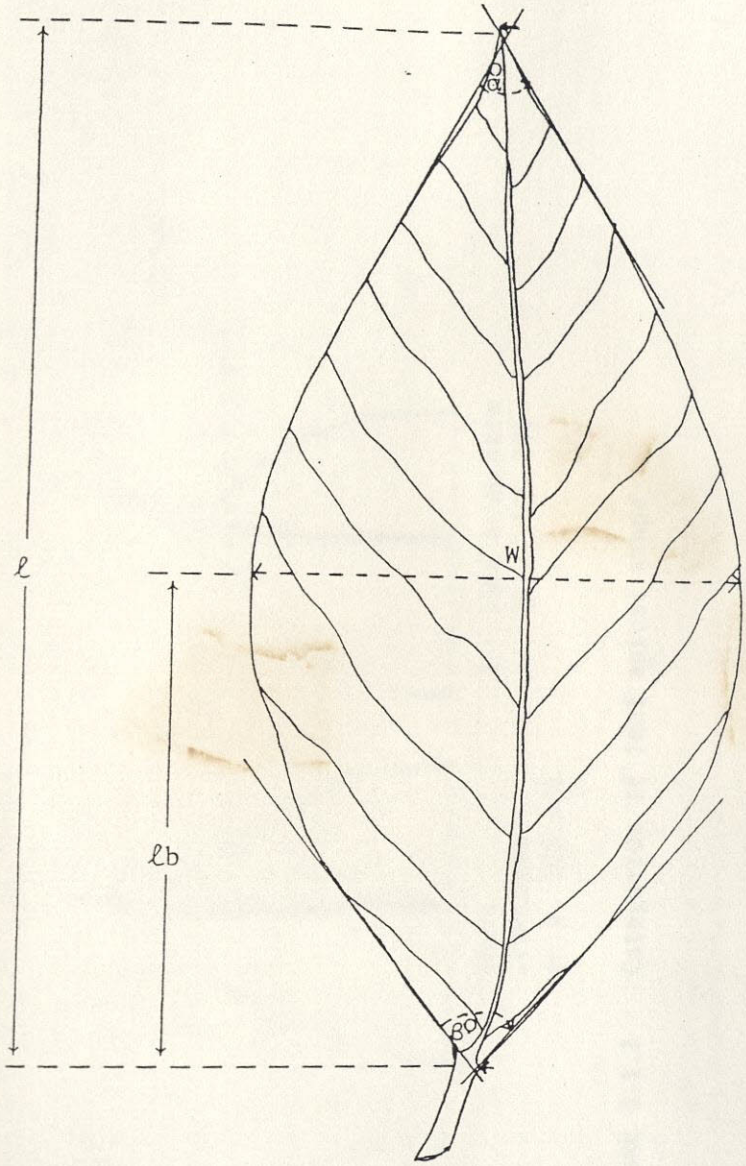
In the laboratory, the leaves were placed on a white drawing board and measured, using a clear, millimetre ruler, from apex to base, at the point of insertion in the petiole (refer to figure 3.1.1). The width of the widest point was then recorded, care being taken to avoid bias, which can occur (Asomaning and Lockard (1963)). The length (basal) from the base of the leaf to the widest point was noted (refer to figure 3.1.1) and the ratio of the total length of the leaf to the width was derived.

The outline of the apical region of the leaf was traced onto the board as closely as possible. The apical angle was then measured by drawing tangents at

the upper border of the lamina, at the point of major contact between the insertion of the apex or the drip tip at the lamina (refer to figure 3.1.1). Apex shape was observed according to the convention shown in figure 3.1.2.

The basal shape was categorized according to the convention given in Figure 3.1.3. The basal angle of the leaf was found by drawing the tangents from each side through the point of union of the petiole and the lamina and the first curvature from the base of the lamina (refer to figures 3.1.1 and 3.1.3). Subsequently, the ratio of the basal to the apical angle (ABR) was derived. The presence of a pulvinus at the point of contact between the base of the lamina and the petiole was also noted.

Plate 2 Flush colour categories 3, 5, 7 for T. cacao (0 or no anthocyanin is present in the accession "Catongo")



KEY:

α^{o} - apical angle

β^{o} - basal angle

ℓ - length

W - width at widest point

ℓb - length from base to widest point

FIGURE 3.1.1 Diagrammatic representation of leaf measurements

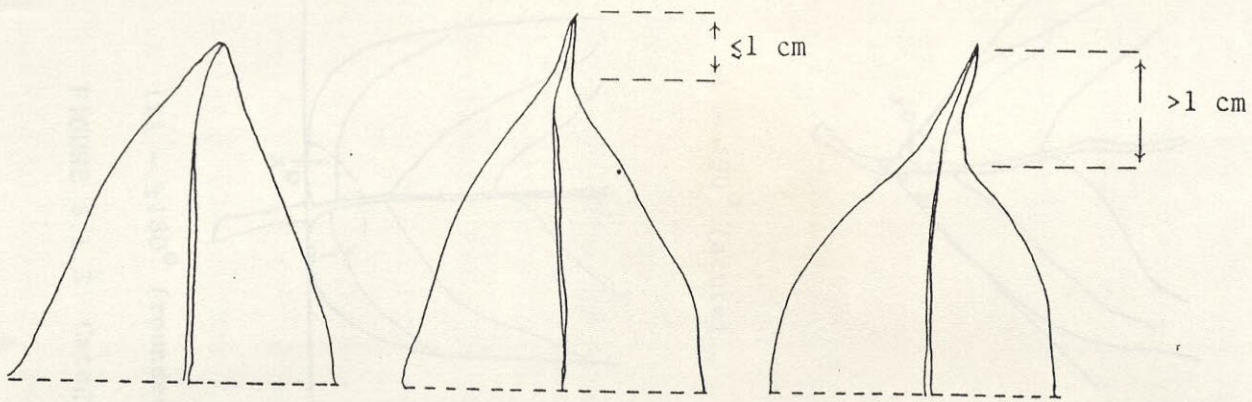
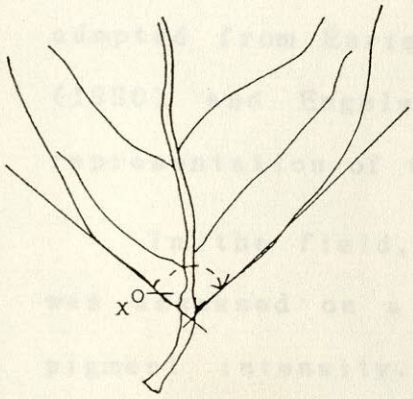
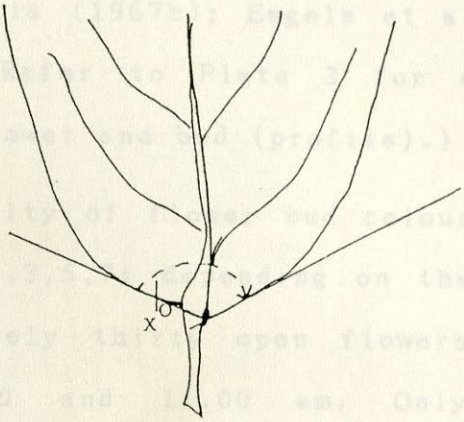


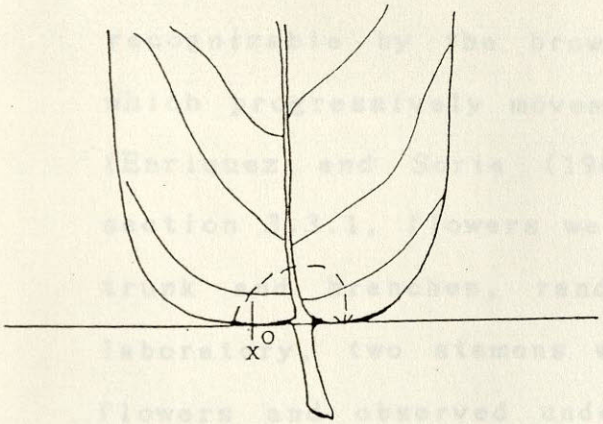
FIGURE 3.1.2 Categories of leaf apical shape



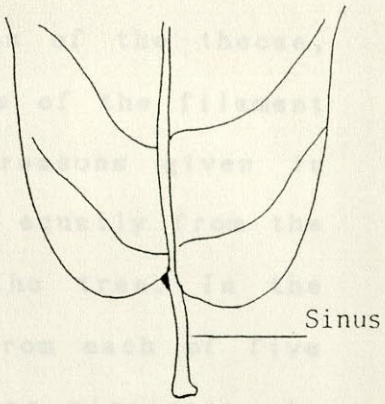
i - ≤90° (acute)



ii - >90° (obtuse)



iii - ±180° (rounded)



iv - cordate

FIGURE 3.1.3 Categories of leaf basal shape

3.1.4.0 RECORDING FLOWER DESCRIPTORS

3.1.4.1 METHOD

The methodology employed in flower collection was adopted from Enriquez and Soria (1967b); Engels et al (1980) and Engels (1981). (Refer to Plate 3 for a representation of the cocoa flower and bud (profile).)

In the field, the intensity of flower bud colour was assessed on a scale of 0,3,5,7; depending on the pigment intensity. Approximately thirty open flowers were collected between 7.00 and 11.00 am. Only disease-free flowers with pearl-coloured thecae were used. Flowers open for more than twenty-four hours are recognizable by the brown colouration of the thecae, which progressively moves to the base of the filament (Enriquez and Soria (1967a)). For reasons given in section 3.3.1, flowers were collected equally from the trunk and branches, randomly over the tree. In the laboratory, two stamens were taken from each of five flowers and observed under a binocular microscope to see whether there was a reddish area between the thecae. This was also assessed on a numerical scale as above. The colour of the stamen filament was observed. One staminode was removed from each of the fifteen flowers and measured from apex to base.

Plate 3 The cocoa flower and bud (profile)

A petal was then randomly chosen and removed from each of the fifteen flowers. The disposition of the anthers was noted (figure 3.1.4). The hood was stripped off from the petal and then the ligule was separated from the ribbon, cutting at the exact point of colour change or where the widening of the ligule begins (refer to figure 3.1.5) The ligules were covered with a microscope cover-slip and their lengths and widths were measured with a mm ruler. The colouration of the ligules was also observed. The ribbons and guidelines were then measured.

Sepal reflexion was observed i.e. whether the sepals were horizontal or curved backwards (refer to plate 4). Two sepals were carefully removed with forceps from each of the fifteen flowers, placed with their abaxial surfaces up under a cover-slip and their lengths and widths measured. Subsequently, the sepal length to sepal width ratio was derived. The presence or absence of glandular hairs on the adaxial and abaxial surfaces and the presence of pigment were recorded (refer to plate 5).

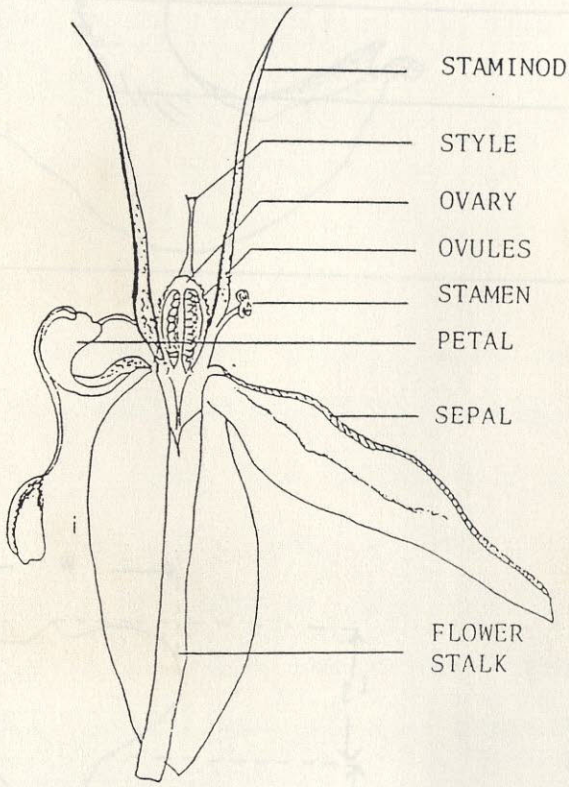
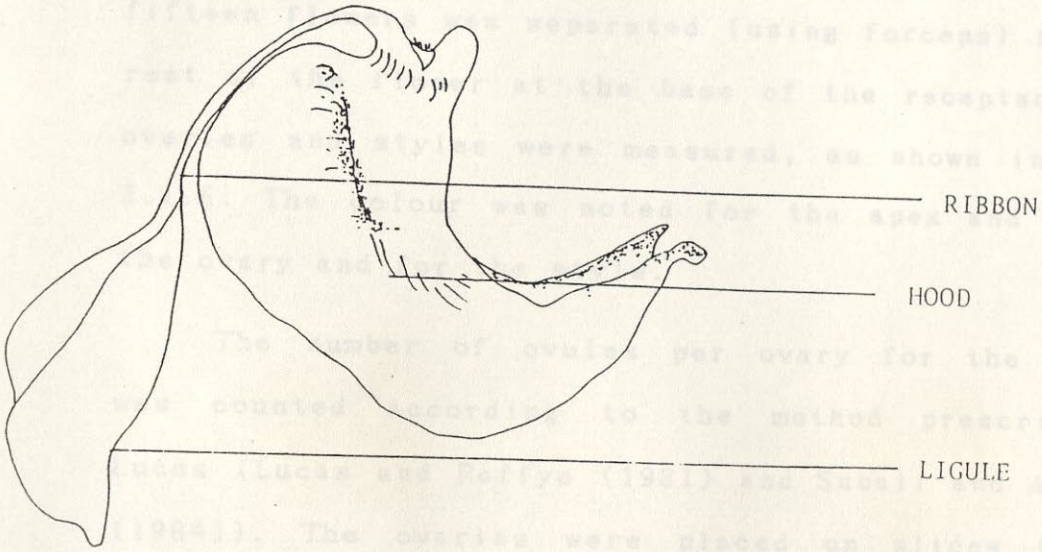


FIGURE 3.1.4 Longitudinal section of the flower of Theobroma cacao L.



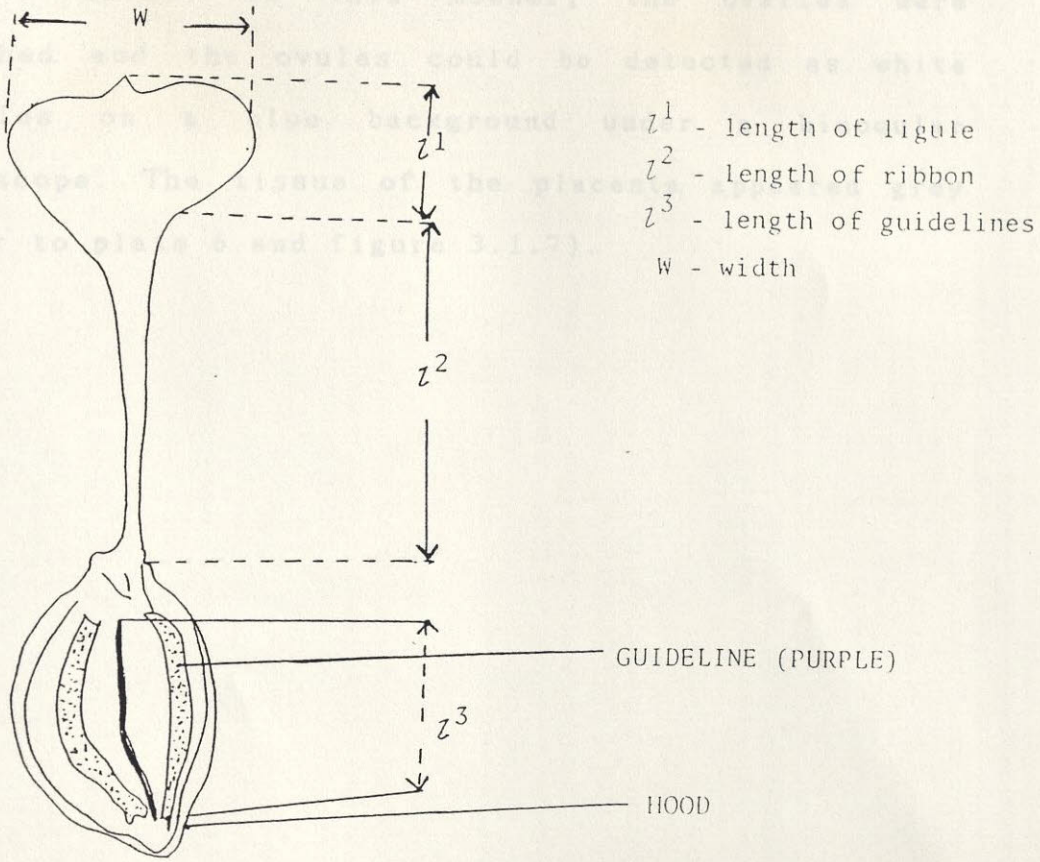


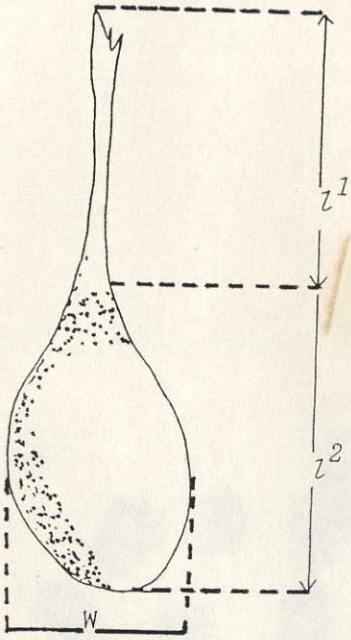
FIGURE 3.1.5 : Cacao flower petal and components; ligule, ribbon and hood

The gynoecium (stigma, style and ovary) of each of fifteen flowers was separated (using forceps) from the rest of the flower at the base of the receptacle. The ovaries and styles were measured, as shown in figure 3.1.6. The colour was noted for the apex and base of the ovary and for the style.

The number of ovules per ovary for the flowers was counted according to the method prescribed by Lucas (Lucas and Reffye (1981) and Subali and Abdullah (1984)). The ovaries were placed on slides (one to three per slide). They were covered with a drop of blue-black ink and the slide was then covered with another slide. In this manner, the ovaries were squashed and the ovules could be detected as white globules on a blue background under a binocular microscope. The tissue of the placenta appeared grey (refer to plate 6 and figure 3.1.7).

Plate 4 Sepal reflexion in the cocoa flower

Plate 5 The range of expression of anthocyanin intensity in the sepals of cocoa flowers (0-absent, 3-slight, 5-intermediate, 7-intense)

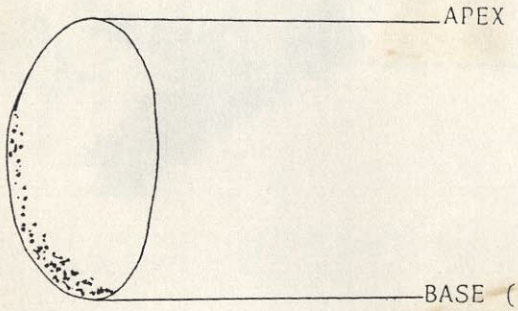


l^1 - length of style

l^2 - length of ovary

W - width of ovary at widest point

OVARY



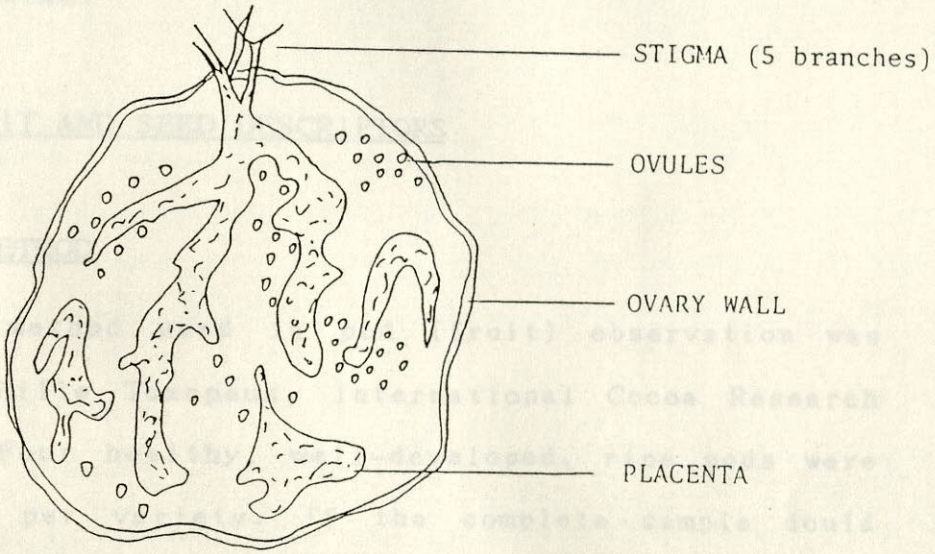
Mag x 16

FIGURE 3.1.6 Diagrammatic representation of ovary and style measurements

Plate 6 Squashed ovary, as seen under a light microscope

(Mx :x100)

OVARY SQUASH



Mag x 25

FIGURE 3.1.7 Diagram showing squashed ovary

The colouration of the pedicel of the flowers was recorded, as well as that of the abscission zone and base. The latter is the point of attachment to the receptacle and the abscission zone is the point of attachment to the tree trunk. The pedicel lengths were then measured.

3.1.5 FRUIT AND SEED DESCRIPTORS

3.1.5.1 METHOD

The method used in pod (fruit) observation was that of Hille Toxopeus, International Cocoa Research Project. Four healthy, well-developed, ripe pods were harvested per variety. If the complete sample could not be obtained at one picking, every effort was made to reach the full complement within one harvest period. Pods were considered mature when the pulp surrounding the beans (seeds) was semi-liquid and before the beans sprouted.

The weights of the individual pods were recorded, using a Sartorius balance (2000 gm capacity), after the pods had been assessed for size, shape, shell hardness and colour and their lengths and widths taken (refer to figure 3.1.8a-d and plates 7-10). The pod length to width ratio was subsequently derived.

The pods were then opened and the wet bean mass

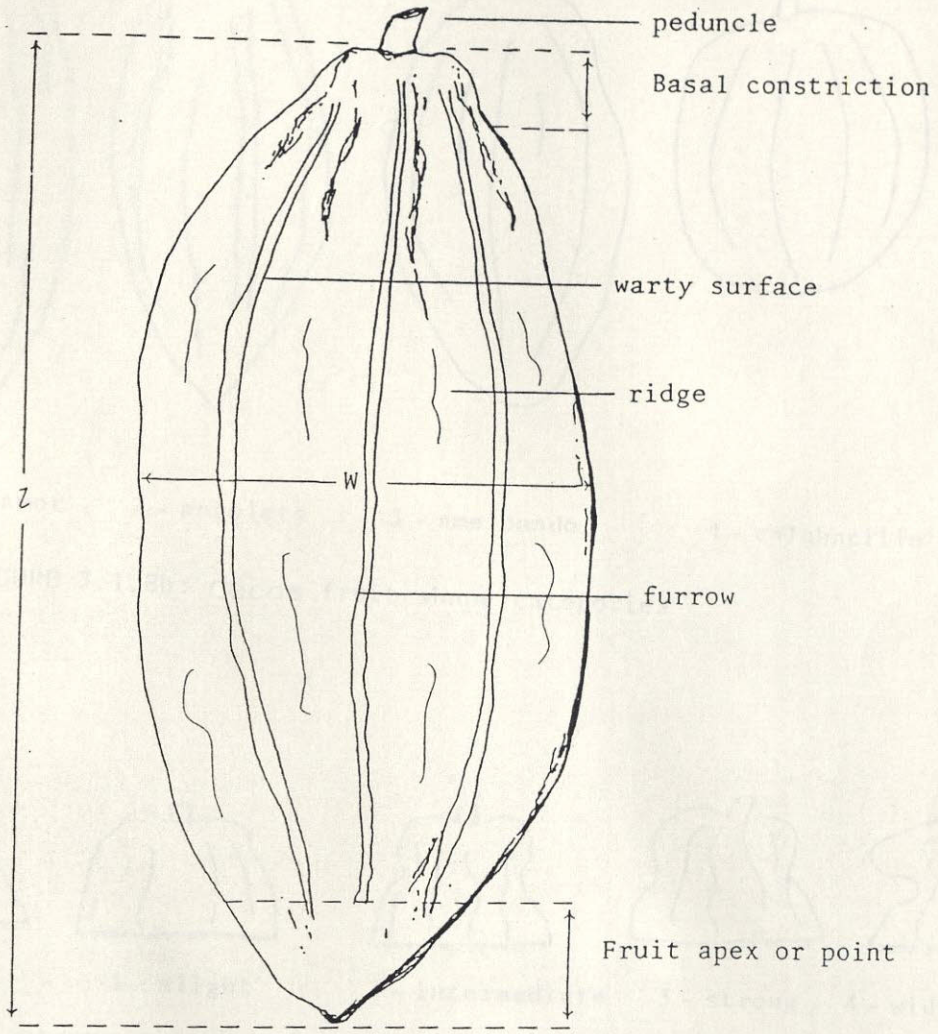


FIGURE 3.1.8a Fruit morphology of *T. cacao* L. and measurements taken

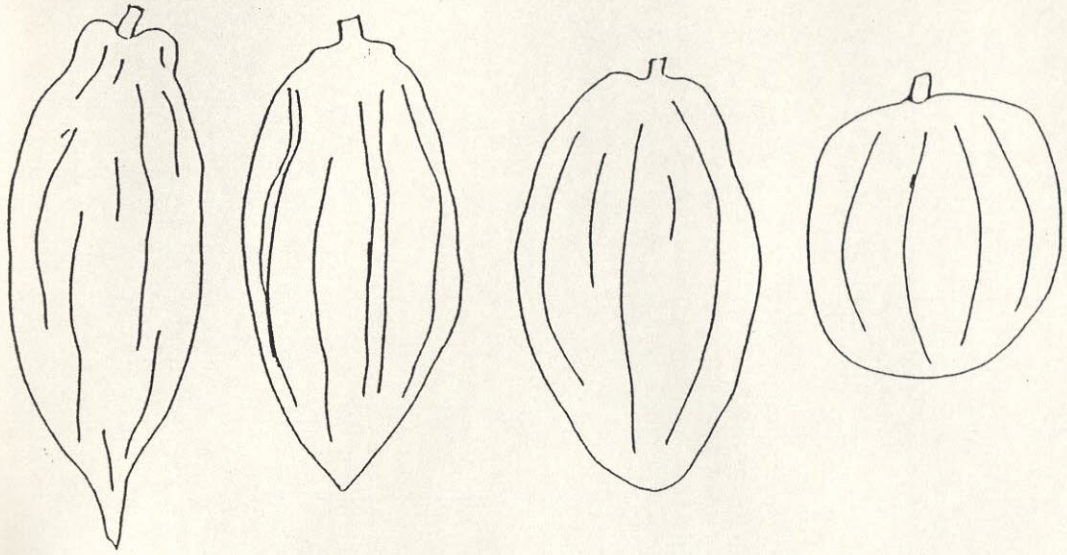


FIGURE 3.1.8b: Cocoa fruit shape categories

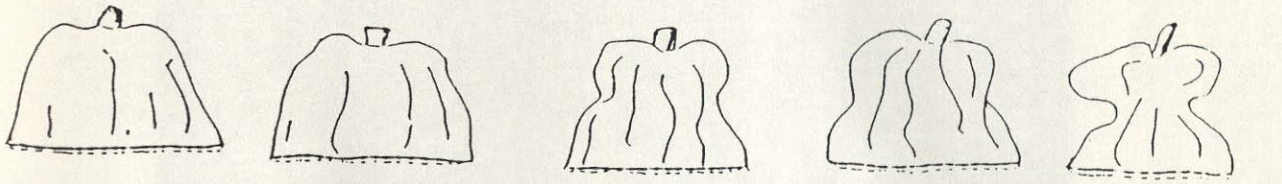


FIGURE 3.1.8c: Cocoa fruit basal constriction categories

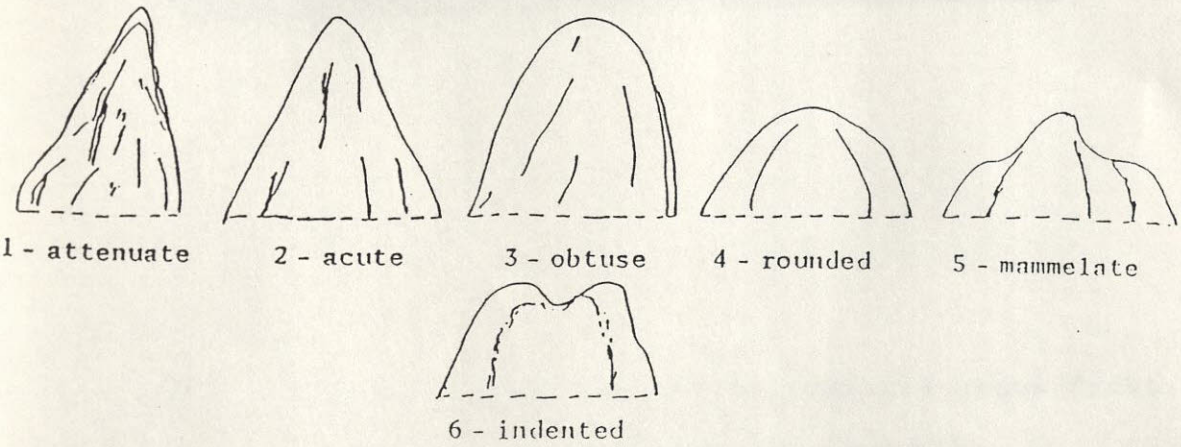


FIGURE 3.1.8d: Cocoa fruit apex shape categories

Plate 7 Colour categories of the immature cocoa fruit: (green (1), reddish (2), red (3))

Plate 8 Colour categories of the mature cocoa fruit: (0-yellow / no anthocyanin, 3-slight anthocyanin, 5-intermediate anthocyanin, 7-intense anthocyanin)

Plate 9 Fruit shape categories : (ICS 95-cundeamor, ICS-angoleta, IMC-amelonado, Catango-calabacillo)

Plate 10 Fruit surface texture (roughness) categories : (smooth (0), slight (3), intermediate (5), rough (7))

Cross Section of a Mature Pod

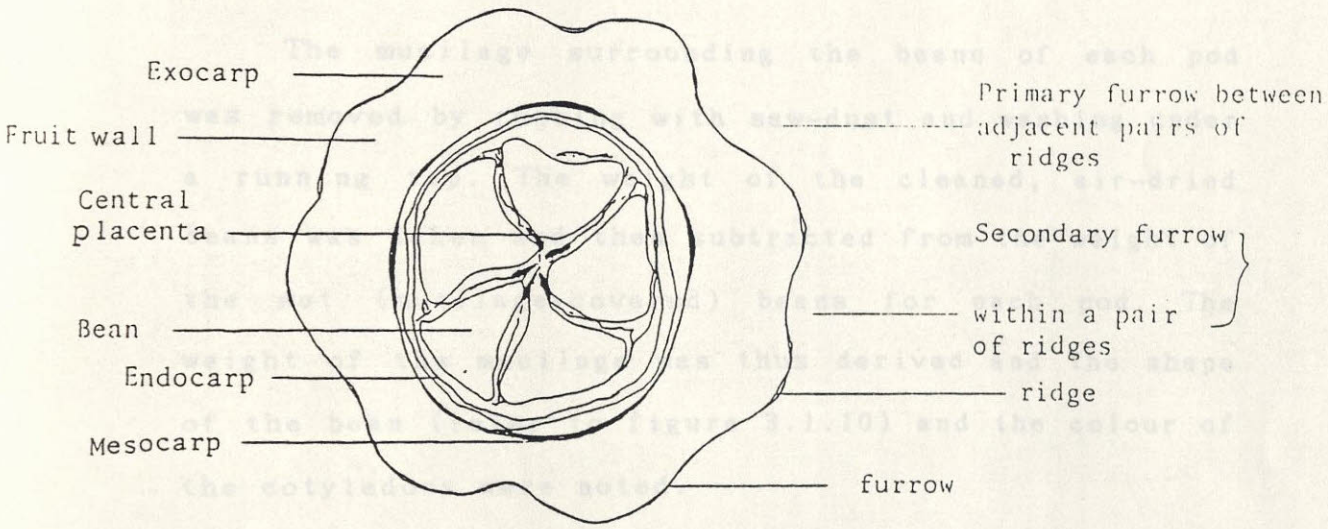


FIGURE 3.1.9 Transverse section of the cocoa fruit showing the Central Placenta and other components

removed and separated from the central placenta (refer to figure 3.1.9). The weight of the central placenta was found and then that of the mass of wet seeds per pod. The husk (shell) weight was recorded, as well as the fruit wall thickness, the furrow depths and the mesocarp thickness.The number of beans per pod was counted (excluding flats) and the colour of the pulp noted: whether it was pale yellow or cream, creamish or white.

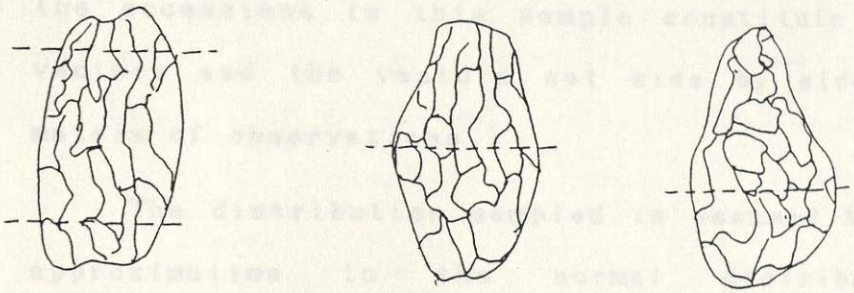
The mucilage surrounding the beans of each pod was removed by rubbing with saw-dust and washing under a running tap. The weight of the cleaned, air-dried beans was taken and then subtracted from the weight of the wet (mucilage-covered) beans for each pod. The weight of the mucilage was thus derived and the shape of the bean (refer to figure 3.1.10) and the colour of the cotyledons were noted.

Twenty beans, randomly selected from each pod, were then put together to dry in an oven at 105^{o}C for twenty-four hours. The weight of the eighty oven-dried beans was determined thereafter.

The testas or seed-coats were peeled off the dried beans and the weight of the eighty oven-dried, peeled beans was found. The weight of the testas was then calculated and the testa percentage derived.

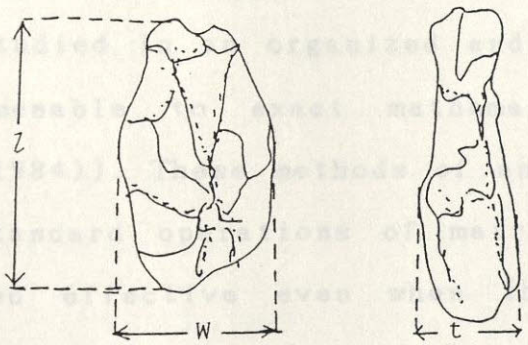
The individual weights of the eighty peeled beans

were then recorded and their lengths, widths and thicknesses measured using vernier callipers (refer to figure 3.1.11).



1 - oblong 2 - elliptic 3 - ovate

FIGURE 3.1.10 Cocoa seed shape categories



l - length
W - width .
t - thickness

FIGURE 3.1.11 Measurements taken from the cocoa seed

3.1.6 MATHEMATICAL ASSUMPTIONS APPLIED IN ANALYZING THE DATA

Multivariate statistical analyses based on the normal distribution were employed to analyze this multivariate dataset. (The set of observations on all the accessions in this sample constitute a sample of vectors and the vectors set side by side comprise a matrix of observations.)

The distribution sampled is assumed to be a close approximation to the normal distribution. This probabilistic model approximates the distribution of continuous measurements in many sampled populations such as these.

Multivariate statistical techniques based on the normal distribution are extensively developed, can be studied in an organized and systematic manner and are amenable to exact mathematical treatment (Anderson (1984)). These methods of analysis are mainly based on standard operations of matrix algebra and are useful and effective even when the distibution sampled is only approximately normal.

3.2 AN ASSESSMENT OF THE USEFULNESS OF CERTAIN COCOA DESCRIPTORS

3.2.1 INTRODUCTION

The importance of proper documentation and the value of descriptor lists in genebanks were explained in section 2.1. To facilitate the identification of accessions, characterization data (refer to section 2.1.1) must include highly heritable, stable and easily measured characters. With regard to cocoa, qualitative (morphological) characters such as fruit colour and shape, which have little agronomic value, tend to be more highly heritable than quantitative (metric) characters (such as pod (fruit) weight, bean (seed) weight and number) and are therefore taxonomically important (Engels (1986)). However, the latter usually have higher agro-economic value. Thus it is important to examine their usefulness in the characterization of cocoa germplasm and in breeding.

During this study, an attempt was made to assess the usefulness in differentiating between clones of certain quantitative leaf, flower and fruit descriptors of cocoa. This was done using Analysis of Variance (ANOVA) to determine the intraclonal and interclonal variability of individual descriptors. An approximately normal distribution of the data was

assumed (refer to section 3.1.6).

Descriptors with high interclonal and relatively low intraclonal variability are considered as most discriminative (Enriquez and Soria (1967a) and Engels (1981)). Among these descriptors, those which are not very difficult to measure or observe are favoured.

3.2.2 MATERIALS AND METHODS

Leaf and fruit data for fifty-five clones and flower data for fifty-three clones were collated at St. Augustine. The clones involved in this study belonged to the following families: EET (Ecuador), E (Ecuador), ICS (Trinidad), IMC (Peru), SCA (Peru) and SPA (Colombia). There is thus a representation of clones of fairly diverse origins; a suggested condition for such a study according to Enriquez and Soria (1967a).

The number of observations recorded for the leaf descriptors was fifteen. Thirty observations were made for sepal length, width and pedicel length and fifteen for the other flower descriptors. There were four observations for all the fruit characters except individual bean weight, bean length, width and thickness, for which eighty observations were made.

The ANOVA procedure employed to achieve the stated objectives was that of SAS Version 82.3 (SAS

(Statistical Analysis System) Institute Inc., North Carolina, U.S.A). This analysis was applied to the raw data, which was collected as described in Section 3.1. The model being tested by ANOVA is that any variation in the expression of the individual descriptors is due to genetic effects i.e. those of clone or family (refer to appendix 4). This may be expressed by the following equation:

Variation in a given variable (descriptor) is genetic variation which can be represented as follows;

$$y_{ik} = \mu + d_i + \epsilon_{ik} \qquad 3.2.1$$

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.

However, variation due to other factors such as environment and genotype-environment interaction is not included in this model. Since the data was collected at one location, variation due to the above factors is expected to be low or insignificant. Only differing degrees of shade, resulting from the different positions of the trees, and the "edge effect", which may lead to varying temperature, light

and wind conditions, are expected.

The possible consequences of these factors are explored in Section 3.5.

The R square value (R^2) calculated is used to measure how much variation in the dependent variable can be accounted for by the model. The larger the value of R^2 the better the model's fit.

The F value tests how well the model as a whole, after adjusting for the mean, accounts for the dependent variable's behaviour. (If the significance probability is small this indicates significance.)

The coefficient of variation (c.v.) is used to describe the amount of variation in the population. (Snedecor and Cochran (1967)). A very large c.v may indicate that there has been experimental error or that there is much inherent variability in the expression of a given character.

3.2.3 RESULTS

Almost all the variables studied were found to show very 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, for all but two cases. The latter involved interfamilial variation for ovary diameter and washed bean weight, whose

Table 3.2.1 ANOVA results for the St. Augustine data.

VARIABLE*	MEAN (S.E.)	TOTAL df	F	R^2	CV
TL (cm)	24.8 (2.5)	824	9.5	0.40	9.9
BL (cm)	11.9 (2.3)	824	4.8	0.25	19.6
W (cm)	8.8 (1.0)	824	6.5	0.31	11.2
LWR	2.9 (0.2)	824	9.7	0.40	8.4
AA (°)	62.8 (6.0)	824	16.1	0.53	9.5
BA (°)	105.2 (8.3)	824	70.7	0.83	7.9
SW (mm)	2.2 (0.16)	1589	51.9	0.64	7.2
SL (mm)	7.7 (0.34)	1589	97.8	0.76	4.5
LIL (mm)	3.0 (0.21)	794	43.9	0.75	7.2
LW (mm)	2.3 (0.23)	794	29.9	0.68	9.9
GL (mm)	2.4 (0.21)	794	54.8	0.79	8.8
RL (mm)	3.1 (0.23)	794	42.8	0.79	7.3
STL (mm)	5.7 (0.28)	794	54.0	0.79	4.9
OL (mm)	1.8 (0.14)	794	27.6	0.66	7.6
OD (mm)	1.0 (0.08)	794	5.6	0.28	7.5
ON	41.4 (3.9)	794	24.5	0.67	9.6
STYL (mm)	2.1 (0.14)	794	24.3	0.63	6.6
PL (mm)	14.5 (1.4)	1589	35.2	0.54	9.5
POL (cm)	16.9 (1.7)	219	9.4	0.75	9.8
POW (cm)	8.2 (0.54)	219	10.7	0.78	6.5
PWT (mm)	12.3 (1.14)	219	27.1	0.89	9.3
FD1 (mm)	6.3 (0.77)	219	42.0	0.94	12.2
FD2 (mm)	4.3 (0.6)	219	53.0	0.95	13.9
POWT (g)	481.6 (113)	219	7.4	0.71	23.5
PLW (g)	8.9 (3.3)	219	7.9	0.72	37.7
WBW (g)	118.6 (27.4)	219	7.1	0.69	23.1
HW (g)	355.6 (85.2)	219	9.0	0.75	23.9
WABW (g)	91.9 (31.3)	219	3.0	0.49	34.1
MUW (g)	27.4 (9.9)	219	9.7	0.76	36.5
BEN	42.4 (8.1)	219	3.6	0.54	19.0
BW (g)	1.1 (0.18)	4399	243.9	0.75	17.1
BL (cm)	2.2 (0.16)	4399	156.5	0.66	7.4
BEW (cm)	1.1 (0.13)	4399	150.2	0.67	11.6
BET (cm)	0.6 (0.09)	4399	100.3	0.59	15.3

S.E. = standard error of the sample mean.

The model degrees of freedom (df) (n-1) was 54 and 52 for leaf and fruit descriptors, and flower descriptors, respectively.

Error df = Total df - Model df.

All F values were significant at the 0.1% level.

*Refer to the glossary for an explanation of the acronyms

F values had significance probabilities of 0.02 and 0.001, respectively.

The results in Table 3.2.1 indicate that the ANOVA model does not account for the variation in leaf descriptors as well as it does for the other descriptors.

This is concluded from the relatively low R^2 values for the leaf descriptors; less than 0.5. However, the leaf apical and basal angles did have R^2 values greater than 0.5.

The R^2 values for all flower and fruit variables were greater than 0.5. The exception was ovary diameter, which had an R^2 value of 0.3.

The measure of variation within the population for all the variables was relatively low i.e. the c.v. was less than 40.0. This satisfies the stipulations of Snedecor and Cochran (1967).

The c.v. tended to be higher for the leaf variables than for the flower variables. This supports the findings by earlier workers such as Enriquez and Soria (1967a and c) and Asomaning and Lockard (1963). The c.v. was comparatively high for most of the fruit descriptors. Thirteen out of fifteen of these variables had c.v.'s greater than 10.0. The largest was 37.7 for placenta weight.

Variables with c.v.'s greater than 10.0 were basal leaf length, leaf width, fruit mesocarp thickness, primary furrow depth, secondary furrow depth, pod weight, placenta weight, wet bean weight, husk weight, washed bean weight, mucilage weight, bean number, bean weight, bean width, and bean thickness.

3.2.4 DISCUSSION

From a consideration of the significance of F values in Table 3.2.1, this study indicates that all the descriptors included are useful identifiers for Theobroma cacao L. germplasm collected in North Trinidad. The descriptors satisfy the conditions of Engels (1981) and Enriquez and Soria (1967a) of showing high variation between clones and a comparatively insignificant variation within clones. The univariate analyses of variance revealed significant differences between these accessions and groups (families) of accessions collected from Peru, Colombia and Ecuador.

However, the R^2 and c.v. values must be scrutinized. All the flower descriptors had a c.v. of less than 10%. All the leaf descriptors, except that for basal leaf length and leaf width, also had c.v.'s of less than 10%. Only four of the fruit descriptors; pod length, pod width, pod wall thickness and bean

length, satisfied this condition, however. According to Enriquez and Soria (1967a), a c.v. of 10% is acceptable for a character to be used confidently in description. This prerequisite may be used to determine the order of usefulness of the descriptors.

Another criterion that may be used in assessing the reliability of descriptors is the R^2 value, defined in section 3.2.2. If this value is closer to one than to zero, then the variation expressed for any given descriptor has a genetic basis.

The leaf descriptors, except the apical and basal angles, all have an R^2 value less than 0.5. This is surprising because of the high significance probabilities associated with them. However, the variability of these descriptors may thus be better explained using environmental factors along with a genetic one. Goodall (1947) and Asomaning and Lockard (1963) have also recognized the role of environment in determining the expression of leaf characters. The effectiveness of leaf descriptors as distinguishing characteristics is therefore questionable. However, the basal and apical angles have been shown to be better differentiating characteristics than linear leaf criteria. Furthermore, the ratio of both linear dimensions appeared to be of slightly greater descriptive value than the original measurements. (R^2 =

0.404 as compared to R^2 < 0.4) Asomaning and Lockard (1963) made similar observations.

It may be concluded that leaf descriptors should not be used exclusively in descriptor lists.

Almost all the flower descriptors seem to be of great value. This has also been found by Ostendorf (1956); Engels (1981); and Enriquez and Soria (1967a). The R^2 values and c.v.'s obtained in this study support this.

Ostendorf (1956) and Engels (1981) commented on the usefulness of ovary diameter as a descriptor. This study does not clearly support this finding. Although the c.v. was less than 10%, the R^2 value of 0.3 was low.

Enriquez and Soria (1967a) concluded that the length of the main guidelines was the most reliable flower descriptor. In this study, it had the highest R^2 value (0.8), along with staminode length, and a c.v. of 8.8. However, the latter descriptor had a lower c.v. (4.9) and so the finding of Enriquez and Soria (1967a) is only partially supported.

Bartley (1964) noted that ovule number "varies considerably depending on variety." Martinson (1976) found neither season or position of the flower on the tree to have any significant influence on the ovule

number. However, she did find that the number was not constant throughout the year. The results of this study support the first observation. The effect of season on the number of ovules was not investigated. The ovule numbers recorded were, however, not as high as those mentioned in the literature (fifty for trinitarios and as much as seventy-seven for forasteros). Therefore it may be important to note where and when the flowers were collected and to exert care when counting the ovules.

Seed number had an R^2 value of 0.5 and a c.v. of 19.0 compared to an R^2 value of 0.7 and a c.v. of 9.6 for ovule number. The latter descriptor is more reliable. Its value was also noted by Lopez et al (1988), who described it as a better indicator of yield than the number of seeds per fruit, which they found to be affected by environment.

The sample sizes used for flower collection are relatively moderate compared to those largely recommended for pods (Engels (1981)). This facilitates collection. The measurement of these descriptors does involve laboratory procedures, but their inclusion in descriptor lists is validated by this study.

Indeed, Ostendorf (1956) proposed that the cocoa flower can be used as the "most effective character for distinguishing between clones." This study

strongly supports this statement.

Pods are described as the most important organs of cocoa because of their commercial value (Cheesman (1944); Wood (1975); Cope, in Simmonds (1976); Soria (1977)). It may thus be important that the pod descriptors showed significant variation between the clones studied. However, there was considerable variation in the population studied for most fruit descriptors. This was also found by workers including Engels (1981); Enriquez and Soria (1966); Ostendorf (1956); Pound (1933); Ruinard (1964); and Soria (1977). Engels (1981) calculated c.v.'s between 5.4 and 35.4.

Toxopeus (1981) concluded that bean characters were more constant than external fruit characters. There was no clear support for this observation from the results of this study.

It appears that no reliable conclusions can be reached by "judging the insides of pods alone", as was stated by Hart (1893), cited by Pound (1932).

This study indicates that fruit wall thickness, pod length, diameter or width, and total weight are reliable pod descriptors, as was found by Ostendorf (1956); and Enriquez and Soria (1966) (refer to table 3.2.1 for the R^2 values and c.v.'s). Pod width was found to be slightly less variable than pod length.

Pound (1932) had a similar finding. Despite very high R^2 values, all the other external pod descriptors may not be considered very acceptable identifiers because of their high c.v.'s.

Among the bean descriptors, bean weight had the highest R^2 value (0.8). Kuppers (1953) concluded that this descriptor is the most dependable index of bean size. Pound (1932) also remarked on its usefulness as an identifier. The c.v. in table 3.2.1 (17.0) appears to detract from the validity of these findings.

Bean number and thickness had the lowest R^2 values among the bean descriptors. However, these values exceeded 0.5. The c.v.'s for both descriptors were less than 20%. Thus they may be useful identifiers, as was found by Glendinning (1963).

Bean length was the only bean descriptor with a c.v. less than 10% and with a R^2 value of 0.7, it thus may be the best bean descriptor. Ghosh (1973) and Pound (1933) found linear bean descriptors to be less variable than bean number. This study supports the above observation. In order of usefulness, this study ranks bean descriptors as bean length, bean weight, bean width, bean thickness, and bean number.

3.2.5 SUMMARY

(1) All the descriptors showed significant

variation between individual clones (within families) and between families of clones. This was inferred from the F values ($P <$ or $= 0.0001$). (The reliability of the descriptors is tempered by the size of c.v. and the R^2 values.) Thus they are useful for differentiating among the numerous accessions studied. The populations involved were of diverse origins (refer to Table 2.1) and it may be useful to conduct a similar study using fewer, very closely related accessions to determine which descriptors are discriminative, under such conditions. These descriptors will be most useful taxonomically.

(2) The flower descriptors (quantitative) may be described as being the most reliable cocoa descriptors from this study. The staminode length and guideline length are two of the best identifiers.

(3) Pod (fruit) and bean (seed) descriptors were found to be discriminatory (high R^2 values), but they were rather variable within the population, as a whole. This detracts from their descriptive value. Bean length, fruit length, fruit width and fruit wall thickness were the most reliable fruit descriptors. The use of larger sample sizes should reduce the c.v.'s of the other fruit descriptors.

(4) Leaf descriptors showed less variability within the population than pod descriptors. However,

the low R^2 values for all leaf descriptors, except basal angle (0.8) and apical angle (0.5), indicate that factors, apart from genetic ones, were responsible for the variation displayed between clones. These descriptors should thus not be used exclusively.

(5) In order to fully test the reliability of these descriptors, it is necessary to evaluate their heritability and the impact of environment (if any) on their expression (refer to section 2.1.1). The former task should be performed by a breeder and has been done to some extent previously by workers such as Pound (1933 and 1935) and Engels (1986) and is described by Soria (1977) and Bartley (1981). An attempt was made during this study to assess whether the quantitative descriptors are stable under different environmental conditions (refer to section 3.5).

3.3. THE DETERMINATION OF RELATIONSHIPS WITHIN GROUPS OF COCOA DESCRIPTORS

3.3.1 INTRODUCTION

3.3.1.1 GENERAL

In order to further evaluate the usefulness of the cocoa descriptors under study, it was necessary to establish whether any correlations exist between them. The existence of such correlations might enable some descriptors to be disregarded because of their association with other more reliable descriptors.

Engels et al (1980) found that the peduncle colour of flowers, pigmentation of the inferior part of the ovary, pigmentation of the superior part of the style and the colour of the ridges and furrows of ripe pods were correlated. Engels (1983a) found that the discriminative values (Dw) for seed length, seed width, seed width/length ratio, wet seed weight, dry seed weight and sepal length were "diminished" because of the correlation of these descriptors with style length, a reliable and easily measured descriptor.

Subali and Abdullah (1984) found a very significant correlation between ovule length and width and the number of seeds per pod, mean dry seed weight, mean seed length, width and thickness. Since ovule

number is a very stable character, controlled by genetic factors (Lucas and Reffye (1981)) and the variation in the number of seeds per pod is due mainly to environmental effects (Toxopeus and Wessel (1970) and Atanda (1972)), to pollen source (Toxopeus and Jacob (1970)) and to the availability of pollination agents (Soria (1981)), then the ovule descriptors are more valuable.

Correlation studies on fruit or pod and seed descriptors supply a better understanding of fruit growth and may be of interest for selection work (Eskes et al (1977)).

3.3.1.2 CORRELATION COEFFICIENTS

Correlation coefficients can be used to determine taxonomic resemblance, as described by Sneath and Sokal (1973); and Legendre and Rogers (1972). By definition, they range from -1 to +1. The coefficient computed between operational taxonomic units (OTUS), "j" and "k" is defined as follows:

$$r_{jk} = \frac{\sum_{i=1}^{n} (x_{ji} - \bar{x}_j)(x_{ki} - \bar{x}_k)}{\sqrt{\sum_{i=1}^{n} (x_{ji} - \bar{x}_j)^2 \sum_{i=1}^{n} (x_{ki} - \bar{x}_k)^2}} \qquad 3.3.1$$

where x_{ji} = value of observation i in OTU j

and $\bar{x}_j$ = mean of all values for OTU j.

n = number of taxonomic units (e.g.clones sampled).

The coefficient, given above, is the Pearson product-moment correlation coefficient, which is most frequently employed in numerical taxonomy (Morishima (1969)) and was used in this study.

3.3.1.3 THE NATURE OF CORRELATIONS

A correlation found between two characters, in a study like this may be explained either as a valuable correlation, corresponding to a correlation between characters, the expression of which would be regulated by environmental factors, or as an undesirable redundancy. The latter may be due to the same structure being unintentionally described twice (Legendre and Rogers (1972)); to the fact that one character is a refinement of the other or to the correlation's dependence on the influence of environmental factors. Biological knowledge of the material under study is thus vital in sorting out the significance of correlations.

3.3.2 MATERIALS AND METHODS

Data was collected at two sites; the St. Augustine Campus and the St. Joseph Field Station. Fifty-three clones representing six families were studied at St. Augustine and thirty-one clones involving four families (ICS, IMC, SCA and SPA) at St.

Joseph using the methodology described in Section 3.1. Mean values were used to calculate the correlation coefficients between variables.

The correlation coefficients were calculated using the Correlation Procedure of SAS Version 82.3. Three individual analyses were carried out on quantitative leaf, flower and fruit descriptors. The manner of data collection, outlined in Section 3.1, made it statistically unsound to analyze the data collectively since it would be impossible to separate within and between tree correlations.

3.3.3 RESULTS AND DISCUSSION

Table 3.3.1 shows the significant correlation coefficients found to exist between certain leaf descriptors of cacao. The results for the St. Augustine and St. Joseph data suggest that it is not necessary to measure both total and basal leaf lengths. This may be an example of the redundancy referred to by Legendre and Rogers (1972).

The linear leaf measurements are highly correlated with one another at both sites. The angular measurements are correlated with one another in St. Joseph, but are not in St. Augustine. The correlations between the angular leaf descriptors at St. Joseph were significant at the 0.05 level.

At both sites, one angular leaf measurement; basal angle, was significantly correlated with at least one linear descriptor. The latter was total length in St. Augustine and total and basal lengths and leaf width in St. Joseph.

Many significant correlations were found among the flower descriptors. Sepal length and sepal width; sepal length and ligule length; sepal length and ribbon length; staminode length and ribbon length; staminode length and sepal length; and staminode length and peduncle length were pairs of descriptors, which all showed significant correlations at the both sites (refer to Table 3.3.2). These are most probably genetic correlations and the characters involved are not redundant or refinements of others (refer to section 3.3.1.2).

Table 3.3.1 Correlations between pairs of leaf descriptors*1 based on clonal means of St Augustine and St Joseph data.

DESCRIPTORS		PCC	DESCRIPTORS		PCC
ST AUGUSTINE			ST JOSEPH		
TL	W	0.62***	TL	W	0.70***
			BL	W	0.59**
TL	ABR	0.35*	BL	BA	0.43*
TL	BA	0.37*	TL	BA	0.40*
BL	W	0.66***			
			W	BA	0.51*
			BA	AA	0.73***

Prob > |R| under H_o : RHO = 0 * 5% significance level

** 1% significance level *** 0.1% significance level

PCC Pearson Correlation Coefficient *1Refer to the glossary

Table 3.3.2 Correlations between pairs of flower descriptors based on clonal means of the St Augustine and St Joseph data.

DESCRIPTORS		PCC	DESCRIPTORS		PCC
ST AUGUSTINE			ST JOSEPH		
SL	SW	0.30*	SL	SW	0.60**
SL	LIL	0.60***	SL	LIL	0.56**
SL	RL	0.48**	SL	RL	0.50**
STL	SL	0.47**	STL	SL	0.58**
STL	PL	0.36*	STL	PL	0.58**
SW	LW	0.48**	SW	RL	0.48**
SL	LW	0.45**	AVPL	LIL	0.64***
LIL	LW	0.49**	AVPL	RL	0.70***
STL	RL	0.45**	AVPL	GL	0.70***
SW	OL	0.45**	STL	SW	0.64***
STL	AVPL	0.49***	STL	RL	0.70**
ON	AVPL	-0.48**	ON	LW	0.66***
STL	PL	0.36**	ON	GL	0.70***
OL	STYL	0.46**			

Prob > |R| under H_o : RHO = 0 * - significance at the 5% level

** - significance at the 1% level *** - significance at the 0.1% level

Significant correlations between the pod and bean descriptors are given in Table 3.3.3. There were several correlations, that occurred at only one site. These could not be purely genetic since they were subject to environmental effects. However, many correlations occurred at both of the sites studied and are considered to be controlled by genotype.

Pod length was positively correlated with five other pod descriptors (refer to Table 3.3.3). Pod width was correlated to six other descriptors, one being bean length. There were three correlations involving pod wall thickness; two involving mesocarp thickness; one for primary furrow depth; nine for pod weight; three for placenta weight; eight for seed weight; four for mucilage weight; eleven for husk weight, including those with bean length, bean number and bean weight; and four for washed bean weight. Bean number was correlated with two other descriptors. Bean length had nine correlations with other fruit descriptors. Bean width was correlated with four other bean descriptors. Bean thickness had no correlations. There were five correlations involving bean weight and four for peeled bean weight.

Pod length was correlated with pod width and pod weight, among other descriptors. The correlation with pod weight was also found by Ostendorf (1956).

Table 3.3.3 Correlations between pairs of fruit and seed descriptors based on clonal means of the St. Augustine (St Aug) and St. Joseph (St Jos) data.

DESCRIPTORS		PCC ST AUG	PCC ST JOS
POL	POW	0.50***	0.56**
POL	PLWR	0.69***	0.36*
POL	POWT	0.68***	0.69***
POL	HW	0.68***	0.68***
POL	WABW	0.50***	0.47**
POW	PWT	0.54***	0.80***
POW	POWT	0.90***	0.60***
POW	WBW	0.67***	0.67***
POW	HW	0.89***	0.86***
PWT	MESOTH	0.67***	0.62***
PWT	POWT	0.56***	0.77***
PWT	HW	0.66***	0.78***
MESOTH	HW	0.50***	0.55**
PFD	SFD	0.86***	0.89***
POWT	PLW	0.64***	0.66***
POWT	WBW	0.74***	0.83***
POWT	HW	0.96***	0.99***
POWT	WABW	0.50***	0.70***
PLW	HW	0.55***	0.66***
WBW	HW	0.58***	0.74***
WBW	WABW	0.79***	0.94***
MUW	POWT	0.66***	0.78***
MUW	WBW	0.75***	0.80***
MUW	HW	0.55***	0.70***
BL	POW	0.60***	0.83***
BL	POWT	0.60***	0.70***
BL	WABW	0.58***	0.56***
DBWT	PBWT	0.90***	0.99***
DBWT	BEW	0.60***	0.67***
PBWT	BW	0.98***	0.90***
PBWT	BL	0.65***	0.70***
PBWT	BEW	0.64***	0.67***
HW	BEN	0.33*	0.43*
HW	BW	0.30*	0.57**

Prob > |R| under H_o : RHO = 0

* -significance at the 5 % level

** -significance at the 1% level *** -significance at the 0.1 % level

It seems reasonable to suggest that pod length is a good indicator of pod size and weight. Its inclusion in a descriptor list may allow the exclusion of the descriptors with which it is correlated. The results of Section 3.2 already indicated that it might be one of the most reliable pod descriptors.

Pod weight showed obvious correlations with the weights of individual pod components such as placenta weight, wet bean weight, husk weight and washed bean weight. The second correlation was also found by Atanda (1972); Ostendorf (1956) and Ruinard (1964). The weighing of all these pod components may not be of any additional value.

Bean length, a reliable descriptor according to the results in Section 3.2, was correlated with pod weight, individual components of pod weight, pod width, average bean weight and bean width. Its inclusion in a descriptor list for cocoa is recommended and may preclude that of related descriptors.

Bean number was correlated with wet bean weight (a finding also of Eskes et al (1977) and Ostendorf (1956)) and to husk weight. It could diminish the need for including the latter characters in the descriptor list.

The correlation between total dried bean weight

(eighty beans) and average bean weight was also found by Eskes et al (1977); Kuppers (1953); Ruinard (1961); Toxopeus and Wessel (1970) and Van der Knaap (1953). It may be unnecessary to measure both characters.

As expected, average peeled bean weight was correlated with total peeled bean weight; a finding shared with Ostendorf (1956). However, there was an unexpected lack of correlation between the latter and the bean testa percentage. A negative correlation was expected since the bean surface should increase less than the bean volume with increasing bean weight (Eskes et al (1977); Alvarado and Bullard (1961)).

Mature pod and bean length have been found to be correlated with many of the other pod and bean descriptors. Indeed, from this limited study, they may even be described as useful in representing all the physical attributes of the cocoa fruit. Their inclusion in a cocoa descriptor list is thus recommended.

There is no reference to correlations among leaf descriptors in the literature and thus no basis for comparing the results of this study. The latter produced several findings, which may warrant further investigations, perhaps using other cocoa populations. However, the results indicate that certain leaf dimensions such as total leaf length and apical and

basal angles are useful quantitative, leaf descriptors. Asomaning and Lockard (1963) also made this observation. Since total leaf length is correlated to basal angle and leaf width, its inclusion in a descriptor list may preclude that of the latter two descriptors.

In Section 3.2, all the floral descriptors were found to be useful. Staminode length and guideline length were among the most useful. The results of this correlation analysis revealed that staminode length and sepal length were correlated with several other descriptors at both sites studied. Since the relationships between these descriptors must almost certainly be genetic, they are valuable. No correlation was found between sepal length and style length and because correlation analysis was not performed using all the descriptors collectively, the findings of Engels (1983a) concerning the importance of style length would have to be verified using Principal Component Analysis (PCA).

Pod and bean descriptors were seen to form groups of closely related descriptors. This is supported by the work of Engels (1983c); Glendinning (1963), Subali and Abdullah (1984) and Ruinard (1961). Bean size (length, width) was more indicative of dry cocoa production (total peeled bean weight) per fruit than

was bean number. This agrees with the findings of Kuppers (1953); Lachenaud (1984) and Ruinard (1961). Pod dimensions were correlated with wet bean production per fruit, but not with dried cocoa production.

Bean number per pod was found not to correlate between clones with pod size (length, width or weight). This was also found by Engels (1983c), who did find a positive correlation between the two within clones, however. Glendinning (1963); and Toxopeus and Jacob (1970) found a correlation between the two descriptors between clones.

This study can be useful in determining a subset of descriptors that most adequately represents the phenotypic diversity in the populations studied. This subset can then be used to perform multivariate analyses, such as Cluster Analysis (refer to Section 3.6). Burley and Barrows (1972), cited by Engels (1983c); and Goodman (1968) recommend the use of only a few characters in these analyses. The performance of correlation analysis can also be useful to the breeder endeavouring to select superior combinations of characters. Thus a study, such as the one described in this chapter, is very valuable.

3.3.4 SUMMARY

A subset of valuable descriptors, that is indicated by this study, includes total leaf length, leaf apical angle, petal length, staminode length, sepal length, ovule number, pod or fruit weight, pod or fruit length, total bean (seed) weight, individual bean length and bean width.

3.4 THE DETERMINATION OF A SUBSET OF USEFUL COCOA DESCRIPTORS USING PRINCIPAL COMPONENT ANALYSIS

3.4.1. INTRODUCTION

In order to identify a subset of cocoa descriptors, which convey the main features of the data collated for the clones observed at St Augustine, Principal Component Analysis (PCA) was performed. Engels (1983c) used Factor Analysis for a similar purpose. With both techniques, there is a danger that the scale of the data matrix will affect the outcome. Standardization of the data can offset this problem (Macabe (1984)).

Using PCA, an attempt was made to reduce the number of quantitative descriptors to less than p, (where p is equal to the total number of descriptors (variables) under study) without losing significant information in the process.

The PCA linearly transforms the variables (x_1, x_2,...., x_p) to new variables y_1,...., y_p, which are referred to as the principal components. The resulting linear combination, which has maximal variance is sought, since a large variance separates out the observations (accessions), making it easy to detect the differences between them. The observations, $x^{(i)}$,

or the vector of the measurements for the ith accession or individual, denoted by $x_{i1}, x_{i2}, \ldots, x_{ip}$, are transformed to corresponding principal component scores $y^{(i)} = (y_{i1}, \ldots . y_{ip})$ (Mardia et al (1979) and Krzanowski (1987)).

3.4.1.1 REDUCING THE DIMENSIONS OF THE DATA

Dimensionality reduction is effected if a subset (q) of the principal components conveys most of the sample information inherent in the total number (p) of original variables. The original observations, $x^{(i)}$, can be replaced by the first q elements of the corresponding principal component scores. If the subset (q) contains two principal components, then a plot of their scores can be made and would approximately show the relative groupings of the accessions under study (Krzanowski (1987)).

The principal components are computed from the covariance or correlation matrix. When all the original variables are highly variable, the principal components are evaluated for the transformed or standardized variables, each of which has a mean of zero and a variance of one. The correlation matrix can be used when the variables have to be transformed.

Procrustes Analysis may then be used as a cross-

validatory technique to determine how well the subset/s of data capture the group structure of the complete data. Such a model-based approach will remove the subjectivity involved in trying to select variables based on the coefficients in the first few principal components. The principal component plots of the data for the full complement and the selected subset of variables can be compared by finding the sum of squared differences between corresponding points (representing the various accessions) of the two configurations (Krzanowski (1987)).

3.4.2 METHOD

The data set subjected to PCA contained the average values of fifty-three accessions for thirty-five quantitative variables (refer to Table 3.6.1). All the variables included in the study could not be used because of the limitations of computer memory. Consequently, the thirty-three qualitative variables were excluded. Since the quantitative variables were highly variable, PCA was effected by finding the latent roots (eigenvalues) and vectors of the correlation matrix of the data. The vector loadings (weights) of all the principal components were derived and the corresponding contributions of each variable are specified (as in Table 3.4.2).

Table 3.4.1 Percentage variation attributed to the first 10 principal components for 35 quantitative variables.

Component	1	2	3	4	5
Percentage	23.22	17.00	10.57	6.52	5.55
Component	6	7	8	9	10
Percentage	4.85	4.28	3.21	2.93	2.82

3.4.3 RESULTS

The dimensionality of the data could not be taken as two since the first two principal components did not comprise more than 46% of the total variance expressed (refer to table 3.4.1). The first six components (with latent roots all greater than unity) accounted for about 76% of the total variance whereas the first two components should have done this if the sample information were to be represented accurately in two dimensions.

Therefore PCA did not achieve the stated objective of this investigation i.e. of grouping the variables into about four main components with eigenvalues greater than 1 (refer to section 3.4.1.2), as recommended by Jolliffe (1973). The information given by all the descriptors cannot be conveyed by a

small subset of descriptors.

However, a consideration of the vector loadings, relative to the first and second principal components, may give some indication of the major sources of diversity among the accessions (refer to table 3.4.2), but no definite inferences can be made in this instance.

Table 3.4.2 Latent vector loadings of the 1st and 2nd principal components for 21 of the quantitative variables.

Variable*	Latent Vectors loadings (weights)	
	Component 1	Component 2
TL	-0.097	-0.335
W	-0.041	-0.204
AA	0.060	0.067
BA	-0.003	-0.345
SW	-0.103	-0.289
SL	-0.155	-0.003
APLL	-0.142	0.218
STL	-0.114	-0.054
OL	0.037	-0.264
ON	-0.006	-0.298
STYL	0.012	-0.230
POL	-0.301	0.259
POW	-0.293	0.274
POWT	-0.355	0.071
WBW	-0.241	0.231
DBWT	-0.305	-0.033
WABW	-0.311	0.025
BW	-0.316	-0.201
BEN	-0.146	-0.299
BL	-0.339	-0.199
BEW	-0.359	-0.059

* refer to the glossary for an explanation of the acronyms

3.4.4 DISCUSSION

For the sample of accessions studied, PCA was not useful in revealing a small subset of reliable quantitative descriptors. The variation in this germplasm collection has to be attributed to more than four of the quantitative variables.

An indirect approach may be taken to test the usefulness of the subset of descriptors obtained using the correlation procedure described in section 3.3. A comparison of the groupings (clusters) revealed by performing Cluster Analysis using all the descriptors and then a subset/s should indicate whether the subset/s can be used reliably for the purpose of classification of the accessions under study. Such an approach is explained in section 3.6.

Information obtained through PCA may assist plant breeders in identifying a limited number of highly divergent accessions or populations to be selected for further studies (Veronesi and Falcinelli (1988)).

Although the latter was not an objective of this investigation, some interesting results were obtained (refer to table 3.4.3 and figure 3.4.1). Twelve accessions or clones appear to be distinct from the others. The former represent five of the six families (populations) studied (refer to Table 1.1). This suggests that geographical origin is linked to the

Table 3.4.3 Most divergent accessions according to the 1st and 2nd principal component scores.

Accession	1st pcp	2nd pcp
EET 156	-0.42	-1.44
EET 95	-1.75	-2.21
ICS 40	-2.91	3.15
ICS 85	1.61	1.30
ICS 89	3.45	1.87
ICS 98	2.02	3.30
IMC 3	2.06	-0.25
IMC 83	-4.33	-1.69
IMC 61	-0.90	-2.94
IMC 65	-0.60	-2.21
SCA 9	5.06	-0.46
SPA 5	3.39	0.73

pcp - principal component score

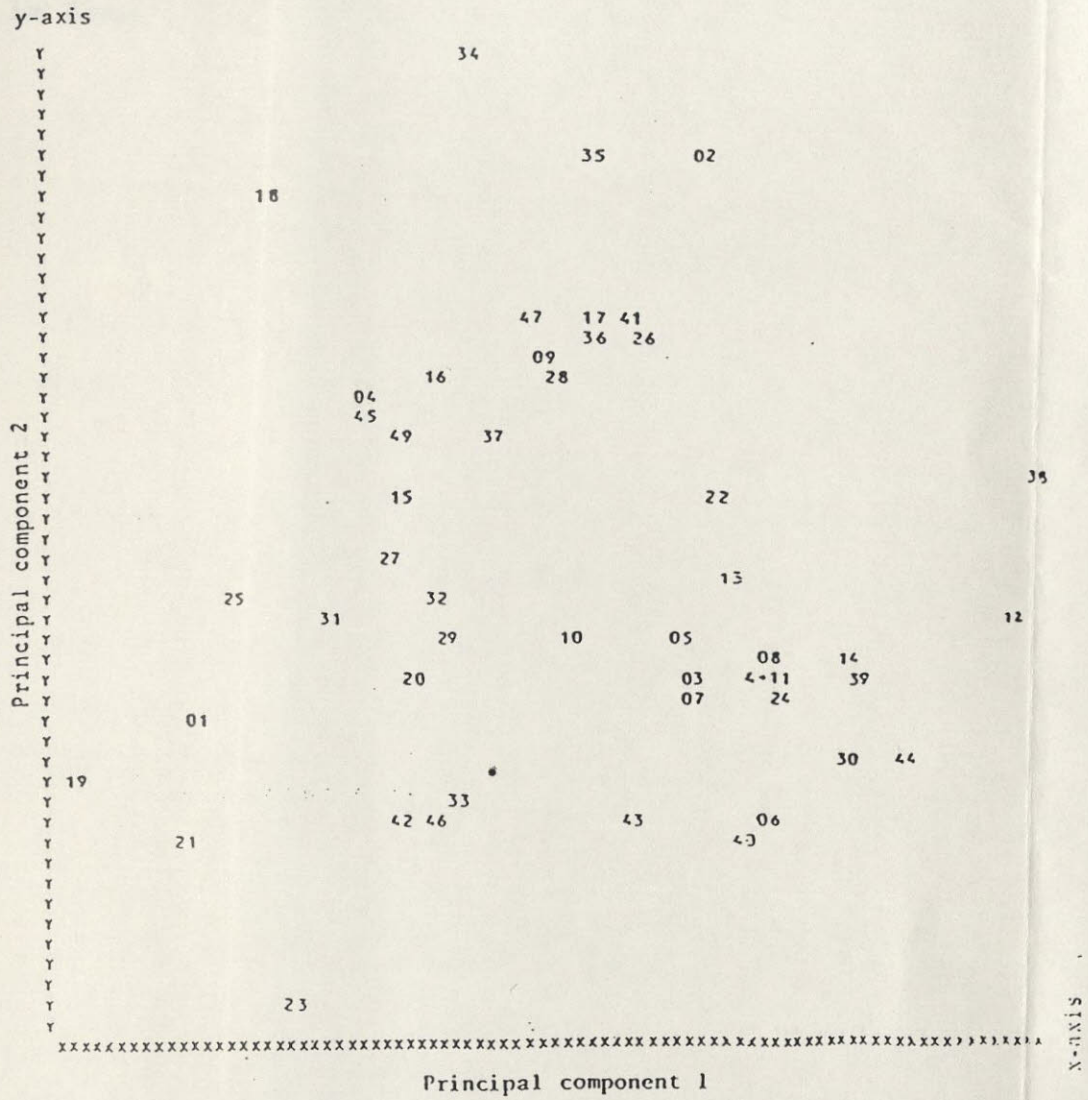


FIGURE 3.4.1 Forty-nine cocoa accessions (numbered) exhibited against the first two principal components computed using thirty-five quantitative variables

Key for Figure 3.4.1 (the accessions represented by numbers in figure 3.4.1)

Accession	Number	Accession	Number
EET 95	1	EET 156	2
EET 272	3	EET 338	4
EET 395	5	EET 400	6
ICS 1	7	ICS 6	8
ICS 16	9	ICS 22	10
ICS 39	11	ICS 40	12
ICS 43	13	ICS 60	14
ICS 75	15	ICS 79	16
ICS 84	17	ICS 65	18
ICS 89	19	ICS 95	20
ICS 98	21	ICS 100	22
IMC 3	23	IMC 5	24
IMC 6	25	IMC 9	26
IMC 16	27	IMC 23	28
IMC 45	29	IMC 49	30
IMC 61	31	IMC 55	32
IMC 67	33	IMC 61	34
IMC 65	35	IMC 67	36
IMC 78	37	IMC 83	38
IMC 85	39	IMC 94	40
IMC 96	41	IMC 103	42
IMC 107	43	SCA 6	44
SCA 8	45	SCA 9	46
SCA 11	47	SCA 12	48
SCA 19	49		

genetic diversity displayed. Cluster Analysis could be used to confirm the validity of this inference andthis is done in section 3.6.

3.5 AN ASSESSMENT OF THE EFFECT OF ENVIRONMENT ON THE PHENOTYPIC EXPRESSION OF TWENTY COCOA CLONES

3.5.1 INTRODUCTION

The relationship between genotype and environment is of great interest to plant breeders (Allard and Bradshaw (1964); Freeman (1973); Hill (1975); Hardwick and Wood (1972); and Simmonds (1981)). It is important for them to know whether preferred genotypes are stable under slightly differing environments before they make selections. When a descriptor list is being compiled, it is important to note the environmental conditions under which sampling is done and to be aware of the impact of environment on the expression of the descriptors. As outlined in section 2.1.1, reliable descriptors are stable under differing environmental conditions.

In this study, an attempt was made to determine the significance of the effect of environment (site) on the phenotypic expression of some Theobroma cacao L. clones. A detailed study of individual environmental effects such as those of soil and climate was not done. However, many such studies have already been conducted at the Imperial College of Tropical Agriculture (now U.W.I), (albeit under controlled conditions) as is described subsequently.

The effects of genotype (G) and environment (E) were separated in this study by the use of variance components. However, no analysis of Genotype-Environment (GE) interaction was attempted as this would require a more detailed study.

3.5.2 MATERIALS AND METHODS

The data analysed in this investigation were collected as outlined in Section 3.1 and represented twenty clones from four families, common to the St. Augustine and St. Joseph sites, which are 5.6 kilometers apart (refer to appendices 1 and 2a and b). The study may be limited since it has been stated, that the more diverse genotypes that are used, the more accurately the environment is expected to be assessed.

Care was taken to ensure that the methodology employed was consistent over genotypes and between sites. Since vegetative growth and seed growth and development have been shown to display seasonal behaviour (Alvim (1967); Are and Atanda (1972); Toxopeus and Wessel (1970)), the data were collected during the same season or harvest period at both sites under study.

In Section 3.2, an account was given of the assessment of intraclonal variability in the data

collected in St. Augustine. The variability was not significant. Thus the data in this study was analysed by Multivariate Analysis of Variance (MANOVA) using means for each site (refer to appendix 5 for an example of the MANOVA output). This is similar to the method of Freeman and Perkins (1971).

The model used in this investigation to test the effect of environment is :

Phenotypic variation = genotype effect (family + clone or "number" (family)) + environmental (site) effect + random error or mathematically as :

$$y_{ij} = \mu + G_i + E_j + \epsilon_{ij} \qquad 3.5.1$$

The variable, y_{ij} of the i^{th} genotype, in the j^{th} environment, is regarded as being comprised by the general mean, μ, a genotype effect, G_i; an environmental effect, E_j; and random error, ϵ_{ij}.

The hypothesis (H_o) being tested is that there is no significant difference between the means of individual variables at the two sites.

3.5.3 RESULTS

3.5.3.1 A COMPARISON OF THE ENVIRONMENTAL FACTORS EXISTING AT THE TWO SITES UNDER STUDY

The total annual rainfall, over the seven years

prior to 1985, was consistently higher in St. Augustine area. The exception was in 1983 (refer to Table 3.5.1). Unfortunately, mean annual maximum temperature, relative humidity and hours of sunshine data were not available for St. Augustine over this period. However, seasonal temperature variation in Trinidad is only about 2-3°C in the mean (Bacon and Cooper (1981)). Diurnally, the variations are greater; 10-15°C. The absence of the aforementioned temperature records may not be critical.

Some relative humidity data were recorded in St. Augustine and St. Joseph for four months each, in 1984 and 1985. (The data were collected between 8.00 am and 10.30 am.) Thus some comparison may be made between the two sites. There are no significant differences between them (refer to Table 3.5.2).

Of the weather data recorded, only rainfall differences can be referenced to explain any phenotypic variation in the clones. The slightly higher precipitation in St. Augustine may result in greater vegetative growth and abundance of flowering there.

Soil data for the two sites is given in Table 3.5.3 The soil samples were taken at a depth of 0-60 ins below the surface, in all but one instance (Refer to Table 3.5.3). Among the features, which seem to

Table 3.5.1 Temperature and total annual rainfall data for St. Augustine (St Aug) and St. Joseph (St Jos). (After Soil Sciences Dept., U.W.I.)

	Mean Maximum Temperature (°C)		Annual Rainfall (mm)	
Year	St Aug	St Jos (SD)	St Aug	St. Jos
1979	_	31.1(0.50)	2432	2292
1980	_	31.9(1.69)	2180	1944
1981	_	31.5(0.58)	2615	2015
1982	_	31.0(0.66)	2010	1776
1983	_	32.0(0.84)	1577	1798
1984	_	31.4(0.69)	1895	1818
1985	_	31.0(0.60)	1885	1848

SD standard deviation

Table 3.5.2 Relative Humidity Data for St. Augustine and St Joseph.

Month	Year	Relative Humidity (%)	
		St Aug	St Jos
August	1984	93	96
September	1984	93	95
October	1984	74	88
December	1984	71	70
Annual Mean	1984	83	87
January	1985	75	68
February	1985	55	57
March	1985	70	67
April	1985	74	71
Annual Mean	1985	68	66

Table 3.5.3 Soil Data for the two sites

(After Brown and Bally (1970))

Factors	Site		
	St Aug Series	St Jos Series	Minimum
	(Mean(SD))	(Mean(SD))	Requirement
1) Drainage	Well drained	Well drained	
2) Topography	Flat	Flat	
3) Soil type (0-60 ins)*	Clay loam	Sandy loam	
4) pH	6.02(0.29)	6.62(0.34)	6.5
5) Coarse sand (% oven dried soil)**	7.83(3.66)	10.83(10.42)	
6) Fine sand	20.33(2.8)	63.50(10.86)	
7) Silt	23.33(2.42)	11.50(3.99)	
8) Clay	49.00(5.62)	13.33(4.46)	
9) Ca (m.e./100g oven-dried soil.)	6.78(4.55)	3.17(0.75)	8
10) Mg	0.72(0.54)	1.93(2.2)	2.00
11) K	0.043(0.04)	0.003(0.005)	0.240
12) Na	0.500(0.47)	0.013(0.015)	
13) C/N ratio (0-20 ins depth)	6.23(1.47)	8.16(0.65)	9.50

* same for 3) - 13). ** same for 5) - 13). SD -standard deviation

differ significantly, are the percentage of sand and clay. The St. Augustine series is a clay loam and the St. Joseph series a sandy loam.

The level of calcium (per 100 gm. of oven-dried soil) is different between sites.That of the St. Augustine series is 6.78 m.e./100g oven-dried soil and St. Joseph is 3.17 (Brown and Bally (1970)). This should be compared to the limits of adequacy for cocoa clay soils of 8 m.e.*/100g oven-dried soil (Bacon and Cooper (1981)).

The level of magnesium at St. Augustine (0.72 m.e.*/100 gm. oven-dried soil) is lower compared to that at St. Joseph (1.93 m.e./100 g oven-dried soil). Both levels are below the limits of adequacy (2.0) proposed
by Bacon and Cooper (1981). The effects of low magnesium may be more detrimental in St. Joseph because the soil is sandy.

The Fe/N ratio is higher at St. Joseph (8.2 me/100 g oven-dried soil) than at St. Augustine (5.1 me/100 g oven-dried soil), in the first three inches (Bacon and Cooper (1981)). These levels are below the limits of adequacy (9.5 in the top 0.6 inches).

The shade trees planted at the two sites are also different. In St. Augustine, shade is provided by Immortelle (Erythrina poeppigiana) trees and banana

(Musa sapientum) cultivars. Pine (Pinus caribaea) and Immortelle trees and banana plants provide shade at the St. Joseph site. The pine trees account for the heavy peripheral shade existing at the latter site.

3.5.3.2 Differences in the expression of descriptors between sites

The MANOVA performed revealed that 24% of the descriptors studied varied significantly between sites. Of these, 17% were leaf characters and the remaining 7% were floral. Thus the environment did seem to have a greater impact on the expression of vegetative characters than on reproductive ones.

3.5.3.2.1 Variation in the expression of leaf descriptors between sites

The differences between the mean leaf values recorded at the two sites were significant for all the descriptors measured. The variation was significant at the $P \leq 0.001$ level for total leaf length, basal leaf length and leaf width. It was significant at the $P \leq 0.05$ level for the remaining leaf descriptors; apical to basal angle ratio, basal angle, length to width ratio and apical angle. The results are shown in Table 3.5.4. The model (refer to 3.4.2) tested to explain the variation in the expression of leaf characters at the two sites was effective. This is

Table 3.5.4 MANOVA results for the leaf data.

Variable (unit)	Mean (S.E.)	F (model)	R^2	C.V.	F (site)
TLL(cm)	24.1(1.5)	4.7^{**}	0.81	6.3	42.8^{***}
BLL(cm)	11.5(1.3)	2.7^{*}	0.73	11.1	28.9^{***}
LW(cm)	8.5(0.6)	3.1^{*}	0.79	6.6	23.6^{***}
LWR	2.8 (0.1)	2.3^{*}	0.72	4.4	4.8^{*}
LAA(°)	57.8(8.2)	0.9^{ns}	0.51	14.2	4.7^{*}
LBA(°)	107.5(9.7)	5.5^{**}	0.88	9.0	8.9^{*}
ABR	1.9 (0.3)	2.2^{*}	0.71	14.8	20.9^{**}

* significant at the 5% level.

** significant at the 1% level.

*** significant at the 0.1% level.

total df = 39, model df = 20, error df = 19; site df = 1, family df = 3, number(family) df = 16.

Refer to the glossary for an explanation of the acronyms.

evident from the R^2 values. The inter-familial variation was significant for all the leaf descriptors except apical angle. The genotype-phenotype relationship is thus illustrated i.e. the expression of the leaf descriptors has a genetic basis.

3.5.3.2.2 Variation in flower descriptors between sites

Three of the thirteen flower descriptors observed varied between sites. Two were significant at the $P \leq 0.05$ level. They were sepal length and ribbon length. Staminode length showed significant variation between sites at the 0.01 level. No flower descriptors differed significantly between the sites at the $P \leq 0.001$ level. It appears that the variation, that exists, is not highly significant.

The model tested did account for variation in the phenotype of the clones between sites (refer to Table 3.5.5 for the R^2 values.) The inter-familial variation was significant at the 5% level for all the floral descriptors, except ovary length, ovary diameter and style length.

3.5.3.2.3 Variation in fruit descriptors between sites

There was no significant environmental effect on the expression of all the measured fruit descriptors

Table 3.5.5 MANOVA results for the flower data.

Variable (unit)	Mean (S.E.)	F (Model)	R^2	C.V.	F (Site)
SW (mm)	2.3 (0.2)	1.7^{ns}	0.64	10.0	1.2^{ns}
SL (mm)	7.8 (0.6)	2.7^{*}	0.69	7.0	7.7^{*}
R	3.4 (0.3)	2.0^{ns}	0.68	7.5	1.9^{ns}
LL (mm)	2.9 (0.3)	1.8^{ns}	0.65	11.4	0.4^{ns}
LW (mm)	2.3 ((0.2)	2.0^{ns}	0.68	10.3	1.0^{ns}
RL (mm)	3.3 (0.3)	4.5^{**}	0.83	9.1	4.5^{*}
GL (mm)	2.5 (0.3)	2.3^{ns}	0.70	13.4	1.0^{ns}
AVPL (mm)	8.6 (0.7)	2.3^{*}	0.71	8.2	2.8^{ns}
STL (mm)	5.8 (0.4)	3.5^{*}	0.79	6.69	8.3^{*}
OVL (mm)	1.9 (0.2)	0.6^{ns}	0.40	10.9	0.1^{ns}
OVD (mm)	1.04 (0.1)	1.96^{ns}	0.67	4.9	0.4^{ns}
ON	43.9 (4.2)	2.5^{*}	0.73	9.5	0.1^{ns}
STYL (mm)	2.1 (0.2)	1.39^{ns}	0.59	7.1	4.4^{ns}
PEL (mm)	14.6 (1.4)	2.6^{*}	0.74	9.6	0.4^{ns}

total df = 39, model df = 20, error df = 19

site df = 1, family df = 3, number (family) df = 16

* 5% level of significance

** 1% level of significance *** 0.1 % level of significance

Table 3.5.6 Manova results for the fruit data.

Variable (unit)	Mean (S.E.)	F (Model)	R^2	C.V.	F (Site)
POL (cm)	6.4 (1.1)	5.3^{**}	0.85	6.7	0.5^{ns}
POW (cm)	8.2 (0.4)	12.9^{***}	0.90	4.7	0.3^{ns}
PLWR	2.1 (0.1)	3.2^{*}	0.80	6.9	1.5^{ns}
PWT(mm)	12.3 (1.8)	5.8^{**}	0.80	14.6	1.0^{ns}
MESOTH(mm)	3.6 (1.3)	2.4^{*}	0.70	35.5	0.2^{ns}
FD1(mm)	6.4 (1.4)	3.1^{*}	0.77	21.9	2.0^{ns}
FD2(mm)	4.6 (1.3)	2.9^{*}	0.75	27.8	7.7^{*}
POWT(g)	487.6 (77.1)	10.4^{***}	0.92	15.8	1.1^{ns}
PLW (g)	10.0 (2.6)	5.2^{**}	0.85	26.3	2.0^{ns}
WBW (g)	118.5 (21.8)	4.5^{**}	0.83	18.4	3.1^{ns}
HW (g)	358.1 (59.6)	11.9^{***}	0.90	16.6	0.3^{ns}
WABW (g)	89.9 (23.3)	1.6^{ns}	0.63	25.9	0.1^{ns}
MUW (g)	29.8 (9.7)	4.5^{**}	0.83	32.8	4.2^{ns}
BEN	44.9 (4.8)	2.6^{*}	0.73	10.6	0.6^{ns}
DBWT(g)	78.9 (7.5)	10.5^{***}	0.92	9.5	2.4^{ns}
PBWT(g)	70.2 (7.5)	9.0^{***}	0.90	10.7	3.4^{ns}
SCWT (g)	8.8 (3.0)	1.34^{ns}	0.58	33.6	0.9^{ns}
SCPE (%)	11.4 (3.9)	1.0^{ns}	0.51	34.3	2.7^{ns}
BW (g)	0.9 (0.1)	7.3^{***}	0.89	11.5	3.0^{ns}
BL (cm)	2.1 (0.1)	8.5^{***}	0.70	5.6	2.1^{ns}
BEW (cm)	1.1 (0.1)	2.4^{*}	0.70	13.4	2.1^{ns}
BET (cm)	0.59 (0.1)	3.2^{*}	0.77	11.8	0.1^{ns}

total df = 39, model df = 20, error df = 19 site df = 1, family df = 3, number(family) df = 16

* 5% level of significance ** 1% level of significance *** 0.1% level of significance

except one; secondary furrow depth (refer to Table 3.5.6). The significance was at the $P< 0.05$ level.The effect of genotype (family) on phenotypic expression was marked. Only primary and secondary furrow depths did not vary significantly between families.

3.5.4 DISCUSSION

The results of this study indicate that the variability of cocoa leaves is explained by both genotypic and environmental effects. Enriquez and Soria (1967c); Goodall (1947); Ostendorf (1956) and Asomaning and Lockard (1963) are among workers, who commented on the high intraclonal variability displayed by the growth habit and leaf characteristics of cocoa. They partly attributed this variability to environmental factors. Asomaning and Lockard (1963) noted significant variation in leaf characteristics within trees and reported that leaves exposed to more light are smaller than those produced in shade. Goodall (1947) found highly significant differences in leaf area within the same tree, which could be attributed to photoperiod.

Several factors may contribute to a difference in the water and nutrient uptake by the plants and consequently to a difference in the leaf sizes at the two sites. The soil types differ to some extent, as

shown in Table 3.5.3. The spacings between blocks or fields of trees are not the same at both sites (refer to table 3.5.7a). There may be different rates of evapo-transpiration in peripheral trees because of the varying exposure to light and wind (refer to Table 3.5.7b for light intensity data). In addition, the monthly rainfall amounts are also slightly different at the two sites, as shown in Table 3.5.1, and the density of undergrowth is not uniform.

Table 3.5.7a shows that the spacings between trees are larger in St. Joseph than in St. Augustine. Furthermore, the trees in St. Joseph (twenty-one years old when the data were collated) are four years younger than those in St. Augustine. Their canopies may be narrower and may allow more light penetration within the blocks. Enriquez and Soria (1967c) cited the density of the shade canopy as one of the probable causes of the variability in leaf characters. The above factors allow greater light penetration of the canopy in St. Joseph resulting in lower relative humidity. There was denser undergrowth in St. Joseph during the period of study. This may have led to increased competition for nutrients and water.

Table 3.5.7a Spacings between trees at the sites studied.

St Aug		St Jos	
Field	Spacing (ft)	Block	Spacing (ft)
9	5 x 5	1	12 x 8
10	5 x 5	5	12 x 8
11	5 x 5	6	12 x 8
12	5 x 8	7	12 x 8
14	10 x 6	12	12 x 8
16	4 x 6	17	12 x 8
17	5 x 4	18	12 x 8
		19	12 x 8

Table 3.5.7b Mean Light intensity values in one field at each site.

St Aug (5x5)		St Jos (12x8)	
Location	Light intensity**	Location	Light intensity
S B	12.79*	S B	0.06
E B	0.24	E B	0.34
W B	0.35	W B	0.39
N B	3.36	N B	6.1
Centre	0.04	Centre	0.05

S B - South Boundary. E B - East Boundary.

W B - West Boundary. N B - North Boundary. Centre- middle of field.

* This periphery is bordered by a fairly wide path, facilitating light penetration.

**Light intensity is measured in photons.

The leaves from St. Joseph were significantly smaller than those from St. Augustine (refer to Tables 3.5.4 and 3.5.8 and figure 3.5.1). This was expected because of the higher competition for water and nutrients and the relatively lower total annual rainfall for St. Joseph.

The three (out of thirteen) flower descriptors, which differed significantly between sites; sepal length, ribbon length, and staminode length have means, which are shown in Tables 3.5.5 and 3.5.9 and figure 3.5.2. It can be concluded that most of these characters are not affected appreciably by any environmental difference, that exists between the two sites. Temperature and rainfall are important for the occurrence of flowering (Alvim (1967)). However, it has not been shown whether these and other factors affect the size of flowers. These results indicate that, whatever the effects the factors have, they are not very marked under these conditions.

Alvim (1967) and Wood (1975) state that when there is no great seasonal difference in temperature and rainfall, cocoa behaves as an "over-flowering" plant. Alvim also reported that flowering is reduced by a "slight decrease in temperature and day-length." The number of flowers per cushion and abundancy of cushions may thus be more affected by weather changes

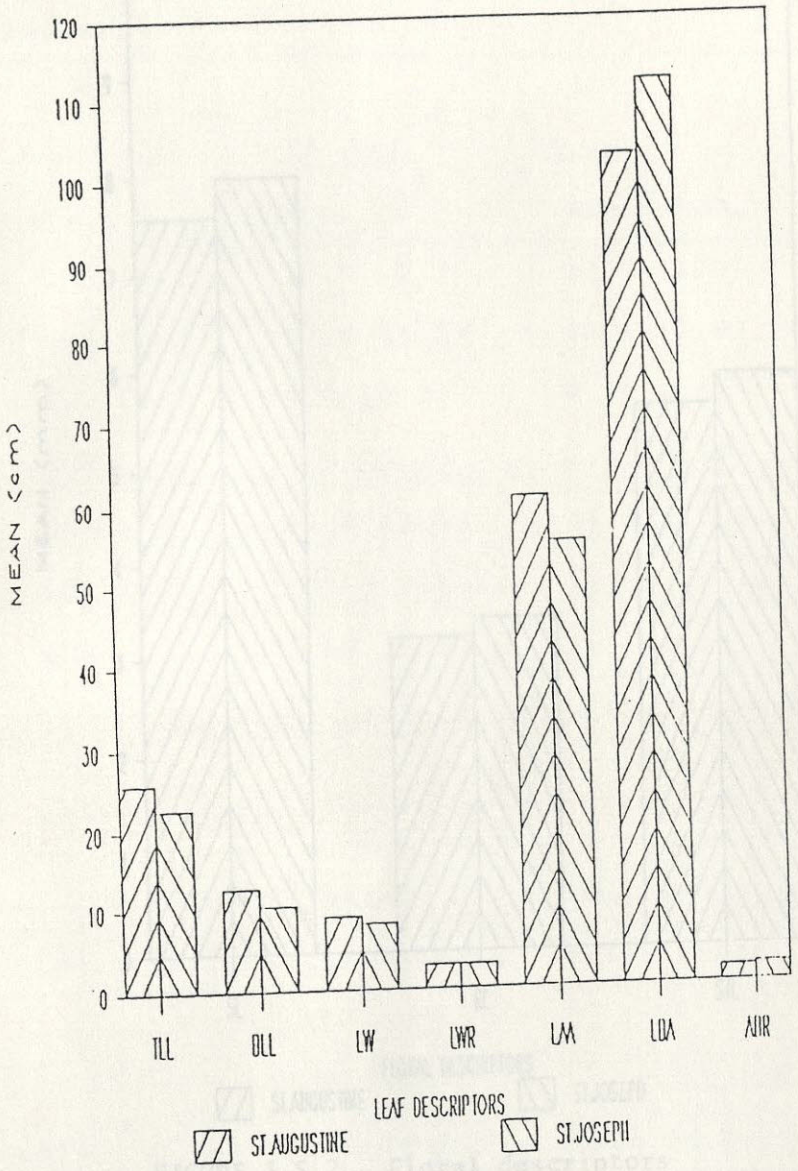


FIGURE 3.5.1 Leaf descriptors St. Augustine vs. St. Joseph

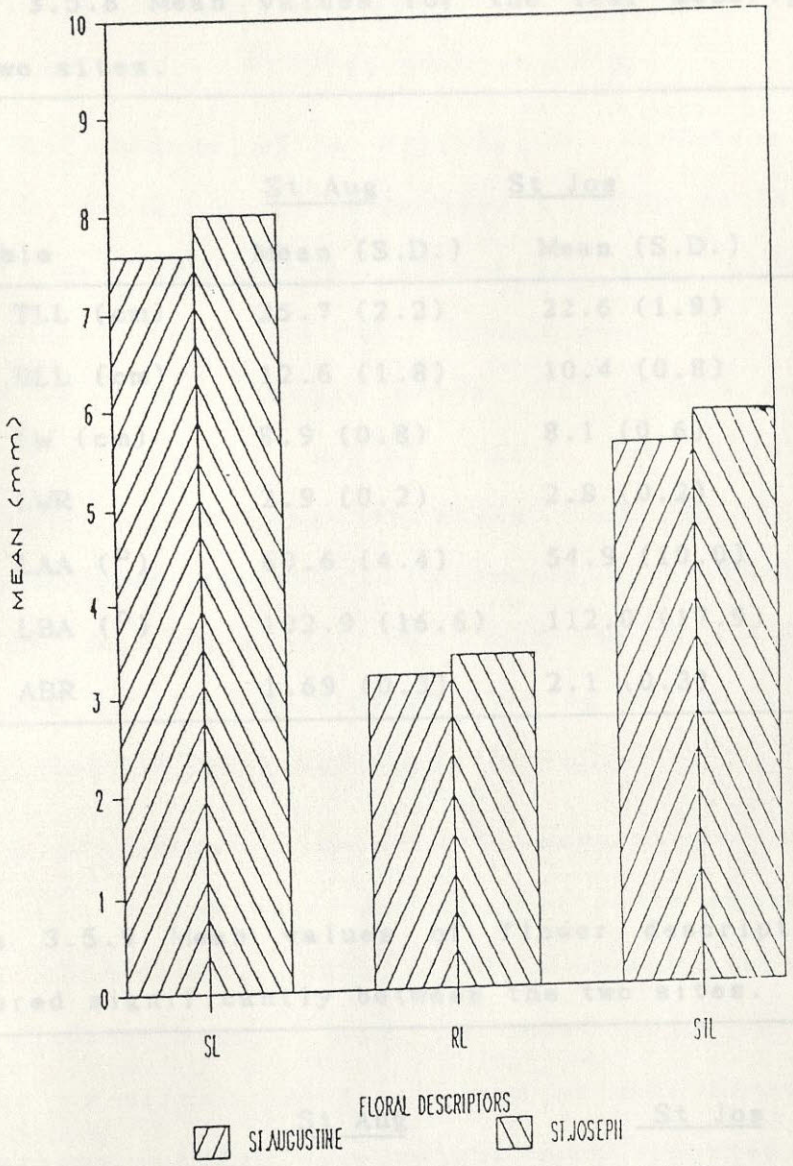


FIGURE 3.5.2 Floral descriptors
St. Augustine vs St. Joseph

Table 3.5.8 Mean values for the leaf descriptors at the two sites.

Variable	St Aug Mean (S.D.)	St Jos Mean (S.D.)
TLL (cm)	25.7 (2.2)	22.6 (1.9)
BLL (cm)	12.6 (1.8)	10.4 (0.8)
LW (cm)	8.9 (0.8)	8.1 (0.6)
LWR	2.9 (0.2)	2.8 (0.2)
LAA (°)	60.6 (4.4)	54.9 (10.0)
LBA (°)	102.9 (16.6)	112.0 (17.9)
ABR	1.69 (0.3)	2.1 (0.3)

Table 3.5.9 Mean values of flower descriptors that differed significantly between the two sites.

Variable	St Aug Mean (S.D.)	St Jos Mean (S.D.)
SL (mm)	7.6 (0.6)	8.0 (0.7)
RL (mm)	3.2 (0.5)	3.4 (0.5)
STL (mm)	5.6 (0.6)	5.9 (0.6)

than flower dimensions. The latter may be described as more stable under varying environments.

The absence of a significant variation between the two sites in the phenotype of pods could signify that the conditions affecting pod growth and development are fairly homogeneous in the St. Augustine - St. Joseph areas. The single descriptor (secondary furrow depth), that was significantly different between the two sites (at $P<0.05$), may be less buffered against environmental differences. (The concept of buffering is discussed by Allard and Bradshaw (1964).) A similar finding was made by Pound (1932) and Enriquez and Soria (1966).

A considerable amount of research has been done to elucidate how environment affects the expression of fruit characters. Ghosh (1973) proposed that variety and growing season were the most important factors likely to affect numerical bean values. However, wide variations in mean bean weight were reported when the same progenies were planted under different environmental conditions. Ruinard (1964) also stated that growing and maturing conditions, created by location, climate and pests (along with genetic factors) accounted for variation in bean weight. Van der Knaap (1953) found that edaphic conditions had an important effect on bean size. Toxopeus and Wessel

(1970) saw a decrease in average bean number with the age of the tree, related to a changing distribution of pods in relation to the position of the tree.

The previous findings demonstrate the effect of environment on the phenotypic expression of fruit characteristics. A more detailed study in Trinidad may be required to observe these effects.

3.5.5 SUMMARY

1) The two sites studied did not differ greatly, but the clones studied may be more buffered against these environmental effects with respect to their reproductive features. A study of more distinctly different sites and the roles of specific and additive environmental effects is perhaps warranted.

2) The most important indication from this study is that floral and pod descriptors appear to be more stable under varying environmental conditions than vegetative or leaf descriptors. Similar observations have been made by Enriquez and Soria (1967a and c), Engels et al (1980) and Ostendorf (1956). This is a significant finding since it must be considered during international collaboration. Comparisons of the same accessions at different locations or in different countries should not be made without a consideration of the environmental conditions.

3) It is therefore useful to record the growth

environment of plants, whose features are being assessed for the purpose of descriptor list compilation. This has also been stressed by many workers including Erskine and Williams (1980), who made this observation specifically for seed-propagated crops.

3.6 RELATIONSHIPS BETWEEN COCOA CLONES

3.6.1 INTRODUCTION

The objectives of this investigation were to characterize or elucidate the relationships among the clones observed at St Augustine using all the descriptors recorded, and then to determine the smallest subset of descriptors that could do this effectively. It was hoped that this subset would contain the most useful descriptors, which would be recommended for inclusion in a concise descriptor list.

It was also hoped that the investigation would reveal whether geographic origin is reflected by the genetic relationships between the accessions studied or whether the accessions from the Amazon Basin (refer to Appendix 3) represent 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 (refer to tables 1.1 and 2.1). 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 SCA and IMC clones originated in Peru (Posnette, in Toxopeus (1981)) and are forastero (refer to section 2.2.1.1). This material was described as unique by Posnette (1981), the IMC clones being uniform in fruit and vegetative characters. The SPA clones, which are from Colombia, also display forastero characteristics. It is not clear whether they are "amazon forasteros" or "forastero trinitarios".

The EET and E clones from Ecuador are the product of hybridization. More variability is expected within such a population.

The sixth population, comprising Imperial College Selections (ICS) evolved in Trinidad and is also the product of hybridization. These sixteen accessions were selected by Pound in 1932 and are "trinitarios" (refer to section 2.2.1.1).

3.6.1.1 ENVIRONMENTAL CONDITIONS AT THE COLLECTION SITES

This germplasm, collected by Pound, was obtained from the bordering areas of Colombia, Ecuador and Peru. In Colombia, the areas surveyed by Pound were the Upper Putomayo and Caqueta valleys (altitude of 1000 metres). He collected in the Upper Napo and

Putomayo valleys in Ecuador and near Rios Maranon and Amazonas in Peru.

The soils at the Colombian and Ecuadorian sites are alluvial and that in Peru is described as of a "good structure" and rich in potassium, nitrogen and potash. The Trinitarios in Trinidad probably developed on silty clay soil.

The mean annual rainfall in Colombia was 1000 mm per year in 1958 (Anon, 1959). The mean maximum temperature was 24°C and the relative humidity was 50-90%. In Ecuador, the mean annual rainfall was 1200 mm, the mean maximum temperature was 22-27°C and the relative humidity was 70-91%. An average mean annual rainfall of 1200mm was recorded in Peru along with an average maximum temperature of 24°C. No relative humidity data were given. The rainfall in Trinidad was about 1200mm, the average maximum temperature was 30-35°C and the relative humidity was about 75%.

These figures allow some comparisons of the collecting sites to be made, but may not reflect the existing conditions at the time of collection. However, such data may be used with more detailed geographical information to partially explain the evolution of distinct genotypes in the Amazon Basin. Since most of this germplasm was collected in valleys, the populations were geographically isolated from one

another. Consequently, the process of speciation, which results in the emergence of divergent populations via natural selection and mutation, was favoured. The presence of marked genetic diversity within the group of accessions under study would support this hypothesis.

3.6.1.2 THE IMPACT OF ENVIRONMENT IN THIS STUDY

The impact of environment, on the expression of the characters observed in this study, may obscure the real relationships among the clones and the degree of genetic diversity observed within the group. However, large differences in field conditions at the St. Augustine site were not apparent and this impact should be minimal (refer to section 3.5).

3.6.1.3 CLUSTER ANALYSIS

Cluster Analysis, a multivariate technique for identification in numerical taxonomy, was employed to achieve the aforementioned objectives (refer to section 3.6.1). It is a procedure that assigns observations into groups such that each group reveals some distinct characteristic of the sample (Sneath and Sokal (1973); Orloci (1978) and Mardia et al (1979)). Specimens may be placed into hierarchical groups, indicating the various levels of relationships between

the objects, which form clusters (Rao (1952) and Edwards and Cavalli-Sforza (1965)). (Descriptors could be clustered by clones to determine correlations between them (Sokal and Sneath (1973) and Engels (1983c))).

The similarities and differences between the accessions can be represented in a simple tree diagram or by a dendrogram, which provides a scale showing at what level of similarity units or clusters are fused (Spencer (1984)).

The clustering procedure involves the calculation of a similarity measure between all the pairs of objects in the study. Individuals or groups are fused when their average similarity is greatest (Orloci (1978)). The clusters become more and more inclusive as the similarity level drops and the procedure stops when all the objects form a single cluster (Legendre and Rogers (1972)).

The hierarchical, average-linkage clustering technique, first proposed by Sokal and Michener, (Sokal and Sneath (1973)) was used in this investigation and begins by forming one cluster for each unit (clone) in the analysis. The two closest clusters are combined into one cluster, then the two closest of the new set of clusters are combined into a single cluster and so on. This technique allows the

formation of non-overlapping groups (Le Gendre and Rogers (1972)).

The hierarchical tree does not itself provide a classification. This can be derived by cutting the dendrogram at some arbitrary level of similarity. Each cluster then consists of samples occurring on the same detached branch of the dendrogram (Genstat 5 Committee (1988)). The one-dimensional representation of the relationships by the dendrogram may not be enough to recognize distance patterns (Morishima (1969)).

3.6.2 METHOD

Hierarchical average-linkage cluster analysis was used to examine how :

i) all the descriptors i.e. thirty-five quantitative*1 and thirty-three qualitative*2 ones

ii) the thirty-five quantitative*1 descriptors

iii) twenty-one quantitative descriptors*3

iv) the thirty-three qualitative*2

and

v) a subset of thirteen qualitative and twenty-one quantitative*3 descriptors

classify the fifty-three cacao clones studied at St. Augustine. A mainframe cluster analysis package (CLUS), belonging to the Caribbean Agricultural

Table 3.6.1 The descriptors* used in clustering the fifty-three clones observed at St Augustine.

GROUP ii	GROUP iv	GROUP iii* and v
TL, W, AA, BA	AS, BS, PS	AS, BS, PS
SW, SL, LW,	FC, BC, SC, SP	FC, SC, FLC
STL, OL, OD	GR, LC, RC	PAC, PEC, POSH
ON, STYL, PL	TC, OBC, OAC	PAF, PST, BEC
POW, POL, PWT	STL, FLC, AD	BES, (TL, W
FD1, FD2, POWT	PAC, PAPC, PEC	AA, BA, SW
PLW, WBW, DBWT	PRCI, PFCI	SL, APLL, STL
PBWT, SCWT, BW	PRCM, POS	OL, ON, STYL
BL, BEW, BET	POSH, PBC	POWT, WBW, WABW
HW, WABW	PAF, PST	MUW, BEN, DBWT
MUW, BEN	PFD, PFS	BW, BL, BEW
APLL, MESOTH	HH, PUC	POL, POW)*
	BEC, BES	

* refer to the glossary

the group iii) descriptors are enclosed within parentheses in column 3.

Research and Development Institute (CARDI) library, was used to analyse the data sets.

The groups of descriptors (ii-v) are listed in table 3.6.1. All the qualitative descriptors recorded in this study were used. Nine of the forty-four quantitative descriptors observed were excluded from group (ii). These were all refinements of other descriptors included in the analysis. This conforms with the recommendation that only characters which provide independent information on taxa be used in clustering. Group (iv) contained a subset of descriptors, which was indicated to be reliable by the preceding analyses.

The variables used are disparate and the data were standardized before analysis, as recommended by Krzanowski (1987). The distances between pairs of clones were calculated using similarity matrices. The "euclidean distance" or ED was the dissimilarity criterion or distance measure. The latter is defined as follows:

Let x be an (n x p) data matrix with rows $x'_1, \ldots . x'_n$ (where n = total number of variables and p is the number of units) then the euclidean distance between the accessions or points x_i and x_j is d_{ij} where

$$d^2_{ij} = \sum^p (x_{ik} - x_{jk})^2 = \| x_i - x_j \|^2 \qquad 3.6.1$$

3.6.3 RESULTS AND DISCUSSION

The results of the clustering procedures are depicted in the dendrograms below (refer to figures 3.6.1 - 3.6.5).

Clustering the accessions using the full complement of variables (sixty-eight) revealed that the group is genetically diverse. Several distinct clusters were formed. The clusters formed by most of the clones of Amazon origin were homogeneous. Therefore these accessions can be differentiated according to their geographical origins.

Seven clusters were formed where the accessions were separated by an euclidean distance (ED) of 1.13 or less, corresponding to about a 50% phenon (where a 50% phenon connotes a group of accessions affiliated at no lower than 50% on the similarity scale used in the analysis (Sneath and Sokal (1973))) (refer to figure 3.6.1).

Thirteen of the twenty-one IMC clones formed a tight cluster at the aforementioned level of similarity (refer to figure 3.6.1). Six of the other IMC clones were clustered in correspondence to an ED of 1.25 or less and the last two were widely separated in the dendrogram. IMC 55 did not belong to any cluster and must be distinct and worthy of special attention. The work of Sirju-Charran et al

(unpublished) also indicated that homogeneity exists within the IMC population. The thirteen IMC clones they studied were classed as two types according to their isoperoxidase banding patterns.

SCA 8, 6, 19 and 9 were grouped together in correspondence to an ED of 1.13 or less. Two of the SPA clones (SPA 10 and 9) were clustered together (ED = 1.14), but were widely displaced from SPA 5. The relationships between the latter Amazonian clones might have been more clearly elucidated if more SPA clones were included in this study.

The hybrid populations were in scattered groups demonstrating a pattern of segregation. Two of the six EET clones were clustered together when an ED of 1.13 or less was applied. The ICS clones were grouped in three distinct clusters in correspondence to an ED of 1.25 or less. ICS 6, 43, 60, 100, 39 and 40, all criollo types (Lockwood and Gyamfi (1979)) with anthocyanin-free pods, formed one cluster. The accessions ICS 16, 79, 75 and 85, which have intermediately pigmented pods, were grouped together. The typical trinitario types, ICS 95 and 98, with intensely pigmented pods, were grouped in the same cluster. The consequences of hybridization viz increased heterogeneity, was demonstrated by these clustering patterns.

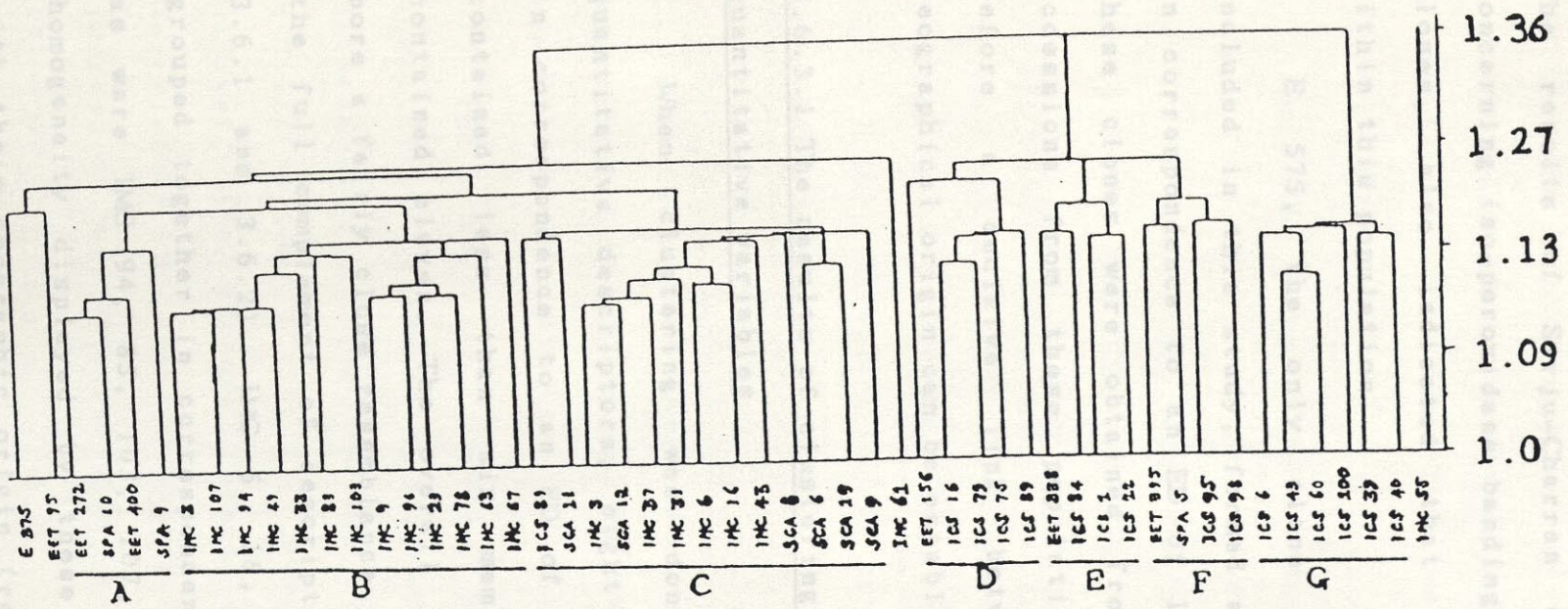


FIGURE 3.6.1 Dendrogram showing relationships between 53 cacao clones after cluster analysis on the basis of the standardised variable-group method using 68 (quantitative and qualitative) varieties. (The clusters of clones on the horizontal axis are numbered and correspond to about a 50% phenon. The ordinate is the euclidean distance between clones.)

The results of Sirju-Charran et al (unpublished), concerning iso-peroxidase banding patterns of some EET clones, also indicated that heterogeneity exists within this population.

E 575, the only clone from its population included in this study, formed a cluster with EET 95 in correspondence to an ED of 1.29 or less. Both of these clones were obtained from Ecuador, but more accessions from these populations must be studied before a decisive link between population and geographical origin can be established.

3.6.3.1 The results of clustering using thirty-five quantitative variables

When clustering was done using thirty-five quantitative descriptors, eight clusters were formed in correspondence to an ED of 1.16. Seven of these contained less than six members and the other contained eleven. The overall pattern of clustering bore a fairly close resemblance to that obtained using the full complement of descriptors (refer to figures 3.6.1 and 3.6.2). IMC 6, 16, 45, 51 and 57 were grouped together in correspondence to an ED of 1.16, as were IMC 94, 85, 103, 107, and 49. The genetic homogeneity displayed by these accessions coincided with their geographic origin (refer to figure 3.6.2).

IMC 61 and 55 remained unclustered at ED = 1.48, as was the case when clustering was done using all the descriptors (and the EDs were 1.27 and 1.36, respectively).

The ICS clones (the products of hybridization) formed four clusters, one of which was removed from the three remaining, adjacent ones (refer to figure 3.6.2). ICS 89, 98, and 95 clustered together in correspondence to an ED of 1.16. The three adjacent clusters contained ICS 1, 22, 84 and 100; ICS 16, 79, 85 and 75; and ICS 39, 40, 43, and 60, respectively (ED = 1.16). This clustering pattern was also similar to that obtained using the full complement of descriptors.

The EET, SPA and SCA clones were scattered throughout the dendrogram. As in figure 3.6.1, EET 272 and EET 400 were clustered together (ED = 1.16).

The analysis based on the thirty-five quantitative characters represents the relationships between some of the IMC and ICS clones in a manner similar to that of the full complement of descriptors. However, the phenons formed using this subset of descriptors are not distinctly homogeneous. A general impression of heterogeneity within the clusters is obtained.

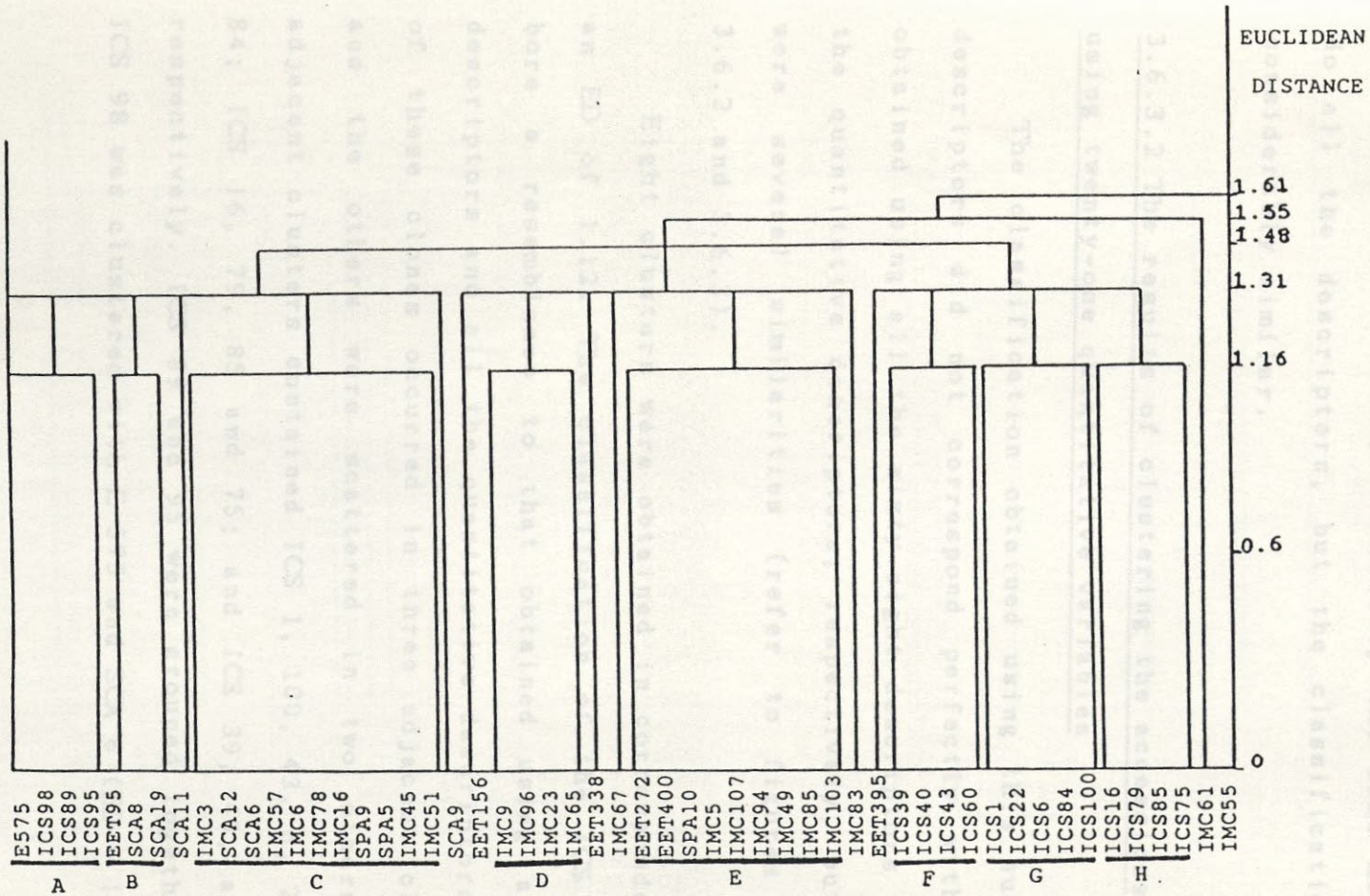


FIGURE 3.6.2 Dendrogram showing relationships between 53 cocoa clones after cluster analysis on the basis of the standardized variable-group method using 35 quantitative descriptors

These quantitative descriptors do not classify the accessions under study in exactly the same manner as do all the descriptors, but the classifications are considerably similar.

3.6.3.2 The results of clustering the accessions using twenty-one quantitative variables

The classification obtained using this subset of descriptors did not correspond perfectly with those obtained using all the sixty-eight descriptors and all the quantitative descriptors, respectively, but there were several similarities (refer to figures 3.6.1, 3.6.2 and 3.6.3).

Eight clusters were obtained in correspondence to an ED of 1.12. The classification of the ICS clones bore a resemblance to that obtained using all the descriptors and all the quantitative descriptors. Most of these clones occurred in three adjacent clusters and the others were scattered in two others. The adjacent clusters contained ICS 1, 100, 43, 6, 22, and 84; ICS 16, 79, 85 and 75; and ICS 39, 40, and 60, respectively. ICS 89 and 95 were grouped together and ICS 98 was clustered with E 575 and SCA 6 (ED = 1.12).

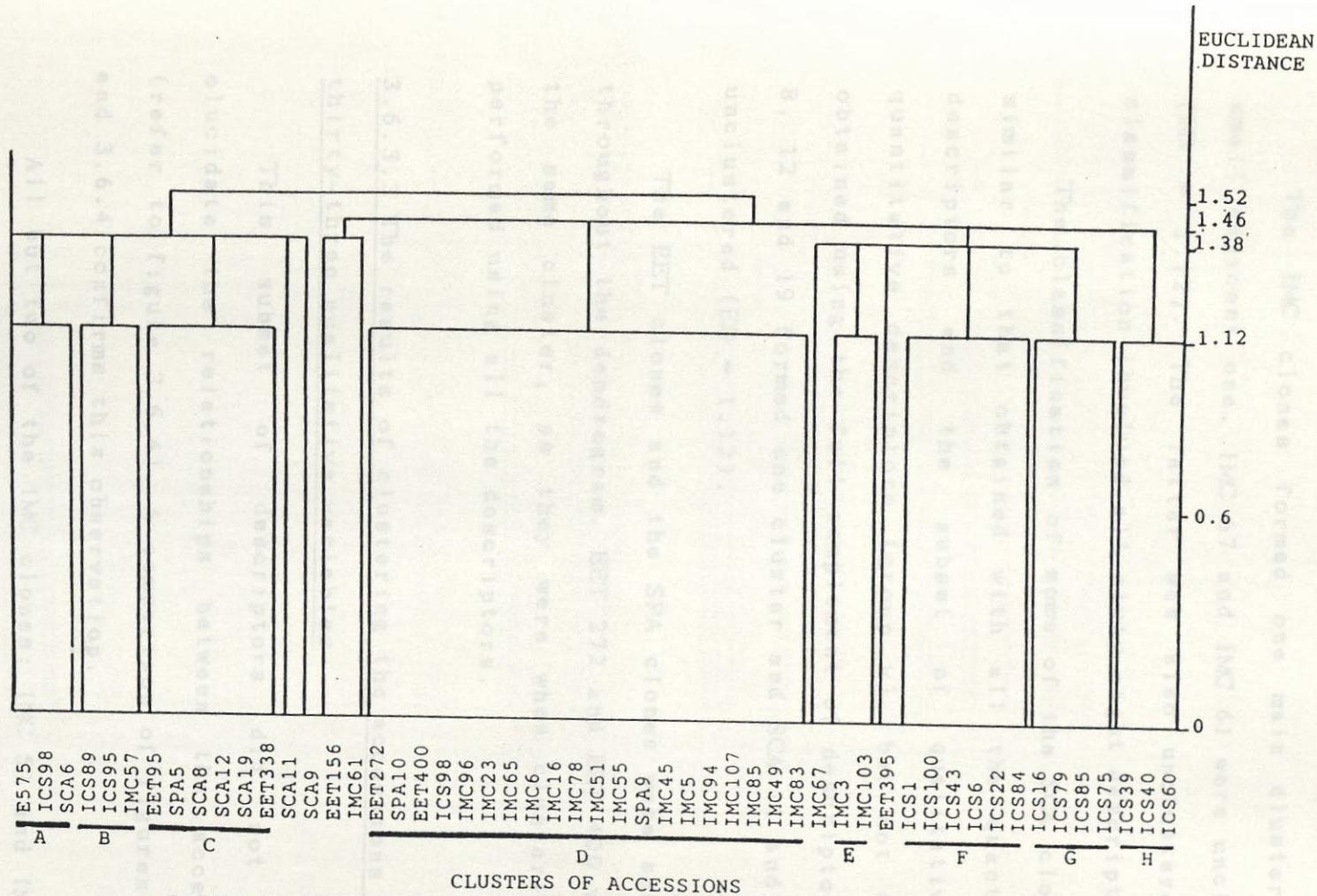


FIGURE 3.6.3 Dendrogram showing the relationships between 53 cocoa clones after cluster analysis on the basis of a standardized variable-group method using 21 quantitative descriptors

The IMC clones formed one main cluster and a small adjacent one. IMC 67 and IMC 61 were unclustered (ED - 1.12). The latter was also unclustered when classification involved all sixty-eight descriptors.

The classification of some of the SCA clones was similar to that obtained with all the quantitative descriptors and the subset of qualitative and quantitative descriptors (group v), but not to that obtained using the full complement of descriptors. SCA 8, 12 and 19 formed one cluster and SCA 11 and 9 were unclustered (ED - 1.12).

The EET clones and the SPA clones were scattered throughout the dendrogram. EET 272 and EET 400 were in the same cluster, as they were when clustering was performed using all the descriptors.

3.6.3.3 The results of clustering the accessions using thirty-three qualitative variables

This subset of descriptors did not fully elucidate the relationships between the accessions (refer to figure 3.6.4). A comparison of figures 3.6.1 and 3.6.4 confirms this observation.

All but two of the IMC clones; IMC 51 and IMC 61, were grouped in one large cluster. The others; IMC 3, 5, 65, 94, 103, 57, 78, 85,9, 78, 96, 55, 49, 107, 23, 45, 67, 16 and 6 formed one cluster with SCA 12, 8 and

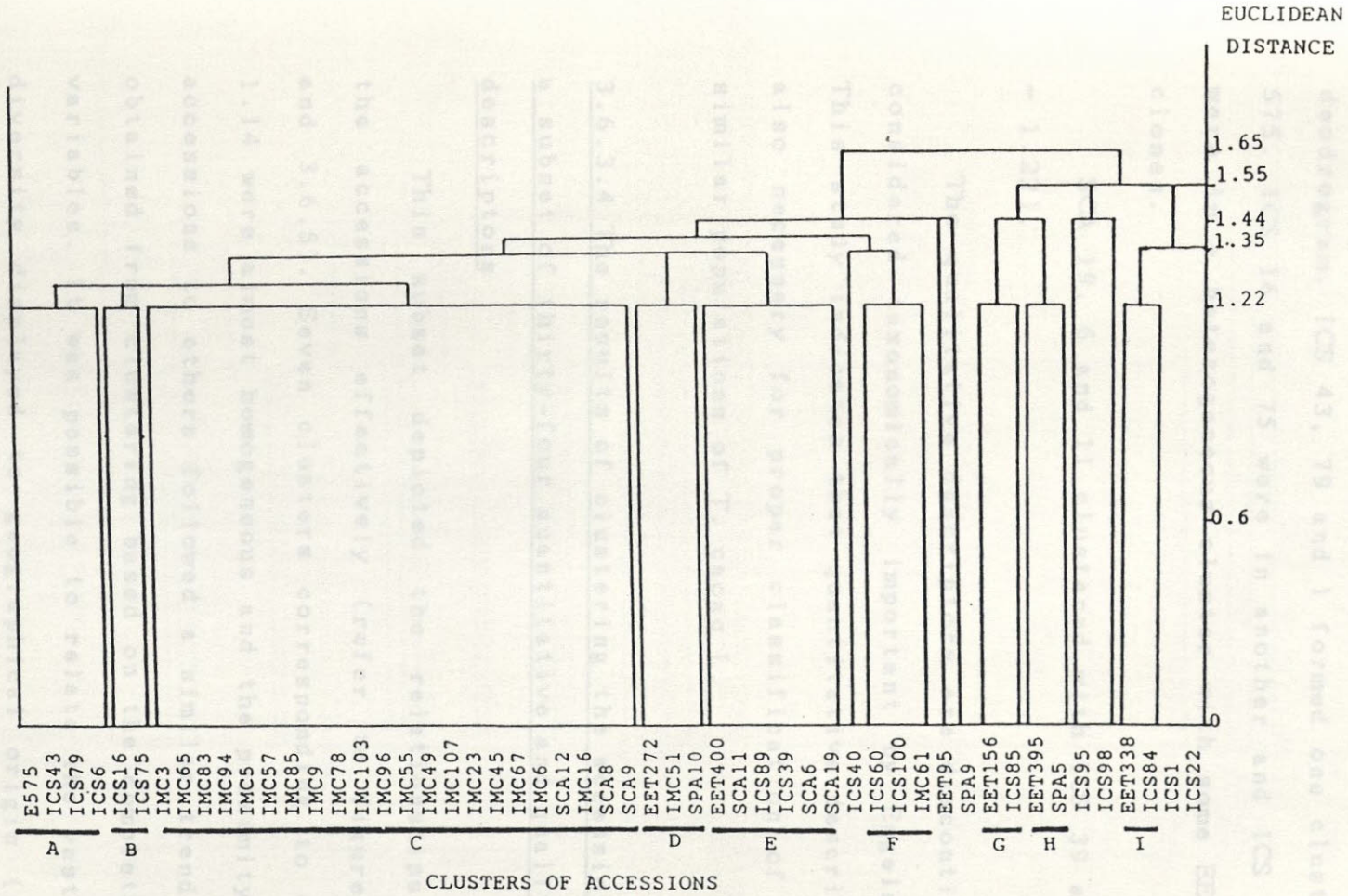


Figure 3.6.4 Dendrogram showing the relationships between 53 clones after cluster analysis on the basis of a standardized variable-group method using 33 qualitative descriptors

9 (ED - 1.22).

The ICS accessions were scattered throughout the dendrogram. ICS 43, 79 and 1 formed one cluster with E 575. ICS 16 and 75 were in another and ICS 39 and 89 were in a heterogeneous cluster with some EET and SCA clones.

SCA 19, 6 and 11 clustered with ICS 39 and 89 (ED - 1.22).

The qualitative descriptors are discontinuous and considered taxonomically important by Engels (1986). This study indicates that quantitative descriptors are also necessary for proper classification of this and similar populations of T. cacao L.

3.6.3.4 The results of clustering the accessions using a subset of thirty-four quantitative and qualitative descriptors

This subset depicted the relationships between the accessions effectively (refer to figures 3.6.1. and 3.6.5). Seven clusters corresponding to an ED of 1.14 were almost homogeneous and the proximity of some accessions to others followed a similar trend to that obtained from clustering based on the complete set of variables. It was possible to relate the vast genetic diversity displayed to geographical origin (refer to figure 3.6.5).

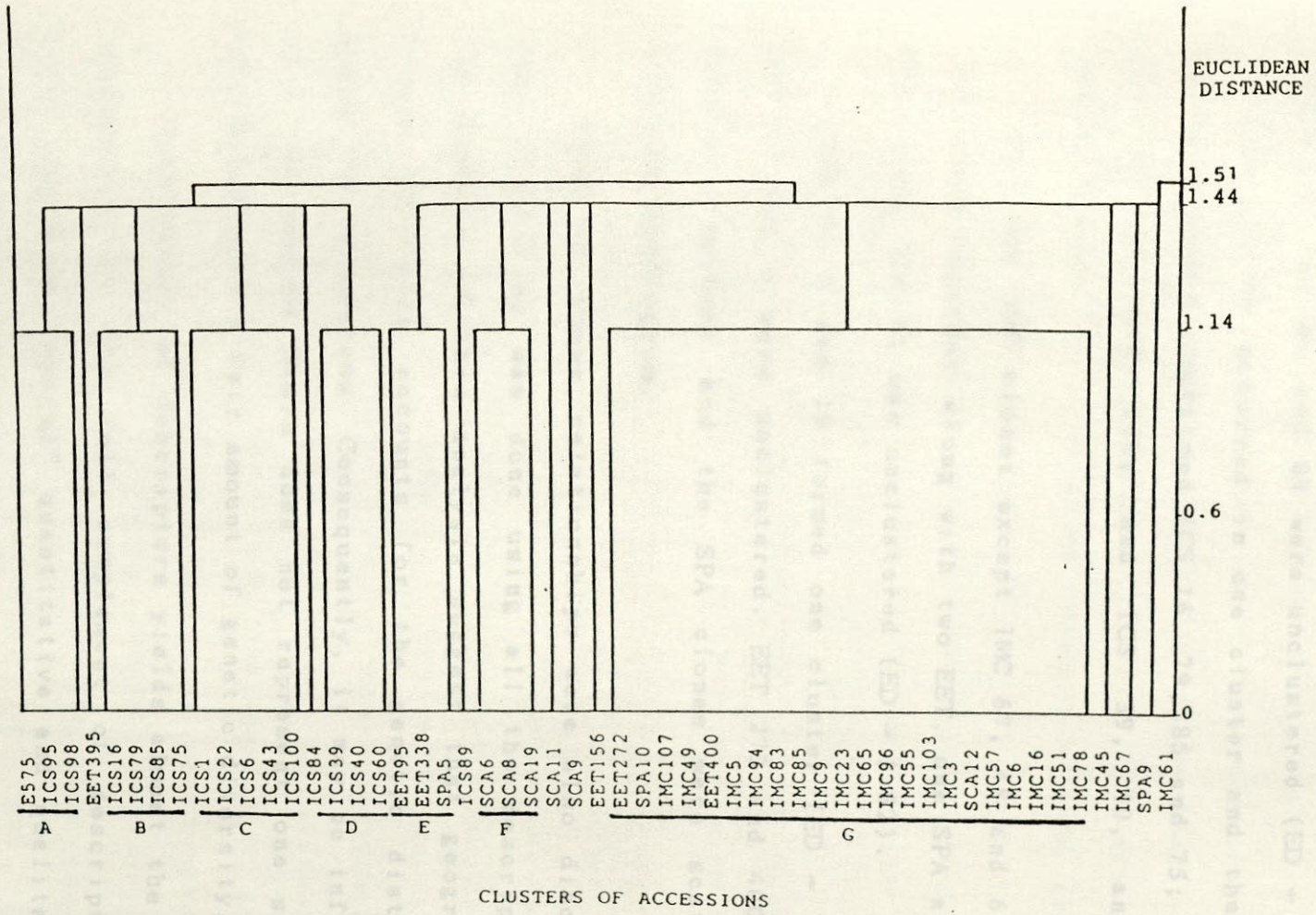


Figure 3.6.5 Dendrogram showing the relationships between 53 clones after cluster analysis on the basis of a standardized variable-group method using 34 qualitative and quantitative descriptors

The ICS clones were scattered in four adjacent clusters. ICS 84 and 89 were unclustered (ED = 1.14). ICS 95 and 98 occurred in one cluster and the other three clusters contained ICS 16, 79, 85 and 75; ICS 1, 22, 6, 43 and 100; and ICS 39, 40, and 60, respectively.

All the IMC clones except IMC 67, 45 and 61 were clustered together along with two EET, one SPA and one SCA clone. IMC 61 was unclustered (ED = 1.52).

SCA 6, 8 and 19 formed one cluster (ED = 1.14). SCA 11 and 9 were unclustered. EET 272 and 400 were grouped together and the SPA clones were scattered over the dendrogram.

Most of these relationships were also discerned when clustering was done using all the descriptors. The results of this 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.

This subset of descriptors yields almost the same information as the full complement of descriptors. Since the most "useful" quantitative and qualitative descriptors are included in this data set, it is valid to recommend them for inclusion in a concise cocoa descriptor list.

3.6.4 GENERAL CONCLUSION

Cluster Analysis proved to be a useful multivariate technique for elucidating the relationships between the accessions studied and for assessing the discriminant capacity of subsets of descriptors. From a breeding perspective, the presence of a fair amount of diversity within this germplasm collection is indicative of its value in breeding programmes. A detailed study of the mean trait values for each population (refer to Appendix 6) should reveal which groups of accessions display desirable characteristics. However, this was not within the scope of this study.

The investigation indicates that as many quantitative and qualitative characteristics as possible be included in the description of similar populations of T. cacao L. This will facilitate detailed inspections of inter-relationships. Clustering with the full complement of sixty-eight descriptors revealed the link between the groups formed and the geographical origin of the accessions under study (refer to Figure 3.6.1). However, the subset of quantitative and qualitative data and the quantitative descriptors (group v and ii), respectively, in Table 3.6.1) also demonstrated this relationship (refer to figures 3.6.2 and 3.6.5). Thus

valuable information was not lost when all the data were not employed in clustering.

The subset of quantitative and qualitative descriptors (group v) is recommended for inclusion in a concise descriptor list for cocoa to be used in the ICG,T. It may be adequate to identify very distinct groups of accessions. However, it may not differentiate between individual accessions or fulfil the range of taxonomic and breeding requirements in this or other cocoa genebanks. Therefore, it is advisable to collect as exhaustive a list of descriptor information as is feasible.

3.7 AN EXPERIMENT TO MEASURE THE INTENSITY OF PIGMENT IN THE SEPALS OF SOME COCOA CLONES USING SPECTROPHOTOMETRY

3.7.1 INTRODUCTION

The purpose of this investigation was to assess the feasibility of using a quantitative method for determining the colour of the sepals of T. cacao L. clones instead of the presently used qualitative one (refer to section 3.1). Such a method may prove useful in the compilation of a cocoa descriptor list (refer to section 2.4). To achieve the stated objective, the extent to which variability (in the intensity of the pigment in the sepals) is related to the genealogy of the clones had to be established.

Anthocyanins have been found to be responsible for the purple colouration of the staminodes, guidelines, ribbons, sepals and other parts of the cocoa flower. The anthocyanin content of plant organs has been found to vary, depending on factors such as genealogy, growth conditions, the physiological state of the plant, the size and position of the organs on the plant and the application of chemicals such as fertilisers (Blank (1958) and Harborne (1965)).

Scogin (1979) examined the cocoa flower and

found two anthocyanin pigments; Cyanidin (Cy) 3-galactoside and Cy 3- arabinoside. Lawrence et al (1938), cited by Chatt (1953) proposed that the anthocyanin pigment, Cy 3-glycoside could also be present in young leaves, flowers and the pod wall and is responsible for the pigmentation of cacao beans (refer to appendix 7 for the molecular configuration).

3.7.1.1 SPECTROPHOTOMETRIC ANALYSIS OF ANTHOCYANINS IN COCOA

Quantitative analyses of anthocyanins can be achieved by spectrophotometric examination of extracts prepared using ethanol or methanol, containing 1M hydrochloric acid (97:3 v/v). (Pigment expression is often pH dependent (Draper (1976)).) The hydrochloric acid stabilizes the pigments and lowers the pH to a level where the absorbance of the anthocyanin is at maximum (Fuleki and Francis (1968)).

Spectrophotometric examination of the pigment in a given solvent involves the determination of the absorption coefficient of the pigment at a given wavelength (Fuleki and Francis (1967)). This is possible because the anthocyanins have an absorption maximum (λmax.) in the 510-550 nm region (Harborne (1958) cited by Swain, in Goodwin (1965)). This is very different from the λmax. of other common phenolics

and the group of compounds with absorption maxima nearest this range is the flavenoids, with λmax. in the 350-380 nm region (Sondheimer et al (1948), cited by Fuleki and Francis (1968)).

Precautions must be taken when performing spectrophotometry because the absorption maximum and molar absorptivity used in simple methods are not only markedly affected by pH, but also by solvent, the presence of certain metals, impurities such as waxes and resins and co-pigments, the time of standing and temperature (Fuleki and Francis (1968); Harborne (1963); Jurd, (1962); cited by Fuleki and Francis (1968); and Imbert (1968)). The λmax. decreases successively in acidified ethanol, methanol, and water (Jurd (1962)).

Since it is difficult to prepare pure crystalline anthocyanin, in sufficient quantities to allow several weighings under optimal conditions (Fuleki and Francis (1968)), no attempt was made in this study to determine the absolute quantities of anthocyanin present in solutions.

3.7.2 MATERIALS AND METHODS

The procedure adopted for this investigation is similar to that of Harborne and Sherratt (1961). It differs in that interfering substances, such as

cinnamic acid, which may affect the λmax., were not removed. In addition, relative absorbances and not absolute concentrations were recorded. The former would suffice for the purpose of this study.

In preparation for spectrophotometric analysis, the sepals of fresh cacao flowers were brushed clean and then separated from the rest of the flower using forceps. This was repeated for forty clones. Approximately fifteen flowers per clone were randomly selected to yield 0.10g of sepals. The samples were weighed using a Sartorius 1219 MP balance (60.000g capacity) within twenty minutes of picking and the pigment was dissolved by placing the sepals in five mls of solvent (1% methanol (1.5 molar)- concentrated hydrochloric acid (97:3, v/v)) (refer to Plate 11). The solutions were stored for twenty-four hours in the dark, at 0°C. The pigment absorbances were then estimated by spectrophotometric analysis at the absorption maximum obtained by scanning with a Perkin Elmer Lambda 3a uv\vis spectrophotometer (190-900 nm wavelength range and 0.301-3.000A absorbance range) (refer to Appendix 8).

For each of the clones studied, five samples were analysed so that some indication of between and within clonal variation could be obtained using analysis of variance.

Plate 11 Variation in the colour of extracts from the sepals of three cocoa clones

3.7.3 RESULTS AND DISCUSSION

The absorption maximum obtained for the pigment in this study was 417 nm. No indication was given of there being any other major pigment in the visible range. However, two secondary peaks were obtained when a scan of ICS 95 extracts was done; one was at 652 nm and the other at 550 nm (refer to Table 3.7.1). These wavelengths were closer to those cited by Harborne (1963) for anthocyanins. The absorption maximum obtained was similar to that of flavonol glucoside (400-420 nm) (Harborne and Sherratt (1961)) and to the distinct shoulder (at 410-450 nm) to the main absorption peak in spectra of anthocyanins, like those of cocoa, with the 5- hydroxyl group free (Jurd (1962)). The fact that the λmax. obtained was so low compared to those associated with anthocyanins might be due to the presence of impurities, interfering substances or a co-pigment(s) in the solutions. A subsequent investigation by some other researchers revealed that a solvent such as petroleum ether removes such substances and the resulting λmax. was 540 nm.

During this study, a primary peak was also obtained in the ultraviolet range, at 240 nm wavelength (refer to Table 3.7.1).

The absorbances obtained for the clones at 417 nm

Table 3.7.1 Absorption maxima of the pigment extracted from a number of cocoa clones.

	Visible range		Ultraviolet range	
Clone	λmax.	2° peaks	λmax.	2° peaks
IMC 67 a)	415		235	275
IMC 67 b)	417		240	270
SCA 8 a)	417		243	277
SCA 8 b)	417		226	277
SCA 11	420	636	240	275
ICS 1 a)	415	640, 522	240	277
ICS 1 b)	416	440	240	270
ICS 95 a)	417*1	652, 526	241*2	277*2
ICS 95 b)	422*1	643, 550	240*2	280*2
ICS 95 c)			241*3	319*3
ICS 95 d)			208*4	277*4

*1 - dilution 0 (0.1g sample/5ml solvent)

*2 - dilution 1:3 v/v (*1:solvent)

*3 - dilution 1:18 v/v (*1:solvent)

*4 - dilution 1:59 v/v (*1:solvent).

λmax. is the wavelength at which maximum absorbance occurred.

The recorder speed was 20 nm/cm

The mode for scanning was the absorbance mode.

The scan speed was 120 nm/min.

The clones scanned represent the visible range of pigment intensity in this study.

Table 3.7.2 Pigment intensity in the sepals of forty cocoa clones (measured spectrophotometrically at 417 nm wavelength and by visual assessment (VA))

Clone	Mean Absorbance	VA	Clone	Mean Absorbance	VA
E 575	0.27	3	IMC 6	0.23	5
EET 272	0.15	0	IMC 9	0.22	5
EET 338	0.36	5	IMC 49	0.18	0
EET 395	0.21	3	IMC 61	0.19	3
EET 400	0.27	3	IMC 65	0.22	3
ICS 1	0.36	5	IMC 67	0.18	0
ICS 6	0.33	5	IMC 83	0.18	0
ICS 16	0.30	5	IMC 98	0.24	0
ICS 39	0.29	3	IMC 103	0.18	3
ICS 40	0.23	3	IMC 107	0.18	3
ICS 43	0.23	3	SCA 6	0.20	0
ICS 53	0.37	5	SCA 8	0.25	3
ICS 60	0.15	3	SCA 9	0.19	0
ICS 61	0.13	3	SCA 11	0.23	3
ICS 84	0.35	5	SCA 12	0.13	0
ICS 89	0.32	5	SCA 19	0.18	0
ICS 95	0.39	7	SPA 5	0.34	7
ICS 100	0.27	5	SPA 7	0.16	0
IMC 3	0.24	3	SPA 9	0.17	0
IMC 5	0.22	3	SPA 10	0.12	0

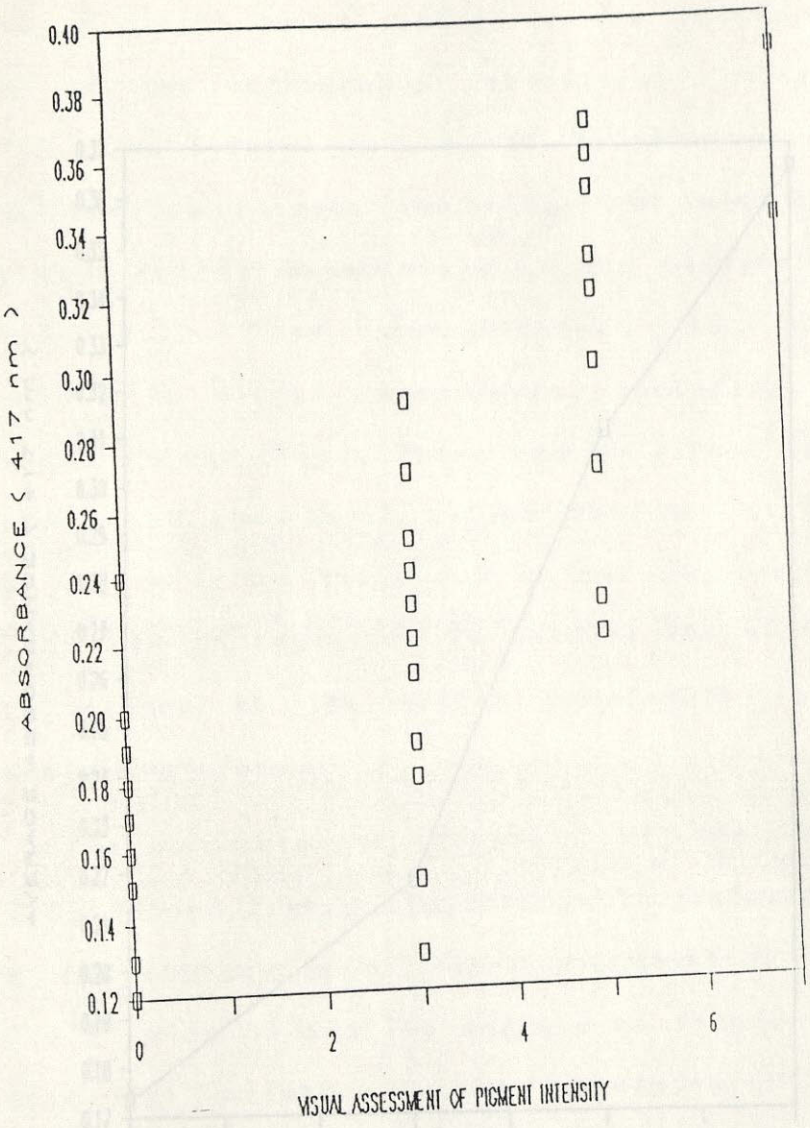


FIGURE 3.7.1a Absorbance values vs visual assessments

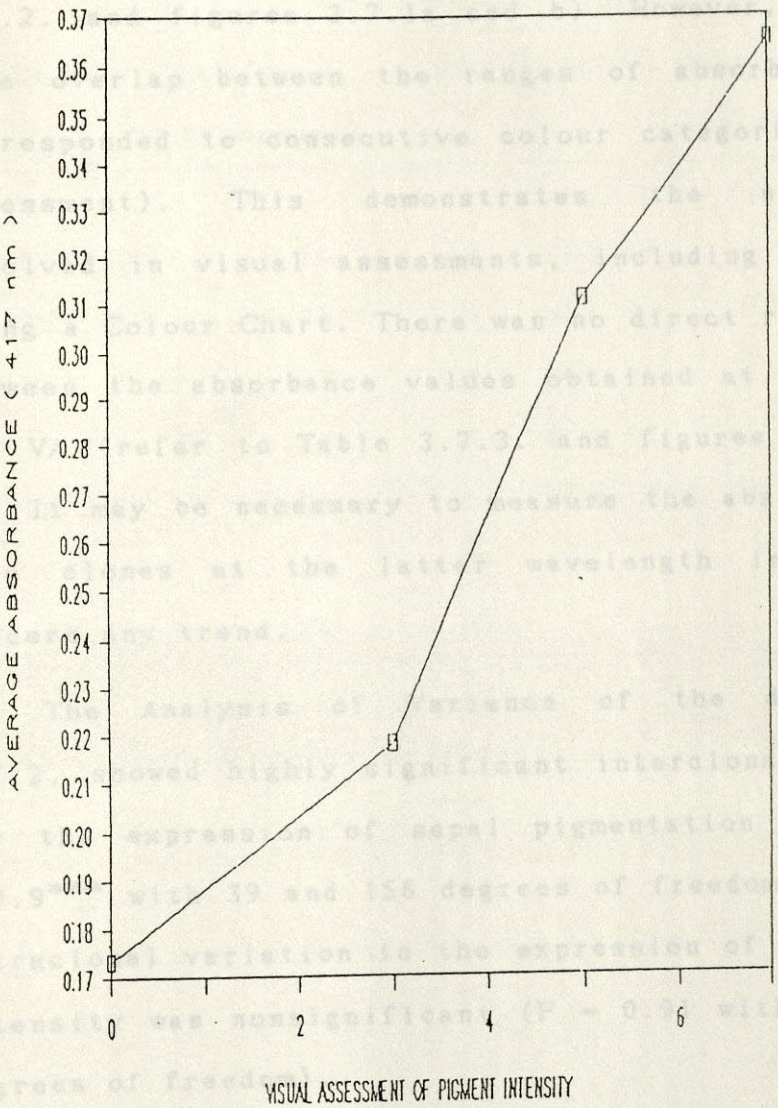


FIGURE 3.7.1b Average absorbance vs visual assessment

bore a direct relationship to the visual assessment (VA) of the anthocyanin intensities (refer to Table 3.7.2. and figures 3.7.1a and b). However, there was some overlap between the ranges of absorbances that corresponded to consecutive colour categories (visual assessment). This demonstrates the subjectivity involved in visual assessments, including those made using a Colour Chart. There was no direct relationship between the absorbance values obtained at 240 nm and the VA (refer to Table 3.7.3. and figures 3.7.2a and b). It may be necessary to measure the absorbances of more clones at the latter wavelength in order to discern any trend.

The Analysis of Variance of the data, Table 3.7.2, showed highly significant interclonal variation for the expression of sepal pigmentation (F value = 110.9*** with 39 and 156 degrees of freedom (DF)). The intraclonal variation in the expression of the pigment intensity was nonsignificant (F = 0.91 with 4 and 156 degrees of freedom).

Table 3.7.3 Pigment intensity in the sepals of twelve cocoa clones (measured at 240 nm wavelength).

CLONE	Mean Absorbance	VA	CLONE	Mean Absorbance	VA
EET 272	1.78	0	IMC 67	0.85	0
EET 338	1.88	5	SCA 6	0.90	0
EET 400	1.87	3	SCA 11	0.71	3
ICS 1	1.04	5	SCA 12	0.87	0
ICS 6	1.11	5	SCA 19	0.83	0
ICS 39	1.04	3			
ICS 95	1.09	7			

VA - visual assessment of the intensity of sepal pigmentation.

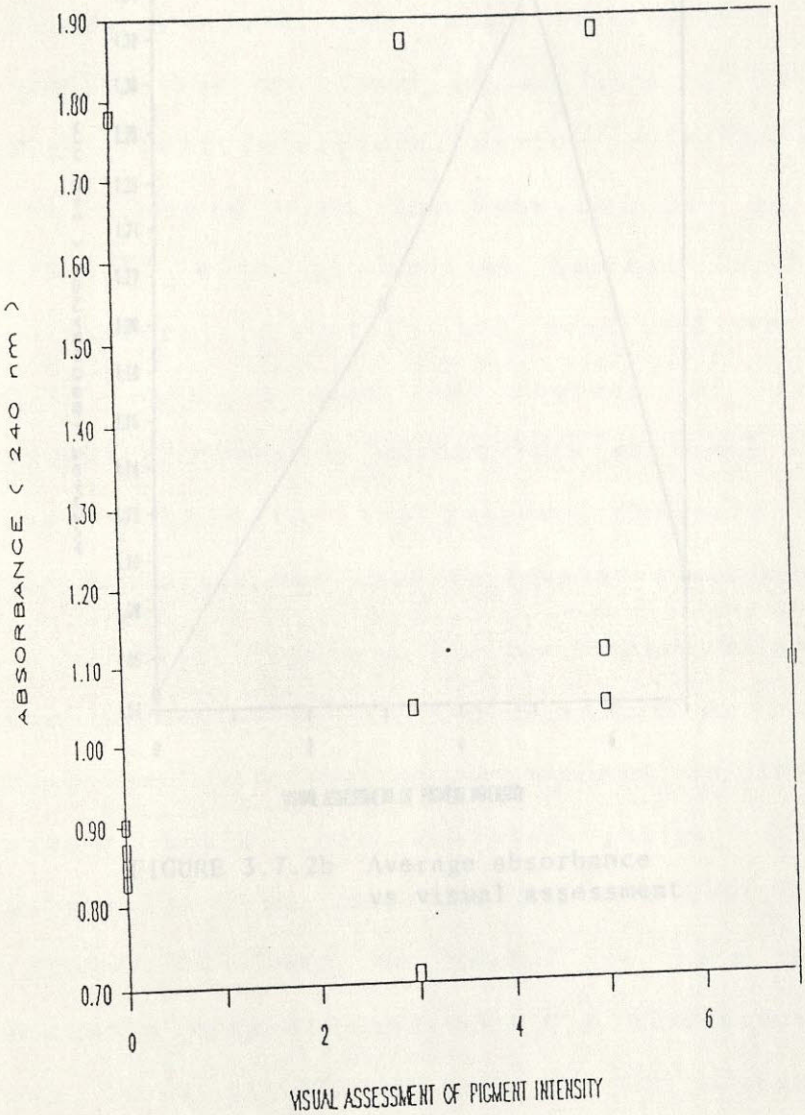


FIGURE 3.7.2a Absorbance values vs visual assessments

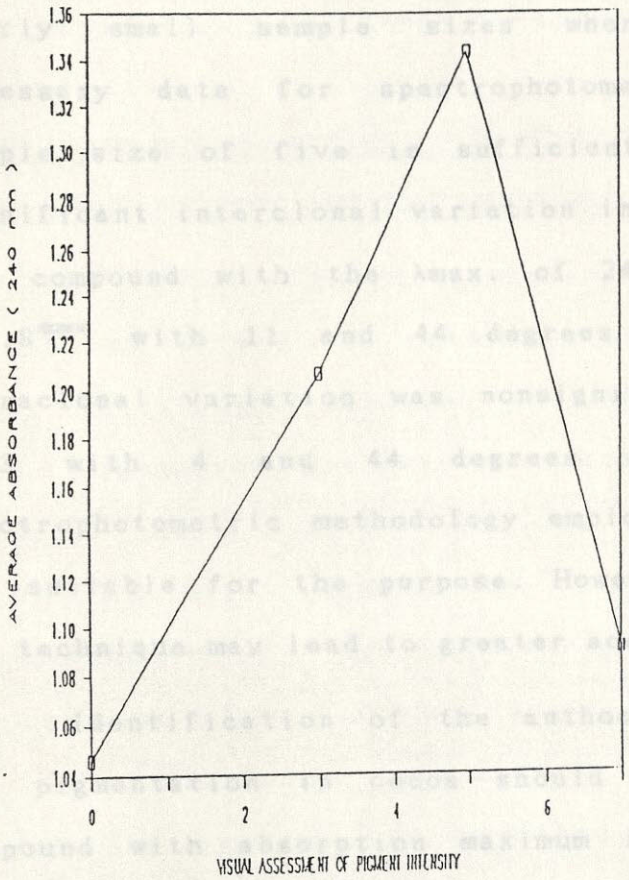


FIGURE 3.7.2b Average absorbance vs visual assessment

The relatively low degree of intraclonal variation indicates that it is acceptable to use fairly small sample sizes when collecting the necessary data for spectrophotometric analyses. A sample size of five is sufficient. There was also significant interclonal variation in the expression of the compound with the λmax. of 240 nm. (F ratio - 215.8*** with 11 and 44 degrees of freedom). The intraclonal variation was nonsignificant (F ratio - 0.73 with 4 and 44 degrees of freedom). The spectrophotometric methodology employed in this study was suitable for the purpose. However, refinement of the technique may lead to greater accuracy.

Identification of the anthocyanins responsible for pigmentation in cocoa should be continued. The compound with absorption maximum in the ultraviolet range could be isolated using chromatographic separation and purification (Hayashi, in Geissman (1962)). It may be useful to note that for the pigments cyanidin and peonidin glycosides, the maxima was found at 281-282 nm, in the ultraviolet region (Fuleki and Francis (1968)). Colourless flavones and flavonols are also perceived in the ultraviolet range (Mabry, in Harborne and Swain (1969); Harborne (1973a); Wellenweber, in Harborne and Williams (1982)). They are invariably present with the coloured anthocyanins in flower petals (Asen et al (1972),

cited by Harborne (1973a)) and are important co-pigments, being essential for the full expression of anthocyanin colour in floral tissues.

3.7.3.1 THE DETERMINATION OF A RELATIONSHIP BETWEEN THE VISIBLE PIGMENT AND THE INVISIBLE COMPOUND

The above spectrophotometric investigation prompted an attempt to determine whether there is a linear relationship between the two main compounds found. Regression analysis was used to achieve the objective using data for twelve clones, whose absorbances were recorded at 240 nm and 417 nm wavelengths.

The mean absorbance values ranged from 0.71 to 1.88 and 0.13 to 0.39, respectively (refer to Table 3.7.4). The analysis of variance performed on the regression data yielded the following results (refer to Table 3.7.5). The regression sum of squares due to fitting the model:

$$y = \alpha + \beta x + \epsilon, \qquad 3.7.1$$

where α is y without the effect of x, β is the slope between x and y and ϵ is the residual; the distance of each point from the straight line,

was not significant. (Refer to table 3.7.4 for the x and y values.)

Table 3.7.4 Absorbances of twelve cocoa clones' sepal extracts at 240 nm and 417 nm wavelengths.

CLONE	MEAN ABSORBANCE AT 240 nm (x)	MEAN ABSORBANCE AT 417 nm (y)
EET 272	1.78	0.15
EET 338	1.88	0.36
EET 400	1.87	0.27
ICS 1	1.04	0.36
ICS 6	1.11	0.33
ICS 39	1.04	0.29
ICS 95	1.09	0.39
IMC 67	0.85	0.18
SCA 6	0.90	0.20
SCA 11	0.71	0.23
SCA 12	0.87	0.13
SCA 19	0.83	0.18

Table 3.7.5 Results of ANOVA of the regression model data.

SOURCE	DEGREES OF FREEDOM	SUMS OF SQUARES	MEANSQUARE	F RATIO
Regression	1	0.67	0.67	3.40^{ns}
Error	10	1.98	0.198	
Total	11	2.66		

ns = not significant.

The intensity of the pigment cannot be used to predict the concentration of the compound, (perceived in the uv range) which might be a co-pigment (refer to figure 3.7.3). The coefficient of determination (R^2) of the model tested was very low (0.25) and further supports this finding.

Despite the absence of a significant relationship between the aforementioned compounds in the clones studied, no conclusion can be made with certainty until more clones are examined.

3.7.4 CONCLUSION

The spectrophotometric determination of pigment intensity in several cocoa clones proved to be useful. Pigment intensity may have considerable discriminatory power with regard to cacao clones. It is recommended that a quantitative measure of pigment intensity in the sepals be included in cocoa descriptor lists since even careful visual assessment of pigment intensity may involve subjectivity.

Visual assessment of the intensity of anthocyanin in the cacao flush, floral components and fruit is quickly and easily done using a colour chart. The quantitative assessment of the pigment by spectrophotometry (or colorimetry) is superior because it excludes subjectivity, but its practicability has

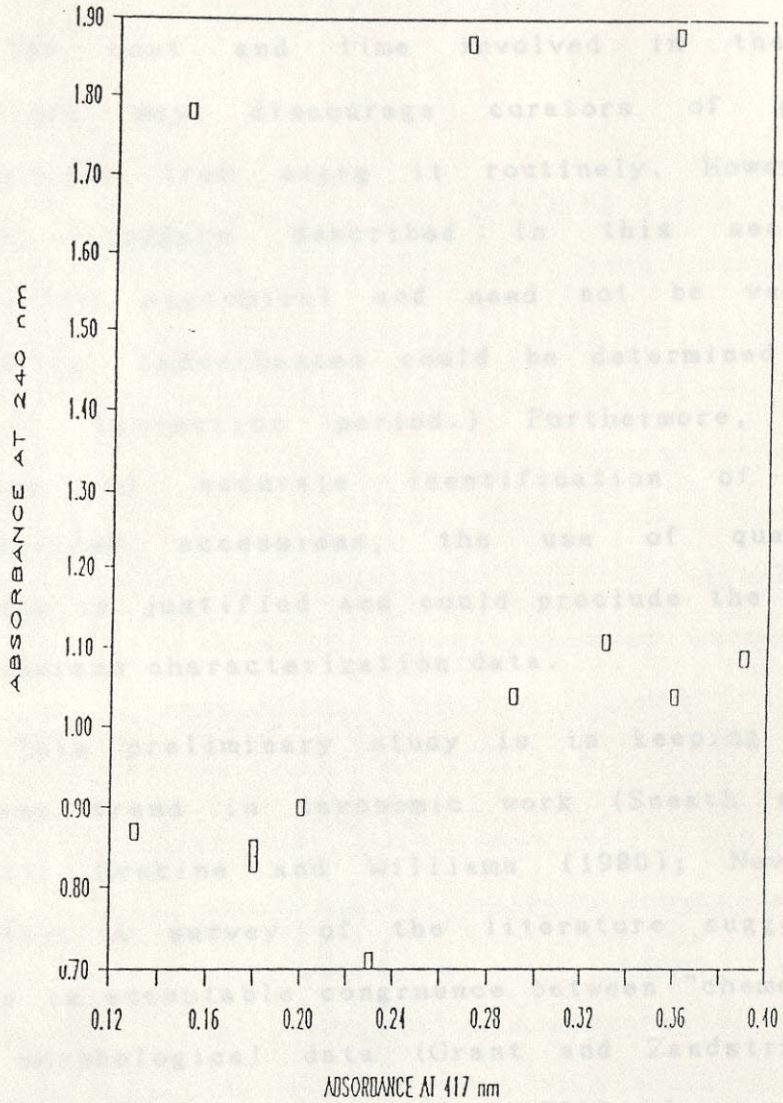


FIGURE 3.7.3 Absorbance at 240 nm vs 417 nm

to be considered.

The cost and time involved in the latter technique may discourage curators of germplasm collections from using it routinely. However, the crude procedure described in this section is reasonably economical and need not be very time-consuming. (Absorbances could be determined after a shorter extraction period.) Furthermore, for the purpose of accurate identification of old or mislabelled accessions, the use of quantitative methods is justified and could preclude the collation of numerous characterization data.

This preliminary study is in keeping with the present trend in taxonomic work (Sneath and Sokal (1973); Erskine and Williams (1980); Nevo et al (1984)). A survey of the literature suggests that there is acceptable congruence between "chemotaxonomy" and morphological data (Grant and Zandstra (1968), cited by Sneath and Sokal (1973)). It is envisaged that such methods will become increasingly important in the description of germplasm (refer to section 2.4).

4.0 GENERAL DISCUSSION AND CONCLUSION

4.1 INTRODUCTION

This investigation was designed to assess the usefulness of the majority of cocoa descriptors, included in the IBPGR Descriptor List for cocoa, using germplasm from the ICG,T, and was in accordance with the goal of the IBPGR to promote international usage of a standardized list. The study also attempted to determine the applicability of a standardized descriptor list for the description of germplasm of different geographical origin, under differing environmental conditions.

The international acceptance of a standardized descriptor list would ensure uniform documentation of information pertaining to *T. cacao* L. and consequently, the universal interpretation of the germplasm data. It is hoped that this would ultimately result in improved cocoa research in such areas as breeding, pathology and agronomy. The time and effort involved in storage and retrieval of information would be reduced. The researchers would be able to evaluate material obtained under a range of environmental conditions through international collaboration.

4.2 THE USEFULNESS OF THE DESCRIPTORS STUDIED, AS DETERMINED BY ANALYSES PERFORMED

The results of Analysis of Variance of the individual descriptors and tests of the stability of the individual descriptors in two slightly different environments suggest 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 more genetic control being exerted on the reproductive characters in the process of evolution. These results correspond to those of Ostendorf (1956), who proposed that the cocoa flower is the most effective character for distinguishing between clones.

A comparison of the mean values for the descriptors, obtained under different environmental conditions, and the results of Correlation Analysis indicate that several quantitative descriptors are more valuable. These descriptors include total leaf length, leaf apical angle, staminode, petal and sepal lengths, ovule number, pod weight and length, total bean weight and individual bean length and width. They are recommended for quick identification of mislabelled or unknown accessions. However, Cluster Analysis of the data indicates that for the purposes

of classification and documentation of the diversity of the germplasm, it is better to employ as many quantitative and qualitative descriptors as is practicable. These can be selected based on their heritability, stability under differing environmental conditions and suitability for easy observation.

The value of the qualitative (highly heritable) characters (Engels (1986)) has been demonstrated by their importance in the accurate classification of the fifty-three accessions described in St. Augustine. A determination of the discriminative values of these descriptors may be useful to further establish the importance of these descriptors, but since they are of little agro-economic value, and are thus not very important in breeding, no attempt was made to perform such an investigation during this study.

However, careful assessment of the qualitative descriptors revealed that flush colour, pulvinus size, the intensity of anthocyanin in the various parts of the flower and fruit and mucilage colour in the fruit may be useful for differentiating between the clones. Other characters, such as the presence of pulvini, anther disposition and sepal reflexion, did not contribute much useful information about these accessions, since very limited variability was displayed.

The pigment intensity of the leaf flushes and flower sepals varied somewhat on different parts of the tree and may be affected by environment. This variability was more marked in the leaf flushes. This underlines the importance of recording the extant environmental conditions and of random selection of material from standardized positions on the tree, when collating qualitative data. Furthermore, some of the subjectivity involved in recording these characters can be removed by quantification. Pigment intensity can be assessed using spectrophotometry, as outlined in section 3.7, or colorimetry. The financial and time constraints involved should be justified by increased accuracy.

Biochemical descriptors such as the banding pattern of various iso-enzymes may be useful inclusions (Brown (1983)) for the existing cocoa descriptor list, which consists of mainly morphogenic descriptors.

4.3 SUMMARY OF FINDINGS

1) The results of this study show that the present cocoa descriptor list is useful and effective for cocoa germplasm description.

2) As large a subset of descriptors, as practical, is recommended for classification of such

germplasm collections.

3) However, to reduce the time and cost involved in documentation, eleven quantitative and about six qualitative descriptors are recommended for identification of unknown material. These include pod weight and length, total bean weight, bean length and width, staminode, petal and sepal lengths, ovule number, total leaf length, leaf apical angle, flush colour, pulvinus size, anthocyanin intensity in organs such as sepals and mucilage colour.

4) Detailed environmental information should accompany the morphological data.

5) It may be beneficial to incorporate some type of biochemical data in the list, which may serve to "fingerprint" accessions.

4.4 RECOMMENDATIONS FOR FURTHER STUDY

1) The design of a field study to assess genotype-environment interaction and its contribution to the phenotypic variation displayed by a range of cocoa accessions and to determine the specific and additive effects of different environmental factors should be undertaken. Murray (1967) reported the difficulties involved in such a time-consuming study of the broadsense heritabilities of the descriptors. However, the results would contribute much to the

classification of cocoa germplasm.

2) Discriminant Analysis, based on a collection of distinctly diverse accessions, would be useful to further test the discriminatory power of individual descriptors and facilitate future identification of accessions.

REFERENCES

Allard R.N. and Bradshaw A.D. (1964). Implications of genotype-enviromental interactions in applied plant breeding. *Crop Science* 4 : 503-507.

Allen J.B. (1983). In search of the perfect cocoa bean. *New Scientist* 3 :293-296.

Allen J.B. (1984). Strategies and methods for collecting *Theobroma*. *Plant Genetic Resources Newsletter* 57 :8-14.

Allen J.B. and Lass R.A. (1983). London Cocoa Trade Amazon Project. Final Report Phase 1. *Cocoa Growers' Bulletin* (Special Issue) 34 :1-72.

Almeida de C.M.V.C. and Almeida de C.F.G. (1987). The collection of wild cacao in the state of Rondonia, Brazil. *Revista Theobroma* 17 :65-92.

Altieri M. and Merrick L. (1987). In situ conservation of crop genetic resources through maintenance of traditional farming systems. *Economic Botany* 41 :86.

Alvarado, R. and Bullard E.T. (1961). Variation of bean characters in hybrid cacao progenies. In

Proceedings of the American Society for Horticultural Science, Caribbean Region 5 : 105-111.

Alvim P. de T. (1967). Ecophysiology of the cacao tree. In *Proceedings of the Conference Internationale sur les recherches agronomiques cacaoyeres*, Abidjan, Ivory Coast, 15-20 Nov. 1965 :23-25.

Alvim P. de T. (1977). Ecological and physiological determinants of cacao yield. In *Proceedings of the Fifth International Cocoa Research Conference*, Ibadan, Nigeria, 1-9 Sept. 1975 :25-37.

Amponsah J.D. (1973). Studies in pod size variation. *Report of the Cocoa Research Institute Ghana 1971-1972* :201-203.

Amponsah J.D. (1977). The cropping pattern of cocoa and resistance to black pod disease caused by *Phytophthora palmivora* (Butl.) Butl. In *Proceedings of the Fifth International Cocoa Research Conference*, Ibadan, Nigeria, 1-9 Sept. 1975 :72-78.

Anderson T.W. (1984). *An Introduction to Multivariate Statistical Analysis*. 2nd ed.. John Wiley and Sons, 675pp.

Andrews D.J. and Hardwick R.C. (1982). A database management system for information retrieval and

documentation of experiments for plant breeders. *Euphytica* 31 (1982) :281-285.

Anon. (1952). A cacao collecting expedition to the Amazon Valley in Colombia. *Tropical Agriculture* (Trinidad) 29 :2.

Anon. (1959). World cacao survey. *FAO Technical Bulletin* (draft) FAO/59/1/268, 127 pp.

Are L.A. and Atanda O.A. (1972). Seasonal influence on some yield factors in four varieties of *Theobroma cacao* L. *Tropical Agriculture* (Trinidad) 49 :161-170.

Are L.A. and Gwyne-Jones D.R.G. (1974). *Cacao in West-Africa*. Ibadan: Oxford University Press, 146pp.

Asomaning A. and Lockard G. (1963). Note on estimation of leaf areas of cocoa from leaf length data. *Canadian Journal of Plant Science* 43 :243-245.

Atanda O.A. (1972). Correlation studies in Theobroma cacao L.. *Turrialba* 22, 81-89.

Atkinson M.D., Withers L.A. and Simpson M.J.A. (1986). Characterisation of cacao germplasm using isoenzyme markers 1. A preliminary survey of diversity using

starch gel electrophoresis and standardisation of the procedure. *Euphytica* 35 :741-750.

Bacon P.R. and Cooper G.C. (1981). *The natural resources of Trinidad and Tobago*. London: Arnold, 223pp.

Baker R.E.D., Cope F.W., Holliday P.C., Bartley B.G.D. and Taylor D.J. (1954). The Anglo-Colombian Cacao collecting Expedition. (Imperial College of Tropical Agriculture, Trinidad) *Annual Report on Cacao Research 1953* :8-18.

Balasimha D., Subramonian N. and Subbaiah C.C. (1985). Leaf characteristics in cocoa (*Theobroma cacao* L.) accessions. *Cafe', Cacao, The'* 29 :95-98.

Bartley B.G.D. (1964). Genetic studies. (Imperial College of Tropical Agriculture, Trinidad) *Annual Report on Cacao Research 1963:* 32.

Bartley B.G.D. (1971). Procedures for the selection of varieties for commercial planting. In *Proceedings of the Third International Cocoa Research Conference*, Accra, Ghana, 23-29 Nov. 1969 :584-589.

Bartley B.G.D. (1981). Global concepts for genetic resources and breeding in cacao. In *Proceedings of the*

Seventh International Cacao Research Conference, Doula, Cameroun, 4-12 Nov. 1979 :519-525.

Bartley B.G.D. and Chalmers W.S. (1971). Cacao germplasm collection at U.W.I.. *Plant Genetic Resources Newsletter* 26 :2-4.

Beauchamp E.T. (1982). The London Cocoa Trade Amazon Project. *Plant Genetic Resources Newsletter* 50 :32-35.

Blank F. (1958). Anthocyanins, flavones, xanthones. *Handbuch der Pflanzenphysiologie* 10: 300-353.

Brown W.L. (1983). Genetic diversity and genetic vunerability - an appraisal. *Economic Botany* 37 :4-12.

Brown C.B. and Bally G.S. (1970). *Land capability survey of Trinidad and Tobago No.3 Soils of Northern Trinidad and Tobago.* Port of Spain: Government Printery, 63.

Budin K.Z. (1973). Introduction and documentation of genetic resources. *Trudy po Prikladnoi Botanike, Genetike i Selektsii* 49 :200-210.

Burt R.L., Edye L.A., Williams W.T., Grof B. and Nicholson C.H.L. (1971). Numerical analysis of variation patterns in the genus *Stylosanthes* as an aid to

plant introduction and assessment. *Australian Journal of Agricultural Research* 22 :737-757.

Burt R.L., Pengelly B.C. and Williams W.I. (1980). Network analysis of genetic resources data 111. *Agroecosystems* 6 :119-127.

Chalmers W.S. (1972). The conservation of wild cacao populations - Plant breeders' most urgent task. In *Proceedings of the Fourth International Cocoa Research Conference*, St. Augustine, Trinidad, 8-18 Jan., 1972 :114-118.

Chalmers W.S. and Murray D.B. (1973). Las Hermanas Progeny Trials No.2-20 (1962). *Annual Report on Cocoa Research 1973* :7-24.

Chassevent F. and Ornano M.D. (1966). La de'termination photome'trique des pigments du cacao. *Cafe', Cacao, The'* (Paris) 10 :243-266.

Chatt E.M. (1953). *Cocoa - cultivation, processing, analysis*. New York: Interscience Publishers, 302pp.

Cheesman E.E. (1932). The Economic botany of cacao. A critical survey of the literature to the end of 1930. *Tropical Agriculture* (Trinidad) 9 (6) Supplement :1-16.

Cheesman E.E. (1944). Notes on the nomenclature, Classification and possible relationships of cacao populations. *Tropical Agriculture* (Trinidad) 21 :144-159.

Ciferri R. and Ciferri F. (1957). The evolution of cultivated cacao. *Evolution* 11 :381-397.

Cook A.B. (1987). Review of production and consumption. *Cocoa Grower's Bulletin* 38 :2-4.

Cook O.F. (1916). Branching and flowering habits of cacao and patashte. In *Contributions to the U.S. National Herbarium* 1 :609-625.

Cope F.W. (1976). Cacao *Theobroma cacao* L. (Sterculiaceae). In *Evolution of Crop Plants*. (Ed. Simmonds, N.W.). London: Longman, 285-289.

Creech J.L. and Reitz L.P. (1971). Plant germplasm now and for tomorrow. *Advances in Agronomy* 23 :1-49.

Crovello T. (1969). Effects of change of characters and of number of characters in numerical taxonomy. *American Midland Naturalist* 81 :68-86.

Cuatrecasas J. (1964). Cacao and its allies - a taxonomic revision. In *Contributions to the U.S. National*

Herbarium 35 : 375-614.

Cunningham R.K. and Lamb J. (1959). A cocoa shade and manurial experiment at WACRI, Ghana 1. *Journal of Horticultural Science* 34 :14-22.

Damania A.B., Porceddu E. and Jackson M.T. (1983). A rapid method for the evaluation of variation in germplasm collections of cereals using polyacrylamide gel electrophoresis. *Euphytica* 32 :877-883.

Dambroth M. (1979). Technical aspects of information management and means of communication in Plant Genetic Resources work for a future utilization of genetic material in Plant Breeding. In *Proceedings of a colloquium* held in Braunschweig on 7th and 8th Nov. 1978 :48-71.

Draper S.R. (1976). *Biochemical analysis in crop science*. London: Oxford University Press, 34-39.

Edwards A.W.F. and Cavalli-Sforza L.L (1965). A method for cluster analysis. *Biometrics* 21 :362-375.

Edwards D.F. (1973a). Seasonal variation in pod and bean characters. *Report of the Cocoa Research Institute of Ghana 1971-1972* :172.

Engels J.M.M. (1981). *Genetic resources of cacao. Catalogue of CATIE collection*. (Technical Bulletin No.7) Turrialba, Costa Rica: CATIE Plant Genetic Resources Unit, 196 pp.

Engels J.M.M. (1983a). A systematic description of cacao clones 1. The discriminative value of quantitative characteristics. *Euphytica* 32 :377-385.

Engels J.M.M. (1983b). A systematic description of cacao clones 11. The discriminative value of qualitative characteristics and the practical compatability of the discriminative value of quantitative and qualitative descriptors. *Euphytica* 32 :387-395.

Engels J.M.M. (1983c). A systematic description of cacao clones 111. Relationships between clones, between characteristics and some consequences for the cacao breeding. *Euphytica* 32 (3) :719-733.

Engels J.M.M. (1984). A systematic description of cacao clones 4. Some evidence of tetraploid inheritance. *Cafe', Cacao, The'* 28 :95-102.

Engels J.M.M. (1986). The systematic description of cacao clones and its significance for taxonomy and

plant breeding. Doctoral thesis. Agricultural University, Wageningen, 125 pp.

Engels J.M.M., Bartley B.G.D., and Enriquez G.A. (1980). Cacao descriptors, their states and modus operandi. *Turrialba* 30 :209-218.

Enriquez C.G. (1969). Tamano optimo de la meustra para hacer estudios biometricos em plantitas de cacao. (T. cacao L.) In *Proceedings of the Second International Cocoa Research Conference*, Bahia, Brasil, 19-26 Nov. 1967 :120-123.

Enriquez C.G. and Soria V.J. (1966). Estudio de la variabilidad de varias caracteristicas de las mazorcas de cacao (*Theobroma cacao* L.). *Fitotecnia Latino americana* 3 (1 & 2) :99-118.

Enriquez C.G. and Soria V.J. (1967a). Seleccion y estudio de los caracteres utiles de la flor para la identificacion y descripcion de cultivares de cacao. *Cacao* (Costa Rica) 12 :8-16.

Enriquez C.G. and Soria V.J. (1967b). *Cacao cultivars register*. Turrialba, Costa Rica: IICA Teaching and Research Center, 550 pp.

Enriquez C.G. and Soria V.J. (1967c). A study of certain leaf characteristics of cacao. *Tropical Agriculture* (Trinidad) 44 :117-123.

Enriquez C.G. and Soria V.J. (1968). The variability of certain bean characters of cacao. (*Theobroma cacao* L.) *Euphytica* 17 :114-120.

Enriquez G. and Soria V.J. (1981). Mejoramiento genetico para resistencia a cinco enfermedades de cacao. In *Proceedings of the Sixth International Cocoa Research Conference,* Caracas, Venezuela, November 1977 :41-56.

Enriquez G.A., Quiros S. and Lopez O. (1988). Characteristics and phytogenetic relationships of cacao pods and beans in cultivars from the collection at Turrialba, Costa Rica. In *Proceedings of the Tenth International Cacao Research Conference*, Santo Domingo, Dominican Republic, 17-23 May 1987 :593-598.

Erskine W.T. and Williams J.T. (1980). The principles, problems and responsibilities of the preliminary evaluation of genetic resources samples of seed-propagated crops. *Plant Genetic Resource Newsletter* 41 :19-32.

Eskes A.B., Beek M.A. and Toxopeus H. (1977). Variation and correlations of some pod and bean values

in cacao (*Theobroma cacao* L.) with reference to the parental effects on fruit setting and bean number. *Turrialba* 27 :193-198.

Esquivel O. and Soria V.J. (1962). Recuento del numero de ovulos en differentes tipos de cacao. *Cacao* (Costa Rica) 7 (3) :9.

Estabrook K.G. and Rogers D. (1966). A general method for taxonomical description for a computed similarity measure. *Bioscience* 16 :789-793.

Evans H. and Fennah K.G. (1953). Investigations on the mineral nutrition of cacao. *A Report on Cacao Research, 1945-1951.* (Imperial College of Tropical Agriculture, Trinidad) :38-52.

Fennah R.G. (1957). An analysis of yield variation in a sample of cacao trees. *A Report on Cacao Research, 1955-56.* (Imperial College of Tropical Agriculture, Trinidad) :9-20.

Fisher R.A. (1936). Use of multiple measurements in taxonomic problems. *Annual Eugenics* 7 :179-184.

Frankel O.H. (1970). Genetic conservation of plants useful to man. *Biological Conservation* 2 :162-170.

Frankel O.H. (1975). Genetic conservation in perspective. In *Crop Genetic resources for today and tomorrow.* (IBP Handbook No. 2) (Eds. Frankel O.H. and Hawkes J.G.). Cambridge: Cambridge University Press 1975 :469-489.

Frankel O.H. and Hawkes J.G. eds. (1975). *Crop genetic resources for today and tommorrow*. (IBP Handbook No. 2) Cambridge: Cambridge University Press. xix, 492pp.

Freeman G.H. (1973). Statistical methods for the analysis of G.E. interaction. *Heredity* 31 :339-354.

Freeman G.H. and Perkins J.M. (1971). Environment and genotype-environment components of variability. *Heredity* 27 :15-23.

Fuleki T. and Francis F.J. (1967). Standards for Quantitative Analysis of anthocyanins. In *Proceedings of the American Society for Horticulture and Science* 91 :894-897.

Fuleki T. and Francis F.J. (1968). Quantitative methods for anthocyanins 1. Extraction and determination of total anthocyanin in cranberries. *Journal of Food Science* 33 :72-77.

Geissman T.A. (ed.) (1962). *The chemistry of flavonoid compounds*. Oxford: Pergamon Press Inc., 666pp.

Genstat 5 Committee (1988). *Genstat 5 Reference Manual*. (Eds. Lane P.W. and Payne R.W.) Oxford: Clarendon Press, 735pp.

Ghosh B.N. (1973). Physical properties of cocoa beans. *Turrialba* 23 :438-443.

Ghosh B.N. (1976). The shape and size of cocoa beans. *Turrialba* 26 :134-138.

Glendinning D.R. (1961). Problems in the assessment of cocoa varieties. *Ghana Journal of Science* 6 :38-43.

Glendinning D.R. (1963). The inheritance of bean size, pod size and number of beans per pod in cocoa (*T. cacao* L.) with a note on bean shape. *Euphytica* 12 :311-322.

Glendinning D.R. and Martinson V. (1967). Fat contents, bean sizes and shell percentages. *Annual Report of the Cocoa Research Institute (Ghana)* 1965-1966 :87-89.

Goodall D.W. (1947). Diurnal changes in the area of cacao leaves. *Annals of Botany* (NS) 11 :449-451.

Goodall D.W. and Posnette A.F. (1947). A morphological change induced in leaves of *Theobroma cacao* L. by mineral deficiency. *Nature* 159 :542-544.

Goodman M.M. (1968). The races of maize : 11. Use of multivariate analysis of variance to measure morphological similarity. *Crop Science* 8 :693-698.

Greenwood M. and Posnette A.F. (1950). The growth flushes of cocoa. *Journal of Horticultural Science* 25 :164-174.

Gregory P.H., (Ed.) (1974). *Phytophthora* disease of cocoa. London: Longman Group, 348pp.

Gregory P.H. (1977). Problems of seeds and selected crop plants: cocoa (*T. cacao* L.). In *Plant Health and quarantine in the international transfer of genetic resources.* (Eds. Hewitt, H. and Chiarappa L.) Cleveland: CRC Press :119-124.

Harborne J.B. (1963). Distribution of anthocyanins in higher plants. In *Chemical Plant taxonomy.* (Ed. Swain, T.). London: Academic Press, 359-387.

Harborne J.B. (1965). Flavonoids: distribution and contribution to plant colour. In *Chemistry and Biochemistry of plant pigments.* (Ed. Goodwin, T.). London: Academic

Press, 247-276.

Harborne J.B. (1973a). Flavonoids. In *Phytochemistry. Organic metabolites.* Vol. 11. The Hague: Van Nostrand Reinhold Company, 344 pp.

Harborne J.B. (1973b). Phenolic compounds. In *Phytochemical methods: a guide to modern techniques of plant analysis.* London: Chapman and Hall, 59-67.

Harborne J.B. and Sherratt H.S.A. (1961). Plant Polyphenols 3. Flavonoids in genotypes of *Primula sinensis.* *Biochemistry Journal* 78 :298-306.

Harborne J.B. and Williams C.A. (1982). Flavone and flavonol glycosides. In *The flavonoids: advances in research.* (Eds. Harborne J.B. and Mabry T.J.). London: Chapman and Hall, 261-311.

Hardwick R.C. and Wood J.T. (1972). Regression methods for studying GE interactions. *Heredity* 28 :209-222.

Hardy F. (1960). *Cacao manual.* Turrialba, Costa Rica: Inter-American Institute of Agricultural Sciences, 395pp.

Harlan J. (1975). Our vanishing genetic resources.

Science 188 :618.

Hayashi K. (1962). The anthocyanins. In *The chemistry of flavonoid compounds* (Ed. Geissman T.A.). Oxford: Pergamon Press Inc., 248-285.

Hill J. (1975). Genotype-environment interactions - a challenge to plant breeding. *Journal of Agricultural Science* 85 :477-493.

Hondelmann W. (1974). Safeguarding the plant genetic resources of the developing countries. *Plant Research and Development* 1 :7-11.

Hrazdina G. (1982). Anthocyanins. In *The flavonoids: advances in research.* (Eds. Harborne J.B. and Mabry T.J.). London: Chapman and Hall, 135-186.

IBPGR Secretariat (Rome) (1981). *Report :IBPGR Working Group on genetic resources of cocoa.* (ACP :IBPGR/80/56 March 1981) Rome: IBPGR, 28pp.

IBPGR Cocoa Working Group (1984). *Report of the Second Meeting*, 21-22 October 1983, Arlington, Va, U.S.A., Rome: IBPGR Secretariat, 34pp.

IBPGR (1989). *Annual Report* 1988. Rome: IBPGR

Secretariat, 87pp.

Illoh H.C. and Olorode O. (1991). Numerical taxonomic studies of mango (*Mangifera indica* L.) varieties in Nigeria. *Euphytica* 51 :197-205.

Iltis H.H., Kolterman D.A. and Benz B.F. (1986). Accurate documentation of germplasm: the lost Guatemalan Teosinites (Zea, Gramineae) *Economic Botany* 40, 1986 :69-77.

Imbert M.P. (1968). Anthocyanin pigments and other polyphenolic compounds of the yam, the sweet potato and some members of the Convolculaceace family. MSc Thesis, University of The West-Indies, St. Augustine, Trinidad, 135pp.

Jacob V.J. (1969). Studies on the number of ovules per ovary in relation to the number of beans per pod. *Annual Report of the Cocoa Research Institute of Nigeria,* 1967-68 :60-62.

Jacob V.J. and Atanda O.A. (1973). Comparative field pod values of West African Amelonado and Amazon cacao in Nigeria. *Ghana Journal of Science* 13 :72-76.

Jain S.K. (1975). Genetic resources. In *Crop genetic resources for today and tomorrow.* (Ed. Frankel O.H. and

Hawkes J.G.) (IBP Handbook 2) Cambridge: Cambridge University Press, 379-396.

Jain S.K. (1979). Biosystematic studies of populations in germplasm collections. In *Proceedings of the Conference on broadening the genetic base of crops*. Wageningen, Netherlands, 3-7th July, 1978. (Ed. Zeven A.C. and van Harten A.M.). The Hague: Centre for Agricultural Publications and Documentation, 1979, 103-110.

Jaradat A. A. (1991). Levels of phenotypic variation for development traits in landrace genotypes of durum wheat (*Triticum turgidum* ssp. *Turgidum* L. conv. *durum* (Desf.) MK.) from Jordan. *Euphytica* 51 :265-271.

Joshi B.S. and Hardas M.W. (1976). The proposed seed bank for plant genetic resources in India. *Seed Research* 4 :167-173.

Jurd L. (1962). Spectral properties of flavonoid compounds. In *The chemistry of flavonoid compounds*. (Ed. Geissman T.A.). Oxford: Pergamon Press Inc., 107-155.

Kennedy A.J.K. (1985). The International Cocoa Genebank, Trinidad : its role in future cocoa research and development. In *Proceedings of the Ninth International Cocoa Research Conference*, Lome', Togo, 12-18 February 1984 :63-

67.

Konzak C.F. (1972). Progress toward internationally standardised documentation of genetic resource collections from plant exploration. *Agronomy Abstracts* 13 :13 (Z5073.A281A1).

Konzak C.F. and Dietz S.M. (1969). Documentation for the conservation, management and use of plant genetic resources. *Economic Botany* 23 :299-308.

Krzanowski W.J. (1987). Selection of variables to preserve multivariate data structure, using Principal components. *Applied Statistics* 36 :22-33.

Kuppers J.R. (1953). Some biometric observations on cacao fruit. *Science* 117 :354-355.

Lachenaud P. (1984). A method for evaluating fresh seed yield, which can be applied to completely randomized trials. *Cafe', Cacao, The'* 28 :83-88.

Lass R.A. and Wood G.A.R. (eds) (1985). Cocoa production : present constraints and priorities for research. In *World Bank Technical Paper* 39. Washington, D.C.: The International Bank for reconstruction and development/ The World Bank, 95pp.

Lawrence E. (1975). Future in the past. *Nature* 258 :278-279.

Lawrence T. (ed) (1984). *Collection of crop germplasm -the first ten years, 1974-84.* Rome: IBPGR Executive Secretariat, 119pp.

Legendre P. and Rogers D.J. (1972). Characters and clustering in taxonomy :a synthesis of two taximetric procedures. *Taxon* 21 :567-606.

Leon J. (1960). Taxonomy of cacao and related genera. In *Cacao Manual.* (Ed. Hardy F.) Turrialba, Costa Rica: Inter-American Institute of Agricultural Sciences, 338-357.

Leon J. (1974). Germplasm collections in Central America. *Plant Genetic Resources Newsletter* 26 :10-19.

Lockwood G. (1976). Determination of wet-dry weight conversion ratios. *Annual Report Cocoa Research Institute, Ghana* 1973-1974 :171-73

Lockwood G. (1985). Genetic resources of the cocoa plant. *Span* 28 :14-16.

Lockwood G. and Edwards D.F. (1980). Determination of

pod and bean characters in progeny trials with cocoa. *Tropical Agriculture* (Trinidad) 57 :289-300.

Lockwood G. and Gyamfi M.M. (1979). The Crig Cocoa Germplasm collection with notes on codes used in the Breeding Programme at Tafo and elsewhere. *The Cocoa Research Institute, Ghana Technical Bulletin* No. 10 :1-62.

Lopez O.B. and Enriquez G.A. (1988). Inheritance of dry weight and bean shape in cacao. In *Proceedings of the Tenth International Cocoa Research Conference,* Santo Domingo, Dominican Republic, 17-23 May, 1987, 691-694.

Lucas P. and Reffye P.H. de (1981). Assessments of genetic parameters for some cacao tree clones. In *Proceedings of the Seventh International Cacao Conference,* Douala, Cameroon, 4-7 Nov., 1979, 541-543.

Mabry T.J. (1969). The ultraviolet and nuclear magnetic resonance analysis of flavonoids. In *Perspectives in Phytochemistry.* (Ed. Harborne J.T. and Swain T.) London: Academic Press, 1-44.

Mardia K.V., Kent J.T. and Bibby J.M. (1979). *Multivariate analysis.* London: Academic Press, 281-325.

Martinson V.A. (1976). Studies on seed development.

Annual Report of the Cocoa Research Institute of Ghana, 1973-1974 :174-176.

Martinson V.A. (1976). Ovule numbers. *Annual Report of the Cocoa Research Institute Ghana* ,1973-1974 :175.

McCabe G.P.(1984). Principal variables. *Technometrics* 26 :137-144.

McDonald J.A. (1932). An Environmental study of the cacao tree. *Annual Report of Cocoa Research* 1931-1945. (Imperial College of Tropical Agriculture, Trinidad) :29-43.

McDonald J.A. (1932). A survey of some Trinidad cacao soils. *Annual Report of Cacao Research* 1931-1945. (Imperial College of Tropical Agriculture, Trinidad) :36-41.

Miller J.F. (1982). Development of a data management information system for a germplasm collection of flax. *Canadian Journal of Plant Science* 62 :827-830.

Miller L. (1973). Isolation and structural determination of flavonoids. In *Phytochemistry 11.* (Ed. Miller L.) New York: Van Nostrand Reinhold, 357-358.

Miri R.K. (1980). Genetic resources of Wheat. *Indian*

Journal of Genetics and Plant Breeding 40 :27-33.

Mora Urpi J. (1958). Notas sobre el posible origen y la variabilidad del cacao cultivado en America Tropical. *Turrialba* 8 :34-43.

Morishima H. (1969). Phenetic similarity and phylogenetic relationships among strains of *Oryza perennis* estimated by methods of numerical taxonomy. *Evolution* 23 :429-443.

Morrison D.F. (1976). *Multivariate statistical methods.* New York: McGraw Hill, 415pp.

Murray D.B. (1952). Mineral nutrition of *T. cacao. A report on Cacao Research,* 1945-1951. (Imperial College of Tropical Agriculture, Trinidad) :77-78.

Murray D.B. (1967). Factors affecting the shade-nutrition interaction in cacao. In *Proceedings of Conference Internationale sur les recherches agronomiques cacaoyeres,* Abidjan, Ivory Coast, 15-20 Nov. 1965, 109-115.

Muttscheller R.W., Gonsalves C. and Toyer A. (1987). Establishment of the ICG,T. *Annual Report Cocoa Research Unit, Trinidad* 1984-1986 :12.

Negassa N. (1986). Estimates of phenotypic diversity and breeding potential of Ethiopian wheat. *Hereditas* 104 :41-48.

Nevo E., Beiles A. Gutterman Y. Storch N. and Kaplan D. (1984). Genetic resources of wild cereals in Israel and vicinity 1. Phenotypic variation within and between populations of wild wheat, *Triticum dicoccoides*. *Euphytica* 33 :717-735.

Ng N.Q. (1982). Genetic resources programme of IITA. *Genetic Resources Newsletter* 49 :26-28.

Onyiliagha J.C. (1986). Numerical analysis of variation among Nigerian *Dioscorea rotundata* accessions. *Euphytica* 35 :413-419.

Orloci L. (1978). *Multivariate analysis in vegetation research*. The Hague: D.W. Junk, ix, 451pp.

Ostendorf F.W. (1956). Identifying characters for cacao clones. Reuniao do Comite' Tecnico Interamericano do cacao (6a, Salvador, Bahia, Brasil). *Trabajos presentados* :89-110.

Paterson A.W. and Reed R.L. (1943). Variation in the size of Trinidad cacao beans and methods of its

assessment. *Tropical Agriculture* (Trinidad) 11 :252-261.

Pearce S.C. (1953). Field experiments with fruit trees and other perennial plants. *Technical Communication Commonwealth Bureau of Horticultural Plantation Crops* 23 :1-182.

Plucknett D.L., Smith N.J.H., Williams J.T. and Murthi Anishetty N. (1983). Crop Germplasm Conservation and Developing Countries. *Science* 220 :163-169.

Porter W.M. and Smith D.H. (1982). Detection of Identification Errors in germplasm collections. *Crop Science* 22 :701-703.

Posnette A.F. (1981). Note on the origin of Scavina. In *Archives of Cocoa Research* 1. *Cocoa populations and Witches' Broom disease in South America.* (Ed. Toxopeus, H.) Washington : International Office of Cocoa and Chocolate and The American Cocoa Research Institute , 13.

Pound F.J. (1932). The Genetic Constitution of cacao crops 1. *Annual Report of Cacao Research* 1931-1945. (Imperial College of Tropical Agriculture, Trinidad) :10-22.

Pound F.J. (1933). The Genetic Constitution of cacao crops 11. *Annual Report of Cacao Research* 1931-1945. (Imperial College of Tropical Agriculture, Trinidad)

:9-26.

Pound F.J. (1934). A preliminary survey of pigment factors in cacao. *Annual Report of Cacao Research* 1931-1945. (Imperial College of Tropical Agriculture, Trinidad) :11-15.

Pound F.J. (1938). Cacao and Witches' Broom disease of South America with notes on other species of *Theobroma*. In *Report on a visit to Ecuador, the Amazon Valley and Colombia*, April 1937 - April 1938. Port-of Spain: Yuille's Printerie, 58pp.

Rao C.R. (1952). *Advanced statistical methods in Biometric research*. New York: Wiley, 390pp.

Reffye Ph. de, Parvais J.P., Mossu G. and Lucas P. (1981). Influence des modalites de la pollinsation sur la recolte du cacaoyer. In *Proceedings of the Seventh International Cocoa Research Conference*, Douala, Cameroun, 4-7 Nov. 1979, 545-552.

Reyes H.E., Perez A.Z., De Reyes L.C., de J. Wagner O.M. (1973). *Catalogo de cultivares de cacao (Theobroma cacao* L.). Venezuela: Ministerio de Agricultura y Cria, 131pp.

Risi C., J. and Galwey N.W. (1989). The pattern of genetic diversity in the Andean grain crop *quinoa (Chenopodium quinoa* Willd). 1. Associations between characteristics. *Euphytica* 41 :147-162.

Risi C., J. and Galwey N.W. (1989). The pattern of genetic diversity in the Andean grain crop *quinoa (Chenopodium quinoa* Willd). 11. Multivariate methods. *Euphytica* 41 :135-145.

Rogers D. and Flemming H. (1964). A computer program for classifying plants 11. A numerical handling of non-numerical data. *Bioscience* 14 :15-28.

Rogers P.J., Snoad B. and Seidewitz L. (1975). Documentation for genetic resources centres. In *Crop genetic resources for today and tomorrow.* (IBP Handbook 2) (Eds. Frankel O.H. and Hawkes J.G.) Cambridge: Cambridge University Press, 399-405.

Rohlf F.J. and Sokal R.R (1967). Taxonomic structure from randomly and systematically scanned biological images. *Systematic Zoology* 16 :246-260.

Ruinard J. (1961). Variability of various pod characters as a factor in cacao selection. *Euphytica* 10 :134-146.

Ruinard J. (1964). Seasonal influences upon the pod weight of cacao and their consequences for selection. *Euphytica* 13 :19-23.

Schilling E.E. and Heiser C.B. (1976). Re-examination of a numerical study of *Solanum* species and hybrids. *TAXON* 25 :451-562.

Scogin R. (1979). Anthocyanins of the Sterculiaceae. *Biochemical Systematics and Ecology* 7 :35-36.

Seidewitz L (1978a). Establishment of a database for genebank purposes. In *Factual Databanks in agriculture* (Commission of the European Communities) Wageningen: Centre for Agricultural Publishing and Documentation, 39-41.

Seidewitz L. (1978b). Database concepts for a rational handling of information in relation to plant genetically valuable material. In *Technical aspects of information management and means of communication in Plant Genetic Resources work for a future utilization of genetic material in Plant Breeding : Proceedings on a colloquium held in Braunschweig* on 7-8 November 1978.

Shivaarajan V.V. (1984). *Introduction to the principles of taxonomy*. New Delhi: Oxford and IBH, 295pp.

Simmonds N.W. (1981). Genotype (G), Environment (E) and GE components of crop yields. *Experimental Agriculture* 17 :355-362.

Singh H.B. and Hardas M.W. (1969). Collection and evaluation of plant germplasm. *Indian Journal of Genetics and Plant Breeding* 29 :379-385.

Sirju-Charran G., Hosein F. and Johnson E. (unpublished). Classification of cocoa germplasm using isoenzyme analysis. *Quarterly Report of The Cocoa Research Unit, Trinidad,* 1989 :1-5.

Smyth A.J. (1966). The selection of soil for cocoa. *FAO soils Bulletin* 5 :75pp.

Sneath P.H.A. and Sokal R.R. (1973). *Numerical Taxonomy.* San Francisco, CA: W.H. Freeman and Co., 573 pp.

Snedecor G.W. and Cochran W.G. (1967). *Statistical Methods.* 6th ed. Iowa: Iowa State University Press.

Sokal R.R. and Rohlf F.J. (1969). *Biometry. The principles and practice of statistics in biological research.* San Francisco, CA: W.H. Freeman and Company, 776 pp.

Soria V. de J. (1969). Tendencias de la variabilidad

de algunas caracteristicas de los frutos, flores y semillas en los cacao nativos de la hoya amazonica. *Cacao* (1970) 15 :16-17.

Soria V. de J. (1975). Recent cacao collecting expeditions. In *Crop genetic resources for today and tomorrow.* (IBP Handbook 2) New York: Cambridge University Press, 175-179.

Soria V. de J. (1977). The genetics and breeding of cacao (*Theobroma cacao* L.). In *Proceedings of the Fifth International Cocoa Research Conference,* Ibadan, Nigeria, 1-9 Sept. 1975 :18-24.

Soria V. de J. (1981). Insectos pollinizadores : Forcipomyia Metodos para aumentar a pollinizacao e seus ejeitos sobre a producao. In *Proceedings of the Seventh International Cocoa Research Conference,* Douala, Cameroun, 4-12 Nov. 1979 :497-506.

Soria V. de J. and Esquivel O. (1969). Algunos resultados del Programa de mejoramiento Genetico de Cacao en el IICA, Turrialba. In *Proceedings of the Second International Cocoa Research Conference* Bahia, Brasil, 19-26 Nov. 1967 :35-42.

Soria V. de J. and Esquivel O. (1970). Relationship

between precocity, growth and yield in cacao. *Turrialba* 20 :193-197.

Soria V. de J., Camp F. and Paez G. (1974). Parental influence on some cacao clones on the yield performance of their progenies. *Turrialba* 24 :58-65.

Soria V. de J., Siva P. and Chapman R.K. (1983). Influence of floral pigmentation on field pollination rates in some cultivated varieties of *T. cacao* L. : some effects on yield. *Revista Theobroma* 13 :141-149.

Spencer R. (1984). A pattern recognition program in Basic. *Byte* September 1984 :129.

Subali S. and Abdullah I. (1984). Preliminary studies on cacao ovules in relation to bean number and bean size. In *Proceedings of the International Conference on cocoa and coconuts*, Kuala Lumpur, Malaysia, 1984 :9pp.

Suzuki S. and Kamagai K. (1981). Present status of genetic resource information management in Japan. *JARQ* 15 :75-84.

Swain T. (1965). Nature and properties of flavonoids. In *Chemistry and biochemistry of plant pigments*. (Ed. Goodwin T.) London: Academic Press :211-228.

Sykes J.T. (1975). Tree crops. In *Crop genetic resources for today and tomorrow.* (IBP Handbook No. 2) (Eds. Frankel O.H. and Hawkes J.G.) Cambridge: Cambridge University Press, 123-137.

Tai G. (1977). Data organization and analysis - use of the computer in potato breeding research. Utilization of the genetic resources of the potato 11. In *Report of the Planning Conference* 1977. International Potato Centre Lima, Peru, 115-119.

Toxopeus H. (1969). Cacao (*Theobroma cacao* L.). In *Outlines of Perennial Crop Breeding in the Tropics. (Miscellaneous Papers 4)* (Eds. Ferwerda F.P. and Wit F.) Wageningen: Veenman, 79-109.

Toxopeus H. (1981). Cocoa populations and Witches' Broom disease in South America. *Archives of cocoa research* 1 (8) :7-12.

Toxopeus H. and Jacob V.J. (1969). Pod and bean value studies on hand-pollinated pods. *Annual Report of the Cocoa Research Institute of Nigeria* 1967-1968 :49-51.

Toxopeus H. and Jacob V.J. (1970). Studies on pod and bean values of *Theobroma cacao* L. in Nigeria 11. Number of beans per pod with special reference to the natural

pollination process. *Netherland Journal of Agricultural Science* 18 :188-195.

Toxopeus H. and Wessel M. (1970). Studies on pod and bean values of *Theobroma cacao* L. in Nigeria 1. Environmental effects on West African Amelonado with particular attention to annual rainfall distribution. *Netherland Journal of Agricultural Science* 18 :132-139.

Urquhart D.H. (1961). *Cocoa.* 2nd ed. London: Longmans, 293pp.

Van der Knaap W.P. (1953). General considerations regarding the factors that affect the productive capacity of cocoa clones. *Archief worde Koffiecultuur* 17 :121-140.

Van Hall C.J.J. (1914). *Cocoa.* London: Macmillan and Company Ltd., 65.

Van Hall C.J.J. (1932). *Cacao.* 2nd ed. London: Macmillan and Company, 514pp.

Vello F., Garcia S.R., and Magalhaes W.S. (1971). Characteristics of the principal cacao varieties grown in Bahia. *Revista Theobroma* 1 :10.

Vernon A.J. (1968). Edge effects in cocoa shade and manurial experiments. *Journal of Horticultural Science* 43 :147-152.

Veronesi F. and Falcinelli M. (1988). Evaluation of an Italian germplasm collection of *Festuca arundinacea* Schreb, through a multivariate analysis. *Euphytica* 38 :211-220.

Wakeling S.A. (1990). Review of production, consumption, stocks and prices. *Cocoa Grower's Bulletin* 43 :2-3.

Williams J.T. (1981). Cacao genetic resources in Latin America. *Plant Genetic Resources Newsletter* 45 :20-22.

Williams J.T. (1984). A decade of crop genetic resources research. In *Crop genetic resources: conservation and evaluation.* (Eds. Holden J.H.W. and Williams J.T.) London: George Allen and Unwin Ltd., 1-16.

Williams W.T., Burt K.L. and Lance G.N. (1979). A method for establishing character inter-relations in plant collections and its possible application to plant improvement programmes. *Euphytica* 29 :625-633.

Wollenweber E. (1982). Flavones and flavonols. In *The flavonoids: advances in research.* (Eds. Harborne J.B. and

Mabry T.J.) London: Chapman and Hall, 189-245.

Wood G.A.R. (1959). *Cocoa-growing in Venezuela, Colombia and Ecuador. With notes on three cocoa diseases.* Bournville, TX Cadbury Brothers Ltd., 57pp.

Wood G.A.R. (1975). *Cocoa.* (Tropical Agriculture Series). 3rd ed. London: Longman, 292 pp.

Wood G.A.R. and Lass R.A. (1985). *Cocoa.* 4th ed. London: Longman, 620pp.

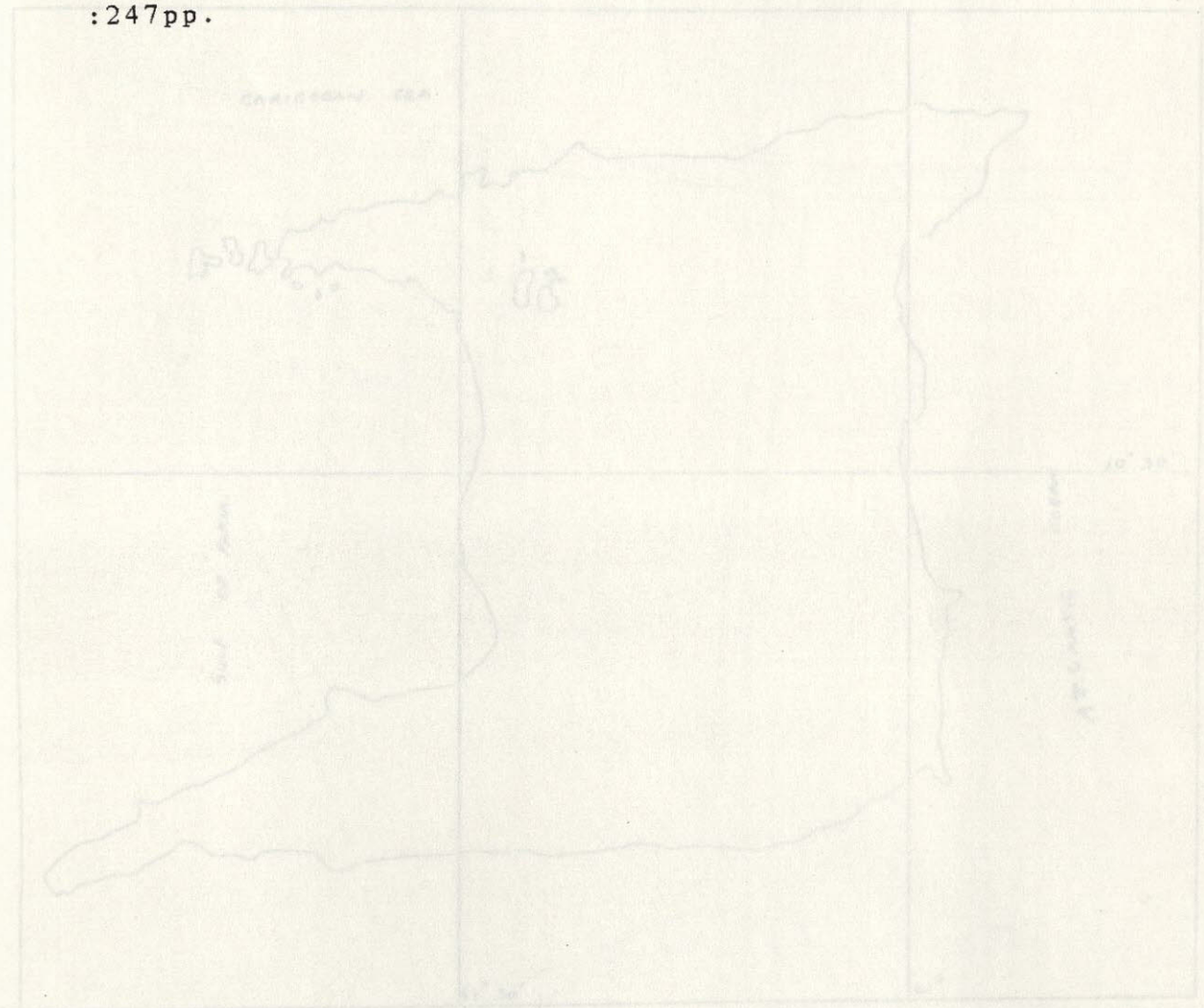
Yidana J.A., Kennedy A.J. and Withers L.A. (1988). Variation in peroxidase isozymes of cocoa (*Theobroma cacao* L.). In *Proceedings of the Tenth International Cocoa Research Conference,* Santo Domingo, Dominican Republic, 17-23 May 1987 :719-723.

Young A., Erickson E.H., Strand M. and Erickson B.J. (1987). Pollination Biology of *Theobroma* and *Herrania* (Sterculiaceae)- 1. Floral Biology. *Insect Science Applications* 8 :151-164.

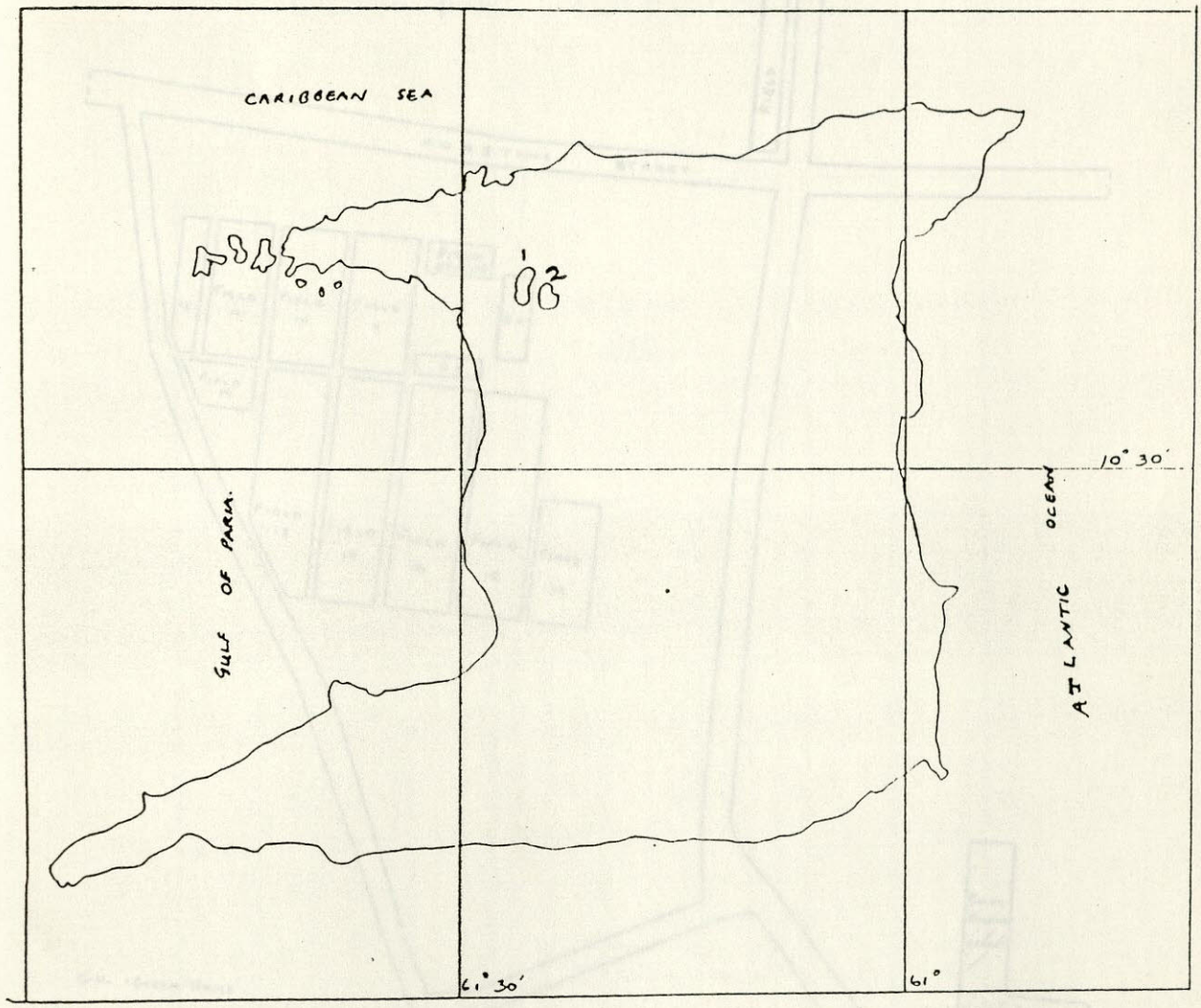
Yndyaard F. (1982). A documentation system for the Nordic Genebank. *Genetic Resources Newsletter* 49 :35-36.

Zeven A.C. and van Harten A.M. (Eds.) (1978). In

Proceedings of the Conference on broadening the genetic base of crops, Wageningen, Netherlands, 3-7 July, 1978. Centre for Agricultural Publishing and Documentation, 1979 :247pp.



APPENDIX 1: Map of Trinidad showing the sites where data were collected; St. Augustine and St. Joseph

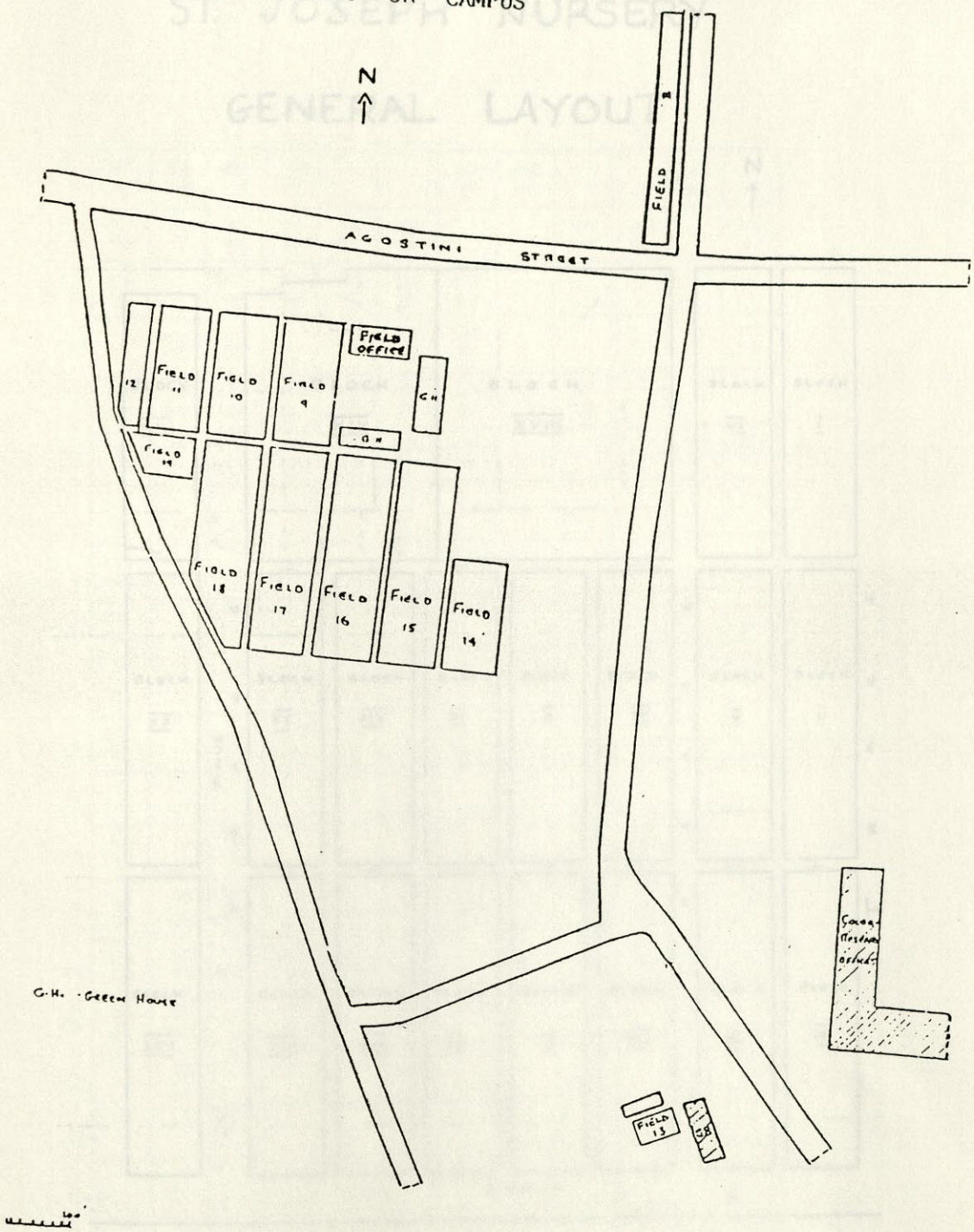


KEY

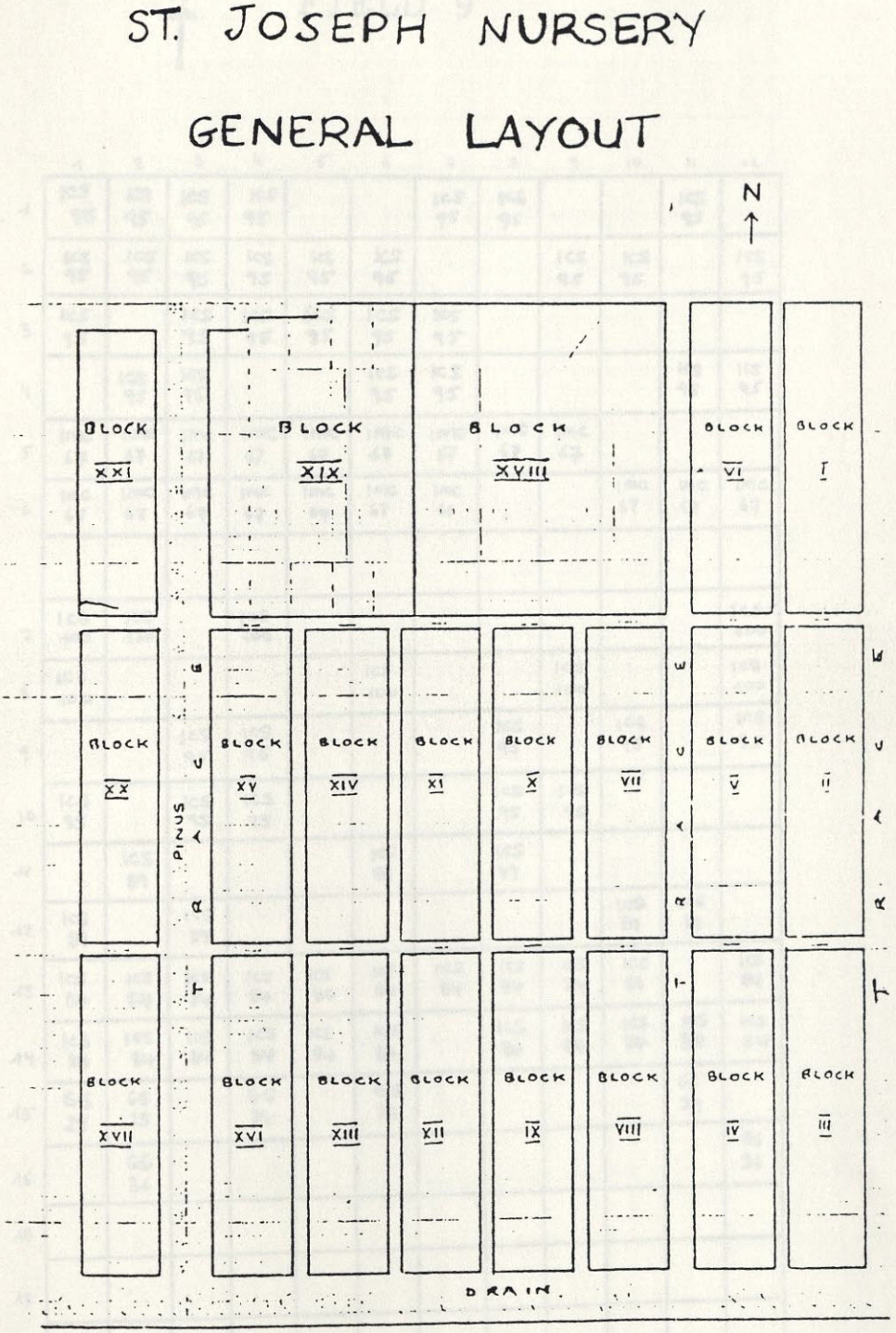
1 St. Joseph
2 St. Augustine

APPENDIX 2a Field layout at St. Augustine

COCOA FIELDS ON CAMPUS



APPENDIX 2b: Field layout of the St. Joseph site



FIELD 9

	1	2	3	4	5	6	7	8	9	10	11	12
1	ICS 95	ICS 95	ICS 95	ICS 95			ICS 95	ICS 95			ICS 95	
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3	ICS 95		ICS 95	ICS 95	ICS 95	ICS 95	ICS 95					
4		ICS 95	ICS 95			ICS 95	ICS 95				ICS 95	ICS 95
5	IMC 67	IMC 67	IMC 67	IMC 67	IMC 67	IMC 67	IMC 67	IMC 67	IMC 67			
6	IMC 67	IMC 67	IMC 67	IMC 67	IMC 67	IMC 67	IMC 67			IMC 67	IMC 67	IMC 67
7	ICS 100	ICS 100		ICS 100								ICS 100
8	ICS 100					ICS 100			ICS 100			ICS 100
9			ICS 95	ICS 95				ICS 95		ICS 95		ICS 95
10	ICS 95		ICS 95	ICS 95				ICS 95	ICS 95			
11		ICS 89				ICS 89		ICS 89				
12	ICS 89		ICS 89							ICS 89	ICS 89	
13	ICS 84	ICS 84	ICS 84	ICS 84	ICS 84	ICS 84	ICS 84	ICS 84	ICS 84	ICS 84		ICS 84
14	ICS 84	ICS 84	ICS 84	ICS 84	ICS 84	ICS 84		ICS 84	ICS 84	ICS 84	ICS 84	ICS 84
15	GS 29	GS 29		GS 29		GS 29					GS 29	
16		GS 36										GS 36
17												
18												
19												

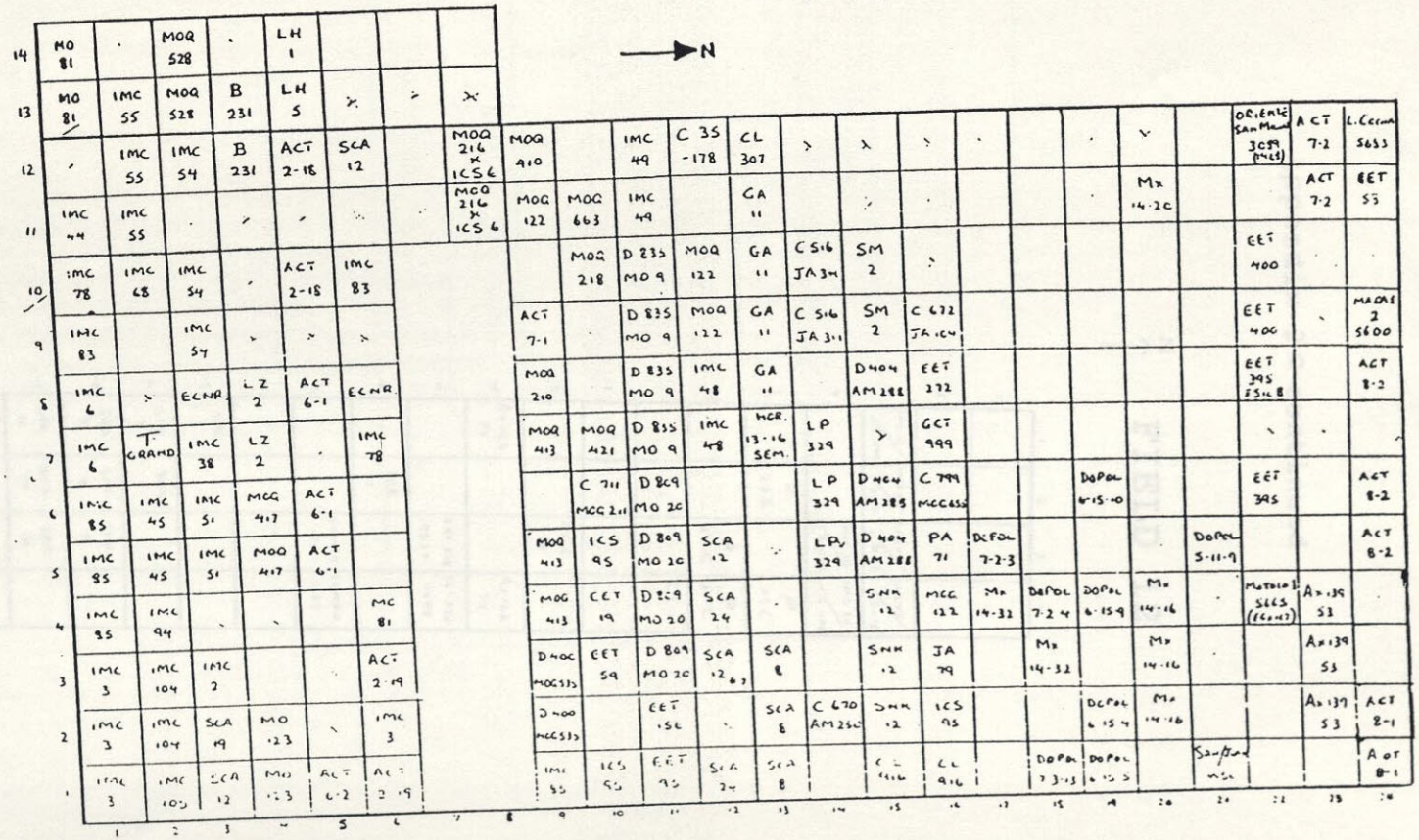
FIELD 10

N

	1	2	3	4	5	6	7	8	9	10	11	12
1		ICS 1	ICS 1	ICS 1	ICS 1			ICS 1	ICS 1	ICS 1		
2						ICS 8	ICS 8				ICS 6	
3					ICS 22	ICS 22		ICS 22	ICS 16	ICS 16	ICS 16	ICS 16
4												
5	ICS 43	ICS 43	ICS 43	ICS 43		ICS 40	ICS 40					
6					ICS 47	ICS 47						
7			ICS 61	ICS 61				ICS 63				
8						ICS 60	ICS 60	ICS 60	ICS 60	ICS 60	ICS 60	ICS 60
9	ICS 75	ICS 75		ICS 75								ICS 70
10	ICS 85				ICS 84	ICS 84	ICS 84	ICS 84	ICS 79	ICS 79	ICS 79	
11					NL		ICS 89		ICS 87	ICS 87	ICS 87	ICS 87
12	ICS 95			ICS 95	ICS 95	ICS 95				ICS 95		ICS 95
13	ICS 100	ICS 100	ICS 100	ICS 100	ICS 98	ICS 98		ICS 98		ICS 91		
14							RT 18	RT 18	IMC 67	IMC 67	IMC 67	IMC 67
15												
16	NL	NL	NL				NL				E 575	
17			E 575					E 575		E 575	E 575	
18							NL	H. CAMA.	H CAMA.	SC 75 H. MARIAS & H EQUATE	H MARIAS & H CONTRC	
19	SC 14 H. ALGI. & H. KANU.	H. KANU	SC 8 H. ALGI & H. NITRO		SC 8 H ALGI & H. NITRO	NL	NL	H. CAMA	H CAMA	SC 75 H. MARIAS & H EQUATE		
20	NL	NL	NL	OCT 998/55	H. NITRO & H MARIAS	SC 80 H KANU & H MARIAS	SC 80 H KANU & H MARIAS			SC 20 H ALGI & H CONTRC	H MARIAS & H CONTRC	
21	OCT. 998/76	NL		NL	H MARIAS	H CAMA	H EQUATE	H MARIAS		H CONTRC	H CONTRC	
22	NL	NL	OCT 998/28	OCT 998/54		SC 66, 9	T			SC 27 H PUR & SMO	SC 27 H PUR & SMO	
23	OCT. 998/66	NL	OCT 998/50	OCT 998/96	H. SMO				NL			
24	SC 89 H. KANU & H. BALO	SC 87 H KANU H BALO		H. SMO								

LEGEND
NL - NOT LABELED

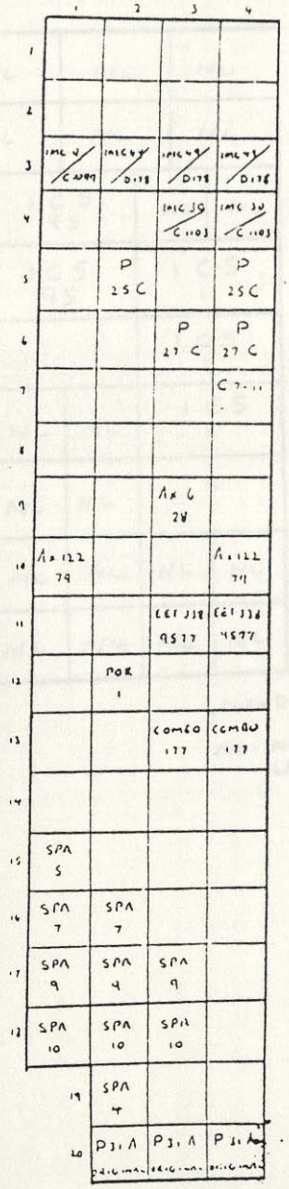
FIELD 11



Appendix 2c continued

N

FIELD 12



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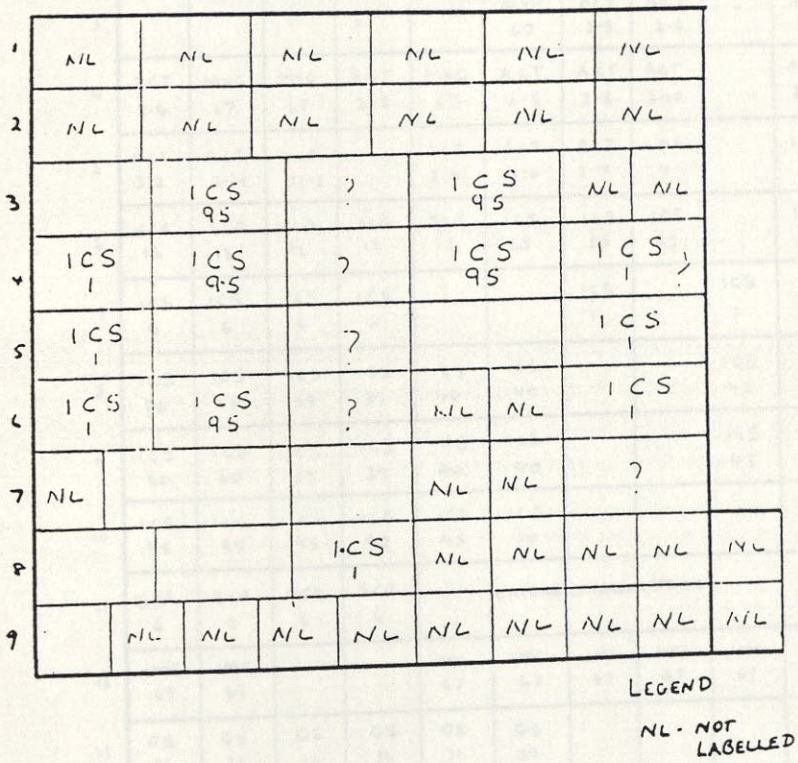
FIELD 13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2 NL NL NL NL NL NL
3 ICS 95 ? ICS 95 NL NL
4 ICS 1 ICS 95 ? ICS 95 ICS 1 ?
5 ICS 1 ? ICS 1
6 ICS 1 ICS 95 ? NL NL ICS 1
7 NL NL NL ?
8 1CS 1 NL NL NL NL NL
9 NL NL NL NL NL NL NL NL NL
LEGEND
NL - NOT LABELLED

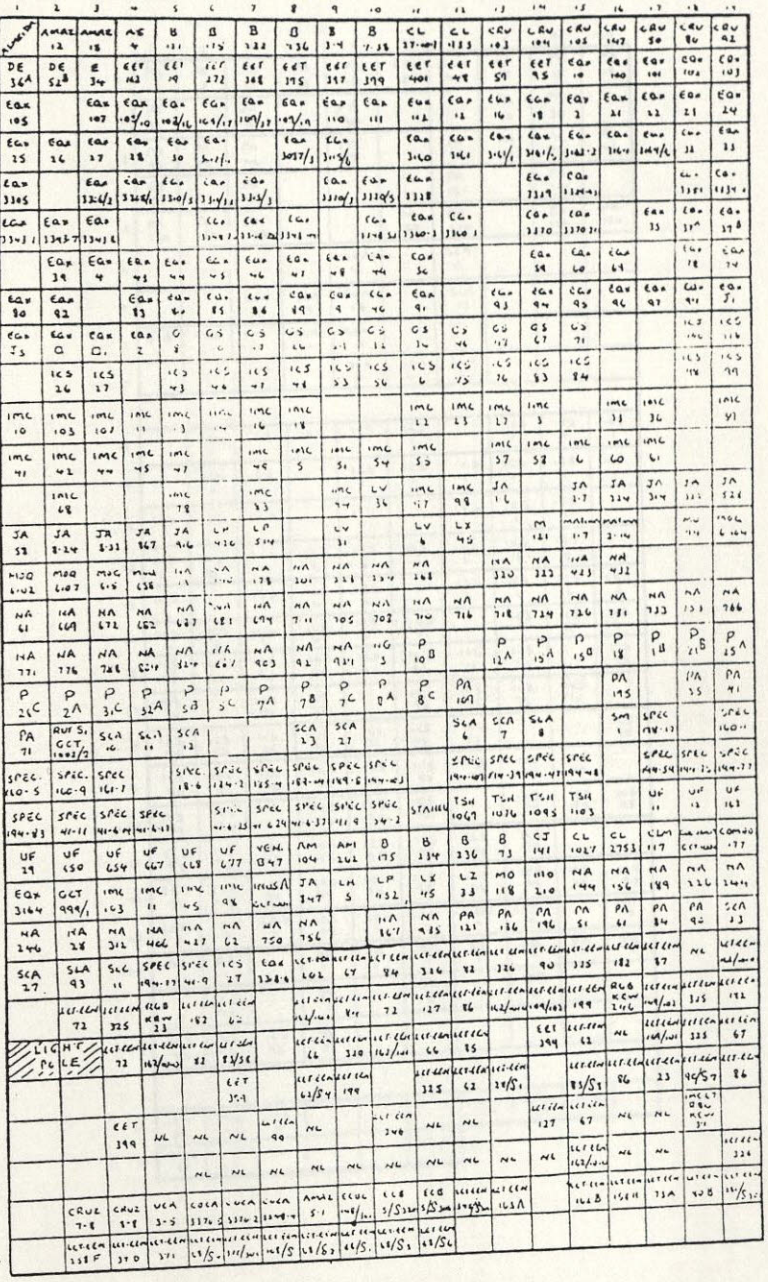
Appendix 2 c continued

FIELD 14

	1	2	3	4	5	6	7	8	9	10
1	ACT 1-1	ACT 1-1	ACT 1-1		MXC 67	ACT 1-5	ACT 1-7	MXC 67	ACT 1-8	MXC 67
2	MXC 67	ACT 1-9			ACT 1-10				ACT 2-3	ACT 2-3
3						MXC 67	ACT 2-5	ACT 2-6		ACT 2-6
4	ACT 2-6	MXC 67	MXC 67	ACT 2-8	MXC 67	ACT 2-8	ACT 2-8	ACT 2-10		ACT 3-2
5	ACT 3-2	ACT 2-14	ACT 2-13		ACT 3 5	ACT 3-6	ACT 2-7	LAFI 7		LAFI 7
6	SCA 12	SCA 12	SCA 12	SCA 12	SCA 12	ICS 65	ICS 65	ICS 65		ICS 65
7	ICS 6	ICS 6	ICS 6	ICS 6			ICS 1		ICS 1	
8	ICS 60	ICS 60	ICS 39	ICS 39	ICS 40	ICS 40			ICS 43	
9	ICS 60	ICS 60	ICS 39	ICS 39	ICS 40	ICS 40			ICS 43	ICS 43
10	ICS 95	ICS 95	ICS 95	ICS 95	ICS 95	ICS 70				
11	SCA 6	SCA 6	SCA 6	SCA 6		CATONGO	CATONGO	CATONGO		
12	IMC 67	IMC 67			IMC 67	IMC 67	IMC 67	IMC 67	IMC 67	IMC 67
13	GS 36	GS 36	GS 36	GS 36	GS 36	GS 29				

Appendix 2 c continued

FIELD 15



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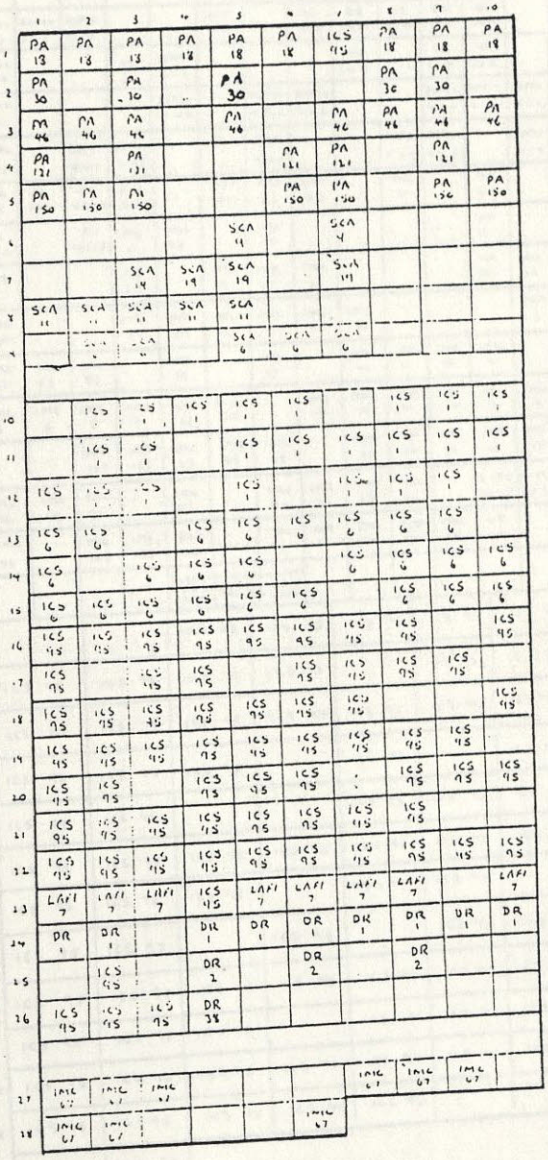
FIELD 16



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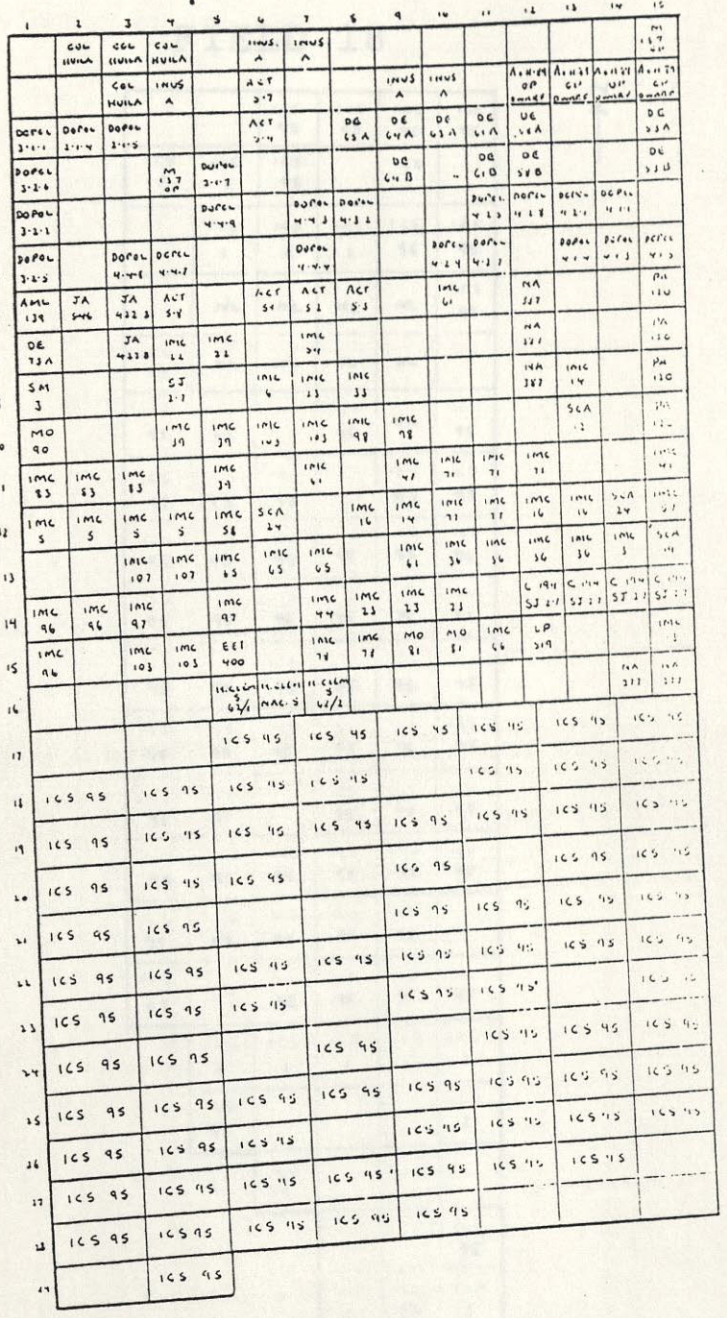
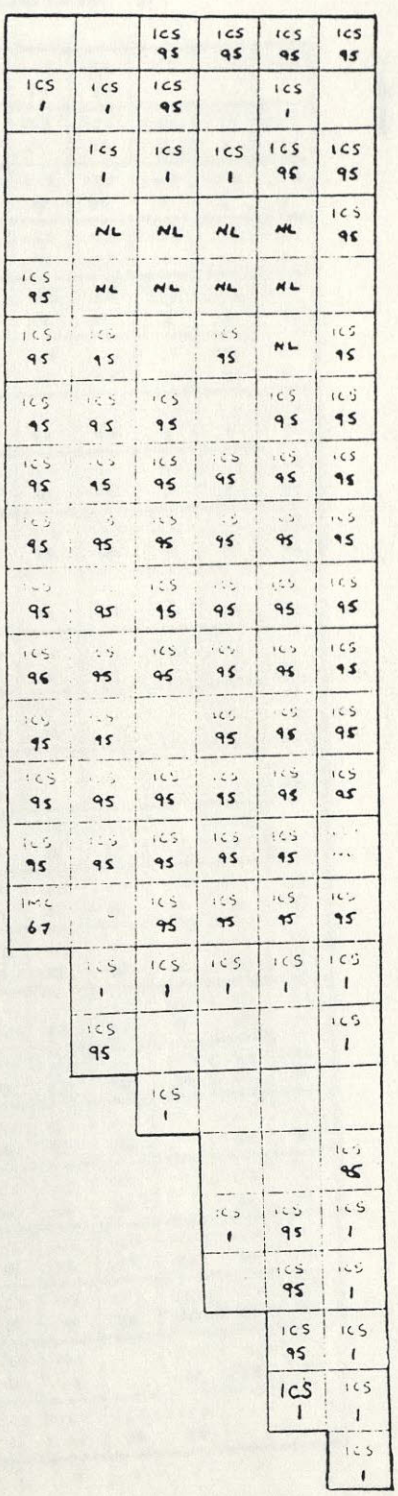


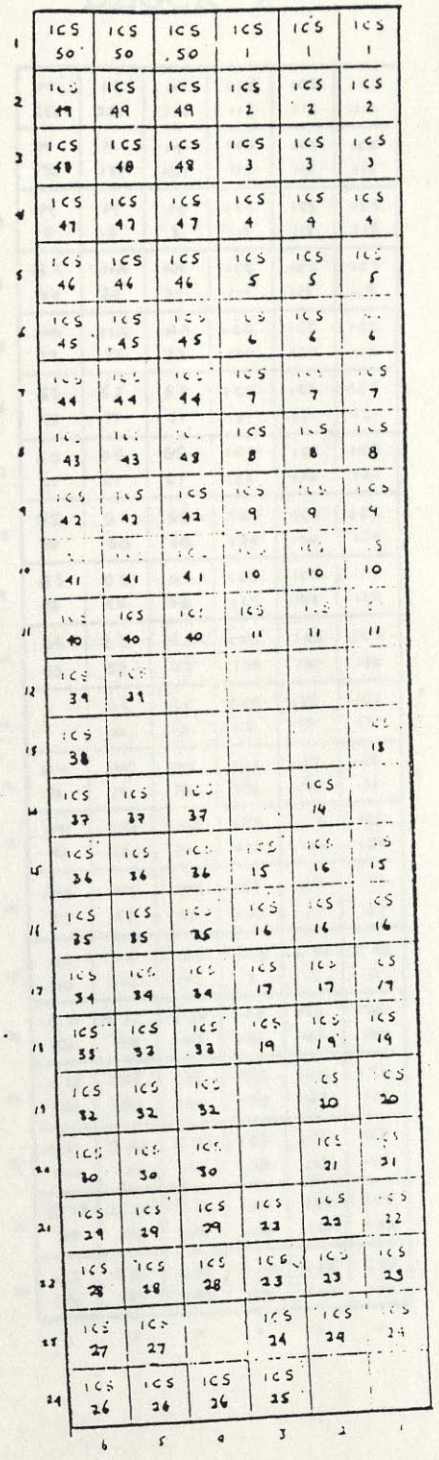
Appendix 2 c, continued

FIELD 18



Appendix 2d St. Joseph field plans

BLOCK 1



Appendix 2d continued

BLOCK 2

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1	M 252	M 252	M 252	ICS 113	ICS 113	ICS 113
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3	M 8	M 8	M 8	ICS 118	ICS 118	ICS 118
4	NA 34	NA 34	NA 34	ICS 119	ICS 119	ICS 119
5	NA 32	NA 32	NA 32	ICS 120	ICS 120	ICS 120
6	GS 77	GS 77	GS 77	ICS 121	ICS 121	ICS 121
7	GS 67	GS 67	GS 67	ICS 122	ICS 122	ICS 122
8	GS 50	GS 50	GS 50	ICS 124	ICS 124	ICS 124
9	GS 48	GS 48	GS 48	ICS 129	ICS 129	ICS 129
10	GS 32	GS 32	GS 32	ICS 130	ICS 130	ICS 130
11		GS 10	GS 10	ICS 131	ICS 131	ICS 131
12	IMC 76	IMC X	IMC 76	ICS 132	ICS 132	ICS 132
13	IMC 67	IMC 67	IMC 67	ICS 134	ICS 134	ICS 134
14	IMC 53	IMC 53	IMC 53	ICS 135	ICS [illegible]	ICS 135
15	ICS 142	ICS 142	ICS 14[illegible]	C 35 2	C 35 [illegible]	C 35 2
16	C 35 163	C 35 163	C 35 43	ICS 136	ICS 136	ICS 136
17	C 35 150	C 35 150	C 35 150	ICS 137	ICS 137	ICS 137
18	C 35 144	C 35 144	C 35 144	ICS 138	ICS 138	ICS 138
19	C 35 128	C 35 135	C 35 128	ICS 140	ICS 140	ICS 140
20	KS 142	C 34 269	C 35 139	C 35 114	C 35 114	C 35 114
	1	6	5	4	3	2	1

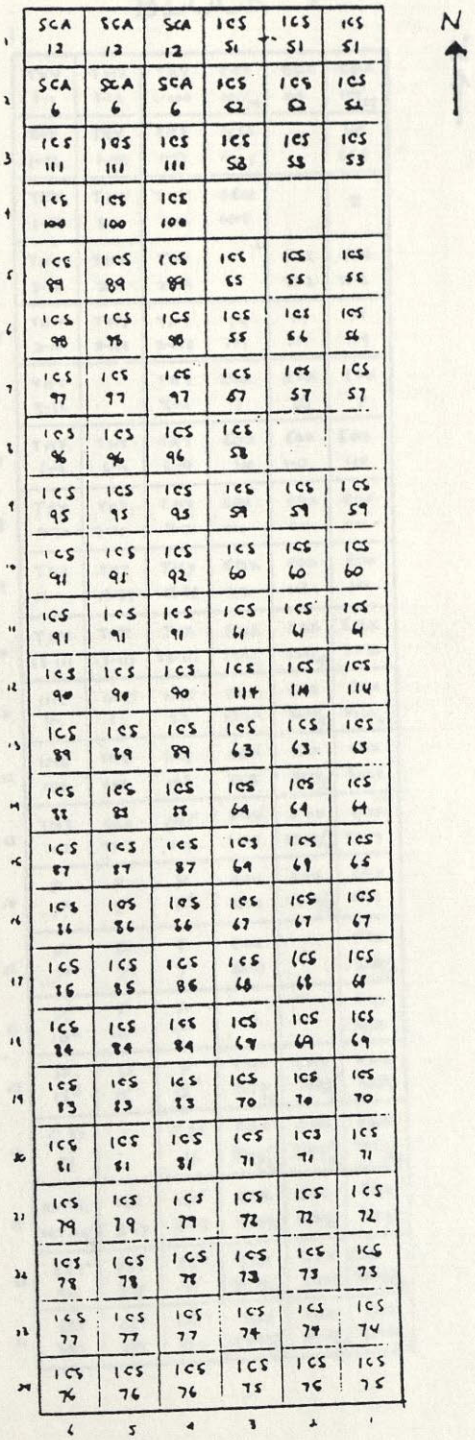
Appendix 2d continued

BLOCK 4

	6	5	4	3	2	1
1	EQX 24	EQX 24	EQX 88	EQX 23	EQX 24	EQX 23
2	EQX 28	EQX 28	EQX 28	EQX 27	EQX 27	EQX 27
3	EQX 91	EQX 32	EQX 32	EQX 31	EQX 31	EQX 31
4	EQX 36	EQX 96	EQX 36	EQX 33	EQX 35	EQX 35
5	EQX 37	EQX 37	EQX 37	EQX 37	EQX 37	EQX 37
6	EQX 89	EQX 89	EQX 89	EQX 91	EQX 91	EQX 91
7	EQX 100	EQX 52	EQX 52	EQX 52	EQX 52	EQX 52
8	EQX 92	EQX 92	EQX 92	EQX 91	EQX 91	EQX 91
9	EQX 92	EQX 100	EQX 100	EQX 100	EQX 92	EQX 54
10	EQX 94	EQX 94	EQX 94	EQX 93	EQX 93	EQX 93
11	EQX 82			EQX 103	EQX 105	EQX 103
12	EQX 95	EQX 95	EQX 95	EQX 59	EQX 100	EQX 100
13	EQX 100	EQX 100	EQX 2	EQX 96	EQX 96	EQX 96
14	EQX 98	EQX 2	EQX 98	EQX 100	EQX 100	EQX 60
15	EQX 99	EQX 99	EQX 99	EQX 97	EQX 97	EQX 97
16	EQX 105	EQX 66	EQX 106	EQX 105	EQX 105	EQX 105
17	EQX 90	EQX 90	EQX 68	EQX 97	EQX 97	EQX 97
18	EQX 69	EQX 69	EQX 69	EQX 69	EQX 90	EQX 67
19	EQX 78	EQX 78	EQX 78	EQX 78	EQX 78	EQX 78
20	EET 98	EET 98	EET 98	EET 19	EET 19	EET 19
21	EET 399	EET 399	EET 399	EET 162	EET 162	EET 162

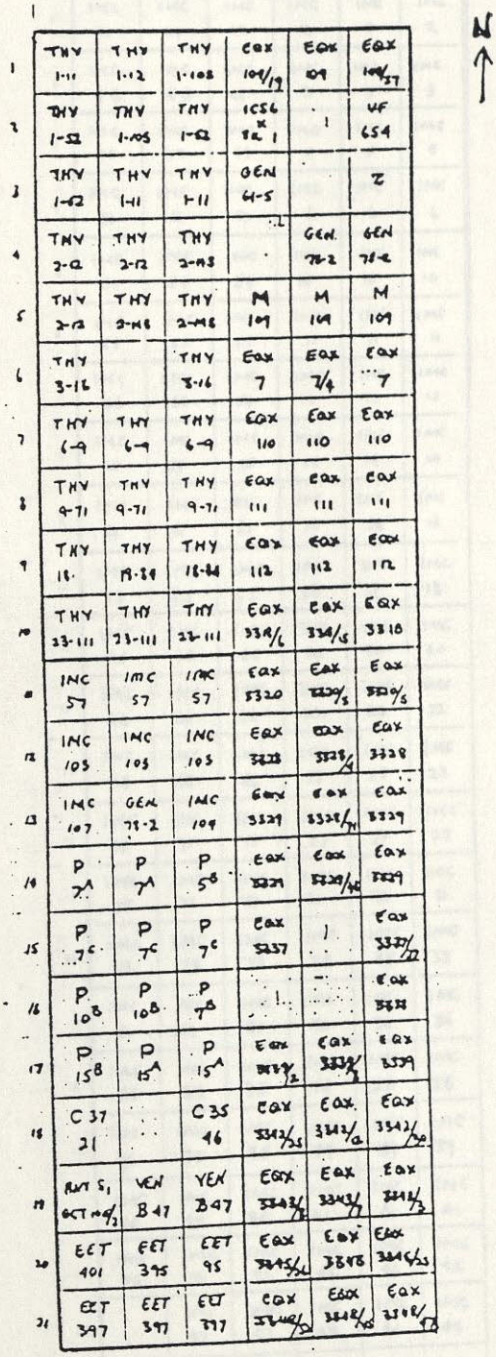
Appendix 2d continued

BLOCK 6



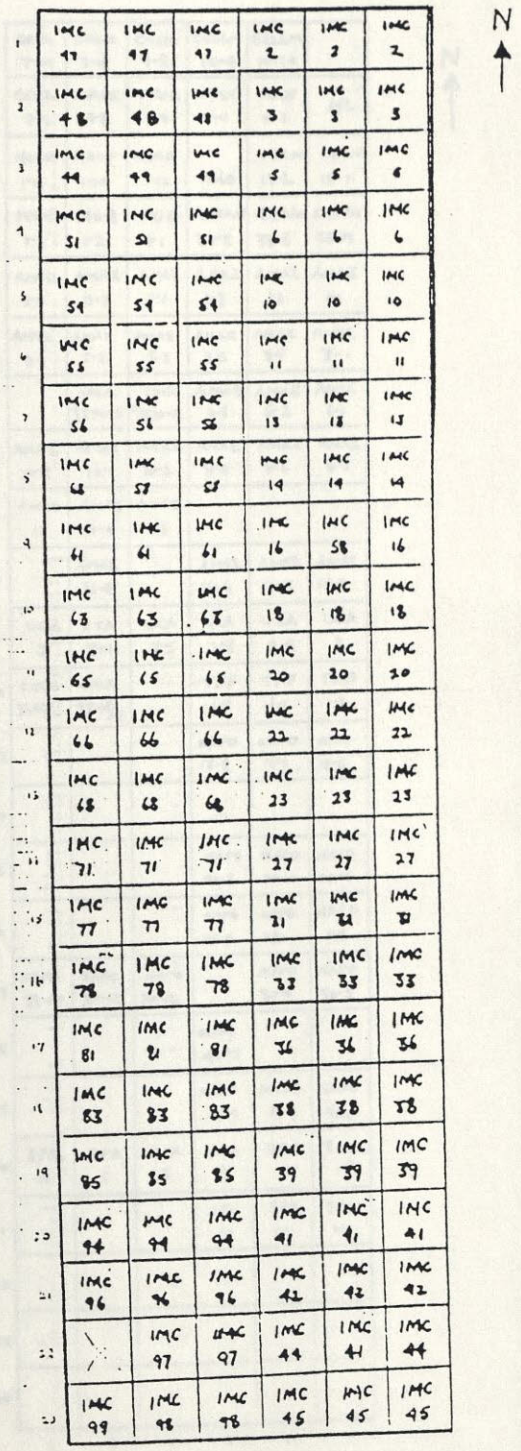
Appendix 2d continued

BLOCK 9



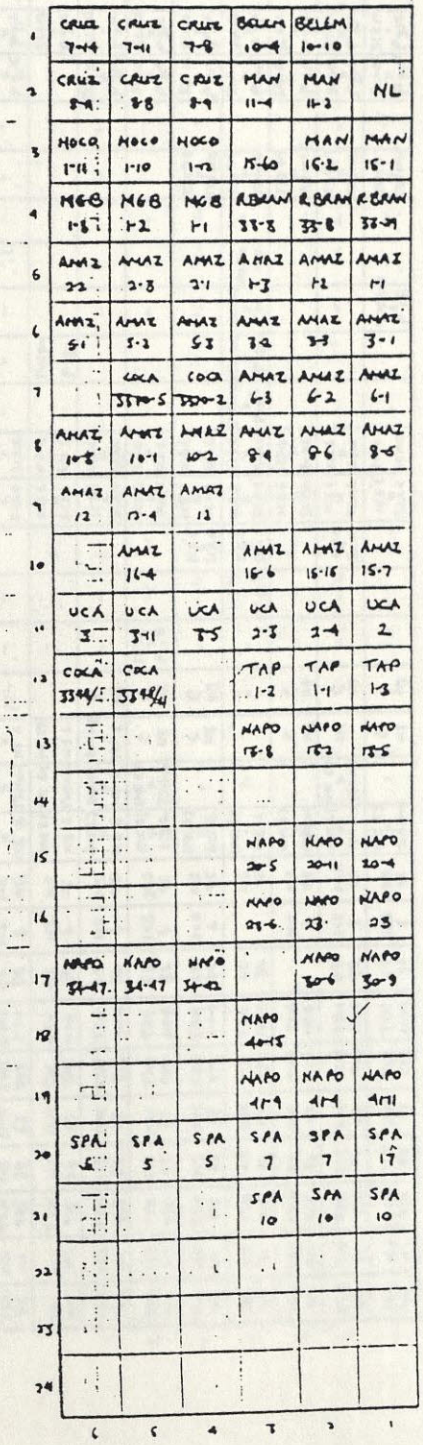
Appendix 2.d continued

BLOCK 12



Appendix 2d continued

BLOCK 17



Appendix 2 d continued

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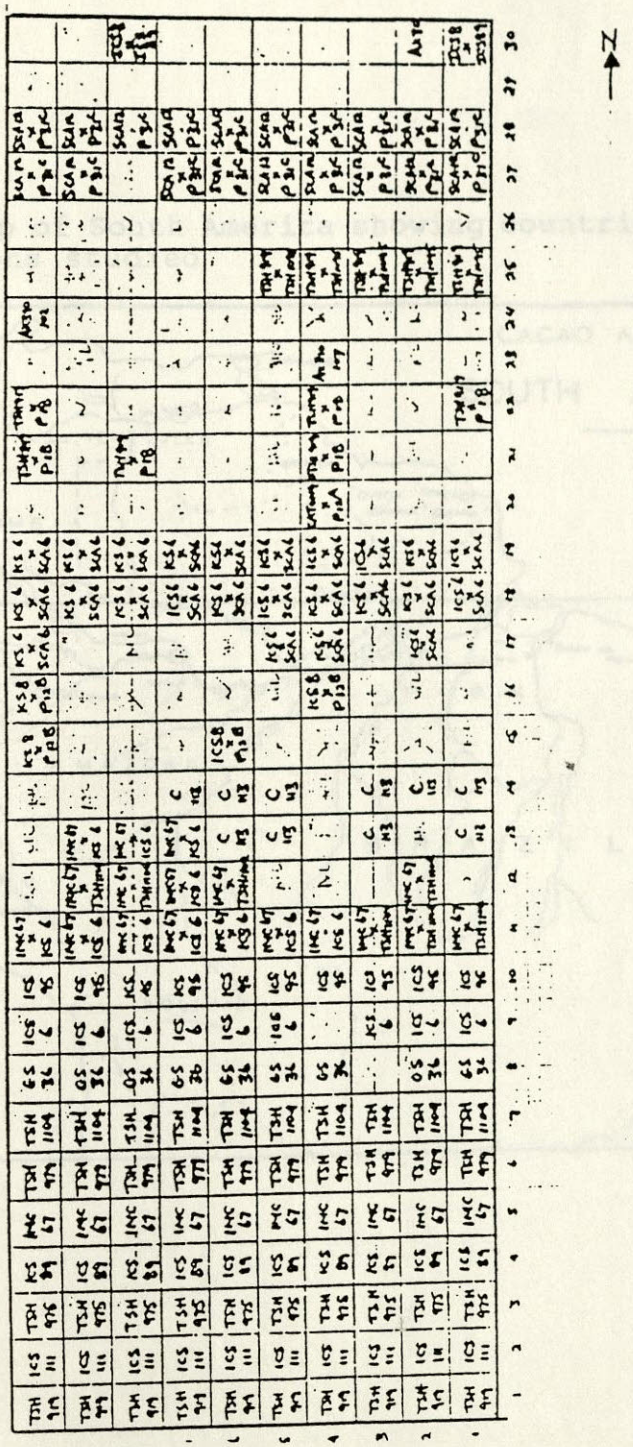
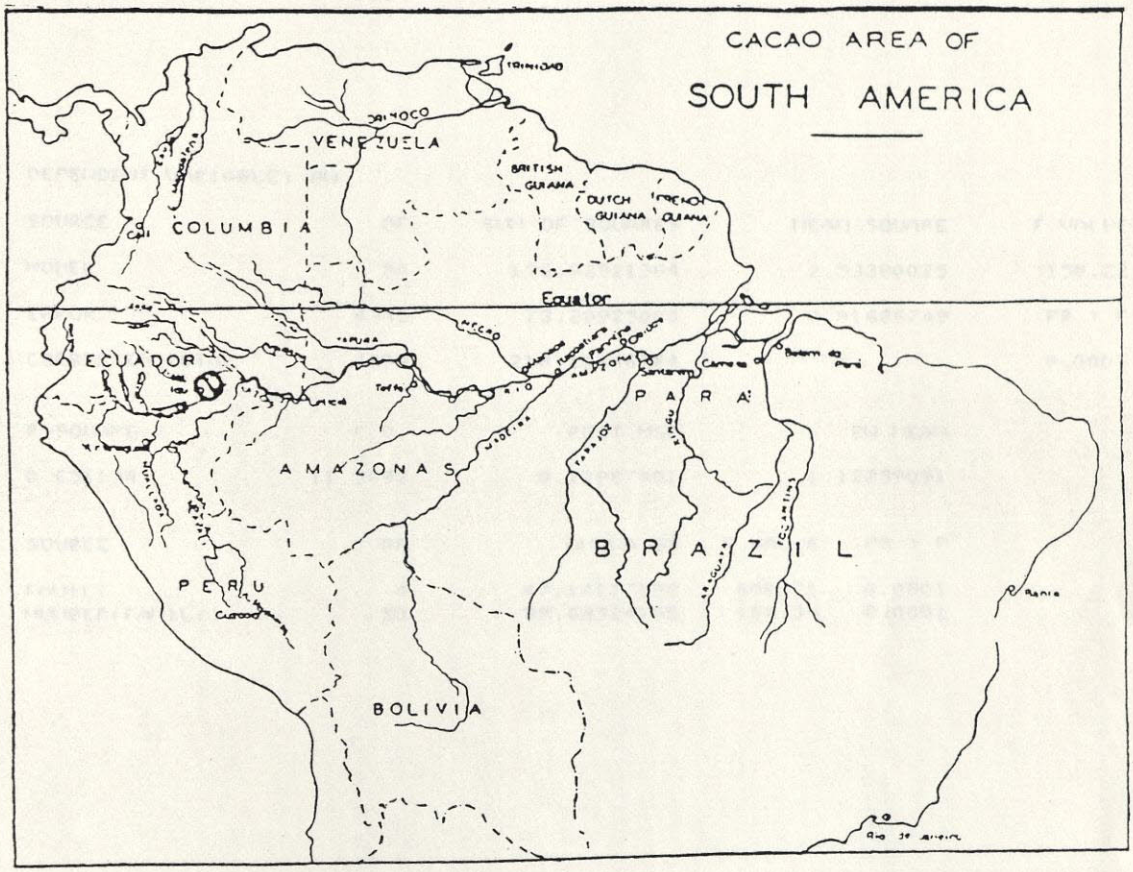


APPENDIX 3 Map of South America showing countries of origin of the populations studied



APPENDIX 4: An example of the ANOVA output

DEPENDENT VARIABLE: BW

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	54	136.82521364	2.53380025	150.22
ERROR	4345	73.28925000	0.01686749	PR > F
CORRECTED TOTAL	4399	210.11446364		0.0001

R-SQUARE	C.V.	ROOT MSE	BW MEAN
0.651194	11.5692	0.12987491	1.12259091

SOURCE	DF	ANOVA SS	F VALUE	PR > F
FAMILY	4	47.14137198	698.71	0.0001
NUMBER(FAMILY)	50	89.68324165	106.34	0.0001

APPENDIX 5: An example of the manova output

MANOVA OF FRUIT DES

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: PL

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR) F	R-SQUARE	C.V.
MODEL	20	125.95900000	6.29795000	5.25	0.0003 **	0.846705	6.6853
ERROR	19	22.80475000	1.20025000		ROOT MSE		PL MEAN
CORRECTED TOTAL	39	148.76375000			1.09555922		16.38750000

SOURCE	DF	TYPE I SS	F VALUE	PR) F	DF	TYPE III SS	F VALUE	PR) F
SITE	1	0.60025000	0.50	0.4880	1	0.60025000	0.50	0.4880
FAMILY	3	53.42420455	14.84	0.0001	3	53.42420455	14.84	0.0001
NUMBER(FAMILY)	16	71.93454545	3.75	0.0036	16	71.93454545	3.75	0.0036

APPENDIX 6: MEAN TRAIT VALUES AND STANDARD ERRORS FOR SIXTY-EIGHT COCOA DESCRIPTORS, OBSERVED FOR FIFTY-THREE ACCESSIONS IN ST. AUGUSTINE

DESCRIPTORS		POPULATION				
	E Mean(S.E.)	EET Mean (S.E.)	ICS Mean (S.E.)	IMC Mean (S.E.)	SCA Mean (S.E.)	SPA Mean (S.E.)
1 TL (cm)	21.6 (0)	25.1 (0.6)	23.6 (0.4)	26.3 (0.4)	23.3 (0.1)	25.3 (0.2)
2 W (cm)	8.5 (0)	8.9 (0.2)	8.7 (0.2)	9.0 (0.1)	8.2 (0.3)	9.4 (0.2)
3 AA (°)	54.2 (0)	64.1 (1.1)	66.5 (2.1)	60.9 (1.0)	60.8 (2.0)	63.7 (0.3)
4 BA (°)	81.9 (0)	118.7 (3.6)	88.6 (3.4)	114.3 (3.03)	96.3 (5.7)	119.9 (0.8)
5 ABR	1.5 (0)	1.9 (0.6)	1.3 (0.6)	1.9 (0.6)	1.6 (0.8)	1.9 (0.3)
6 SW (mm)	2.00 (0)	2.21 (0.82)	2.15 (0.31)	2.33 (0.54)	2.08 (0.51)	2.23 (0.11)
7 SL (mm)	6.8 (0)	8.3 (0.2)	7.9 (0.1)	7.6 (0.2)	7.0 (0.2)	7.7 (0.3)
8 LW (mm)	2.03 (0)	2.32 (0.14)	2.44 (0.83)	2.37 (0.73)	2.02 (0.14)	2.18 (0.83)
9 APLL (mm)	7.7 (0)	8.4 (0.3)	9.0 (0.2)	8.2 (0.1)	8.1 (0.4)	8.0 (0.3)
10 STL (mm)	5.2 (0)	5.6 (0.2)	5.8 (0.2)	5.8 (0.7)	5.3 (0.4)	5.2 (0.1)
11 OL (mm)	1.43 (0)	1.84 (0.68)	1.76 (0.52)	1.89 (0.33)	1.86 (0.94)	1.97 (0.38)
12 OD (mm)	1.00 (0)	1.02 (0.13)	1.03 (0.14)	1.04 (0.10)	1.04 (0.19)	1.00 (0.0)
13 ON	36.0 (0)	38.7 (1.3)	37.4 (1.0)	44.6 (1.2)	43.2 (2.1)	43.3 (1.7)
14 STYL (mm)	1.6 (0)	2.22 (0.14)	2.00 (0.23)	2.08 (0.28)	2.07 (0.58)	2.08 (0.83)
15 PL (mm)	12.8 (0)	14.9 (0.4)	14.4 (0.4)	14.8 (0.4)	13.8 (0.8)	13.9 (0.3)
16 DBWT (g)	113.9 (0)	87.6 (6.8)	120.0 (7.0)	82.8 (2.8)	59.7 (3.2)	95.9 (9.1)

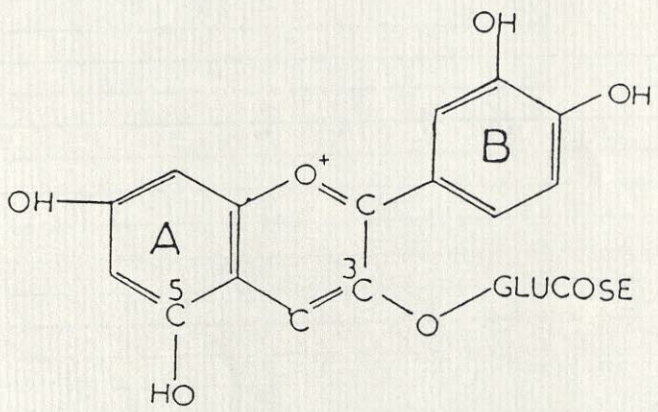
APPENDIX 6 Cont'd

DESCRIPTORS	POPULATION					
	E Mean(S.E.)	EET Mean (S.E.)	ICS Mean (S.E.)	IMC Mean (S.E.)	SCA Mean (S.E.)	SPA Mean (S.E.)
17 PBWT (g)	101.5 (0)	80.3 (7.2)	108.3 (6.2)	75.1 (2.6)	53.1 (2.8)	83.1 (5.7)
18 SCWT (g)	12.4 (0)	7.4 (0.8)	11.7 (1.1)	10.6 (2.7)	6.6 (1.4)	12.8 (3.4)
19 BW (g)	1.3 (0)	0.97 (0.92)	1.37 (0.84)	0.94 (0.33)	0.67 (0.42)	1.03 (0.88)
20 BL (mm)	2.4 (0)	2.13 (0.95)	2.31 (0.43)	2.24 (0.43)	1.85 (0.43)	1.96 (0.12)
21 BEW (mm)	1.3 (0)	1.20 (0.14)	1.24 (0.27)	1.07 (0.25)	0.93 (0.33)	1.10 (0.57)
22 BET (mm)	0.5 (0)	0.66 (0.42)	0.64 (0.22)	0.58 (0.19)	0.57 (0.61)	0.67 (0.33)
23 POL (cm)	15.7 (0.0)	16.8 (0.7)	17.3 (0.9)	17.2 (0.4)	15.1 (0.6)	15.5 (0.9)
24 POW (cm)	7.4 (0.0)	8.2 (0.5)	8.2 (0.1)	8.7 (0.2)	7.0 (0.2)	7.9 (0.5)
25 PWT (mm)	10.4 (0.0)	11.2 (1.7)	12.2 (0.6)	13.4 (0.6)	10.6 (0.5)	9.5 (0.7)
26 MESOTH(mm)	2.50 (0.0)	2.92 (0.63)	2.73 (0.18)	4.00 (0.35)	2.73 (0.53)	3.46 (0.33)
27 FD1 (mm)	6.0 (0.0)	5.58 (0.54)	7.86 (0.64)	5.79 (0.57)	6.43 (0.69)	3.67 (0.89)
28 FD2 (mm)	4.9 (0.0)	4.18 (0.45)	5.16 (0.65)	4.09 (0.49)	4.07 (0.42)	2.20 (0.95)
29 POWT (g)	337.8 (0.0)	448.1 (63.2)	474.8 (30.3)	560.3 (32.7)	297.6 (25.2)	412.5 (81.9)
30 PLW (g)	4.5 (0.0)	7.45 (2.21)	8.52 (1.05)	10.18 (0.99)	5.47 (0.88)	11.37 (5.03)
31 WBW (g)	118.0 (0.0)	118.1 (16.3)	126.4 (10.0)	127.4 (6.4)	75.5 (6.2)	104.7 (31.1)
32 HW (g)	208.8 (0.0)	319.7 (57.2)	348.5 (23.03)	420.8 (28.3)	218.9 (28.6)	294.9 (45.7)
33 WABW (g)	107.2 (0.0)	87.9 (10.0)	103.4 (7.4)	91.4 (4.2)	76.3 (17.6)	74.0 (16.6)

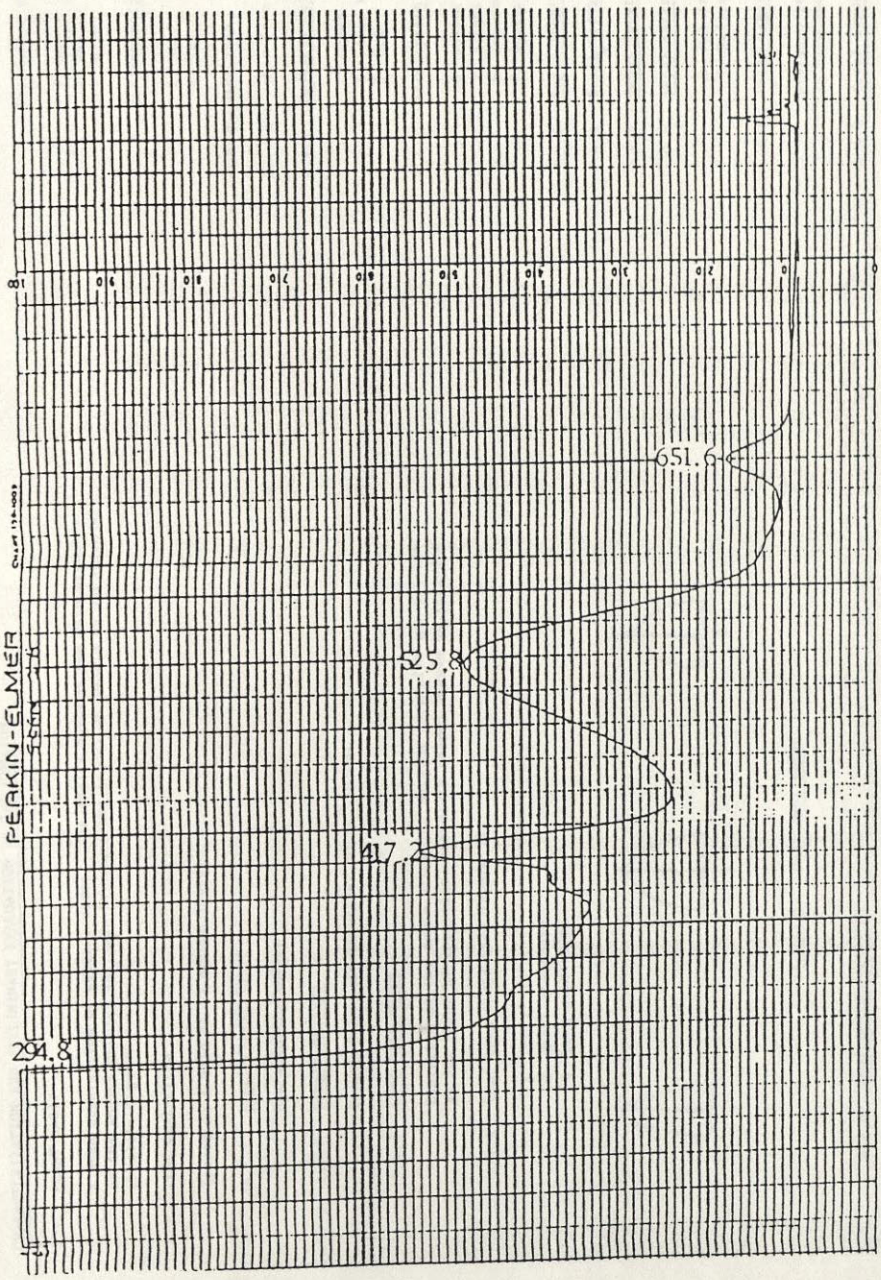
DESCRIPTORS	POPULATION					
	E Mean(S.E.)	EET Mean (S.E.)	ICS Mean (S.E.)	IMC Mean (S.E.)	SCA Mean (S.E.)	SPA Mean (S.E.)
34 MUW (g)	10.9 (0.0)	31.1 (7.5)	21.7 (2.7)	34.3 (3.5)	14.5 (2.1)	30.7 (14.8)
35 BEN	41.0 (0)	41.7 (1.7)	37.0 (1.2)	48.7 (1.4)	39.3 (2.4)	37.7 (6.9)
36 AS	2.0 (0.0)	2.7 (0.2)	2.6 (0.1)	2.9 (0.8)	2.8 (0.2)	3.0 (0.0)
37 BS	1.0 (0.0)	2.0 (0)	1.4 (0.1)	2.0 (0.5)	1.3 (0.2)	2.7 (0.7)
38 PS	5.0 (0.0)	5.3 (0.6)	4.0 (0.3)	5.5 (0.3)	4.3 (0.4)	2.7 (1.5)
39 FC	3.0 (0.0)	4.0 (0.4)	3.4 (0.6)	4.0 (0.2)	3.6 (0.4)	4.3 (1.3)
40 BC	3.0 (0.0)	4.0 (1.3)	2.3 (0.6)	3.0 (0.4)	0 (0.0)	3.0 (2.0)
41 SC	3.0 (0.0)	3.3 (1.1)	3.1 (0.6)	2.5 (0.4)	1.5 (0.7)	2.3 (2.3)
42 SP	0 (0.0)	0.8 (1.7)	0.6 (0.1)	0.7 (0.1)	1.0 (0.0)	0.7 (0.3)
43 GR	1.0 (0.0)	0.7 (0.2)	0.9 (0.6)	1.0 (0.5)	0.8 (0.2)	0.7 (0.3)
44 LC	5.0 (0.0)	2.7 (1.0)	1.8 (0.4)	2.9 (0.3)	0.5 (0.5)	3.7 (0.7)
45 RC	0 (0.0)	1.0 (0.6)	1.3 (0.4)	2.5 (0.5)	0.5 (0.5)	0 (0.0)
46 TC	0 (0.0)	2.7 (0.9)	0.4 (0.3)	0.1 (0.1)	0.8 (0.8)	4.7 (2.3)
47 OBC	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
48 OAC	0 (0.0)	1.7 (1.0)	1.4 (0.4)	0 (0.0)	0 (0.0)	2.3 (2.3)
49 STL	0 (0.0)	0.5 (0.5)	0.4 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)
50 FLC	0 (0.0)	1.7 (1.0)	0 (0.0)	3.6 (0.5)	0 (0.0)	3.3 (1.7)

DESCRIPTORS	POPULATION					
	E Mean(S.E.)	EET Mean (S.E.)	ICS Mean (S.E.)	IMC Mean (S.E.)	SCA Mean (S.E.)	SPA Mean (S.E.)
51 AD	3.0 (0.0)	3.0 (0.0)	3.0 (0.0)	3.0 (0.0)	3.0 (0.0)	3.0 (0.0)
52 PAC	7.0 (0.0)	3.2 (0.9)	3.4 (0.7)	3.2 (0.5)	1.1 (1.1)	5.0 (1.2)
53 PAPC	7.0 (0.0)	1.5 (0.7)	0.8 (0.3)	1.0 (0.4)	0 (0.0)	2.3 (2.3)
54 PEC	3.0 (0.0)	2.0 (0.4)	1.9 (0.2)	2.0 (0.1)	1.3 (0.2)	1.7 (0.2)
55 PRCI	1.0 (0.0)	1.3 (0.2)	1.8 (0.3)	1.0 (0.0)	1.0 (0.0)	1.7 (0.7)
56 PFCI	0 (0.0)	1.7 (1.0)	1.6 (0.7)	0 (0.0)	0 (0.0)	2.3 (2.3)
57 PRCM	0 (0.0)	2.5 (1.1)	2.6 (0.8)	0 (0.0)	0 (0.0)	1.7 (1.7)
58 POS	5 (0.0)	4.7 (0.3)	4.5 (0.2)	5.0 (0.0)	4.3 (0.4)	5.0 (0.0)
59 POSH	2.0 (0.0)	2.3 (0.2)	1.9 (0.2)	2.6 (0.1)	2.0 (0.0)	2.3 (0.3)
60 PBC	2.0 (0.0)	2.7 (0.5)	2.1 (0.4)	1.7 (0.1)	2.0 (0.6)	1.3 (0.3)
61 PAF	3.0 (0.0)	3.3 (0.6)	1.9 (0.2)	3.1 (0.2)	3.3 (0.3)	2.3 (0.7)
62 PST	7.0 (0.0)	5.0 (0.5)	5.6 (0.4)	4.4 (0.3)	4.3 (0.4)	5.0 (0.0)
63 PFD	2.0 (0.0	1.8 (0.2)	1.9 (0.9)	2.0 (0.5)	2.0 (0.0)	2.0 (0.0)
64 PFS	2.0 (0.0)	2.2 (0.2)	2.2 (0.2)	2.4 (0.1)	1.8 (0.2)	2.7 (0.3)
65 HH	7.0 (0.0)	6.3 (0.4)	6.5 (0.2)	6.8 (0.1)	6.0 (0.4)	6.3 (0.6)
66 PUC	2.0 (0.0)	1.5 (0.2)	1.4 (0.2)	1.9 (0.1)	1.3 (0.2)	1.3 (0.3)
67 BEC	3.0 (0.0)	3.8 (0.5)	2.9 (0.2)	4.5 (0.1)	4.7 (0.2)	4.0 (0.6)
68 BES	2.0 (0.0)	2.5 (0.3)	2.5 (0.2)	2.7 (0.2)	2.7 (0.3)	2.3 (0.7)

APPENDIX 7: The molecular configuration of cyanidin 3-glycoside



APPENDIX 8: Results of a scan for the λ max. of sepal extracts using a Perkin-Elmer 52 A Spectrophotometer



4. CHARACTERIZATION AND PRELIMINARY EVALUATION BY THE CURATOR

4.1 PLANT HABIT

A mean observation of several trees. The angles between two main branches or the angles between the main branches and the trunk should be recorded as absolute figures

4.2 LEAF CHARACTERS

Scored as a mean of 15 second leaves

4.2.1 Length/width ratio

Use absolute values

4.2.2 Length of leaf

Length from base to widest point, in cm.

4.2.3 Leaf base shape

Expressed as the angle which the margins form with the petiole at its point of insertion. Absolute values should be recorded

4.2.4 Leaf apex shape (see Figure 3)

1 Acute
2 Short acuminate
3 Long acuminate

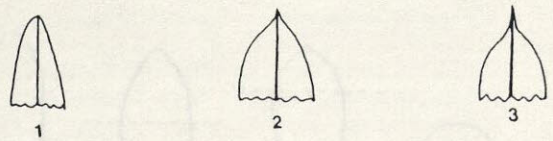


Figure 3. Leaf apex shape

4.2.5 Leaf petiole pulvini

Presence or absence of pulvini

0 Pulvini absent
+ Pulvini present

4.3 FLOWERING CHARACTERS

4.3.1 Flowering intensity

The number of flowers per cushion (sample size 300)

3 Low
5 Medium
7 High

4.3.2 Self-compatibility

0 No
+ Yes

4.4 FLOWER CHARACTERS

Data are taken from five feshly opened flowers

4.4.1 Peduncle colour

1 Green
2 Green with red
3 Red

4.4.2 Anthocyanin in outer sepal

0 Absent
3 Slight
5 Intermediate
7 Intense

4.4.3 Sepal length in millimetres (sample size 20)

4.4.4 Sepal width at widest point in millimetres (sample size 20)

4.4.5 Sepal length/width ratio

4.4.6 Sepal reflexion

0 Not reflexed (i.e. horizontal)
1 Reflexed (i.e. curved backwards)

4.4.7 Petal ligule length

Distance in millimetres between point of insertion of isthmus in hood and apex of ligule (sample size 15)

4.4.8 Petal ligule width at widest point in millimetres

4.4.9 Anthocyanin in stamen filament

0 Absent
3 Light
5 Intermediate
7 Intense

4.4.10 Staminode length in millimetres (sample size 10)

4.4.11 Ovary length in millimetres (sample size 15)

4.4.12 Ovary width at widest point in millimetres (sample size 10)

4.4.13 Number of ovules per ovary

The absolute numbers in 5 ovaries

4.4.14 Style length in millimetres (sample size 10)

4.5 FRUIT CHARACTERS

4.5.1 Fruit shape

The character recorded during collection[1/] can be amplified by more detailed description of mature fruits as below (see Figure 4)

1 Oblong
2 Elliptic
3 Obovate
4 Orbicular
5 Oblate

(lines represent greatest width)

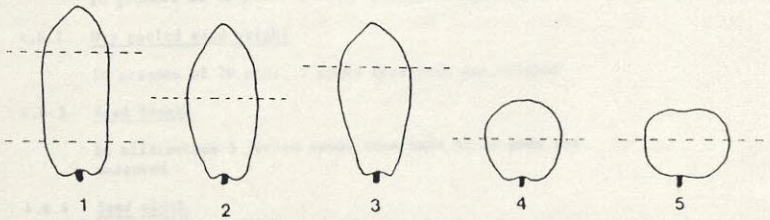


Figure 4. Fruit shape

4.5.2 Fruit length in centimetres (sample size 35)

4.5.3 Fruit width at widest point in centimetres (sample size 35)

4.5.4 Weight of whole pod in grammes (sample size 35)

1/ Curators should include in the data base the characters recorded during collection

4.5.5 Fruit primary furrow depth

The depth of the furrow between a pair of ridges

3 Superficial
5 Intermediate
7 Deep

4.5.6 Fruit wall thickness

Measurements made after removal of endocarp. Sample size 35

4.5.6.1 Husk hardness

3 Soft
5 Intermediate
7 Hard

4.5.6.2 Ridge depth in millimetres

4.5.6.3 Primary furrow depth in millimetres

4.5.6.4 Secondary furrow depth in millimetres

4.5.6.5 Mesocarp stony layer

3 Thin
5 Intermediate
7 Thick

4.5.7 Anthocyanin intensity of ridges

Scored on unripe fruits

0 Absent
3 Light
5 Intermediate
7 Intense

4.5.8 Anthocyanin intensity of primary furrows

0 Absent
3 Light
5 Intermediate
7 Intense

4.5.9 Anthocyanin intensity of ripe fruits

0 Absent (yellow)
3 Light
5 Intermediate
7 Intense

4.5.10 Period of maturity

The days from pollination to maturity

3 Short (150 days)
5 Intermediate (151-170 days)
7 Long (171 days)

4.5.11 Weight of husk

In grammes oven dry weight. Sample size 20 pods

4.5.12 Wet weight of beans

Mean weight in grammes per pod. Sample size 20 pods

4.5.13 Minimum number of seeds in a ripe pod

Sample size 20 pods

4.5.14 Average number of seeds in a ripe pod

Sample size 20 pods

4.6 SEED CHARACTERS

4.6.1 Dry cleaned unpeeled seed weight

In grammes of 20 pods, 5 seeds from each are weighed

4.6.2 Dry peeled seed weight

In grammes of 20 pods, 5 seeds from each are weighed

4.6.3 Seed length

In millimetres 5 peeled seeds from each of 20 pods are measured

4.6.4 Seed width

In millimetres 5 peeled seeds from each of 20 pods are measured

4.6.5 Seed thickness

In millimetres 5 peeled seeds from each of 20 pods are measured

4.6.6 Seed shape in longitudinal section (see Figure 5)

1 Oblong
2 Elliptic
3 Ovate

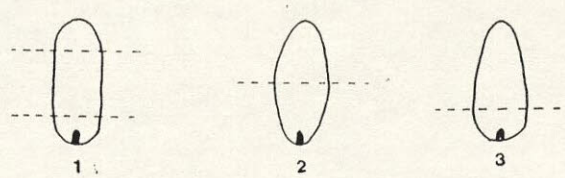


Figure 5. Seed shape in longitudinal section

PESTS AND DISEASES REACTION

It is not proposed that curators of collections carry out screening for pests and diseases, this being a task for the breeders as part of further evaluation. Nonetheless data should be fed back to the curators who maintain the primary material.