

**PERFORMANCE ANALYSIS OF SELECTED
ACCESSIONS OF COCOA
(*Theobroma cacao* L.)**

By

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(2011-11-116)

THESIS

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requirement for the degree of**

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**DEPARTMENT OF PLANT BREEDING AND GENETICS
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2013**

DECLARATION

I hereby declare that this thesis entitled “**Performance analysis of selected accessions of cocoa (*Theobroma cacao* L.)**” is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Introduction

1. INTRODUCTION

Cocoa (*Theobroma cacao* L.) originally belonging to the family Sterculiaceae (Purseglove, 1974) and at present included in the recently expanded family Malvaceae (Alverson *et al.*, 1999), is an important beverage crop grown all around the world for the delicious chocolates. It is indigenous to the tropical humid forests on the lower eastern equatorial slopes of the Andes in South America (Amma, 2010).

The term ‘cocoa’ is believed to have been derived from the word ‘cacahoatl’ used by the Aztec Indians of the high Mexican plateau to represent the seeds of this plant. According to their belief cocoa was brought to earth by the God ‘Quetzacoatl’ (the plumed serpent) whom they called as ‘xocolatl’. Hence, cocoa is considered to have a divine origin and is popularly known as the ‘Food of Gods’. It was from ‘xocolatl’ the word ‘chocolate’ was derived. It may probably be with this legend in mind that Linnaeus gave the name *Theobroma cacao* to the cultivated cocoa plant using the Greek words *theos* meaning Gods and *broma* meaning food (Mossu, 1992).

Cocoa, the only source of chocolate, is cultivated in 58 tropical countries of the world for its nibs (Amma *et al.*, 2011). The nibs form the source of energy rich and nutritious chocolates as well as an array of products. The large scale cultivation of cocoa started in India in 1970’s (Nair *et al.*, 2002) and now it is widely grown as an intercrop in Kerala, Tamil Nadu, Andhra Pradesh and Karnataka (Apshara, *et al.*, 2011). The spreading cultivation of cocoa has necessitated the production of large number of high yielding hybrids with pest and disease resistance suited to different agro-climatic conditions.

The main centre for cocoa research in India is Cocoa Research Centre (CRC) of Kerala Agricultural University, Vellanikkara. This centre is maintaining a large germplasm collection of cocoa. However, clustering of these germplasm accessions

based on morphological, biochemical or molecular markers has not yet been attempted. At present parents for hybridization programmes in cocoa are selected based on the yield potential as well as pest and disease resistance. Hence, many of the hybrid progenies evolved are not showing the desirable level of superiority and are to be discarded during the later stages of evaluation resulting in a slow progress in the improvement programmes in this perennial crop. Lack of genetic divergence among the selected parental lines may be a reason for this. The success of any hybridization programme, particularly in perennials depends upon the proper selection of parental lines.

It was in this background the present study entitled ‘Performance analysis of selected accessions of cocoa (*Theobroma cacao* L.)’ which forms part of an ongoing project ‘Germplasm collection and maintenance of cocoa’ at Cocoa Research Centre was taken up with the following objectives:

- i. To evaluate and characterize the selected accessions of cocoa using morphological and biochemical markers
- ii. To assess the genetic diversity among these selected accessions

Review of Literature

2. REVIEW OF LITERATURE

Biodiversity in *Theobroma*

The genus *Theobroma* presently includes 22 species organized in six sections (Cuatrecasas, 1964) (Table 1). Three species are considered as cultivated, although other species are used as timber, sweetening agents, refreshments or as medicine. The three cultivated species include *T. cacao*, the cocoa used worldwide as beverage, chocolate etc, *T. bicolor*, the ‘patashte’ of Chiapas, Mexico (Martinez, 1959) and *T. grandiflorum*, the ‘cupuassu’ of Brazil, used for juice and chocolate (Velho *et al.*, 1990; Giacometti, 1992). In addition, the pulp of the following species are edible: *T. canumanense* and *T. subincanum* (Giacometti, 1992). The use of wild species of *Theobroma* and *Herrania* as medicinal plants in Colombia has been reported by Barriga (1992).

Diversity in cultivated cocoa types was revised by Hunter (1990), Lanaud (1987) and Toxopeus (1989). Different groups of cocoa were recognized by scholars on the basis of pod and seed morphology: Cundeamor, Amelonado, Angoleta and Calabacillo. Later, two major groups were recognized by Cheesman (1944): the Criollos and the Forasteros (considered as two subspecies within *T. cacao* by Cuatrecasas (1964)). Each of them is further subdivided based on geographic origin. The Criollos are divided into Central American and South American Criollos and the Forasteros into Amazonian Forasteros and Trinitarios. Amazonian Forasteros are subdivided into two groups, the Lower Amazon and the Upper Amazon Forasteros, the latter being morphologically more variable (Toxopeus, 1989). Trinitarios are hybrids of Criollo and Forastero and are indigenous to Trinidad and Tobago (Cheesman, 1944). Criollo and Trinitario beans are collectively known as “fine or flavor” cocoa because of its premium quality and high demand among manufactures of fine chocolates (Mooledhar, 1995).

Table 1. List of *Theobroma* speices

Sl. No.	Section	Species
1.	Rhytidocarpus	<i>T. bicolor</i>
2.	Oreanthes	<i>T. sylvestre</i> <i>T. speciosum</i> <i>T. velutinum</i> <i>T. glaucum</i> <i>T. bernouillii</i>
3.	Theobroma	<i>T. cacao</i>
4.	Telmatocarpus	<i>T. gileri</i> <i>T. microcarpum</i>
5.	Glossopetalum	<i>T. cirmolinae</i> <i>T. stipulatum</i> <i>T. simiarum</i> <i>T. chocoense</i> <i>T. angustifolium</i> <i>T. grandiflorum</i> <i>T. obovatum</i> <i>T. sinuosum</i> <i>T. canumanense</i> <i>T. subincanum</i> <i>T. hylaeum</i> <i>T. nemorale</i>
6.	Andropetalum	<i>T. mammosum</i>

eographic distribution

The genus *Theobroma* is distributed exclusively in the Neotropics, from 20° north latitude to 15° south latitude, that is from Chiapas and Yucatan in Mexico (Gomez-Pompa *et al.*, 1990) down to Beni in Bolivia (Cuatrecasas, 1964). Most species are found in South America, although some species are distributed in Central America (Debouck, 1992). A form of *T. cacao* subsp. *cacao* seems to occur wild in Yucatan and in Chiapas, Mexico (Gomez-Pompa *et al.*, 1990). One cannot discard the role of ancient and modern Maya indigenous people in its distribution and the possibility that they might have contributed to the domesticated stocks of cultivated cocoa (Lanaud, 1987; Gomez-Pompa *et al.*, 1990).

It is now generally accepted that cocoa (*T. cacao*) is a diploid species of South American origin (Amm, 2010). Although native to the humid tropical regions of the northern parts of South America and the northern parts of Central America (Cheesman, 1944; Cuatrecasas, 1964; Bartley, 2005; Motamayor *et al.*, 2008), the largest cultivation of cocoa is found in West and Central Africa accounting for about 70 percent of the world's cocoa output (ICCO, 2010). The eight cocoa producing countries at present in the order of annual production are Cote d'Ivoire, Ghana, Indonesia, Nigeria, Cameroon, Brazil, Ecuador and Malaysia. These countries account for 90 percent of the total production of cocoa in the world (UNCTAD, 2010).

Genetic diversity

Genetic diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions and enables changes in the genetic composition to cope up with the changes in the environment. Genetic markers used for detecting genetic differences among individuals are grouped into phenotypic or morphological markers, biochemical markers and molecular markers (Aikpokpodion, 2012).

Morphological characterization

Morphological characterization is well known for its applicability to derive economic and breeding gains from germplasm collections and families of related accessions (Hawkes, 1983; Brown *et al.*, 1989; Iwaro *et al.*, 2003; Bekele *et al.*, 2006). Several studies were carried out using morpho-agronomic characteristics of pods, seeds and flowers to elucidate population structure and genetic diversity of cocoa populations (Engles, 1992; Bekele and Bekele, 1996; Aikpokpodion, 2010).

For the purposes of characterization, identification and measurement of diversity a number of qualitative as well as quantitative descriptors are proposed. A comprehensive list of descriptors was compiled by Engles *et al.* (1980) and this formed the basis for the IPGRI recommended descriptor list (Engles, 1992). In 1981, IPGRI Working Group on the Genetic Resources of Cocoa proposed an exhaustive list of 65 cocoa descriptors (Anon, 1981). This descriptor list has been adopted internationally and is used to characterize cocoa germplasm at CATIE, Costa Rica (Engles, 1981; Enriquez and Soria, 1981). Allen (1988) used pod surface texture, pod apex form, anthocyanin intensity on unripe pod ridges and ratios between measurements such as ratio of pod diameter to pod length to describe the wild materials collected from Ecuador. Phillips and Enriquez (1988) also used a shortened list of 26 descriptors for cataloguing of accessions from CATIE. A final set of traits for characterization and preliminary evaluation of accessions based on discriminative value, genetic background and taxonomic importance was recommended by Engles in 1992. CIRAD scientists observed that floral descriptors are useful taxonomically as found by ICGT (Bekele, 1993; Bekele *et al.*, 1994). In 1998, Bekele and Butler proposed a short list of morphological and agronomic descriptors which is adopted by CRU.

One hundred and fifty five clones of wild cocoa trees in French Guiana were characterized with floral descriptors like width of sepal, petal and ligule, length of gynoecium and number of ovules per ovary and Mahalanobis distances between the

populations were calculated. In this study Lachenaud *et al.* (1999) noted substantial genotypic variability in the Guianan material for the floral descriptors.

Cocoa trees in north-west Guyana were characterized by Chesny (2001) using qualitative and quantitative morphological traits in the descriptor list prepared by Cocoa Research Unit. The study revealed wide phenotypic diversity and established the presence of fine flavor cocoa among the on-farm cocoa types sampled.

In a study to determine the morphological diversity existing in cocoa farms in relation to that in gene bank accessions, a total of 300 farm accessions (FA) were selected from two major cocoa producing areas (Southern and Western) of Cameroon. Seventeen qualitative and quantitative descriptors related to leaf, flower, pod and seed were considered. Considerable morphological variation was observed within FA and gene bank accessions for the qualitative characters evaluated. The quantitative traits showed spatial differentiation between western and southern FA and a closer relationship between gene bank and some farm accessions (Efombagn *et al.*, 2009).

To determine the phenotypic variation among cocoa types grown in Nigeria, 17 agro-morphological traits were studied in 184 accessions collected from farmers' field and field gene banks. Fruit and bean traits of Upper Amazon Forasteros observed in farmers' accessions provided evidence of a shift from previously grown local 'West African Amelonado' from the Lower Amazon Forastero population (Aikpokpodion *et al.*, 2010).

Silva *et al.*, 2011, analyzed the genetic diversity, spatial genetic structure (SGS) and mating system at the hierarchical levels of fruits and individuals as well as pollen dispersal patterns in a population of *Theobroma cacao* in Brazil and provided the fundamental information required to establish long-term *ex situ* genetic conservation.

Based on 35 quantitative and 13 qualitative traits, the morphological variability of four wild species, one semi-cultivated and one cultivated species of *Theobroma* were

characterized. Large variation was observed for all morphological traits evaluated. Multivariate analysis also indicated significant differences between *Theobroma* species for individual or grouped morphological fruit traits (Santos *et al.*, 2012).

Pod and bean index

Cocoa varieties with high seed index are of better economic value for chocolate industries (Ruinard, 1961). Glendinning (1963) conducted studies on the genetics of yield and yield attributes and indicated that the number and size of beans in cocoa are highly heritable traits. Enriquez and Soria (1966) revealed that yield expressed as dry or wet weight of the bean is a highly variable character. The dry weight varied from 0.5g to 2.5g per seed. High variability was observed for weight of bean even within a single pod of cocoa. Their study also showed that the thickness of ridge and depth of furrow of the pods are very descriptive characters and are partially affected by the environment. The inheritance of fruit size was studied by Soria *et al.*, 1974, and estimated the heritability for fruit length as 55 percent, for fruit diameter as 63 percent and total weight as 57 percent indicating that these are highly transmissible characters. In 1975, Soria reported great variation in fruit characteristics like length, diameter, total weight, weight of the husk and weight of seeds in each pod.

In 1982, Engles analyzed the phenetic relationships between 32 cocoa clones and the results were compared with their genetic relationships. From this study, he concluded that selection for seed size could lead to higher dry cocoa production per fruit than selection solely for seed number per fruit.

The study on the effect of season on pod and bean characters of cocoa indicated that the pod weight, TSS and bean weight was low and pulp percentage was high in wet season as compared with the dry season (Bopaiah and Bhat, 1989). Cilas *et al.*, 1989, based on a study with 20 clones belonging to Upper Amazon, Amelonado and Trinitario types reported that bean size was extremely variable and tended to be greatest in Trinitario types. Average bean weight per 100 fermented dried beans ranged from

212.6g for clone UF 667 (Trinitario) to 67.5g for SCA 6 (Upper Amazon). It was reported that seeds originating from the fruit apex were shorter and free from flat beans. Flat beans were found only in the apical areas of some fruits of varieties EET 400 and SPA 9 (Mora, 1989). Among the characters evaluated by Francis (1998) the highest variability was observed for yield of dry beans/tree as well as precocity of bearing and the lowest for pod width and bean width.

Bekele *et al.*, 2008, characterized 1464 accessions using morphological descriptors and identified promising Trinitario accessions in terms of bean size, pod index (less than 20), pod wall thickness and pale cotyledon colour. In this study, they found that the five of the seven Trinitario groups *viz.*, GA, GDL, GS, ICS and TRD, had appreciably lower mean pod index (PI) values. The mean cotyledon weights for DOM, GA, GDL, GS, ICS, MAR and TRD were 0.94g, 1.02g, 1.22g, 1.11g, 1.14g, 0.89g and 1.03g, respectively.

Cocoa genotypes with bean weight higher than 1g are considered to be superior (Monterio *et al.*, 2009). Dried beans of 14 genotypes of cocoa were evaluated for their bean values (Oyedokun *et al.*, 2011) and these genotypes were found to be significantly different from each other with respect to weight of single bean, bean length, width, thickness, 100 bean weight, ratio of bean length to width, bean length to thickness and bean width to thickness. Morphological characterization of 20 TSH varieties based on 23 fruit and 12 floral traits carried out by Maharaj *et al.* (2011) revealed that the characteristics of economic interest *viz.*, bean number, cotyledon weight and pod index (PI) ranged from 42.2 to 61.4, 0.74 to 1.49g, 12 to 26.1, respectively. Based on studies on 23 selected accessions of cocoa Minimol *et al.*, 2011, observed the influence of fruit apex on fruit shape. The results of the study also showed that 12 among 23 were with angoleta type fruits, seven were with calabacillo type fruits and three were with cundeamor type fruits. One accession, RED 127 alone was having amelonado type fruits. With respect to fruit apex, eleven accessions were obtuse tipped, seven acute, four mammelate and one round tipped.

Biochemical characterization

Cocoa butter is the major commercial product from the seeds of *Theobroma cacao* L. Cocoa seeds contains more fat than any other major oil crop other than coconut (Luhs and Friedt, 1994). Fat content directly influence the value of commercial seeds, representing the increased cost of grinding during processing of seeds with low fat content (Duncan and Veldsman, 1994). In cocoa, several workers like Ronning & Schnell (1994) and Warren (1994) used isozyme systems to explore genetic diversity among cocoa populations. The average fat content in unfermented seeds of 490 accessions evaluated by Pires *et al.* (1998) was found to be 53.2 percent, ranging from 45.4 percent in CC 57 to 60.3 percent in NA 312.

Polyphenols accounting for 12-18 percent of the whole bean weight is associated with the flavor and colour of chocolate (Kim and Keeney, 1984). The reactions of polyphenol with sugar and amino acids contribute flavor and colour to cocoa beans whereas the alkaloids contribute to the bitterness (Lehrian and Patterson, 1983; Afoakwa and Paterson, 2010). Nazaruddin *et al.* (2006) reported that the total polyphenols ranged from 45 to 52 mg/g in cocoa liquor, 34 to 60 mg/g in beans, and 20 to 62 mg/g in powder. The place of origin as well as the method of processing are found to influence the antioxidant polyphenol content of cocoa products (Jalil and Ismail, 2008). Afoakwa *et al.*, 2012, conducted investigations to elucidate changes in total polyphenols during fermentation of Ghanaian cocoa beans and noted reduction in its concentration with increase in the storage and fermentation time.

Molecular characterization

In cocoa, the use of molecular markers was first reported by Wilde *et al.* (1992). Russel *et al.*, 1993; Laurent *et al.*, 1994; Lerceteau *et al.*, 1997 and Whitkus *et al.*, 1998 used Random Amplified Polymorphism DNA (RAPD) to study relationships among cocoa groups. N'Goran *et al.* (1994) analyzed the genetic diversity of 106 genotypes in Cote d'Ivoire belonging to various morpho-geographic groups using 49 repeatable

polymorphic RAPD products. This study revealed clear structures for Forastero and Criollo groups with clear cut differentiation between Upper and Lower Amazon Forasteros. Lerceteau *et al.*, 1997, analyzed the genetic diversity of Ecuadorian Nacional clones, Forastero, Trinitario and Criollo clones using 43 genomic probes and found that within-group genetic diversity was almost identical in Forastero, Trinitario and Criollo. The results also indicated that the populations of Amazon Forasteros and Criollos evaluated were highly diverse and that Criollo and Trinitario populations showed some overlapping.

Lerceteau *et al.*, 1997; N’Goran *et al.*, 2000; Motamayor and Lanaud, 2000 and Motamayor *et al.*, 2002 used RFLPs to determine the genetic diversity of cocoa populations. Microsatellites are recommended as an international standard for defining genetic identity and were widely used in the study of genetic diversity of cocoa genetic resources (Saunders *et al.*, 2000; Zhang *et al.*, 2006; Aikpokpodion *et al.*, 2009, 2010).

Efombagn *et al.* (2006) assessed 194 cocoa accessions collected from farms in Southern Cameroon and 71 Trinitario and Upper Amazon clones available in gene banks using 13 SSR markers. The genetic differentiation and similarities were analyzed and 282 alleles were detected within all the populations studied. Their study also showed that the farmers’ planting materials were not highly diverse but were genetically close to the parental genotypes available in gene banks. However, some promising genetically distant Upper Amazon clones (T-clones) were also identified. In 2008, Efombagn *et al.* analyzed the genetic diversity of 400 accessions collected from farms and gene banks using the 12 microsatellite markers and revealed a total of 125 alleles, 113 of which were present in the farm accession group (FA). They also found the presence of admixture in farmers’ fields.

To assess genetic diversity of cocoa types in Cote d’Ivoire 12 microsatellites were used by Pokou *et al.* (2009). Results revealed the presence of considerable diversity in farmers’ accessions reflecting large hybridization between local Amelonado types and Upper Amazon types.

In order to assess the genetic diversity of cocoa types grown, 377 accessions were collected from all cocoa growing regions of Ghana (Opoku *et al.*, 2007) and analyzed using 17 microsatellite markers. Genetic diversity indices indicated that average gene diversity was high in all populations and the highest was recorded in accessions from breeders' and parental collections.

Cluster analysis

Engles (1986) carried out cluster analysis as well as principal component analysis using 39 characters in a group of 294 cultivars and found that the distribution of these cultivars corresponded roughly to the traditional classifications into Criollo, Forastero and their subdivisions.

Hundred accessions from the germplasm maintained at International Cocoa Gene Bank, Trinidad were characterized by Bekele and Bekele (1996) for phenetic diversity with morphological descriptors and associations among them were examined by hierarchical average linkage cluster analysis. Cluster analysis indicated rich phenetic diversity in this sample of germplasm. At 75 percent level of similarity, nine accessions remained ungrouped and the remaining accessions grouped into 11 clusters. The observed link between geographic origin and accession grouping suggested that it is necessary to collect and conserve germplasm representing a broad geographic range.

Dias *et al.*, 1997, investigated stability of the genetic divergence among five non-commercial cocoa cultivars at advanced ages for over a five-year period and performed cluster analysis on five yield components measured on harvests from each crop year and on the data pooled over five years. The comparison of D^2 values and of clusters based on pooled analysis with D^2 values and clusters obtained from each year showed a stable clustering pattern in the most favorable years. In the same year, Santos *et al.* (1997) quantified multivariate phenetic divergence among SIC and SIAL series clones by cluster and principal component analyses. SIC 17 and SIAL 244 clones showed the highest divergence (3.05). SIC 18 and SIC 765 clones formed the highest

similar pair (0.33) based on the Euclidean distance matrix. Tocher's and single linkage methods applied to this matrix identified four clusters.

In 2011, Maharaj *et al.* carried out cluster analysis using 15 quantitative variables to study the relationships among 20 Trinidad Selected Hybrids (TSH) cocoa cultivars and five parental types. This study revealed that SCA 6, ICS 95 and ICS 1 were very distinct from the TSH progeny. SCA 6 was very distinct even at the lowest level of similarity. The two TSH types in this group had descriptive fruit values which were similar to the parental types and possessed strong IMC 67 ancestry.

Oyedokun *et al.*, 2011, employed Principal Component Analysis (PCA) to identify the distinguishing traits and to group the 14 genotypes based on similarities. PCA grouped the 14 genotypes into four distinct clusters. Clusters I and II had a membership of five and seven genotypes with a mean bean weight of 1.07g and 1.02g respectively. Clusters III and IV had G1 and G8 as single members. They also showed an outstanding bean weight of 1.12g and 1.30g respectively.

Pests and Diseases

Black pod disease was first noticed in Guyana and West Indies and referred as black cocoa (Jenman and Harrison, 1897). It was reported for the first time from India in 1965 (Ramakrishnan and Thankappan). *Phytophthora palmivora* has been found to be the causal organism of this disease (Chandramohan, 1979). At present it is prevalent in all cocoa growing countries (Zentmyer, 1988). Chandramohan, 1982, indicated that Nigerian collections exhibit certain degree of tolerance against black pod disease. Muthulakshmi *et al.*, 2011, laid out an experiment on the management of cocoa pod rot and stem canker caused by *Phytophthora palmivora* using ecofriendly biocontrol agents with six treatment combinations. The treatment T3 -*P. fluorescens* + *Trichoderma viridae* talc formulation + 2kg of FYM per tree was observed to be

effective in reducing pod rot incidence. However, the highest pod yield and dry bean yield were recorded by the treatment T4 – *P. fluorescens* liquid formulation.

Azhar, 1988, identified pod wall hardness as a phenotypic factor associated with resistance to the pod borer (*Conopomorpha cramerella*). This can be due to increased mortality of the larvae.

Tea mosquito bugs are serious pests of cocoa worldwide causing an yield loss as high as 75 percent. Estimates of crop loss attributed to damage by *Helopeltis* are highly variable and depend on factors such as agricultural practices, control methods, locality, climate and the varieties and insect species involved (Alagar and Subaharan, 2011).

Rodents form another group of major pests in almost all cocoa growing countries of the world (Everard, 1968; Stapley, 1972; Taylor, 1972; Williams, 1973; Gratz and Arata, 1975). Abraham and Padmanabhan reported rodent damage in cocoa as early as 1967 in India. Among them the black rat (*Rattus rattus*), the Western Ghats squirrel (*Funambulus tristriatus*) and the South Indian palm squirrel (*F. palmarum*) are considered to be the major ones (Bhat, 1978; Abraham and Remamony, 1979; Advani, 1984). Together they are reported to cause 29 to 52 percent damage to the standing crop in different areas of South India (Abraham and Padmanabhan, 1967; Bhat, 1978; Abraham *et al.*, 1979 and Bhat, 1981). Abraham *et al.*, 1979, suggested that timely harvest of mature pods could reduce the squirrel damage in cocoa. The damage can be reduced from 52 to 25 percent just by increasing the number of harvests from 12 to 21 per year. He also noticed that covering the cocoa pods with gunny bags or polythene covers smeared with bitumen was very effective in reducing squirrel damage. The rats usually damage cocoa pods near the stalk portion whereas squirrels damage the central part of the pod (Bhat, 1980). Bhat also noticed that the damage caused by rats can be reduced by poison baiting or by trapping (2011). He also suggested single catch ‘live’ traps for controlling squirrels.

Materials & Methods

3. MATERIALS AND METHODS

The present study entitled 'Performance analysis of selected accessions of cocoa (*Theobroma cacao* L.)' was carried out in the Department of Plant Breeding and Genetics, College of Horticulture and the Cocoa Research Centre, Vellanikkara during the period 2011-2013.

Fifty clonal accessions of cocoa comprising of 40 exotic and 10 indigenous ones selected from the germplasm maintained by Cocoa Research Centre, Vellanikkara, formed the material for the study. The selected accessions laid out in RBD with three replications have already reached the steady bearing stage. The details of these accessions are presented in Table 2.

3.1 Morphological characterization

Both qualitative and quantitative characters were considered for morphological evaluation. The descriptor list developed by Bekele and Butler (2000) was used for recording the observations. The descriptor and the descriptor states are presented in Table 3. The morphological descriptors are useful in selecting suitable accessions for breeding programmes (Engles *et al.*, 1980).

3.1.1 Qualitative evaluation

For qualitative evaluation observations on 13 qualitative characters were recorded. Since the colour of staminode and hardness of husk were uniform in all accessions, they were not included for further analysis. The genetic associations among the accessions were estimated by Jaccard's similarity coefficients (Jaccard, 1908) using NTSYS pc version 2.1 (Rohlf, 1992). Cluster analysis was performed based on the similarity matrix and dendrograms were constructed by unweighted pair-group method (UPGMA) (Sneath and Sokal, 1973) for the exotic and indigenous accessions.

Table 2. The accessions of cocoa used for the study

Sl. No.	Code for accession	Accession	Derivation	Source of collection
Exotic accessions				
1	Exo 1	SC 10	Santa Cruz	Brazil
2	Exo 2	COCA 3370-3	COCA river	Ecuador
3	Exo 3	AMAZ 10-1	AMAZonas	Ecuador
4	Exo 4	BE 3	Belen	Brazil
5	Exo 5	AMAZ 15	AMAZonas	Ecuador
6	Exo 6	AMAZ 6-3	AMAZonas	Ecuador
7	Exo 7	AMAZ 3-2	AMAZonas	Ecuador
8	Exo 8	PINA	-	French Guiana
9	Exo 9	B7 B2	Borne	French Guiana
10	Exo 10	PA 56	PARinari	Peru
11	Exo 11	DOM 4	DOMinica	Trinidad & Tobago
12	Exo 12	KER 2 E	River KERininton	French Guiana
13	Exo 13	R (10) (MEX)	Rosario Izapa Mexico	Mexico
14	Exo 14	B7 B4	Borne	French Guiana
15	Exo 15	UF 677	United Fruit Selections	Costa Rica
16	Exo 16	GDL 3	GuaDeLoupe	Trinidad & Tobago
17	Exo 17	B5-7	Balao	Ecuador
18	Exo 18	MAR 9	MARTinique	Trinidad & Tobago
19	Exo 19	CLM 90	CLementina farm Mixed	Ecuador
20	Exo 20	R (39) (MEX)	Rosario Izapa Mexico	Mexico
21	Exo 21	B7 B5	Borne	French Guiana
22	Exo 22	DOM 25	DOMinica	Trinidad & Tobago
23	Exo 23	KER 9	River KERininton	French Guiana
24	Exo 24	LV 28	Large Vuelta	Ecuador

25	Exo 25	B7 A6	Borne	French Guiana
26	Exo 26	GU 310	Guyana	French Guiana
27	Exo 27	EET 400	Estacion Experimental Tropical	Ecuador
28	Exo 28	IMC 16	Iquitos Mixed Calabacillo	Ecuador
29	Exo 29	EET 397	Estacion Experimental Tropical	Ecuador
30	Exo 30	ICS 95	Imperial College Selections	Trinidad & Tobago
31	Exo 31	IMC 67	Iquitos Mixed Calabacillo	Peru
32	Exo 32	SCA 6	SCAvina	Peru
33	Exo 33	PA 137	PARinari	Peru
34	Exo 34	RB 33/3	Rio Branco	Brazil
35	Exo 35	SPEC 160-9	SPECimen	Columbia
36	Exo 36	EQX 3348-44	EQuator crosses	Ecuador
37	Exo 37	PUCALA 1	-	Peru
38	Exo 38	IMC 54	Iquitos Mixed Calabacillo	Peru
39	Exo 39	IMC 14	Iquitos Mixed Calabacillo	Peru
40	Exo 40	Criollo	-	Costa Rica
Indigenous accessions				
41	Ind 1	Calicut local 1	-	Calicut
42	Ind 2	Calicut local 2	-	Calicut
43	Ind 3	Konni local 1	-	Konni
44	Ind 4	Konni local 2	-	Konni
45	Ind 5	Konni local 3	-	Konni
46	Ind 6	Konni local 4	-	Konni
47	Ind 7	Konni local 5	-	Konni
48	Ind 8	Thodupuzha local 1	-	Thodupuzha
49	Ind 9	Thodupuzha local 2	-	Thodupuzha
50	Ind 10	Thodupuzha local 3	-	Thodupuzha

Table 3. Descriptor and descriptor states used for recording observations

Sl. No.	Character	Descriptor state	Description
1	Flush colour	0	Absent (green)
		3	Slight (greenish red)
2	Length of pedicel (cm)	-	
3	Colour of pedicel	1	Green
		2	Reddish
4	Diameter of flower (cm)	-	
5	Length of sepal (cm)	-	
6	Width of sepal (cm)	-	
7	Colour of sepal	1	Cream
		2	Greenish cream
8	Length of petal (cm)	-	
9	Width of petal (cm)	-	
10	Colour of petal	1	Cream
		2	Greenish cream
11	Number of staminodes	-	
12	Length of staminodes (cm)	-	
13	Colour of staminodes	1	Slight
		3	Intermediate
14	Number of stamens	-	
15	Length of stamens (cm)	-	
16	Colour of stamen	1	Cream
		2	Greenish cream
17	Length of ovary (cm)	-	
18	Width of ovary (cm)	-	
19	Length of style (cm)	-	
20	Pod shape	1	Cundeamor
		2	Angoleta

21	Ridge & furrow colour	0 3	Absent (green) Slight (greenish yellow)
		1 2	Attenuate Acute
23	Pod basal constriction	0 1	Absent Slight
24	Husk hardness (cm) (difference between ridge and	3 5	Soft (<1) Intermediate (1-1.5)
25	Pod rugosity	0 3	Absent Slight
		1 2	White Grey
27	Number of beans/pod	-	
28	Number of flat beans/pod	-	
29	Length of pod (cm)	-	
30	Breadth of pod (cm)		
31	Furrow thickness (cm)	-	
32	Ridge thickness (cm)	-	
33	Total wet bean weight/pod (g)	-	
34	Wet weight of peeled bean (g)	-	
35	Dry weight of peeled bean (g)	-	
36	Length of peeled bean (mm)	-	
37	Breadth of peeled bean (mm)	-	
38	Thickness of peeled bean (mm)	-	
39	TSS (%)	-	

3.1.2 Quantitative evaluation

Quantitative evaluation was based on 26 quantitative characters. Leica EZ 4D model microscope was used for taking floral measurements like length of pedicel, diameter of flower, length and width of sepal, petal and ovary as well as length of staminode, stamen and style (Plate 1). The length and width of pods were measured using a measuring device fabricated by Cocoa Research Centre (Plate 2). The thickness of husk was taken with the help of vernier calipers (Plate 3). Analysis of variance was done for each of these 26 quantitative characters observed. All the characters except diameter of flower, width of sepal, width of petal, number of staminodes, number of stamen, length of ovary, and width of ovary showed significant difference between the exotic and indigenous groups. Hence, further analysis was carried out separately for exotic and indigenous groups using the 19 characters having significant difference.

The descriptive statistics *viz.*, mean, standard deviation, standard error, phenotypic coefficient of variation, genotypic coefficient of variation, heritability and genetic gain for these 19 quantitative characters were worked out separately for exotic as well as indigenous groups. Clustering of the accessions was done and the genetic divergence was computed for exotic and indigenous groups following the D^2 statistics developed by Mahalanobis (1936).

3.2 Comparison of qualitative and quantitative clustering patterns

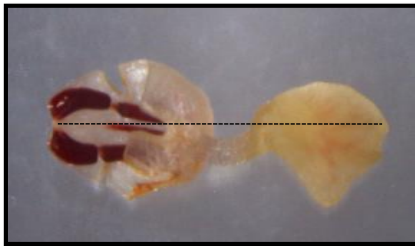
For each qualitative cluster, the percentages of accessions distributed into various quantitative clusters were worked out for exotic and indigenous groups. This was done to find out the homology between qualitative and quantitative clustering patterns.



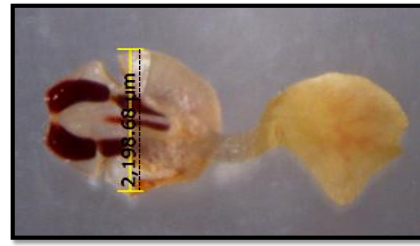
Length of pedicel



Diameter of flower



Length of petal



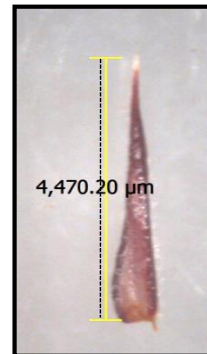
Width of petal



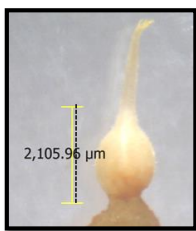
Length of sepal



Width of sepal



Length of staminode



Length of ovary



Width of ovary



Length of style



Length of stamen

Plate 1. Floral measurements in cocoa



Length of pod



Width of pod

Plate 2. Measurement of length and width of pod



Plate 3. Measurement of husk thickness

3.3 Biochemical characterization

The selected accessions were evaluated based on biochemical parameters also. For biochemical evaluation, fat and total polyphenol contents were estimated following standard procedures.

Fifteen ripe pods were harvested from each selected accession of cocoa. The ripeness and maturity levels of pods were considered for harvesting. The pods were then split open and beans of pods from each accession were pooled. Twenty beans were then selected at random from each accession. After removing the outer slimy layer the beans were dried to moisture content below 8 percent either by sun drying or by oven drying. Under normal sunny weather drying could be completed in 6-7 days. The dry beans were then ground to fine powder using laboratory grinder and the powder was stored in polyethylene bottles for analysis.

3.3.1 Determination of fat

Cocoa nibs were defatted by extracting the fat with petroleum ether (40-60°C) in a soxhlet extraction apparatus (Sadasivam and Manickam, 1996). Ten grams of the powdered sample wrapped in blotting paper was put in the extraction tube of soxhlet extraction apparatus. The total fat present in the sample was extracted along with the siphoning of petroleum ether and was collected in pre-weighed flask of the apparatus. The petroleum ether in the extract was evaporated to dryness. The cream coloured fat left behind after the evaporation of solvent was weighed and expressed as percentage.

3.3.2 Determination of polyphenol

The powdered defatted bean samples were used for the estimation of total polyphenol content. The defatted samples were extracted exhaustively with methanol in a soxhlet extractor. Phenolic constituents in the extract were then estimated colorimetrically by the Folin-Ciocalteu procedure (Sadasivam and Manickam, 1996). The procedure followed is presented below in detail.

Exactly 250 mg of sample was weighed into a micro soxhlet extractor. The sample was extracted initially using 10 ml petroleum ether (b.p. 40-60°C) for about 30 minutes to remove the fatty matter. The extract was discarded and extraction was continued for another 30 min. with 10 ml methanol. The extract was transferred quantitatively to a 50 ml beaker and solvent evaporated off on a steam bath. The beaker was cooled and the contents were reconstituted in exactly 5 ml of methanol. The extract was taken in an eppendorf tube, centrifuged at 10000 rpm for one minute and stored until analysis.

The detector was calibrated for quantification of total polyphenols by the following procedure. The total polyphenols in extracts were assayed in terms of catechin taken as the reference. A series of standard solutions of catechin ranging from 0 to 150 ppm were prepared by suitably diluting the aliquots of a standard solution of catechin prepared by dissolving weighed amount of catechin in measured amount of methanol.

10µl of each of the concentrations were transferred into separate 10 ml test tubes and diluted with 190µl distilled water followed by 500µl Folin-Ciocalteu reagent. After 3 minutes 2 ml of 20 percent Na₂CO₃ solution was added, mixed well and the test tube was placed in a boiling water bath. After exactly one minute the tubes were removed from the bath and cooled to room temperature under a stream of cold water. The blue colour of the solution was read at 750 nm in a spectrophotometer against reagent blank.

A curve was plotted with concentration of catechin solution pipetted and the absorbance of the corresponding blue coloured solution. From the linear part of the graph, the equation connecting concentration of catechin in the extract analyzed and the corresponding absorbance readings was established.

For estimation of phenols in methanolic plant extracts, 10µl extract was pipetted out into a 10 ml test tube and blue colour was developed in the same manner as done in case of standards and the absorbance was read at 750 nm in a spectrophotometer against

reagent blank. Concentration of phenols in the extract (expressed as catechin) was worked out by substituting the absorbance value thus obtained in the calibration equation developed for the purpose.

3.3.3 Clustering based on biochemical characters

The total fat and total polyphenol content in the samples were calculated and expressed as percent. Jaccard's similarity coefficients among the selected accessions based on fat and total polyphenol contents were worked out using NTSYS pc version 2.1 (Rohlf, 1992). Based on the similarity matrix cluster analysis was performed and dendrogram was constructed by unweighted pair-group method (UPGMA) by Sneath and Sokal, 1973, for the exotic and indigenous accessions.

3.4 Scoring of pests, diseases and rodents

A preliminary scoring of all the accessions was also done for their susceptibility to pests, diseases and rodents (rats and squirrels). Every month ten pods were selected at random from each accession and any infestation if present was recorded. This was continued for one year and the results were expressed as percentage of the total number of pods observed.

3.5 Development of statistical key for the accessions

The key qualitative and quantitative characters were identified. Qualitative characters which were highly variable and easily identifiable were taken as key qualitative characters. The quantitative characters of commercial importance in cocoa were selected as key quantitative characters. Using these key qualitative and quantitative characters the statistical key was developed which can serve as a preliminary tool for predicting the performance of various accessions.

Results & Discussion

4. RESULTS & DISCUSSION

Fifty clonal accessions of cocoa were used for the present investigation. Among these 40 were exotic. Twelve of these exotic accessions belonged to Ecuador, two to Mexico, one to Columbia, eight to French Guiana, three to Brazil, seven to Peru, five to Trinidad & Tobago and two to Costa Rica. The results of the present investigation on the performance of these 50 selected accessions are presented below.

4.1 Morphological characterization

Both qualitative and quantitative characters were used for evaluating the accessions morphologically. Morphological characterization is well known in its applicability to derive economic and breeding gains from germplasm collections and families of related accessions (Hawkes, 1983; Brown *et al.*, 1989; Iwaro *et al.*, 2003; Bekele *et al.*, 2006).

4.1.1 Qualitative evaluation

The observations on qualitative characters are presented in Table 4 and 5. Wide variability was present among the accessions for 11 out of the 13 qualitative characters. The qualitative characters *viz.*, colour of staminodes and hardness of husk were uniform in all the accessions, intense red and intermediate respectively. Hence, these two characters were not included in further analysis.

Four types of flush colour *viz.*, green, greenish red, reddish green and red were observed in the accessions evaluated. The results are presented in Table 4 and Plate 4. Majority of the accessions evaluated were having reddish green flush followed by red. However, in one accession, COCA 3370-3, alone the flush colour was green.

The floral characters of different accessions evaluated are presented in Table 4. Twenty seven accessions were having green pedicel, greenish cream sepals, cream petals and cream stamens. Similarly different combinations of pedicel, sepal, petal and

Table 4. Flush & floral qualitative characters of 50 accessions of cocoa

Accessions	Flush colour	Pediceal colour	Sepal colour	Petal colour	Stamen colour
SC 10	Greenish red	Green	Greenish cream	Cream	Cream
COCA 3370-3	Green	Green	Greenish cream	Cream	Reddish
AMAZ 10-1	Red	Green	Reddish	Cream	Cream
BE 3	Reddish green	Green	Greenish cream	Cream	Cream
AMAZ 15	Red	Green	Greenish cream	Cream	Greenish cream
AMAZ 6-3	Red	Green	Greenish cream	Cream	Reddish
AMAZ 3-2	Red	Green	Greenish cream	Cream	Cream
PINA	Reddish green	Green	Greenish cream	Cream	Cream
B7 B2	Reddish green	Green	Greenish cream	Cream	Cream
PA 56	Greenish red	Green	Greenish cream	Cream	Cream
DOM 4	Reddish green	Green	Greenish cream	Cream	Cream
KER 2E	Reddish green	Green	Greenish cream	Cream	Cream
R (10) (MEX)	Reddish green	Green	Greenish cream	Cream	Cream
B7 B4	Reddish green	Green	Greenish cream	Cream	Cream
UF 677	Reddish green	Green	Greenish cream	Cream	Cream
GDL 3	Red	Green	Greenish cream	Cream	Cream
B5-7	Greenish red	Green	Greenish cream	Cream	Cream
MAR 9	Red	Green	Greenish cream	Cream	Cream
CLM 90	Red	Green	Reddish	Cream	Cream
R (39) (MEX)	Red	Reddish	Greenish cream	Cream	Cream
B7 B5	Red	Green	Greenish cream	Cream	Cream
DOM 25	Greenish red	Green	Greenish cream	Cream	Cream
KER 9	Red	Green	Greenish cream	Greenish Cream	Cream
LV 28	Red	Reddish	Greenish cream	Cream	Cream

B7 A6	Red	Green	Cream	Cream	Reddish
GU 310	Red	Green	Greenish cream	Cream	Cream
EET 400	Red	Green	Greenish cream	Cream	Reddish
IMC 16	Reddish green	Green	Reddish	Reddish	Cream
EET 397	Reddish green	Green	Greenish cream	Cream	Reddish
ICS 95	Red	Green	Reddish	Cream	Cream
IMC 67	Reddish green	Green	Cream	Cream	Cream
SCA 6	Reddish green	Green	Greenish cream	Cream	Reddish
PA 137	Reddish green	Green	Greenish cream	Cream	Cream
RB 33/3	Greenish red	Green	Greenish cream	Cream	Reddish
SPEC 160-9	Reddish green	Green	Greenish cream	Cream	Cream
EQX-3348-44	Reddish green	Green	Greenish cream	Cream	Cream
PUCALA 1	Red	Green	Greenish cream	Greenish Cream	Cream
IMC 54	Reddish green	Green	Reddish	Cream	Cream
IMC 14	Reddish green	Green	Greenish cream	Cream	Cream
Criollo	Red	Reddish	Reddish	Reddish	Reddish
Calicut local 1	Reddish green	Green	Greenish cream	Cream	Cream
Calicut local 2	Greenish red	Green	Greenish cream	Cream	Cream
Konni local 1	Red	Green	Greenish cream	Cream	Cream
Konni local 2	Reddish green	Green	Greenish cream	Cream	Reddish
Konni local 3	Reddish green	Green	Greenish cream	Cream	Cream
Konni local 4	Reddish green	Green	Cream	Cream	Reddish
Konni local 5	Red	Reddish	Greenish cream	Reddish	Reddish
Thodupuzha local 1	Red	Green	Greenish cream	Greenish Cream	Cream
Thodupuzha local 2	Red	Green	Greenish cream	Cream	Cream
Thodupuzha local 3	Reddish green	Green	Reddish	Reddish	Reddish



Absent (Green)



Slight (Greenish red)



Intermediate (Reddish green)



Intense (Red)

Plate 4. Descriptor states for flush colour

stamen colours were observed in different accessions. However, in the case of exotic accession Criollo all these floral parts were reddish in colour.

Pod colour, pod shape, pod basal constriction, pod rugosity and cotyledon colour showed variability among the accessions (Table 5). The unripe pods were observed to be green in majority of accessions except EET 397, Criollo, Konni local 5 and Thodupuzha local 3. In EET 397 pods were greenish red where as in Konni local 5 and Thodupuzha local 3 it was reddish green. The accession Criollo alone had red coloured pods.

The features of different types of pods have already been described by Wood and Lass in 1955 (Plate 5). According to them, cundeamor types are characterized by the presence of pods which are deeply ridged and warty as well as having bottle neck. Pods are square shaped at the stalk end and devoid of bottleneck in angoleta type. Smooth, melon shaped pods with blunt end, shallow furrows and slight bottle neck are the features of amelonado types. Calabacillo types are small, nearly spherical in shape with a point at its apex. Criollo types show resemblance to cundeamor types. Pods though warty like cundeamor are characterized with attenuate apex in criollo types. Among the five descriptor states for pod shape only four types were observed in the present study. Forty four accessions were having cundeamor shaped pods. The pod shape was angoleta in UF 677, SPEC 160-9, EQX-3348-44 and amelonado in PA 137, PUCALA 1, Calicut local 1 and 2. The pod apex was acute in all the accessions except Criollo where it was attenuate (Plate 6).

Pod basal constriction may or may not be present in the accessions. If present it can be slight, intermediate or strong (Plate 7). In the accessions UF 677, SPEC 160-9 and EQX-3348-44 the pod basal constriction was absent. Sixty six percent of the accessions exhibited intermediate basal constriction. Thirteen accessions were having slight basal constriction. The accession B7 B5 alone had strong basal constriction.

Table 5. Pod qualitative characters of 50 accessions of cocoa

Accessions	Unripe pod colour	Pod shape	Pod base	Pod apex	Rugosity	Bean colour
SC 10	Green	Cundeamor	Slight	Acute	Intense	Mixed
COCA 3370-3	Green	Cundeamor	Intermediate	Acute	Intense	Medium purple
AMAZ 10-1	Green	Cundeamor	Intermediate	Acute	Absent	Dark purple
BE 3	Green	Cundeamor	Slight	Acute	Intermediate	Medium purple
AMAZ 15	Green	Cundeamor	Intermediate	Acute	Slight	Mixed
AMAZ 6-3	Green	Cundeamor	Intermediate	Acute	Slight	Mixed
AMAZ 3-2	Green	Cundeamor	Intermediate	Acute	Intermediate	Medium purple
PINA	Green	Cundeamor	Intermediate	Acute	Intermediate	Dark purple
B7 B2	Green	Cundeamor	Intermediate	Acute	Intermediate	Medium purple
PA 56	Green	Cundeamor	Intermediate	Acute	Absent	Dark purple
DOM 4	Green	Cundeamor	Intermediate	Acute	Slight	Dark purple
KER 2E	Green	Cundeamor	Slight	Acute	Intermediate	Dark purple
R (10) (MEX)	Green	Cundeamor	Intermediate	Acute	Intermediate	Mixed
B7 B4	Green	Cundeamor	Intermediate	Acute	Intense	Dark purple
UF 677	Green	Angoleta	Absent	Acute	Intermediate	Medium purple
GDL 3	Green	Cundeamor	Intermediate	Obtuse	Intermediate	Dark purple
B5-7	Green	Cundeamor	Intermediate	Acute	Intense	Dark purple
MAR 9	Green	Cundeamor	Intermediate	Acute	Slight	Medium purple
CLM 90	Green	Cundeamor	Slight	Acute	Absent	Medium purple
R (39) (MEX)	Green	Cundeamor	Intermediate	Acute	Intense	Medium purple
B7 B5	Green	Cundeamor	Strong	Acute	Intermediate	Medium purple
DOM 25	Green	Cundeamor	Intermediate	Acute	Absent	Dark purple
KER 9	Green	Cundeamor	Intermediate	Acute	Intense	Medium purple
LV 28	Green	Cundeamor	Intermediate	Acute	Intense	Dark purple
B7 A6	Green	Cundeamor	Intermediate	Acute	Intense	Dark purple

GU 310	Green	Cundeamor	Intermediate	Acute	Intense	Mixed
EET 400	Green	Cundeamor	Slight	Acute	Intense	Medium purple
IMC 16	Green	Cundeamor	Slight	Acute	Slight	Medium purple
EET 397	Greenish Red	Cundeamor	Intermediate	Acute	Intermediate	Dark purple
ICS 95	Green	Cundeamor	Intermediate	Acute	Intense	Dark purple
IMC 67	Green	Cundeamor	Slight	Acute	Intermediate	Light purple
SCA 6	Green	Cundeamor	Slight	Acute	Intermediate	Dark purple
PA 137	Green	Amelonado	Slight	Acute	Slight	Dark purple
RB 33/3	Green	Cundeamor	Intermediate	Acute	Slight	Dark purple
SPEC 160-9	Green	Angoleta	Absent	Acute	Intermediate	Dark purple
EQX-3348-44	Green	Angoleta	Absent	Acute	Absent	Light purple
PUCALA 1	Green	Amelonado	Slight	Acute	Slight	Dark purple
IMC 54	Green	Cundeamor	Intermediate	Acute	Intermediate	Dark purple
IMC 14	Green	Cundeamor	Intermediate	Acute	Slight	Dark purple
Criollo	Red	Criollo	Intermediate	Attenuate	Intense	Mixed
Calicut local 1	Green	Amelonado	Slight	Acute	Slight	Medium purple
Calicut local 2	Green	Amelonado	Slight	Acute	Absent	Medium purple
Konni local 1	Green	Cundeamor	Slight	Acute	Slight	Medium purple
Konni local 2	Green	Cundeamor	Intermediate	Acute	Intense	Dark purple
Konni local 3	Green	Cundeamor	Intermediate	Acute	Slight	Medium purple
Konni local 4	Green	Cundeamor	Intermediate	Acute	Slight	Mixed
Konni local 5	Reddish green	Cundeamor	Intermediate	Acute	Intermediate	Dark purple
Thodupuzha local 1	Green	Cundeamor	Intermediate	Acute	Intermediate	Medium purple
Thodupuzha local 2	Green	Cundeamor	Slight	Acute	Intermediate	Dark purple
Thodupuzha local 3	Reddish green	Cundeamor	Slight	Acute	Slight	Dark purple



Cundeamor



Angoleta



Amelonado



Calabacillo



Criollo

Plate 5. Descriptor states for pod shape



Attenuate



Acute



Obtuse



Rounded



Mammelate

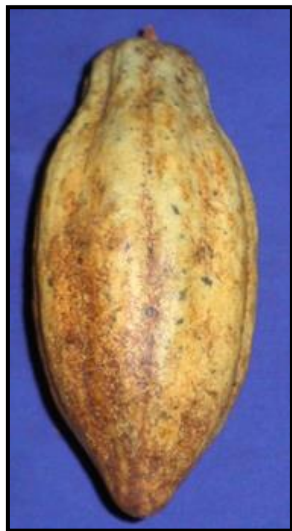
Plate 6. Descriptor states for pod apex



Absent



Slight



Intermediate



Strong

Plate 7. Descriptor states for pod basal constriction

Pod surface was smooth (rugosity absent) in the accessions DOM 25, AMAZ 10-1, PA 56, CLM 90, EQX-3348-44 and Calicut local 2. Intermediate rugosity was observed in 17 accessions followed by slight rugosity in 14 and intense rugosity in 13 accessions (Plate 8).

Accessions COCA 3370-3, AMAZ 3-2, B7 B 2, R (39) (MEX), MAR 9, KER 9, Konni local 3 and Thodupuzha local 1 were having cundeamor type pods with intermediate basal constriction and acute apex. The typical criollo type pods with intermediate constriction at the base and attenuate apex were observed in the exotic accession Criollo.

In the descriptor, only six states *viz.*, white, grey, light purple, medium purple, dark purple and mottled are given for cotyledon colour. However, in the present investigation a mixture of different coloured beans was also observed and was represented by the grade seven (mixed) (Plate 9). The accessions SC 10, GU 310, AMAZ 15, AMAZ 6-3, R (10) (MEX), Criollo and Konni local 4 exhibited the presence of mixture of beans having cotyledons ranging in colour from white to dark purple. From Table 4 it can also be seen that there is variability in bean colour among the five local accessions from Konni. Similarly Thodupuzha local 1 was found to be different from 2 and 3 in bean colour.

4.1.1.1 Clustering based on qualitative characters

Agglomerative hierarchical clustering was performed based on the Jaccard's similarity coefficient using the UPGMA method with 11 qualitative characters. The dendrogram was then constructed for exotic and indigenous accessions (Fig. 1 and 2 respectively).

Clustering of exotic accessions

The 40 exotic accessions used in the present study were grouped into nine clusters at 70 percent similarity level (Fig. 1). The nine clusters obtained along with the



Absent



Slight



Intermediate



Strong

Plate 8. Descriptor states for pod rugosity



Light purple



Medium purple



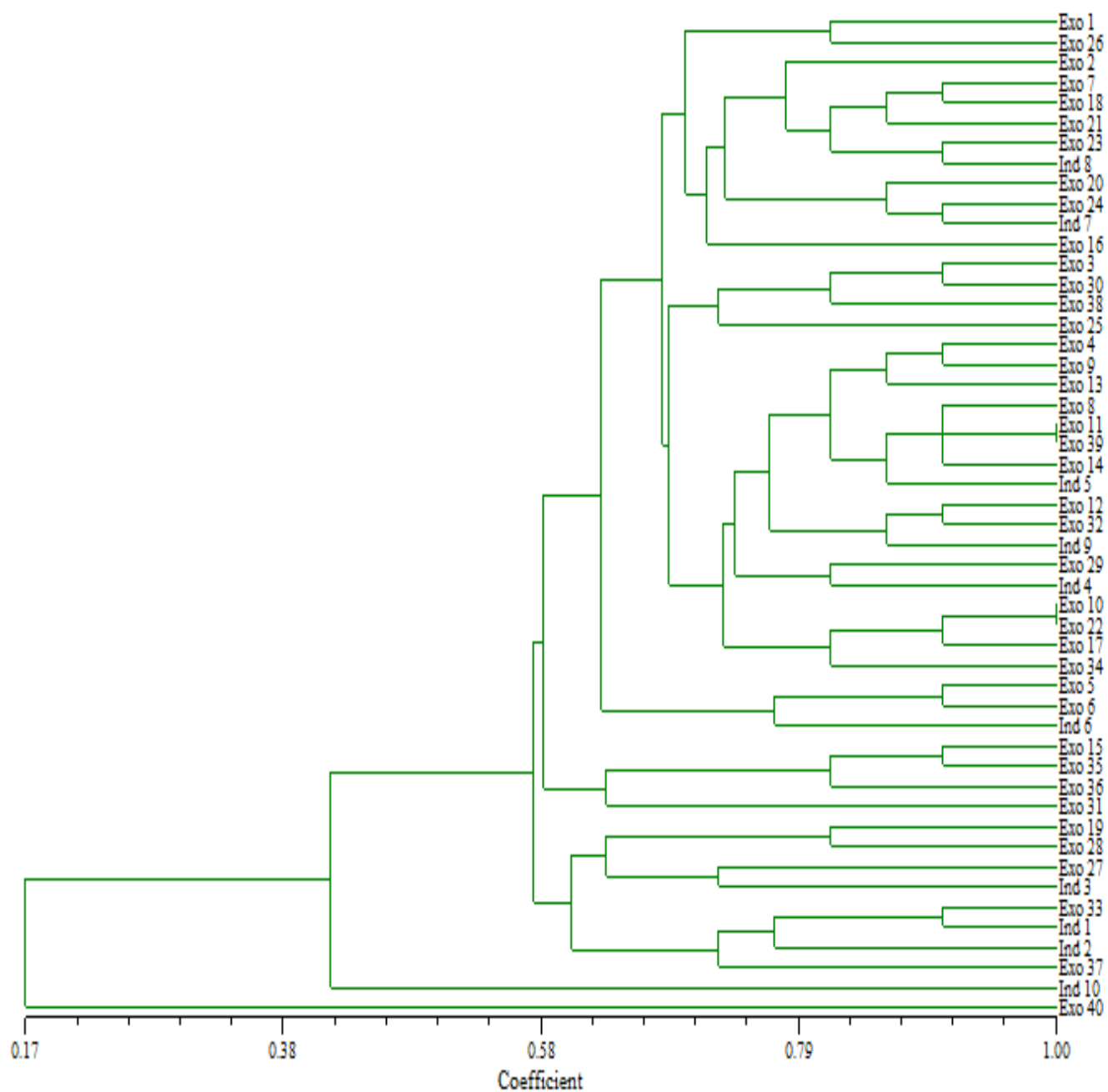
Mixed beans



Dark purple

Plate 9. Descriptor states for cotyledon colour

Fig. 1. Dendrogram based on similarity coefficient among exotic accessions of cocoa



accessions included in each cluster are presented in Table 6. The accessions SC 10, GU 310, AMAZ 15 and AMAZ 6-3 were grouped in cluster I. Cluster II included accessions COCA 3370-3, KER 9, AMAZ 3-2, MAR 9, B7 B 5, R (39) (MEX), LV 28 and EET 400. Among the nine clusters, Cluster IV was the biggest one with 16 accessions. Clusters VIII and IX included single accession each. The accession IMC 67 was included in cluster VIII and Criollo in cluster IX. The accession IMC 67 which fell in a separate cluster was morphologically distinct from the other accessions with its cream coloured sepals and petals, cundeamor type pods having intermediate rugosity and light purple beans. However, Madell (2005) reported that this accession produces flattish dark purple beans. The accession Criollo was distinct from all the exotic accessions evaluated by its reddish pedicel, sepals, petals and stamens as well as with its typical criollo type pods having attenuate apex. However, the pods produced by this accession were found to contain beans of different colours. The typical Criollo types are characterized by the presence of white beans alone as reported by Wood (1975). Hence, the presence of mixed beans in the pods of this accession indicates the variability developed by natural out crossing.

From the dendrogram (Fig. 1) it can be seen that the accessions DOM 4 and IMC 14 belonging to cluster IV were exactly identical (cent percent similarity). Similarly accessions PA 56 and DOM 25 falling in the same qualitative cluster were also identical.

Clustering of indigenous accessions

The 10 indigenous accessions evaluated were grouped into five clusters at 70 percent similarity level (Fig. 2). These clusters along with the accessions included in each cluster are presented in Table 7. The clusters I and II were having three accessions each. Konni local 2 and 4 were found to fall in cluster IV. The accessions Konni local 1 and Thodupuzha local 3 formed two distinct clusters, cluster II and V respectively indicating that they are distinct in qualitative features.

Table 6. Clustering based on qualitative characters of exotic accessions of cocoa

Cluster No.	No. of accessions	Code of accessions	Name of accessions
I	4	Exo 1 Exo 5 Exo 6 Exo 26	SC 10 AMAZ 15 AMAZ 6-3 GU 310
II	8	Exo 2 Exo 7 Exo 18 Exo 21 Exo 20 Exo 23 Exo 24 Exo 27	COCA 3370-3 AMAZ 3-2 MAR 9 B7 B5 R (39) (MEX) KER 9 LV 28 EET 400
III	3	Exo 3 Exo 25 Exo 30	AMAZ 10-1 B7 A6 ICS 95
IV	16	Exo 4 Exo 8 Exo 9 Exo 10 Exo 11 Exo 12 Exo 13 Exo 14 Exo 16 Exo 17 Exo 22 Exo 29 Exo 32 Exo 34 Exo 38 Exo 39	BE 3 PINA B7 B2 PA 56 DOM 4 KER 2E R (10) (MEX) B7 B4 GDL 3 B5-7 DOM 25 EET 397 SCA 6 RB 33/3 IMC 54 IMC 14
V	3	Exo 15 Exo 35 Exo 36	UF 677 SPEC 160-9 EQX-3348-44
VI	2	Exo 33 Exo 37	PA 137 PUCALA 1
VII	2	Exo 19 Exo 28	CLM 90 IMC 16
VIII	1	Exo 31	IMC 67
IX	1	Exo 40	Criollo

Fig. 2. Dendrogram based on similarity coefficient among indigenous accessions of cocoa

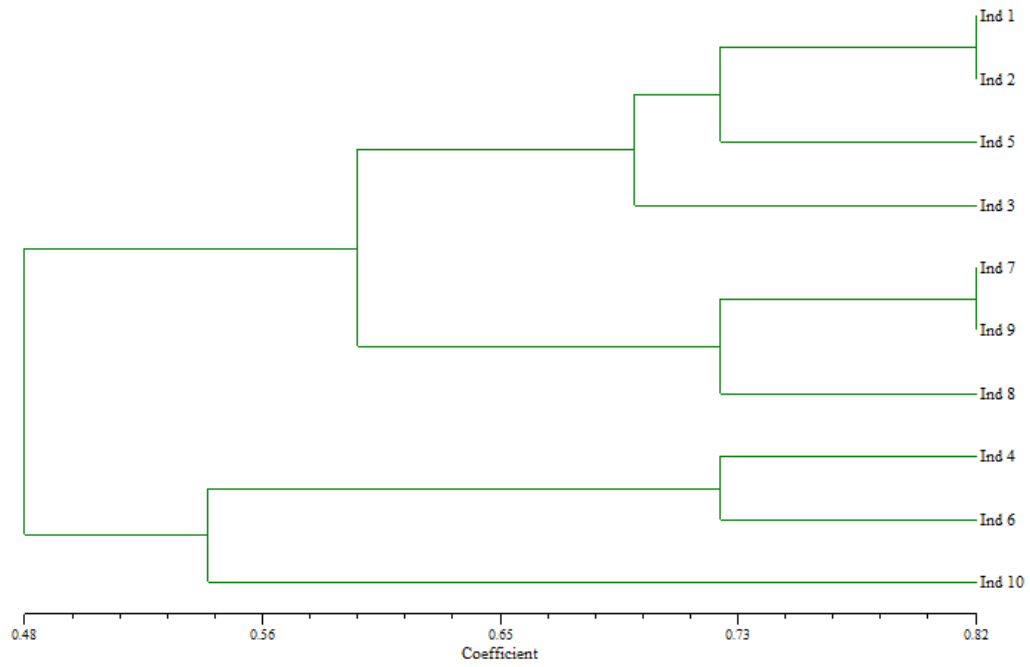


Table 7. Clustering based on qualitative characters of indigenous accessions of cocoa

Cluster No.	No. of accessions	Code of accessions	Name of accessions
I	3	Ind 1	Calicut Local 1
		Ind 2	Calicut Local 2
		Ind 5	Konni Local 3
II	1	Ind 3	Konni Local 1
III	3	Ind 7	Konni Local 5
		Ind 8	Thodupuzha Local 2
		Ind 9	Thodupuzha Local 1
IV	2	Ind 4	Konni Local 2
		Ind 6	Konni Local 4
V	1	Ind 10	Thodupuzha Local 3

It can also be seen from Table 7 that among the indigenous accessions from Konni, Konni local 2 and 4 fell in the same cluster indicating their similarity. However, Konni local 1, 3 and 5 were in different clusters indicating that they are distinct among themselves. Calicut local 1 and 2 were in the same cluster showing that they are similar in qualitative characters. Among the Thodupuzha accessions, Thodupuzha local 3 can be considered as distinct from the other two *viz.*, Thodupuzha local 1 and 2 at qualitative level.

4.1.2 Quantitative evaluation

Analysis of variance was carried out for each of the 26 quantitative characters observed in the 50 accessions of cocoa. All the characters except diameter of flower, width of sepal, width of petal, number of staminodes, number of stamen, length of ovary and width of ovary showed significant difference between the exotic and indigenous groups. Hence, further analysis was carried out separately for exotic and indigenous groups using the 19 characters showing significant variation.

The mean values of floral, pod and bean quantitative characters for exotic and indigenous accessions of cocoa are presented in Tables 8a, 8b, 9a, 9b, 10a and 10b respectively. Variations expressed by the accessions in terms of both floral and pod quantitative traits were very high. This reflects the heterogeneity expected within these accessions.

The significant floral quantitative characters *viz.*, pedicel length, sepal length, petal length, stamen length and style length ranged from 0.65cm to 1.85cm, 0.42cm to 0.83cm, 0.51cm to 0.96cm, 0.15cm to 0.23cm and 0.10cm to 0.32cm respectively among the exotic accessions (Table 8a). In the case of indigenous accessions the respective ranges were 1.12cm to 1.69cm, 0.53cm to 0.82cm, 0.58cm to 0.99cm, 0.15cm to 0.19cm and 0.17cm to 0.25cm (Table 8b). According to Mossu (1992), each flower is supported by a pedicel of 1 to 3 cm long. This report is not in agreement with the present observation (Table 8a).

Table 8a. Mean values of floral characters of exotic accessions of cocoa

Accessions	Pedicel length (cm)	Flower diameter (cm)	Sepal length (cm)	Sepal width (cm)	Petal length (cm)	Petal width (cm)	Staminode length (cm)	Stamen length (cm)	Ovary length (cm)	Ovary width (cm)	Style length (cm)
Exotic accessions											
SC 10	1.54	1.03	0.52	0.19	0.64	0.20	0.52	0.22	0.17	0.11	0.21
COCA 3370-3	1.19	1.03	0.63	0.17	0.70	0.18	0.46	0.15	0.16	0.11	0.20
AMAZ 10-1	1.35	1.04	0.57	0.18	0.63	0.19	0.57	0.23	0.12	0.12	0.18
BE 3	1.59	1.10	0.64	0.17	0.63	0.18	0.37	0.20	0.13	0.09	0.21
AMAZ 15	1.37	1.11	0.62	0.17	0.68	0.19	0.52	0.20	0.12	0.09	0.19
AMAZ 6-3	1.15	1.14	0.71	0.19	0.71	0.20	0.44	0.20	0.10	0.09	0.18
AMAZ 3-2	1.08	1.07	0.58	0.17	0.68	0.17	0.56	0.19	0.15	0.10	0.18
PINA	1.29	1.02	0.60	0.17	0.79	0.17	0.51	0.21	0.13	0.09	0.19
B7 B2	1.03	1.07	0.48	0.17	0.57	0.17	0.45	0.17	0.11	0.09	0.18
PA 56	1.26	0.94	0.62	0.16	0.69	0.16	0.51	0.19	0.12	0.10	0.20
DOM 4	1.47	1.21	0.70	0.19	0.72	0.16	0.55	0.20	0.19	0.10	0.20
KER 2 E	1.08	1.03	0.58	0.17	0.69	0.17	0.46	0.23	0.13	0.10	0.20
R (10) (MEX)	1.13	1.13	0.55	0.17	0.68	0.17	0.54	0.17	0.12	0.10	0.18
B7 B4	1.42	1.23	0.56	0.17	0.69	0.19	0.53	0.18	0.16	0.10	0.18
UF 677	1.49	0.95	0.65	0.19	0.76	0.19	0.57	0.15	0.17	0.10	0.20
GDL 3	0.83	1.09	0.53	0.17	0.65	0.13	0.36	0.18	0.08	0.09	0.12
B5-7	0.78	1.26	0.54	0.17	0.56	0.17	0.41	0.16	0.11	0.10	0.14
MAR 9	1.09	1.35	0.63	0.17	0.69	0.13	0.52	0.17	0.14	0.09	0.17
CLM 90	0.94	0.93	0.45	0.12	0.55	0.13	0.41	0.16	0.15	0.11	0.16
R (39) (MEX)	0.67	0.73	0.78	0.16	0.51	0.12	0.38	0.16	0.11	0.10	0.11
B7 B5	0.95	1.13	0.55	0.11	0.52	0.16	0.41	0.16	0.10	0.10	0.10
DOM 25	0.65	0.76	0.52	0.12	0.63	0.12	0.49	0.17	0.13	0.09	0.13

KER 9	0.73	0.89	0.62	0.11	0.63	0.13	0.44	0.17	0.12	0.10	0.17
LV 28	0.94	0.72	0.63	0.14	0.64	0.14	0.47	0.17	0.15	0.11	0.18
B7 A6	1.75	0.85	0.66	0.18	0.89	0.17	0.52	0.19	0.17	0.11	0.21
GU 310	1.07	0.62	0.52	0.17	0.71	0.17	0.50	0.19	0.12	0.10	0.18
EET 400	1.43	0.67	0.59	0.15	0.81	0.16	0.52	0.18	0.13	0.11	0.18
IMC 16	1.77	0.99	0.71	0.18	0.96	0.19	0.52	0.18	0.11	0.10	0.17
EET 397	1.63	0.89	0.69	0.17	0.83	0.17	0.53	0.21	0.15	0.10	0.32
ICS 95	1.85	1.13	0.83	0.18	0.89	0.16	0.47	0.16	0.18	0.10	0.19
IMC 67	1.76	1.19	0.66	0.22	0.92	0.16	0.49	0.21	0.16	0.09	0.21
SCA 6	1.70	1.33	0.65	0.17	0.72	0.16	0.51	0.21	0.19	0.12	0.19
PA 137	1.61	1.12	0.69	0.19	0.78	0.18	0.49	0.19	0.13	0.10	0.23
RB 33/3	1.67	1.23	0.55	0.14	0.83	0.16	0.53	0.18	0.13	0.10	0.22
SPEC 160-9	1.28	1.04	0.42	0.15	0.71	0.16	0.51	0.18	0.12	0.09	0.23
EQX-3348-44	0.69	0.69	0.50	0.17	0.58	0.13	0.50	0.19	0.14	0.09	0.16
PUCALA 1	1.68	1.26	0.67	0.17	0.73	0.18	0.51	0.20	0.15	0.11	0.20
IMC 54	1.50	1.08	0.56	0.17	0.74	0.18	0.53	0.21	0.15	0.11	0.18
IMC 14	1.35	0.67	0.55	0.17	0.76	0.18	0.48	0.16	0.12	0.10	0.21
Criollo	1.59	1.14	0.62	0.17	0.81	0.17	0.51	0.18	0.14	0.11	0.19
CD	0.11	NS	0.07	NS	0.05	NS	NS	0.02	NS	NS	0.05

Table 8b. Mean values of floral characters of indigenous accessions of cocoa

Accessions	Pedicle length (cm)	Flower diameter (cm)	Sepal length (cm)	Sepal width (cm)	Petal length (cm)	Petal width (cm)	Staminode length (cm)	Stamen length (cm)	Ovary length (cm)	Ovary width (cm)	Style length (cm)
Indigenous accessions											
Calicut local 1	1.68	1.24	0.82	0.18	0.88	0.16	0.53	0.18	0.12	0.10	0.25
Calicut local 2	1.69	1.24	0.67	0.18	0.80	0.17	0.49	0.17	0.13	0.11	0.25
Konni local 1	1.63	0.96	0.64	0.17	0.78	0.18	0.49	0.19	0.17	0.11	0.22
Konni local 2	1.54	1.15	0.76	0.16	0.99	0.18	0.55	0.19	0.15	0.11	0.21
Konni local 3	1.12	1.11	0.62	0.16	0.83	0.16	0.51	0.16	0.13	0.11	0.19
Konni local 4	1.45	1.10	0.67	0.16	0.82	0.16	0.49	0.18	0.11	0.11	0.22
Konni local 5	1.22	0.99	0.53	0.18	0.58	0.18	0.42	0.20	0.10	0.09	0.18
Thodupuzha local 1	1.44	0.91	0.67	0.17	0.93	0.14	0.50	0.15	0.13	0.09	0.19
Thodupuzha local 2	1.45	0.79	0.69	0.17	0.65	0.18	0.49	0.17	0.15	0.10	0.18
Thodupuzha local 3	1.41	0.97	0.68	0.18	0.84	0.14	0.49	0.15	0.19	0.12	0.17
CD	0.21	NS	0.24	NS	0.07	NS	NS	0.02	NS	NS	0.02

Among the exotic accessions, COCA 3370-3 was having the highest pod weight, pod length, pod breadth, ridge thickness and furrow thickness. However, its wet bean weight accounted for only 14.79 percent of the pod weight. This shows that the pod weight is not the indicator of the total wet bean weight. The observations also revealed that the thickness of the husk has a significant role in deciding the pod weight. The total wet bean weight was the lowest in the accession KER 2E (42.21g) which accounted for 13.25 percent of its pod weight (318.67g). The highest wet bean content was recorded by the accession R (39) (MEX) (33.82%) followed by R (10) (MEX) (33.26%) (Table 9a). Among the indigenous types, Konni local 2 was the best performer for these traits (Table 9b).

The characters of economic interest *viz.*, pod weight, number of beans/pod, dry weight of peeled bean, percentage of flat beans/pod and pod index ranged from 318.67g to 1268.33g, 22.93 to 49.27, 0.58g to 1.72g, 0.00% to 12.60% and 12 to 49 respectively among the exotic accessions. The corresponding traits in the case of indigenous accessions ranged from 416.67g to 719.33g, 31.60 to 46.27, 0.93g to 1.52g, 0.75% to 7.86% and 17 to 34 respectively. Pound (1932) and Enriquez and Soria (1966) revealed that yield expressed as dry or wet weight of bean is a highly variable character. However, the thickness of ridges and furrows in the pods are very descriptive characters. Their study has also shown that the dry weight of single bean varied from 0.5g to 2.5g. However, in the present study dry weight of single bean ranged from 0.58g to 1.72g (Table 10a). This can be due to the difference in the accessions used for the study.

The most interesting economic part of this crop is the beans. The bean size is one of the most important components of yield in cocoa (Soria, 1978). Morphological and structural characteristics of beans often exhibit large and high discriminatory variations within the species (Adewale *et al.*, 2010). Physical properties of the beans are important in the determination of their shapes (Balkaya and Odabas, 2002; Adewale *et al.*, 2010) and are very important as inferential factors or components in determining

Table 9a. Mean values of pod characters of exotic accessions of cocoa

Accessions	Pod length (cm)	Pod breadth (cm)	Ridge thickness (cm)	Furrow thickness (cm)	Pod wt. (g)	Total wet bean wt. (g)	Wet bean wt./ pod wt. (%)	TSS (%)
Exotic accessions								
SC 10	18.99	8.38	1.34	0.88	552.67	150.19	27.18	18.07
COCA 3370-3	23.63	10.47	1.97	1.78	1268.33	187.57	14.79	16.67
AMAZ 10-1	14.60	8.60	1.25	1.08	503.33	125.67	24.97	20.67
BE 3	17.01	9.20	1.54	1.22	531.33	132.20	24.88	18.67
AMAZ 15	18.71	8.47	1.38	1.08	576.67	150.22	26.05	16.84
AMAZ 6-3	17.97	8.35	1.31	1.10	520.00	110.90	21.33	17.59
AMAZ 3-2	17.93	7.95	1.30	1.13	483.33	124.46	25.75	16.60
PINA	18.41	8.54	1.23	1.06	541.67	146.59	27.06	19.00
B7 B2	16.55	7.88	1.31	0.93	392.67	135.47	34.50	19.91
PA 56	15.18	7.88	1.23	1.01	402.67	116.74	28.99	19.40
DOM 4	14.79	7.85	1.15	0.95	403.00	136.25	33.81	18.40
KER 2 E	11.93	7.53	1.37	1.08	318.67	42.21	13.25	18.07
R (10) (MEX)	18.93	7.05	1.09	0.86	396.00	131.70	33.26	18.34
B7 B4	16.84	8.78	1.15	0.97	496.00	114.57	23.10	20.53
UF 677	17.73	8.67	1.16	0.95	527.33	158.73	30.10	23.03
GDL 3	15.39	9.71	1.59	1.11	645.00	148.44	23.01	18.47
B5-7	16.79	9.29	2.15	1.74	788.67	98.99	12.55	21.53
MAR 9	16.27	9.20	1.67	1.34	621.33	129.99	20.92	17.93
CLM 90	16.53	8.55	1.13	0.99	580.33	115.09	19.83	19.40
R (39) (MEX)	16.56	7.67	1.09	0.95	400.00	135.26	33.82	18.20
B7 B5	14.23	7.63	1.37	0.95	389.33	87.32	22.43	18.83

DOM 25	14.80	8.38	1.25	1.07	504.33	124.48	24.68	18.97
KER 9	14.15	7.73	1.20	0.99	417.33	102.77	24.63	17.60
LV 28	15.65	8.77	1.28	1.03	532.33	128.97	24.23	19.31
B7 A6	13.77	7.02	1.09	0.85	322.00	95.98	29.81	20.73
GU 310	18.91	8.47	1.41	1.13	594.00	152.83	25.73	21.60
EET 400	18.98	9.47	1.63	1.23	797.67	206.08	25.84	17.27
IMC 16	16.10	8.17	1.45	1.19	795.33	149.22	18.76	20.34
EET 397	18.61	8.51	1.21	1.01	563.67	144.84	25.70	18.73
ICS 95	18.82	8.38	1.36	1.14	552.67	129.20	23.38	19.67
IMC 67	22.06	9.06	1.51	1.05	734.67	186.80	25.43	20.27
SCA 6	16.41	7.81	1.24	0.95	423.00	95.24	22.52	19.00
PA 137	15.41	9.03	1.15	0.95	436.33	99.77	22.87	22.67
RB 33/3	16.04	7.81	1.46	1.23	375.33	64.59	17.21	20.60
SPEC 160-9	18.53	8.95	1.15	0.99	534.67	152.01	28.43	19.60
EQX-3348-44	17.47	8.32	1.13	1.02	408.67	75.25	18.41	21.20
PUCALA 1	15.33	8.46	1.17	1.01	395.33	107.52	27.20	16.67
IMC 54	16.49	8.41	1.25	1.05	451.33	118.07	26.16	20.73
IMC 14	17.73	8.01	1.04	0.91	395.33	96.10	24.31	20.93
Criollo	18.32	7.98	1.32	0.85	474.67	119.21	25.11	26.53
CD	0.89	0.63	0.11	0.15	56.49	23.68	-	1.34

Table 9b. Mean values of pod characters of indigenous accessions of cocoa

Accessions	Pod length (cm)	Pod breadth (cm)	Ridge thickness (cm)	Furrow thickness (cm)	Pod wt. (g)	Total wet bean wt. (g)	Wet bean wt./ pod wt. (%)	TSS (%)
Indigenous accessions								
Calicut local 1	16.01	7.99	1.19	1.00	503.67	128.07	25.43	18.32
Calicut local 2	15.65	8.49	1.01	0.88	530.00	192.06	36.24	17.60
Konni local 1	16.53	8.4	1.28	1.04	531.33	113.66	21.39	16.74
Konni local 2	18.45	9.67	1.54	1.11	719.33	149.21	20.74	19.47
Konni local 3	16.54	7.98	1.28	1.12	476.00	108.53	22.80	20.47
Konni local 4	14.23	8.43	1.15	1.05	448.67	107.33	23.92	18.40
Konni local 5	16.87	8.71	1.29	1.05	562.00	146.84	26.13	17.87
Thodupuzha local 1	17.13	7.83	1.20	0.75	466.67	122.18	26.18	19.63
Thodupuzha local 2	17.77	7.80	1.35	1.11	495.00	109.68	22.16	20.93
Thodupuzha local 3	14.15	8.08	1.44	1.08	416.67	89.17	21.40	17.73
CD	1.07	0.62	0.21	0.08	55.37	18.43	-	1.29

yield (Omokhafa and Alike, 2004), protein and oil content (Kaushik *et al.*, 2007) in many crops. Some bean characters *viz.*, seed size and fat content are related to the quality of the cocoa beans (Monteiro *et al.*, 2009).

According to the international standards the dry weight of peeled bean must be 0.8g or more. Among the evaluated accessions Criollo, KER 9 and CLM 90 failed to satisfy this international standard. The unfertilized ovules are considered as developing into flat beans in cocoa. Presence of flat beans is an undesirable character in cocoa and the crop improvement programmes in cocoa aims to reduce the number of flat beans/pod. The flat bean content was the highest in accession LV 28 (12.20%) followed by AMAZ 6-3 (10.42%) (Table 10a). Flat beans were absent in the pods of accession EQX 3348-44 (Table 10a). Among the indigenous types flat bean content was the highest in Thodupuzha local 1 (7.86%) and the lowest in Calicut local 2 (0.75%) (Table 10b). Mora (1989) reported that flat beans were few and are located in the apical areas of pods of the accession EET 400. The results of the present study are in agreement with this report.

The pod index (PI) value indicates the number of pods required to produce 1kg of dry cocoa without testa (peeled bean) (Maharaj *et al.*, 2011). Low PI values are associated with high yield potential. Among the accessions evaluated R (10) (MEX) was found to be a desirable one with low PI value (12) and flat bean content/pod (0.81%) coupled with high number of beans/pod (49.20) and dry weight of peeled bean (1.68g). This was followed by EET 400 having a PI of 15 (Table 10a). The indigenous accession Konni local 4 also exhibited low PI value (17) (Table 10b). For breeding purposes, a PI value of less than 15 is very desirable (Pound, 1932). R (10) (MEX), EET 400, UF 677 and Konni local 4 were having the lowest PI values along with high dry weight of peeled bean. This result suggests that dry weight of peeled bean contributes more than number of beans per pod to the PI.

Table 10a. Mean values of bean characters of exotic accessions of cocoa

Accessions	Peeled bean (mm)			No. of beans/pod	Flat beans/pod (%)	Wt. of peeled bean (g)		Pod index value
	Length	Breadth	Thickness			Wet	Dry	
Exotic accessions								
SC 10	19.77	11.03	5.95	33.66	3.25	1.77	1.53	19
COCA 3370-3	19.30	9.14	6.77	30.66	4.16	1.75	1.37	24
AMAZ 10-1	16.96	8.02	4.10	39.00	2.18	1.23	0.83	31
BE 3	15.71	8.89	4.78	35.73	3.43	1.34	0.94	30
AMAZ 15	18.24	9.43	7.25	36.13	1.45	1.63	1.25	22
AMAZ 6-3	20.90	10.25	4.70	33.27	10.42	1.72	1.39	22
AMAZ 3-2	15.78	8.65	5.45	35.13	2.96	1.33	1.01	28
PINA	17.28	9.82	4.89	42.33	1.56	1.41	1.07	22
B7 B2	18.49	10.65	6.51	36.47	3.70	1.88	1.49	18
PA 56	16.56	9.55	3.95	38.13	4.68	1.29	0.89	30
DOM 4	15.56	9.01	4.88	24.07	0.82	1.39	0.92	45
KER 2 E	13.89	8.84	4.17	41.67	0.64	1.39	0.75	32
R(10) (MEX)	21.29	12.57	6.29	49.20	0.81	1.95	1.68	12
B7 B4	17.42	9.39	7.30	37.87	0.53	1.59	1.26	21
UF 677	20.82	11.95	4.61	42.80	1.09	1.76	1.35	17
GDL 3	17.43	10.49	4.96	29.60	0.67	1.54	1.18	29
B5-7	18.83	8.89	5.96	34.87	1.89	1.70	1.16	25
MAR 9	17.71	10.25	5.44	38.27	1.03	1.64	1.28	20
CLM 90	14.37	7.62	3.22	42.73	1.54	1.36	0.71	33
R (39) (MEX)	18.24	10.79	5.89	32.27	2.03	1.74	1.21	26
B7 B5	16.98	9.43	5.65	40.27	0.81	1.43	0.97	26

DOM 25	15.68	8.29	5.28	33.07	0.39	1.30	0.93	33
KER 9	15.72	7.84	3.90	38.53	1.71	1.34	0.71	37
LV 28	19.63	11.76	4.37	35.60	12.60	1.66	1.16	24
B7 A6	16.09	8.83	4.02	39.20	1.01	1.33	0.87	29
GU 310	19.02	9.76	4.29	37.93	1.74	1.55	1.18	22
EET 400	20.90	9.92	6.78	47.07	0.15	1.72	1.37	15
IMC 16	19.12	9.69	4.05	41.20	1.13	1.41	1.01	24
EET 397	17.22	9.69	5.36	36.93	0.73	1.53	1.03	26
ICS 95	18.68	11.44	5.56	40.80	0.49	1.67	1.35	18
IMC 67	17.18	9.18	4.15	36.80	1.42	1.43	1.10	25
SCA 6	18.34	7.63	4.77	37.20	1.92	1.35	0.81	33
PA 137	20.22	9.69	5.01	31.07	1.49	1.35	1.07	30
RB 33/3	21.36	9.82	3.20	39.80	0.50	1.41	1.07	24
SPEC 160-9	18.34	10.48	5.79	22.93	0.82	2.00	1.72	25
EQX-3348-44	18.48	9.09	3.36	44.80	0.00	1.39	0.88	25
PUCALA 1	15.51	9.76	3.81	48.27	0.14	1.53	1.03	20
IMC 54	17.81	8.02	4.36	49.27	0.14	1.33	0.73	28
IMC 14	18.12	8.89	2.85	49.07	0.41	1.45	1.13	18
Criollo	12.83	7.05	2.25	35.53	2.92	1.17	0.58	49
CD	1.45	0.77	0.56	5.97	0.65	0.14	0.13	5.41

Table 10b. Mean values of bean characters of indigenous accessions of cocoa

Accessions	Peeled bean (mm)			No. of beans/pod	Flat beans/pod (%)	Wt. of peeled bean (g)		Pod index value
	Length	Breadth	Thickness			Wet	Dry	
Indigenous accessions								
Calicut local 1	17.83	10.23	5.16	35.87	1.46	1.54	1.19	23
Calicut local 2	23.42	11.54	5.29	43.80	0.75	1.48	1.12	20
Konni local 1	18.35	9.92	4.48	40.27	3.20	1.39	1.07	23
Konni local 2	19.96	10.44	5.05	37.67	4.87	1.79	1.42	19
Konni local 3	16.35	9.79	4.98	36.80	1.08	1.35	1.03	26
Konni local 4	16.91	7.86	4.74	39.33	1.01	1.84	1.52	17
Konni local 5	20.44	10.69	5.64	40.07	3.84	1.62	1.34	19
Thodupuzha local 1	18.11	10.30	5.37	32.00	7.86	1.52	1.17	27
Thodupuzha local 2	15.29	8.29	5.23	46.27	3.04	1.45	0.93	23
Thodupuzha local 3	14.10	8.36	4.77	31.60	4.13	1.36	0.93	34
CD	1.28	0.81	0.52	3.17	1.23	0.11	0.14	4.32

Nair *et al.*, 1990, evaluated nine accessions of cocoa for yield and related characters and found that ICS clones are better performers with respect to number of pods/ plant and bean yield.

The descriptive statistics *viz.*, range (minimum & maximum), mean, standard deviation (SD), standard error (SE), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (H^2) and genetic gain (GG) of the 19 quantitative characters of exotic and indigenous accessions are presented in Table 10 and 11 respectively. Similar investigation was also done by Apshara and Nair (2001) using 44 accessions.

The phenotypic coefficient of variation of floral quantitative traits for exotic accessions ranged from 10.75 percent to 27.29 percent, for stamen and pedicel length respectively (Table 11). The variations observed among floral quantitative traits for indigenous accessions were of a lower magnitude (Table 12) ranging from 11.56 percent to 15.64 percent with respect to the five traits, pedicel length, sepal length, petal length, stamen length and style length. This further supports the use of floral traits for taxonomic studies as found by Engles (1981) and Bekele *et al.* (1994).

In the case of exotic accessions all the floral characters except length of style had high heritability (Table 11). However, for indigenous ones, high heritability was observed for all the floral characters (Table 12). This indicates the low influence of environment on these characters. Among the floral characters of exotic accessions length of pedicel showed high GCV (26.73%), heritability (95.93%) and GG (53.94%) indicating its scope for use in selection programmes (Table 11)

Among the pod and bean characters of exotic accessions evaluated, number of flat beans/pod exhibited maximum variability as indicated by its high PCV (131.68%) and GCV (121.68%). The high amount of variability coupled with high heritability (85.38%) of flat beans/ pod indicates the scope for selection based on this character. From this table, it is also clear that the selection based on this character can bring about

Table 11. Descriptive statistics of exotic accessions of cocoa

Character	Range		Mean	SD	SE	PCV (%)	GCV (%)	H ² (%)	GG (%)
	Minimum	Maximum							
Length of pedicel (cm)	0.65	1.85	1.285	0.35	0.054	27.29	26.73	95.93	53.94
Length of sepal (cm)	0.42	0.83	0.602	0.08	0.013	15.46	13.56	76.92	24.50
Length of petal (cm)	0.51	0.96	0.708	0.11	0.016	15.47	14.81	91.67	29.22
Length of stamen (cm)	0.15	0.23	0.186	0.02	0.003	10.75	9.31	75.00	16.61
Length of style (cm)	0.10	0.32	0.188	0.04	0.005	23.79	16.82	50.00	24.50
Weight of pod (g)	318.67	1268.33	526.17	171.25	27.077	27.28	32.32	81.93	46.04
Length of pod (cm)	11.93	23.63	16.963	2.19	0.346	13.17	12.77	94.05	25.51
Breadth of pod (cm)	7.02	10.47	8.41	0.69	0.193	9.11	7.86	74.60	13.99
Ridge thickness (cm)	1.04	2.15	1.328	0.23	0.036	18.03	17.23	91.28	33.90
Furrow thickness (cm)	0.85	1.78	1.071	0.19	0.030	19.14	17.72	85.71	33.79
Total wet wt. of bean (g)	42.21	206.08	125.88	31.33	4.95	13.43	24.69	86.47	23.92
No. of beans/pod	22.93	49.27	37.98	6.10	0.964	17.36	14.37	68.51	24.51
No. of flat beans/pod	0.00	5.13	0.792	0.98	0.154	131.68	121.68	85.38	231.61
Length of peeled bean (mm)	12.83	21.36	17.79	2.03	0.322	13.16	11.21	94.04	25.51
Breadth of peeled bean (mm)	7.05	12.57	9.537	1.22	0.193	13.43	12.49	86.47	23.93
Thickness of peeled bean (mm)	2.25	7.30	4.897	1.19	0.188	25.03	24.03	92.15	47.51
Wet wt. of peeled bean (g)	1.17	2.00	1.52	0.20	0.322	14.17	13.05	84.89	24.78
Dry wt. of peeled bean (g)	0.58	1.72	1.099	0.27	0.042	25.03	24.02	92.07	47.47
TSS (%)	16.60	26.53	19.214	1.97	0.311	9.34	8.30	78.97	15.19

*PCV & GCV (Sivasubramanian & Madhavamenon, 1973) - Low: Less than 10%, Moderate: 10-20%, High: More than 20%

*H² (Johnson *et al.*, 1955) - Low: Less than 30%, Moderate: 30-60%, High: More than 60%

*GG (Johnson *et al.*, 1955) - Low: Less than 10%, Moderate: 10-20%, High: More than 20%

Table 12. Descriptive statistics of indigenous accessions of cocoa

Character	Range		Mean	SD	SE	PCV (%)	GCV (%)	h ² (%)	GG (%)
	Minimum	Maximum							
Length of pedicel (cm)	1.12	1.69	1.463	0.18	0.058	14.45	11.77	66.42	19.77
Length of sepal (cm)	0.53	0.82	0.68	0.77	0.024	12.88	11.07	73.91	19.61
Length of petal (cm)	0.58	0.99	0.809	0.12	0.038	15.64	14.63	87.50	28.18
Length of stamen (cm)	0.15	0.19	0.173	0.02	0.005	11.56	10.01	75.00	17.86
Length of style (cm)	0.17	0.25	0.206	0.03	0.008	13.15	12.22	86.36	23.39
Weight of pod (g)	416.67	719.33	514.93	83.60	26.44	17.03	15.82	86.26	30.27
Length of pod (cm)	14.15	18.45	16.33	1.39	0.438	9.04	8.19	82.08	15.29
Breadth of pod (cm)	7.80	9.67	8.34	0.56	0.176	7.58	6.20	66.78	10.44
Ridge thickness (cm)	1.01	1.54	1.273	0.15	0.047	14.13	10.34	53.61	15.60
Furrow thickness (cm)	0.70	1.18	1.02	0.12	0.038	12.27	11.46	87.23	22.05
Total wet wt. of bean (g)	89.10	192.05	126.67	29.40	9.297	24.23	22.67	87.57	43.72
No. of beans/pod	31.60	46.27	38.36	4.65	1.471	12.75	11.80	85.57	22.48
No. of flat beans/pod	0.33	2.80	1.24	0.99	0.313	93.61	73.18	61.11	117.84
Length of peeled bean (mm)	14.10	23.42	18.07	2.70	0.854	15.33	14.76	92.64	29.26
Breadth of peeled bean (mm)	7.86	11.54	9.74	1.19	0.376	12.86	11.90	85.65	22.68
Thickness of peeled bean (mm)	4.48	5.64	5.071	0.34	0.107	8.35	5.73	47.03	8.09
Wet wt. of peeled bean (g)	1.35	1.84	1.53	0.17	0.536	11.53	10.78	87.23	20.74
Dry wt. of peeled bean (g)	0.93	1.52	1.099	0.20	0.063	57.36	56.85	98.24	116.07
TSS (%)	16.74	20.93	19.214	1.35	0.427	7.74	6.66	74.09	11.81

more than 200 percent improvement in the population (Table 11). All the pod and bean characters exhibited high heritability as per the classification proposed by Johnson *et al.* (1955).

In the case of indigenous types number of flat beans/pod as well as dry weight of single bean exhibited high PCV (93.61% & 57.36% respectively) and GCV (73.18% & 56.85% respectively) (Table 12). Selection based on these characters is expected to bring about more than 100 percent improvement in the population. The heritability values for all pod and bean quantitative characters were high (>60 %) except for thickness of single bean and ridge thickness. This indicates the very low influence of environment on these characters.

Both exotic and indigenous accessions showed high GCV (>20%), PCV (>20%), heritability (>60%) and GG (>20%) for most of the characters evaluated. Among the pod and bean quantitative characters studied in both exotic and indigenous groups, dry weight of single bean exhibited the highest heritability and number of flat beans/pod showed highest genetic gain. Hence, the selection based on these characters will be very effective in improving the population.

Glendinning (1963) also observed high heritability for number and size of beans in cocoa. High heritability for dry weight of single bean was reported by Kumaran and Amma (1981). The inheritance of pod size in cocoa was studied by Soria *et al.* (1974) and observed that the heritability for pod length was 55 percent. They also reported great variation in fruit characters like length, diameter, total weight and weight of husk. Similar results are obtained in the present study also.

4.1.2.1 Clustering based on quantitative characters

Cluster analysis was performed for exotic and indigenous accessions using 19 quantitative characters by D^2 statistics. Engles (1986) also carried out cluster analysis using 39 quantitative characters to group 294 cultivars.

Clustering of exotic accessions

The clustering of exotic accessions using D^2 statistics taking into consideration 19 quantitative characters resulted in seven clusters. The accessions included in each cluster are presented in Table 13. The accessions MAR 9, LV 28, B7 A6, ICS 95, PUCALA 1, IMC 54 and Criollo were found to fall in the same cluster. The cluster II included the highest number of accessions. The accessions BE 3, DOM 4, B7 B4, R (10) (MEX), EET 400, IMC 16, SCA 6 and SPEC 160-9 were falling in cluster III. Clusters IV and VI were having 5 accessions each. The accession B7 B 2 was clearly distinct from other exotic accessions as indicated by its position alone in cluster V.

The intra and inter cluster distances are presented in Table 14. The distances between different cluster centroids are represented in Fig. 3. The maximum inter cluster distance was observed between clusters I and V (33763.40) indicating that the accessions belonging to these clusters are genetically distinct and can be used in hybridization programme for getting maximum vigour. The minimum inter cluster distance was observed between clusters II and III (1309.67). This indicates the close genetic association of the accessions belonging to these clusters.

Clustering of indigenous accessions

Cluster analysis of indigenous accessions based on 19 quantitative characters revealed that the 10 indigenous accessions grouped into three different clusters. The accessions included in each cluster are presented in Table 15. The accessions Konni local 2, Thodupuzha local 1, 2 and 3 were present in cluster I. Calicut local 1 and Konni local 3 were found in cluster II. Cluster III included remaining four accessions.

The intra and inter cluster distances are presented in Table 16. The distances between different cluster centroids are represented in Fig. 4. The maximum inter cluster distance observed was between clusters I and II (148447.44) and the minimum was between clusters I and III (34369.20).

Table 13. Clustering based on quantitative characters of exotic varieties of cocoa

Cluster No.	No. of accessions	Code of accessions	Name of accessions
I	7	Exo 18 Exo 24 Exo 25 Exo 30 Exo 37 Exo 38 Exo 40	MAR 9 LV 28 B7 A6 ICS 95 PUCALA 1 IMC 54 Criollo
II	10	Exo 1 Exo 3 Exo 10 Exo 12 Exo 15 Exo 21 Exo 23 Exo 29 Exo 31 Exo 39	SC 10 AMAZ 10-1 PA 56 KER 2 E UF 677 B7 B5 KER 9 EET 397 IMC 67 IMC 14
III	8	Exo 4 Exo 11 Exo 13 Exo 14 Exo 27 Exo 28 Exo 32 Exo 35	BE 3 DOM 4 R (10) (MEX) B7 B4 EET 400 IMC 16 SCA 6 SPEC 160-9
IV	5	Exo 5 Exo 6 Exo 8 Exo 17 Exo 34	AMAZ 15 AMAZ 6-3 PINA B5-7 RB 33/3
V	1	Exo 9	B7 B2
VI	5	Exo 2 Exo 7 Exo 16 Exo 20 Exo 26	COCA 3370-3 AMAZ 3-2 GDL 3 R (39) (MEX) GU 310
VII	4	Exo 19 Exo 22 Exo 23 Exo 36	CLM 90 DOM 25 PA 137 EQX-3348-44

Table 14. Intra and inter cluster distances between clusters of exotic accessions of cocoa

Cluster	I	II	III	IV	V	VI	VII
I	732.55						
II	3043.63	502.02					
III	7252.66	1309.67	486.86				
IV	16436.79	1421.86	3980.55	501.18			
V	33763.40	5170.66	9765.47	2086.03	1602.19		
VI	1307.26	2875.44	1008.03	6831.68	14135.43	373.86	
VII	11453.94	10416.21	5701.57	17457.30	28305.06	2920.73	579.02

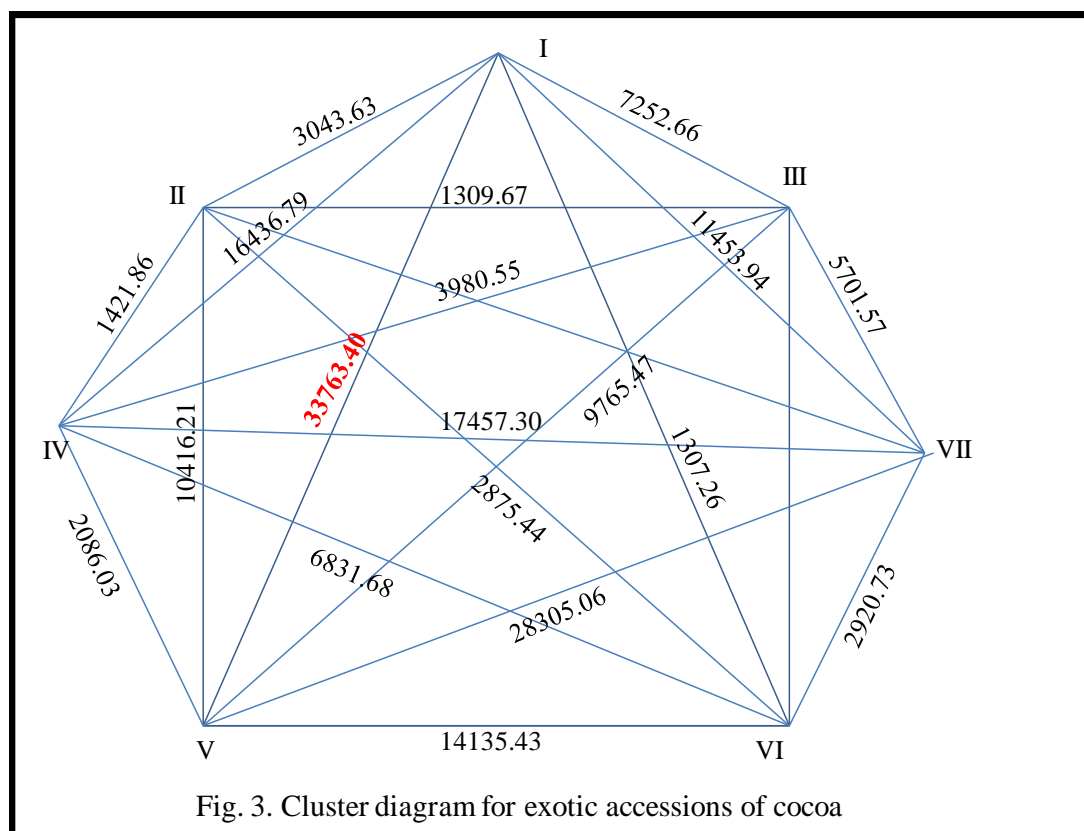
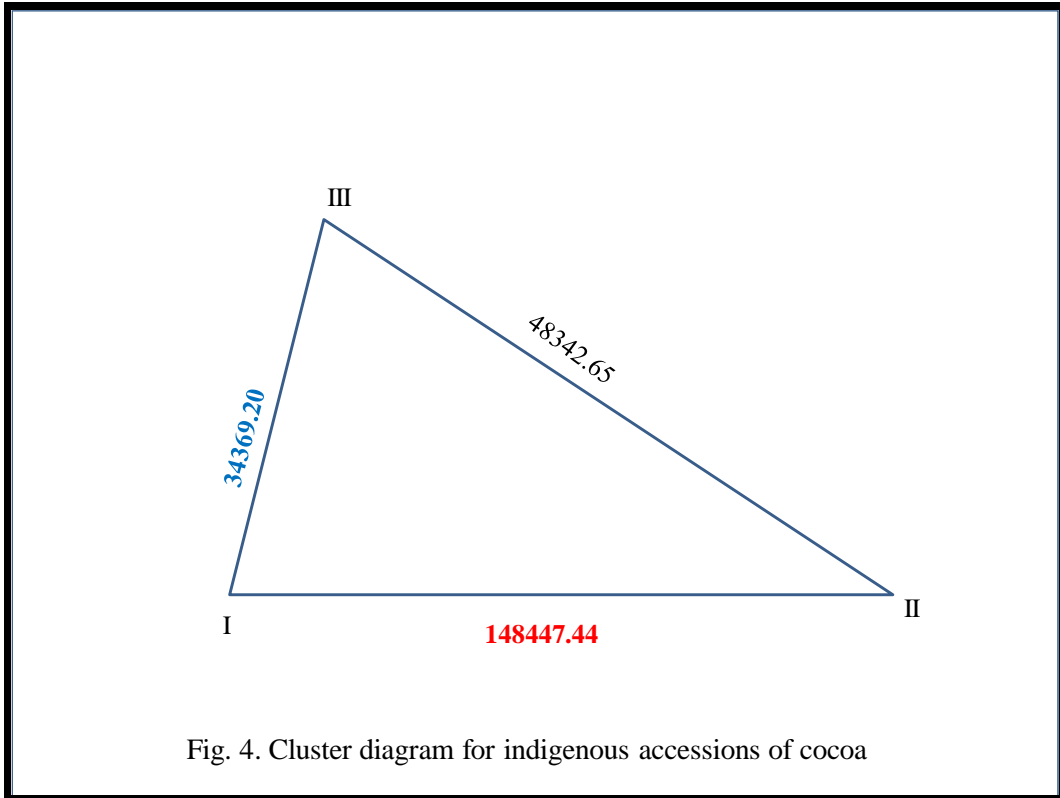


Table 15. Clustering based on quantitative characters of indigenous accessions of cocoa

Cluster No.	No. of accessions	Code of accessions	Name f accessions
I	4	Ind 4 Ind 8 Ind 9 Ind 10	Konni Local 2 Thodupuzha Local 1 Thodupuzha Local 2 Thodupuzha Local 3
II	2	Ind 1 Ind 5	Calicut Local 1 Konni Local 3
III	4	Ind 2 Ind 3 Ind 6 Ind 7	Calicut Local 2 Konni Local 1 Konni Local 4 Konni Local 5

Table 16. Intra and inter cluster distances between clusters of indigenous accessions of cocoa

Cluster	I	II	III
I	6189.66		
II	148447.44	2225.96	
III	34369.20	48342.65	7967.45



Unlike in the case of qualitative characters all the accessions from Thodupuzha were found to be included in the same cluster. This points to the fact that even though Thodupuzha local 3 is different from the other two in qualitative characters, they are identical at quantitative level. However, Konni local 1, 2 and 3 belonged to different clusters as in morphological clustering. This indicates that these accessions are qualitatively as well as quantitatively distinct.

4.2 Comparison of qualitative and quantitative clustering patterns

To find out the homology between the qualitative and quantitative clustering patterns, the distribution of accessions belonging to each qualitative cluster in different quantitative clusters was worked for exotic and indigenous groups and presented in Tables 17 and 18.

Exotic accessions

In the case of exotic accessions the analysis based on qualitative characters resulted in nine clusters and that based on quantitative characters resulted in seven clusters. The parallelism between the qualitative and quantitative clustering patterns is presented in Table 17. From Table 17 it can be seen that 50 percent of accessions of qualitative cluster I fell in quantitative cluster IV.

In the case of qualitative cluster II 25 percent of accessions each were distributed in quantitative clusters I and II. The remaining 12.5 and 37.5 percent of accessions were distributed in quantitative clusters III and IV respectively. Nearly 66.6 percent of the accessions of qualitative cluster III were found to be fall in quantitative cluster I. The 16 accessions in qualitative cluster IV were found to be distributed in seven different quantitative clusters, I to VII. In this case, maximum number (31.25%) of accessions fell in quantitative cluster III (Table 17). The accessions in qualitative cluster V were found to be equally distributed in the quantitative cluster II, III and VII. Similarly the accessions in qualitative clusters VI and VII were also found to be equally distributed in two different quantitative clusters.

Table 17. Homology between the qualitative and quantitative clustering patterns of exotic accessions of cocoa

Qualitative cluster	No. of accessions	Quantitative clusters						
		I	II	III	IV	V	VI	VII
I	4	-	25.00	-	50.00	-	25.00	-
II	8	25.00	25.00	12.50	-	-	37.50	-
III	3	66.66	33.33	-	-	-	-	-
IV	16	6.25	25.00	31.25	18.75	6.25	6.25	6.25
V	3	-	33.33	33.33	-	-	-	33.33
VI	2	50.00	-	-	-	-	-	50.00
VII	2	-	-	50.00	-	-	-	50.00
VIII	1	-	100.00	-	-	-	-	-
IX	1	100.00	-	-	-	-	-	-

Table 18. Homology between the qualitative and quantitative clustering patterns of indigenous accessions of cocoa

Qualitative cluster	No. of accessions	Quantitative clusters		
		I	II	III
I	3	-	66.66	33.33
II	1	-	-	100.00
III	3	66.66	-	33.33
IV	2	50.00	-	50.00
V	1	100.00	-	-

Indigenous accessions

The analysis based on qualitative characters of indigenous accessions resulted in five clusters and that based on quantitative characters resulted in three clusters. To find out the parallelism between these clustering patterns, the distribution of accessions of each qualitative cluster in different quantitative cluster was worked out and presented in Table 18.

From the Table 18 it can be clearly seen that 66.66 and 33.33 percent of accessions of qualitative cluster I were falling respectively into quantitative clusters II and III. Quantitative cluster I was having 66.66 and 50 percent of accessions of qualitative clusters III and IV.

The number of clusters formed based on qualitative characters and quantitative characters were different. In majority of cases accessions belonging to a single qualitative cluster were found to be falling in different quantitative clusters. This indicates that even though these accessions appear to be similar at qualitative level they are different at quantitative level.

4.3 Biochemical Characterization

Biochemical evaluation of accessions based on fat and total polyphenol contents was done following standard procedures.

4.3.1 Fat content

The Table 19 summarizes the fat contents of 50 accessions of cocoa. Significant difference was observed among the accessions based on the Analysis of Variance.

The average fat content among the 50 accessions was 51.14 percent, ranging from 40 percent (Criollo) to 60 percent (MAR 9, B7 B2 and Calicut local 2). The distribution of fat content within the accessions is shown in Fig. 5. The differences among the accessions were highly significant. These significant differences indicated

Table 19. Fat content in 50 accessions of cocoa

Name of accessions	Fat content (%)
SC 10	38
COCA 3370-3	57
AMAZ 10-1	58
BE 3	42
AMAZ 15	53
AMAZ 6-3	57
AMAZ 3-2	59
PINA	33
B 7B2	61
PA 56	42
DOM 4	21
KER 2 E	45
R (10) (MEX)	32
B 7 B4	37
UF 677	58
GDL 3	55
B5-7	46
MAR 9	60
CLM 90	44
R (39) (MEX)	54
B 7 B5	39
DOM 25	58
KER 9	40
LV 28	71
B7 A6	64
GU 310	73
EET 400	33
IMC 16	26
EET 397	20
ICS 95	44
IMC 67	49
SCA 6	40
PA 137	47

RB 33/3	42
SPEC 160-9	51
EQX-3348-44	70
PUCALA 1	36
IMC 54	42
IMC 14	38
Criollo	40
Calicut local 1	63
Calicut local 2	60
Konni local 1	73
Konni local 2	48
Konni local 3	57
Konni local 4	44
Konni local 5	26
Thodupuzha local 1	46
Thodupuzha local 2	42
Thodupuzha local 3	55
CD	1.99

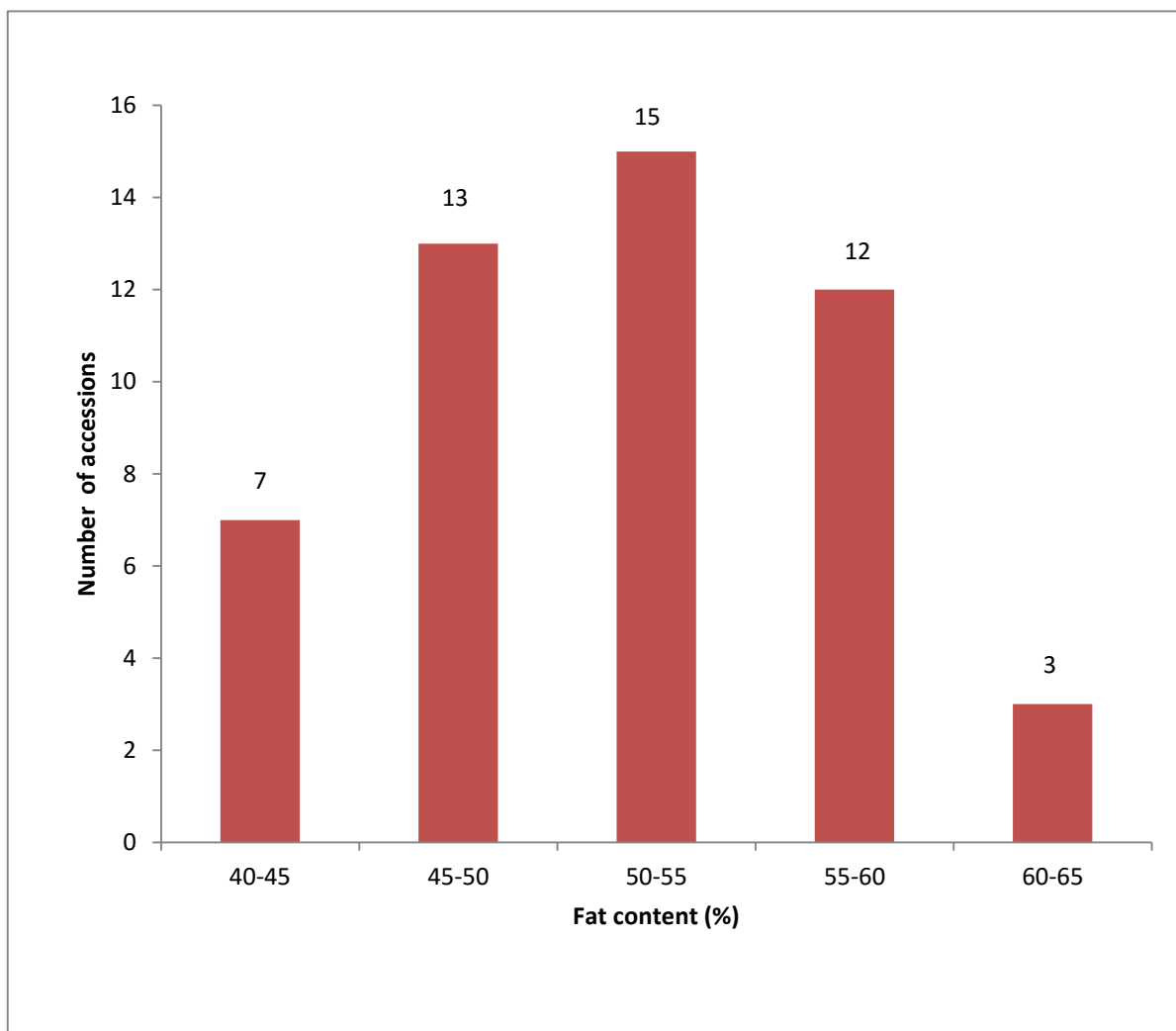


Fig. 5. Distribution of fat content in 50 accessions of cocoa

the availability of genetic variability for selection for high fat content

In the case of accessions from Konni, the fat content ranged from 44 percent to 57 percent. 30 percent of the accessions were having a fat content between 50-55 percent. Nearly 26 percent of the accessions each had a fat content between 45-50 percent and 55-60 percent (Fig. 6).

High fat content of cocoa beans is an important criterion to obtain the characteristic flavor and aromatic qualities of chocolate (Mossu, 1992). So the cocoa beans from the accessions like MAR 9, B7 B2 and Calicut local 2 with high fat content can be selected for making chocolates.

4.3.2 Polyphenol estimation

Total contents of polyphenolic compounds in the cocoa bean extracts of the selected accessions were determined following to the Folin-Ciocalteau procedure (Sadasivam and Manickam, 1996) and are presented in Table 20. Analysis of Variance showed highly significant difference among accessions. The distribution of total polyphenol content in 50 accessions of cocoa is shown in Fig. 6.

From the Table 20 it can be seen that IMC 54 had the lowest (2.25%) and B7 B 2 had the highest total polyphenol content (9.09%).

Polyphenols, comprising 12-18 percent of the whole bean weight, have long been associated with the flavor and colour of chocolate (Kim and Keeney, 1984). Nazaruddin *et al.* (2006) reported that the total polyphenols ranged from 34-60 mg/g in beans. In the present study total polyphenol content of the unfermented cocoa samples ranged from 22.55 mg/g to 90.85 mg/g.

Cocoa with too high polyphenols content is undesirable as this will impart bitterness and astringency to the final product and mask the characteristic chocolate flavor of chocolate. However, polyphenols could also contribute different level of

Table 20. Total polyphenol content in the beans of 50 accessions of cocoa

Name of accession	Total polyphenol content (%)
SC 10	4.26
COCA 3370-3	3.26
AMAZ 10-1	4.33
BE 3	5.56
AMAZ 15	6.89
AMAZ 6-3	7.16
AMAZ 3-2	3.82
PINA	7.11
B7B2	9.09
PA 56	4.19
DOM 4	5.16
KER 2 E	4.02
R (10) (MEX)	5.64
B 7 B4	5.77
UF 677	4.28
GDL 3	3.16
B5-7	7.19
MAR 9	2.84
CLM 90	6.66
R (39) (MEX)	3.26
B 7 B5	4.22
DOM 25	6.00
KER 9	4.69
LV 28	2.60
B7 A6	3.06
GU 310	4.19
EET 400	6.08
IMC 16	5.28
EET 397	4.50
ICS 95	2.39
IMC 67	4.93
SCA 6	5.05
PA 137	6.30

RB 33/3	7.22
SPEC 160-9	5.59
EQX-3348-44	6.59
PUCALA 1	2.69
IMC 54	2.25
IMC 14	4.84
Criollo	3.19
Calicut local 1	3.05
Calicut local 2	5.06
Konni local 1	5.59
Konni local 2	7.70
Konni local 3	3.49
Konni local 4	6.03
Konni local 5	4.85
Thodupuzha local 1	7.53
Thodupuzha local 2	6.89
Thodupuzha local 3	6.45
CD	0.05

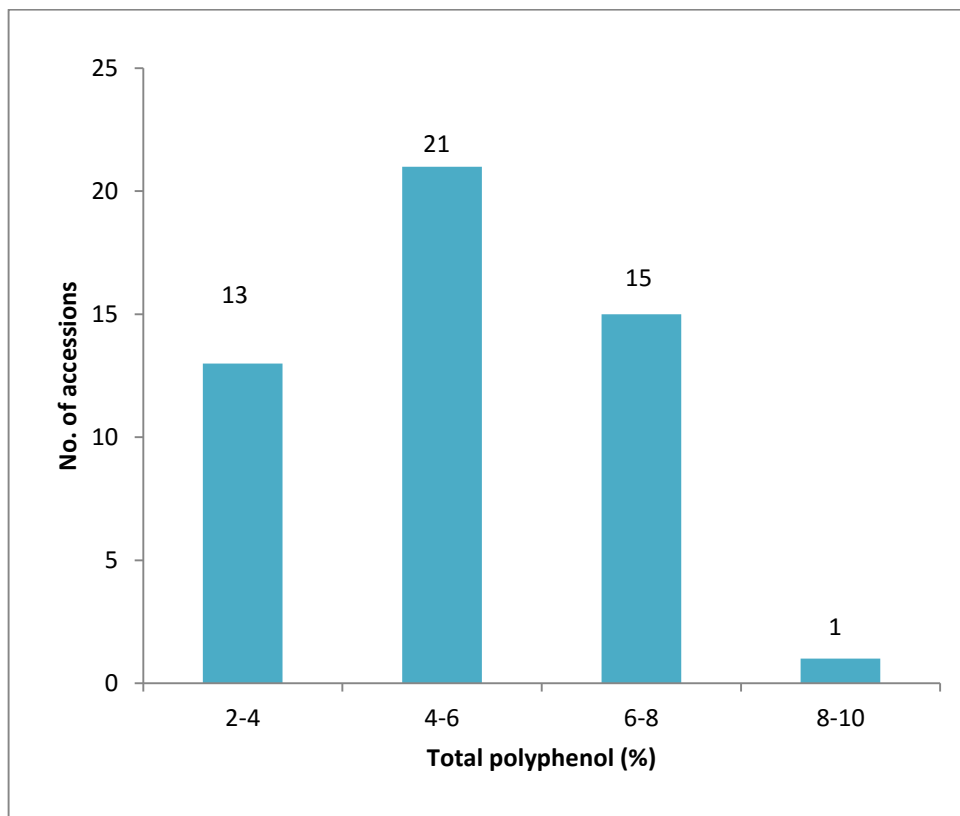


Fig. 6. Distribution of total polyphenol content in 50 accessions of cocoa

antioxidant property beneficial to human health depending on the content. Hence, the accessions with high polyphenol content can be selected for medicinal purposes.

Agglomerative hierarchical clustering was performed using the Jaccard's similarity co-efficient matrix by unweighted pair group method (Sneath & Sokal, 1973) and the resulting dendrogram is presented in Fig. 7. As depicted in the Fig. 7 it can be seen that the accessions are highly variable based on fat and total polyphenol content. Even at one percent similarity level, majority of accessions were found to be remaining as independent units.

4.4 Scoring for pests, diseases and rodents

Mealy bug and tea mosquito bug were the major pests and black pod was the major disease affecting the pods resulting in yield loss. Besides, rodents like rats and squirrels also caused damage to the pods. The percentage of infestation by each pest, disease and rodent are presented in Table 21.

Among the accessions evaluated eleven accessions *viz.*, PINA, B7 B2, PA 56, KER 2E, SCA 6, B7 A6, EQX-3348-44, IMC 54, Konni local 1, 2, 4 and Thodupuzha local 3 were seen infected by mealy bug (Table 21) (Plate 10). The percentage of infection was more in Konni local 2 (22.5%) followed by SCA 6 (20%).

The exotic accessions COCA 3370-3, BE 3, PINA, DOM 4, R (10) (MEX), B7 B4, UF 677, B5-7, GDL 3, CLM 90, DOM 25, R (39) (MEX), GU 310, EET 400, IMC 16, EET 397, IMC 67, PA 137, IMC 54, SPEC 160-9, Criollo and indigenous accessions except Konni local 4 and 5 and Thodupuzha local 3 were found to be free from tea mosquito attack (Table 21). The highest percentage of tea mosquito attack was observed in the accession ICS 95 (52.5%) (Plate 11). The estimates of crop loss due to *Helopeltis* (tea mosquito) are variable and depend upon factors such as agricultural practices, control methods, locality, climate and the varieties and insect species involved (Alagar and Subaharan, 2011).

Fig. 7. Dendrogram based on biochemical characters

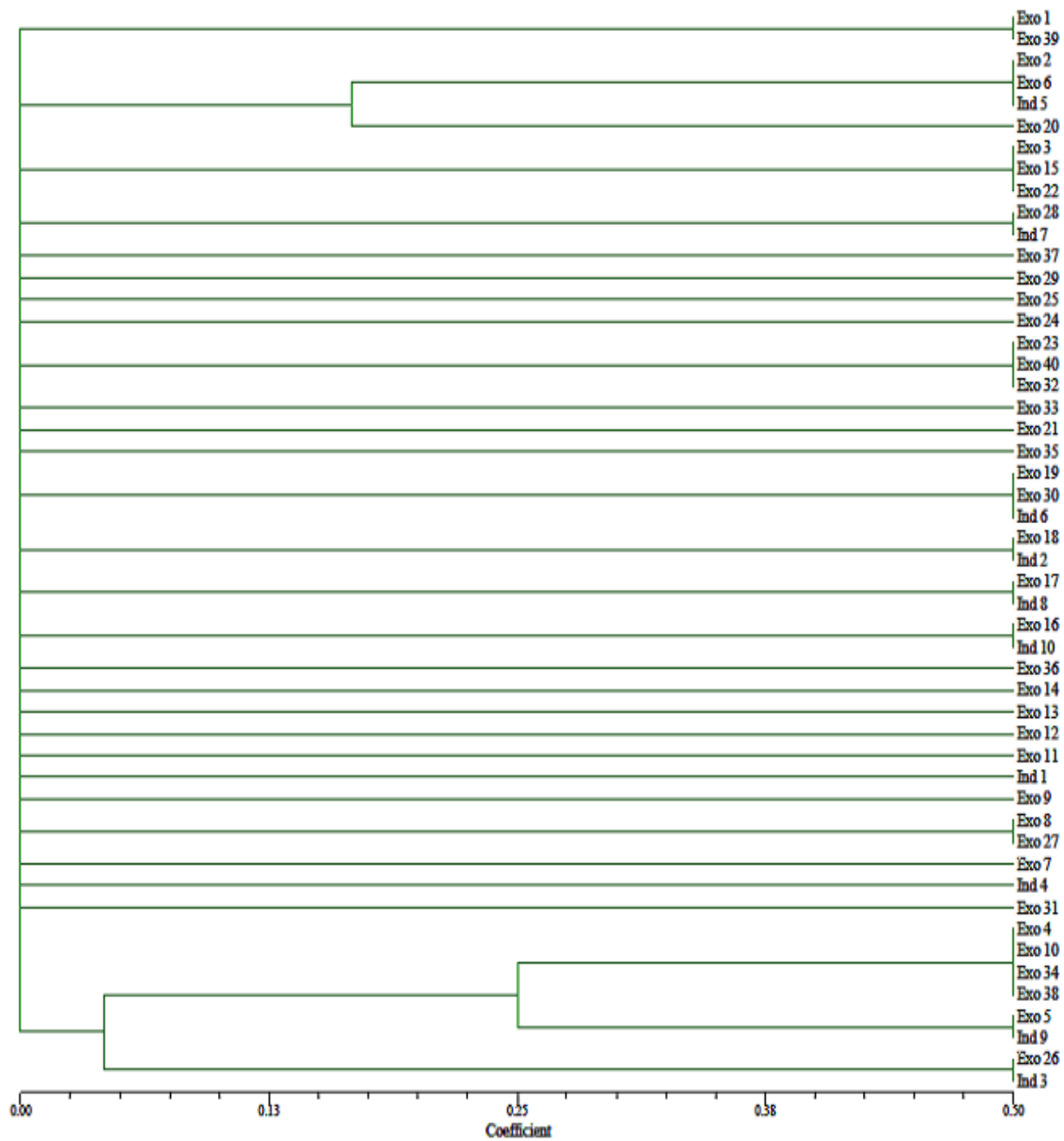


Table 21. Percentage of infestation

Accessions	Mealy bug (%)	Tea mosquito bug (%)	Black pod (%)	Squirrel (%)	Rat (%)	Caterpillar (%)
SC 10	-	30	25	-	-	-
COCA 3370-3	-	-	-	-	-	-
AMAZ 10-1	-	15	97.5	-	60	2.5
BE 3	-	-	-	-	2.5	-
AMAZ 15	-	25	35	-	7.5	-
AMAZ 6-3	-	7.5	17.5	-	15	-
AMAZ 3-2	-	2.5	50	-	7.5	-
PINA	5	-	95	2.5	2.5	2.5
B7 B2	2.5	7.5	57.5	10	65	-
PA 56	5	10	40	5	35	-
DOM 4	-	-	10	25	50	-
KER 2E	12.5	22.5	65	2.5	17.5	12.5
R (10) (MEX)	-	-	-	10	2.5	-
B7 B4	-	-	12.5	10	42.5	5
UF 677	-	-	2.5	2.5	55	-
GDL 3	-	-	5	12.5	22.5	-
B5-7	-	-	-	2.5	5	-
MAR 9	-	7.5	10	10	5	-
CLM 90	-	-	12.5	-	15	17.5
R (39) (MEX)	-	-	12.5	-	-	-
B7 B5	-	25	47.5	2.5	15	-
DOM 25	-	-	-	-	2.5	-
KER 9	-	7.5	30	-	20	-
LV 28	-	5	2.5	-	-	-
B7 A6	5	50	7	-	-	-
GU 310	-	-	25	7.5	25	-
EET 400	-	-	25	7.5	72.5	-
IMC 16	-	-	30	-	2.5	-
EET 397	-	-	10	-	7.5	-
ICS 95	-	52.5	10	-	7.5	-
IMC 67	-	-	15	-	37.5	-
SCA 6	20	7.5	32.5	-	37.5	-
PA 137	-	-	12.5	-	17.5	-
RB 33/3	-	2.5	22.5	2.5	10	-
SPEC 160-9	-	-	22.5	5	25	-

EQX-3348-44	2.5	5	22.5	5	7.5	-
PUCALA 1	-	7.5	20	-	27.5	-
IMC 54	5	-	12.5	10	12.5	-
IMC 14	-	5	25	-	12.5	-
Criollo	-	-	22.5	-	12.5	-
Calicut local 1	-	-	12.5	-	-	2.5
Calicut local 2	-	-	2.5	-	-	-
Konni local 1	2.5	-	35	-	32.5	-
Konni local 2	22.5	-	42.5	-	7.5	-
Konni local 3	-	-	42.5	-	15	-
Konni local 4	5	5	27.5	-	5	2.5
Konni local 5	-	10	25	-	52.5	-
Thodupuzha local 1	-	-	72.5	-	2.5	-
Thodupuzha local 2	-	-	57.5	-	15	-
Thodupuzha local 3	5	12.5	-	-	30	-

Black pod (pod rot) caused by *Phytophthora palmivora* was the major disease affecting almost all the selected accessions. The highest percentage of infection was observed on the accession AMAZ 10-1 (97.5%) (Plate 12). The incidence of black pod was absent in COCA 3370-3, BE 3, R (10) (MEX), B5-7, DOM 25 and Thodupuzha local 3. Padwick (1956) made an estimate of the loss caused by *Phytophthora*. Medeiros (1977) reported that nearly 30 percent of the world cocoa crop is lost due to this disease. Lawrence (1978) screened fifty-one cultivars for *P. palmivora* resistance and found that SCA 6 had promising degree of resistance. However, in the present study SCA 6 showed a percentage infestation of 32.5.

Rodents are the major pests of cocoa in almost all cocoa growing countries of the world (Everard, 1968). In this study, majority of accessions evaluated were susceptible to rat attack. The accession EET 400 showed the highest susceptibility (72.5%) to damage by rats. The two indigenous accessions, Calicut local 1 and 2 were free from rat attack. Squirrel attack was limited to accessions PINA, B7 B2, PA 56, DOM 4, KER 2E, R (10) (MEX), UF 677, B7 B4, GDL 3, B5-7, MAR 9, B7 B5, GU 310, EET 400, RB 33/3, SPEC 160-9, EQX-3348-44, IMC 54. All the indigenous accessions were observed to be resistant to squirrel attack. Bhat (1980) suggested that Indian squirrels (*Funambulus* spp.) tend to make oval holes centrally or terminally (Plate 13) and rats (*Rattus rattus*) round holes near the stalk (Plate 14). Most damage in the Ivory Coast has been shown to be caused by squirrels followed by rats (Bellier and Lefevre 1968). But in the present investigation more damage was caused by rats than squirrels.

In addition to these pests, disease and rodents, caterpillar attack was also observed in a few of the accessions viz., AMAZ 10-1, PINA, KER 2E, B7 B4, CLM 90, Calicut local 1, Konni local 4. This was identified as the caterpillar (cocoa bollworm) of green moth, *Earias biplaga*, and was found to devour the pericarp of unripe pods of cocoa (Plate 15). The accession CLM 90 was the most susceptible one (Table 21). In Ivory Coast, Decazy and Coulibaly (1982) studied the susceptibility of twelve clones of



Plate 10. Mealy bug attack



Plate 11. Tea mosquito bug attack



Plate 12. Black pod



Plate 13. Squirrel attack



Plate 15. Caterpillar attack



Plate 14. Rat attack

cocoa to *Earias biplaga* and noted that clone UF 677 as most infected. However, in the present study no infection was observed on accession UF 677.

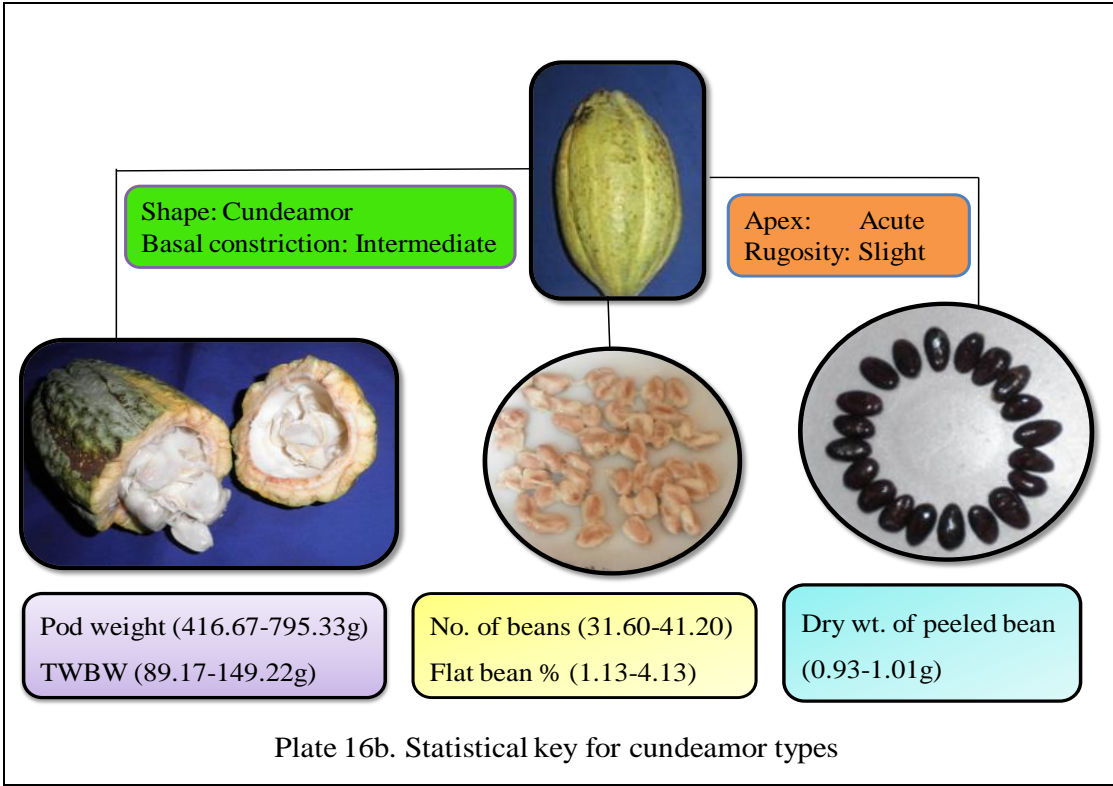
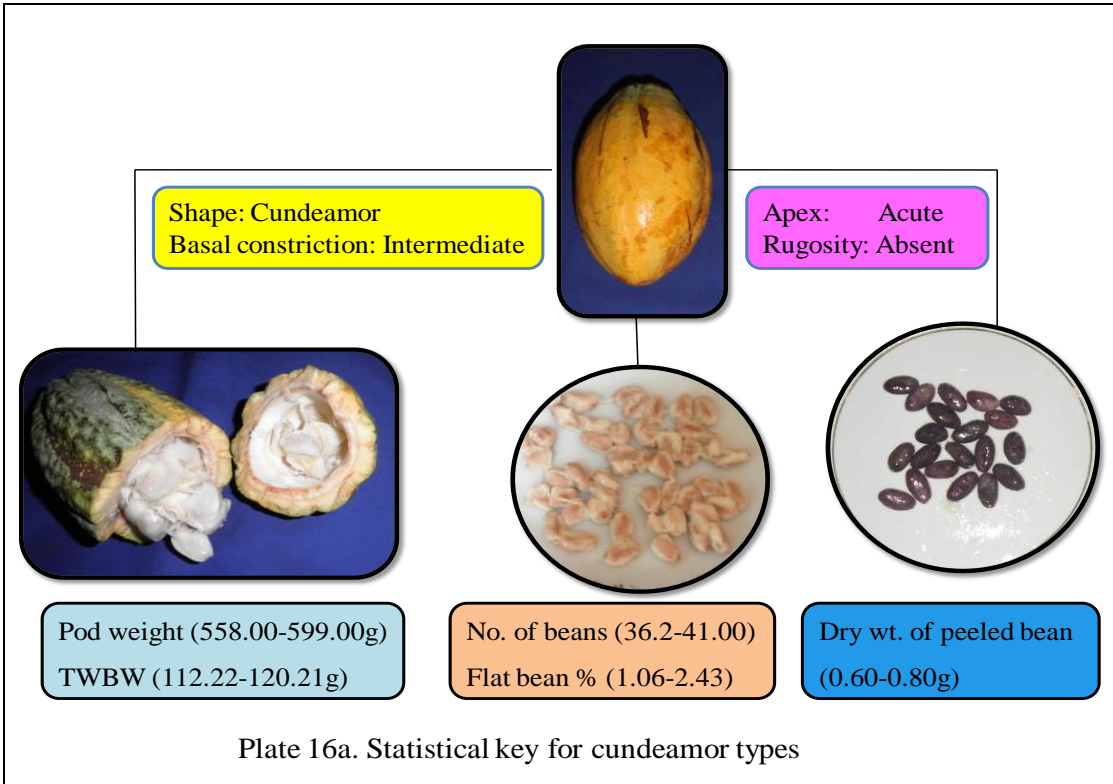
Among the accessions evaluated COCA 3370-3 having high husk thickness was found to be tolerant to the major pests, diseases and rodents affecting the pods of cocoa and can serve as donor parent in resistance breeding programme.

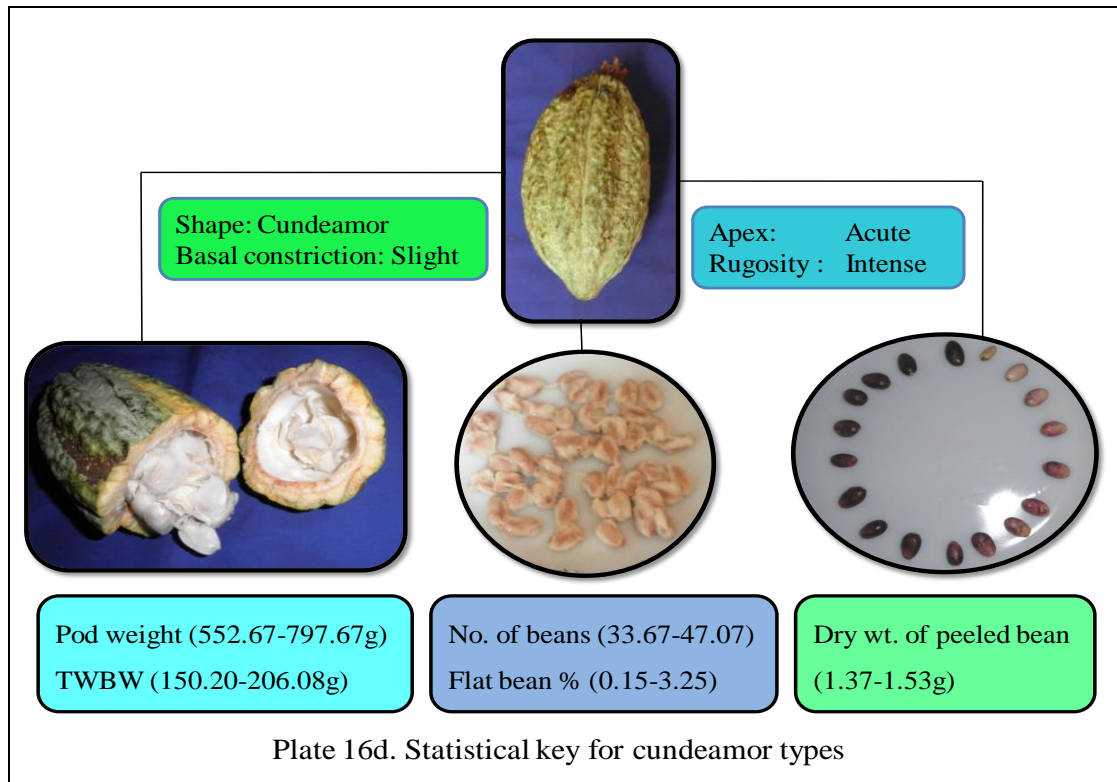
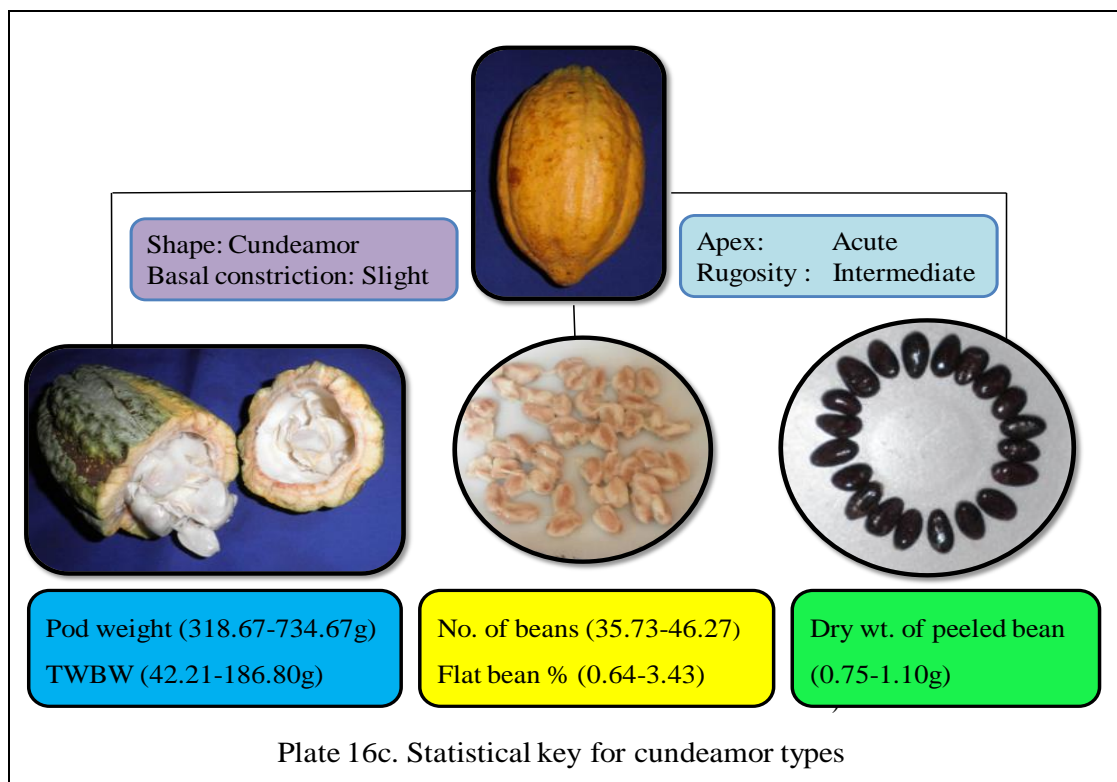
4.5 Statistical key

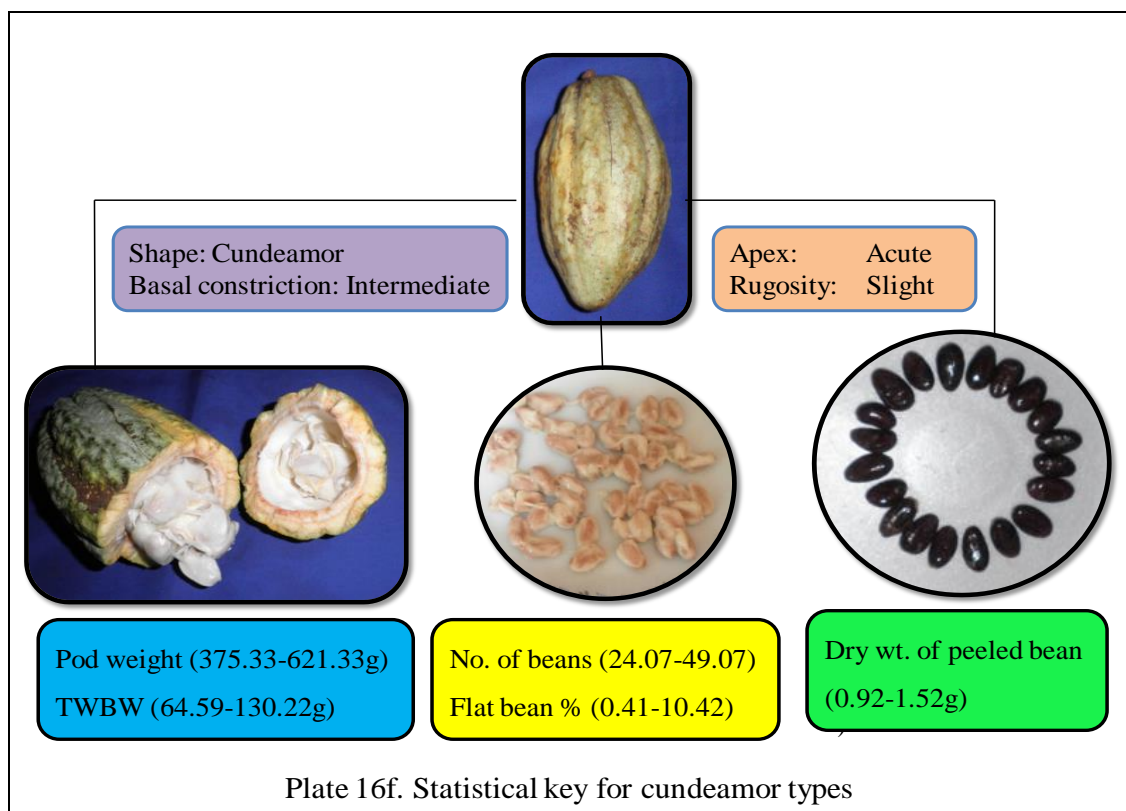
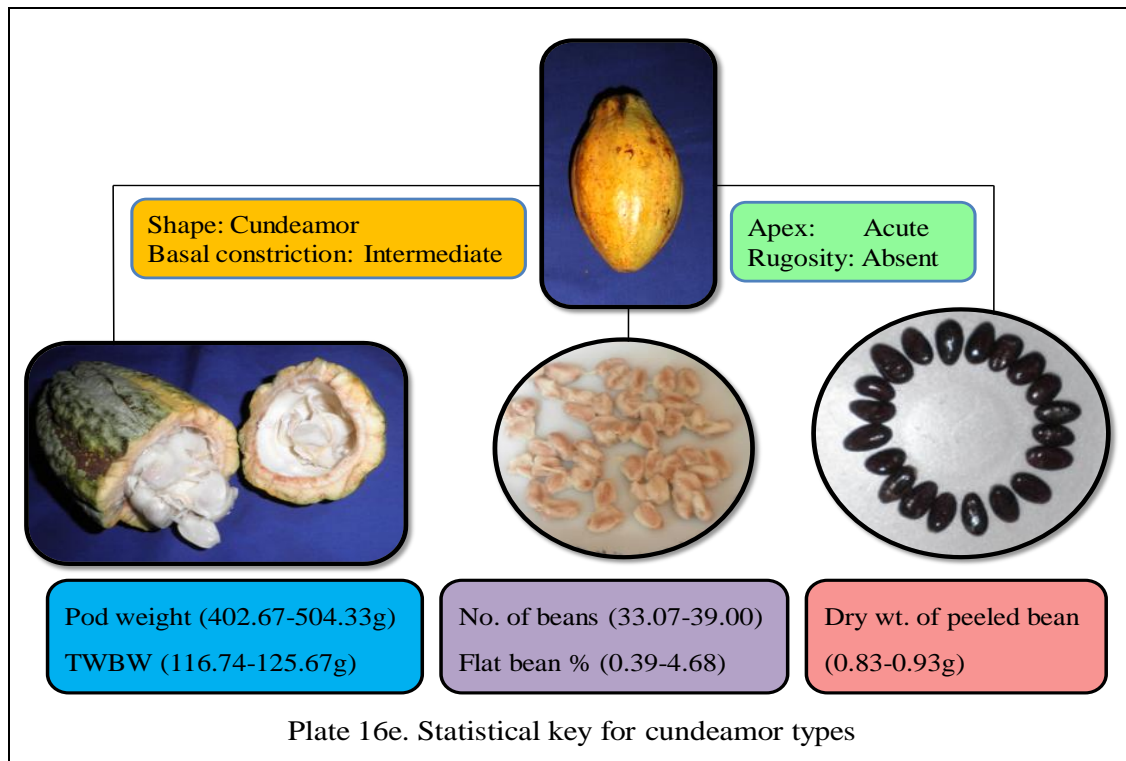
The key qualitative and quantitative characters were selected. Pod shape, pod apex, pod basal constriction and pod rugosity which were highly variable and easily identifiable were selected as key qualitative characters. The commercially important characters *viz.*, pod weight, total wet bean weight (TWBW), number of beans/pod, percentage of flat beans/pod and dry weight of peeled bean were identified as key quantitative characters. Using these key characters, statistical key was developed. If the combinations of key qualitative characters are known, it is possible to predict the range of key quantitative characters. For example, if the combination of key qualitative characters are cundeamor shaped pods with slight basal constriction, acute apex and intense rugosity, we can predict the approximate range for pod weight, total wet bean weight (TWBW), number of beans/pod, percentage of flat beans/pod and dry weight of peeled bean as shown in Plate 16a. The statistical key developed for different combinations of key qualitative characters in cundeamor types are presented in Plates 16a to 16i. Similarly the keys developed for angoleta types are presented in Plates 17a and 17b. Plates 18a and 18b shows the keys developed for Amelonado types. The key for criollo type is presented in Plate 19. Thus a statistical key can serve as a preliminary tool for predicting the performance of an accession of cocoa.

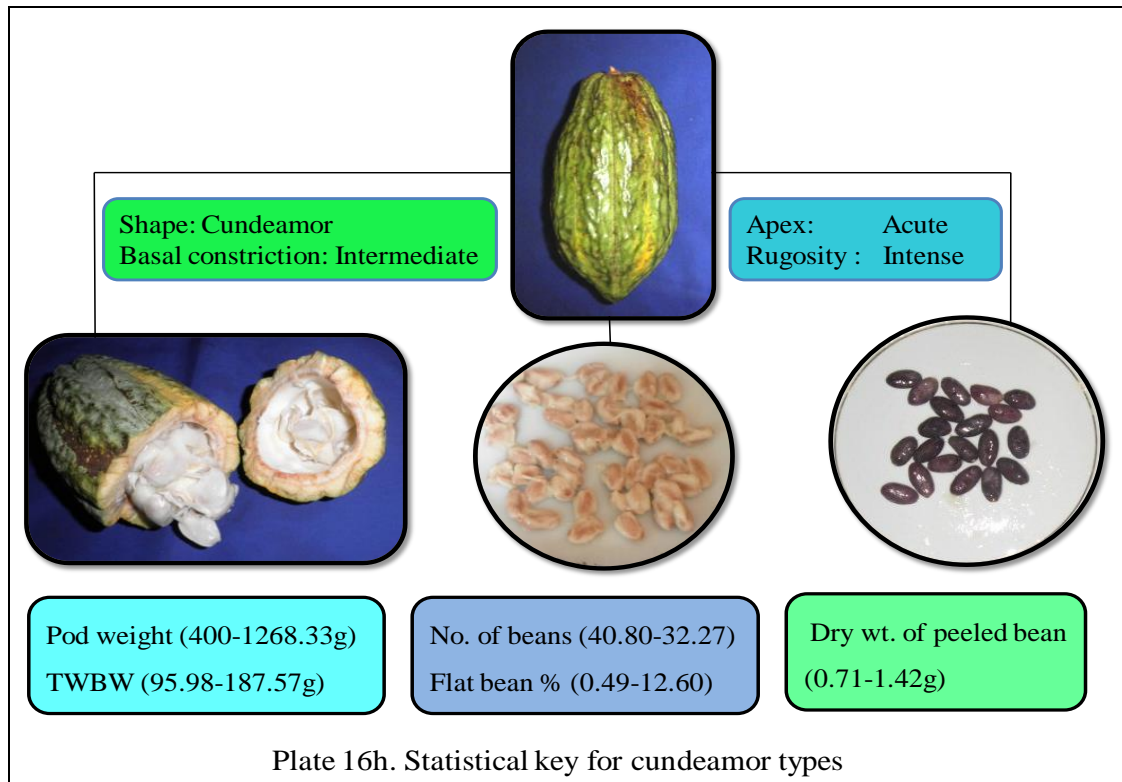
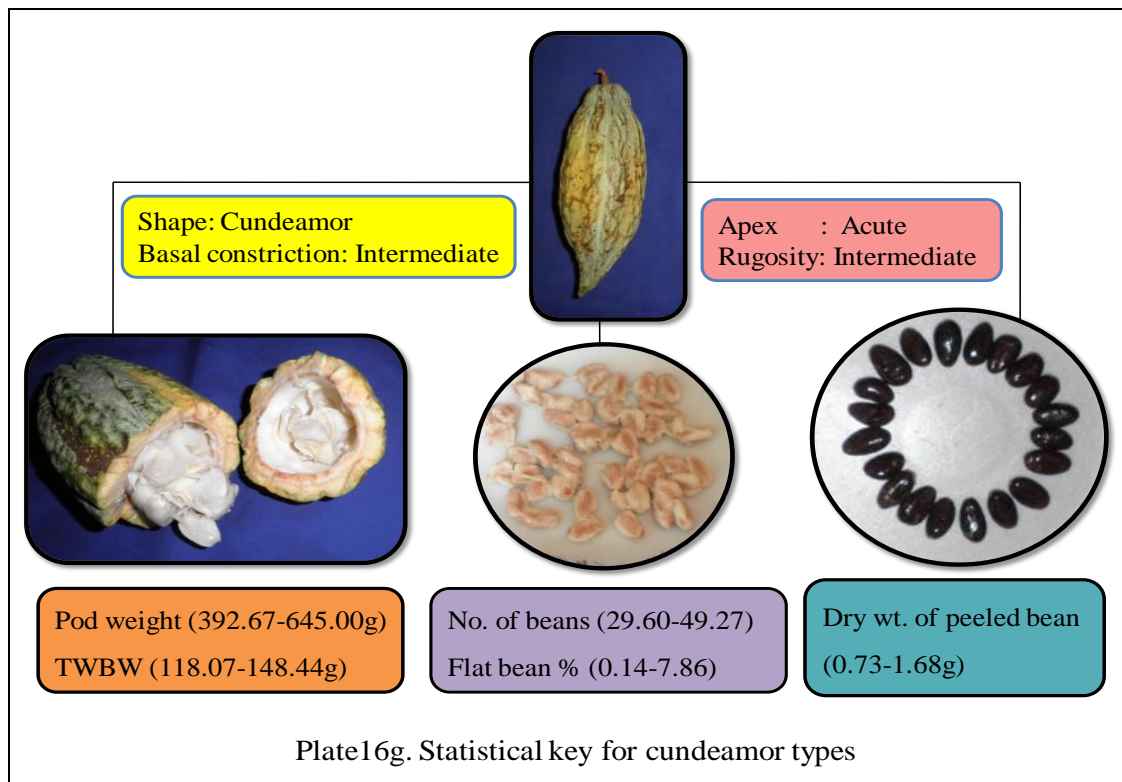
4.6 Future line of work

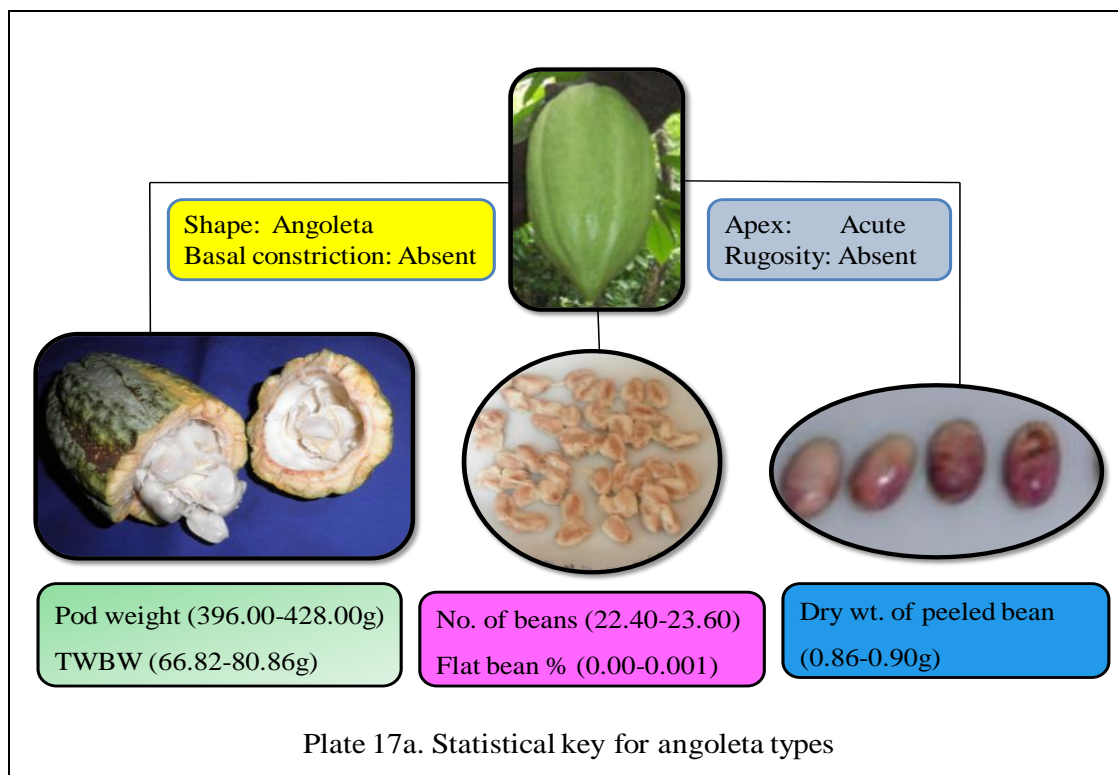
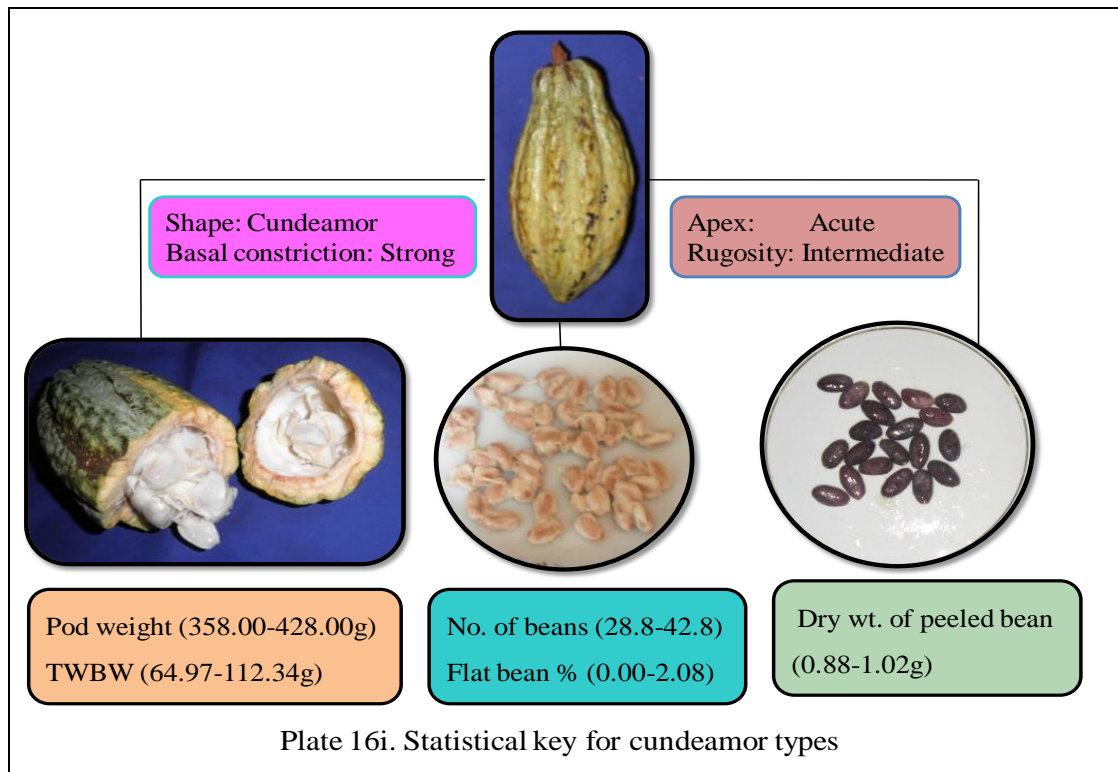
- I. In the present investigation, the characterization of 50 accessions was done based on morphological and biochemical markers alone. This can be further

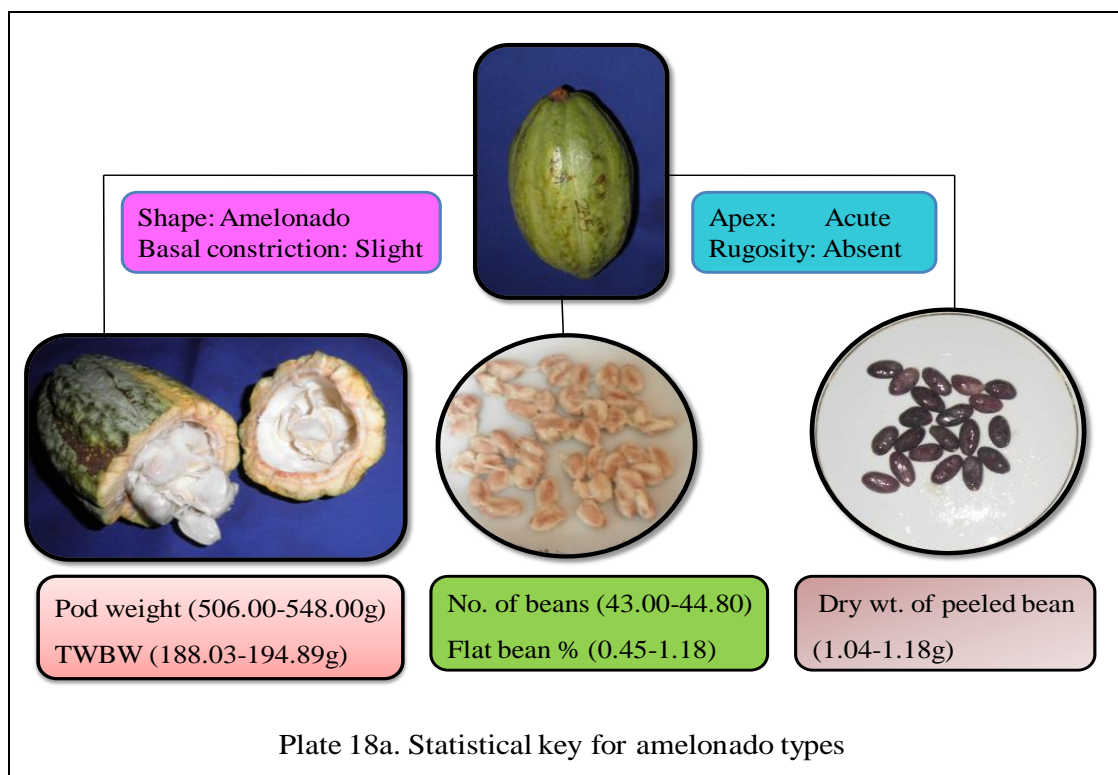
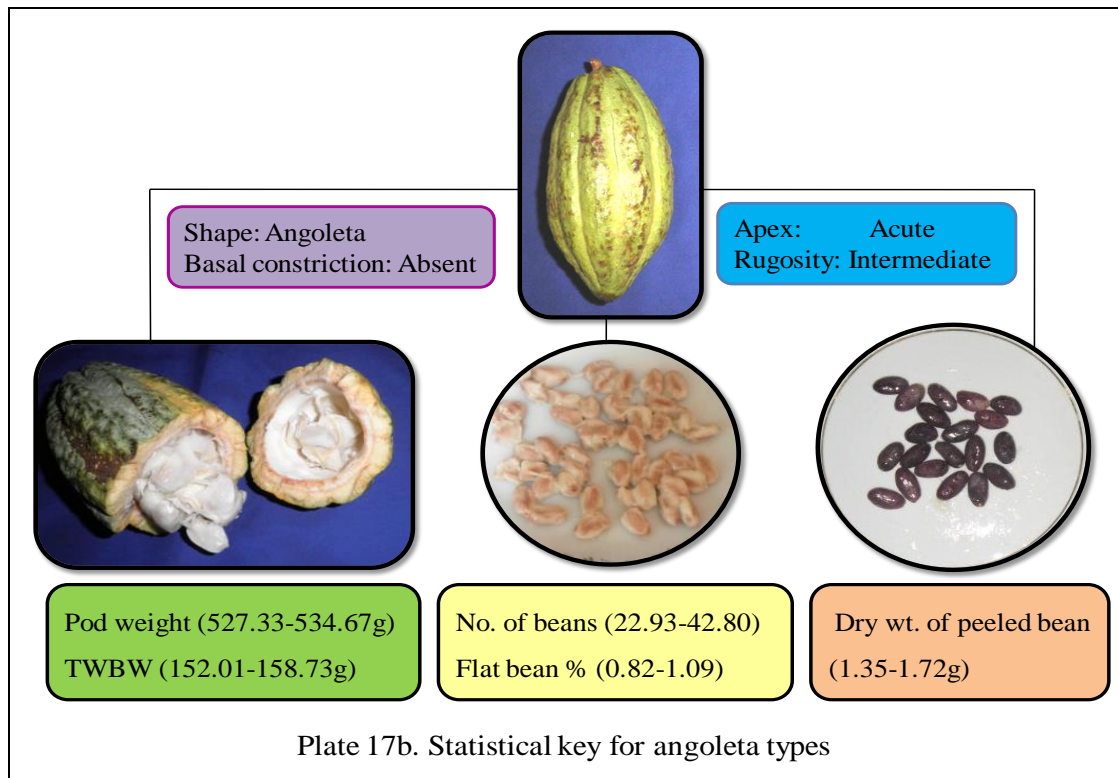


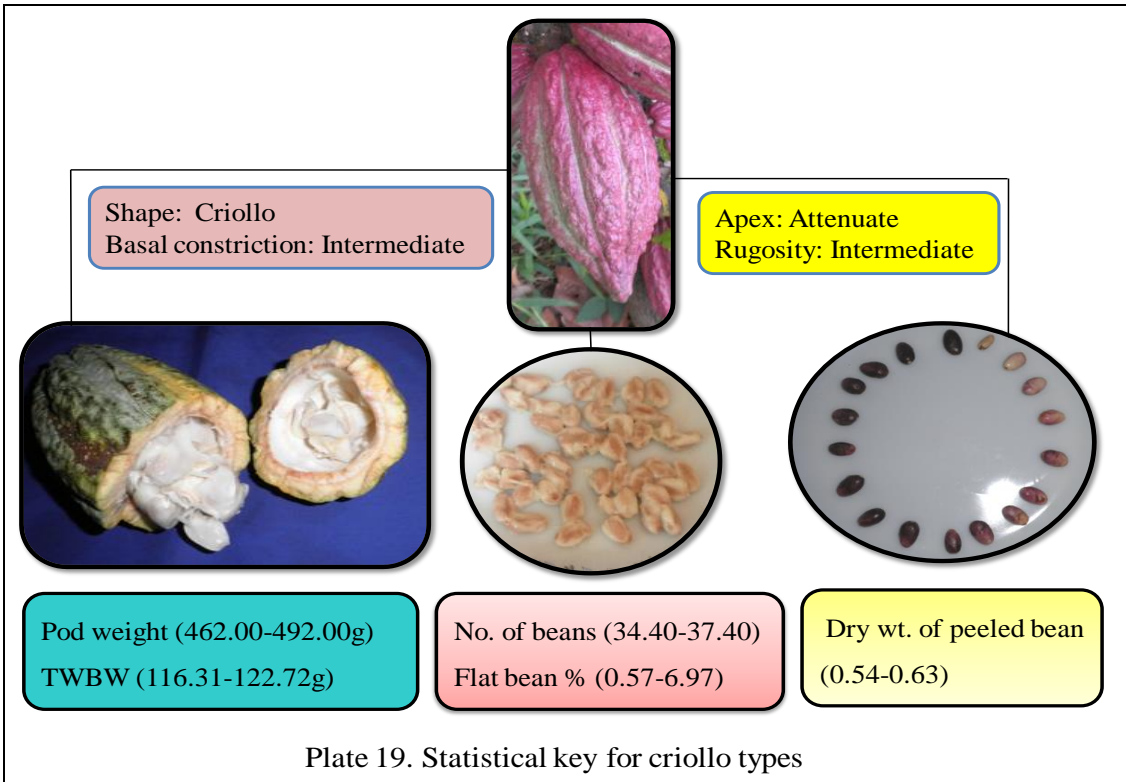
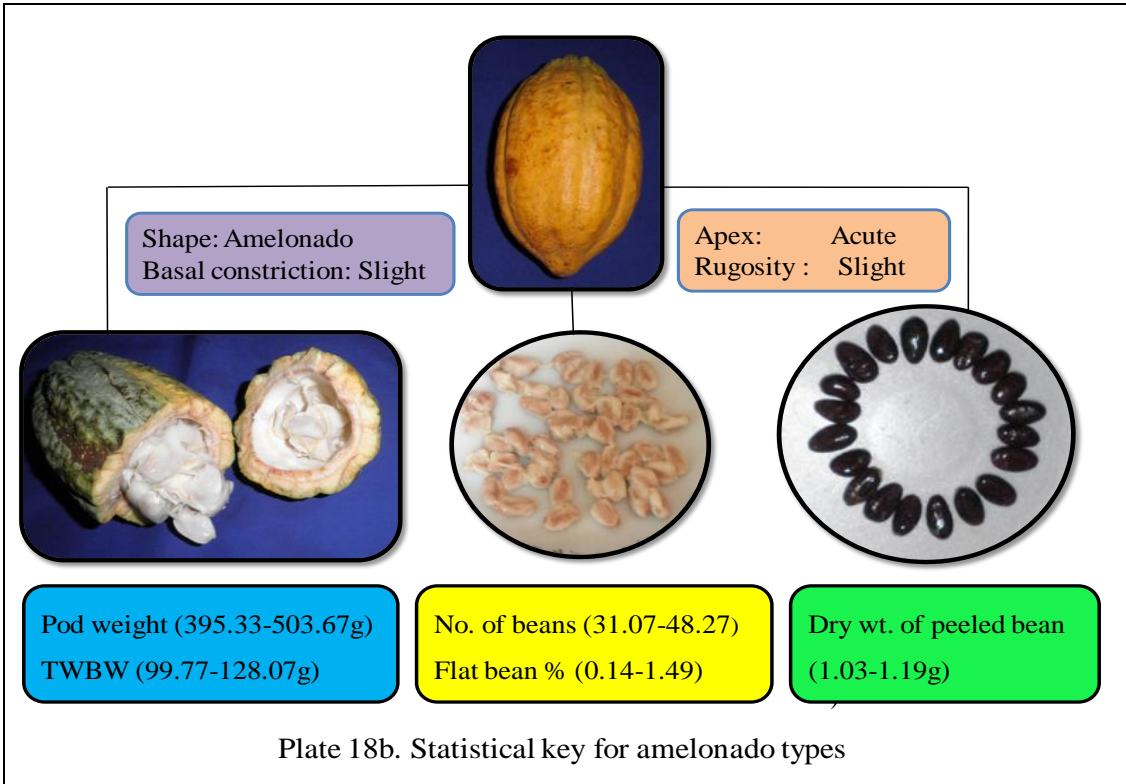












strengthened by the use of molecular markers. Molecular characterization allows the direct assessment of genetic variation at DNA level.

- II. Self and cross compatibility are common in cocoa. So the cross compatibility of the accessions belonging to the divergent clusters identified has to be undertaken.
- III. In the present study, total polyphenol contents of different accessions were estimated. Detailed study of the polyphenol profile of different accessions has to be taken up.
- IV. Flat beans in cocoa are considered to be developing from unfertilized ovules. Detailed study regarding the use of flat beans for the development of monoploids has to be taken up which will be useful in the production of inbred lines in this perennial crop.

Summary

5. SUMMARY

The study entitled, “Performance analysis of selected accessions of cocoa (*Theobroma cacao* L.)” was carried out in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during the period 2011-2013. The objectives of the study were to evaluate and characterize the selected accessions of cocoa using morphological and biochemical markers and to assess the genetic diversity among these accessions. Fifty clonal accessions of cocoa selected from the germplasm maintained by Cocoa Research Centre, Vellanikkara served as the material for the study. The selected accessions already in the full bearing stage included 40 exotic ones belonging to diverse countries of origin as well as ten indigenous ones. For morphological evaluation of these accessions 13 qualitative and 26 quantitative characters were considered. The descriptor list developed by Bekele and Butler (2000) was used for recording the observations.

Wide variability was observed among the accessions for eleven out of the 13 qualitative characters. The two characters *viz.*, colour of staminodes and hardness of husk were uniform in all the accessions and hence, were not included for further analysis. Reddish green coloured flush was present in majority of the accessions. However, in one accession, COCA 3370-3, alone the flush colour was green. Different combinations of pedicel, sepal, petal and stamen colours were observed in different accessions. However, in the case of exotic accession Criollo all these floral parts were reddish in colour. Pod colour, pod shape, pod basal constriction, pod rugosity and cotyledon colour also showed wide range of variability among the accessions. The unripe pods were observed to be green in majority of accessions except EET 397, Criollo, Konni local 5 and Thodupuzha local 3. Accessions COCA 3370-3, AMAZ 3-2, B7 B2, R (39) (MEX), MAR 9, KER 9, Konni local 3 and Thodupuzha local 1 were having cundeamor type pods with intermediate basal constriction and acute apex. The typical criollo type pods with intermediate constriction at the base and attenuate apex were observed in the exotic accession Criollo. In the present investigation the

accessions SC 10, GU 310, AMAZ 15, AMAZ 6-3, R (10) (MEX), Criollo and Konni local 4 exhibited the presence of mixture of beans having cotyledons ranging in colour from white to dark purple.

All the quantitative characters except diameter of flower, width of sepal, width of petal, number of staminodes, number of stamen, length of ovary and width of ovary showed significant difference between the exotic and indigenous groups. Hence, further analysis was carried out separately for exotic and indigenous groups using the 19 characters having significant difference.

The significant floral quantitative characters *viz.*, pedicel length, sepal length, petal length, stamen length and style length ranged from 0.65cm to 1.85cm, 0.42cm to 0.83cm, 0.51cm to 0.96cm, 0.15cm to 0.23cm and 0.10cm to 0.32cm respectively among the exotic accessions. In the case of indigenous accessions the respective ranges were 1.12cm to 1.69cm, 0.53cm to 0.82cm, 0.58cm to 0.99cm, 0.15cm to 0.19cm and 0.17cm to 0.25cm

Among the exotic accessions, COCA 3370-3 was having the highest pod weight, pod length, pod breadth, ridge thickness and furrow thickness. The total wet bean weight was the lowest in the accession KER 2E (42.21g). The highest wet bean content was recorded by the accession R (39) (MEX) (33.82%) followed by R (10) (MEX) (33.26%). Among the indigenous types, Konni local 2 was the best performer for these traits. The characters of economic interest *viz.*, pod weight, number of beans/pod, dry weight of peeled bean, percentage of flat beans/pod and pod index ranged from 318.67g to 1268.33g, 22.93 to 49.27, 0.58g to 1.72g, 0.00 to 12.60 percent and 12 to 49 respectively among the exotic accessions. The corresponding traits in the case of indigenous accessions ranged from 416.67g to 719.33g, 31.60 to 46.27, 0.93g to 1.52g, 0.75 to 7.86 percent and 17 to 34 respectively.

Among the evaluated accessions Criollo, KER 9 and CLM 90 failed to satisfy the international standard of 0.8g for the dry weight of peeled bean. The presence of flat

beans is considered as an undesirable character in cocoa. The flat bean content among the exotic accessions was the highest in LV 28 (12.20%) and was absent in EQX 3348-44. Among the indigenous types flat bean content was the highest in Thodupuzha local 1 (7.86%) and the lowest in Calicut local 2 (0.75%). Among the accessions evaluated R (10) (MEX) was found to be a desirable one with low pod index (PI) value (12) and flat bean content/pod (0.81%) coupled with high number of beans/pod (49.20) and dry weight of peeled bean (1.68g). This was followed by EET 400 having a PI of 15 (Table 10a). The indigenous accession Konni local 4 also exhibited a low PI value (17).

Both exotic and indigenous accessions showed high GCV, PCV, heritability and GG for most of the characters evaluated. In both exotic and indigenous groups, among the pod and bean quantitative characters studied, dry weight of single bean exhibited the highest heritability and number of flat beans/pod showed the highest genetic gain. Hence, the selection programmes based on these characters will be very effective in improving the populations.

The cluster analysis based on qualitative characters following the unweighted pair group method suggested by Sneath and Sokal, 1973, resulted in 9 clusters for exotic accessions and 5 clusters for indigenous ones at 70 percent similarity level. Clustering of the accessions using 19 quantitative characters following D^2 statistics developed by Mahalanobis (1936) resulted in five and three clusters respectively for exotic and indigenous groups. Among the exotic types, the maximum genetic divergence was observed among the accessions falling in quantitative cluster I and V as indicated by the highest inter cluster distance (33763.40). In the case of indigenous ones maximum divergence was found among the accessions falling in cluster I and II (148447.4).

The accessions belonging to same qualitative cluster were found to fall in different quantitative clusters indicating that even though they are similar at qualitative level they are different at quantitative level.

For biochemical evaluation fat and total polyphenol contents were estimated following procedures suggested by Sadasivam and Manickam (1996). The fat content ranged from 40 to 60 percent and total polyphenol content from 2.25 percent to 9.09 percent. Majority of accessions were remaining as independent units even at one percent level of similarity in the cluster analysis based on biochemical characters following the unweighted pair group method suggested by Sneath and Sokal, 1973.

Mealy bug and tea mosquito bug were the major pests and black pod was the major disease affecting the pods. Besides, rodents like rats and squirrels also caused damage to the pods. The accessions varied in their susceptibility to pests, diseases and rodents. Among the accessions evaluated, COCA 3370-3 was found to be tolerant to the major pests, diseases and rodents affecting the pods and can serve as donor parent in resistance breeding programme.

Statistical key was developed by identifying the key qualitative and quantitative characters. Pod shape, pod apex, pod basal constriction and pod rugosity which were highly variable and easily identifiable were selected as key qualitative characters. The commercially important characters *viz.*, pod weight, total wet bean weight (TWBW), number of beans/pod, percentage of flat beans/pod and dry weight of peeled bean were identified as key quantitative characters. Statistical keys were developed for different combinations of key qualitative characters which can serve as a preliminary tool for predicting the performance of an accession of cocoa.

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**PERFORMANCE ANALYSIS OF SELECTED
ACCESSIONS OF COCOA
(*Theobroma cacao* L.)**

By

ASNA A.C.

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ABSTRACT OF THE THESIS

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ABSTRACT

The spreading cultivation of cocoa as an intercrop in states like Kerala, Tamil Nadu, Andhra Pradesh and Karnataka necessitated the development of high yielding hybrids with pest and disease resistance and adaptability to various agro climatic zones. The success of any hybridization programme particularly in perennials depends on the proper selection of parental lines having genetic divergence.

The present study entitled “Performance analysis of selected accessions of cocoa (*Theobroma cacao* L.)” was taken up in this background at COH, Vellanikkara during 2011-13 to evaluate and characterize the selected cocoa accessions and to assess the genetic divergence among them. Fifty accessions of cocoa comprising of both exotic and indigenous ones which are in the full bearing stage and maintained at Cocoa Research Centre, Vellanikkara formed the material for the study. These accessions were subjected to morphological and biochemical evaluation. The morphological evaluation based on 13 qualitative and 25 quantitative characters was done using the descriptor developed by Bekele and Butler (2000). Fat and total polyphenol contents were estimated following standard procedures for biochemical characterization. The clustering of the accessions based on these characters was done by unweighted pair group method (Sneath & Sokal, 1973) and the genetic divergence was estimated by D^2 statistics developed by Mahalanobis (1936).

Wide variability was observed among the accessions for all the qualitative traits except colour of staminodes and hardness of husk. Variations expressed by the accessions in terms of both floral and pod quantitative traits were also high. Among the exotic accessions, COCA 3370-3 was having the highest pod weight, pod length, pod breadth, ridge thickness and furrow thickness. However, its wet bean weight accounted only for 14.79 percent of the pod weight. Among the indigenous types, Konni local 2 was the best performer for these traits.

The characters of economic interest *viz.*, pod weight, number of beans/pod, dry weight of peeled bean, percentage of flat beans/pod and pod index ranged from 318.67g to 1268.33g, 22.93 to 49.27, 0.58g to 1.72g, 0.00 to 12.60 percent and 12 to 49 respectively among the exotic accessions. The corresponding traits in the case of indigenous accessions ranged from 416.67g to 719.33g, 31.60 to 46.27, 0.93g to 1.52g, 0.75 to 7.86 percent and 17 to 34 respectively. Among the accessions evaluated R (10) (MEX) was found to be a desirable one with low pod index value (12) and flat bean content/pod (0.81%) coupled with high number of beans/pod (49.20) and dry weight of peeled bean (1.68g). This is followed by EET 400 having a pod index of 15. The exotic accessions Criollo, KER 9 and CLM 90 failed to satisfy the international standard for dry weight of peeled bean *viz.*, 0.8g or more. The fat content ranged from 40 percent to 60 percent and total polyphenol content from 2.25 percent to 9.09 percent.

The cluster analysis based on qualitative and quantitative characters resulted in nine and seven clusters respectively for exotic accessions and five and three clusters respectively for indigenous ones. The accessions belonging to same qualitative cluster were found to fall in different quantitative clusters indicating that even though they are similar at qualitative level they are different at quantitative level. With respect to biochemical characters majority of accessions were remaining as independent units even at one percent similarity level and hence could not be clustered.

Among the exotic types, the maximum genetic divergence was observed among the accessions falling in quantitative cluster I and V as indicated by the highest inter cluster distance (33763.40). In the case of indigenous ones maximum divergence was found among the accessions falling in cluster I and II (148447.4).

Mealy bug and tea mosquito bug were the major pests and black pod was the major disease affecting the pods resulting in yield loss. Besides, rodents like rats and squirrels also caused damage to the pods. Among the accessions evaluated, COCA 3370-3 having high husk thickness was found to be tolerant to the major pests

and diseases affecting the pods and can serve as donor parent in resistance breeding programme.

A statistical key was developed using key qualitative and quantitative characters which can serve as a preliminary tool for predicting the performance of the accessions.