

173934

EVALUATION OF SELECTED COCOA (*Theobroma cacao* L.) HYBRIDS BRED FOR QUALITY

By

AJMAL P.M.

(2014-12-135)



THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Horticulture

(PLANTATION CROPS AND SPICES)

Faculty of Agriculture

Kerala Agricultural University, Thrissur



DEPARTMENT OF PLANTATION CROPS AND SPICES

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680656

KERALA, INDIA


2016

DECLARATION

I hereby declare that this thesis entitled “**Evaluation of selected cocoa (*Theobroma cacao* L.) hybrids bred for quality**” 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.

Vellanikkara

Date: 25.10.2016



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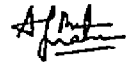
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Vellanikkara

Date: 25.10.2016



Ajmal P.M.

2014-12-135

CERTIFICATE

Certified that this thesis entitled “**Evaluation of selected cocoa (*Theobroma cacao* L.) hybrids bred for quality**” is a record of research work done independently by **Ajmal P.M. (2014-12-135)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.



Vellanikkara

Date: 25.10.16

Dr. B. Suma

Chairman, Advisory Committee

Professor

Dept. of Plantation Crops and Spices

College of Horticulture,

Vellanikkara

CERTIFICATE

We, the undersigned members of the advisory committee of **Mr. Ajmal P.M.** (2014-12-135) a candidate for the degree of **Master of Science in Horticulture** with major field in **Plantation Crops and Spices**, agree that this thesis entitled **“Evaluation of selected cocoa (*Theobroma cacao* L.) hybrids bred for quality”** may be submitted by **Mr. Ajmal P.M.**, in partial fulfilment of the requirement for the degree.



Dr. B. Suma

(Chairman, Advisory Committee)

Professor

Dept. of Plantation Crops and Spices

College of Horticulture, Vellanikkara



Dr. V. S. Sujatha

(Member, Advisory Committee)

Professor and Head

Dept. of Plantation Crops and Spices

College of Horticulture, Vellanikkara



Dr. Minimol J. S.

(Member, Advisory Committee)

Assistant Professor

Plant Breeding and Genetics

Cocoa Research Centre, Vellanikkara



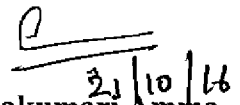
Smt. Meagle Joseph

(Member, Advisory Committee)

Associate professor

Dept. of Processing Technology

College of Horticulture, Vellanikkara



Dr. S. Prasannakumari Amma

(External Examiner)

Professor (Retd.)

College of Horticulture, Vellanikkara

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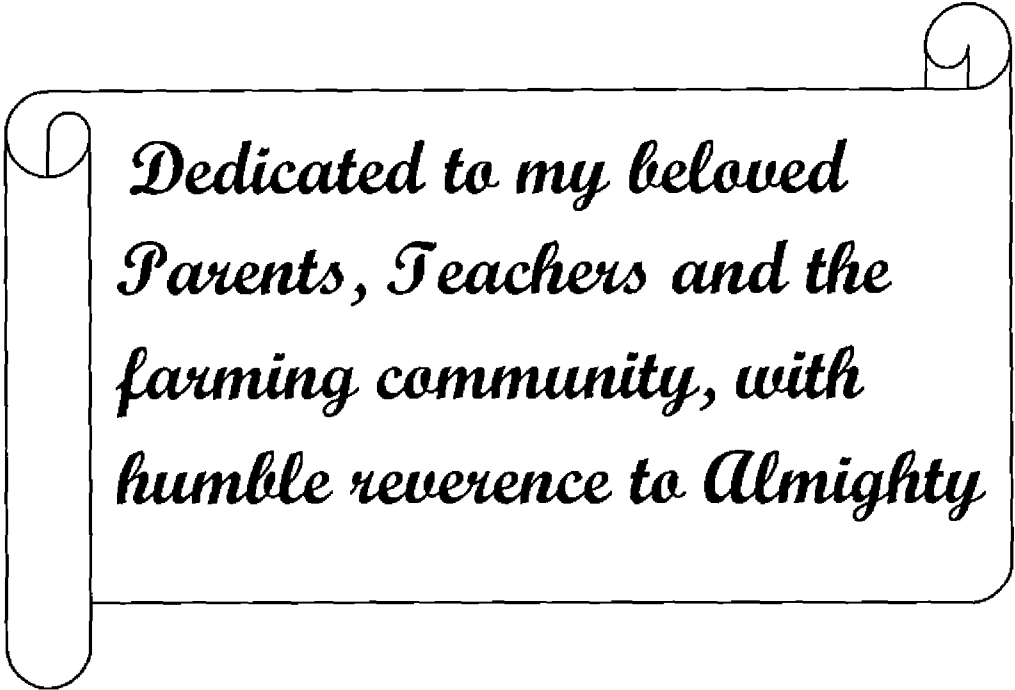
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*Dedicated to my beloved
Parents, Teachers and the
farming community, with
humble reverence to Almighty*

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Introduction



1. INTRODUCTION

Cocoa (*Theobroma cacao* L.) is an important beverage crop formerly included in the family Sterculiaceae (Purseglove, 1974) and at present reclassified into the expanded family Malvaceae (Alverson *et al.*, 1999). It is a perennial crop originated from the tropical humid rain forests on the lower eastern equatorial slopes of the Andes in South America (Wood and Lass, 1985). Cocoa was domesticated approximately 3000 years ago in Central America and introduced to India by 1970s, (Nair *et al.*, 2002) and at present it is widely cultivated in South Indian states like Kerala, Karnataka, Andhra Pradesh and Tamil Nadu in an area of 78,000 ha with total production of 16,050 MT. The highest productivity was reported from Kerala with 785 kg/ha. At present, the average productivity of cocoa in India is reported to be 475 Kg/ha (DCCD, 2016). Price determined for cocoa beans directly depend on the quality of beans.

The cocoa beans were consumed by Mayan and Aztec Indians of the high Mexican plateau and likely by the Olmec Indians in 1500 – 4000 BC. Today it is an important component of the economy of many producers and processor countries. Olmecs used the name “kakawa,” and it was believed that, they were the first to grow cocoa as a domestic crop (Coe and Coe, 1996). The term cocoa has been derived from the word ‘cacahoatl’ which was earlier used by the Aztec Indians. According to Aztec mythology, God ‘quetzacoatl’ whom they called as ‘xocolatl’ brought the cocoa to the earth. It is popularly known as ‘The Food of Gods’ because of it’s divine origin. Also the term chocolate was derived from the word ‘xocolatl’ (Mossu, 1992).

Theobroma cacao L., is commercially the most important among the 22 species of the genus *Theobroma* due to the value of its seeds (Bartley, 2005). The most important economic part of the cocoa crop is optimally fermented and dried beans, which is the only source of chocolate flavour (Amma *et al.*, 2011). Consumers have shown an increased interest for high quality chocolate and dark chocolate containing a higher percentage of cocoa. Therefore, the quality of beans has a great importance

while considering the market value. The quality of cocoa beans depends on many factors such as genotype, agronomic management, soil factors, climatic conditions and the post-harvest technology. Genotype influences flavour, quality and intensity of chocolate (Brito *et al.*, 2000).

Cocoa can be mainly classified into three types, namely Criollo, Forastero and Trinitario. This classification is based on morphology, geographical origin, genetic characters and flavour quality attributes of the cocoa seeds (Motamayor *et al.*, 2002). The Criollo cocoa variety is having a nearly unique and homozygous genotype, which is the first cultivable variety of cocoa in the world and it provides fine flavour chocolate. However, due to its poor agronomic performance and disease susceptibility, it is very difficult to cultivate. Nowadays, its cultivation is very much limited to Central America and a few regions in Asia (Thompson *et al.*, 2007). Forastero types are now cultivated in more than 80 percent of total cocoa plantations because of its high yield and resistance to pests and diseases (Marita *et al.*, 2001). Trinitario types are hybrids, which originated recently by the natural crossing between Criollo and Forastero (Motamayor, 2001).

Based on quality, beans from Criollo and Trinitario types are generally known as “fine or flavour” cocoa and these types having high demand among chocolate manufacturers because of its premium quality (Mooleedhar, 1995). At present fine cocoa production is estimated to be less than five percent of the world’s total cocoa production, due to the low productivity and disease susceptibility of the traditional fine flavour cocoa varieties. Therefore, breeding for improved Criollo varieties is important for the sustainable production of fine-flavour cocoa.

Considering the importance of quality of cocoa beans for chocolate production, hybridization programme was initiated at Cocoa Research Center (CRC), Kerala Agricultural University, Vellanikkara during 2004 for the development of varieties with beans of superior quality, which is a character of Criollo type along with high

yield and disease tolerance, which are the characters of Forastero type. Parental lines identified as having superior quality were crossed with high yielders along with disease tolerance in order to develop man made Trinitario types with the superior characters of both Criollo and Forastero types. The hybrid progenies from the compatible crosses were field planted and evaluated for their yield. In the present study, thirty hybrids were selected based on their initial performance in the field and further evaluated for qualitative and quantitative morphological traits, biochemical parameters, quality parameters and sensory attributes with the aim of developing of varieties with superior quality traits and preferable sensory attributes along with high vigour.

In this background the present study entitled ‘Evaluation of selected cocoa (*Theobroma cacao* L.) hybrids bred for quality’ which forms a part of the ongoing project at Cocoa Research Center (CRC) was taken up with the following objectives:

- i. To evaluate and characterize the morphological, qualitative and quantitative characters of pod and bean of the cocoa hybrids
- ii. To assess the biochemical and qualitative parameters of the beans of the cocoa hybrids
- iii. To evaluate the organoleptic characters of the chocolates made from the hybrids
- iv. To select the superior hybrids with premium quality along with high vigour and disease tolerance for further development of a variety

Review of literature



2. REVIEW OF LITERATURE

Cocoa (*Theobroma cacao* L.) is a perennial crop, on which the thriving chocolate industry is very much dependent. It was originated from the Amazon basin and indigenous to tropical areas of South and Central America. At present genus *Theobroma* is classified into six sections which include 22 species (Cuatrecasas, 1964). Among all the 22 species, *Theobroma cacao* is the only species which is now commercially cultivating and it is characterized by large genetic diversity (Bartley, 2005; Motamayor *et al.*, 2008). Cocoa beans are generally used to produce chocolate and several intermediate products are also popular such as cocoa liquor, cocoa butter, cocoa cake and raw cocoa powder. Cocoa powder can be used for flavouring biscuits, other dairy products, cakes and drinks (Frost *et al.*, 2011).

It is necessary to evaluate physical, bio-chemical and organoleptic attributes, which influences the cocoa bean quality regarding the genotype and the environment (Bucheli *et al.*, 2001). Genotype influences flavour quality and intensity of chocolate and also determines the amount of precursors and the enzymatic activities, thus contributing to flavour formation (Luna *et al.*, 2002; Counet *et al.*, 2004; Taylor and Roberts, 2004). Clapperton *et al.*, (1994) reported that “flavour” attributes of cocoa bean partly dependent on the genotype and it can be used as a selection criteria for further crop improvement programme. In addition to that cocoa flavour intensity, acidity, bitterness, astringency, fat content and bean count are very much dependent on the genotype.

2.1 Types of cocoa and the effect of genotype on cocoa bean flavours

Traditionally, cocoa can be classified into three types, namely Criollo, Forastero and Trinitario based on genetics, morphology, geographical origin and flavour quality attributes (Cheesman, 1944). Cocoa pods vary with varieties in different qualitative aspects like size, colour, appearance and shape. The typical characters of

Criollo types are small and elongated pod, intense rugosity, red or yellow coloured pod, deeply furrowed pod surface with ten ridges and furrows, slight pod basal constriction, attenuate pod apex form, white cotyledon colour, large bean size, increased dry bean weight and low husk thickness. Forastero type pods are generally thick walled, moderately sized, smooth textured, green coloured with bulbous or round shaped (Wood and Lass, 1985). Trinitario types are natural hybrids developed by crossing between Criollo and Forastero types and are indigenous to Trinidad and Tobago (Cheesman 1944). Trinitario types have red or yellow coloured pod and sometimes it can be orange or purple coloured with warty or smooth skin and elongated pods (Wood and Lass 1985).

Among the three cocoa types, Forastero is considered as one with low quality, Trinitario with intermediate quality and the Criollo having high quality (Ciferri and Ciferri, 1957). The selection procedure for Criollo type is based on phenotypic traits like sweet pulp, white beans, elongated pods and high quality based on sensory attributes (Engels, 1983). The Criollo beans are white to ivory or have a very pale purple colour, due to the presence of an anthocyanin inhibitor gene (Fowler, 1999).

Criollo and Trinitario types are generally known as ‘fine or flavour’ cocoa based on quality. They have a very high demand among the chocolate manufacturers because of its high quality which fetch premium prices in the world market and they are used for the production of fine chocolates (Mooleedhar, 1995). Fine cocoas are characterized as aromatic and smoother (Luna *et al.*, 2002). Criollo beans are nutty and floral in flavour, Trinitario are acidic and fruity in flavour and Forastero is generally known as bulk cocoa with bitter and astringent flavour (Afoakwa, 2010).

Criollo cocoa was cultivated during the pre-Columbian and colonial period in Latin America and it is characterized by premium quality when compared to Forastero types, but low performance in yield and vigour (Cheesman, 1944). At present, red pigmented fruits, a characteristic trait of Criollo and Trinitario types are controlled by

a single dominant gene. However, they are not popular in Nigeria. This could be due to limited use of Criollo and Trinitario clones in Nigerian cocoa breeding programme (Bartley, 2005). Eventhough West African Amelonado cocoa types shows less vigour, it possess attractive flavours to chocolate manufacturers. However they were replaced by Upper Amazon Forastero types because of high vigour, so there is a need to retain the characteristic flavour quality profile of Criollo through breeding programmes (Aikpokpodion, 2010).

Cocoa trees grown in some parts of America are generally characterized by high quality beans due to its sensory attributes and also due to its Criollo origin (Smith, 1999). Most of the cultivated genotypes with larger seed size having Criollo or Trinitario as their ancestors (Motamayor *et al.*, 2002). Genetics, environmental and post-harvest processing factors have a direct impact on the characters, which leads to the development of high quality chocolate (Voight, 2013) and among these, genetic factor is the most important one.

Three primary cocoa types: Forastero (bulk grade), Criollo (fine grade) and Trinitario (fine grade) showed wide variations in flavor quality (Awua, 2002; Amoye, 2006). Fine or flavour cocoa is produced from Criollo or Trinitario types, while bulk cocoa is produced from Forastero types and the fine cocoa fetch high prices than bulk cocoa (Donovan, 2006). Trinidad selected hybrids have been widely cultivating in Trinidad estates and they are producing well known hundred percent fine flavour beans of premium Trinitario origin. The flavour attributes of Trinidad hybrids are linked to genetic factor (Abdul Karimu *et al.*, 2003).

2.2 Pod and bean traits

Bean yield as well as disease resistance are the traits that receive the most attention of cocoa breeders. However but some emphasis has to be given on bean quality viz flavor and chemical composition. Yield in terms of dry bean weight in cocoa

can be measured by taking the weight of the dry beans produced per plant or unit area. Number of pods per tree or unit area, number of beans per pod and weight of individual beans are the three main components of yield (Wood and Lass, 1985).

There are a number of qualitative as well as quantitative descriptors proposed for pod and bean characterization. In cocoa, morphological descriptors are helpful for the breeders to select superior genotypes for the breeding programme (Engles *et al.*, 1980). Engles *et al.*, 1980, compiled several descriptors and IPGRI Working Group on the Genetic Resources of Cocoa had a list of 65 cocoa descriptors based on this (Anonymous, 1981). These descriptors were internationally approved and used at CATIE, Costa Rica to characterize cocoa germplasm (Engles, 1981; Enriques and Soria, 1981).

Engles (1986) evaluated several cocoa germplasm accessions to study morphological variation in flowers, fruits and leaves and classified them into two morphological groups. The first group comprised of Criollo and Trinitario types and second group with Forastero types and a continuous variation was observed between the two groups due to some genetic factors. Later this was confirmed by N'Goran (1994) using pod and bean characters. Based on pod and bean characters scholars identified different groups of cocoa: Cundeamor, Angoleta, Criollo, Amelonado and Calabacillo (Marita *et al.*, 2001 and Sounigo *et al.*, 2003).

Cocoa types with high seed index (weight of 1000 seeds) are of better economic value and increased demand for chocolate industries (Ruinard, 1961). Enriquez and Soria (1966) observed high variability in weight among the beans of a single pod and also recorded high variability in yield when expressed as dry or wet weight of bean per pod. Soria *et al.*, (1975) studied the inheritance pattern of pod size and estimated that there is 55 percent heritability for pod length, 63 percent for pod diameter and 57 percent for total pod weight, which indicates that these characters are highly

inheritable. He also reported high variability in pod characters like length, diameter, total weight, husk weight and weight of individual beans per pod.

Cilas *et al.*, 1989, conducted a study among twenty clones belonging to Amelonado and Trinitario types to find the variation in bean size and observed that Trinitario types showed maximum variability. They also observed that seeds originated from fruit apex have smaller seeds and they are free from flat beans. Napitupulu (1992) evaluated seedlings of open pollinated biclonal and hand pollinated hybrids, synthetic variety no. 3 and F3 Amazon in trials conducted during 1987-91 in Adolina and Tinjowan, North Sumatra. He reported that variations in yield and related characters are highly heritable. According to him selection should be based on bean quality to improve the yield.

Francis (1998) reported that yield, dry beans per tree and precocity of bearing the characters with highest variability and pod and bean width with the lowest. Chesny (2001) characterized cocoa trees based on qualitative and quantitative morphological characters based on the descriptor list provided by Cocoa Research Unit in North-West Guyana and they observed wide phenotypic variation among the morphological characters and also found the presence of fine flavour cocoa among the types analysed.

Alvarez *et al.*, (2003) studied morphology of pod and quality parameters of cocoa mucilage in different accessions collected from Chuao, Cuyagua and Cumboto states of Venezuela. The results revealed variations in different characters like fresh bean colour, bean shape, fruit texture and mucilage content among the genotypes collected from each area. Qualitative characters like colour of mature pod, pod basal constriction, pod surface texture and quantitative characters like weight of the bean, and number and length of the bean were the most prominent descriptors which can be used for morphological characterization (Bekele *et al.*, 2004).

Bekele *et al.*, 2008, identified promising Trinitario types based on the characters like bean size, husk thickness, cotyledon colour and pod index. The mean seed dry weight for the accessions DOM, GA, GDL, GS, ICS, MAR and TRD were 0.94 g, 1.02 g, 1.22 g, 1.11 g, 1.14 g, 0.89 g and 1.03 g respectively. Ramos *et al.*, (2004) conducted a study on morphological characters of Criollo populations from Gausare and Andean foothills in Western Venezuela. He studied 40 morphological characters in relation with flowers, pods and beans. The Gausare population was characterized by the rigidity of fruit surface, roundness of seeds with white cotyledons. The Andean foothills population was characterized by the intensity of pigmentation and smoothness of the fruit surface.

The morphological traits of the seeds are helpful to determine the shape of the seed (Balkaya and Odabas, 2002), polygenic traits like yield (Omokhafa and Alike, 2004), protein and fat content (Kaushik *et al.*, 2007). Daymond and Hadley, (2008) evaluated five cocoa genotypes; Amelonado, AMAZ 15/15, SCA 6, SPEC 54/1 and UF 376 to study the effects of light and temperature on fruit growth and development and found a negative relationship between temperature and bean size in Amelonado and UF 676. Mean seed weight of cocoa genotypes with greater than one gram are considered to be of superior quality (Monteiro *et al.*, 2009).

Efombagn *et al.*, (2009) conducted a study on morphological variation in cocoa among farm accessions (300) and field gene bank accessions (77) in Cameroon using 17 qualitative and quantitative characters related to leaf, flower, pod and bean. They found variation in pod characters like length, width, weight, apex form, shape, rugosity, colour, husk thickness and basal constriction of the pod and seed characters like number, length, width, dry weight and colour of the seed. Among the 300 farm accessions evaluated, the average pod length, pod width and pod weight reported as 14.8 cm, 7.0 cm and 510.6 g respectively. Also seed characters like mean seed length,

mean seed width, mean seed number and mean single dry bean weight were reported as 23.8 mm, 13.1 mm, 40.5 and 0.92 g respectively.

Apshara *et al.*, (2009) evaluated the pod and bean characters of 44 Nigerian cocoa clones for their growth and yield performance, which are being conserved in the field gene banks of Central Plantation Crops Research Institute, Regional station, Vittal, Karnataka. They identified the clones; NC-37, NC-23, NC- 26, NC-50, NC-20, NC-51, NC-27 and NC-25 as heavy bearers with an average of 61.9, 55.3, 49.4, 48.4, 45.1, 44.2, 43.9 and 43.0 pods per tree per year respectively. Also these clones are reported with high dry bean yield of more than one kilogram per tree per year, single bean weight of greater than one gram, shelling percentage of 10-15 percent and fat content of more than 50 percent which will make them suitable for chocolate industries.

Quantitative characters like seed size, seed shell percent and fat content are very much correlated with the quality of cocoa beans (Monteiro *et al.*, 2009). Cilas *et al.*, (2010) evaluated 200 clones to study the variations among some economically important traits and revealed that number of seeds varied from 3 to 63 per pod and seed weight varied from 0.3 to 2.8 g.

Aikpokpodion, (2010) evaluated 184 accessions of cocoa collected from farmers field (138 accessions) and field gene bank (46 accessions) in Nigeria using 17 agro-morphological traits and he observed that the most important characters which showed variability among the genotypes were quantitative bean characters like dry bean weight, nib weight, fresh bean weight, cotyledon length and cotyledon width and qualitative fruit characters like basal constriction, apex form, ridge colour, fruit shape, flush colour and cotyledon shape.

Cilas *et al.*, (2010) conducted an inheritance study in more than 200 cocoa clones about number of beans per pod and weight of beans per pod. They observed high heritability for mean bean weight per pod. Oyedokun *et al.* (2011) evaluated 14

cocoa hybrids to study the phenotypic variability among beans and revealed that hybrids were significantly different in characters like single bean weight, bean length, width, thickness, bean length to width ratio, length to thickness ratio and width to thickness ratio.

Maharaj *et al.*, (2011) studied important economic traits among 20 Trinidad Selected Hybrids (TSH). They reported that mean bean number ranged from 42.2 to 61.4 and weight of cotyledon ranged from 0.74 to 1.49 g. The coefficient of variation for fruit characters observed in the range of 5.4 percent to 16.6 percent. More than 50 percent of TSH showed Angoleta shape. The pod apex form showed wide variation from attenuate to indented and pod rugosity mostly characterized as intermediate to intense.

Adewale *et al.*, (2013) evaluated twenty four hybrids to study diversity among genotypes for phenotypic traits and observed a wide variation in the morphological characters; pod weight (0.43-0.86 kg), pod length (15.9-27.96 cm), pod thickness (1.026-5.71 cm), number of beans per pod (20-51) and bean weight per pod (0.017-0.41 kg). Positive and significant correlation was found between pod weight and length and also between pod girth and number of beans per pod.

Velayutham *et al.*, (2013) evaluated 151 cocoa accessions from farmer's field to study the variability among them and to identify superior accessions for high yield and quality for further crop improvement programme based on morphological, pod, bean and yield characters. They identified 15 promising trees based on important economic traits like dry bean yield per tree (> 2.4 kg), number of pods per tree (>60), number of beans per pod (>35) and single dry bean weight (>1 g). It was reported that pod characters like pod length, pod weight, pod ridge thickness and pod furrow thickness varied in the range of 10.20 cm to 20.10 cm, 221.1 g to 815 g, 8.2 mm to 19 mm and 6.2 mm to 15.6 mm with a mean value of 15.11 cm, 427.4 g, 12.86 mm and 9.36 mm respectively. Bean characters were also studied and number of pods, wet bean

weight per pod and single dry bean weight reported in the range of 25.50 to 50.50, 73.79 to 210.5 g and 0.59 to 1.72 g with a mean value of 39.45, 121.4 g and 1 g respectively.

Bekele *et al.*, (2004) evaluated 600 cocoa accessions including Forastero, Trinitario and Refractario types from the International Cocoa Genebank, Trinidad and they revealed that Trinitario types were found to be with lowest mean pod index (no. of pods required to get 1 kg dry beans) of 23.3. Either cotyledon weight of more than one gram and bean number almost equal to 40 or moderate cotyledon weight (0.9 g) and high bean number (> 40) will result in lower pod index value. Peeling ratio was observed in the range of 27.10 percent to 82.40 percent and dry matter recovery in the range between 47.70 percent and 78.30 percent through the study conducted among 25 hybrids (Rubeena, 2015). A study conducted by Vasudevan *et al.*, (2011) on heterosis of hybrids in cocoa showed positive and significant values in several hybrids for pod value (PV), pod index (PI), efficiency index (EI) and conversion index (CI).

2.3 Hybrids and heterosis

An extensive research is needed for the development of hybrids and also for the production of hybrid seeds. Hybrids were evolved by the crosses made between selected parents. The progenies thus developed should be evaluated in farmer's field. The whole process requires at least twelve years. Cocoa breeding programme mainly aims at the development of hybrids with increased yield in terms of dry cocoa bean weight without sacrificing the quality of beans. Several traits are characterized for assessing the yield and quality of beans. (Wood and lass, 1985).

Identifying superior plants, their development into clones and the exploitation of hybrid vigour were the approaches considered for cocoa improvement programme (Christian, 2003). Dias *et al.*, (2003) reported that hybridization programme for the production of superior hybrids have increased cocoa productivity. Hybrid exhibits wide

adaptability and better performance for yield and its components when compared to local cultivars (Dias *et al.*, 1998). Dias and Kageyama (1996) realized heterosis in hybrids through evaluation of genetic divergence between cocoa parent cultivars using D^2 of Mahalanobis distance.

2.4 Diversity analysis

Aikpokpodion (2010) evaluated 184 accessions of cocoa from farmer's field (138 accessions) and field gene bank (46 accessions) in Nigeria using 17 agromorphological traits and cluster analysis was done to explore the relationship among the accessions. The variation observed through the evaluation of accessions classified into six clusters. Accessions with large bean size, fruit length and width, high dry and fresh bean weight were classified under cluster III showed the characteristics of Trinitario and Upper Amazon derived varieties. Accessions with less bean weight, cotyledon length and width, small fruit size were included in cluster II which showed the characteristics of Amelonado. Accessions with intermediate pod and bean characters formed under cluster I revealed that hybridization could have taken place between Amelonado and Upper Amazon varieties. Cluster I and II mostly related to Cundeamor and Amelonado and cluster III mostly related to Angoleta type.

Principal component (PC) analysis of these traits revealed that first PC axis showed 24.5 percent variation for bean traits like dry bean weight, fresh bean weight, cotyledon length and width. Second PC axis showed 10.8 percent variation for fruit traits like fruit length, fruit shape, rugosity and apex form. It was reported that fruit length is positively correlated with rugosity and a negative correlation was observed for fruit shape and apex form.

Oyedokun *et al.*, 2011, carried out principal component (PC) analysis to categorize 14 genotypes based on similarities and grouped into four distinct clusters. Five genotypes were grouped under cluster I and seven genotypes under cluster II with

mean bean weight reported as 1.07 g and 1.02 g respectively. Cluster III and IV had one member each with an excellent bean weight of 1.12 g and 1.30 g respectively.

Engles (1986) evaluated 294 cultivars using 39 characters and cluster analysis as well as principal component analysis were done, revealed that cultivars were clustered under traditional classifications like Criollo, Forastero and their subdivisions. Santos *et al.*, (1997) carried out multivariate phenetic divergence among SIC and SIAL series clones through cluster and principal component analysis revealed that SIC 17 and SIAL 244 clones showed the highest divergence (3.05) and SIC 18 and SIC 765 clones showed highest similarity (0.33).

Maharaj *et al.*, (2011) carried out cluster analysis to study the relationship among 20 Trinidad Selected Hybrids (TSH) and five parents using 15 quantitative traits. SCA 6, ICS 95 and ICS 1 clones showed distinct characters from TSH progeny. The two TSH types within this group had showed descriptive fruit values and they are similar to the parental types.

2.5 Biochemical characterization.

Cocoa bean consists of two cotyledons, referred as nibs and also an embryo, which are all enclosed in a shell and two types of cells are present in the cotyledon; storage or parenchyma cells and bigger pigmented cells. Former one containing fat globules, protein bodies and starch granules, and later one containing polyphenols and methyl xanthines (Del Boca, 1962; Biehl *et al.*, 1977). Mora and Bullard, (1961) studied the variations in bean characters of hybrid cocoa progenies. They found that there is a positive correlation between bean size and fat content at five percent significant level and did not find any significant difference in flavour and fermentation rate among the hybrids. Criollo crosses or Criollo genotypes or Trinitario types were estimated for fat content and observed an average fat content of 53 percent (Wood and Lass, 1985).

Cocoa butter extracted from cocoa seed is one of the major products which is commercially produced and cocoa seed contains higher amount of fat (Luhs and Friedt, 1994). Pires *et al.*, (1998) evaluated the fat content of the unfermented cocoa beans in 490 accessions and reported that the average fat content to be 53.2 per cent, with a lower value of 45.4 percent in accession CC 57 and a higher value of 60.3 percent in NA 312. The lower the size of beans, the lesser will be the percentage of fat. The bean biochemical compounds interact each other through fermentation process results in the formation of cocoa flavor quality (Amin *et al.*, 2002). Cocoa butter, protein, polyphenols and alkaloids like theobromine, theophylline and caffeine are the major biochemical components present in the beans (Taylor, 2002; Luna *et al.*, 2002 and Counet *et al.*, 2004).

Rossini *et al.*, (2011) evaluated the major biochemical components in beans of selected clones and found that cocoa fat content depends largely on the genotype used. In cocoa butter, triglycerides are the main component accounting about 97 percent of the total composition and free fatty acids, mono- and diglycerides, phospholipids, glycolipids and un-saponifiable matter are the remaining fractions. Fat content observed in the range of 50.4-53.35 percent and 52.27-55.21 percent in fermented and unfermented cocoa beans respectively (Afoakwa, 2013).

Polyphenols present in the seed are responsible for the flavour and colour of chocolate. Based on the compounds present, polyphenols can be classified into three main groups; catechins or flavan-3-ols, anthocyanins and proanthocyanidins. Polyphenol and alkaloid contributes 14-20 percent of total weight of bean and are responsible for the quality of cocoa beans (Kim and Keeney, 1983). Kim and Keeney (1984) reported that total polyphenols in unfermented cocoa beans ranged from 40.0 mg GAE/g to 84.2 mg GAE/g which varies with geographical origin and also with respect to the cocoa varieties.

Total polyphenol content in Criollo type has been reported to be two by third of the amount of polyphenol present in Forastero types (Lange and Fincke, 1970). Chocolates produced from Criollo type contain higher amount of aromatic compounds and flavour precursors while it was lower in Forastero variety (Keeney 1972). The Criollo type generally shows lower total polyphenol content because anthocyanin content is less compared to other varieties, which is a type of polyphenol. Also, they found concentration of (-)-epicatechin ranged from 2.66 mg/g to 16.52 mg/g in cocoa unfermented beans collected from various countries and six percent polyphenols reported in air dried unfermented fat free cocoa beans.

The (-)-epicatechin is the main catechin present in cocoa beans, which contributes up to 35 percent (Kim and Keeney, 1984). The antioxidant properties of polyphenols in cocoa beans protect them against damage and diseases. The major polyphenolic compounds present in cocoa seeds are catechins (3-6 percent), leucocyanidins (2.5 percent) and tannins (2-3.5 percent) (Kyi *et al.*, 2005). It was reported that genetic factor can cause much variation (four fold difference) in polyphenolic content of fresh cocoa beans (Nazaruddin *et al.*, 2006 and Rodriguez-Campos *et al.*, 2011).

According to Niemenak *et al.*, (2006) total acid, reducing sugar, theobromine and total polyphenol content were the biochemical characters responsible for the quality parameters *viz.*, flavour and aroma. Nib acidity, flavour precursors (proteins and reducing sugars) and free fatty acids were the biochemical characters which affect the economic value as well as the quality of cocoa beans. The total phenol content has been reported in the range between 67 and 149 mg/g and from 101 to 144 mg/g in freshly harvested and two day fermented cocoa beans respectively.

Total polyphenol content was reported within a range of 45 to 52 mg/g in cocoa liquor, 34 to 60 mg/g in cocoa beans and 20 to 62 mg/g in cocoa powder (Nazaruddin *et al.*, 2006). Thus the polyphenols are astringent and bitter, its content should not be

much higher in cocoa beans to get a good flavour (Afoakwa, 2010). Polyphenols react with sugar and amino acids which will contribute to colour and flavour in cocoa beans and alkaloids present in cocoa beans were the factor responsible for bitterness and astringency (Afoakwa and Paterson, 2010). Polyphenolic compounds present in cocoa beans provide health benefits (Djousse *et al.*, 2011). Investigation on the relationship between polyphenols and other chemical compounds revealed significant correlation between polyphenols and pH, concentration of O-dihydric phenols and iodine value. Iodine value serve as an index for determining hardness of cocoa butter fat (Afoakwa *et al.*, 2012)

Total alkaloid content present in dry fat free beans was reported to be in the range of 23.7 to 49.7 mg/g with an average value of 37 mg/g (Jalal and Collin, 1976). Major alkaloid present in cocoa beans is theobromine with an average value of 2.9 percent (Aremu *et al.*, 1995). Luna *et al.*, (2002) conducted a study in Ecuadorian cocoa samples to find out the relationship between the genotypes and the biochemical estimation like polyphenols, alkaloids, organic acids and sugars which involved in the development of aroma and flavour and also the primary sensory attributes like bitterness, astringency, fruity and floral notes. The results revealed a positive correlation between polyphenols and astringency and bitterness, also found a negative correlation with fruity flavour. The major alkaloids present in cocoa beans are caffeine (0.1-0.8 percent), theobromine (2.5-3.2 percent) and theophylline (Osman *et al.*, 2004). Stark *et al.*, (2006) reported that alkaloids present in the beans are the major compounds which contribute to the bitter taste.

Studies carried out by Davrieux, *et al.*, (2004) showed the existence of a relationship between the methylxanthine content with cocoa genotype. Criollo has lower theobromine concentration and higher caffeine content, whereas Forastero has more theobromine content and less caffeine concentration. During the fermentation process, bitter flavour developed is fundamentally determined by the concentration of

theobromine and caffeine. Alkaloid compounds like methyl xanthine, caffeine, theobromine and polyphenolic compounds like anthocyanidins and flavanzols (catechin and epicatechin) result in astringency and bitterness in cocoa (Camu *et al.*, 2008).

The percent protein present in cocoa bean was reported in the range of 15-20 percent in which albumin constituted about 52 percent of total protein and globulin fraction about 43 percent (Spencer and Hodge, 1992). Protein breaks down molecules like peptides and hydrophobic free amino acids by the action of aspartic proteinase and carboxypeptidase enzymes when react with fructose and glucose will develop characteristic cocoa flavour. Albumin and globulin are the two major fractions of protein in which albumin contributes about 52 percent of total protein (Voigt *et al.*, 1993) and globulin accounts for 43 percent (Dodo and Furtek, 1994).

Liendo *et al.*, 1997, evaluated high quality Venezuelan criollo cocoa beans for biochemical characters which are mostly accepted due to excellent aroma and flavour revealed that fat content, moisture content and protein content in cocoa beans varied from 46 percent to 56 percent, 4.35 percent to 7.06 percent and 14.69 percent to 20.50 percent respectively. High quality cocoa beans are proposed to contain 8-14 mg/g dry matter of total amino acids (Rohsius *et al.*, 2006). The storage proteins and carbohydrates present in seeds will break down by the enzymes, yielding peptides, free amino acids and reducing sugars, which will aid in the development of chocolate aroma precursors (Schwan and Wheals, 2004; Afoakwa *et al.*, 2008).

Total protein in cocoa beans reported in a range between 16 to 22 percent (Afoakwa, 2013); and 15.2 to 19.8 percent (Afoakwa, 2010; Aremu *et al.*, 1995). Brito *et al.*, (2001) reported that amino acid concentration developed through proteolysis is very important while considering the flavour compounds present in chocolate. During fermentation albumin will not be degraded and globulin will undergo degradation which results in the formation of flavour precursors (Afoakwa, 2010).

Bertazzo *et al.*, (2011) reported that protein content varied in defatted cocoa beans from 11.8 g/100 g from the Dominican Republic to 15.7 g/100 g in beans from the Ivory Coast. During the fermentation process polyphenols will be subjected to oxidation due to the activity of polyphenol oxidase and it will be condensed to high molecular weight tannins and their interaction with protein will improve the quality of cocoa beans for the production of chocolate (Afoakwa *et al.*, 2012).

Chocolate aroma developed in the beans derived from seed endogenous compounds like storage proteins and carbohydrates and fine or flavour cocoa characterized by fruity aroma has been linked to pulp of beans (Kadow *et al.*, 2013). Total fat, total acidity, total phenols, phenolic acids, organic acids, heavy metals, amino acids, caffeine, theobromine, pH, sugars and macro and micro nutrients were the main variables included in the cocoa quality index for the Forastero cocoa beans (Araujo, *et al.*, 2014).

2.6 Total soluble solids, pH and Moisture content

Alvarez *et al.*, (2003) analyzed total soluble solids (TSS) and pH of seed mucilage in cocoa genotypes collected from Chuao, Cuyagua and Cumboto States, Venezuela. They revealed that TSS measured from different zones varied significantly. It is ranged from 19.89-22.26 percent in Chuao genotypes, 14.48-17.52 percent in Cuyagua genotypes and 7.83-15.68 percent in Cumboto genotypes and the pH value did not show much variation in genotypes from different zones ranged from 3.03-3.09, 3.01-3.68, 3.36 -3.76 for Chuao, Cuyaga and Cumboto respectively.

Beans with higher pH (5.5–5.8) after fermentation are characterized as not fully fermented with low fermentation index and cut test score. Cocoa beans with lower (4.75–5.19) and higher pH (5.50–5.80) scored lower chocolate flavor and higher off-flavor notes respectively and chocolate prepared from beans with intermediate pH (5.20– 5.49) scored higher chocolate flavour. A very low pH of cocoa beans after

fermentation are considered as low quality. Sensory evaluation was carried out on the chocolates prepared from cocoa beans with low pH and high pH revealed that both showed lower notes of chocolate flavour and higher off flavour notes. Chocolate made from cocoa beans with intermediate pH showed higher notes of chocolate flavour and low off flavour notes (Jinap *et al.*, 1995).

According to Whitefield, (2005) pH value of fermented dried cocoa beans reported in the range of 5.00 to 5.72. The pH value in between 5.5 to 5.8 are considered to be unfermented and pH value in between 4.75 to 5.19 are considered to be well fermented. Beans with low pH value reported as acidic and pH above 7 have been over fermented (Schwan and Wheals, 2004). Decrease in the pH value during fermentation is due to diffusion of acids produced by lactic acid and acetic acid bacteria into the cocoa beans (Afoakwa *et al.*, 2008). High nib acidity adversely affects the quality of cocoa beans. After drying pH value attained in between 5 to 5.5 would lead to improved chocolate flavour (Afoakwa *et al.*, 2015).

Optimum moisture content in dried beans would not be more than 7.5 percent. Moulds will develop when moisture content would go above 8 percent within the beans, while when it go beyond 8 percent, it would be brittle in nature (Galvez *et al.*, 2007 and Ndukwu, 2009). Fermented beans will have 53 to 55 percent moisture content initially and this will create an unsuitable environment for storage, so it has to be brought down to 6 percent moisture. Moisture content in cocoa dried beans would be in the range of 3.89-4.95 percent, which is lower than the acceptable limits (6-7 percent) for long term storage of beans (Fowler, 2009). Reduction in drying process has great influence on the rate of browning reaction during roasting process (Afoakwa *et al.*, 2014). Lower moisture content was ensured to cease all the microbial and enzymatic reactions in the beans.

2.7 Fermentation index and fermentation recovery

Fermentation is one of the most important processes which involves in the development of characteristic cocoa flavour from precursors. Optimum fermentation will aid in the improvement of quality of cocoa products and it results in the development of flavour and reduction in sourness, astringency and bitterness through several biochemical reactions in the beans (Meyer *et al.*, 1989; Biehl *et al.*, 1990).

According to Afoakwa, (2010) the time taken for fermentation of cocoa beans is very much dependent on its polyphenol content. Fermentation process takes more time when polyphenol content is higher in beans because it is difficult to break down them into smaller compounds. He reported that Forastero cocoa beans take more time for complete fermentation (5-6 days) and Criollo type requires only 1-3 days. During the fermentation and drying process several volatile and nonvolatile compounds released were considered as an indicative of cocoa bean quality (Campos *et al.*, 2011).

According to the quality standards, brown colour indicates the end point of fermentation. This colour change is widely used to assess the flavour profile of cocoa. Cut test and fermentation index are the important tools to check the quality of beans based on changes in cotyledon colour through fermentation (del-Boca, 1962). Cut test is an important tool to measure the final quality of cured beans (Sadasivam and Manickam, 1996). Assematt *et al.*, (2005) evaluated Guinian cocoa populations for bean quality using cut test revealed no mould attack, insect damaged or germinated beans and very small quantities of slaty beans. Also less than one percent of flat beans and less than 20 percent of purple coloured beans were observed after fermentation, which indicates good quality and can be graded them as category 1 of ISO quality classification.

In order to check the quality of cocoa beans, cut test can be used, in which beans are taken randomly from a sample and record the defected beans and cotyledon colour.

Purple colour of cotyledon can be considered as unfermented and brown colour as fermented. The highest mean fermentation through cut test scoring was 55.53 percent during April-May and 63.76 percent during October- November season. The average recovery percent of cured beans (Sun dried) was reported as 39.09 percent (Sunil Kumar *et al.*, 2008).

Afoakwa *et al.*, 2008, reported that fermentation process leads to the breakdown of outer mucilage and results in the death of cotyledon. This will make some biochemical reactions inside the beans, which aids in the development of flavour precursors (aminoacids, peptides and sugars) and also reduction in bitterness and astringency (Thompson *et al.*, 2007; Kratz *et al.*, 2009).

2.8 Sensory evaluation

Sensory analysis was carried out to find strength of the taste by using a 9-point hedonic scale ranging from “much too weak” to “much too strong” (Meilgaard *et al.*, 1987). Chocolates produced from cocoa beans have evolved as one of the highest popular non basic food and it was widely accepted throughout the world because of its texture, sensory stimulus and mood created by its consumption (Macht and Dettmer, 2006). Inorder to produce chocolate with low cocoa percent without affecting flavour characteristics, cocoa butter percent could be increased to get an optimum mouth feel (Afoakwa *et al.*, 2007).

Luna *et al.*, (2002) carried out sensory analysis in chocolates prepared from Ecuadorian cocoa samples observed a positive correlation between brown colour and sensory characters like chocolate odour (0.98), toasted odour (0.98), bitterness (0.94), toasted flavour (0.97), chocolate flavour (0.87) and firmness (0.88) and a negative correlation (-0.97) was observed between firmness and melting quality.

Camu *et al.*, (2008) carried out sensory analysis on chocolates prepared from beans taken from seven separate heaps and found that heap 6 showed highest score for

sensory attributes like intensity, acidity and after taste (lemon acid). Heap 2 and 4 showed highest score for floweriness but low after taste intensity. Heap 5 with the highest score for fruitiness with almost no bitterness and Heap 7 showed highest score for cocoa flavour. Based on mouth feel, chocolates with low cocoa content are recognized as melting and creamy, while high cocoa content as dry, mealy and sticky. Afoakwa *et al.*, (2009) reported that molecules such as alcohols, ketones, aldehydes, esters and pyrazines were associated with sweet and nutty odour, which are highly desirable compounds and could lead to the production of high quality chocolates.

Leite, *et al.*, (2013) evaluated the chocolates prepared from two cultivars, PH 16 and SR 162. PH 16 was developed through crossing between Criollo and Forastero parents and SR 162 was a mutant variety. They assessed the acceptability and identified the cultivars with high sensory quality. The results revealed variation among sensory attributes of chocolate. The chocolates made from PH 16 cultivar characterized by dark brown colour, intensive flavour, chocolate odour and soft texture, indicated good quality sensory attributes and the chocolates made from SR 162 cultivar were characterized by higher sweetness and good melting quality. A conventional cultivar was taken as the standard, which showed intermediate sensory attributes. All the three cultivars showed good sensory acceptance in appearance, odour, flavour, texture and global quality.

Ovando *et al.*, (2015) evaluated 7 representative samples of a total of 45 cocoa bean samples for the odour and taste profile in Southern Mexico by trained panelists. Taste attributes like sweetness, bitterness, acidity and astringency and nine characters related to odour were evaluated. The results revealed that, the sample G7 recorded with high scores for sweet taste (2.85), nutty odour (3.39), lower acidity (1.23) and less bitter taste (3.23). G3 sample was characterized with lower astringent taste and higher chocolate flavour (2.48). Based on sensory descriptors, high quality cocoa beans were

associated with sweet taste, chocolate and hazelnut odour and less bitterness and low quality cocoa beans with bitter taste and off odour.

2.9 Pests and Diseases

Cacao black pod, an economically serious problem throughout the world, where cocoa is grown, causing significant pod losses of up to 30 percent and results in the death of 10 percent of the trees annually (Matos *et al.*, 1998). Black pod disease generally called as black cocoa was first reported from Guyana and West Indies (Jenman and Harrison, 1897). In India it was first reported in 1965 (Ramakrishnan and Thankappan, 1965) and the causal organism for black pod disease was reported as *Phytophthora palmivora* (Chandramohan, 1979). Chandramohan (1982) observed certain level of tolerance in Nigerian cocoa collections against black pod disease.

Pods or cherelles can be infected at any location, infection mostly occurs at the tip or stem end of the pod and more frequently on pods close to the soil. Firm, spreading, chocolate-brown lesion affects the whole pod. When husk become infected, *Phytophthora sp.* enter inside the pod and results in discoloration and shrivelling of the cocoa beans. Later infected pods became black and mummified (Deberdt *et al.*, 2008). Prabha and Chandramohan (2011) conducted a survey in Southern states of India to find the occurrence of major diseases of cocoa revealed that *Phytophthora* diseases were the most important one which causes great economic loss. Among the *Phytophthora* diseases black pod disease caused by *Phytophthora palmivora* was mostly noticed. In Kerala black pod incidence was reported as 90.75percent of the gardens surveyed. Vascular streak die back disease incidence was reported as 17.8 percent of the gardens surveyed in Kerala. Cherelle wilt, Colletotrichum pod rot, chupon blight, twig dieback, white thread blight, horse hair blight and pink disease were also reported but not arised as a serious problem.

It was reported that 35 percent yield loss in cocoa was due to pests and diseases in which pests account for 25 percent and diseases account for 10 percent. Tea mosquito bugs (*Helopeltis* sp.) are reported as serious pests throughout the world with yield loss of more than 75 percent. The adults and nymphs of *Helopeltis* sp. will feed on the pods. The pests suck juices from pods aid in the development of brown water-soaked lesions. Secondary infections through the lesions results in crop loss. Damage caused by them is highly variable and depend on several factors like agricultural practices, locality, climate, control measures, varieties and species involved (Alagar and Subaharan, 2011).

Mealy bugs contributes about 40 percent yield loss among the insect pests. The adult and young ones of mealy bugs feed on the tender shoots, cushions, flowers and pods through sucking the sap, as a result cushion will abort. Stem borer is a polyphagous pest which accounts for 8 percent loss in cocoa. Caterpillars bore into the branches and trunks of trees. The aerial portion above the point of entry of the pest dries up. Adults and young ones of aphids feed on the tender leaves, succulent stem and flowers. Heavy infestation results in premature shedding of flowers and stunting of stem-tip. Red banded thrips will feed on tender leaves, surfaces of cherelles and immature pods results in feeding marks (Khader, 2005).

Rodents, another important group of major pests reported from almost all cocoa growing countries (Taylor, 1972; Williams, 1973; Gratz and Arata, 1975). Abraham and Padmanabhan (1967) reported rat damage in the cocoa pod from India as early in 1967. In cocoa plantations, a heavy damage by rodents of about 75 per cent has been reported (Advani, 1982). Black rat (*Rattus rattus*), the Western Ghat squirrel (*Funambulus tristriatus*) and the South Indian palm squirrel (*F. Palmarum*) are the major rodent pests which causes damage to cocoa pods and beans (Bhat, 1978; Abraham and Remamony, 1979; Advani, 1984). Timely harvest of mature pods reduced squirrel attack from 52 to 25 percent just through increased pod harvest from

12 to 21 per year (Abraham *et al.*, 1979). He also suggested covering of cocoa pods with gunny bags or bitumen smeared polythene cover, which will be very effective. Bhat, (1980) noticed that squirrels attack the central part of the pod and rat attacks near the stalk end of the pod. He suggested poison bait trap for the control of rats and single catch live traps for the control of squirrels.

Materials and methods



3. MATERIALS AND METHODS

The present study entitled ‘Evaluation of selected cocoa (*Theobroma cacao* L.) hybrids bred for quality’ was carried out in the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during the period 2014-2016.

Considering the importance of quality of cocoa beans for chocolate production, hybridization programme was initiated at Cocoa Research Center (CRC), Kerala Agricultural University (KAU) during 2004. High quality Criollo types were selected and crossed with high yielding Forastero types with moderate disease tolerance. As a result, 240 hybrids were established in the field during 2005-06 (Plate 1). These hybrids were observed for their general vigour and yield. Thirty high yielders were selected in the present study for further morphological, qualitative, quantitative, biochemical and quality parameters evaluation. Organoleptic evaluation of chocolates prepared from selected hybrids were also carried out and compared with the chocolates prepared from KAU released CCRP varieties (CCRP 1 to 9) and commercial chocolate ‘chocolate 4 u’ released from KAU. List of hybrids used in the study and their parentage are presented in table 1.

3.1 Morphological characterization

The descriptor developed by Bekele and Butler (2000) was used for taking the morphological observations. Both quantitative and qualitative characters of pod and bean were observed for morphological characterization. The morphological descriptors are useful in selecting superior genotypes for further crop improvement programme (Engles *et al.*, 1980). For morphological characterization of pods and beans five pods were collected from each hybrid to record the observation. A total of 150 pods was collected from 30 hybrids during the period from October to December and evaluated based on the descriptor. Statistical analysis was carried out using Completely Randomized Design (CRD). Husk was split opened to evaluate bean characters and the outer mucilage was peeled using forceps to record peeled bean observations.

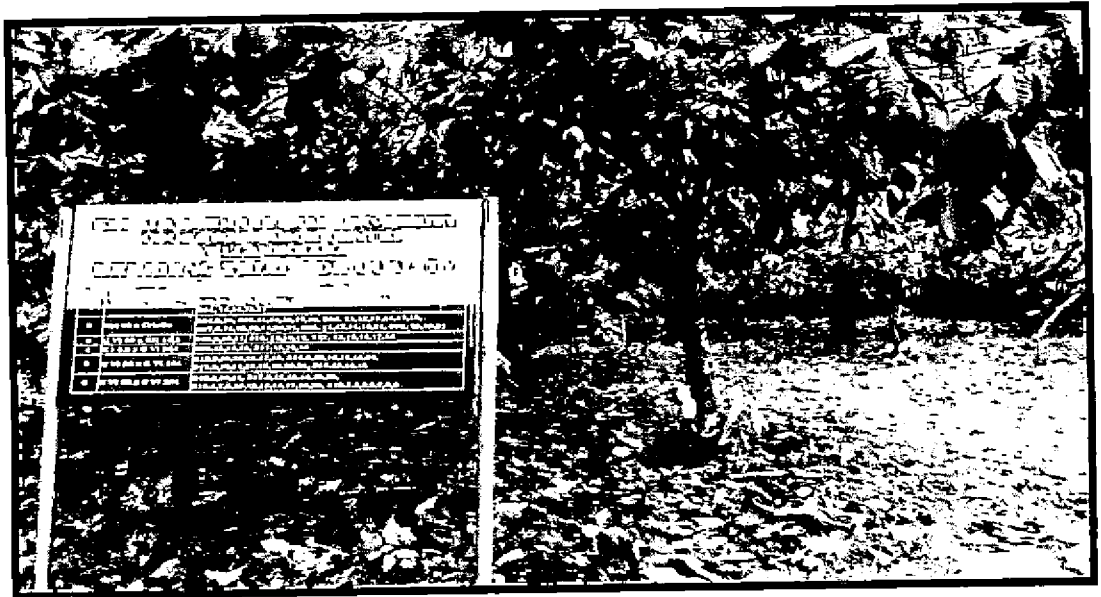


Plate 1. Field view

Table 1. The hybrid progenies and their parentage

SI. No.	Hybrid stand No.	Hybrid name	Parentage
1	303.9	Hyb.1	G IV 18.5 X Criollo
2	303.10	Hyb.2	G IV 18.5 X Criollo
3	303.11	Hyb.3	G IV 18.5 X Criollo
4	303.13	Hyb.4	G IV 18.5 X Criollo
5	306.11	Hyb.5	G VI 55 X Criollo
6	307.9	Hyb.6	G VI 55 X Criollo
7	307.11	Hyb.7	G VI 55 X Criollo
8	307.12	Hyb.8	G VI 55 X Criollo
9	307.13	Hyb.9	G VI 55 X Criollo
10	307.20	Hyb.10	G VI 55 X Criollo
11	307.21	Hyb.11	G VI 55 X Criollo
12	308.9	Hyb.12	G VI 55 X Criollo
13	308.11	Hyb.13	G VI 55 X Criollo
14	308.21	Hyb.14	G VI 55 X Criollo
15	309.9	Hyb.15	G VI 55 X Criollo
16	309.10	Hyb.16	G VI 56 X G II 14.3
17	309.16	Hyb.17	G VI 55 X Criollo
18	309.20	Hyb.18	VSD 13.10 X G VI 51
19	309.21	Hyb.19	G VI 55 X Criollo
20	310.12	Hyb.20	VSD 13.10 X G VI 50
21	311.9	Hyb.21	VSD 13.8 X G VI 50
22	311.18	Hyb.22	VSD 23.17 X G VI 51
23	312.10	Hyb.23	VSD 27.1 X G VI 50
24	312.11	Hyb.24	VSD 27.1 X G VI 50
25	314.11	Hyb.25	VSD 13.10 X G VI 50
26	315.9	Hyb.26	G VI 55 X G VI 144
27	315.10	Hyb.27	G VI 55 X G VI 144
28	316.9	Hyb.28	G VI 55 X G VI 264
29	316.10	Hyb.29	G VI 55 X G VI 264
30	318.8	Hyb.30	G VI 55 X G VI 264

3.1.1 Quantitative evaluation of pod and beans

Quantitative evaluation was carried out based on 17 quantitative characters.

1. Pod weight

Five pods were taken from each hybrid and calculated the mean value. It is measured using a standard weighing balance and expressed in grams.

2. Number of beans per pod

3. Number of flat beans per pod

4. Number of ridges per pod

5. Number of furrows per pod

6. Length of pod (cm)

7. Breadth of pod (cm)

The length and breadth of the pod measured by using a measuring device fabricated by Cocoa Research Center.

8. Furrow thickness (cm)

9. Ridge thickness (cm)

The husk thickness measured by taking the average of ridge thickness and furrow thickness which was measured with the help of a vernier caliper.

10. Total wet bean weight per pod (g)

11. Unpeeled wet bean weight (g)

12. Peeled wet bean weight (g)

13. Peeled dry bean weight (g)

14. Single bean dry weight (g)

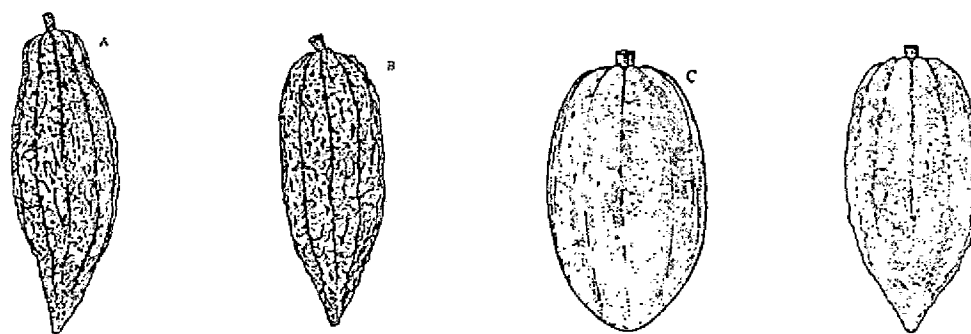
15. Single bean length (mm)
16. Single bean breadth (mm)
17. Single bean thickness (mm)

For the bean observations, five pods from each tree was taken, split opened and beans were bulked. Twenty beans selected randomly, peeled using a forceps and used for further observations.

3.1.2 Qualitative evaluation of pod and beans

Qualitative evaluation was carried out by recording eight qualitative characters; pod shape, ridge colour, pod apex form, pod basal constriction, colour of ripe and unripe pod, husk hardness, pod rugosity and colour of bean (cotyledon colour) were the important qualitative characters recorded using the descriptor given by Bekele and Butler, 2000.

3.1.2.1 Pod shape



Cundeamor

Criollo

Amelonado

Angoleta

Descriptor states and description

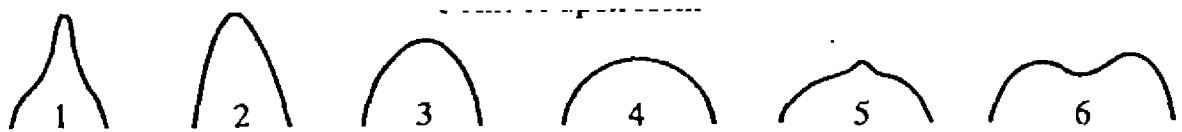
- 1 = Cundeamor - characterized by bottle neck
- 2 = Angoleta - deeply ridged, warty and square at the stalk end
- 3 = Amelonado - characterized by slight bottle neck, smooth and shallow

furrows and melon shaped with blunt end

4 = Calabacillo - Spherical and small in shape

5 = Criollo - Intense surface with acute apex

3.2.1.2 Pod apex form



1 = Attenuate

2 = Acute

3 = Obtuse

4 = Rounded

5 = Mammelate

6 = Indented

3.1.2.3 Pod basal constriction



0 = Absent

1 = Slight

2 = Intermediate

3 = Strong

4 = Wide shoulder

3.1.2.4 Pod rugosity

0 = Absent

3 = Slight

5 = Intermediate

7 = Intense

3.1.2.5 Colour of ripe pod (Ridge and furrow colour)

0 = Absent (Green)

3 = Slight (Greenish yellow)

5 = Intermediate (Yellowish green)

7 = Intense (Yellow)

3.1.2.6 Colour of unripe pod

3 = Light

5 = Intermediate

7 = Purplish green

9 = Dark green

3.1.2.7 Bean colour

The outer mucilage was removed using forceps and observed the cotyledon colour.

1 = White

2 = Grey

3 = Light purple

4 = Medium purple

5 = Dark purple

6 = Mottled

7 = Mixed

3.1.3 Economic characters

3.1.3.1 Yield

It is the number of pods harvested in a year from a tree.

3.1.3.2 Pod Value

It is the dry weight of beans per pod. It can be measured by multiplying dry weight per bean with number of beans per pod (Toxopeus and Jacob, 1970). It is expressed in grams.

3.1.3.3 Pod Index (P. I.)

It indicates the number of pods required to get 1 kg of dried beans (Morera *et al.*, 1991) and it can be measured using the following formula.

$$P. I. = 1000g \div \text{pod value (g)}$$

3.1.3.4 Efficiency Index (E. I.)

It indicates the pod weight required to produce 1 g dry bean (Jacob and Atanda, 1971)

$$E. I. = \frac{\text{Pod weight}}{\text{Pod value}}$$

3.1.3.5 Conversion Index (C. I.)

It is defined as the amount of dry bean obtained from a given amount of wet bean. It can be computed by using the following formula.

$$C. I. = \frac{\text{Pod value (g)}}{\text{Wet bean weight per pod (g)}}$$

3.1.3.6 Peeling ratio

It is the percent of bean weight obtained after peeling.

$$\text{Peeling ratio} = \frac{\text{Peeled wet bean weight (g)}}{\text{Unpeeled wet bean weight (g)}} \times 100$$

3.1.3.7 Dry matter recovery

It is the bean weight obtained after drying and expressed in percent.

$$\text{Dry matter recovery} = \frac{\text{Dry bean weight (g)}}{\text{Wet bean weight (g)}} \times 100$$

3.1.3.8 Flat bean percent

It is the number of flat beans present among total number of beans per pod and it is expressed in percent.

3.1.4 Genetic parameters

Genetic parameters like Genotypic Coefficient of Variation (GCV), Phenotypic Coefficient of Variation (PCV) (Sivasubramanian and Madhavamenon, 1973), Heritability (h^2) and Genetic Advance (GA) (Johnson *et al.*, 1955) were estimated.

Relative Heterosis (RH), Standard Heterosis (SH) and Heterobeltiosis were also computed using standard procedures (Briggle, 1963; Hayes *et al.*, 1965).

3.1.4.1 Coefficient of variation

Coefficient of variation for the characters at genotypic and phenotypic levels were computed.

3.1.4.2 Genotypic Coefficient of Variation (GCV)

$$\text{GCV} = \frac{\sigma_g}{\text{Grand mean}} \times 100$$

Where, σ_g is genotypic standard deviation

3.1.4.3 Phenotypic Coefficient of Variation (PCV)

$$PCV = \frac{\sigma_p}{\text{Grand mean}} \times 100$$

Where, σ_p is phenotypic standard deviation

The PCV and GCV value were ranked as described by Sivasubramanian and Madhavamenon (1973).

0 – 10% - low

10.1 – 20% - moderate

> 20% - high

3.1.4.4 Heritability (H^2)

$$\text{Heritability} = \frac{V_g}{V_p} \times 100$$

Where, V_g is genotypic variance and V_p is phenotypic variance.

Range of heritability was classified by Robinson *et al.*, 1949

0-30% - low

31-60% - moderate

61% and above – high

3.1.4.5 Genetic Advance (GA)

$$GA = k \sigma_p H^2$$

Where, $k = 2.06$, a constant

σ_p = phenotypic standard deviation

3.1.4.6 Genetic Gain (GG)

$$GG = \frac{GA}{\text{General mean}} \times 100$$

Where, GA = Genetic advance

GA value was categorized by Johnson *et al.*, 1955.

0 – 10% - low

10.1 – 20% - moderate

> 20% - high

3.1.5 Estimation of heterosis

Heterosis was estimated based on better parent, mid-parent and standard parent

3.1.5.1 Relative Heterosis (RH)

Superiority over mid parental value was calculated.

$$RH = \frac{F1 - \text{Mid parental value}}{\text{Mid parental value}} \times 100$$

3.1.5.2 Heterobeltiosis

Superiority over better parent was estimated.

$$\text{Heterobeltiosis} = \frac{F1 - \text{Better parent}}{\text{Better parent}} \times 100$$

To test the significant difference, critical difference (CD) was worked out. CD value was calculated using standard error of difference as given below (Briggle, 1963).

$$\begin{aligned} CD (0.05) / (0.01) &= t_{e'} (0.05) / (0.01) \times \sqrt{2MSE/r} \\ &= t_{e'} (0.05) / (0.01) \times SE \end{aligned}$$

Where, $t_{e'}$ - critical value at 5% significance or at 1% level

MSE - Error mean square

r = number of replications

SE - standard error between two means

3.1.5.3 Standard Heterosis (SH)

Superiority over standard variety was calculated

$$SH = \frac{F1 - \text{Check variety}}{\text{Mid parental value}} \times 100$$

3.2 Quality parameters/ biochemical characterization

Thirty hybrids were evaluated for biochemical characterization. Fat content, total phenol content, protein content, total alkaloid content and total soluble solids were estimated following standard procedures.

Sample preparation

Five ripened pods were harvested from each of the hybrid based on ripeness and maturity indices. Pod husk was split opened and beans were scooped out. Beans from all the pods were pooled for analysis. From this 20 beans were selected randomly. The beans were dried under sun or by using an oven until the moisture reached below 8 percent. The drying was completed under sun within six to seven days. The dried beans were then ground to fine powder using laboratory grinder and the powder was tightly packed, labelled and stored for further biochemical analysis.

3.2.1 Fat estimation

Method: Soxhlet apparatus method

Materials required: Cocoa bean powder: 10 g
Petroleum ether (40-60°C)
Blotting paper

Procedure: Cocoa nibs were defatted to extract the fat with petroleum ether (40-60°C) in a soxhlet apparatus (Sadasivam and Manickam, 1996). Ten grams of cocoa bean powder was wrapped in a blotting paper and tied with twine. The sample was placed in the extraction tube of soxhlet apparatus. The fat present in the cocoa powder was extracted through siphoning of petroleum ether through the apparatus and fat got settled at the bottom of the flask along with a little amount of petroleum ether. This was transferred to a pre-weighed beaker and kept open for the petroleum ether to evaporate. The cream coloured substances left behind after the evaporation of solvent was the fat and it was weighed and expressed as percentage.

3.2.2 Total phenol estimation

Method: Folin- Ciocalteau (FC) reagent method

Required: Powdered sample- 500 mg
Ethanol (80 percent)
Na₂CO₃ (20 percent)
FC reagent
Catechol – 100 mg

The powdered and defatted cocoa bean powder was used for the estimation of total polyphenols. The defatted samples were extracted exhaustively with ethanol. The total phenols in the extract then estimated by Folin- Ciocalteau reagent method developed by Malick and Singh (1980). The procedure followed was detailed below.

Exactly 500 mg of powdered defatted sample was taken and ground it with 80 percent ethanol using mortar and pistle and centrifuged at 10,000 rpm for 20 minutes. The supernatant was collected in a beaker and the remaining residue settled down was re extracted with five times the volume of 80 percent ethanol. Again centrifuged and the supernatant was collected and pooled in the beaker. Then supernatant was allowed to evaporate. Five milli litre water was poured to the residue to dissolve the phenols in it. Pipetted out 0.2 ml of the solution into a test tube and then made up the volume to 3 ml using distilled water followed by the addition of 0.5 ml of Folin-Ciocalteau reagent. Kept it for three minutes and added 2 ml of 20 percent Na_2CO_3 solution and mixed well. The test tubes were kept in a boiling water bath exactly for one minute and after that cooled it to room temperature and incubated at room temperature for 60 minutes for colour development. A blue coloured complex, molybdenum blue was formed as the phenol undergoes a complex redox reaction with phosphomolibdic acid present in Folin-Ciocalteau reagent in alkaline medium. Absorbance was read at 650 nm.

The detector was calibrated for quantification of total phenols using following procedure. The total phenols in the extracts were assayed in terms of catechin taken as the reference. 100 mg of catechol dissolved in 100 ml of distilled water was taken as stock solution. Working standards were prepared from this. Pipetted out 1 ml aliquot from the stock solution into a 10 ml standard flask and made up the volume. For the measurement of absorbance value, pipetted out 0.2 ml from this to a test tube and made up the volume to 3 ml with distilled water followed by the addition of 0.5 ml of Folin-Ciocalteau reagent. Kept it for three minutes and 2 ml of 20 percent Na_2CO_3 solution was added and mixed thoroughly. The absorbance was read at 650 nm.

Concentration of phenols present in the extract was worked out by substituting the absorbance value, thus obtained in the calibration equation. The total phenol content was calculated as mg catechol equivalent of phenol per gram sample and expressed it as percent.

$$\text{Total phenol} = \frac{\text{OD sample}}{\text{OD standard}} \times \frac{\text{Conc.of standard}}{\text{Vol.of sample}} \times 100$$

Where, OD sample = absorbance value of sample

OD standard = absorbance value of standard

3.2.3 Protein estimation

Method: Lowry's method

Materials: Reagent A (Sodium carbonate 2% in 0.1 N NaOH)

Reagent B (Copper sulphate 0.5% in potassium sodium tartarate)

Reagent C (Mix 50 ml reagent A and 1 ml Reagent B)

Reagent D (Folin - Ciocalteu reagent)

Protein standard solution (0.2 mg/ml)

Test sample solution (100 mg)

The Lowry's method of protein estimation was described by Lowry *et al.*, (1951). The phenolic group of tyrosine and tryptophan residues (amino acids) present in protein will produce a blue colour when react with Folin-Ciocalteu reagent which consists of sodium tungstate, molybdate and phosphate. The maximum absorption in the region of 660 nm wavelength. The intensity of colour directly depends on the amount of protein present in the sample.

0.1 g of the defatted powdered sample was ground using 10 ml tris buffer, centrifuged and filtered. Exactly 0.1 ml taken from this and made up to 1 ml in a test tube. Five ml reagent C was added to this and mixed well. Kept it for 10 minutes. Then 0.5 ml Folin Ciocalteu (FC) reagent was added, mixed well and kept it for 30 minutes in darkness for blue colour development. The absorbance was read at 660 nm. The intensity of colour depends on the amount of protein in the sample. The total protein was calculated as mg protein/100ml sample and expressed it as percent.

In order to calibrate the instrument, exactly 0.1 g albumin bovine fraction powder was dissolved in 100 ml water. From this 20 ml was taken and made up to 100 ml. This is the protein standard. From this, working standards were prepared; 0.1, 0.2, 0.4, 0.6, 0.8, 1 ml and made up the volume to 1 ml with distilled water in test tubes. Five ml of reagent C was added to each test tube. Mixed well and kept it for 10 minutes. Then added 0.5 ml FC reagent to each test tube and kept for 30 minutes in darkness at room temperature till blue colour developed. The absorbance was read at 660 nm and the instrument was calibrated.

$$\text{Protein content} = \frac{\text{OD sample}}{\text{OD standard}} \times \frac{\text{Conc.of standard}}{\text{Vol.of sample}} \times 100$$

Where, OD sample = absorbance value of sample

OD standard = absorbance value of standard

3.2.4 Total alkaloid estimation

Method: Harborne method

Materials: Powdered sample-2 g

Ethanol

Acetic acid

Ammonium hydroxide

Two grams of defatted bean powder was weighed and taken in a 250 ml beaker containing 80 ml of 10 percent acetic acid in ethanol. Kept covered and allowed it to stand for four hours. Filtered the extract and concentrated on a water bath to bring down to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered using Whatman filter paper. The residue was dried, weighed and expressed as total alkaloid content in percent (Harborne, 1973).

3.2.5 Total Soluble Solids (TSS)

The total soluble solids (TSS) of the mucilage of cocoa seed was measured using refractometer (Alvarez *et al.*, 2003). To measure the TSS, the daylight plate was lifted up and the mucilage content was placed on top of the prism assembly. Then the daylight plate is closed so that the mucilage content spreads across the entire surface of the prism without any air bubbles or dry spots. The refractometer was held in the direction of natural light source and when looked through the eyepiece, a circular field with markings and a partition with blue colour in the top and white below was found. The partition line indicates the TSS value and expressed it as degree brix.

3.2.6 Scoring of quantitative characters

The mean of the quantitative characters was scored and ranked using Duncans Multiple Range Test (DMRT). Based on this score, hybrids were ranked in order to select the superior hybrids.

The selected hybrids were further evaluated for fermentation index, fermentation recovery, pH and moisture content. The KAU released varieties (CCRP 1, 2, 3, 4, 5, 6, 7, 8 and 9) were also evaluated for the same to make comparison with selected hybrids. Then chocolates were prepared from the selected hybrids and KAU released CCRP varieties and further evaluated for organoleptic evaluation based on 9 point Hedonic scale.

3.2.7 Fermentation index

The hybrids were screened based on morphological and biochemical evaluation and superior hybrids were selected with premium quality. The pods were harvested from the selected hybrids and subjected to fermentation in order to find out the degree of fermentation (Fermentation index) using cut test (Sadasivam and Manickam, 1996).

Fermentation was carried out in bamboo basket. Ripened pods were harvested, split opened and 2 kg of beans were collected. The beans were tightly kept inside the

basket and covered the top with banana leaves and kept on an elevated surface to facilitate the exudate to flow off. After 24 hours the basket was covered with sack and a weight was placed above it. Cocoa beans were turned twice at 48 and 96 hours to ensure uniform fermentation. Fermentation was completed within 7 days.

Hundred seeds from the fermented lot of each hybrid was taken and cut longitudinally with a sharp knife and observed the cotyledon colour by placing on a white back ground. Based on the colour, beans were characterized into fully fermented, partially fermented, not fermented, slaty and mouldy. White colour at center or full dark brown colour indicated as fully fermented, partly pink colour or brown colour across and along margin indicated as partially fermented beans, fully purple colour indicated as not fermented, dark black colour indicated as slaty beans. The value is expressed in percent based on the number of beans recorded under each category.

3.2.8 Fermentation recovery

Ripened pods were harvested, split opened and 2 kg of beans were collected. Fermentation was carried out using bamboo basket method. After fermentation the beans were dried and dry weight was recorded. Then fermentation recovery was estimated and expressed in percent.

$$\text{Fermentation Recovery} = \frac{\text{Dry weight after fermentation (g)}}{\text{Fresh weight before fermentation (g)}} \times 100$$

3.2.9 pH estimation

pH of selected hybrids after fermentation was recorded. Five gram samples of beans was homogenized for 30 s in 100 ml of hot distilled water and vacuum filtered through Whatman filter paper. A 25 ml aliquot was pipetted into a beaker and the pH was measured using a pH meter (AOAC, 2005).

3.2.10 Moisture content

Moisture content of the selected hybrids after fermentation and drying was measured using moisture meter. Five gram powdered sample was used for the estimation and the moisture content was expressed in percent (AOAC, 2005).

3.3 Chocolate preparation

Chocolates were prepared from the selected hybrids for further organoleptic evaluation. Chocolates were also prepared from KAU released varieties; CCRP1, 2, 3, 4, 5, 6, 7, 8 and 9 as standards. For chocolate preparation, the dried beans were cleaned to remove any foreign materials and to separate the small or broken beans. After that alkalization was done by using 0.1 sodium bicarbonate in order to neutralize the beans and to improve the colour and flavour. Then beans were roasted in hot air (120-125°C) for two minutes and immediately after roasting the shells were separated by a process called kibbling. After that cocoa nibs (shelled cocoa beans) were ground to get mass or liquor and milk chocolates were prepared by the procedure standardized by KAU (Amma *et al.*, 2004). The moulded, tempered chocolate was wrapped in aluminum foil and maintained at 7°C until evaluation.

Ingredients

- Cocoa powder: 200g
- Cocoa butter: 250g
- Milk powder: 250g
- Powdered sugar: 500g

3.4 Sensory evaluation

Organoleptic evaluation of chocolates prepared from the selected hybrids and the standards (KAU released varieties) were performed by a panel of thirty judges. Appearance, colour, flavour, texture, odour, taste, after taste and overall acceptability were the characters evaluated based on nine point Hedonic scale (Jinap *et al.*, 1995).

The chocolates were brought to room temperature prior to sensory evaluation. Chocolate samples for evaluation were prepared by cutting the chocolate bar into squares. A maximum of three chocolate samples were evaluated in each session to reduce perception fatigue. Warm water was used for rinsing the mouth in between sample tasting. The panel members performed a multiple comparison test for flavour preference. They were characterized into inferior flavour, medium flavour and superior flavour based on 9 point Hedonic scale (scale of 1-9); scale 1 to 4 indicates inferior flavour (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly), scale 5 indicates medium flavour (5 = neither like nor dislike) and scale 6 to 9 indicates superior flavour (6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely). Hedonic values was converted to rank scores and rank was analyzed by using Kendall's coefficient of concordance.

3.5 Pests and disease scoring

Scoring of pests and diseases were carried out by observing the pod throughout the three seasons.

$$\text{Pests/ disease score} = \frac{\text{No.of infected pods/tree}}{\text{Total no.of pods /tree}} \times 100$$

3.6 Statistical analysis

3.6.1 Analysis of variance

Analysis of variance of quantitative characters and biochemical characters on 30 hybrids were carried out using WASP (Web Agri Stat Package) software.

3.6.2 Cluster analysis

The genetic associations among the genotypes based on qualitative characters of pod and bean was measured by Jaccard's similarity coefficients (Jaccard, 1908) using NTSYS pc version 2.1 (Rohlf, 1992). Cluster analysis was carried out based on similarity matrix and constructed a dendrogram by Unweighted Pair- Group Method

(UPGMA) (Sneath and Sokal, 1973). Clustering of hybrids based on quantitative characters and genetic divergence among and between clusters were carried out by using D^2 statistics developed by Mahalanobis (1936).

3.6.3 Correlation studies

It was used to study the nature and relationship among various traits. The relationship among qualitative characters was studied by using Spearman coefficient (non-parametric) and the association among quantitative characters was studied by Pearson coefficient (Parametric).

3.6.4 Path coefficient analysis

Through path coefficient analysis the correlation between a particular cause and effect will be partitioned into direct and indirect effects of the various causal factors on the effect factor. The technique was suggested by Wright (1921) and Li (1955) using the formula given by Dewey and Lu (1959) (Table 2).

Table 2. Path analysis range

Scale	Effect
0.00 - 0.09	Negligible
0.10 - 0.19	Low
0.2 - 0.29	Moderate
0.30 - 1.00	High
More than 1	Very high

Results



4. RESULTS

The study entitled 'Evaluation of selected cocoa (*Theobroma cacao* L.) hybrids bred for quality' was conducted to evaluate the hybrids derived as a result of hybridization programme designed for improving quality of cocoa at Cocoa Research Centre (CRC), Vellanikkara during 2004. High quality Criollo types were selected and crossed with high yielding Forastero types with moderate disease tolerance. As a result, 240 hybrids were established in the field during 2005-06. These hybrids were observed for their general vigour and yield. Thirty high yielders were selected based on their initial performance. The results thus obtained through evaluation of thirty hybrids based on their qualitative and quantitative pod and bean characters, biochemical parameters, quality parameters and sensory attributes are presented below.

Evaluation of cocoa hybrids

4.1 Morphological characterization

Morphological observations on distinguishable quantitative and qualitative characters were recorded on five pods collected from each hybrid using the descriptor developed by Bekele and Butler, (2000).

4.1.1 Qualitative evaluation

The observations on qualitative characters are described in Table 3. Qualitative evaluation was carried out by recording seven qualitative characters. Pod shape, pod apex form, pod basal constriction, colour of ripe and unripe pod, pod rugosity and colour of bean (cotyledon colour) were the important qualitative characters measured. All the qualitative characters showed wide variation among the hybrids.

4.1.1a Pod shape

The hybrids evaluated classified under four different pod shapes such as cundeamor, amelonado, criollo and angoleta (Plate 2). Out of the thirty hybrids, sixteen hybrids (53%) showed angoleta shape (oval). Criollo shape was observed in nine



Criollo



Angoleta



Amelonado



Cundeamor

Plate 2. Descriptor states for pod shape

hybrids (30%), which were characterized by acute apex with slight basal constriction in most of the hybrids and attenuate apex in a few. The hybrids; Hyb.1, Hyb.11, Hyb.14 and Hyb.15 showed cundeamor (ridged and with bottle neck) shape characterized by intense rugosity. Amelonado shape (melon) was observed in Hyb.19 which was characterized by obtuse apex and slight basal constriction.

4.1.1b Pod apex form

Pod apex form was classified into six forms according to the descriptor. Three types were only observed in the study; acute, attenuate and obtuse (Plate 3). Sixteen hybrids (56%) exhibited acute apex form, nine hybrids (30%) with obtuse apex form and four hybrids with attenuate apex form.

4.1.1c Pod basal constriction

Pod basal constriction was classified into slight, intermediate, strong and absent (Plate 4). Seventy three percent of the hybrids showed slight pod basal constriction. Six hybrids showed intermediate basal constriction and it was absent in Hyb.21 and Hyb.25.

4.1.1d Ripe and unripe pod colour

Colour of ripe and unripe pod (Plate 5 and Plate 6) showed variation among the hybrids. Purplish yellow colour in ripe pods and purplish green colour in unripe pods observed in hybrids; Hyb.8, Hyb.12, Hyb.16 and Hyb.22. Forty three percent of the hybrids observed with greenish yellow colour in pod when ripe and 33 percent with yellowish green when ripe. The remaining three hybrids showed yellow pod colour when ripe. Forty percent of the hybrids were characterized by light green colour and twenty six percent showed dark green colour when it was unripe. The remaining hybrids expressed intermediate green colour when it was unripe.



Rounded



Attenuate



Obtuse



Acute

Plate 3. Descriptor states for pod apex form



Absent



Slight



Intermediate



Strong

Plate 4. Descriptor states for pod basal constriction



Absent (Green)



Slight (Greenish)



Intermediate (Yellowish green)



Intense (Yellow)

Plate 5. Descriptor states for colour of ripe pods



Light green



Intermediate green



Dark green



Purplish green

Plate 6. Descriptor states for colour of unripe pods

Table 3. Qualitative pod and bean characters of hybrids

Hybrids	Pod Shape	Colour of ripe pod	Colour of unripe pod	Pod apex	Pod basal constriction	Rugosity	Bean colour
Hyb.1	cundeamor	yellow	light green	acute	slight	intense	Medium purple
Hyb.2	criollo	yellowish green	light green	attenuate	slight	medium	dark purple
Hyb.3	angoleta	yellowish green	intermediate green	attenuate	intermediate	medium	medium purple
Hyb.4	angoleta	yellowish green	light green	acute	slight	intense	mixed
Hyb.5	angoleta	yellow	dark green	obtuse	slight	medium	dark purple
Hyb.6	angoleta	yellowish green	light green	acute	slight	medium	dark purple
Hyb.7	criollo	yellowish green	light green	acute	slight	medium	mixed
Hyb.8	criollo	purplish yellow	purplish green	acute	slight	medium	mixed
Hyb.9	criollo	yellowish green	intermediate green	acute	slight	medium	white
Hyb.10	criollo	yellowish green	light green	acute	slight	intense	light
Hyb.11	cundeamor	greenish yellow	intermediate green	acute	intermediate	medium	mixed
Hyb.12	criollo	purplish yellow	purplish green	acute	slight	medium	mixed
Hyb.13	angoleta	yellow	light green	obtuse	slight	medium	white
Hyb.14	cundeamor	yellowish green	dark green	acute	intermediate	intense	mixed
Hyb.15	cundeamor	greenish yellow	light green	attenuate	intermediate	intense	mixed

Hyb.16	angoleta	purplish yellow	purplish green	obtuse	intermediate	slight	white
Hyb.17	criollo	yellowish green	light green	acute	slight	medium	white
Hyb.18	angoleta	yellowish green	intermediate green	obtuse	slight	medium	mixed
Hyb.19	amelonado	greenish yellow	light green	obtuse	slight	medium	dark purple
Hyb.20	criollo	greenish yellow	dark green	attenuate	intermediate	medium	light
Hyb.21	angoleta	greenish yellow	intermediate green	acute	absent	slight	mixed
Hyb.22	angoleta	purplish yellow	purplish green	obtuse	slight	medium	mixed
Hyb.23	angoleta	greenish yellow	dark green	acute	slight	slight	light
Hyb.24	angoleta	greenish yellow	light green	obtuse	slight	slight	dark purple
Hyb.25	angoleta	greenish yellow	dark green	acute	absent	slight	mixed
Hyb.26	angoleta	greenish yellow	dark green	acute	slight	slight	dark purple
Hyb.27	angoleta	greenish yellow	light green	obtuse	slight	slight	mixed
Hyb.28	angoleta	greenish yellow	intermediate green	obtuse	slight	slight	mixed
Hyb.29	criollo	greenish yellow	dark green	acute	slight	slight	mixed
Hyb.30	angoleta	greenish yellow	dark green	acute	slight	slight	mixed

4.1.1e Pod rugosity

Pod rugosity (Plate 7) is the smoothness of the surface of pod. Fifty percent of the hybrids observed with medium rugosity and thirty six percent with slight rugosity. The hybrids; Hyb.4, Hyb.10, Hyb.14 and Hyb.15 showed intense rugosity on the pod surface.

4.1.1f Cotyledon colour

In the descriptor, six colours were described for bean colour *viz.*, white, grey, light purple, medium purple, dark purple and mottled. In the present study, wide variation was observed in the colour of cotyledon between the hybrids (Plate 8). Variation of colour from white to dark purple was also observed within the seeds of the same hybrid and it is represented as mixed colour and given a descriptor value of 7. Fifteen hybrids were characterized with mixed beans, six with dark purple and three with light purple. The hybrids; Hyb.1 and Hyb.3 observed with medium purple colour and the hybrids; Hyb.9, Hyb.13, Hyb.16 and Hyb.17 expressed with white cotyledon colour, which is the typical character of Criollo.

4.1.1.1 Correlation among qualitative characters

Correlation studies were carried out in qualitative characters among thirty hybrids and it is presented in the Table 4. The study revealed that pod apex is negatively and significantly correlated with pod basal constriction (-0.366). It indicates that when pod apex form is attenuate, basal constriction will be intermediate and when pod apex is acute then basal constriction will be intermediate or slight. Also when pod apex is obtuse, basal constriction will be slight or intermediate.

Pod basal constriction is positively correlated with rugosity of pod surface (0.384). It indicates that when basal constriction is absent, rugosity will be slight and when it is slight, rugosity of pod surface will be slight or intermediate. Similarly intermediate basal constriction results in intermediate or strong rugosity.



Slight



Medium



Intense

Plate 7. Descriptor states for pod rugosity



White



Light purple



Medium purple



Dark purple

Plate 8. Descriptor states for cotyledon colour

Table 4. Correlation among qualitative characters

	PS	RPC	UPC	PA	PBC	RS	CC
PS	1						
RPC	0.194	1					
UPC	0.045	0.036	1				
PA	-0.162	-0.035	0.088	1			
PBC	-0.261	0.097	0.101	-0.366(*)	1		
RS	0.112	0.35	-0.238	-0.283	0.384(*)	1	
CC	0.213	-0.006	-0.002	-0.154	-0.079	-0.056	1

*Correlation is significant at 0.05 level

PS - Pod shape; RPC – Ripe pod colour; UPC – Unripe pod colour; PA - Pod apex; PBC - Pod basal constriction; RS – Rugosity; CC - Cotyledon colour

4.1.1.2 Clustering based on qualitative characters

Agglomerative hierarchical clustering based on Jaccard's similarity coefficient was done using the UPGMA method with 6 qualitative characters. Dendrogram was constructed and presented in Fig. 1.

Thirty hybrids were grouped into ten clusters at 50 percent similarity level based on seven qualitative characters. Cluster along with the hybrids are presented in Table 5. Cluster II comprises of 7 hybrids; Hyb.2, Hyb.4, Hyb.6, Hyb.7, Hyb.9, Hyb.10 and Hyb.17, which is the largest cluster formed. Cluster VI formed with 6 hybrids and they were characterized with similar qualitative characters. In this cluster, both angoleta and criollo pod shape observed along with greenish yellow ripened pod, acute apex form, slight basal constriction and smooth pod surface.

Two clusters were formed with four hybrids each; Cluster IV and V. The hybrids; Hyb.19, Hyb.24, Hyb.27 and Hyb.30 were formed under cluster V, which was characterized by angoleta shape with greenish yellow ripe pod colour, light green unripe pod colour, obtuse apex form, slight basal constriction and dark purple or mixed bean colour. Members of cluster IX include Hyb.11, Hyb.14 and Hyb.15. Cluster VII, VIII and X were formed by one hybrid each. Cluster III comprises of Hyb.8 and Hyb.12 and they are identical with respect to qualitative characters.

Fig. 1 Dendrogram based on qualitative characters

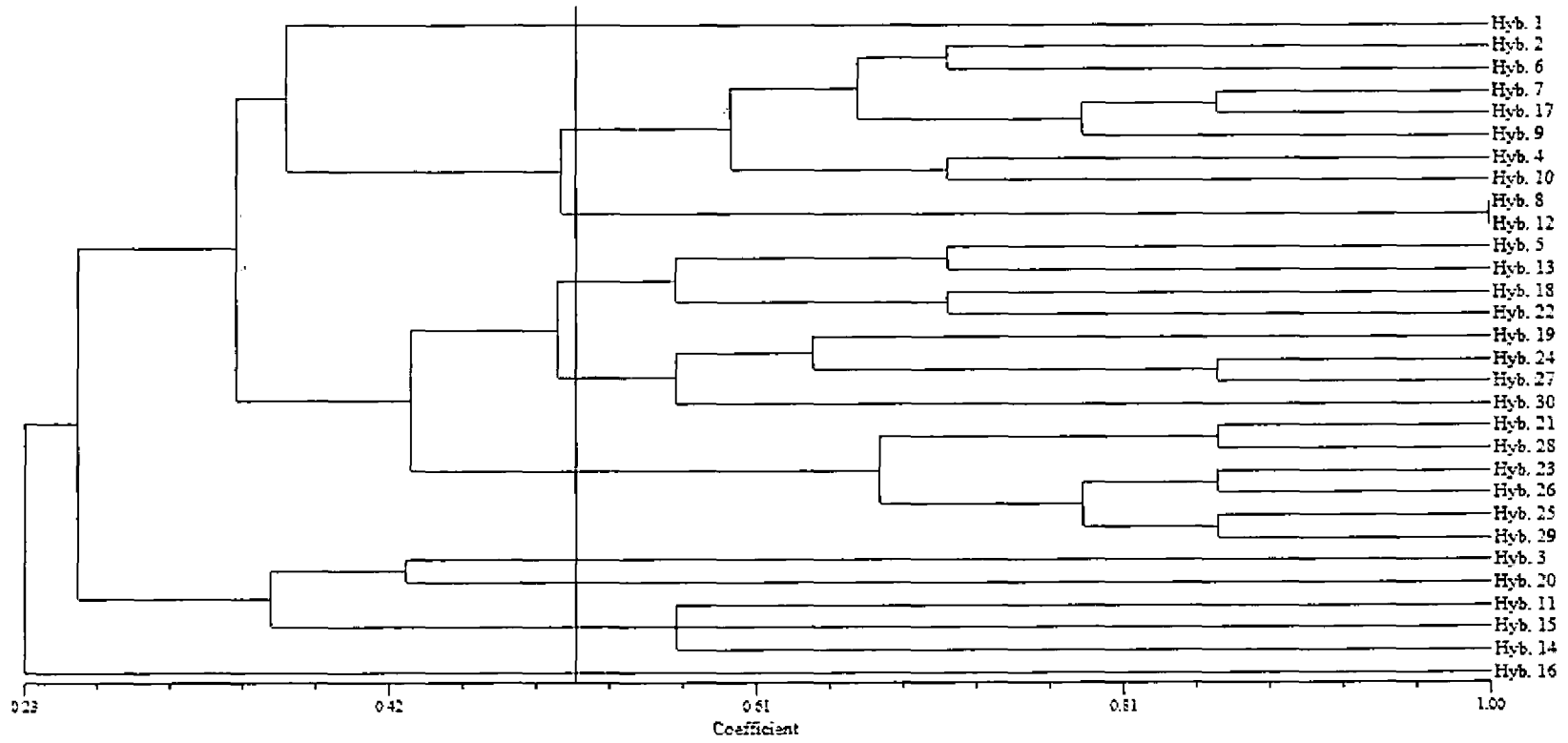


Table 5. Clustering based on qualitative characters

Cluster No.	No. of hybrids	Hybrid no.
Cluster I	1	Hyb.1
Cluster II	7	Hyb.2, Hyb.6, Hyb.7, Hyb.9, Hyb.17, Hyb.4, Hyb.10
Cluster III	2	Hyb.8, Hyb.12
Cluster IV	4	Hyb.5, Hyb.13, Hyb.18, Hyb.22
Cluster V	4	Hyb.19, Hyb.24, Hyb.27, Hyb.30
Cluster VI	6	Hyb.21, Hyb.23, Hyb.25, Hyb.26, Hyb.28, Hyb.29
Cluster VII	1	Hyb.3
Cluster VIII	1	Hyb.20
Cluster IX	3	Hyb.11, Hyb.15, Hyb.14
Cluster X	1	Hyb.16

4.1.2 Quantitative evaluation

4.1.2.1 Pod characters

The mean values of pod characters of hybrids are presented in Table 6. All the pod characters showed significant variation among the hybrids except number of ridges and furrows. Analysis of variance was carried out and it was found that significant difference was observed in pod characters among hybrids.

4.1.2.1a Pod weight (g)

A significant difference was expressed for pod weight among the thirty hybrids. The hybrids; Hyb.10, Hyb.12 and Hyb.21 were observed with pod weight of 685 g, 670.92 g and 684 g respectively, which is higher than other hybrids. Pod weight was ranged between 249.64 g and 685 g among the thirty hybrids. In the hybrids; Hyb.2 (249.64 g) and Hyb.3 (259.26 g) values for pod weight were minimum.

4.1.2.1b Pod length (cm)

Pod length was observed maximum in the Hyb.11 (20.22 cm) followed by the Hyb.17 with a pod length of 19.92 cm. Pod length was recorded in the range of maximum value of 20.22 cm to a minimum value of 12.56.

4.1.2.1c Pod breadth (cm)

The highest pod breadth was recorded in Hyb.19 (9.46 cm) followed by the hybrids; Hyb.17 (9.16 cm) and Hyb.16 (9.14 cm) respectively. The least pod breadth (6.54 cm) was recorded in Hyb.2.

4.1.2.1d Number of ridges and furrows

Number of ridges and furrows did not expressed any significant difference among the hybrids. Except hybrids; Hyb.9 and Hyb.11, all the other hybrids exhibited ten ridges and furrows.

4.1.2.1e Number of beans per pod

Average number of beans per pod was observed in the range of 28.80 in Hyb.3 to 53 in Hyb.26. The Hyb.26 (53) showed maximum number of beans per pod followed by the Hyb.30 (49.20).

4.1.2.1f Wet bean weight per pod (g)

The highest wet bean weight per pod was recorded in the Hyb.21 (185.72 g) followed by Hyb.30 (178.6 g), Hyb.9 (175 g) and Hyb.12 (174.54 g). The total wet bean weight character showed 13.24% coefficient of variation. The Hyb.2 was observed with least wet bean weight (74.38 g).

The pod characters were scored using DMRT technique and ranked based on the score obtained. The score obtained for all the pod characters are presented in Appendix I. The hybrids; Hyb.12, Hyb.10, Hyb.17, Hyb.22, Hyb.29 and Hyb.11 were selected as superior ones.

Table 6. Mean values of pod characters of hybrids

Hybrids	Pod weight (g)	Pod length (cm)	Pod breadth (cm)	No. of ridges and furrows	No. of beans/pod	Total wet bean weight (g)	Total score	Rank
Hyb.1	468.06	13.96	8.36	10.00	42.40	141.70	32.0	17
Hyb.2	249.64	14.08	6.54	10.00	45.40	74.38	48.0	26
Hyb.3	259.26	12.56	7.70	10.00	28.80	59.08	51.0	28
Hyb.4	313.10	16.16	7.40	10.00	39.40	82.08	47.0	25
Hyb.5	415.30	14.66	7.68	10.00	43.60	98.08	41.0	22
Hyb.6	530.70	16.12	8.90	10.00	45.00	164.36	19.5	8
Hyb.7	508.66	17.68	8.02	10.00	45.80	157.60	24.0	9
Hyb.8	354.00	15.60	7.14	10.00	47.00	120.96	41.0	22
Hyb.9	406.60	16.00	7.52	9.00	45.60	175.00	28.5	13
Hyb.10	685.60	19.00	9.18	10.00	43.00	171.46	12.0	2
Hyb.11	569.00	20.22	8.48	9.00	44.20	159.62	16.3	5
Hyb.12	670.92	18.46	9.02	10.00	47.80	174.54	9.0	1
Hyb.13	300.84	13.10	7.46	10.00	34.40	77.80	50.0	27
Hyb.14	460.96	19.72	7.80	10.00	35.40	97.98	37.5	19
Hyb.15	421.60	18.60	7.38	10.00	45.20	152.84	31.0	15
Hyb.16	553.02	16.02	9.14	10.00	39.60	138.22	25.5	11
Hyb.17	636.82	19.92	9.16	10.00	43.80	153.70	14.0	3
Hyb.18	407.22	14.56	8.26	10.00	41.20	121.82	39.0	20
Hyb.19	601.22	15.98	9.46	10.00	43.00	163.80	16.8	6
Hyb.20	299.40	14.18	7.46	10.00	41.80	122.92	44.0	24
Hyb.21	684.00	15.96	9.06	10.00	43.80	185.72	14.0	3
Hyb.22	396.60	13.86	8.04	10.00	38.00	98.80	43.5	23
Hyb.23	405.00	14.14	7.62	10.00	41.80	131.26	39.5	21
Hyb.24	530.00	15.54	7.98	10.00	43.60	143.34	29.5	14
Hyb.25	500.00	15.50	9.00	10.00	43.20	147.72	24.5	10
Hyb.26	454.00	15.64	7.66	10.00	53.00	127.16	31.5	16
Hyb.27	400.00	13.84	7.80	10.00	47.80	135.70	34.5	18
Hyb.28	520.00	15.32	8.60	10.00	44.80	134.60	27.0	12
Hyb.29	612.00	17.06	8.28	10.00	48.80	165.60	16.0	4
Hyb.30	566.00	14.70	8.54	10.00	49.20	178.60	17.0	7
C. D. (0.05)	75.98	1.90	0.71	N.S.	4.92	22.19		
CV (%)	12.97	9.60	7.00	N.S.	9.18	13.24		

Table 7. Mean values of low value preferred pod characters

Hybrids	Husk thickness (cm)	No. of flat beans	Flat bean percent	Total score	Rank
Hyb.1	0.90	1.0	2.40	14.5	7
Hyb.2	0.81	2.4	5.44	14.0	8
Hyb.3	0.89	2.8	9.39	8.5	16
Hyb.4	0.80	2.4	6.11	14.0	8
Hyb.5	0.87	0.8	1.78	16.5	5
Hyb.6	0.94	1.2	2.72	14.5	7
Hyb.7	0.97	1.0	2.18	13.5	9
Hyb.8	0.83	1.4	2.98	16.5	5
Hyb.9	0.76	1.2	2.64	18.5	2
Hyb.10	1.00	1.0	2.32	12.5	11
Hyb.11	0.98	1.4	3.13	11.5	13
Hyb.12	0.93	0.8	1.67	15.0	6
Hyb.13	0.84	2.8	8.78	11.5	13
Hyb.14	0.92	1.0	2.80	14.0	8
Hyb.15	0.70	0.4	0.91	20.5	1
Hyb.16	1.09	2.8	7.53	5.5	17
Hyb.17	0.98	0.6	1.41	12.5	11
Hyb.18	0.93	0.8	1.96	15.0	6
Hyb.19	1.15	3.8	9.53	3.0	18
Hyb.20	1.00	1.2	2.85	12.5	11
Hyb.21	1.00	0.8	1.85	13.5	9
Hyb.22	0.85	2.8	7.41	12.0	12
Hyb.23	0.67	2.0	4.89	18.0	3
Hyb.24	0.96	2.2	5.01	11.0	14
Hyb.25	1.07	1.8	4.18	10.5	15
Hyb.26	1.08	0.4	0.80	13.0	10
Hyb.27	0.78	2.0	4.22	17.0	4
Hyb.28	1.00	1.6	3.66	12.0	12
Hyb.29	0.94	1.8	3.71	13.5	9
Hyb.30	0.97	1.6	3.25	12.5	11
CD(0.05)	0.16	1.46			
CV (%)	13.56	73.99			

4.1.2.2 Low value preferred pod characters

Husk thickness and no. of flat beans per pod are the characters which require minimum value. The mean values of low value preferred pod characters of hybrids are presented in Table 7. Analysis of variance was carried out and it was found that significant difference was observed among the hybrids.

4.1.2.2a Husk thickness (cm)

The husk thickness value should be lower for the selection. Husk thickness was calculated by taking the average of ridge and furrow thickness (Plate 9). Among the thirty hybrids, Hyb.23 (0.67 cm), Hyb.15 (0.70 cm) and Hyb.9 (0.76 cm) were scored with least husk thickness. The Hyb.19 recorded with highest husk thickness (1.15 cm). Husk thickness below 1 cm is the desirable character.

4.1.2.2b Number of flat beans per pod

The number of flat beans per pod should be minimum for the criteria of selection. Average value of flat beans was observed in the range of 0.4 to 3.8 per pod. The Hyb.19 was observed with nine percent flat beans in the pod, which is the highest among hybrids and the Hyb.26 with only 0.8 percent flat beans in the pod. The Hyb.15 was also observed with low number of flat beans per pod.

The low value preferred pod characters were scored using DMRT technique and ranked to select the superior hybrids among the thirty hybrids based on the score obtained. The score obtained for all the low value preferred pod characters are presented in Appendix II. Among the hybrids; Hyb.15 ranked as first followed by the hybrids; Hyb.9, Hyb.23, Hyb.27, Hyb.8 and Hyb.5 in second, third, fourth, fifth and sixth position respectively based on the score obtained.

4.1.2.3 Bean characters

The mean values for bean characters are presented in Table 8. Analysis of variance with bean characters was carried out and found that all the bean characters showed significant difference among the thirty hybrids.



Pod length



Pod breadth



Peeling



Husk thickness

Plate 9. Quantitative evaluation

4.1.2.3a Unpeeled wet bean weight (g)

The highest unpeeled wet bean weight was observed in Hyb.10 (91.16 g) followed by the Hyb.6 and Hyb.11 with 80.08 g and 76.20 g respectively. Lowest unpeeled bean weight was observed in the Hyb.2 (30.36 g).

4.1.2.3b Peeled wet bean weight (g)

The outer mucilage was removed using forceps and 20 beans were taken as the standard for calculating the weight of peeled beans. The Hyb.11 was recorded with highest peeled wet bean weight (41.82 g). The peeled wet bean weight was observed in the range of 15.34 g to 41.82 g.

4.1.2.3c Peeled dry bean weight (g)

Dry bean weight of peeled twenty beans was recorded. Among the thirty hybrids; Hyb.11 was observed with highest dry bean weight (30.29 g) followed by the hybrids; Hyb.17, Hyb.21, Hyb.6, Hyb.10, Hyb.30 and Hyb.12 with 24.22 g, 23.12 g, 22.94 g, 22.82 g, 22.38 g and 22.34 g respectively. The least dry bean weight was showed by the Hyb.2 (30.36 g).

The bean characters were scored using DMRT technique and ranked to select the superior hybrids among the thirty hybrids based on the score obtained. The score obtained for all the bean characters are presented in Appendix III. The hybrids; Hyb.11, Hyb.6, Hyb.21, Hyb.17, Hyb.12, Hyb.10 and Hyb.15 showed the superior bean characters.

4.1.2.4 Single bean characters

The mean values for single bean characters are presented in Table 9. Analysis of variance with bean characters was carried out and found that all the bean characters showed significant difference among the thirty hybrids.

4.1.2.4a Unpeeled single bean weight (g)

The unpeeled weight of single bean was recorded and observed that Hyb.10 exhibited with highest unpeeled bean weight (4.56 g) followed by the hybrids; Hyb.11 and Hyb.6 with 3.81 g and 4 g respectively. The Hyb.2 (1.52 g) and Hyb.3 (1.93 g) was with low unpeeled single bean weight.

4.1.2.4b Peeled single bean wet weight (g)

The average values of 20 peeled bean weight was estimated and found that Hyb.11 showed highest weight with 2.09 g, followed by the hybrids; Hyb.18 with 1.97 g and Hyb.6 with 1.96 g.

4.1.2.4c Peeled single bean dry weight (g)

Peeled single bean dry weight is an important economic character considered for the selection of hybrids. It ranged from 0.51 g in Hyb.2 to 1.48 g in Hyb.11. The hybrids; Hyb.11, Hyb.17 and Hyb.23 exhibited more than 1.2 g dry bean weight. A total of 25 hybrids exhibited single dry bean weight of more than 0.8 g, which is considered as the desirable criteria for selection. The hybrids; Hyb.2, Hyb.3, Hyb.4, Hyb.20 and Hyb.27 were observed with a dry bean weight of less than 0.8 g, which is an undesirable character.

4.1.2.4d Peeled bean length (cm)

The highest peeled bean length was expressed by Hyb.11 with 2.21 cm and lowest in Hyb.2 with 1.39 cm.

4.1.2.4e Peeled bean breadth (cm)

The Hyb.11 showed highest value for peeled bean breadth with 1.31 cm, while lowest in Hyb.2 with 0.63 cm.

4.1.2.4f Peeled bean width (cm)

Among the thirty hybrids peeled bean thickness was found highest in Hyb.24 with 0.72 cm and lowest in Hyb.3 with 0.44 cm.

The single bean characters were scored and ranked based on the score obtained using DMRT technique. The score obtained for all the single bean characters are presented in Appendix IV. According to the ranking, hybrids; Hyb.11, Hyb.6, Hyb.17, Hyb.24 and Hyb.12 were selected as superior hybrids.

Table 8. Mean values of bean characters of hybrids

Hybrids	Unpeeled bean weight (g) (20 beans)	Peeled bean weight (g) (20 seeds)	Peeled bean dry weight (g) (20 seeds)	Total score	Rank
Hyb.1	55.16	30.28	21.48	22.0	13
Hyb.2	30.36	15.34	9.96	41.0	26
Hyb.3	38.56	17.02	12.32	39.5	25
Hyb.4	45.34	22.54	14.30	36.0	24
Hyb.5	46.20	21.42	17.26	34.0	22
Hyb.6	80.08	39.12	22.94	6.5	2
Hyb.7	69.00	30.66	20.94	16.5	10
Hyb.8	48.26	25.34	17.18	32.0	20
Hyb.9	71.06	32.54	21.92	13.5	8
Hyb.10	91.16	30.56	22.82	11.5	5
Hyb.11	76.20	41.82	30.29	3.5	1
Hyb.12	70.26	35.44	22.34	11.5	5
Hyb.13	42.56	22.14	16.68	35.5	23
Hyb.14	57.52	24.66	18.84	28.0	16
Hyb.15	73.32	33.76	22.54	12.5	6
Hyb.16	69.78	27.24	18.26	21.5	12
Hyb.17	71.56	35.14	24.22	10.0	4
Hyb.18	60.84	39.38	21.58	13.0	7
Hyb.19	65.42	28.46	18.94	21.5	12
Hyb.20	61.60	22.74	14.68	30.5	19
Hyb.21	75.50	38.12	23.12	8.0	3
Hyb.22	47.30	26.52	19.28	29.5	18
Hyb.23	61.66	28.62	19.20	22.0	13
Hyb.24	74.34	31.50	19.46	15.5	9
Hyb.25	63.84	27.80	16.56	24.5	14
Hyb.26	49.14	24.04	16.52	33.5	21
Hyb.27	50.74	27.68	16.12	29.0	17
Hyb.28	67.70	23.26	17.02	27.0	15
Hyb.29	54.14	30.52	20.96	22.0	13
Hyb.30	69.54	28.66	22.38	17.0	11
CD(0.05)	9.66	3.36	1.36		
CV (%)	12.73	9.43	5.69		

Table 9. Mean values of single bean characters of hybrids

Hybrids	Unpeeled single bean wet weight (g)	Peeled single bean wet weight (g)	Peeled single bean dry weight (g)	Peeled bean length (cm)	Peeled bean breadth (cm)	Peeled bean width (cm)	Total score	Rank
Hyb.1	2.76	1.51	1.04	1.93	1.09	0.48	37.0	12
Hyb.2	1.52	0.77	0.51	1.39	0.63	0.44	68.0	24
Hyb.3	1.93	0.85	0.62	1.54	1.00	0.35	64.5	23
Hyb.4	2.27	1.13	0.79	1.46	0.94	0.45	58.0	22
Hyb.5	2.31	1.07	1.02	1.74	1.10	0.47	49.5	19
Hyb.6	4.00	1.96	1.11	2.19	1.21	0.54	15.0	2
Hyb.7	3.45	1.53	1.07	1.66	1.23	0.36	33.0	10
Hyb.8	2.41	1.27	0.84	1.84	1.02	0.44	51.5	20
Hyb.9	3.55	1.63	1.13	1.81	1.09	0.37	32.0	9
Hyb.10	4.56	1.53	1.06	2.21	1.14	0.45	26.0	5
Hyb.11	3.81	2.09	1.48	2.46	1.31	0.48	9.0	1
Hyb.12	3.51	1.77	1.11	2.44	1.09	0.45	24.5	4
Hyb.13	2.13	1.11	0.86	1.58	0.93	0.44	58.0	22
Hyb.14	2.88	1.23	0.96	1.39	1.00	0.54	48.5	18
Hyb.15	3.67	1.69	1.13	1.67	0.89	0.56	28.5	7
Hyb.16	3.49	1.36	1.19	2.02	1.19	0.44	28.0	6
Hyb.17	3.58	1.76	1.24	2.08	1.11	0.44	22.5	3
Hyb.18	3.04	1.97	1.07	2.03	1.07	0.48	28.0	6
Hyb.19	3.27	1.42	0.97	2.07	0.99	0.45	40.5	14
Hyb.20	3.08	1.14	0.69	1.74	1.09	0.39	51.5	20
Hyb.21	3.78	1.91	1.09	1.57	0.90	0.45	32.0	9
Hyb.22	2.37	1.33	0.98	1.65	1.06	0.44	48.0	17
Hyb.23	3.08	1.43	1.26	1.72	0.97	0.41	38.0	13
Hyb.24	3.72	1.58	1.03	1.63	1.29	0.72	24.5	4
Hyb.25	3.19	1.39	0.83	1.67	0.84	0.42	49.5	19
Hyb.26	2.46	1.20	0.95	1.68	0.80	0.45	57.5	21
Hyb.27	2.54	1.38	0.79	1.99	1.09	0.52	44.0	15
Hyb.28	3.39	1.16	1.03	1.67	0.95	0.43	47.5	16
Hyb.29	2.71	1.53	1.09	2.09	1.18	0.69	29.0	8
Hyb.30	3.48	1.43	1.17	1.64	0.98	0.45	34.5	11
CD(0.05)	0.48	0.17	0.07	0.51	0.06	0.04		
CV (%)	12.73	9.43	12.06	45.33	9.76	15.53		

4.1.2.5 Economic characters of the hybrids

Yield in terms of number of pods/ tree/ year and other economic characters derived from the primary data were worked out using standard formula and the results are explained in table 10.

4.1.2.5a Yield (No. of pods/ tree/ year)

The yield data of the year 2015-16 reported that Hyb.6 yielded 111 pods /tree/ year and it was the highest among the thirty hybrids, followed by the hybrids; Hyb.7, Hyb.15 and Hyb.11 with 108, 107 and 105 pods/ tree/ year respectively. The lowest yield was reported in the Hyb.2 with 63 pods/ tree/ year.

4.1.2.5b Wet bean weight/ pod weight (%)

The Hyb.9 showed highest wet bean weight/ pod weight (43.04 %) among the thirty hybrids. The hybrids; Hyb.20 (41.06 %), Hyb.15 (36.25 %) and Hyb.8 (34.17 %) were also observed with high wet bean weight/ pod weight.

4.1.2.5c Dry matter recovery (%)

Dry matter recovery is the weight of beans obtained after drying. It was observed that Hyb.5 with highest recovery after drying. Dry matter recovery was observed in the range between 81.20 % and 54.81 %. The hybrids; Hyb.30 (78.14 %), Hyb.14 (76.70 %) and Hyb.13 (76.22 %) also exhibited high dry matter recovery percent.

4.1.2.5d Peeling ratio (%)

Peeling ratio is the weight obtained after peeling. Among the thirty hybrids, peeling ratio was highest in the Hyb.18 (64.75 %) followed by the hybrids; Hyb.1, Hyb.22 and Hyb.29 with 56.85 %, 56.56 % and 56.37 % respectively.

4.1.2.5e Pod value (g)

Pod value is the dry bean weight obtained per pod. It was observed in the range from 17.71 g in Hyb.3 to 65.20 g in Hyb.11.

4.1.2.5f Pod index

Pod index is the number of pods required to get 1 kg of dried beans. It should be minimum for the hybrids based on the selection criteria. It was observed minimum in Hyb.11 (15.34). The hybrids; Hyb.30 (17.45), Hyb.17 (18.41) and Hyb.12 (18.85) also showed low pod index value.

4.1.2.5g Efficiency index

Efficiency index indicates the pod weight required to produce 1 g dry bean. Efficiency index should also be minimum for the hybrids. The least value for efficiency index was expressed in Hyb.23 (7.72) followed by the hybrids; Hyb.9 (7.93), Hyb.15 (8.29) and Hyb.11 (8.73).

4.1.2.5h Conversion index

Conversion index can be defined as the amount of dry bean weight obtained from a given amount of wet bean weight. Conversion index was found maximum in Hyb.5 (0.45) followed by Hyb.11 (0.41). The least value was recorded in Hyb.20 (0.23).

Table 10. Economic characters of the hybrids

Hybrids	Yield (no. of pods/tree/year)	Wet bean weight/pod weight (%)	Dry matter recovery (%)	Peeling ratio (%)	Pod value (g)	Pod index	Efficiency index	Conversion index
Hyb.1	88	30.27	71.62	56.85	43.88	22.79	10.67	0.31
Hyb.2	63	29.79	65.67	50.44	22.93	43.62	10.89	0.31
Hyb.3	75	22.79	72.72	45.38	17.71	56.46	14.64	0.30
Hyb.4	68	26.22	65.48	50.16	30.93	32.33	10.12	0.38
Hyb.5	90	23.62	81.20	46.05	44.25	22.60	9.38	0.45
Hyb.6	111	30.97	58.69	49.34	49.95	20.02	10.62	0.30
Hyb.7	108	30.98	68.69	44.88	48.78	20.50	10.43	0.31
Hyb.8	71	34.17	68.12	52.64	39.48	25.33	8.97	0.33
Hyb.9	104	43.04	67.49	45.81	51.30	19.49	7.93	0.29
Hyb.10	88	25.01	75.29	33.90	45.37	22.04	15.11	0.26
Hyb.11	105	28.05	73.20	55.00	65.20	15.34	8.73	0.41
Hyb.12	87	26.02	63.14	50.82	53.06	18.85	12.65	0.30
Hyb.13	101	25.86	76.22	52.75	29.58	33.80	10.17	0.38
Hyb.14	101	21.26	76.70	43.60	33.98	29.43	13.56	0.35
Hyb.15	107	36.25	66.81	46.08	50.85	19.67	8.29	0.33
Hyb.16	100	24.99	67.22	39.02	46.93	21.31	11.78	0.34
Hyb.17	105	24.14	68.93	49.35	54.31	18.41	11.73	0.35
Hyb.18	104	29.92	54.81	64.75	44.08	22.68	9.24	0.36
Hyb.19	85	27.24	66.68	43.86	41.71	23.98	14.41	0.25
Hyb.20	74	41.06	64.56	38.74	28.63	34.92	10.46	0.23
Hyb.21	100	27.15	60.67	50.53	47.74	20.95	14.33	0.26
Hyb.22	88	24.91	72.77	56.56	37.05	26.99	10.70	0.38
Hyb.23	82	32.41	67.13	46.40	52.46	19.06	7.72	0.40
Hyb.24	102	27.05	61.88	42.37	44.69	22.38	11.86	0.31
Hyb.25	82	29.54	59.66	43.78	35.86	27.89	13.94	0.24
Hyb.26	79	28.01	68.69	48.93	50.09	19.97	9.06	0.39
Hyb.27	88	33.93	61.21	54.37	37.76	26.48	10.59	0.28
Hyb.28	92	25.88	73.20	34.60	45.92	21.78	11.32	0.34
Hyb.29	81	27.06	68.70	56.37	52.95	18.89	11.56	0.32
Hyb.30	84	31.55	78.14	41.22	57.32	17.45	9.87	0.32

4.1.2.6 Cluster analysis based on quantitative traits

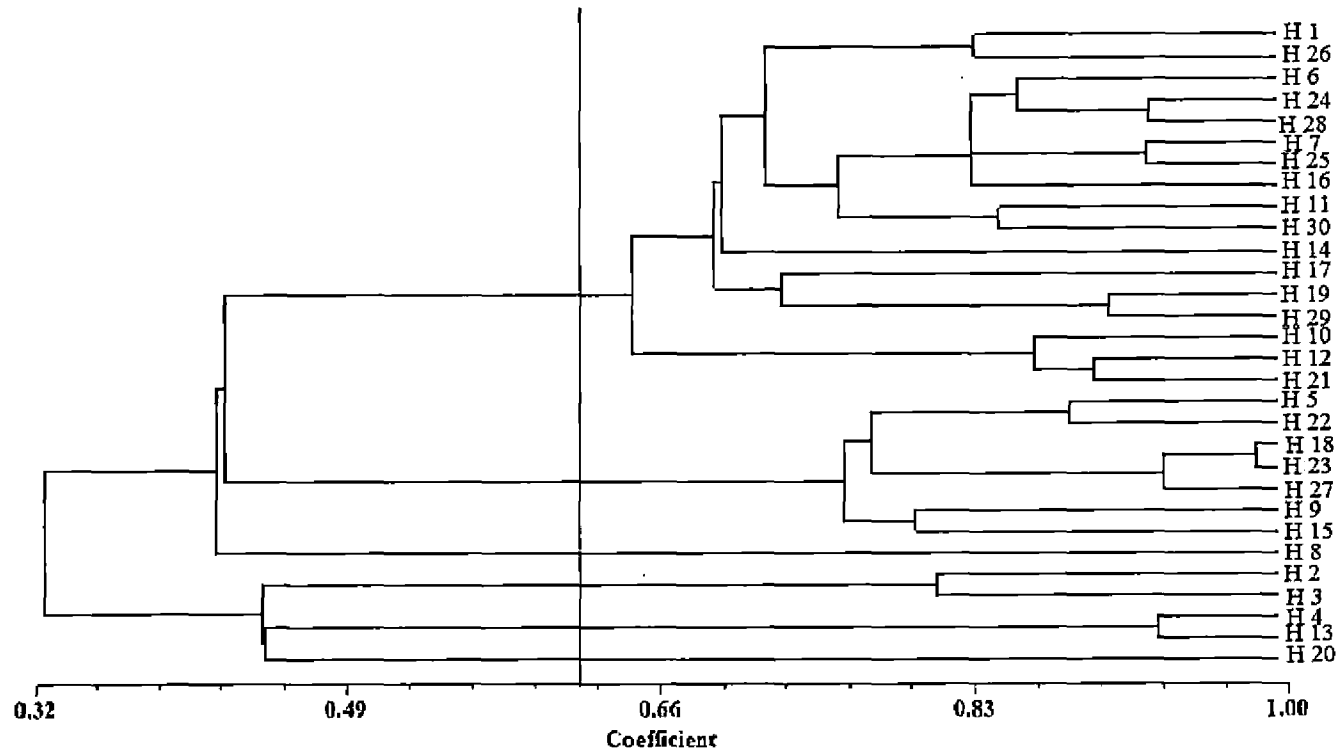
Cluster analysis was done based on Jaccard's similarity coefficient using UPGMA method with quantitative data (Fig. 2) and result obtained in such a way is represented in the form of dendrogram.

Thirty hybrids evaluated were grouped into six clusters at 60 per cent similarity level. These clusters along with their cluster members are presented in Table 11. Cluster I is with maximum members of seventeen hybrids. Cluster II had seven hybrids; Hyb.5, Hyb.22, Hyb.18, Hyb.23, Hyb.27, Hyb.9, Hyb.15. They were similar with respect to quantitative characters. Cluster IV and V are with two members each. Hyb.8 fall in cluster III and Hyb.20 in cluster VI respectively. They are distinct from other hybrids

Table 11. Clustering based on quantitative characters

Cluster No.	No. of Hybrids	Name of Hybrids
Cluster I	17	Hyb.1, Hyb.26, Hyb.6, Hyb.24, Hyb.28, Hyb.7, Hyb.25, Hyb.16, Hyb.11, Hyb.30, Hyb.14, Hyb.17, Hyb.19, Hyb.29, Hyb.10, Hyb.12, Hyb.21
Cluster II	7	Hyb.5, Hyb.22, Hyb.18, Hyb.23, Hyb.27, Hyb.9, Hyb.15
Cluster III	1	Hyb.8
Cluster IV	2	Hyb.2, Hyb.3
Cluster V	2	Hyb.4, Hyb.13
Cluster VI	1	Hyb.20

Fig.2 Dendrogram based on quantitative characters



4.1.2.7 Comparison of qualitative and quantitative clustering patterns

Homology between qualitative and quantitative clustering pattern was worked out for the hybrids studied and it is presented in Table 12.

The analysis based on qualitative characters resulted in ten clusters and that based on quantitative clustering resulted in six clusters. Maximum homology in qualitative and quantitative characters were observed between members in cluster VI of quantitative character. The six members were distributed only in two clusters; cluster I (83.33%) and cluster II (16.6%). Seven members present in cluster II of qualitative characters were split and distributed under cluster I, II, IV and V with a percentage distribution of 57.1% in cluster I and 14% each in cluster III, IV and V. This indicated that even though they are morphologically similar in qualitative character, they showed wide variation in quantitative characters.

Table 12. Comparison of qualitative and quantitative cluster data

Qualitative clusters	No. of hybrids	Quantitative clusters					
		Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	1	100	-	-	-	-	-
Cluster II	7	57.1	14	-	14	14	-
Cluster III	2	50	-	50	-	-	-
Cluster IV	4	-	75	-	-	25	-
Cluster V	4	75	25	-	-	-	-
Cluster VI	6	83.33	16.6	-	-	-	-
Cluster VII	1	-	-	-	100	-	-
Cluster VIII	1	-	-	-	-	-	100
Cluster IX	3	66.6	33.3	-	-	-	-
Cluster X	1	100	-	-	-	-	-

4.1.2.8 Clustering of quantitative characters based on D² statistics

Cluster analysis of thirty hybrids was carried out using 17 quantitative characters by D² statistics. Hybrids were classified into six clusters based on D² statistics. The hybrids included under each cluster are presented in Table 13. Cluster I was found biggest among other clusters which includes 9 hybrids and these hybrids were similar based on qualitative characters. Cluster V includes only two hybrids; Hyb.11 and Hyb.17. Cluster II and cluster VI were having four hybrids each and cluster III with six hybrids.

The inter and intra cluster distance are presented in table 14. Based on the genetic distance, cluster diagram was drawn and it is presented in figure 2. The inter cluster distance was observed maximum between cluster II and cluster V (233.90). The inter cluster distance between cluster I and cluster V (118.29) was also found significant indicating that divergent hybrids placed under these clusters can be effectively crossed between each other for further crop improvement. Intra cluster distance was observed maximum in cluster V indicating that two hybrids under this cluster were divergent to certain extent even though they are grouped together.

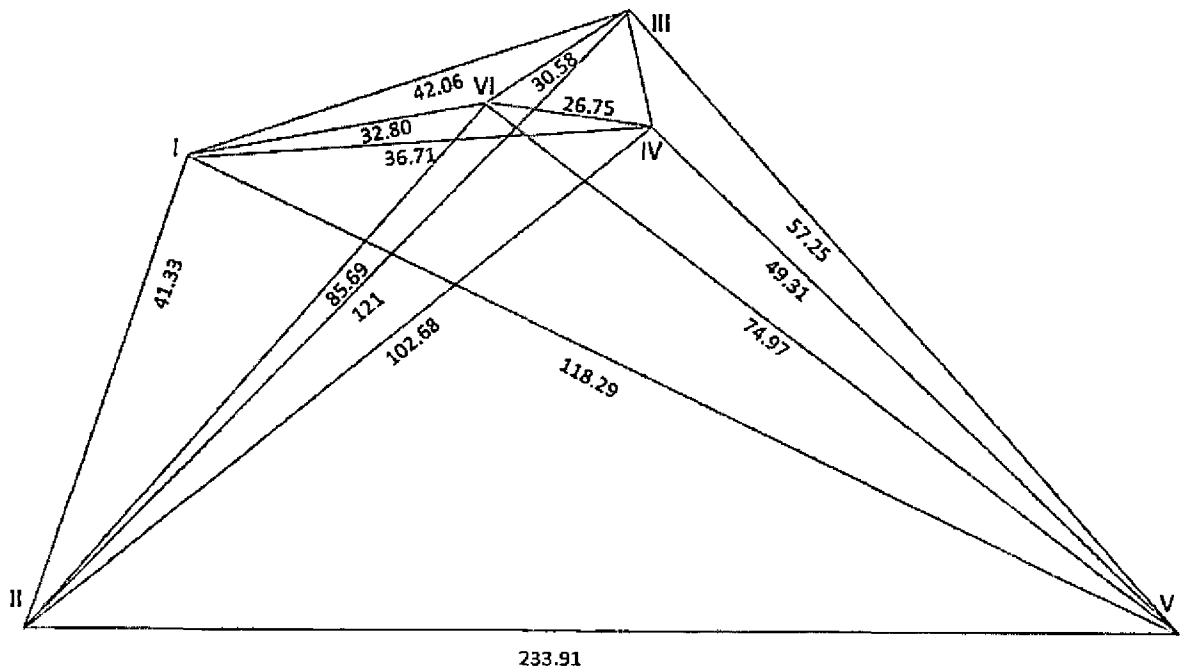
Table 13. Clustering of quantitative characters based on D² statistics

Cluster No.	No. of hybrids	Hybrid no.
Cluster I	9	Hyb.5, Hyb.8, Hyb.13, Hyb.22, Hyb.23, Hyb.25, Hyb.26, Hyb.27, Hyb.28
Cluster II	4	Hyb.2, Hyb.3, Hyb.4, Hyb.20
Cluster III	6	Hyb.1, Hyb.12, Hyb.19, Hyb.21, Hyb.29, Hyb.30
Cluster IV	5	Hyb.6, Hyb.7, Hyb.9, Hyb.15, Hyb.18
Cluster V	2	Hyb.11, Hyb.17
Cluster VI	4	Hyb.10, Hyb.14, Hyb.16, Hyb.24

Table 14. Inter and intra cluster distance based on quantitative characters

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	14.84					
Cluster II	41.33	26.10				
Cluster III	42.06	121.00	15.07			
Cluster IV	36.71	102.68	25.96	12.67		
Cluster V	118.29	233.91	57.25	49.31	39.11	
Cluster VI	32.80	85.69	30.58	26.75	74.97	25.21

Fig 3. Cluster diagram based on inter cluster distance



4.1.3 Descriptive statistics

The descriptive statistics was computed through range (maximum and minimum), mean, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (H^2), genetic advance (GA) and genetic gain (GG) for 15 pod and bean quantitative characters and they are presented in the Table 15.

4.1.3.1 PCV and GCV

The pod and bean characters studied among thirty hybrids revealed that number of flat beans/pod exhibited high variability through the value of PCV (85.27 %) and GCV (74.26 %). Through the computation of PCV of pod and bean characters, it was found that number of flat beans/pod exhibited maximum phenotypic variability followed by seed length (51.28%), seed weight (41.45%) and seed width (37.32%). High GCV was observed for traits like pod weight (25.79%), number of flat beans per pod (41.91%), total wet bean weight per pod (24.55%), peeled (20.59%) and unpeeled bean (21.88%) weight per pod, single bean wet (21.90%), dry weight (39.60%) and single seed length (23.87%), breadth (27.94%) and width (34.06%). Medium PCV and GCV observed for pod length (15.47% and 12.13%), husk thickness (17.74% and 11.32%) and number of beans per pod (13.74% and 10.23%). Low PCV and GCV exhibited for TSS (8.78% and 6.60%). For pod breadth, medium PCV (10.91%) was exhibited but with low GCV (8.42%). Characters exhibited high GCV, gave maximum potential for selection between the hybrids based on these values.

4.1.3.2 Heritability

Among the pod and bean characters of thirty hybrids, peeled bean weight showed high heritability (92.91%) followed by single bean dry weight (91.28%), seed breadth (89.36%), seed width (83.33%), pod weight (79.82%) and wet bean weight (77.48%). All these characters were classified under high heritability. Low heritability was observed with no. of flat beans/pod (24.16%) and bean length (21.66%).

Table 15. Descriptive statistics

Characters	Range		Mean	PCV (%)	GCV (%)	H ² (%)	GA (%)	GG (%)
	Max.	Min.						
Pod wt. (g)	685.60	249.00	472.65	28.87	25.79	79.82	224.33	47.46
Pod length (cm)	19.00	12.56	15.94	15.47	12.13	61.51	3.12	19.60
Pod breadth (cm)	9.46	6.54	8.15	10.91	8.42	59.60	1.09	13.40
Husk thickness (cm)	1.15	0.67	0.93	17.74	11.32	40.74	0.14	14.89
No. of beans/pod	53.00	28.80	43.21	13.74	10.23	55.43	6.78	15.69
No. of flat beans/pod	3.80	0.40	1.59	85.27	41.91	24.16	0.68	42.44
Total wet bean wt./pod (g)	185.72	59.08	135.21	27.89	24.55	77.48	60.19	44.51
Unpeeled bean wt./20 beans (g)	91.16	30.36	61.27	25.31	21.88	74.71	23.87	38.96
Peeled bean wt./20 beans (g)	30.92	9.96	19.34	21.36	20.59	92.91	7.90	40.87
Single wet bean wt. (g)	4.51	1.55	3.06	25.28	21.90	75.00	1.20	39.06
Single dry bean wt. (g)	1.47	0.51	1.00	41.45	39.60	91.28	0.78	77.94
Bean length (cm)	2.46	1.38	1.82	51.28	23.87	21.66	0.42	22.88
Bean breadth (cm)	1.31	0.63	1.04	29.56	27.94	89.36	0.56	54.42
Bean width (cm)	0.72	0.35	0.46	37.32	34.06	83.33	0.30	64.06
TSS (° brix)	22.00	17.00	20.00	8.78	6.60	56.52	2.04	10.22

GCV and PCV : 0 – 10% - low, 10.1 – 20% - moderate, > 20% - high

Heritability : 0-30 % - low, 31-60% - moderate, 61% and above -high

GA and GG : 0 – 10% - low, 10.1 – 20% - moderate, > 20% - high

4.1.3.3 Genetic gain and genetic advance

The single seed dry weight showed a maximum value for genetic gain (77.94%) among the quantitative characters followed by bean width (64.06%), bean breadth (54.42%), pod weight (47.46 %) and wet bean weight (44.51%). All these characters were classified under high genetic gain and TSS was observed with medium genetic gain (10.22%). Among the quantitative characters pod weight was observed with maximum genetic advance (224.33) followed by wet bean weight (60.19). The characters like single seed dry bean weight, bean width, bean breadth, pod weight and wet bean weight with high heritability and genetic gain indicating that there will be considerable improvement over population, if these characters are considered as selection criteria.

4.1.4 Heterotic effect of pod and bean characters of hybrids

Heterotic effect of pod and bean characters of hybrids such as Relative Heterosis (RH), Standard Heterosis (SH) and Heterobeltiosis were computed using standard methods. CCRP 8 was taken as check variety to compute standard heterosis. The observations on pod and bean characters of CCRP 8 and the parents of the hybrids used to compute relative heterosis and heterobeltiosis are presented in Table 16. Several hybrids exhibited positive heterotic effects for various pod and bean characters.

4.1.4.1 Relative Heterosis (%)

The Relative Heterosis (RH) is calculated to find the vigour over mid parental value. The RH for important pod characters like pod weight, pod length, pod breadth, husk thickness, no. of beans per pod and total wet bean weight per pod are presented in Table 17 and RH for bean characters like single bean dry weight, length, breadth and width are presented in Table 21. The highest RH for pod length was exhibited by Hyb.11 (14.17%) followed by Hyb.14 (11.35%). Pod length showed significant positive RH in hybrids; Hyb.10, Hyb.11, Hyb.12, Hyb.14, Hyb.15, Hyb.16, Hyb.17 and Hyb.29. The RH for pod breadth was exhibited maximum in Hyb.16 (16.43%)

and minimum in Hyb.8 (-14.90%). The highest RH for pod weight was exhibited by Hyb.21 (64.62%) followed by Hyb.16 (50.07%). Relative heterosis for husk thickness was highly negative and significant in Hyb.15 (-35.78%) and highest positive and significant value in Hyb.16 (18.48%). The RH observed for no. of beans was highest in Hyb.26 (26.19%) followed by the Hyb.2 (24.38%). It was observed lowest in the Hyb.3 (-21.10%).

Relative heterosis for wet bean weight/ pod exhibited high positive and significant value in Hyb.16 (47.04%) and the hybrids; Hyb.30 (47%) and Hyb.16 (47.04%) also showed high positive and significant RH. For single seed length and seed weight, Hyb.1 showed high positive and significant RH (56.28% and 95.28% respectively) followed by the Hyb.11 (75.5% and 45.6% respectively). The RH observed for seed breadth and seed width was highest in Hyb.1 (52.45%) (Table 20). The RH was positive and significant for all the pod characters except husk thickness in Hyb.10 and Hyb.17.

Table 16. Pod and bean characters of the parents and check used for estimating heterosis

Parents/check	Pod length (cm)	Pod breadth (cm)	Pod weight (g)	Husk thickness (cm)	Wet bean weight/pod (g)	No. of beans/pod	Single bean dry weight (g)	Bean length (cm)	Bean breadth (cm)	Bean width (cm)
CCRP 8 (Check variety)	15.9	8.8	495.0	1.0	125.0	41.0	0.9	1.7	1.0	0.4
G IV 18.5	14.5	6.9	390.0	0.9	103.5	38.0	0.5	1.2	0.7	0.3
Criollo	18.3	8.0	474.0	1.1	119.2	35.0	0.6	1.3	0.7	0.2
G VI 55	17.1	8.8	495.0	1.1	128.0	44.0	1.1	2.1	1.0	0.5
G VI 56	17.6	8.7	526.0	1.1	128.0	46.0	1.0	2.3	1.2	0.8
G II 14.3	12.3	7.0	211.0	0.7	60.0	39.0	0.5	1.5	0.8	0.4
G VI 51	18.2	8.6	553.0	1.1	193.0	42.0	1.2	2.5	1.4	0.7
G VI 50	18.3	8.4	505.0	1.0	152.0	39.0	1.1	2.2	1.2	0.5
G VI 144	13.8	7.0	375.0	1.1	123.0	40.0	0.6	2.0	0.8	0.4
G VI 264	14.5	7.2	430.0	1.0	115.0	42.0	0.7	1.8	0.9	0.4
VSD 13.10	15.6	7.7	331.0	1.0	99.6	42.0	0.6	1.7	1.0	0.5
VSD 13.8	17.0	7.5	326.0	1.0	106.0	40.0	0.6	1.9	1.0	0.5
VSD 23.17	14.7	7.5	312.0	0.9	90.0	47.0	0.8	2.1	1.1	0.6
VSD 27.1	15.1	8.1	408.0	1.0	102.0	43.0	0.7	1.9	1.1	0.5
VSD 13.10	15.6	7.7	331.0	1.0	99.6	42.0	0.6	1.7	1.0	0.5

Table 17. Relative heterosis (%) for pod characters of hybrids

Hybrids	Pod length	Pod breadth	Pod weight	Husk thickness	Wet bean weight/pod	No. of beans/pod
Hyb.1	-14.01*	12.37*	8.35	-9.09*	27.25*	16.16*
Hyb.2	-13.27*	-12.10*	-42.21*	-18.18*	-33.20*	24.38*
Hyb.3	-22.64*	3.49*	-39.99*	-10.10*	-46.94*	-21.10*
Hyb.4	-0.46	-0.54*	-27.52*	-19.19*	-26.29*	7.95*
Hyb.5	-17.22*	-8.46*	-14.28	-20.18*	-20.65*	10.38*
Hyb.6	-8.98*	6.08*	9.54	-13.76*	32.97*	13.92*
Hyb.7	-0.17	-4.41*	4.99	-11.01*	27.50*	15.95*
Hyb.8	-11.91*	-14.90*	-26.93*	-23.85*	-2.14	18.99*
Hyb.9	-9.66*	-10.37*	-16.08	-30.28*	41.58*	15.44*
Hyb.10	7.28*	9.42*	41.51*	-4.59*	38.72*	8.86*
Hyb.11	14.17*	1.07*	17.44	-4.59*	29.14*	11.90*
Hyb.12	4.23*	7.51*	38.48*	-14.68*	41.21*	21.01*
Hyb.13	-26.03*	-11.08*	-37.91*	-22.94*	-37.06*	-12.91*
Hyb.14	11.35*	-7.03*	-4.86	-15.60*	-20.73*	-10.38*
Hyb.15	5.03*	-12.04*	-12.98	-35.78*	23.65*	14.43*
Hyb.16	7.16*	16.43*	50.07*	18.48*	47.04*	-6.82*
Hyb.17	5.34*	9.18*	31.44*	-2.75*	24.35*	10.89*
Hyb.18	-13.85*	1.35*	-7.87	-11.85*	-16.73	-1.90
Hyb.19	-9.77*	12.75*	24.09*	5.50*	32.52*	8.86*
Hyb.20	-16.34*	-7.33*	-28.37*	0.50*	-2.29	3.21*
Hyb.21	-9.58*	13.96*	64.62*	0.00	43.97*	10.89*
Hyb.22	-15.74*	-0.12	-8.30	-15.84*	-30.18*	-14.61*
Hyb.23	-15.33*	-7.64*	-11.28	-33.00*	3.35	1.95*
Hyb.24	-6.95*	-3.27*	16.10	-4.00*	12.87*	6.34*
Hyb.25	-8.55*	11.80*	19.62	7.54*	17.42*	6.67*
Hyb.26	1.39*	-3.04*	4.37	0.47*	1.32	26.19*
Hyb.27	-10.28*	-1.27*	-8.05	-27.44*	8.13	13.81*
Hyb.28	-3.04*	7.50*	12.43	-4.76*	10.78*	4.19*
Hyb.29	7.97*	3.50*	32.32*	-10.48*	36.30*	13.49*
Hyb.30	-6.96*	6.75*	22.38	-7.62*	47.00*	14.42*
CD value	0.71	0.27	29.49	0.06	8.61	1.90

* significant at 0.05 level

4.1.4.2 Heterobeltiosis

The heterobeltiosis is calculated to find the vigour of hybrids over their better parent. The heterobeltiosis for pod and bean characters are presented in Table 18. The highest positive and significant value for heterobeltiosis for pod weight was observed in Hyb.10 (38.51 %) followed by the hybrids; Hyb.12 (35.54%) and Hyb.21 (35.45%). In the case of pod length, positive and significant heterobeltiosis value was observed in the hybrids; Hyb.10 (3.71%), Hyb.11 (10.37%), Hyb.14 (7.64%) and Hyb.17 (2.15%). The highest positive and significant heterobeltiosis for pod breadth was exhibited by Hyb.21 (7.86%) and highest significant negative value for husk thickness was exhibited by Hyb.15 (-36.36%).

The highest heterobeltiosis for total wet bean weight was showed by the Hyb.30 (39.53%) followed by Hyb.9 (36.72%) and Hyb.12 (36.36%). Heterobeltiosis for no. of beans was observed with maximum value in the Hyb.26 (20.45%). The highest heterobeltiosis for single seed dry weight (78.45%), seed length (50.78%), seed breadth (49.32%) and seed thickness (92%) was exhibited by the Hyb.1 (Table 19). The hybrids; Hyb.4 (35.34%), Hyb.11 (34.09%) and Hyb.16 (18.50%) also showed significant and high positive heterobeltiosis value for single seed dry weight.

Table 18. Heterobeltiosis (%) for pod characters of hybrids

Hybrids	Pod length	Pod breadth	Pod weight	Husk thickness	Wet bean weight/pod	No. of beans
Hyb.1	-23.80*	4.76*	-1.25	-16.67*	18.87*	11.58*
Hyb.2	-23.14*	-18.05*	-47.33*	-25.00*	-37.61*	19.47*
Hyb.3	-31.44*	-3.51*	-45.30*	-17.59*	-50.44*	-24.21*
Hyb.4	-11.79*	-7.27*	-33.95*	-25.93*	-31.15*	3.68*
Hyb.5	-19.98*	-12.73*	-16.10	-20.91*	-23.38*	-0.91
Hyb.6	-12.01*	1.14*	7.21	-14.55*	28.41*	2.27*
Hyb.7	-3.49*	-8.86*	2.76	-11.82*	23.13*	4.09*
Hyb.8	-14.85*	-18.86*	-28.48	-24.55*	-5.50	6.82*
Hyb.9	-12.66*	-14.55*	-17.86	-30.91*	36.72*	3.64*
Hyb.10	3.71*	4.32*	38.51*	-5.45*	33.95*	-2.27*
Hyb.11	10.37*	-3.64*	14.95	-5.45*	24.70*	0.45
Hyb.12	0.76*	2.50*	35.54*	-15.45*	36.36*	8.64*
Hyb.13	-28.49*	-15.23*	-39.22*	-23.64*	-39.22*	-21.82*
Hyb.14	7.64*	-11.36*	-6.88	-16.36*	-23.45*	-19.55*
Hyb.15	1.53*	-16.14*	-14.83	-36.36*	19.41*	2.73*
Hyb.16	-8.98*	5.06*	5.14	-4.39*	7.98	-10.00*
Hyb.17	2.15*	4.09*	28.65	-3.64*	20.08*	-0.45
Hyb.18	-20.00*	-3.95*	-26.36	-16.96*	-36.88*	-1.90
Hyb.19	-12.77*	7.50*	21.46	4.55*	27.97*	-2.27*
Hyb.20	-22.51*	-11.19*	-40.71*	0.00	-19.13*	-0.48
Hyb.21	-12.79*	7.86*	35.45*	0.00	22.18*	9.50*
Hyb.22	-23.85*	-6.51*	-28.28	-24.11*	-48.81*	-19.15*
Hyb.23	-22.73*	-9.29*	-19.80	-33.00*	-13.64*	-2.79*
Hyb.24	-15.08*	-5.00*	4.95	-4.00*	-5.70	1.40
Hyb.25	-15.30*	7.14*	-0.99	7.00*	-2.82	2.86*
Hyb.26	-8.54*	-12.95*	-8.28	-1.82*	-0.66	20.45*
Hyb.27	-19.06*	-11.36*	-19.19	-29.09*	6.02	8.64*
Hyb.28	-10.41*	-2.27*	5.05	-9.09*	5.16	1.82
Hyb.29	-0.23	-5.91*	23.64	-14.55*	29.38*	10.91*
Hyb.30	-14.04*	-2.95*	14.34	-11.82*	39.53*	11.82*
CD	0.71	0.27	29.49	0.06	8.61	1.90

* significant at 0.05 level

Table 19. Heterobeltiosis (%) for bean characters of hybrids

Hybrids	Single bean dry weight	Seed length	Seed breadth	Seed thickness
Hyb.1	78.45*	50.78*	49.32*	92.00*
Hyb.2	-12.93*	8.20*	-13.70*	74.00*
Hyb.3	6.03*	20.31*	36.30*	40.00*
Hyb.4	35.34*	14.06*	28.77*	80.00*
Hyb.5	-7.73*	-17.38*	10.00*	-7.00*
Hyb.6	0.91*	4.29*	21.00*	8.00*
Hyb.7	-3.18*	-21.19*	22.50*	-28.00*
Hyb.8	-23.64*	-12.38*	2.00*	-13.00*
Hyb.9	2.27*	-14.05*	8.50*	-26.00*
Hyb.10	-4.09*	5.24*	14.00*	-11.00*
Hyb.11	34.09*	17.14*	30.50*	-5.00*
Hyb.12	0.91*	16.19*	8.50*	-11.00*
Hyb.13	-21.82*	-25.00*	-7.00*	-12.00*
Hyb.14	-12.73*	-33.81*	-0.50*	7.00*
Hyb.15	2.27*	-20.71*	-11.50*	12.00*
Hyb.16	18.50*	-12.39*	-0.83*	-45.00*
Hyb.17	12.73*	-1.19*	10.50*	-12.00*
Hyb.18	-10.83*	-18.80*	-23.93*	-32.14*
Hyb.19	-11.82*	-1.43*	-1.50*	-10.00*
Hyb.20	-37.73*	-20.91*	-9.58*	-23.00*
Hyb.21	-0.91*	-28.64*	-25.00*	-11.00*
Hyb.22	-18.75*	-34.20*	-24.29*	-37.14*
Hyb.23	14.09*	-21.82*	-19.17*	-19.00*
Hyb.24	-6.82*	-25.91*	7.50*	43.00*
Hyb.25	-24.55*	-24.09*	-30.42*	-16.00*
Hyb.26	-14.09*	-20.00*	-20.00*	-11.00*
Hyb.27	-28.18*	-5.24*	9.00*	3.00*
Hyb.28	-6.82*	-20.71*	-5.50*	-14.00*
Hyb.29	-1.36*	-0.48*	18.00*	38.00*
Hyb.30	5.91*	-22.14*	-2.00*	-11.00*
CD	0.029	0.197	0.024	0.017

* significant at 0.05 level

4.1.4.3 Standard heterosis (%)

The Standard Heterosis (SH) was calculated to find the vigour over a standard check variety and SH for pod characters is presented in Table 20 and for bean characters in Table 21. The highest positive and significant SH for pod length was exhibited by Hyb.11 (27.41%) and the highest positive and significant SH for pod breadth exhibited by Hyb.19 (8.11%). For the pod weight highest positive and significant SH was observed in Hyb.10 (38.51%) followed by Hyb.21 (38.18%).

Highest negative and significant SH value for husk thickness was observed in Hyb.23 (-33.66%) followed by the Hyb.15 (-30.69%). The Hyb.21 showed highest positive and significant SH for wet bean weight per pod (48.58%) and the hybrids; Hyb.30 (42.88%) and Hyb.9 (40%) were also characterized with high SH. In case of no. of beans, Hyb.26 showed highest significant value for SH (29.27%) followed by Hyb.30 (20%). The SH for single seed dry weight was observed highest in the Hyb.11 (56.91%) and lowest in Hyb.2 (-46.28%). The SH for seed length and seed breadth was observed highest in Hyb.11 (41.38% and 29.21%). For the seed thickness highest positive and significant SH was observed in Hyb.24 (70.24%) followed by Hyb.29 (64.29%).

Table 20. Standard heterosis (%) for pod characters of hybrids

Hybrids	Pod length	Pod breadth	Pod weight	Husk thickness	Wet bean weight/pod	No. of beans
Hyb.1	-12.04*	-4.46*	-5.44	-10.89*	13.36*	3.41*
Hyb.2	-11.28*	-25.26*	-49.57*	-19.80*	-40.50*	10.73*
Hyb.3	-20.86*	-12.00*	-47.62*	-11.88*	-52.74*	-29.76*
Hyb.4	1.83*	-15.43*	-36.75*	-20.79*	-34.34*	-3.90*
Hyb.5	-7.62*	-12.23*	-16.10	-13.86*	-21.54*	6.34*
Hyb.6	1.58*	1.71*	7.21	-6.93*	31.49*	9.76*
Hyb.7	11.41*	-8.34*	2.76	-3.96*	26.08*	11.71*
Hyb.8	-1.70*	-18.40*	-28.48	-17.82*	-3.23	14.63*
Hyb.9	0.82*	-14.06*	-17.86	-24.75*	40.00*	11.22*
Hyb.10	19.72*	4.91*	38.51*	2.97*	37.17*	4.88*
Hyb.11	27.41*	-3.09*	14.95	2.97*	27.70*	7.80*
Hyb.12	16.32*	3.09*	35.54	-7.92*	39.63*	16.59*
Hyb.13	-17.45*	-14.74*	-39.22*	-16.83*	-37.76*	-16.10*
Hyb.14	24.26*	-10.86*	-6.88	-8.91*	-21.62*	-13.66*
Hyb.15	17.20*	-15.66*	-14.83	-30.69*	22.27*	10.24*
Hyb.16	0.95*	4.46*	11.72	7.92*	10.58*	-3.41*
Hyb.17	25.52*	4.69*	28.65	4.95*	22.96*	6.83*
Hyb.18	-8.25*	-5.60*	-17.73	-7.92*	-2.54	0.49
Hyb.19	0.69	8.11*	21.46	13.86*	31.04*	4.88*
Hyb.20	-10.65*	-14.74*	-39.52*	-0.99*	-1.66	1.95*
Hyb.21	0.57	3.54*	38.18*	-0.99*	48.58*	6.83*
Hyb.22	-12.67*	-8.11*	-19.88	-15.84*	-20.96*	-7.32*
Hyb.23	-10.90*	-12.91*	-18.18	-33.66*	5.01	1.95*
Hyb.24	-2.08*	-8.80*	7.07	-4.95*	14.67*	6.34*
Hyb.25	-2.33*	2.86*	1.01	5.94*	18.18*	5.37*
Hyb.26	-1.45*	-12.46*	-8.28	6.93*	1.73	29.27*
Hyb.27	-12.79*	-10.86*	-19.19	-22.77*	8.56	16.59*
Hyb.28	-3.47*	-1.71*	5.05	-0.99*	7.68	9.27*
Hyb.29	7.50*	-5.37*	23.64	-6.93*	32.48*	19.02*
Hyb.30	-7.37*	-2.40*	14.34	-3.96*	42.88*	20.00*
CD value	0.71	0.27	29.49	0.06	8.61	1.90

* significant at 0.05 level

Table 21. Relative Heterosis (RH) and Standard Heterosis (SH) for bean characters

Hybrids	Single bean dry weight		Seed length		Seed breadth		Seed thickness	
	RH (%)	SH (%)	RH (%)	SH (%)	RH (%)	SH (%)	RH (%)	SH (%)
Hyb.1	95.28*	10.11*	56.28*	10.92*	52.45*	7.92*	100.00*	14.29*
Hyb.2	-4.72*	-46.28*	12.15*	-20.40*	-11.89*	-37.62*	81.25*	3.57*
Hyb.3	16.04*	-34.57*	24.70*	-11.49*	39.16*	-1.49*	45.83*	-16.67*
Hyb.4	48.11*	-16.49*	18.22*	-16.09*	31.47*	-6.93*	87.50*	7.14*
Hyb.5	20.83*	7.98*	2.66*	-0.29*	29.41*	8.91*	27.40*	10.71*
Hyb.6	32.14*	18.09*	29.59*	25.86*	42.35*	19.80*	47.95*	28.57*
Hyb.7	26.79*	13.30*	-2.07*	-4.89*	44.12*	21.29*	-1.37*	-14.29*
Hyb.8	0.00	-10.64*	8.88*	5.75*	20.00*	0.99*	19.18*	3.57*
Hyb.9	33.93*	19.68*	6.80*	3.74*	27.65*	7.43*	1.37*	-11.90*
Hyb.10	25.60*	12.23*	30.77*	27.01*	34.12*	12.87*	21.92*	5.95*
Hyb.11	75.60*	56.91*	45.56*	41.38*	53.53*	29.21*	30.14*	13.10*
Hyb.12	32.14*	18.09*	44.38*	40.23*	27.65*	7.43*	21.92*	5.95*
Hyb.13	2.38*	-8.51*	-6.80*	-9.48*	9.41*	-7.92*	20.55*	4.76*
Hyb.14	14.29*	2.13*	-17.75*	-20.11*	17.06*	-1.49*	46.58*	27.38*
Hyb.15	33.93*	19.68*	-1.48*	-4.31*	4.12*	-12.38*	53.42*	33.33*
Hyb.16	61.22*	26.06*	6.05*	15.80*	17.82*	17.82*	-26.67*	4.76*
Hyb.17	47.62*	31.91*	22.78*	19.25*	30.00*	9.41*	20.55*	4.76*
Hyb.18	16.94*	13.83*	-4.02*	16.67*	-11.25*	5.45*	-20.83*	13.10*
Hyb.19	15.48*	3.19*	22.49*	18.97*	15.88*	-2.48*	23.29*	7.14*
Hyb.20	-20.81*	-27.13*	-11.45*	0.00	-1.36*	7.43*	-23.00*	-8.33*
Hyb.21	26.01*	15.96*	-22.47*	-9.77*	-18.18*	-10.89*	-11.00*	5.95*
Hyb.22	-2.50*	3.72*	-28.48*	-5.46*	-14.17*	4.95*	-32.31*	4.76*
Hyb.23	39.44*	33.51*	-16.10*	-1.15*	-15.65*	-3.96*	-19.00*	-3.57*
Hyb.24	13.89*	9.04*	-20.49*	-6.32*	12.17*	27.72*	43.00*	70.24*
Hyb.25	-4.05*	-11.70*	-15.01*	-4.02*	-24.09*	-17.33*	-16.00*	0.00
Hyb.26	9.88*	0.53*	-17.44*	-3.45*	-11.11*	-20.79*	-1.11*	5.95*
Hyb.27	-8.14*	-15.96*	-2.21*	14.37*	21.11*	7.92*	14.44*	22.62*
Hyb.28	12.02*	9.04*	-14.62*	-4.31*	-0.53*	-6.44*	-4.44*	2.38*
Hyb.29	18.58*	15.43*	7.18*	20.11*	24.21*	16.83*	53.33*	64.29*
Hyb.30	27.32*	23.94*	-16.15*	-6.03*	3.16*	-2.97*	-1.11*	5.95*
CD	0.029	0.029	0.197	0.197	0.024	0.024	0.017	0.017

* significant at 0.05 level

4.2 Biochemical/ Quality parameters evaluation

The biochemical and quality parameters evaluation of thirty hybrids were carried out and the results are presented in Table 22. Analysis of variance of biochemical and quality parameters like TSS, fat, total phenol, total alkaloid and protein content were carried out and significant difference was observed among the hybrids.

4.2.1 Total Soluble Solids (TSS)

The TSS evaluated among the thirty hybrids revealed that Hyb.10 recorded a maximum average TSS with a value of 22° brix (Table 22). The hybrids; Hyb.7, Hyb.9, Hyb.17 and Hyb.26 also showed high TSS value of 21.8° brix. Low TSS values was observed in the hybrids; Hyb.3 (17° brix), Hyb.2 (17.80° brix) and Hyb.27 (18° brix). The range of TSS varied from 17 to 22° brix among the thirty hybrids.

4.2.2 Fat content (%)

Fat content was estimated using soxhlet apparatus (Plate 10). Fat content varied among the hybrids and showed significant difference among the thirty hybrids. It is presented in the Table 22. The Hyb.17 showed highest value for fat content (56.5%) followed by Hyb.14 (55.5%). Among the thirty hybrids, fat content was observed in the range from 39% in Hyb.28 to 56.5% in Hyb.17. Fourty six percent of the hybrids exhibited fat content above fifty percent.

4.2.3 Alkaloid content (%)

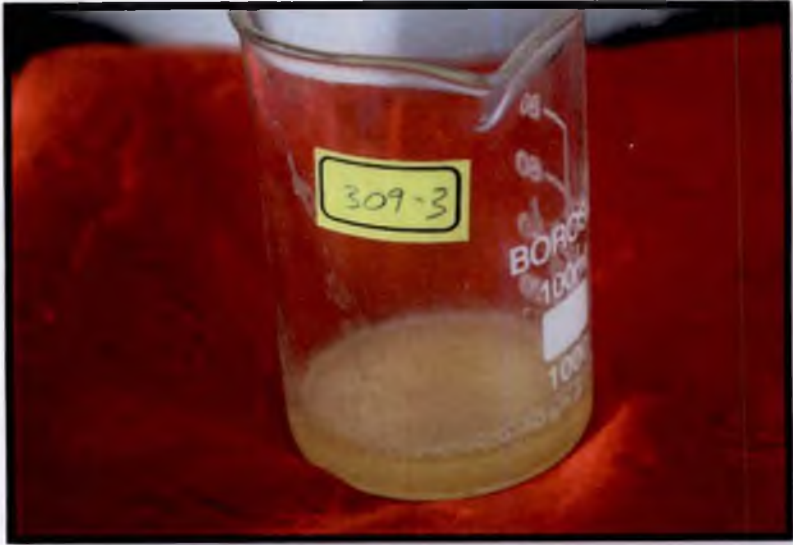
The alkaloid content was observed highest in both Hyb.26 and Hyb.25 with a value of 4.2% each followed by Hyb.13 (4.15%) (Table 22). The lowest alkaloid content was observed in Hyb.4 (2.65%).

4.2.4 Total phenol (%)

The highest total phenol content was exhibited by Hyb.15 (5.45%) followed by hybrids; Hyb.11 (5.40%), Hyb.12 (5.30%), Hyb.14 (5.30%), Hyb.6 (5.25%) and Hyb.20 (5.15%) (Table 22). The lowest value was exhibited by the Hyb.27 (2.95%).



Soxhlet apparatus



Extracted fat

Plate 10. Fat estimation

4.2.5 Protein content (%)

Among the thirty hybrids, Hyb.17 (17.4%) scored highest value for protein content followed by the hybrids; Hyb.11 (17.3%), Hyb.7 (17%) and Hyb.9 (16.95%). The protein content varied from 12.15 % to 17.4% among the hybrids (Table 22).

Based on biochemical evaluation, all the thirty hybrids were scored using DMRT technique and they were ranked based on this score to find the hybrids with superior quality. The score obtained for all the biochemical characters are presented in Appendix V. According to the scores obtained, hybrids; Hyb.30, Hyb.12, Hyb.15, Hyb.7, Hyb.6 and Hyb.11 were selected as superior hybrids with excellent biochemical characters.

Table 22. Mean values of biochemical or quality parameters

Hybrids	TSS (° brix)	Fat (%)	Alkaloid (%)	Total phenol (%)	Protein (%)	Total score	Rank
Hyb.1	20.80	52.5	3.70	5.05	16.75	10.5	6.0
Hyb.2	17.80	42.5	3.45	4.55	13.85	29.0	19.0
Hyb.3	17.00	50.0	4.05	3.60	16.25	24.5	14.0
Hyb.4	18.60	42.0	2.65	4.90	13.85	29.5	20.0
Hyb.5	21.40	53.0	3.80	4.20	15.00	17.0	11.0
Hyb.6	20.80	54.5	3.85	5.25	16.40	9.5	4.0
Hyb.7	21.80	54.0	3.95	4.85	17.00	9.0	3.0
Hyb.8	20.40	47.5	2.90	4.45	16.00	19.5	12.0
Hyb.9	21.80	54.5	3.80	4.85	16.95	10.5	7.0
Hyb.10	22.00	52.5	3.75	4.95	14.70	13.5	9.0
Hyb.11	20.40	54.5	3.85	5.40	17.30	10.0	5.0
Hyb.12	21.40	51.5	3.90	5.30	16.85	8.5	2.0
Hyb.13	19.00	42.5	4.15	3.85	12.95	28.5	18.0
Hyb.14	20.60	55.5	3.45	5.30	14.40	14.5	10.0
Hyb.15	21.40	54.5	3.80	5.45	16.80	8.5	2.0
Hyb.16	18.00	44.0	3.85	4.15	15.00	27.5	17.0
Hyb.17	21.80	56.5	3.95	4.95	17.40	8.0	1.0
Hyb.18	19.00	44.5	3.85	3.85	13.60	29.0	19.0
Hyb.19	19.40	49.0	4.20	4.55	14.30	19.5	12.0
Hyb.20	20.00	43.5	3.40	5.15	14.00	21.0	13.0
Hyb.21	20.80	51.5	3.90	4.50	16.95	12.5	8.0
Hyb.22	18.80	47.5	3.10	4.10	15.00	27.0	15.0
Hyb.23	19.80	43.5	2.95	3.50	13.85	32.0	21.0
Hyb.24	18.20	39.5	3.60	4.45	14.40	29.5	20.0
Hyb.25	19.40	40.0	3.25	2.90	12.70	35.0	22.0
Hyb.26	21.80	43.0	4.20	4.40	13.95	19.5	12.0
Hyb.27	18.00	41.5	3.35	2.95	12.15	37.5	23.0
Hyb.28	19.00	39.0	3.00	4.10	13.55	32.0	21.0
Hyb.29	20.00	44.5	3.65	3.85	13.90	27.0	16.0
Hyb.30	20.80	52.5	4.10	5.10	16.75	8.0	1.0
CD(0.05)	1.43	6.37	0.75	0.48	1.23		
CV (%)	5.78	6.49	10.14	5.32	4.14		

4.2.6 Clustering based on biochemical characters

The cluster analysis was carried out using Jaccard's similarity coefficients matrix by unweighted pair group method (Sneath and Sokal, 1973) and the dendrogram resulted is presented in Fig. 4. From the figure it is clear that all the hybrids remaining as independent clusters except Hyb.12 and Hyb.18, which formed a cluster. Almost all the hybrids were different with respect to biochemical characters and they varied widely one among other.

4.3 Correlation studies

Correlation studies were carried out among quantitative characters in thirty hybrids and it is presented in the Table 23. Correlation studies revealed that pod weight showed significant positive correlation with pod length (0.621), pod breadth (0.857), husk thickness (0.597), number of beans/ pod (0.401), total wet bean weight/ pod (0.825), peeled dry bean weight (0.709) and single dry bean weight (0.674). Pod length showed significant and positive correlation with pod breadth (0.376), total wet bean weight/ pod (0.490), peeled dry bean weight (0.646), single dry bean weight (0.565) and found negative correlation with number of flat beans per pod (-0.463).

The pod breadth exhibited significant and positive correlation with pod weight (0.857), husk thickness (0.719), total wet bean weight/ pod (0.648), peeled dry bean weight (0.548), single dry bean weight (0.521). Husk thickness exhibited significant positive correlation with pod weight (0.597), pod length (0.301) and pod breadth (0.719). Number of beans showed positive and significant correlation with total wet bean weight/ pod (0.624) and observed negative correlation with number of flat beans per pod (-0.454). Number of flat beans per pod observed negative correlation with total wet bean weight/ pod (-0.378), TSS (-0.769) and fat content (-0.459). Single seed dry weight exhibited positive and significant correlation with TSS (0.517), fat content (0.465) and protein content (0.496). Fat content exhibited positive and significant correlation with alkaloid content (0.453), total phenol content (0.654) and protein content (0.825). Alkaloid content exhibited positive and significant correlation with fat (0.453) and protein content (0.388). Protein content showed significant and positive correlation with TSS (0.541), fat content (0.825), alkaloid content (0.388) and phenol content (0.662)

Fig. 4 Dendrogram based on biochemical characters

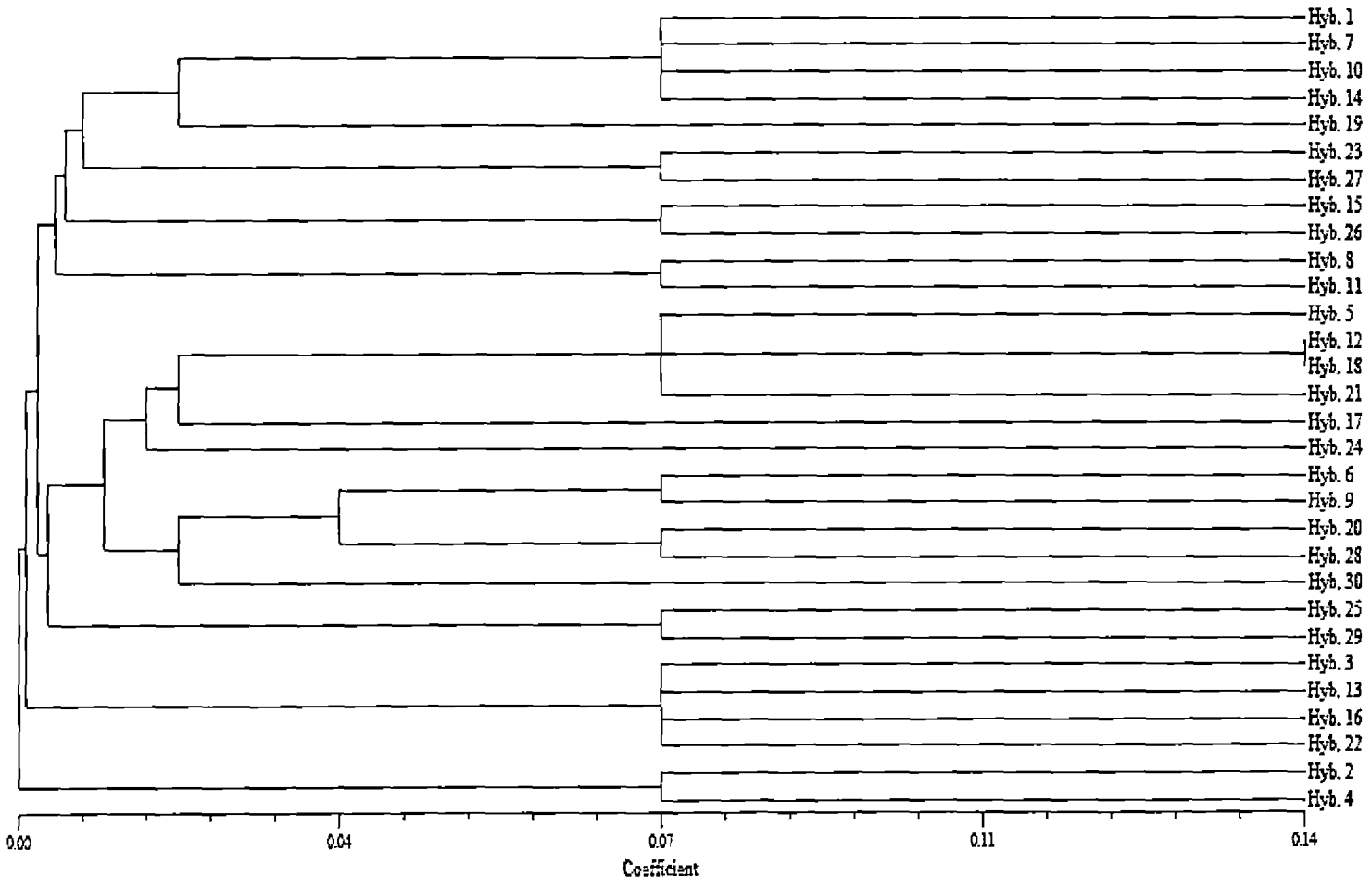


Table 23. Correlation among quantitative characters

	PW	PL	PB	HT	NBP	FBP	WBW	PDBW	SPDBW	TSS	FC	AC	TPC	PC
PW	1													
PL	.621(**)	1												
PB	.857(**)	.376(*)	1											
HT	.597(**)	0.301	.719(**)	1										
NBP	.401(*)	0.247	0.091	0.123	1									
FBP	-0.272	-.463(*)	-0.017	0.011	-.454(*)	1								
WBW	.825(**)	.490(**)	.648(**)	0.355	.624(**)	-.378(*)	1							
PDBW	.709(**)	.646(**)	.548(**)	0.224	0.282	-.446(*)	.741(**)	1						
SPDBW	.674(**)	.565(**)	.521(**)	0.182	0.279	-0.346	.666(**)	.902(**)	1					
TSS	.480(**)	.583(**)	0.215	0.122	.496(**)	-.769(**)	.586(**)	.601(**)	.517(**)	1				
FC	0.351	.549(**)	0.239	0.026	0	-.459(*)	0.36	.620(**)	.465(**)	.674(**)	1			
AC	0.353	0.148	0.35	.427(*)	0.07	-0.147	0.309	0.349	0.263	0.314	.453(*)	1		
TPC	0.262	.593(**)	0.065	0.107	0.17	-.425(*)	0.307	.466(**)	0.324	.573(**)	.654(**)	0.233	1	
PC	0.354	.430(*)	0.222	0.049	0.106	-.403(*)	.430(*)	.610(**)	.496(**)	.541(**)	.825(**)	.388(*)	.662(**)	1

** Correlation is significant at the 0.01 level * Correlation is significant at the 0.05 level

PW - pod weight.; PL – pod length; PB – pod breadth; HT -husk thickness; NBP - no of beans/pod; FBP – no. of flat beans/ pod; WBW - wet bean wt./ pod; PDBW - peeled dry bean wt.; SPDW - single peeled dry bean wt.; TSS – total soluble solids; FC – fat content; AC – alkaloid content; TPC – total phenol content; PC – protein content

4.4 Path coefficient analysis

Path coefficient analysis was carried out to study the direct and indirect effects of total wet bean weight/ pod and its component characters by partitioning the correlation between total wet bean weight/ pod and its component characters into direct and indirect effects. The results of path coefficient analysis is presented in the Table 24 and the path diagram representing direct and indirect effects are presented in Fig.5.

Table 24. Path coefficient analysis of various pod and bean characters on wet bean weight

Character	NB	PBDW	TP	PL	PB	PW
NB	0.412	0.093	0.010	-0.032	0.018	0.125
PBDW	0.116	0.329	0.061	-0.086	0.110	0.221
TP	0.043	0.201	0.100	-0.057	0.044	0.110
PL	0.101	0.213	0.043	-0.133	0.075	0.194
PB	0.03	0.180	0.022	-0.050	0.201	0.267
PW	0.165	0.23	0.035	-0.082	0.172	0.312

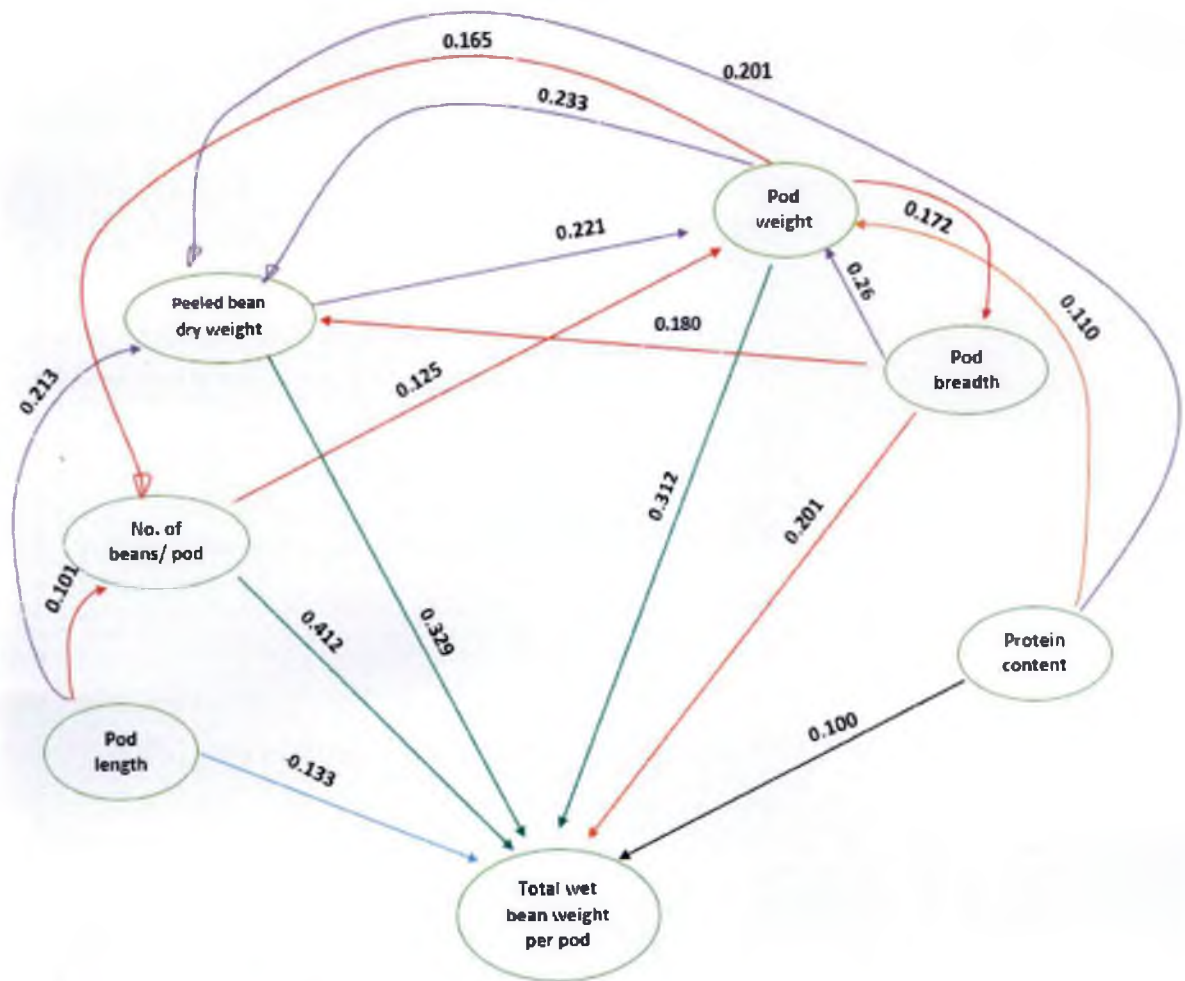
Residual effect = 0.142

NB - No. of beans, PBDW – Peeled bean dry weight, TP – Total protein, PL – Pod length, PB – Pod breadth, PW - Pod weight

4.4.1 Direct effects

The highest positive direct genotypic effects on total wet bean weight per pod was exhibited by number of beans (0.412) and its correlation with wet bean weight per pod was also positive (0.624). Bean dry weight (0.329) and pod weight (0.312) showed high direct effects on wet bean weight per pod. They also exhibited positive correlation with total wet bean weight. Pod breadth showed moderate direct effects (0.201) on total wet bean weight per pod and pod breadth also exhibited positive correlation with total wet bean weight per pod (0.648). Protein content exhibited low direct effects on total wet bean weight per pod (0.100) and its correlation with total wet bean weight was also

Fig. 5 Path diagram



High direct effects █ Moderate direct effects █ Low direct effects █

Negligible direct effects █ High indirect effects █ Moderate indirect effects █

positive (0.43). Pod length (-0.133) exhibited negative direct effects on total wet bean weight per pod, in which alkaloid content showed negligible direct effects.

4.4.2 Indirect effects

Pod weight showed high positive indirect effect on total wet bean weight per pod (0.172) through the moderate positive direct effect of pod breadth (0.201) and exhibited low positive indirect effect on total wet bean weight per pod (0.165) through the high positive direct effect of number of beans per pod (0.412). Pod weight showed moderate positive indirect effect on total wet bean weight per pod (0.233) through the high positive direct effect of peeled bean dry weight (0.329).

Protein content exhibited moderate positive indirect effect on total wet bean weight per pod (0.20) through the high positive direct effect of peeled bean dry weight per pod (0.329) and exhibited low positive indirect effect on total wet bean weight per pod (0.110) through the high positive direct effect of pod weight (0.312).

Pod breadth showed moderate positive indirect effect on total wet bean weight per pod (0.26) through the high positive direct effect of pod weight (0.312) and exhibited low positive indirect effect on total wet bean weight per pod (0.180) through the high positive direct effect of peeled bean weight per pod (0.329).

Number of beans per pod exhibited low positive indirect effect on total wet bean weight per pod (0.125) through the high positive direct effect of pod weight (0.312). Peeled bean dry weight showed low positive indirect effect on total wet bean weight per pod (0.116) through the high positive direct effect of number of beans per pod (0.412).

Pod length showed moderate positive indirect effect on total wet bean weight per pod (0.194) through the high positive direct effect of pod weight (0.312) and exhibited moderate positive indirect effect on total wet bean weight per pod (0.21) through the high positive direct effect of peeled bean weight per pod (0.329). Pod

length also exhibited low positive indirect effect on total wet bean weight per pod (0.101) through the high positive direct effect of number of beans per pod (0.412).

4.5 Screening of hybrids based on quantitative, qualitative and biochemical characters

Based on the performance of thirty hybrids bred for quality through morphological evaluation based on qualitative and quantitative characters of pod and beans and biochemical evaluation, hybrids were ranked and the rank score is presented in Table 25. Based on this rank score, twelve superior hybrids were selected (Table 26) for further evaluations. The KAU released varieties of cocoa (CCRP 1, 2, 3, 4, 5, 6, 7, 8 and 9) were also taken for further evaluation in order to compare their performance with the selected hybrids. The selected hybrids and CCRP varieties were further evaluated for fermentation index, fermentation recovery, pH and moisture content. They were then used for making chocolates and organoleptic evaluation was carried out based on nine point hedonic scale to test the sensory attributes of the chocolates prepared. The sensory attributes of these chocolates were then compared with that of commercial chocolate standardized by KAU.

Table 25. Rank score for various quantitative and biochemical characters

Hybrids	Pod characters rank	Husk thickness and flat bean rank	Bean characters rank	Single bean characters rank	Biochemical parameters rank	Total rank score	Rank based on rank score
Hyb.1	17	7	13	12	6	55	11
Hyb.2	26	8	26	24	19	103	24
Hyb.3	16	8	11	10	8	53	10
Hyb.4	25	8	24	22	20	99	23
Hyb.5	22	5	22	19	11	79	19
Hyb.6	8	7	2	2	4	23	3
Hyb.7	9	9	10	10	3	41	8
Hyb.8	22	5	20	20	12	79	19
Hyb.9	13	2	8	9	7	39	7
Hyb.10	2	11	5	5	9	32	6
Hyb.11	5	13	1	1	5	25	4
Hyb.12	1	6	5	4	2	18	1
Hyb.13	27	13	23	22	18	103	24
Hyb.14	19	8	16	18	10	71	16
Hyb.15	15	1	6	7	2	31	5
Hyb.16	11	17	12	6	17	63	15
Hyb.17	3	11	4	3	1	22	2
Hyb.18	20	6	7	6	19	58	12
Hyb.19	6	18	12	14	12	62	14
Hyb.20	24	11	19	20	13	87	22
Hyb.21	3	9	3	9	8	32	6
Hyb.22	23	12	18	17	15	85	21
Hyb.23	21	3	13	13	21	71	16
Hyb.24	14	14	9	4	20	61	13
Hyb.25	10	15	14	19	22	80	20
Hyb.26	16	10	21	21	12	80	20
Hyb.27	18	4	17	15	23	77	18
Hyb.28	12	12	15	16	21	76	17
Hyb.29	4	9	13	8	16	50	9
Hyb.30	7	11	11	11	1	41	8

Table 26. Selected hybrids based on rank score

SI No.	Selected hybrids
1	Hyb.1
2	Hyb.3
3	Hyb.6
4	Hyb.7
5	Hyb.9
6	Hyb.10
7	Hyb.11
8	Hyb.12
9	Hyb.15
10	Hyb.17
11	Hyb.21
12	Hyb.30

4.6 Fermentation index

Fermentation index was found out for the selected hybrids and also for CCRP varieties and it is presented in Table 27. The beans after fermentation were scored based on the degree of fermentation into fully fermented, partially fermented, not fermented and slaty beans (Plate 11). Fermentation index was observed highest in Hyb.17 with 78% fully fermented beans, 20% partially fermented beans and 1% not fermented and slaty beans each. The hybrids; Hyb.11 and Hyb.12 exhibited 74% fully fermented beans and 24% partially fermented beans.

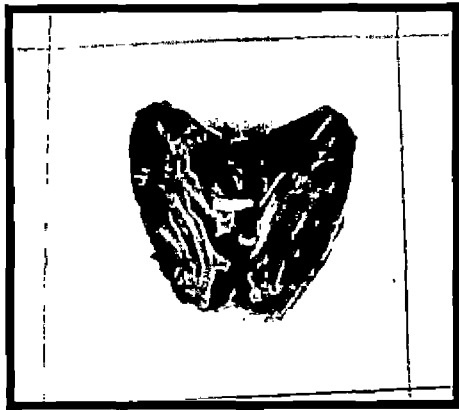
The hybrids; Hyb.1, Hyb.7, Hyb.10, Hyb.11, Hyb.12, Hyb.15, Hyb.17 and Hyb.21 were observed with greater than or equal to 70% fully fermented beans. CCRP 4, 8 and 9 also showed greater than 70% fully fermented beans. CCRP 3 was observed with least fully fermented beans and highest percent in non-fermented beans among all



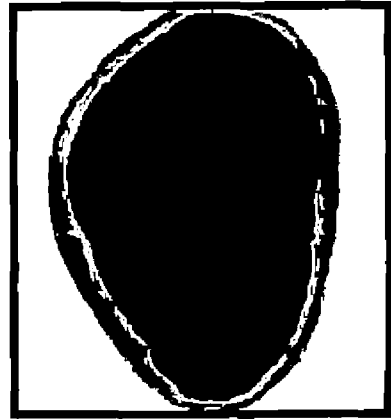
Fully fermented Forastero



Fully fermented



Partially fermented



Slaty beans

Plate 11. Fermentation index score chart



Fully fermented Forastero



Fully fermented



Partially fermented



Slaty beans

Plate 11. Fermentation index score chart

the hybrids and CCRP varieties. Slaty beans was not observed in the hybrids; Hyb.15, Hyb.21 and Hyb.30 and also in CCRP varieties like CCRP 1, 2, 4 and 8.

Table 27. Fermentation index of selected hybrids and CCRP varieties

Hybrids and CCRP varieties	Fully fermented beans score (%)	Partially fermented beans Score (%)	Not fermented beans score (%)	Slaty beans score (%)
Hyb.1	70	28	1	1
Hyb.3	62	36	0	2
Hyb.6	69	28	1	2
Hyb.7	71	28	0	1
Hyb.9	73	24	2	1
Hyb.10	70	29	0	1
Hyb.11	74	24	0	2
Hyb.12	74	24	1	1
Hyb.15	72	27	1	0
Hyb.17	78	20	1	1
Hyb.21	71	28	1	0
Hyb.30	69	29	2	0
CCRP 1	67	32	1	0
CCRP 2	62	37	1	0
CCRP 3	45	49	5	1
CCRP 4	71	27	2	0
CCRP 5	64	35	0	1
CCRP 6	64	35	0	1
CCRP 7	66	32	1	1
CCRP 8	75	24	1	0
CCRP 9	70	28	1	1

4.7 Fermentation recovery

Fermentation recovery was worked out among the selected hybrids and CCRP varieties (CCRP 1 to 9) and it is presented in the Table 28. Fermentation recovery among the selected hybrids was observed maximum in Hyb.6 (41.2%) followed by the hybrids; Hyb.10 (41.1%), Hyb.21 (40.2%) and Hyb.17 (38.7%). Among the CCRP varieties CCRP 8 showed highest fermentation recovery percent (40.1%) followed by the hybrid CCRP 1 (37.5%).

4.8 pH

The value of pH among the hybrids and CCRP varieties are presented in the Table 28 and it ranged from 5.20 in Hyb.10 to 6.10 in the CCRP 3 variety. Almost all the hybrids and CCRP varieties showed a pH range of 5 to 6 except CCRP 3, which was observed with a pH value of 6.10.

4.9 Moisture content

Moisture content among the selected hybrids and CCRP varieties after sun drying was observed below 8 percent.

Table 28. Fermentation recovery and pH

Hybrids and CCRP varieties	Fermentation recovery (%)	pH value
Hyb.1	36.4	5.23
Hyb.3	35.9	5.27
Hyb.6	41.2	5.40
Hyb.7	37.7	5.24
Hyb.9	36.7	5.28
Hyb.10	41.1	5.20
Hyb.11	36.6	5.44
Hyb.12	37.1	5.6
Hyb.15	37.6	5.70
Hyb.17	38.7	5.23
Hyb.21	40.2	5.43
Hyb.30	36.0	5.40
CCRP 1	37.5	5.26
CCRP 2	33.5	5.63
CCRP 3	30.5	6.10
CCRP 4	36.0	5.25
CCRP 5	35.5	5.31
CCRP 6	31.5	5.46
CCRP 7	35.5	5.27
CCRP 8	40.1	5.34
CCRP 9	36.0	5.41

4.10 Sensory evaluation

Sensory evaluation was carried out among the chocolates made from selected hybrids and CCRP varieties (CCRP 1, 2, 3, 4, 5, 6, 7, 8 and 9) based on the attributes like appearance, colour, flavour, texture, taste, odour, after taste and overall acceptability using a nine point hedonic scale which ranged from dislike extremely (1) to like extremely (9). The commercial chocolate standardized by KAU was taken as the standard for comparing the sensory attributes (Plate 12). Hedonic ratings were then converted to rank scores and the rank analysis was carried out by using Kendall's coefficient of concordance and the total score was tabulated from the mean value of each attributes. The mean rank score thus obtained is presented in the Table 29. The score card used and the hedonic scale are presented in Appendix VI.

The highest rank for appearance was recorded in Hyb.1 (16.02) followed by Hyb.17 (15.66). The CCRP 1 (14.56), 2 (14.36) and 9 (14.42) also observed with very good appearance. The CCRP 1(18.82), CCRP 4 (14.84) and CCRP 6 (16.64) showed high mean rank score for colour attribute. The Hyb.17 (15.30) and the standard chocolate (14.58) also scored high value for colour. The highest rank for flavour was recorded in Hyb.6 (19.18) followed by the hybrids; Hyb.9 (17.38), Hyb.10 (14.56), CCRP 9 (14.78) and Hyb.1 (14.42) and this was above the flavour characteristics of commercial chocolate standardized by KAU.

Among the hybrids, the highest rank score for texture was observed in Hyb.6 (19.18) followed by the hybrids; Hyb.12, Hyb.7 and Hyb.11. CCRP 1 and 4 also scored good acceptance for texture. The highest rank for odour was exhibited by Hyb.6 (15.34) followed by the hybrids; Hyb.7 (14.72), Hyb.9 (14.56) and Hyb.11 (14.46). The hybrids; Hyb.9 (18.88), Hyb.6 (15.40), Hyb.7 (15.36), Hyb.11 (15.24), Hyb.12 (14.92) and CCRP 9 (14.48) exhibited high scores with regard to taste, when compared to the standard chocolate.



Chocolate ingredients



Grinder



Chocolate bars



Individual Chocolate bar

Plate 12. Chocolates made for sensory evaluation

Table 29. Mean rank score based on sensory evaluation

Appearance		Colour		Flavour		Texture		Odour		Taste		After taste		Overall acceptability	
Hybrids/ varieties	Mean rank score	Hybrids/ varieties	Mean rank score	Hybrids/ varieties	Mean rank score	Hybrids/ varieties	Mean rank score	Hybrids/ varieties	Mean rank score	Hybrids/ varieties	Mean rank score	Hybrids/ varieties	Mean rank score	Hybrids/ varieties	Mean rank score
Hyb.1	16.02	CCRP 1	18.82	Hyb.6	19.18	Hyb.6	19.18	Hyb.6	15.34	Hyb.9	18.88	Hyb.10	17.78	Hyb.6	18.60
Hyb.17	15.66	CCRP 6	16.64	Hyb.9	17.38	CCRP 1	15.46	Hyb.7	14.72	Hyb.6	15.40	Hyb.6	17.66	Hyb.7	17.22
CCRP 1	14.56	Hyb.17	15.30	Hyb.11	15.56	Hyb.12	15.46	Hyb.9	14.56	Hyb.7	15.36	Hyb.7	16.72	Hyb.10	17.02
CCRP 9	14.42	CCRP 4	14.84	CCRP 9	14.78	Hyb.7	14.72	Hyb.11	14.46	Hyb.11	15.24	CCRP 1	16.06	CCRP 1	15.34
CCRP 2	14.36	Standard	14.58	Hyb.10	14.56	Hyb.11	14.68	CCRP 6	14.30	Hyb.12	14.92	Standard	15.40	Hyb.9	15.04
Hyb.11	13.86	CCRP 2	14.56	Hyb.1	14.42	Hyb.9	14.52	Hyb.1	13.78	CCRP 9	14.48	CCRP 9	15.00	Hyb.30	14.86
CCRP 4	13.52	CCRP 9	14.00	CCRP 1	14.12	CCRP 4	14.42	Hyb.17	13.64	Hyb.30	14.40	Hyb.9	14.54	Hyb.11	14.26
Standard	13.02	Hyb.10	13.22	Hyb.15	14.08	Hyb.30	14.02	CCRP 1	13.08	CCRP 1	14.28	Hyb.11	14.46	CCRP 9	13.96
Hyb.12	13.00	Hyb.15	12.62	Standard	13.76	Hyb.1	13.52	Standard	13.06	Standard	14.08	Hyb.12	13.74	Standard	13.54
Hyb.9	12.94	Hyb.1	12.26	Hyb.7	13.56	CCRP 9	13.04	Hyb.12	12.96	Hyb.10	13.70	Hyb.3	12.82	Hyb.12	13.16
Hyb.10	12.92	Hyb.3	11.04	Hyb.12	13.52	Standard	12.90	Hyb.21	12.88	Hyb.3	12.64	Hyb.1	12.14	Hyb.3	13.14
CCRP 7	12.44	Hyb.12	10.92	Hyb.3	13.40	Hyb.3	12.90	CCRP 2	12.70	Hyb.1	12.36	CCRP 7	11.36	Hyb.1	11.86
CCRP 5	11.88	Hyb.11	10.38	Hyb.30	12.74	CCRP 6	12.78	CCRP 8	12.18	CCRP 6	11.50	Hyb.30	9.76	Hyb.17	11.78
Hyb.7	11.58	Hyb.9	10.12	CCRP 4	12.46	Hyb.10	10.96	CCRP 7	12.12	CCRP 5	11.22	Hyb.15	9.68	CCRP 6	11.60
CCRP 3	11.28	CCRP 5	9.88	CCRP 6	7.86	CCRP 2	8.80	Hyb.15	10.24	CCRP 7	10.26	CCRP 3	8.86	CCRP 4	9.40
CCRP 6	10.62	Hyb.6	9.44	CCRP 5	7.38	Hyb.15	8.38	Hyb.30	9.36	Hyb.15	9.50	CCRP 4	8.44	Hyb.15	8.64
Hyb.6	9.40	Hyb.21	9.06	CCRP 7	7.02	Hyb.21	7.08	Hyb.10	9.10	CCRP 4	9.12	Hyb.17	8.40	CCRP 7	7.96
Hyb.15	8.64	Hyb.7	8.92	CCRP 8	6.68	CCRP 5	6.68	Hyb.3	8.90	Hyb.17	7.62	CCRP 2	7.64	CCRP 2	6.72
Hyb.3	5.96	CCRP 3	7.98	CCRP 2	6.48	CCRP 8	6.54	CCRP 9	7.42	Hyb.21	5.92	CCRP 6	7.00	CCRP 5	6.36
Hyb.30	5.7	CCRP 8	6.68	CCRP 3	5.06	CCRP 7	6.20	CCRP 5	7.18	88.5	5.44	CCRP 8	6.62	CCRP 8	5.42
CCRP 8	5.64	CCRP 7	5.88	Hyb.21	4.94	Hyb.17	6.16	CCRP 4	7.06	CCRP 2	5.16	CCRP 5	4.72	Hyb.21	5.28
Hyb.21	5.58	Hyb.30	5.86	Hyb.17	4.06	CCRP 3	4.60	CCRP 3	3.96	CCRP 3	1.52	Hyb.21	4.20	CCRP 3	1.84
K value	0.142	0.09		0.203		0.139		0.194		0.116		0.084		0.501	

The highest mean rank score for after taste was observed in Hyb.10 (17.78) followed by the hybrids; Hyb.6 (17.66), Hyb.7 (16.72) and CCRP 1 (16.06) and these chocolates scored high preference above the standard chocolate. The CCRP 9 (15) and the Hyb.9 (14.54) also exhibited high after taste score. With regard to overall acceptability, hybrids; Hyb.6 (18.60), Hyb.9 (17.22), Hyb.10 (17.02), Hyb.9 (15.04), Hyb.30 (14.86), Hyb.11 (14.26) and CCRP varieties like CCRP 1 (15.34) and CCRP 9 (13.96) scored high mean rank score.

4.11 Influence of weather on pod characters

Correlation studies were carried out between weather parameters and pod characters and it is presented in Table 30. Weather data used for the correlation is showed in Appendix VII. Pod weight and total wet bean weight per pod is negatively and significantly correlated with maximum temperature and it is positively and significantly correlated with minimum temperature, relative humidity (RH), rainfall and rainy days. Number of beans per pod is negatively and significantly correlated with maximum temperature.

Table 30. Correlation of weather parameters with pod characters

	Max temp.	Min temp.	RH morning	RH evening	Rainfall	Rainy days
Pod weight	-0.340(**)	0.524(**)	0.469(**)	0.492(**)	0.512(**)	0.475(**)
Total wet bean weight	-0.319(**)	0.431(**)	0.372(**)	0.396(**)	0.416(**)	0.378(**)
No. of beans/pod	-0.232(**)	0.158	0.097	0.119	0.14	0.103

**Correlation is significant at the 0.01 level

4.12 Pests and diseases scoring

Pests and diseases were observed among the thirty hybrids for three seasons and the data is presented in Table 31. Generally, the pests and diseases infestation observed from the field is very low. Mealy bug and rat were the major pests and black pod was the major disease affecting the pods which resulted in yield loss (Plate 13). Among the thirty hybrids, maximum infestation through black pod disease caused by *Phytophthora palmivora* was observed in Hyb.2 (15.7%). Black pod disease was not observed in the hybrids; Hyb.5, Hyb.7, Hyb.8, Hyb.10, Hyb.12, Hyb.14, Hyb.16, Hyb.18, Hyb.21 and Hyb.25. Mealy bug infestation was more in Hyb.23 (11.6%) followed by the hybrids; Hyb.23 (11.6%) and Hyb.26 (9.6%). Tea mosquito bug attack was observed more in Hyb.25 (10%) followed by Hyb.19 (8.4%).

Rat attack was observed in several hybrids, in which attack was more in Hyb.4 (15.9%). The hybrids; Hyb.26 (15.7%), Hyb.3 (10.5%) and Hyb.18 (10.5%) also observed with high rat attack. Squirrel attack was more noticed in Hyb.14 (8.4%) followed by the hybrids; Hyb.1 and Hyb.15 with 7.8% attack each. Caterpillar was found only in a few hybrids; Hyb.2, Hyb.3, Hyb.8, Hyb.19 and Hyb.25 and observed only 2.5 to 5.2 percent attack.



Tea mosquito bug



Pod rot



Rat attack



Mealy bug

Plate 13. Major pests and diseases

Table 31. Pests and diseases scoring

Hybrids	Black pod (%)	Mealy bug (%)	Tea mosquito bug (%)	Rat (%)	Squirrel (%)	Caterpillar (%)
Hyb.1	9.3	-	4.2	11.6	7.8	-
Hyb.2	15.7	-	-	7.5	6.9	4.6
Hyb.3	5	7.8	5	10.5	7.5	2.5
Hyb.4	13.9	-	-	15.9	-	-
Hyb.5	-	-	-	12.5	6.9	-
Hyb.6	5	-	1.3	2.5	-	-
Hyb.7	-	-	-	5	-	-
Hyb.8	-	-	5.2	8.4	-	5.2
Hyb.9	5.2	-	-	2.4	5	-
Hyb.10	-	-	-	7.5	-	-
Hyb.11	5	-	-	-	-	-
Hyb.12	-	6.8	4.6	2.1	-	-
Hyb.13	6.9	-	5	-	-	-
Hyb.14	-	-	-	5.2	8.4	-
Hyb.15	5.2	-	-	2.5	7.8	-
Hyb.16	-	6.9	-	5	-	-
Hyb.17	3.4	-	2.2	-	-	-
Hyb.18	-	-	-	10.5	5.2	-
Hyb.19	10.5	-	8.4	4.6	-	2.6
Hyb.20	12.5	-	-	5.5	-	-
Hyb.21	-	7.8	-	2.6	-	-
Hyb.22	6.9	-	-	7.8	-	-
Hyb.23	7.8	11.6	-	2.5	-	-
Hyb.24	13.9	-	-	-	6.3	-
Hyb.25	-	-	10	5.2	-	2.5
Hyb.26	4.6	9.6	-	15.7	-	-
Hyb.27	10	5	-	2.5	6.9	-
Hyb.28	10.5	-	6.3	2.6	-	-
Hyb.29	8.4	7.	-	8.4	5	-
Hyb.30	7.5	-	-	10	-	-

4.13 Selection of potential hybrids for further crop improvement programme

Thirteen superior hybrids were first selected based on quantitative characters and these hybrids were ranked based on the presence of both Criollo (biochemical and quality parameters) and Forastero characters (yield and pests and disease tolerance). Also the preference based on sensory evaluation is also recorded. This is presented in Table 32. The hybrids; Hyb.6, Hyb.11, Hyb.17 and Hyb.7 recorded superior characters of Criollo and Forastero along with high preference among the judges based on sensory evaluation. The hybrids; Hyb.12, Hyb.15 and Hyb.18 recorded superior Criollo characters, but were not much superior with respect to Forastero characters like yield and pests and disease tolerance.

Table 32. Potential hybrids based on Criollo and Forastero character

Hybrids selected based on quantitative characters	Criollo character	Forastero character		Chocolate quality
	Biochemical and quality parameters rank	Yield rank	Pests and disease tolerance rank	Organoleptic evaluation
Hyb.12	2	12	7	
Hyb.6	4	1	3	Highly preferred
Hyb.11	5	4	1	Highly preferred
Hyb.17	1	3	2	Highly preferred
Hyb.10	9	14	4	
Hyb.21	8	11	6	
Hyb.15	2	3	13	
Hyb.9	7	6	5	Highly preferred
Hyb.29	16	16	22	
Hyb.7	3	2	1	Highly preferred
Hyb.18	1	5	14	
Hyb.30	14	14	5	
Hyb.24	20	7	20	

Discussion



5. DISCUSSION

Cocoa (*Theobroma cacao* L.) is a beverage crop which have a great potential for chocolate production. Based on the importance of quality of cocoa beans for chocolate production, selection of superior hybrids with premium quality is very important. A number of qualitative as well as quantitative descriptors were proposed for cocoa pod and bean characterization. In cocoa, morphological descriptors are helpful for the breeders to select superior genotypes for the breeding programme (Engles *et al.*, 1980). Biochemical characters and other quality parameters also have great importance while selecting superior hybrids based on quality. Success of plant breeding greatly depends up on the identification of superior genotypes. It is necessary to evaluate morphological, bio-chemical and organoleptic characters, which influences the cocoa bean quality regarding the genotype and the environment (Bucheli *et al.*, 2001). There is a great scope for selection of hybrids from the genotypes bred for quality for further crop improvement programme.

With this context, the study entitled 'Evaluation of cocoa (*Theobroma cacao* L.) hybrids bred for quality' conducted to evaluate the hybrid progenies derived as a result of hybridization programme initiated at Cocoa Research Centre (CRC), Vellanikkara during 2004 for quality. For this purpose high quality parental lines with superior quality (Criollo type) were crossed with high yielders along with disease resistance traits (Forastero type). Out of the crosses made, thirty hybrids were selected for evaluation based on pod and bean qualitative and quantitative characters, biochemical characters, quality parameters and organoleptic evaluation.

The results pertaining to 'Evaluation of selected cocoa (*Theobroma cacao* L.) hybrids bred for quality' are discussed in this chapter under the following titles.



173934

5.1 Morphological characterization

For morphological characterization, both qualitative as well as quantitative characters were used to evaluate the hybrids. Morphological evaluation is applicable to derive economic and breeding gains from genotypes (Iwaro *et al.*, 2003; Bekele *et al.*, 2006). In cocoa, morphological descriptors are helpful for the breeders to select superior genotypes for the breeding programme (Engles *et al.*, 1980).

The hybrids evaluated in the present study fell under four different pod shapes such as cundeamor, amelonado, criollo and angoleta. Based on pod and bean morphology scholars identified different groups of cocoa: cundeamor, angoleta, amelonado, criollo and calabacillo (Marita *et al.*, 2001 and Sounigo *et al.*, 2003). The features of different shapes of pod have been described by Wood and Lass in 1985. In the present study, criollo shape was characterized by both attenuate apex and acute apex with slight basal constriction, where criollo shape, attenuate apex and slight basal constriction are the true characters of Criollo type (Wood and Lass, 1985).

The cundeamor shape (ridged and with bottle neck) is characterized by the presence of intense pod rugosity, which is a true criollo character. Angoleta shape (similar to amelonado but deeply ridged with square base) was observed in sixteen hybrids. According to the present study, amelonado (melon shaped) shape was characterized by obtuse apex and intermediate basal constriction. The obtuse apex form and intermediate basal constriction is the character of Forastero type. Out of four hybrids with cundeamor shape three expressed acute apex and out of sixteen hybrids with angoleta shape, eight expressed obtuse apex form. The results were in tune with the early study by Minimol *et al.* (2011) stating that fruit shape is influenced by fruit apex and it indicates that fruit shape can be identified by its apex form.

Qualitative characters like pod apex, pod basal constriction, rugosity and cotyledon colour showed wide variability among the hybrids. This observation is on

par with the early findings by Aikpokpodion (2010) and it will help in selection of superior hybrids with quality.

In the present study, a wide variability was observed in morphological characters among the hybrids in relation with qualitative as well as quantitative characters like length, width, weight, apex form, shape, rugosity, colour, husk thickness and basal constriction of the pod and seed characters like number, length, width, dry weight and colour of the seed. The study carried out by Efombagn *et al.*, (2009), Aikpokpodion (2010) and Asna *et al.*, (2014) found wide morphological variation in qualitative and quantitative characters of cocoa related to pod and bean. Therefore, this will be a great scope for the selection of superior hybrids.

The hybrids resulted as a cross of Criollo as one parent showed dark purple, medium purple, light purple and white colour, which is commonly categorized as mixed colour. In the present study, fifteen hybrids expressed mixed bean colour. The presence of mixed bean colour indicates the hybridity between Forastero type with Criollo type. Wood and Lass, (1985) had also reported that mixed type is an indicator of Trinitario *ie* cross between Criollo and Forastero

Wood and Lass (1985) reported that typical Criollo types are characterized by the presence of white cotyledon colour. Hence the presence of white colour in hybrids Hyb.9, Hyb.15, Hyb.16 and Hyb.17 indicated the presence of Criollo characters. The Criollo beans are white to ivory or have a very pale purple colour, due to the presence of an anthocyanin inhibitor gene (Fowler, 1999). White coloured beans were also present under mixed bean colour classification and it was observed in several hybrids. The selection procedure for Criollo type is based on phenotypic traits like sweet pulp, white beans and elongated pods (Engels, 1983). Four hybrids were recorded with intense rugosity, which is a true Criollo character (Wood and Lass, 1985).

The Criollo characters like Criollo pod shape, purplish yellow ripe pod colour, purplish green unripe pod colour, attenuate apex form, slight pod basal constriction, intense rugosity and white and mixed bean colour were observed among the thirty hybrids indicating that there is a transfer of morphological Criollo characters from the parents to the progenies.

Number of ridges and furrows did not show significant difference among the hybrids. Some hybrids expressed deep furrows on pod surface and most of the hybrids exhibited ten ridges and furrows. The hybrid with deeply furrowed and with ten ridges and furrows is a character of Criollo (Wood, and Lass, 1985).

Hybrids exhibited wide variation with respect to quantitative characters and significant differences were observed among the hybrids in terms of pod and bean characters. This indicates the heterogeneity present in the hybrids, which will be a great potential in selection for superior hybrids. Increased vigour in quantitative characters is important while selecting hybrids with respect to economical characters.

Among the thirty hybrids, Hyb.10, Hyb.12 and Hyb.2 recorded pod weight of 685 g, 670.92 g and 684 g respectively, which was higher than other hybrids. Pod weight was observed in the range between 249.64 g and 685 g. However most of the hybrids expressed pod weight more than 350 g, which is the selection criteria recommended by Francies *et al.* (2002). Among the thirty hybrids, pod length was recorded in the range of maximum value of 20.22 cm in Hyb.11 to a minimum value of 12.56 cm in Hyb.3. Pod breadth was observed in the range between 6.54 cm and 9.46 cm. Average number of beans per pod was found in the range of 28.80 to 53. The total wet bean weight was observed highest in Hyb.21 (185.72 g) and least in Hyb.2 (74.38 g). These recorded observations were in tune with the earlier findings by Adewale *et al.*, (2013). The wide variation observed among quantitative characters will help to study the diversity among genotypes for phenotypic traits. Number of pods per

tree, number of beans per pod and weight of individual beans were the three main components of yield (Wood and Lass, 1955).

Among the thirty hybrids, Hyb.10 (685 g) observed with maximum pod weight and the total wet bean weight was observed maximum in Hyb.21 (185.72 g) followed by Hyb.30 (178.6 g). This reveals that the pod weight is not the indicating factor for the total wet bean weight and also the husk thickness has some significance in contributing to the pod weight. In the present study, the wet bean weight and dry bean weight among the thirty hybrids varied significantly. A group of scientists had reported that yield expressed as wet or dry bean weight is highly variable (Pound 1932; Enriquez and Soria, 1966, Rubeena, 2015).

Husk thickness was observed in the range between 0.67 cm in Hyb.23 and 1.15 cm in Hyb.19. This observation is on par with the earlier findings by Velayutham *et al.*, (2013). High husk thickness is considered as an undesirable character. Husk thickness had a significant role in deciding pod weight (Rubeena, 2015). Husk thickness of one cm or less than one cm is the desirable character (Enriquez and Soria, 1966).

The Hyb.19 observed nine percent flat beans in the pod, which was maximum among the hybrids and Hyb.26 with only less than 0.5 percent flat beans in the pod. The unfertilized ovules will develop into flat beans. Presence of flat beans in the pod is indicated as an undesirable character and crop improvement programme aims to reduce the number of flat beans per pod. Less percent of flat beans will be considered as a desirable character in cocoa breeding (Mora, 1989).

Based on the score obtained for pod weight, pod length, pod breadth, no. of beans per pod and total wet bean weight, hybrids; Hyb.12, Hyb.10, Hyb.17, Hyb.22, Hyb.29 and Hyb.11 were selected as superior ones. Also based on husk thickness,

number of flat beans and flat bean percent, the hybrids were ranked and the hybrids; Hyb.15, Hyb.9, Hyb.23, Hyb.27, Hyb.8 and Hyb.5 were observed as superior ones.

Peeled wet bean weight and dry bean weight observed maximum in Hyb.11 even though unpeeled wet bean weight was observed maximum in Hyb.10. This indicates that mucilage weight is more for Hyb.10. Hence unpeeled wet bean weight alone cannot be considered as a good indicator for final dry bean yield.

Enriquez and Soria (1966) observed that the dry bean weight of single bean varied from 0.5 g to 2.5 g. Maharaj *et al.*, (2011) reported dry bean weight in the range of 0.74g to 1.49 g. Velayutham *et al.*, (2013) reported in the range of 0.59 to 1.72 g. In the present study, dry bean weight of single bean was observed in the range of 0.51 g to 1.48 g and this is in the range reported by the scientists. Cilas *et al.*, (1989) reported high variation in bean size in Trinitario types.

In the present study, the bean characters like bean dry weight, length, breadth and width widely varied among the hybrids and Hyb.11 exhibited highest value for bean length, breadth and width. The bean size is one of the most important economic character in cocoa and considered as an important component of yield (Soria, 1978). Morphological and structural characteristics of beans exhibited high variation among the species (Adewale *et al.*, 2010). In the present study also it was observed that there is wide variation in morphological characters and it will help in cocoa breeding programme for selection of superior hybrids.

The hybrids; Hyb.11, Hyb.17 and Hyb.23 exhibited more than 1.2 g single dry bean weight which is a character of Criollo beans (Motamayor *et al.*, 2002). According to the international standards peeled dry bean size must be 0.8g or more for the selection of superior hybrids. In the study, this standard was met by almost all the hybrids except Hyb.2, Hyb.3, Hyb.4, Hyb.20 and Hyb.27. Mean seed wet weight of

cocoa genotypes with greater than one gram are considered to be of superior quality (Monteiro *et al.*, 2009).

In the present study, purplish yellow colour in ripened pods and purplish green colour in unripened pods were observed in hybrids Hyb.8, Hyb.12, Hyb.16 and Hyb.22. Red or purple pigmented colour of pod is the character of Criollo types (Bartley, 2005). It indicates that Criollo characters are imparted on hybrids through crossing, which can be used for further selection.

Based on unpeeled and peeled wet bean weight and peeled dry bean weight, the hybrids were ranked using DMRT technique and the hybrids; Hyb.11, Hyb.6, Hyb.21, Hyb.17, Hyb.12, Hyb.10 and Hyb.15 were observed as superior ones. Also hybrids were ranked based on the single bean characters like seed length, breadth, thickness and dry weight. Based on this ranking hybrids; Hyb.11, Hyb.6, Hyb.17, Hyb.24 and Hyb.12 were found as superior ones.

5.2 Economical characters with respect to pod and bean

The yield data for the year 2015-16 showed that Hyb.6 yielded 111 pods /tree/ year and it is the highest among the thirty hybrids, followed by the hybrids; Hyb.7, Hyb.15 and Hyb.11 with 108, 107 and 105 pods/ tree/ year respectively. The high yield is a character of Forastero type. Pod Index (PI) was observed minimum in Hyb.11 (15). The hybrids; Hyb.30, Hyb.17 and Hyb.12 also showed least pod index value. Pod index means number of pods required to get 1 kg of peeled and dried cocoa beans (Maharaj *et al.*, 2011). The hybrid with high yield potential should be associated with those having a low PI value. The hybrid with PI value less than or almost equal to 15 is suitable for breeding purpose (Pound, 1932). Therefore Hyb.11 (15) is suitable for breeding programme based on PI value.

In the present study, the hybrids having low PI value are showing high dry weight of peeled bean eventhough number of beans per pod are not much high. This

result indicates that dry weight of peeled bean is the important character which contributes to the PI value than the number of beans. And increase in bean count with small bean size is an undesirable character (Rubeena, 2015). Either cotyledon weight of more than one gram and bean number almost equal to 40 or moderate cotyledon weight (0.9g) and high bean number (>40) will result in lower pod index value (Bekele et al., 2004).

Among the thirty hybrids, Hyb.9 showed highest percentage of wet bean weight/ pod weight (43.04 %) followed by the Hyb.20 (41.06%) and total wet bean weight per pod was observed maximum in Hyb.21 (185.72 g) followed by Hyb.30 and highest pod weight was observed in Hyb.10 (685 g) followed by Hyb.21 (684 g). These observations revealed that total pod weight is not the factor to be considered for finding the total wet bean weight. Husk thickness may contribute to the pod weight which should be considered as an undesirable character while selection (Rubeena, 2015).

In the present study peeling ratio was observed highest in Hyb.18 (64.75%) followed by the hybrids; Hyb.1 (56.85%), Hyb.22 (56.56%) and Hyb.29 (56.37%). This indicated that the testa was thickest in Hyb.10 and thinnest in Hyb.18. The dry matter recovery was observed highest in Hyb.5 (81.20%) and it indicated the presence of low water content in the beans. The lowest dry matter recovery was found in Hyb.6 (54.81%) revealed that more water content is present in its beans. Peeling ratio and dry matter recovery was also computed by Rubeena (2015) and the range observed in the present study was on par with the observation made by her.

Efficiency index (EI) indicated the pod weight required to produce 1 g dry bean. The Hyb.23 was observed with least efficiency index (7.72) followed by Hyb.9 (7.93). Efficiency index should be minimum for the hybrids for the selection criteria. Conversion index (CI) indicated the amount of dry bean weight obtained from a given amount of wet bean weight. It should be maximum for the selection of superior hybrids. The Hyb.5 (0.45) observed with maximum CI followed by the Hyb.11 (0.41). EI and

CI values on hybrids were earlier computed by Vasudevan *et al.*, (2011) and the values in the present study was coming under the range observed by them.

Based on the secondary observations discussed above such as yield, wet bean weight/ pod weight, dry matter recovery, peeling ratio, pod value, pod index, efficiency index and conversion index, hybrids; Hyb.1, Hyb.5, Hyb.6, Hyb.7, Hyb.9, Hyb.11, Hyb.12, Hyb.13, Hyb.14, Hyb.15, Hyb.17, Hyb.18, Hyb.20, and Hyb.30 showed superior qualities.

5.3 Clustering

Cluster analysis based on seven qualitative characters resulted in ten clusters and cluster analysis based on quantitative characters resulted in six clusters. The hybrids present in each cluster were similar with respect to qualitative or quantitative characters. Only one hybrid each was present in cluster III and VI, which indicates that, they are distinct from other hybrids.

Aikpokpodion (2010) carried out cluster analysis based on 17 agro morphological traits to explore the relationship between 184 accessions. Hence, in the present study cluster analysis will help to study the relationship between the hybrids and it can be helpful in breeding programme.

The comparison of qualitative and quantitative clusters revealed that even though some hybrids are morphologically similar in qualitative character, they showed wide variation with respect to quantitative characters. Cluster analysis of thirty hybrids using D^2 statistics (Mahalanobis, 1936) based on 17 quantitative characters resulted in six clusters. This has been successfully exploited in biology to determine divergence among populations in terms of 'generalised group distance' (Chandrasekhariah *et al.*, 1963; Murty and Arunachalam, 1966; Murty *et al.*, 1967; Ram and Panwar, 1970). In cocoa D^2 statistics was used by many scientist like Engles (1986) and Asna (2013) for cluster analysis.

In the present study, cluster I was found biggest among other clusters which includes 9 hybrids. This indicates that the hybrids under this cluster is similar with respect to quantitative characters. The hybrids under different clusters are different from each other with respect to quantitative characters. The inter cluster distance was observed maximum between cluster II and cluster V, indicating that divergent hybrids placed under these clusters can be effectively crossed between each other for further crop improvement. Further crossing between the hybrids of diverse cluster can be done to produce double cross hybrids and there by exploiting much more vigour. Intra cluster distance was observed maximum in cluster V indicating that two hybrids under this cluster were divergent to certain extent even though they are grouped together. The minimum inter cluster distance was observed between cluster III and IV, which indicates that there will be a close genetic association between the hybrids present in these clusters. Thirty nine quantitative characters were clustered to group 294 cultivars by Engles (1986). Maharaj *et al.*, (2011) clustered 25 accessions based on 15 quantitative traits to study the relationship among them.

The number of clusters formed based on qualitative and quantitative characters were different. Even though hybrids under single qualitative cluster were found to be falling under different quantitative clusters. This indicates that even though the hybrids are similar based on qualitative characters, they are different based on quantitative characters. Clustering was carried out based on biochemical characters which revealed that the most of the hybrids were distinct each other because they formed under separate clusters. This observation is on par with the observations recorded by Rubeena (2015)

5.4 Descriptive statistics

The descriptive statistics was computed through range (maximum and minimum), mean, standard deviation (SD), standard error (SE), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), environmental

coefficient of variation (ECV), heritability (H^2), genetic advance (GA) and genetic gain (GG) for 15 pod and bean quantitative characters. The descriptive statistics for same quantitative characters was also computed by Apshara and Nair (2001).

Coefficient of variation gives a relative measure of variance among different characters. The total variation arises in a population due to genetical and environmental factors. Hence, there is a need to split the variability into heritable and non heritable components like genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV).

The PCV and GCV were classified by Sivasubramanian and Madhavamenon, (1973) into low (0-10%), moderate (10.1-20%) and high (>20%). High GCV was observed for traits like pod weight, number of flat beans per pod, total wet bean weight per pod, peeled bean weight per pod, single bean wet and dry weight and single seed length, breadth and width. High variability associated with these characters indicates that there is an ample scope for selection. This observations is on par with the earlier findings by Asna *et al.*, (2014). Characters exhibited high GCV, gave maximum potential for selection between the hybrids based on these values. The amount of genetic variation alone will not be much effective to the breeder unless provided with the value of heritability, which is a measure of heritable part of total variation. Heritability is an important character which decides the suitability for selection of a character. High heritability results in high scope for genetic improvement of these characters through selection.

Range of heritability was classified by Robinson *et al.*, (1949) into low (0-30%), moderate (30-60%) and high (>61%). Among the pod characters of thirty hybrids, heritability for pod weight (79.82%), pod length (61.51%) and total wet bean weight per pod (77.48%) were reported to be high. This observation is on par with the findings by Soria *et al.*, (1974).

Among the bean characters of the hybrids, peeled bean weight (92.91%), single bean dry weight (91.28%), bean breadth (89.36%) and bean width (83.33) showed high heritability. This observation is on par with the earlier findings by Cilas *et al.*, (2010). The high heritability obtained in this study indicates that dependence of environmental factors on these phenotypic characters is low. Hence, based on all bean characters will result in improvement of the population. Total no. of flat beans per pod and single bean length showed high PCV and GCV value but observed with low heritability. Therefore this cannot be used in crop improvement programme .Even though parents expressed high GCV, the character will not get transferred to the progeny selection without high heritability and GG.

High heritability for peeled bean weight, breadth and width along with high PCV, GCV and GG imparts a great scope in selection for crop improvement through this character. High heritability for single bean dry weight was reported by Kumaran and Amma (1981). High broad sense heritability is considered as a good indicator of genetic improvement of phenotypic traits (Adewale *et al.*, 2010).

Genetic advance is a measure of genetic gain under selection. The value of genetic advance was categorized by Johnson *et al.*, (1955) into low (0-10%), moderate (10.1-20%) and high (>20%). High genetic advance was observed in most of the quantitative traits like pod weight, no. of flat beans per pod, total wet bean weight per pod, peeled bean weight per pod, single bean wet and dry weight and single seed length, breadth and width. High genetic advance indicates that crop improvement of these traits are possible by selection.

Among the quantitative characters seed dry weight, seed width, seed breadth and pod weight showed high genetic gain among the quantitative characters. These characters also expressed high heritability. High heritability accompanied by high genetic advance and genetic gain is a good indicator of additive gene effect. Hence selection based on these characters will be effective in breeding programme (Minimol

et al., 2014). The characters like single dry bean weight, bean width, bean breadth, pod weight and wet bean weight were with high heritability and genetic gain indicating that there will be considerable improvement over population, if these characters are considered as selection criteria. Genetic gain and genetic advance values for these traits found high in the study conducted by Rubeena (2015).

5.5 Heterosis

Heterosis is the genetical tool to denote the expression of increased vigour. Vigour of hybrids is computed over mid parent, better parent and standard variety. Heterosis can be effectively utilized for the selection based on vigour in economic traits of hybrids (Christian, 2003).

The relative heterosis (RH) was measured to find the vigour over their parents. In the present study, the highest positive and significant relative heterosis observed for pod length was exhibited by Hyb.11 (14.17%); pod breadth by Hyb.16 (16.43%); pod weight Hyb.21 (64.62%); number of beans per pod by Hyb.26 (26.19%); wet bean weight per pod by Hyb.16 (47.04%); single seed length and seed weight by Hyb.1 (56.28% and 95.28% respectively); seed breadth and seed width also by Hyb.1 (52.45% and 100% respectively). These results indicate the presence of high hybrid vigour for different traits among the hybrids with respect to the parents. Those hybrids which showed positive and significant RH can be effectively utilized for breeding programme through selection as it shows high genetic diversity over their parents (Santhosh and Singh, 2006).

Heterobeltiosis was tabulated to measure the hybrid vigour over their better parent. Different hybrids showed high positive and significant heterobeltiosis value for various quantitative traits like yield, pod length, pod breadth, pod weight, total wet bean weight/ pod, number of beans/ pod, single bean dry weight, length, breadth and

width. Those hybrids showed high positive and significant heterobeltiosis value for different traits can be used for selection with respect to vigour.

Standard heterosis (SH) was computed to find the vigour over a standard check variety (Nadarajan and Gunasekaran, 2008). In the present study KAU released CCRP variety CCRP 8 was taken as the standard variety. Several hybrids showed high positive and significant standard heterotic value for different traits like yield (Hyb.6), pod length (Hyb.11), pod breadth (Hyb.19), pod weight (Hyb.10), total wet bean weight/ pod (Hyb.21), number of beans/ pod (Hyb.26), single bean dry weight, length and breadth (Hyb.11) and width (Hyb.24). This indicated that this hybrid can be commercially exploited. Rubeena (2015), also observed high and positive RH and SH value for the above traits.

The relative heterosis and standard heterosis for husk thickness was high positive and significant in hybrids Hyb.16 and Hyb.25 and Hyb.19. High husk thickness is considered as an undesirable character with respect to pod weight in cocoa breeding programme even though thick ridges provide protection against rodents and squirrels. Therefore hybrids which are showing high positive and significant values are not considered for selection (Wood and Lass, 1985).

5.6 Biochemical evaluation

Biochemical parameters in cocoa beans are very important with respect to the quality of cocoa beans. The bean biochemical compounds interact with each other through fermentation process results in the formation of cocoa bean flavor quality (Amin *et al.*, 2002). Cocoa butter, protein, polyphenols and alkaloids like theobromine, theophylline and caffeine are the major biochemical components present in beans. (Taylor, 2002; Luna *et al.*, 2002; Counet *et al.*, 2004). Total fat, total acidity, total phenols, phenolic acids, organic acids, heavy metals, amino acids, caffeine,

theobromine, pH, sugars and macro and micronutrients were the main variables included in the cocoa quality index for the cocoa beans (Araujo, *et al.*, 2014).

The major biochemical components in beans of selected clones found that cocoa fat content in the beans depends largely on the genotypes used (Rossini *et al.*, 2011). In the present study, fat content was observed in the range from 39% in Hyb.28 to 56.5% in Hyb.17. Variation was observed among the thirty hybrids with respect to fat content and it is presented in Figure 6. Afoakwa (2013) observed in the range of 50.4–53.35% and 52.27-55.21% in fermented and unfermented cocoa beans respectively. This data was in the range observed in the present study. Forty six percent of the hybrids showed fat content above fifty percent. Significant difference was found among the hybrids with respect to fat content, which indicated genetic variability among the hybrids and it can be exploited for selection purpose for high fat content.

High fat content is very important with respect to characteristic flavour and aromatic qualities of chocolate and it is a character of Criollo type (Mossu 1992). The fat content in Criollo type recorded in between 49% to 56% (Leindo *et al.*, 1997). Therefore the hybrids; Hyb.28, Hyb.14, Hyb.1, Hyb.5, Hyb.6, Hyb.7, Hyb.9, Hyb.10, Hyb.11, Hyb.15 and Hyb.30 with high fat content can be effectively used for further breeding programme to prepare quality chocolates (Monteiro *et al.*, 2009).

Total polyphenols ranged between 34 to 60 mg/g in unfermented beans (Nazaruddin *et al.*, 2006); 40.0 mg GAE/g to 84.2 mg GAE/g (Kim and Keeney, 1984) and 67 mg/g to 149 mg/g (Niemenak *et al.*, 2006). It was reported that genetic factor can cause much variation (four fold difference) in polyphenolic content of fresh cocoa beans (Nazaruddin *et al.*, 2006; Rodriguez-Campos *et al.*, 2011). In the present study, total phenol content was observed in the range of 29.5 mg/g to 54.5 mg/g and this observation is on par with the observation made by them. Variation was observed among the thirty hybrids with respect to total polyphenol content and it is presented in

Fig 6. Variation in fat content among the hybrids

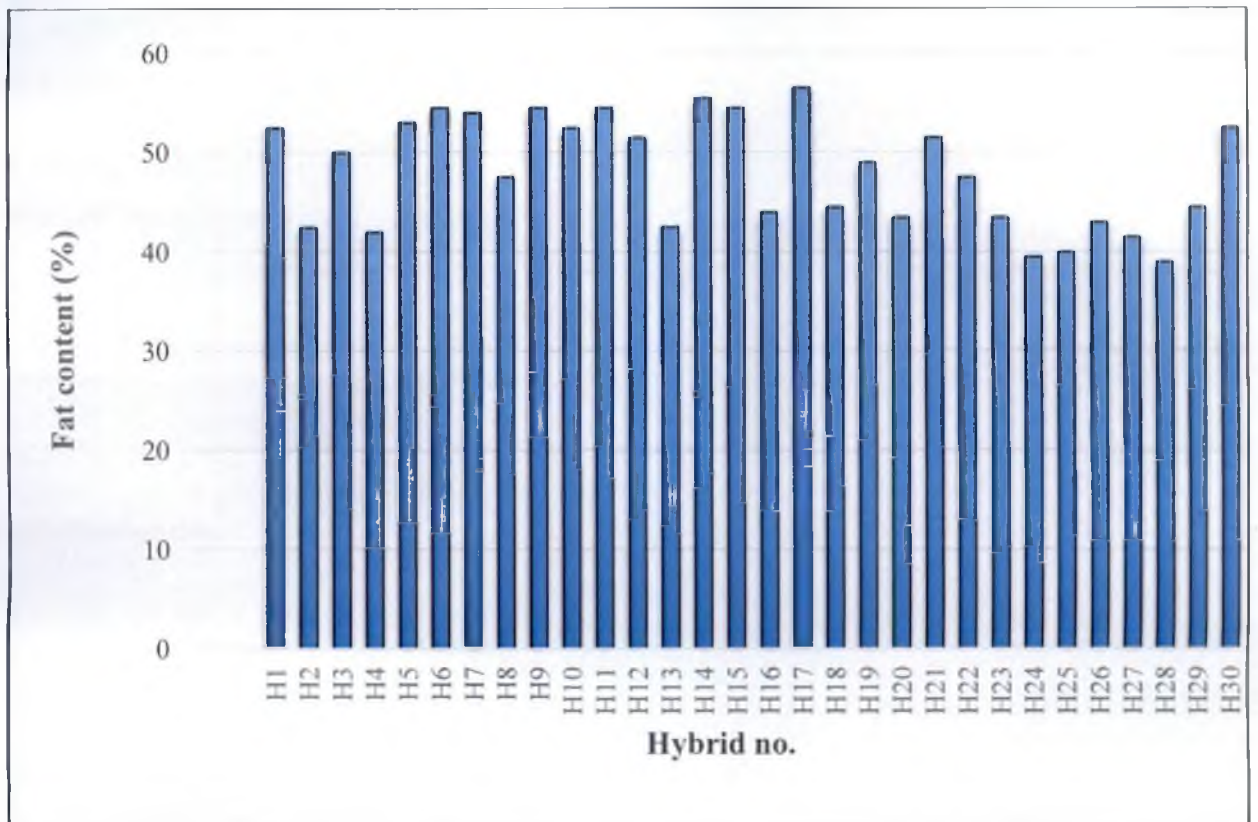
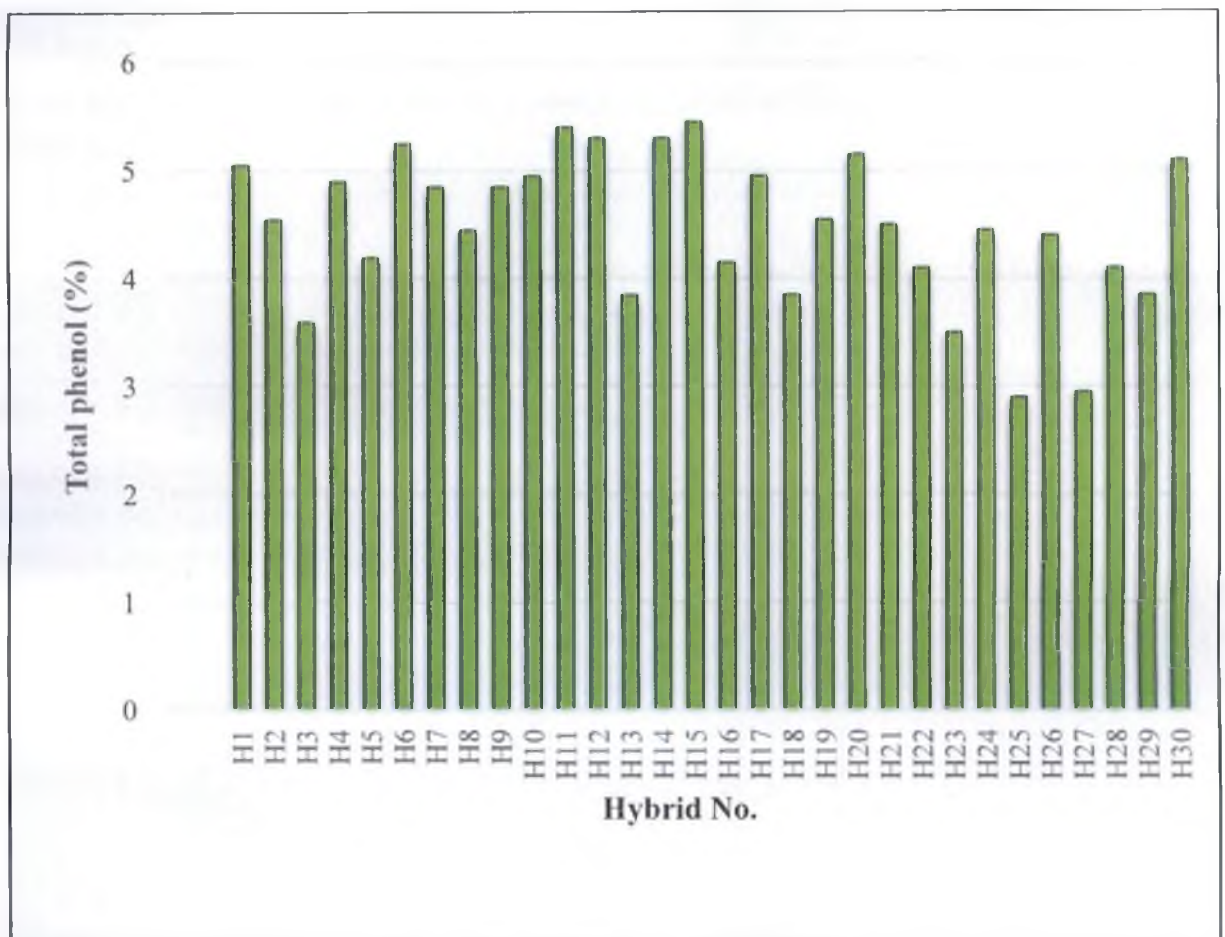


Figure 7. The high polyphenol content impart bitterness and astringency to the cocoa beans and it affects the final quality of chocolate and also the beans requires more time for the fermentation process because it is difficult to break down polyphenols into smaller compounds (Afoakwa, 2010).

Polyphenol is responsible for the flavour and colour of chocolate. If the polyphenol content in cocoa beans is too high or too low then it will be considered as inferior quality. Good quality cocoa beans have an optimum content (39 mg/g to 52 mg/g) of polyphenols (Elwers *et al.*, 2009). In the present study, many of the hybrids like Hyb.11, Hyb.12, Hyb.14, Hyb.6, Hyb.15, and Hyb.19 showed an optimum range, it is due to the Criollo origin which have only two third of the original polyphenol content as in Forastero type (Lange and Fincke, 1970). This will impart good flavour and aroma to the chocolates prepared.

Total alkaloid content present in dry fat free beans is reported to be in the range of 23.7 to 49.7 mg/g with an average value of 37 mg/g (Jalal and Collin, 1976). In the present study alkaloid content was observed in the range of 26.5 mg/g in the hybrid Hyb.3 to 42 mg/g in hybrids Hyb.25 and Hyb.26. This observations was on par with the earlier findings by Jalal and Collin (1976). Variation was observed among the thirty hybrids with respect to alkaloid content and it is presented in Figure 8. High amount of alkaloids present in the beans are the major compounds which contributes to astringency and bitter taste. During the fermentation process, bitter flavour developed is fundamentally determined by the concentration of theobromine and caffeine (Stark *et al.*, 2006). Therefore hybrids with medium level (35 mg/g to 40 mg/g) of alkaloid content were considered to be having premium quality. The hybrids; Hyb.1, Hyb.5, Hyb.6, Hyb.7, Hyb.9, Hyb.10, Hyb.11, Hyb.12, Hyb.15, Hyb.16, Hyb.17, Hyb.18, Hyb.21, Hyb.24 and Hyb.29 were categorized under the range of medium alkaloid content.

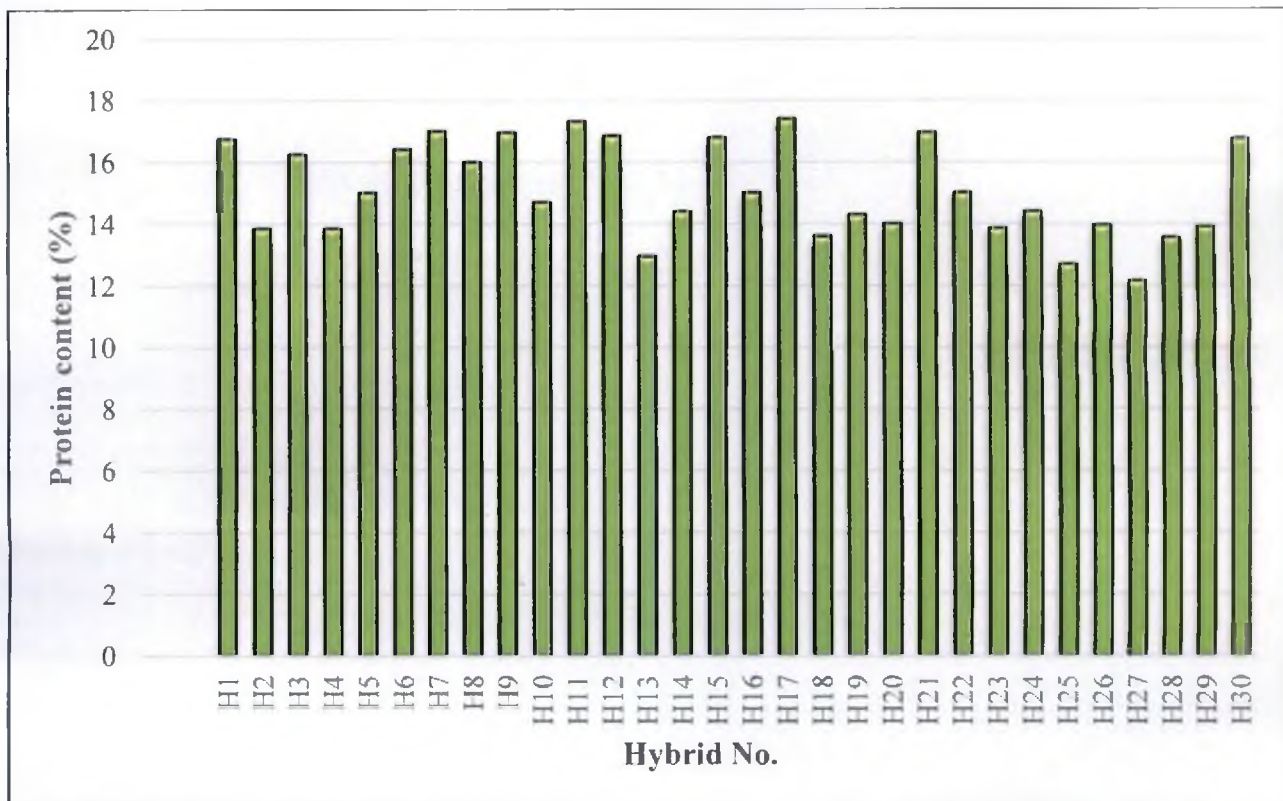
Fig 7. Variation in total phenol content among the hybrids



Among the thirty hybrids; Hyb.1 (16.75%), Hyb.17 (17.4%), Hyb.11 (17.3%), Hyb.7 (17%), Hyb.15 (16.80%), Hyb.9 (16.95%), Hyb.21 (16.95%) and Hyb.30 (16.75%) showed high protein content and in the range of Criollo types. The protein content in Criollo types reported in the range of 14.90% to 20.50% (Leindo *et al.*, 1997). Total protein in cocoa beans reported in a range between 15.2% and 19.8% (Afoakwa, 2008; Aremu *et al.*, 1995); 16% and 22% (Afoakwa, 2013). In the present study protein content was observed in a range from 12.95% to 17.4%. This observation was in the range reported by the scientists. Variation was observed among the thirty hybrids with respect to protein content and it is presented in Figure 9. The storage protein present in seeds was broken down by the enzymes resulting in the formation of peptides and free amino acids which aids in the development of chocolate aroma precursors (Schwan and Wheals, 2004; Afoakwa *et al.*, 2008). Therefore high protein will be considered as a desirable character for selection with respect to premium quality chocolates. Polyphenols will be subjected to oxidation through the activity of polyphenol oxidase and it will be condensed to high molecular weight tannins and their interaction with protein will improve the quality of cocoa beans for the production of chocolate (Afoakwa *et al.*, 2012).

Alvarez *et al.*, (2003) reported total soluble solids (TSS) in a range from 19.89 to 22.26% in Chuao genotypes. In the present study, TSS was found high in the hybrids; Hyb.10, Hyb.7, Hyb.9, Hyb.17 and Hyb.26 and the range of TSS was varied from 17 to 22° brix among the hybrids. This observation is on par with the findings by Alvarez and coworkers. The pulp of beans is responsible for the characteristic cocoa flavour and aroma. It will aid in fast fermentation through the increased action of microorganisms on the pulp (Kadow *et al.*, 2013). Therefore high amount of TSS in hybrids will be correlated with the quality of Criollo cocoa beans. The beans and the surrounding pulp were subjected to microbial activities and result in several biochemical reactions which improves the biochemical quality of cocoa beans (Gill *et al.*, 1984).

Fig 9. Variation in protein content among the hybrids



Based on biochemical characters, hybrids were evaluated and find out that hybrids; Hyb.30, Hyb.12, Hyb.15, Hyb.7, Hyb.6 and Hyb.11 were with superior qualities.

Based on the performance of thirty hybrids bred for quality through various morphological, biochemical and qualitative evaluation, superior hybrids were selected based on DMRT technique; Hyb.1, Hyb.3, Hyb.6, Hyb.7, Hyb.9, Hyb.10, Hyb.11, Hyb.12, Hyb.15, Hyb.17, Hyb.21 and Hyb.30. The KAU released varieties of cocoa (CCRP1, 2, 3, 4, 5, 6, 7, 8 and 9) were also taken for further evaluation in order to compare their performance with the selected hybrids. The selected hybrids and CCRP varieties were further evaluated for fermentation index, fermentation recovery, pH and moisture content. The selected hybrids and CCRP varieties were then used for making chocolates. Organoleptic evaluation was also carried out based on nine point hedonic scale to test the quality of the chocolates prepared.

5.7 Correlation studies

The association of various qualitative traits were studied by Spearman correlation coefficient which provided the information on nature and relationship among qualitative traits. Correlation among qualitative characters revealed that pod apex is negatively and significantly correlated with pod basal constriction (-0.366) and pod basal constriction is positively correlated with rugosity of pod surface (0.384).

The association of various quantitative traits by Pearson coefficient revealed that pod weight showed significant positive correlation with pod length (0.621), pod breadth (0.857), husk thickness (0.597), number of beans/ pod (0.401), total wet bean weight/ pod (0.825), peeled dry bean weight (0.709) and single dry bean weight (0.674). Adewale *et al.*, (2013) reported positive and significant correlation between pod weight and pod length, pod girth and number of beans per pod. This observation is on par with the results obtained in the present study. The correlation between pod

weight, husk thickness and total wet bean weight/ pod indicates that pod weight alone cannot take as a selection criteria with respect to wet bean weight/ pod because husk thickness also contributes some weight to the pod. Therefore wet bean weight per pod can be low even if the pod weight is high.

Number of flat beans per pod observed negative correlation with total wet bean weight/ pod (-0.378), TSS (-0.769) and fat content (-0.459). It indicates that number of flat beans is an undesirable character. Hence during selection not only increase in pod weight but also other characters like husk thickness, wet bean weight per pod, dry bean weight, no. of flat beans etc has to be considered. Francis (1998) also reported that these traits had to be taken into consideration while designing selection criteria for cocoa.

The seed dry weight exhibited positive and significant correlation with TSS (0.517), Fat content (0.465) and protein content (0.496). A positive and significant correlation was found between bean size and fat content at 5 % significant level (Mora and Bullard, 1961). This observation is in concurrent with the result obtained in the present study. In the present study, the hybrids; Hyb.17, Hyb.11, Hyb.23 and Hyb.24 observed with large bean size and they were correlated with high fat content and high protein content. This observation is on par with the observation made by Monteiro *et al.*, (2009).

5.8 Path coefficient analysis

The residual effect (0.142) indicates that almost 86% of the characters which contribute to total wet bean weight/ pod was considered in the study. The highest positive direct genotypic effects on wet bean weight was exhibited by number of beans (0.412) and its correlation with wet bean weight was also positive (0.624) which reveals direct relationship between them and direct selection for this trait will be resulting high

wet bean weight per pod. Dry bean weight (0.329) and pod weight (0.312) also expressed high direct effects on wet bean weight.

Pod weight showed high positive indirect effect on total wet bean weight/ pod (0.172). The result concluded that total wet bean weight of cocoa is highly influenced by number of beans, dry bean weight and wet bean weight.

5.9 Fermentation index and fermentation recovery

The hybrids; Hyb.17, Hyb.11, Hyb.1, Hyb.7, Hyb.10, Hyb.11, Hyb.12, Hyb.15, Hyb.17, Hyb.21 and KAU released CCRP 4, 8 and 9 showed greater than or equal to 70% fermentation index (FI). Sunil Kumar *et al.*, (2008) reported that highest mean FI through cut test score was 63.76%. In the present study, FI of hybrids observed above this range. Optimum FI aid in the improvement of quality of cocoa products and it results in the development of flavour and reduction in sourness, astringency and bitterness through biochemical reactions (Meyer *et al.*, 1989; Biehl *et al.*, 1990). Normally good quality beans will show high FI because it requires less time for maximum fermentation and high FI is the characteristics of Criollo type. Therefore those hybrids with high FI can be selected for chocolate production. When comparison made with the selected hybrids and CCRP released varieties, most of the hybrids showed better performance with respect to FI value.

The hybrids; Hyb.6, Hyb.10, Hyb.21, Hyb.17 and KAU released varieties like CCRP 1 and 8 observed with high fermentation recovery. Fermentation recovery among the hybrids reported in a range from 35.90% to 41.25%. Sunil Kumar *et al.*, (2008) reported that average recovery percent of cured beans (sun dried) was 39.09%. This observation is at par with the data recorded in the present study. Fermentation recovery should be high for the hybrids in terms of economic character. Therefore the hybrids with high fermentation recovery can be selected as superior ones. When

comparison was made with the selected hybrids and CCRP varieties, most of the hybrids showed better performance in fermentation recovery.

5.10 pH and moisture content

In the present study pH ranged from 5.20 in Hyb.10 to 6.10 in the CCRP 3 variety. Whitefield, (2005) reported that pH value of fermented dried cocoa beans was in the range from 5.00 to 5.72. Beans with higher pH (> 5.5) after fermentation are characterized as not fully fermented with low fermentation index and cut test score. A very low pH of cocoa beans after fermentation are considered as with low quality. Chocolate made from cocoa beans with intermediate pH (5-5.5) showed higher notes of chocolate flavour and low off flavour notes (Jinap *et al.*, 1995). Therefore hybrids showed pH value within the intermediate range can be considered as premium quality. The hybrids; Hyb.12 and Hyb.15 exceeded the intermediate pH range slightly while all other hybrids were under intermediate range.

Moisture content was observed below 8 percent among the hybrids and CCRP varieties. Moulds will develop when moisture content would go above 8% within the beans (Galvez *et al.*, 2007 and Ndukwu, 2009). Lower moisture content was ensured to arrest all the microbial and enzymatic reactions in the beans. Moisture content of all the hybrids and CCRP varieties were observed within the acceptable limits, and hence can prevent further damage by mould attack.

5.11 Sensory evaluation

Sensory evaluation was carried out based on sensory attributes like appearance, colour, flavour, texture, taste, odour, after taste and overall acceptability using a nine point hedonic scale (Meilgaard *et al.*, 1987). The chocolates made from the hybrids; Hyb.1, Hyb.3, Hyb.6, Hyb.7, Hyb.9, Hyb.10, Hyb.11 and Hyb.12 were most preferred/accepted by the panelists over the commercial chocolate standardized by KAU because of their appearance, colour, texture, taste and after taste. It indicates that these hybrids

are of Criollo origin. The selection procedure for Criollo type is based on the preference with respect to sensory attributes (Engels, 1983). The fundamental sensory attributes like acceptance, appearance, odour, flavour and texture directly related with the quality of the chocolates (Ovando *et al.*, 2015 and Leite, *et al.*, 2013). Therefore the preferred hybrids based on sensory evaluation can be characterized as having high quality.

The sensory profile of cocoa chocolates made from hybrids which secured the top five positions in the sensory evaluation based on the mean rank and total score with respect to consumer preference was presented in Figure 10. From the figure, it is clear that Hyb.6 with high flavour and texture and Hyb.9 with superior taste.

5.12 Pests and diseases scoring

Generally, the pests and diseases infestation observed from the field is very low with respect to percent attack or infestation. This is a character of Forastero type (Wood and Lass, 1985). The Mealy bug and rat attack were the major pests and black pod was the major disease affected the pods which resulted in yield loss. In the present study, black pod incidence was less and also disease was not observed in the hybrids; Hyb.5, Hyb.7, Hyb.8, Hyb.10, Hyb.12, Hyb.14, Hyb.16, Hyb.18, Hyb.21 and Hyb.25. It may be due to certain level of tolerance (Chandramohan, 1982) and also by the application of *Pseudomonas flourescens* talc formulation along with 2 kg farm yard manure per tree. Muthulakshmi *et al.*, (2011) reported that it was effective in control of black pod disease.

In the present study, mealy bug infestation was more in Hyb.23 (11.6%) followed by the hybrids; Hyb.23 (11.6%) and Hyb.26 (9.6%). Tea mosquito bug attack was observed more in Hyb.25 (10%) followed by Hyb.19 (8.4%). The adult and young ones of mealy bugs feed on the tender shoots, cushions, flowers and pods through sucking the sap, as a result cushion will abort (Khader, 2005)

Fig.10 Sensory profile of top ranked hybrids

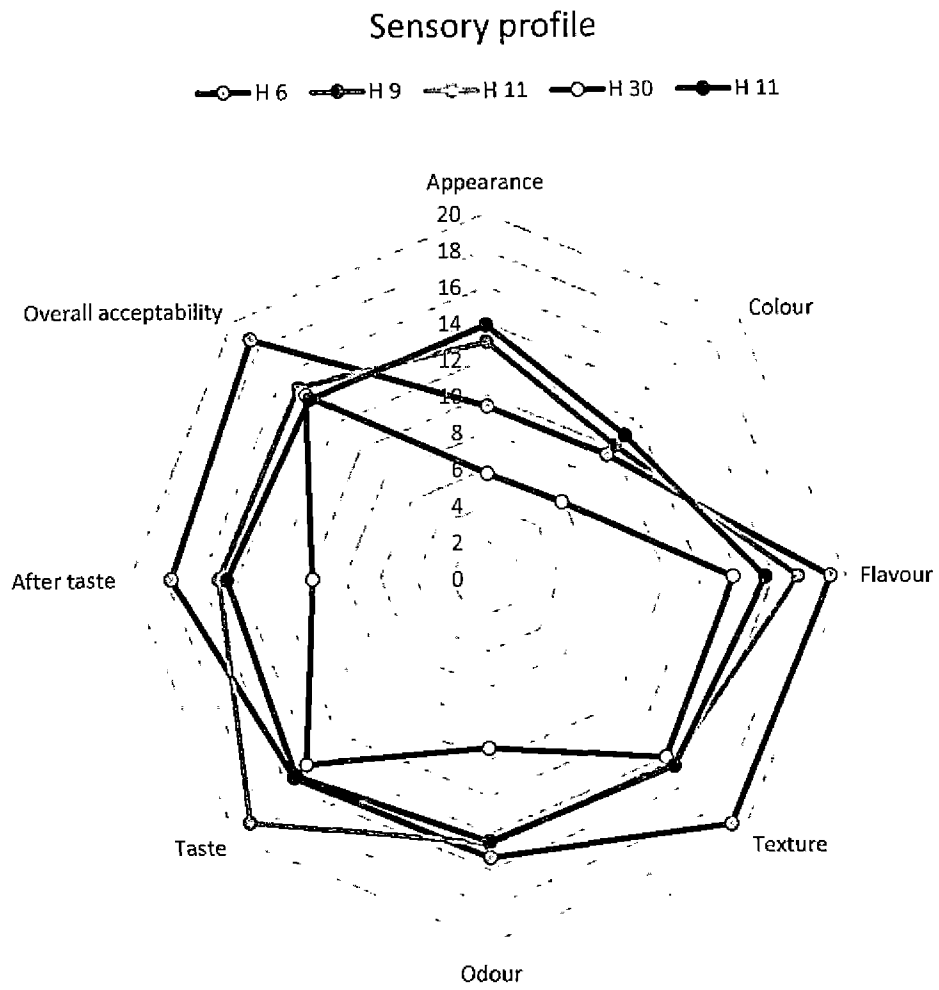
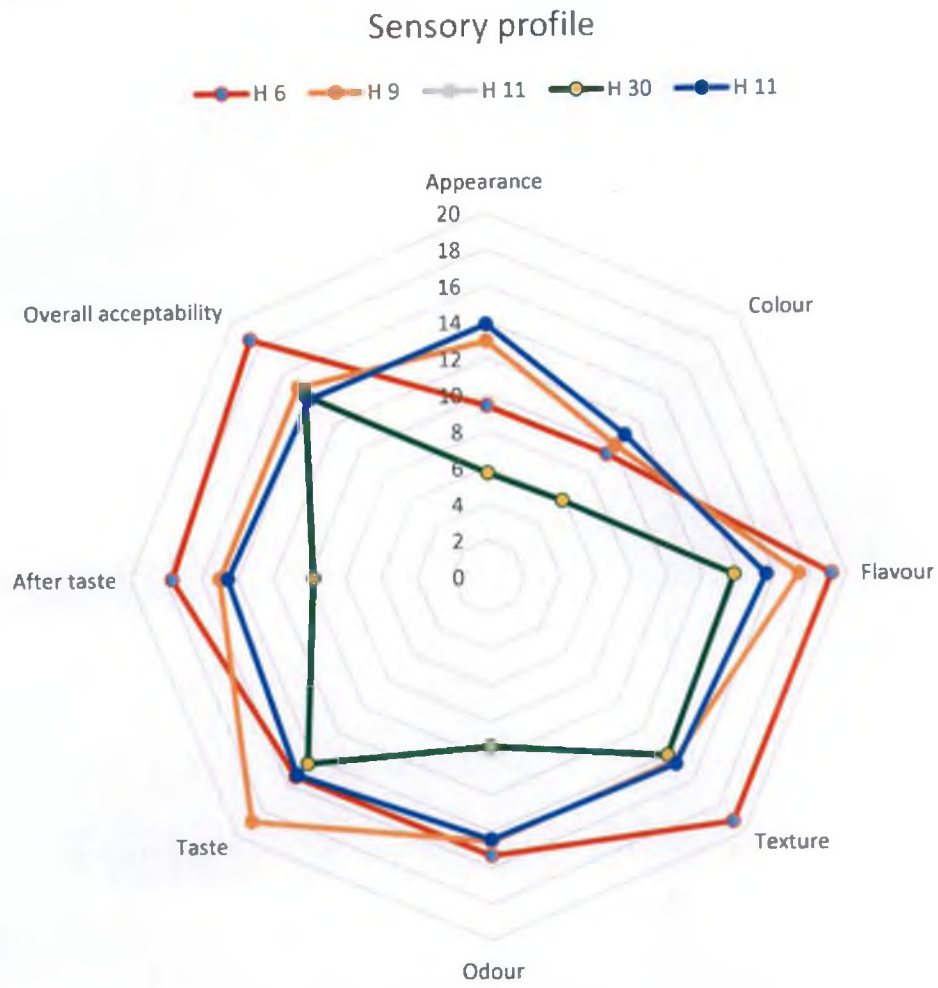


Fig.10 Sensory profile of top ranked hybrids



Rodents are the major pests of cocoa reported from almost all cocoa growing countries (Everard, 1968). In cocoa plantations, a heavy damage by rodents of about 75 per cent has been reported (Advani, 1982). Rat attack was observed in several hybrids, in which attack was more in Hyb.4 (15.9%). The hybrids; Hyb.26 (15.7%), Hyb.3 (10.5%) and Hyb.18 (10.5%) also observed with high rat attack and the hybrids; Hyb.9, Hyb.11, Hyb.12, Hyb.13, Hyb.17, Hyb.24 and Hyb.25 were not exposed to rat attack.

Squirrel attack was more noticed in Hyb.14 (8.4%) followed by the hybrids; Hyb.1 and Hyb.15 with 7.8% attack each. The Indian squirrels (*Funambulus* sp.) usually make oval shaped holes centrally or terminally and rats (*Rattus rattus*) makes round shaped holes near the stalk end (Bhat, 1980). Bellier and Lefevre, (1968) reported that squirrels showed most of the damages to the pod followed by rats. But in the present study, it was found that more damage was caused by rats than squirrels. This results is on par with the observation by Asna, (2013). Timely harvest of pods can prevent the attack by rat and squirrel (Abraham *et al.*, 1979).

Caterpillar was found only in few hybrids like Hyb.2, Hyb.3, Hyb.8, Hyb.19 and Hyb.25 and observed only 2.5 to 5.2 percent attack. It will devour the pericarp of unripe cocoa pods.

5.13 Influence of weather on pod characters

Weather parameters were correlated with pod weight, total wet bean weight/ pod and number of beans/ pod. Pod weight is negatively correlated with maximum temperature and positively correlated with relative humidity and rainfall. It indicates that, when temperature rises pod weight will decrease and when RH and rainfall increases pod weight will also increase. Total wet bean weight/ pod is negatively and significantly correlated with maximum temperature and it is positively and significantly correlated with minimum temperature, relative humidity (RH), rainfall

and rainy days. This observation is on par with the study conducted by Daymond and Hadley, (2008) and Minimol *et al.*, (2015).

5.14 Selection of potential hybrids for further crop improvement programme

Thirteen superior hybrids were first selected based on quantitative characters and these hybrids were ranked based on the presence of both Criollo (biochemical and quality parameters) and Forastero characters (yield and pests and disease tolerance). Also the preference based on sensory evaluation was recorded. The hybrids; Hyb.6, Hyb.11, Hyb.17 and Hyb.7 (Plate 14) were observed with superior characters for both Criollo and Forastero along with high preference among the judges based on sensory evaluation. Therefore these hybrids can be forwarded to comparative yield trial (CYT). The qualitative and quantitative pod and bean characters, economic characters, biochemical and quality parameters of selected hybrids for CYT are presented in Table 33, 34, 35, 36 and 37. The hybrids; Hyb.12, Hyb.15 and Hyb.18 (Plate 15) observed with superior Criollo characters but not much superior with respect to Forastero characters like yield and pests and disease tolerance, hence these hybrids can be used for further breeding programme to include high yield and hardiness by crossing them with Forastero parents.



Hyb.6



Hyb.7



Hyb.11



Hyb.17

Plate 14. Selected hybrids with both superior Criollo and Forastero characters



Hyb.12



Hyb.15



Hyb.18

Plate 15. Selected hybrids with superior Criollo characters

Table 33. Qualitative pod and bean characters of selected hybrids for CYT

Hybrids	Pod Shape	Colour of ripe pod	Colour of unripe pod	Pod apex	Pod basal constriction	Rugosity	Bean colour
Hyb.6	Angoleta	Yellowish green	Light green	Acute	Slight	Medium	Dark purple
Hyb.7	Criollo	Yellowish green	Light green	Acute	Slight	Medium	Mixed
Hyb.11	Cundeamor	Greenish yellow	Intermediate green	Acute	Intermediate	Medium	Mixed
Hyb.17	Criollo	Yellowish green	Light green	Acute	Slight	Medium	White

Table 34. Quantitative pod characters of selected hybrids for CYT

Hybrids	Pod weight (g)	Pod length (cm)	Pod breadth (cm)	No. of ridges and furrows	No. of beans/pod	Total wet bean weight (g)	Husk thickness (cm)	No. of flat beans
Hyb.6	530.70	16.12	8.90	10.00	45.00	164.36	0.94	1.2
Hyb.7	508.66	17.68	8.02	10.00	45.80	157.60	0.97	1.0
Hyb.11	569.00	20.22	8.48	9.00	44.20	159.62	0.98	1.4
Hyb.17	636.82	19.92	9.16	10.00	43.80	153.70	0.98	0.6

Table 35. Quantitative bean characters of selected hybrids for CYT

Hybrids	Unpeeled bean weight (g) (20 beans)	Peeled bean weight (g) (20 seeds)	Peeled bean dry weight (g) (20 seeds)	Unpeeled single bean wet weight (g)	Peeled single bean wet weight (g)	Peeled single bean dry weight (g)	Peeled bean length (cm)	Peeled bean breadth (cm)	Peeled bean width (cm)
Hyb.6	80.08	39.12	22.94	4.00	1.96	1.11	2.19	1.21	0.54
Hyb.7	69.00	30.66	20.94	3.45	1.53	1.07	1.66	1.23	0.36
Hyb.11	76.20	41.82	30.29	3.81	2.09	1.48	2.46	1.31	0.48
Hyb.17	71.56	35.14	24.22	3.58	1.76	1.24	2.08	1.11	0.44

Table 36. Economic characters of selected hybrids for CYT

Hybrids	Yield (no. of pods/tree/year)	Wet bean weight/pod weight (%)	Dry matter recovery (%)	Peeling ratio (%)	Pod value (g)	Pod index	Efficiency index	Conversion index
Hyb.6	111	30.97	58.69	49.34	49.95	20.02	10.62	0.30
Hyb.7	108	30.98	68.69	44.88	48.78	20.50	10.43	0.31
Hyb.11	105	28.05	73.20	55.00	65.20	15.34	8.73	0.41
Hyb.17	105	24.14	68.93	49.35	54.31	18.41	11.73	0.35

Table 37. Biochemical and quality parameters of selected hybrids for CYT

Hybrids	TSS (°brix)	Fat content (%)	Alkaloid content (%)	Total phenol (%)	Protein content (%)	Fermentation index (%)	Fermentation recovery (%)	pH
Hyb.6	20.80	54.5	3.85	5.25	16.40	69	41.2	5.40
Hyb.7	21.80	54.0	3.95	4.85	17.00	71	37.7	5.24
Hyb.11	20.40	54.5	3.85	5.40	17.30	74	36.6	5.44
Hyb.17	21.80	56.5	3.95	4.95	17.40	78	38.7	5.23

Summary



6. SUMMARY

The study entitled “Evaluation of selected cocoa (*Theobroma cacao* L.) hybrids bred for quality” was carried out in the Dept. of Plantation Crops and Spices, College of Horticulture and Cocoa Research Centre, KAU, Vellanikkara during the period 2014-2016. The objective of the study was to identify hybrids with beans of superior quality along with high vigour in yield related parameters and disease tolerance. Thirty hybrids derived as a result of crossing between Forastero type and Criollo type maintained at Cocoa Research Centre, Vellanikkara formed the material for the study. The salient findings are summarized below.

- Morphological characterization was carried out based on qualitative and quantitative characters of beans
- A wide variability was observed among the hybrids for various qualitative characters of pod and bean
- Morphological Criollo characters like purplish green unripe pod colour, purplish yellow ripe pod colour, intense rugosity, slight basal constriction, attenuate apex, deep furrows and white cotyledon colour were observed among the hybrids. This indicated that Criollo characters are transferred from parents to the progenies
- Forastero and Trinitario morphological characters were also found among the hybrids
- The cluster analysis based on qualitative characters resulted in ten clusters at 50 percent similarity level revealed that there is a wide variability among hybrids with respect to qualitative characters
- Significant variability was observed for all the 17 pod and bean quantitative characters studied except for number of ridges and furrows. The wide variability exhibited revealed that there is an ample scope for improvement of the traits through selection
- Twenty five hybrids showed single bean dry weight more than the international standards and five hybrids exhibited above 1.2 g, which is a Criollo character

- The hybrids; Hyb.6, Hyb.7, Hyb.9, Hyb.10, Hyb.11, Hyb.12, Hyb.15, Hyb.17 and Hyb.18 were selected as superior with respect to quantitative characters
- Several hybrids exhibited high yield (no. of pods/ tree/ year) and yield related parameters, which indicates that there is a transfer of Forastero character from parents to the progenies
- The cluster analysis based on quantitative characters using D^2 statistics resulted in 6 clusters and maximum inter cluster distance was observed between cluster II and V, which indicates that there is a wide variation among the hybrids present in these clusters, so there is an ample scope for selection for further breeding programme
- The pod and bean characters like pod weight, total wet bean weight per pod, unpeeled and peeled wet bean weight, single dry bean weight, bean length, breadth and width showed high PCV and GCV along with high heritability and GG which indicates that these characters can be used as selection criteria in further crop improvement programme
- Several hybrids used in the study expressed high RH, SH and heterobeltiosis for different pod and bean characters, it indicated high vigour for the hybrids with respect to the parents and standard variety
- Biochemical and quality parameters like fat, alkaloid, phenol, protein and TSS were estimated and several hybrids expressed superior quality attributes of Criollo
- The hybrids; Hyb.30, Hyb.12, Hyb.15, Hyb.7, Hyb.6 and Hyb.11 were selected as superior ones based on DMRT technique with respect to biochemical and quality parameters
- Correlation studies revealed that bean dry weight and bean size correlated with fat, phenol, alkaloid and protein content
- The hybrids; Hyb.1, Hyb.3, Hyb.6, Hyb.7, Hyb.9, Hyb.10, Hyb.11, Hyb.12, Hyb.15, Hyb.17, Hyb.21 and Hyb.30 were selected as superior hybrids based on qualitative and quantitative characters of pod and bean, biochemical and quality parameters evaluation using DMRT technique

- Fermentation index was observed high among hybrids, which indicated that there is high rate of fermentation and this is reported as a Criollo character
- The chocolates made from the hybrids; Hyb.6; Hyb.7, Hyb.9, Hyb.10, Hyb.11 and Hyb.30 scored high over all acceptability among the judges revealed that they are having high quality sensory attributes
- Pests and diseases incidence was reported low in the field, which is a Forastero character
- The hybrids; Hyb.6, Hyb.11, Hyb.17 and Hyb.7 observed with superior characters of both Criollo and Forastero and the chocolates made from these hybrids showed good preference based on sensory evaluation, so it can be forward to comparative yield trial (CYT) for further evaluation.
- Pod weight, wet bean weight and no. of beans per pod were negatively correlated with maximum temperature
- The hybrids; Hyb.12, Hyb.15 and Hyb.18 exhibited superior Criollo characters but were not superior with respect to Forastero characters like yield, pests and disease tolerance. These can be crossed with hybrids in divergent clusters having superior Forastero characters for further improvement.

Annexures



APPENDIX 1

Score obtained for pod characters

Hybrids	Pod weight score	Pod length score	Pod breadth score	No. of beans per pod score	Total wet bean weight score	Total score	Rank
Hyb.1	7.5	7.0	4.5	6.0	7.0	32.0	17
Hyb.2	12.0	7.0	12.0	4.5	12.5	48.0	26
Hyb.3	12.0	8.0	8.0	10	13.0	51.0	28
Hyb.4	11.5	5.0	10.0	8.0	12.5	47.0	25
Hyb.5	9.0	7.0	8.5	5.0	11.5	41.0	22
Hyb.6	5.0	5.0	2.0	4.5	3.0	19.5	8
Hyb.7	6.0	3.0	7.0	3.5	4.5	24.0	9
Hyb.8	10.5	5.0	11.5	3.5	10.5	41.0	22
Hyb.9	9.5	5.0	8.5	4.0	1.5	28.5	13
Hyb.10	1.0	2.0	1.5	5.5	2.0	12.0	2
Hyb.11	3.3	1.0	4.0	4.5	3.5	16.3	5
Hyb.12	1.0	2.0	2.0	2.5	1.5	9.0	1
Hyb.13	11.5	7.5	9.5	9.0	12.5	50.0	27
Hyb.14	8.0	1.5	7.5	9.0	11.5	37.5	19
Hyb.15	9.0	2.0	10.5	4.5	5.0	31.0	15
Hyb.16	4.0	5.0	1.5	7.5	7.5	25.5	11
Hyb.17	1.5	1.0	1.5	5.0	5.0	14.0	3
Hyb.18	9.5	7.0	6.0	6.5	10.0	39.0	20
Hyb.19	2.3	5.0	1.0	5.5	3.0	16.8	6
Hyb.20	11.5	7.0	9.5	6.0	10.0	44.0	24
Hyb.21	1.0	5.0	2.0	5.0	1.0	14.0	3
Hyb.22	9.5	7.0	7.0	8.5	11.5	43.5	23
Hyb.23	9.5	7.0	8.5	6.0	8.5	39.5	21
Hyb.24	5.0	5.0	7.5	5.0	7.0	29.5	14
Hyb.25	6.5	5.0	2.0	5.0	6.0	24.5	10
Hyb.26	8.0	5.0	8.5	1.0	9.0	31.5	16
Hyb.27	9.5	7.0	7.5	2.5	8.0	34.5	18
Hyb.28	5.5	6.0	3.0	4.5	8.0	27.0	12
Hyb.29	2.0	4.0	5.5	2.0	2.5	16.0	4
Hyb.30	3.5	7.0	3.5	1.5	1.5	17.0	7

APPENDIX 1I

Score obtained for low value preferred pod characters

Hybrids	Husk thickness score	No. of flat beans score	Flat bean percent score	Total score	Rank
Hyb.1	5.0	3.0	6.5	14.5	7
Hyb.2	8.0	2.0	4.0	14.0	8
Hyb.3	5.5	1.5	1.5	8.5	16
Hyb.4	8.5	2.0	3.5	14.0	8
Hyb.5	6.5	3.5	6.5	16.5	5
Hyb.6	5.0	3.0	6.5	14.5	7
Hyb.7	4.0	3.0	6.5	13.5	9
Hyb.8	8.0	3.0	5.5	16.5	5
Hyb.9	9.0	3.0	6.5	18.5	2
Hyb.10	3.0	3.0	6.5	12.5	11
Hyb.11	3.0	3.0	5.5	11.5	13
Hyb.12	5.0	3.5	6.5	15.0	6
Hyb.13	8.0	1.5	2.0	11.5	13
Hyb.14	5.0	3.0	6.0	14.0	8
Hyb.15	9.5	4.0	7.0	20.5	1
Hyb.16	1.5	1.5	2.5	5.5	17
Hyb.17	2.5	3.5	6.5	12.5	11
Hyb.18	5.0	3.5	6.5	15.0	6
Hyb.19	1.0	1.0	1.0	3.0	18
Hyb.20	3.5	3.0	6.0	12.5	11
Hyb.21	3.5	3.5	6.5	13.5	9
Hyb.22	7.5	1.5	3.0	12.0	12
Hyb.23	10	3.0	5.0	18.0	3
Hyb.24	4.0	2.5	4.5	11.0	14
Hyb.25	2.0	3.0	5.5	10.5	15
Hyb.26	2.0	4.0	7.0	13.0	10
Hyb.27	8.5	3.0	5.5	17.0	4
Hyb.28	3.5	3.0	5.5	12.0	12
Hyb.29	5.0	3.0	5.5	13.5	9
Hyb.30	4.0	3.0	5.5	12.5	11

APPENDIX III

Score obtained for bean characters

Hybrids	Unpeeled bean weight (g) (20 beans)	Peeled bean weight (g) (20 seeds)	Peeled bean dry weight (g) (20 seeds)	Total score	Rank
Hyb.1	9.5	8.0	4.5	22.0	13
Hyb.2	14.0	16.0	11.0	41.0	26
Hyb.3	13.5	16.0	10.0	39.5	25
Hyb.4	12.0	15.0	9.0	36.0	24
Hyb.5	11.5	15.0	7.5	34.0	22
Hyb.6	2.0	1.5	3.0	6.5	2
Hyb.7	4.0	7.5	5.0	16.5	10
Hyb.8	11.5	13.0	7.5	32.0	20
Hyb.9	4.0	5.5	4.0	13.5	8
Hyb.10	1.0	7.5	3.0	11.5	5
Hyb.11	1.5	1.0	1.0	3.5	1
Hyb.12	4.0	3.5	4.0	11.5	5
Hyb.13	12.5	15.0	8.0	35.5	23
Hyb.14	8.5	13.5	6.0	28.0	16
Hyb.15	3.5	5.0	4.0	12.5	6
Hyb.16	4.0	11.0	6.5	21.5	12
Hyb.17	4.0	4.0	2.0	10.0	4
Hyb.18	7.5	1.5	4.0	13.0	7
Hyb.19	5.5	10.0	6.0	21.5	12
Hyb.20	7.0	14.5	9.0	30.5	19
Hyb.21	3.0	2.5	2.5	8.0	3
Hyb.22	11.5	12.0	6.0	29.5	18
Hyb.23	7.0	9.0	6.0	22.0	13
Hyb.24	3.0	6.5	6.0	15.5	9
Hyb.25	6.0	10.5	8.0	24.5	14
Hyb.26	11.5	14.0	8.0	33.5	21
Hyb.27	10.5	10.5	8.0	29.0	17
Hyb.28	5.0	14.5	7.5	27.0	15
Hyb.29	9.5	7.5	5.0	22.0	13
Hyb.30	4.0	9.0	4.0	17.0	11

APPENDIX IV

Score obtained for single bean characters

Hybrids	Unpeeled single bean wet weight (g)	Peeled single bean wet weight (g)	Peeled single bean dry weight (g)	Peeled bean length (cm)	Peeled bean breadth (cm)	Peeled bean width (cm)	Total score	Rank
Hyb.1	9.5	7.5	8.0	3.5	5.0	3.5	37.0	12
Hyb.2	14.0	16.0	14.0	6.0	13.0	5.0	68.0	24
Hyb.3	13.5	16.0	13.0	5.0	8.0	9.0	64.5	23
Hyb.4	12.0	14.5	12.0	5.0	9.5	5.0	58.0	22
Hyb.5	11.5	15.0	9.5	4.5	4.5	4.5	49.5	19
Hyb.6	2.0	1.5	5.5	2.0	2.0	2.0	15.0	2
Hyb.7	4.0	7.0	7.0	4.5	2.0	8.5	33.0	10
Hyb.8	11.5	12.0	12.0	4.0	7.0	5.0	51.5	20
Hyb.9	4.0	5.0	5.0	4.5	5.0	8.5	32.0	9
Hyb.10	1.0	7.0	7.5	2.0	3.5	5.0	26.0	5
Hyb.11	1.5	1.0	1.0	1.0	1.0	3.5	9.0	1
Hyb.12	4.0	3.5	5.5	1.5	5.0	5.0	24.5	4
Hyb.13	12.5	14.5	12.0	4.5	9.5	5.0	58.0	22
Hyb.14	8.5	13.0	10.5	5.5	8.0	3.0	48.5	18
Hyb.15	3.5	3.0	5.0	4.5	10.5	2.0	28.5	7
Hyb.16	4.0	10.0	3.0	3.5	2.5	5.0	28.0	6
Hyb.17	4.0	4.0	2.5	2.5	4.5	5.0	22.5	3
Hyb.18	7.5	1.5	7.0	3.0	5.5	3.5	28.0	6
Hyb.19	5.5	9.0	10.0	2.5	8.5	5.0	40.5	14
Hyb.20	7.0	14.0	13.0	4.5	5.0	8.0	51.5	20
Hyb.21	3.0	2.5	6.5	4.5	10.5	5.0	32.0	9
Hyb.22	11.5	11.0	10.0	4.5	6.0	5.0	48.0	17
Hyb.23	7.5	8.5	2.0	4.5	8.5	7.0	38.0	13
Hyb.24	3.0	6.0	9.0	4.5	1.0	1.0	24.5	4
Hyb.25	6.0	9.5	12.0	4.5	11.5	6.0	49.5	19
Hyb.26	11.5	13.5	11.0	4.5	12	5.0	57.5	21
Hyb.27	11.5	9.5	12.0	3.5	5.0	2.5	44.0	15
Hyb.28	5.0	14.0	9.0	4.5	9.5	5.5	47.5	16
Hyb.29	9.5	7.0	6.5	2.5	2.5	1.0	29.0	8
Hyb.30	4.0	8.5	4.0	4.5	8.5	5.0	34.5	11

APPENDIX V

Score obtained for biochemical characters

Hybrids	TSS (° brix)	Fat (%)	Alkaloid (%)	Total phenol (%)	Protein (%)	Total score	Rank
Hyb.1	2.0	1.5	3.5	2.5	1.0	10.5	6
Hyb.2	8.5	6.0	4.0	4.5	6.0	29.0	19
Hyb.3	9.0	2.5	1.5	9.5	2.0	24.5	14
Hyb.4	7.5	6.0	7.0	3.0	6.0	29.5	20
Hyb.5	1.5	1.5	3.5	7.0	3.5	17.0	11
Hyb.6	2.0	1.5	3.0	1.5	1.5	9.5	4
Hyb.7	1.0	1.5	2.0	3.5	1.0	9.0	3
Hyb.8	3.0	4.0	6.5	3.5	2.5	19.5	12
Hyb.9	1.0	1.5	3.5	3.5	1.0	10.5	7
Hyb.10	1.0	1.5	3.5	3.0	4.5	13.5	9
Hyb.11	3.0	1.5	3.0	1.5	1.0	10.0	5
Hyb.12	1.5	2.0	2.5	1.5	1.0	8.5	2
Hyb.13	6.0	5.5	1.5	8.5	7.0	28.5	18
Hyb.14	2.5	1.0	4.0	1.5	5.5	14.5	10
Hyb.15	1.5	1.5	3.5	1.0	1.0	8.5	2
Hyb.16	8.5	5.5	2.5	7.5	3.5	27.5	17
Hyb.17	1.0	1.0	2.0	3.0	1.0	8.0	1
Hyb.18	6.0	5.0	3.0	9.0	6.0	29.0	19
Hyb.19	5.5	3.0	1.0	4.5	5.5	19.5	12
Hyb.20	4.0	5.5	4.0	1.5	6.0	21.0	13
Hyb.21	2.0	2.0	2.5	5.0	1.0	12.5	8
Hyb.22	6.5	4.0	5.0	7.5	4.0	27.0	15
Hyb.23	4.5	5.5	6.0	10	6.0	32.0	21
Hyb.24	8.0	7.0	3.5	5.5	5.5	29.5	20
Hyb.25	5.5	6.5	4.5	11	7.5	35.0	22
Hyb.26	1.0	5.5	1.0	6.0	6.0	19.5	12
Hyb.27	8.5	6.0	4.0	11	8.0	37.5	23
Hyb.28	6.0	7.0	5.5	7.5	6.0	32.0	21
Hyb.29	4.0	5.0	3.5	8.5	6.0	27.0	16
Hyb.30	2.0	1.5	1.5	2.0	1.0	8.0	1

APPENDIX - VI

Score card for sensory evaluation of chocolates made

Name of the judge:

Date:

Samples	Sensory attributes and Score							
	Appearance	Colour	Flavour	Texture	Odour	Taste	After taste	Overall acceptability
Sample 1								
Sample 2								
Sample 3								
Sample 4								
Sample 5								
Sample 6								

9 point Hedonic scale	
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

APPENDIX - VII

Meteorological data during the period of observation (2014 and 2015)

Month	Max. Temp.(°C)	Min. Temp.(°C)	RH morning (%)	RH evening (%)	Rainfall (mm)	Rainy days
October'14	31.9	23.7	93	68	224.6	15
November'14	31.6	23.2	84	60	85.3	5
December'14	31.9	23.2	78	53	9.6	1
January'15	32.5	22.1	75	41	0	0
February'15	34.3	23.0	73	37	0	0
March'15	35.8	24.9	83	44	72	2
April'15	34.0	24.6	89	64	162.2	8
May'15	32.9	24.7	92	68	259	12
June'15	31.0	23.9	94	72	629.8	23
July'15	30.3	23.5	95	74	510.1	23
August'15	31.0	23.7	95	70	320.8	17
September'15	31.9	23.7	93	69	242.2	12
October'15	32.5	24.1	90	68	203.8	15
November'15	31.6	23.8	83	66	151.2	8
December'15	32.3	23.3	78	53	88.3	3

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EVALUATION OF SELECTED COCOA (*Theobroma cacao* L.) HYBRIDS BRED FOR QUALITY

By

AJMAL P.M.

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ABSTRACT OF THE THESIS

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DEPARTMENT OF PLANTATION CROPS AND SPICES

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680656

KERALA, INDIA

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ABSTRACT

Cocoa (*Theobroma cacao* L.) is an important beverage crop belonging to the family Malvaceae. The most important economic part of cocoa is the optimally fermented and dried beans, which is the only source of chocolate flavour. Consumers have shown an increased interest for high quality dark chocolate containing a higher percentage of cocoa. Therefore, the quality of cocoa beans has a great importance while considering the market value. The genetic makeup of an individual cocoa genotype influences flavour, quality and intensity of chocolate.

Cocoa is mainly classified into three types, namely Criollo, Forastero and Trinitario. The Criollo types provide fine flavour chocolate. At present fine cocoa production is very less due to the low productivity and disease susceptibility. More than 80 percent of cocoa plantations are established with Forastero types considering its higher yield and tolerance to pests and diseases. Due to increase in demand for fine cocoa in the market, it is important to have genotypes where quality is combined with hardiness. Hence, hybridization programme was initiated at Cocoa Research Center (CRC), KAU, Vellanikkara during 2004 for the development of varieties with beans of superior quality without sacrificing the yield and disease tolerance. Parental lines identified as having superior quality were crossed with high yielders with disease tolerance.

Thirty hybrid progenies derived from the crosses were selected for the present study based on initial performance. These hybrids were subjected to morphological, biochemical, quality parameters and sensory evaluation. The morphological evaluation based on eight qualitative and seventeen quantitative characters was carried out using the descriptor developed by Bekele and Butler (2000). Biochemical and quality parameters were estimated following standard procedures and organoleptic evaluation was carried out based on nine point hedonic scale.

Variability was observed among the hybrids for all the qualitative characters evaluated. Qualitative Criollo characters were observed among the hybrids indicating the transfer of Criollo characters from the parents to the progenies. Variations expressed by the hybrids in terms of pod and bean quantitative characters were also significant, indicating their heterogeneity.

Analysis of pod and bean characters revealed that the hybrids; Hyb.10, Hyb.21 and Hyb.11 expressed higher values with respect to pod weight (685g), total wet bean weight (185.72g) and dry weight of single bean (1.48g) respectively. The highest values for yield, dry matter recovery and pod index recorded in Hyb.6 (111 pods/ tree/ year), Hyb.5 (81.2%) and Hyb.11 (15) respectively. Analysis of biochemical and quality parameters revealed that the fat content ranged from 39% in Hyb.28 to 56.5% in Hyb.17, total phenol content ranged from 2.95% in Hyb.27 to 5.45% in Hyb.15, protein content ranged from 12.15% in Hyb.27 to 17.4% in Hyb.17 and the alkaloid content ranged from 2.65% in Hyb.4 to 4.2% in Hyb.26. The highest values for TSS, fermentation index and fermentation recovery observed in Hyb.10 (22° brix), Hyb.17 (78%) and Hyb.6 (41.2%) respectively.

Sensory evaluation of the chocolates prepared from the selected hybrids revealed that hybrids; Hyb.6, Hyb.7 and Hyb.10 scored high rank with respect to overall acceptability. Among the thirty hybrids evaluated, hybrids; Hyb.6, Hyb.7, Hyb.11 and Hyb.17 were found to possess with both Criollo and Forastero characters along with premium quality chocolates. Therefore, these hybrids can be further evaluated in comparative yield trial for variety release. Hybrids with superior Criollo characters (Hyb.12, Hyb.15 and Hyb.18) can be further used in breeding programmes.

