GENETIC ANALYSIS OF COCOA (*Theobroma cacao* L.) HYBRIDS AND SCREENING SUPERIOR HYBRIDS FOR MAJOR BIOTIC STRESS

By

SHILPA K. S.

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THESIS

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VELLANIKKARA, THRISSUR – 680 656

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DECLARATION

I, hereby declare that the thesis entitled **"Genetic analysis of cocoa (*Theobroma cacao* L.) hybrids and screening superior hybrids for major biotic stress"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Vellanikkara,
Date: 09-08-2019

Shilpa K. S.
(2017-11-027)

CERTIFICATE

Certified that the thesis entitled **"Genetic analysis of cocoa (*Theobroma cacao* L.) hybrids and screening superior hybrids for major biotic stress"** is a record of research work done independently by **Ms. Shilpa K. S.** 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 her.

Vellanikkara,
Date: 09-08-2019

Dr. Minimol J. S.
(Major advisor)
Associate Professor
Plant Breeding and Genetics,
Cocoa Research Centre,
Vellanikkara.

CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Shilpa K.S.** (2017-11-027) a candidate for the degree of **Master of Science in Agriculture** with major field in **Plant Breeding and Genetics**, agree that this thesis entitled **"Genetic analysis of cocoa (*Theobroma cacao* L.) hybrids and screening superior hybrids for major biotic stress"** may be submitted by Ms. **Shilpa K.S.** in partial fulfilment of the requirement for the degree.

Dr. Minimol J.S.
(Chairman, Advisory Committee)
Associate Professor,
Plant Breeding and Genetics,
Cocoa Research Centre,
Kerala Agricultural University.

Dr. Jiji Joseph
(Member, Advisory Committee)
Professor and Head,
Department of Plant Breeding and Genetics,
College of Horticulture, Vellanikkara.

Dr. Sainamole Kurian P.
(Member, Advisory Committee)
Professor,
Plant Pathology, AICVIP
College of Horticulture, Vellanikkara.

Dr. Gavas Ragesh
(Member, Advisory Committee)
Assistant Professor,
Agricultural Entomology,
Banana Research Station, Kannara,
Kerala Agricultural University.

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TABLES OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

Introduction

1. Introduction

Cocoa (*Theobroma cacao* L.) taxonomically belongs to the family Malvaceae. It is a diploid species with a somatic chromosome number of 20. The botanical name *Theobroma cacao*, was first used by Carolus Linnaeus in the 13th edition of his classic book, Systema Naturae. Theobroma is a Greek word, were 'theos' means 'God' and 'broma' means 'food' thus cocoa came to be known as 'Food of Gods'.

Cocoa is a beverage crop native to Amazon region of South America and has been introduced to India as profitable mixed crop in coconut and arecanut plantations in 1970s. The current area under cocoa is 82,940 ha covering the states of Kerala, Karnataka, Tamil Nadu and Andra Pradesh with an annual production of 18,920 tonnes of cocoa beans (DCCD, 2018).

Cocoa is a crop which is highly influenced by climate change and growing environment and thus makes it necessary to have long term and dynamic breeding programme (Malhotra and Hubali, 2016). Yield improvement was the prime objective of most of the earlier breeding programmes. However, with the onset and spread of many diseases and pests, more emphasis is given for evolving disease and pest tolerant cocoa varieties, without sacrificing yield.

At present, one of the main challenges faced by cocoa growers is *Phytophthora* pod rot caused by *Phytophthora palmivora.* This pathogen causes extensive damage to the flower cushion, leaves, stems, roots, pod and beans (Nyadanu *et al*., 2009).It is a disease of universal occurrence causing nearly 61.4 per cent global crop loss (Nyadanu *et al*., 2012).The damage to the pod due to *Phytophthora* pod rot result in huge economic loss of approximate USD3.8billion (Guiltinan and Becker, 2015). Since this disease is prevalent during rainy season it is very difficult to control using fungicides. Hence, use of resistant varieties is recommended for effective and ecofriendly control.

Tea mosquito bug (TMB) (*Helopeltis* spp.), is a major sucking pest of cocoa, whose incidence became severe during last three years in summer and post monsoon seasons. Among different species *H. theivora* is the most predominant one in cocoa causing damage to young shoots, cherelles and pods (Malhotra and Apshara, 2017).Chemical control is difficult against tea mosquito bug in cocoa because, the pollinator (*Forcipomyia* spp.) of cocoa also belongs to the same family, Miridae. The development and use of tea mosquito bug resistant cocoa varieties is one of the alternatives to chemical control (N'Guessan *et al*., 2004).

Twenty cocoa hybrids which are in the steady bearing stage, selected from the Comparative Yield Trial (CYT), planted during 2008 at Cocoa Research Centre (CRC) farm served as the material for the study. These twenty hybrids were selected based on their general vigour and yield performance. In the present study they were further evaluated for morphological and biochemical characters and screened for major pest and disease resistance with the aim of developing high yielding varieties with resistance to major biotic stresses affecting cocoa.

It was in this background the present study entitled 'Genetic analysis of cocoa (*Theobroma cacao* L.) hybrids and screening superior hybrids for major biotic stress' was taken up with an objectiveto assess genetic potential of cocoa hybrids and to evaluate the reaction of superior ones against *Phytophthora* pod rot and tea mosquito bug (*H. theivora*).

Review of Literature

2. REVIEW OF LITERATURE

Cocoa (*Theobroma cacao* L.), the source of fashionable delicacy chocolate is believed to be originated in the Amazon region of South America. However, it is hard to point out the exact centre of origin of cocoa due to its widespread wild biodiversity. There are different opinions among the researchers regarding the origin of cocoa. According to Van Hall (1914), the centre of origin of cocoa is the region extending from the forests of Amazon to the Orinoco and Tabasco in Southern Mexico. In the view point of Cheesman (1944) cocoa originated in the Upper Amazon near the Colombian–Ecuadorian border, on the eastern flanks of the Andes. Hypothesis of concurrent origin of cocoa in South America and Central America was proposed by Cuatrecasas (1964) and these populations were known as two subspecies, *T. cacao* ssp. *Cacao* and *T. cacao* ssp. *sphaerocarpum*. Schultes (1984) put forward the view that from the Amazon Valley cocoa spread to the north and the west and thus, the domestication of cocoa started in South America and the migrating Indians carried it to Central America and Southern Mexico.

2.1. History and status of cocoa cultivation

Cocoa was first cultivated as a crop by Aztecs and Mayans who were the indigenous populations of South America. They used cocoa beans for the preparation of a bitter drink known as 'Cacahuatl' which they served during their religious ceremonies and from this, the word chocolate originated. In indigenous cultures cocoa beans were so important that it was used as currency in trade, given as post-battle reward and was one of the main items in royal feasts (Amma *et al.*, 2009). When the Spanish conquered New World they discovered the value of cocoa and bought it to their place and added their own innovations to that bitter drink and sweetened it. Thus chocolate become popular among the Spanish and they kept the production process a secret for 100 years. They were not able to hold

the secret forever and it spread to Europe and chocolate drinks became popular among European royal classes. With the arrival of industrial revolution production of cocoa powder became easier and the exclusivity of cocoa was thus lost and chocolate began to appear in different solid forms rather than drinks (World Cocoa Foundation, 2018).

Cocoa had been introduced to India as a profitable mixed crop in coconut and arecanut plantations in 1970s by Cadbury India Private Limited (now renamed as Mondelez International) as part of their commercialization strategy. In 1959, Government of India invited D.H. Urquhart the chief chemist of Cadbury, London to study the possibility of developing cocoa cultivation in India. In his report, he recommended Kerala to be the most suitable state in India to cultivate cocoa on a large scale (Jayasekhar and Ndung'u, 2018). The current area under cocoa in India is 83,000 ha covering the states of Andra Pradesh, Karnataka, Tamil Nadu and Kerala with an annual production of 19,000 metric tonnes of cocoa beans (GOI, 2017). As per DCCD (2018) 18920 tonnes of cocoa is produced in an area of 82940 hectare in India.

2.2. Taxonomy and botanical classification

Theobroma cacao L. was first classified under the family Sterculiaceae (Purseglove, 1974). However, based on the studies using molecular markers it was reclassified and now belongs to the family Malvaceae (Alvenson*et al.*, 1999; Sailaja *et al.*, 2015). *T. cacao* is a diploid species with a somatic chromosome number of 20. The botanical name *Theobroma cacao*, was first used by Carolus Linnaeus in his classic book, Systema Naturae. Theobroma is a Greek word, were 'theos' means 'god' and 'broma' means 'food' thus cocoa came to be known as 'Food of Gods'.

Morris (1882) was the first person who gave the nomenclature of the cultivars of *Theobroma cacao* L. He grouped the cultivars into two classes, Class I

the Cacao Criollo and Class II the Cacao Forestero group. Second class was again divided into eight varieties. Morris classification was modified by Hart (1892) and he gave three classes *viz*. Arecriollo, Forasteroand Calabacillo. Cheesman (1944) grouped cacao into two classes of varieties based on the morphological features and geographical origins, *i.e*. Criollo and Forastero. He put forward the view that Trinitarios may be originated from the mingling of Criollos and Forasteros. Based on the fruit and vegetative characteristics 22 species of cocoa was described by Cuatrecasas (1964) under six sections. Based on pod and seed morphology cocoa can be classified as cundeamor, amelonado, angoleta and calabacillo (Marita *et al*., 2001 and Sounigo *et al*., 2003).

2.3. Cocoa tree

In the centre of origin, cocoa a tropical perennial plant is found to be growing in the lower storery of evergreen Amazon rain forests (Wood and Lass, 2008). Plant generally grows vertically upto 1-2 metres height and after that grows side wise and usually known as jorquetting. Two types of branches are usually observed in cocoa the chupons and fan branches. Chupons are having upright growth habit with spiral leaf arrangement. A fan branch which grows side wise is having an alternate leaf arrangement. Thus growth in cocoa is dimorphic with orthotropic and plagiotropic branches.

Cocoa tree starts to produce flowers from three years after planting under good management conditions (Chaidamsari *et al*., 2006). Cocoa shows typical cauliflorous habit by bearing flowers and fruit on its trunk and branches. Cocoa flowers profusely but only 1-5 per cent of the flowers pollinate successfully and produce pod (Wood and Lass, 1987).

Cocoa is a crop mostly pollinated by female *Forcipomyia* midges (Billes, 1941; Soetardi, 1950; Entwistle, 1957; Saunders, 1958). Being an entomophilic crop out breeding is the most common breeding system and it is difficult to self

pollinate cocoa due to its sporophytic self incompatibility (Cope, 1962). Lachenaud and Oliver (2005) studied 67 cocoa clones from nine populations and proved that majority (92 %) are self incompatible or with slight self incompatibility.

2.4. Morphology characterisation of cocoa leaf, flower pod and bean

Morphology characterisation involves evaluation of both quantitative and qualitative traits and cocoa genotypes exhibits wide variability for these traits. Klug and Cummings (1994) and Sounigo *et al.* (1997) suggested that, morphological and economic characters which are highly heritable are useful for characterisng germplasm in plants. Morphological and agronomic characters of cocoa pod, bean and flowers were used to evaluate diversity of genotypes by many researchers (Bekele and Bekele, 1996; Lachenaud *et al.*, 1999; Lachenaud and Oliver, 2005). Iwaro *et al.* (2003) and Bekele *et al.* (2006) suggested that morphological evaluation is having role in improving economic traits and providing breeding gain from selected cocoa genotypes.

Wood and Lass (1985) and Kochhar (1998) reported that the flush colour of leaves in cocoa ranges from pale green to shades of red. Bartely (2005) reported that colour of young leaves of cocoa is a distinctive character.

The first investigator who suggested the use of flower descriptor to identify cocoa clones was Ostendorf (1954). Floral descriptors are taxonomically important because, they exhibit less coefficient of variation and have high broad sense heritability (Enriquez and Soria, 1967; Engles, 1983; Raboin *et al.*, 1993; Bekele *et al.*, 1994 and Lachenaud *et al.*, 1999). Floral descriptors are now routinely used for characterizing different cocoa germplasms (Engels, 1986; Castro *et al.*, 1989; Bekele *et al.*, 2006; Santos, 2012).

Lachenaud *et al.* (1999) conducted a study to access the worth of floral

descriptor for characterising the diversity among the wild cocoa trees found in French Guiana. 155 clones belonging to 16 populations were used for the study. All genotypes were characterized based on petal ligule width, sepal width, length of gynoecium and number of ovules per ovary. They concluded that genetic distance exists between populations when floral descriptors were considered.

Santos (2012) characterised six Brazilian *Theobroma* spp. based on 32 quantitative and 13 qualitative traits. Among the 32 quantitative characters considered five were floral traits and he came up with a conclusion that average corolla diameter observed in cocoa flowers are about 13.82 cm, sepal length (7.37 cm), ovary length (1.44cm), staminode length (6.63 cm) and style length (2.57cm).

Cuatreasas (1964) reported that, fruits produced in different species belonging to genera *Theobroma*, particularly *T. cacao* exhibits high variability with respect to shape, size and colour. Since then many similar reports are made by different researchers who have worked in cocoa (Subramanian and Balasimha, 1981; Mallika *et al.*, 1996; Maharaj *et al.*, 2006; Apsara *et al.*, 2008).

Bekele and Bekele (2006) evaluated 603 cocoa accessions from International cocoa gene bank for morphological variation. Twenty four morphological traits were considered and the descriptor suggested by Bekele and Butler (2000) was used for the evaluation. They observed that pod length varied from 11.7 cm to 22.6 cm and pod width (6 to 10.5 cm).

Aikpokpodion (2010) conducted a study on 184 cocoa genotypes collected from farmers field and field gene bank of Cocoa Research Institute, Nigeria. The study was performed with an objective to identify the extent of variability between genotypes of farmers field and field gene bank. All the selected genotypes were characterized based on seventeen morphological traits including leaf colour, fruit shape, pod apex form, pod basal constriction, pod rugosity, ridge

pair disposition, cotyledon shape, cotyledon colour, fruit length, fruit width, bean number, cotyledon length, width, fresh bean weight, total dry bean weight and nib weight. He reported that genotypes showed high heterogeneity for these characters and the nine quantitative characters considered can alone explain the 76.7 per cent of the observed phenotypic variation. Minimol *et al.* (2011) conducted a study to identify the influence of pod apex on pod shape in 23 accessions of cocoa and concluded that fruit shape is influenced by fruit apex.

Velayutham *et al.* (2013) selected 151 cocoa tress based on yield, pod and bean characters from major cocoa growing places of Tamil Nadu and conducted morphological evaluation. They observed that pod weight varied from 238 g to 815 g with a mean weight of 427 g, pod length ranged from 10.20 cm to 20.20 cm and mean pod length was 15.11 cm, and rind thickness exhibited a mean value of 1.2 cm and it varied from 0.82 cm to 1.28 cm.

Fallo and Cilas (1998) found out high heritability and additive variance for cocoa bean weight. In general, cocoa pods have twenty to forty beans and they differ in colour like white, yellowish and pink (Bekele and Butler, 2000). Clement *et al.* (2003) stated that in *T. cacao* seed shape, number, length, width, thickness and weight show high variability and it was genetic in origin.

Enriquez and Soria (1966) reported that yield expressed as dry or wet weight of bean is a highly variable character. They also observed high variability for weight of bean even within a single pod. Engles (1982), based on a study of 32 clones of cocoa concluded that selection for seed size could lead to higher cocoa production per fruit than selection for seed number per fruit.

Bekele and Bekele (2006) reported that bean number (17 to 58), cotyledon weight (0.44 to 1.84 g), cotyledon length (1.47 to 2.72 cm), cotyledon width (0.65 to 2.05 cm) and bean weight ranged from 0.51 g to 1.88 g. Pod index an important economic parameter showed a high range of variation (13.6 to 49).

Studies of Velayutham *et al.* (2013) in selected cocoa genotypes confirmed that in cocoa number of beans per pod ranged from 25.5 to 50.5, wet bean weight per pod varied from 73.79 g to 210.5 g and single dry bean weight showed a minimum value of 0.59 g and maximum of 1.71 g. The trees having the following traits; 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) were selected as superior ones in their study.

2.5. Heterosis and hybrid production

At present most commonly adopted method in cocoa breeding is developing hybrids between two distant genotypes. Hybrid vigor between parents showing good combining ability can be readily exploited in cocoa improvement programs to produce high yielding hybrids (Apshara, 2019).

The cultivation of hybrid cocoa seedling led to very high increase in the cocoa production in Brazil (Dias *et al.*, 2003). Hybrid breeding programs have remarkably contributed to improvement in yield, biotic stress resistance, qualities improvement and adaptability (Adewale *et al.*, 2014).

Toxopeus (1969) proposed wide crossing to exploit hybrid vigour in cocoa. Increased yield was observed in progeny obtained from inter-crossing unrelated or partially inbred Forastero cocoa types of Trinitario and Amazon origin. They also confirmed that the bi-parental crosses were more vigorous than their parents in cocoa.

Montserin *et al.* (1957) studied the yield, pod value, bean weight and resistance to *Marasmius perniciosus* (*Crinipellis perniciosa*) of a number of cocoa hybrids which originated from crosses between Amazonian clones and between

Amazonian and Trinitario clones of cocoa. The hybrid seedlings were found to be high yielding and early bearing with satisfactory dry beans weight of more than 1 g. In bi-parental crosses involving diverse cocoa genotypes, general combining abilities was found to be more for characters like yield, vegetative growth, yield efficiency and bean weight (Pang *et al.*, 2006).

2.6. Biochemical properties of cocoa beans

2.6.1. Fat content of cocoa beans

Cocoa beans contain the second highest fat content among crop plants next to coconut (Luhs and Friedt, 1994). Cocoa butter is one of the major by-product of processed cocoa beans and it is a highly demanded raw material in food industry due to its physical, chemical and organoleptic characteristics which is unique and not comparable with any other edible vegetable fat (Lipp and Anklam, 1998). Apart from food industry it is widely used in cosmetic and pharmaceutical industries also.

Fat content has a direct influence on the commercial value of cocoa beans because, low fat content in cocoa beans will increase the cost of grinding a major step in chocolate production (Duncan and Veldsman, 1994; Wood and Lass, 1985).

Pires *et al.* (1998) studied the fat content in dried unfermented seeds of 490 Brazilian accessions and reported that average fat content was 53.2 per cent, ranging from 45.4 per cent in accession CC 57 to 60.3 per cent in accession NA 312. They also identified a significant negative correlation between dry seed yield/ plant and fat content. A complete diallel crossing scheme between three genotypes with high fat content and three with low fat content revealed that pollen has a significant effect in determining fat content of cocoa beans.

A study was conducted to determine the nutritional composition and fatty acids content of cocoa beans of different geographical origin (Ghana and Ecuador). They reported that, major nutrient present in cocoa beans collected from both the geographical origin was fat (>40 %). Ecuadorian cocoa beans contains more total fat content (43.8%) when compared to cocoa beans from Ghana (Moreno *et al.*, 2015).

2.6.2. Total polyphenol content of cocoa beans

Cocoa beans have high phenolic content of about 12–18 per cent in unfermented dried beans (Kim and Keeney, 1984). Phenolics or polyphenols have received considerable attention because of their physiological functions, including antioxidant, antimutagenic and antitumour activities (Saliva *et al.*, 1991; Kono *et al.*, 1995).

About 60 per cent of the total phenolics in raw cocoa beans are flavanol monomers (epicatechin and catechin) and procyanidin oligomers (dimer to decamer) (Dreosti, 2000).

Nazaruddin *et al.* (2006) reported that the total polyphenol content ranged from 34 to 60 mg/g in beans, 45 to 52 mg/ g in cocoa liquor and 20 to 62 mg/ g in cocoa powder. The place of origin as well as the method of processing was found to influence the polyphenol content of cocoa products (Jalil and Ismail, 2008).

Othman *et al.* (2007) investigated antioxidant capacity and total phenol content of cocoa beans from different countries and concluded that they are positively correlated ($r = 0.78$). Storage condition and fermentation influence the polyphenol content of cocoa beans (Afoakwa *et al.*, 2012). Phenol extracts from unfermented dry cocoa beans have higher polyphenol content and stronger antioxidant capacity when compare to partially fermented cocoa beans (Prayoga *et al.*, 2013).

Amudhan and Apshara (2015) compared different clones collected from various places for polyphenol content. Results showed that higher polyphenol content of 136 mg/ g and lower content of 82.4 mg/ g was found in the different clones. Polyphenol content also showed positive correlation with antioxidant capacity (r = 0.439).

Asna and Presannakumari (2016) characterized the cocoa accessions based on phenol content and reported that phenol content in cocoa ranged from 2.25 to 9.09 per cent.

2.6.3. Antioxidant property of cocoa beans

An antioxidant is a substance which, when present at a comparatively low concentration, can significantly delay or prevent oxidation of substrates present in the body (Halliwell and Gutteridge, 2000).

There are reports that phenols are having antioxidant capacity (Rice-Evans *et al*., 1997). The phenols present in the cocoa beans, especially the flavanoids are responsible for the antioxidant activity of cocoa beans (Steinberg *et al*., 2002; Sun and Ho, 2005; Aikpokpodion and Dongo; 2010 and Martinez *et al*., 2012).

According to Lee *et al.* (2003) and Steinberg *et al.* (2003) cocoa products contains greater antioxidant capacity and flavonoids content than tea or red wine.

Essien (2008) determined the antioxidant capacity of cocoa hybrids in comparison to traditional cocoa varieties. The antioxidant capacity of the beans, was determined using the FRAP assay method and it was found to be 12.4 µmol TE/ g for the traditional variety, whilst the values for the hybrids ranged from 21.6 to 45.5 µmol TE/ g.

Aikpokpodion and Dongo (2010) evaluated total antioxidant activity of the phenolic extract from cocoa beans under different days of fermentation, obtained with methanol using Soxhlet apparatus, was then performed using DPPH method. The scavenging properties of the methanolic extract of phenolic pigments from cocoa extract (1:500 diluted in methanol) showed an inhibition percent (IP %) 96 per cent, 93 per cent, 91 per cent, 88 per cent, 84 per cent, and 79 per cent at 1, 2, 3, 4, 5 and 6 days after fermentation.

2.6.4. Mineral content of cocoa beans

Mineral content in cocoa beans is very important for product manufacturing and also for the consumer preference. Olaofe and Onajeta (1987) reported that cocoa samples are good sources of calcium, potassium and sodium.

High calcium content in cocoa beans favours fermentation process (Aremu *et al.*, 1995). As the content of calcium in cocoa beans increase it helps in making the digestion of cocoa butter easy and thus protect against fat gain in human (Zemel *et al.*, 2000; Shahkhalili *et al.*, 2001).Potassium is a vascular tone, necessary for retaining cellular osmolarity and membrane potentials in human (Stipanuk, 2000). Sodium plays a key role in normal nerve and muscle function.

Olaofe *et al.* (1987) conducted experiment to identify the minerals present in cocoa powder. Among 11 cocoa samples analysed mean content of sodium was in the range of 21 µg/ g, potassium 2480 µg/ g and calcium 25.5 µg/ g. Rucker (2009) estimated mineral contents of cocoa beans obtained from different accessions and confirmed that calcium(100 to 180 mg/100g) and potassium(1500 to 2000 mg/ 100g) are present in abundance.

Afoakwa *et al.* (2013) conducted a study to determine the effect of pulp preconditioning on the mineral composition of fermented and unfermented cocoa

bean samples. They reported that in unfermented cocoa beans sodium content was 3.4 mg/ 100 g, calcium 140.2 mg/ 100 g and potassium 2313.1 mg/ 100 g.

2.7. Correlation studies

Mary and Gopalan (2006) suggested that, analysis of variability among the characters used for the study along with knowledge on association of a trait with respect to other trait contributing to yield is important for an effective breeding programme. Magnitude of association between two traits is represented by correlation coefficient. The reason for association between two characters can be due to pleiotrophy or tight linkage between genes controlling those characters.

Yield is a character controlled by polygenes and it is affected by many environmental factors. Correlation studies enable us to have better understanding of yield related factors and thus help in indirect selection for yield (Singh and Narayanan, 1993).

Ruinard (1961) reported positive correlation between pod size and bean size in cocoa. High significant correlation exists between yield and number of pods per tree (Moses, 1979).When pod and bean characters of cocoa were analyzed strong correlation was observed between pod weight and pod length, pod diameter, weight of beans per pod, wet bean weight, weight of peeled beans and number of developed beans (Kumaran and Amma, 1982). Wet bean weight and weight of peeled bean expressed positive correlation with weight of dry beans (Rubeena, 2015). Husk thickness showed low and negative correlation with all pod and bean characters (Veeresh, 2017).

Engels (1983) assessed relationship between the characters of different cocoa clones and reported positive correlation between bean number and pod size, negative correlation between bean number and bean size. Rind thickness showed positive influence over pod width, bean size and bean weight. A positive

correlation was reported between commercial economic yield and pod weight and fresh seed weight (Lachenaud, 1984).

Thondaiman and Rajamani (2014) conducted studies on phenotypic correlation for twenty characters of 151 cocoa trees. They observed phenotypic correlation between tree girth, pod length, pod weight, pod volume, number of beans per pod, wet bean weight per pod, dry bean weight per pod, single wet bean weight and single dry bean weight. Number of pods per tree and polyphenol content exhibited high significant positive correlation with the dry bean yield per tree.

Correlation analysis of cocoa hybrids by Rubeena (2015) reported that the total wet bean weight was positively correlated with number of beans per pod, length and width of bean, wet and dry weight of peeled bean and thickness of the bean.

Several studies revealed low to moderate correlations between phenolic content and antioxidant capacity of cocoa beans (Velioglu *et al.*, 1998; Allaith, 2008). Studies of Veeresh (2017) on *Phytophthora* pod rot infection revealed that there is significant correlation between *Phytophthora* infection and pod morphology.

2.8. Path analysis

The concept of path analysis was introduced by Wright (1921). This method helps to quantify direct effects and indirect effects of independent variables on a dependent variable (Li, 1975; Cruz and Regazzi, 2006).

Almeida *et al.* (1994) estimated the direct and indirect effect of ten traits of twelve cocoa hybrids planted at the experimental station in Medicilandia, Brazil. Number of healthy fruits per tree and weight of dry bean per fruit showed

direct effects on dry bean weight per tree. The single dry bean weight and number of seeds per fruit constituted the main components of weight of dry bean per fruit. Therefore, these traits with high value of direct and indirect effects should be considered as secondary yield components.

Thondaiman and Rajamani (2014) conducted path analysis for twenty characters of 151 clones. Result of the study showed a significant positive effect through pod girth, pod volume, furrow thickness of pod, wet bean weight per pod, dry bean weight per pod, number of pods per tree, per cent of shelling, fat content and polyphenol towards yield.

Veeresh (2017) employed correlation studies and path analysis to study the nature and relationships among the yield attributing characters. It was reported that wet bean weight (g) showed positive correlation with pod weight (g), furrow thickness (cm), pod length (cm), pod breadth (cm), weight of the bean (g) and number of beans per pod. Results of path analysis revealed that total wet bean weight (g) was directly influenced by pod thickness (cm), number of beans per pod, single dry bean weight (g) and wet bean weight (g).

2.9. *Phytophthora* pod rot in cocoa

At present, one of the main challenges faced by cocoa growers is *Phytophthora* pod rot caused by *Phytophthora palmivora.* It is a disease of universal occurrence causing nearly 61.4 per cent global crop loss (Nyadanu *et al.*, 2012). Since this disease is prevalent during rainy season it is very difficult to control using fungicides. Hence, use of resistant varieties is recommended for effective and ecofriendly control.

To confirm the resistance for *Phytophthora* in laboratory detached pod test was identified as the best method in cocoa (Iwaro *et al.,* 2005). Artificial inoculation of pods with non-pricking method was most appropriate method to

check the morphological characters influencing *Phytophthora* pod rot resistance. Whereas, pricking method of pod inoculation is followed to check internal resistance in cocoa (Nyadanu *et al.*, 2012).

Rubiyo and Rivaie (2013) classified the genotypes of cocoa based onresistance and susceptibility to *Phytophthora* pod rot disease caused by *Phytophthora palmivora* using per cent pod area infection. They grouped the genotypes into highly resistant (0- 15 % pod area infection), resistant (15.1-25% pod area infection), moderately resistant (25.1-50 % pod area infection), moderately susceptible (50.1-75 % pod area infection) and susceptible (more than 75 % pod area infection).

Bhavani *et al.* (2007) classified the cocoa genotypes based on the *Phytopthora* pod rot resistance reaction by considering percentage of infection by taking the measurement of lesion size.

2.10. Role of pod husk biochemiclas in providing *Phytopthora* disease resistance

Sporangia of *Phytophthora* species always requires the contact of water to get germinated (Duniway, 1979). If wax is present on pod surface it will repel water, which leads to the formation of droplets and they evaporates easily than a film of water (Grammatikopoulos and Manetas, 1994). Thus cocoa genotypes with high content of wax on pod husk will be having dry pod surface which make them unsuitable for *Phytophthora* (Nyandanu *et al.*, 2012).

Phenolic compounds present in plants are having role in plant defense reponse against biotic stresses (Tan et al., 2004; Omokolo and Boudjeko, 2005) and it was confirmed in different crops including rice, apple, cucumber *etc.*

Conway *et al.* (1994) studied about the relationship between calcium

and diseases resistance and found that presence of calcium in storage tissue enhances the diseases resistance. In order to find out the relationship between potassium and disease resistance, Amtmann *et al.* (2008) conducted a study and revealed that presence of potassium in plants improve the resistance against diseases.

2.11. Tea mosquito bug attacking cocoa

Insects belonging to the family Miridae are serious pests of cocoa worldwide (Entwistle, 1972). Tea mosquito bug (TMB), is a major mirid pest of cocoa, whose incidence became severe during the last three years in summer and post monsoon seasons. Chemical control is difficult against tea mosquito bug in cocoa because, being a highly cross pollinated crop; the pollinator (*Forcipomyia* spp.) of cocoa also belongs to the same family. *Helopeltis antonii*, *H. theivora*, and *H. bradyi* are reported on cocoa in South India. Among the different species *H. theivora* is the most predominant one in cocoa causing damage to young shoots, cherelles and pods (Malhotra and Apshara, 2017).

Feeding lesions produced by tea mosquito bug can kill small cherelles however, older pods may continue to develop even when severely damaged (Miller, 1941). But yield is affected due to malformed pods (Tan, 1974).

Longevity and fecundity of *H. theivora* vary depending on rearing conditions. Tan (1974) recorded a mean adult longevity of 30 days for *H. theivora* raised on cocoa pods in West Malaysia. The same species was reported by Awang *et al.* (1988) to have a mean longevity of 20 days when reared on cocoa pods, but only 6 days when raised on the shoots.

The development and use of tea mosquito bug resistant cocoa varieties is one of the alternatives to chemical control (N'Guessan *et al.*, 2004).Resistance studies against tea mosquito bug in cocoa have mostly concentrated on assessment

of field damage by observing damage on flushes, cherelles, and pods of individual trees and different grade levels of infection on cherelles and pods are assessed to work out the TMB tolerance among genotypes (Apshara, 2018).

Apshara (2013) conducted penetrometer readings for determining the hardness of sclerotic layer, thickness at primary and secondary furrows of pod husk in 100 cocoa genotypes and interpreted the result with reference to tea mosquito bug resistance.

2.12. Regression analysis

Regression model is used to estimate the conditional expectation of the dependent characters. Nyadanu *et al.* (2012) worked out regression analysis on morpho-physiological characters of cocoa and revealed that pod lesion number, lesion size and leaf disc score had 99.2 per cent, 91.2 per cent and 81.3 per cent of variation respectively.

Veeresh (2017) conducted binomial logistic regression and revealed that different phenes like ridge thickness, polyphenol content and calcium content were positively contributing to disease resistance. Whereas, phenes like pod rugosity, pod basal constriction and pod length were negatively correlated with disease resistance. If these phenes are considered for selection, ample increase in the level of resistance will be noticed in the resultant population.

2.13. Diversity analysis

Analysis of relation between genotypes is one of the important components of plant breeding programs. Diversity analysis can be used to determine the variability among the genotypes in a population or between

populations and helps in stratified selection from a breeding population (Ram and Panwar, 1970; Engels, 1986; Asna, 2013; Ajmal,2016; Veeresh, 2017).

Hundred accessions from the germplasm maintained at International cocoa gene bank, Trinidad were characterized by Bekele and Bekele (1996) for phenotypic diversity with morphological descriptors and associations among them were examined by hierarchical cluster analysis. Cluster analysis indicated rich phenotypic diversity in the sample. At 75 per cent level of similarity, nine accessions remained as independent clusters and the remaining accessions were grouped into11 clusters.

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

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

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled 'Genetic analysis of cocoa (*Theobroma cacao* L.) hybrids and screening superior hybrids for major biotic stress' was carried out in the Department of Plant Breeding and Genetics, College of Horticulture and the Cocoa Research Centre (CRC), Vellanikkara during the period 2017-2019.

Twenty cocoa hybrids which are in the steady bearing stage, selected from the comparative yield trial (CYT), planted during 2008 at CRC farm served as the material for the study. These twenty hybrids were selected based on their general vigour and yield performance. In the present study they were further evaluated for morphological and biochemical characters and screened for major pest and disease resistance. CCRP 13 and CCRP 15 were included as check varieties. List of selected hybrids and their parentage are detailed in Table 3.1.

Table 3.1. List of hybrids selected for the study and their parentage

Sl. No.	Hybrids	Parentage
H1	PIV 45.4	$GI_{5.9}$ x $GI_{10.2}$
H2	PIII 2.3	$H_{7.1}$ x $H_{5.3}$
H3	PIV 59.8	$H_{10.1}$ x $H_{6.8}$
H4	SIV 10.11	$GI_{5.9}$ x $GI_{10.2}$
H5	VSDI 10.13	GIV_{126} x $GIV_{18.5}$
H6	SIV 1.10	GIV_{68} x $GI_{5.9}$
H7	PIV 60.9	$GII_{20.4}$ x $GI_{5.9}$
H8	PII 12.11	GIV_{24} x GIV_{51}
H9	SIV 5.15	$GII_{20.4}$ x $GI_{5.9}$
H10	VSDI 33.4	GIV_{148} x $GIV_{18.5}$
H11	VSDI 23.21	GIV_{171} x $GIV_{18.5}$
H12	PIV 58.6	$GII_{20.4}$ x $GI_{5.9}$

Sl. No.	Hybrids	Parentage
H13	PIV 56.9	GIV_{148} x $GIV_{18.5}$
H14	VSDI 30.8	$GIV_{18.8}$ x $GIV_{18.5}$
H15	VSDI 11.11	GVI_{126} x $GIV_{18.5}$
H16	SIV 1.6	GVI_{51} x $GI_{5.9}$
H17	PIV 19.9	$H_{5.3}$ x $H_{6.1}$
H18	PIV 26.8	$H_{7.10}$ x $H_{3.5}$
H19	PIII 15.9	$H_{10.1}$ x $H_{6.8}$
H20	VSDI 29.9	GVI_{188} x GVI_{55}

3.1. Morphological evaluation of the hybrids

For morphological characterisation, the hybrids were evaluated based on quantitative and qualitative characters. Randomized Block Design was used to carry out statistical analysis with three replications. Thirteen pod characters, twelve flower characters, six bean characters and flush colour of leaves were studied. For morphological characterisation of pod and bean, three pods were selected from each replication during the period from September to February. Outer mucilage covering of beans were removed using forceps to evaluate peeled bean characters. Five flowers from each replication was analysed for morphological characterisation of flower. The descriptor developed by Bekele and Butler (2000) was used for recording observations on qualitative characters. The descriptor and descriptor states are presented in Table 3.2.

3.1.1. Evaluation of qualitative characters

3.1.1.1. Flush colour

All the twenty hybrids were evaluated for flush colour as per the descriptor given in Table 3.2.

Table 3.2. Descriptor and descriptor states for qualitative characters

Sl. No.	Characters	Descriptor state	Description
1.	Flush colour	0	Absent (green)
		3	Slight
		5	Intermediate(reddish green)
		7	Intense (red)
2.	Colour of pedicel	1	Green
		2	Reddish
		3	Red
3.	Colour of sepal	1	Cream
		2	Greenish cream
		3	Reddish
		4	Red
4.	Colour of petal	1	Cream
		2	Greenish cream
		3	Reddish
		4	Red
5.	Pod shape	1	Cundeamor
		2	Angoleta
		3	Amelonado
		4	Calabacillo
		5	Criollo
6.	Form of pod apex	1	Attenuate
		2	Acute
		3	Obtuse
		4	Rounded
		5	Mammelate
		6	Indented

Table 3.2. contd.

7.	Pod basal constriction	0	Absent
		1	Slight
		2	Intermediate
		3	Strong
		4	Wide shoulder
8.	Pod rugosity	0	Absent
		3	Slight
		5	Intermediate
		7	Intense
9.	Unripe pod colour	0	Absent
		3	Slight
		5	Intermediate
		7	Purplish green
10.	Colour of ripe pod	0	Absent (green)
		3	Slight (greenish yellow)
		5	Intermediate (yellowish green)
		7	Intense (yellow)
11.	Bean colour	1	White
		2	Grey
		3	Light purple
		4	Medium purple
		5	Dark purple
		6	Mottled
		7	Mixed

3.1.1.2. Flower characters

From each hybrid five flowers were collected and observations on colour of pedicel, sepal and petal were taken as per the descriptor given in Table 3.2.

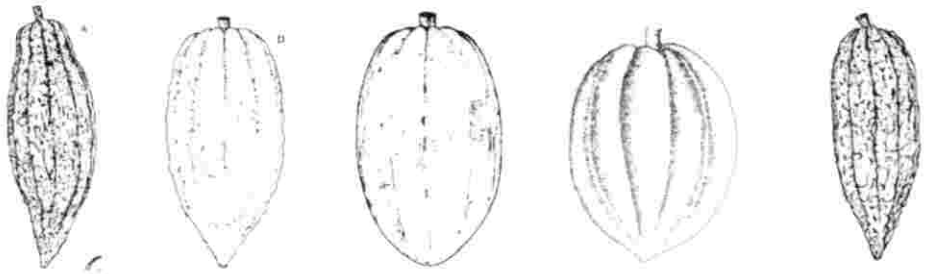
3.1.1.3. Pod and bean characters

Seven characters including shape of the pod, form of pod apex, form of pod basal constriction, colour of ripe and unripe pod, pod rugosity and cotyledon colour of peeled bean were observed. Ten pods were collected from each hybrid and the characters were scored based on the descriptor given in Table 3.2.

3.1.1.3a. Pod shape

As per descriptor (Figure 3.1:), pods were classified as

1. Cundeamor: intensively ridged, warty and characterised by bottle neck
2. Angoleta: deeply ridged and warty with square shape at stalk end
3. Amalelonado: slightly bottle neck, smooth and shallow furrows and melon shaped with blunt ends
4. Calabacillo: small spherical fruits
5. Criollo: deeply ridged surface with acute apex



Cundeamor Angoleta Amelonado Calabacillo Criollo

Figure 3.1. Different shapes of cocoa pods

3.1.1.3b. Pod apex form

As depicted in Figure 3.2. cocoa pods are observed with different forms of pod apex. Observations were recorded for different pod apex in all hybrids included in the study.

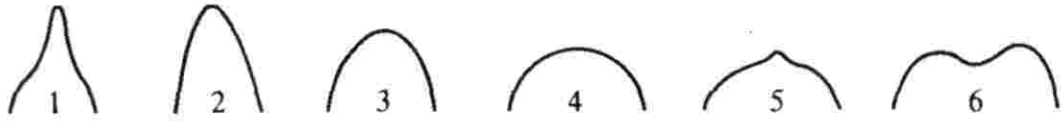


Figure 3.2. Different pod apex forms in cocoa

3.1.1.3c. Pod basal constriction

Observations on the shape of basal constriction of pod were recorded for each hybrid based on the descriptor given in Table 3.2. Naturally observed shape of basal constriction is depicted in Figure 3.3.

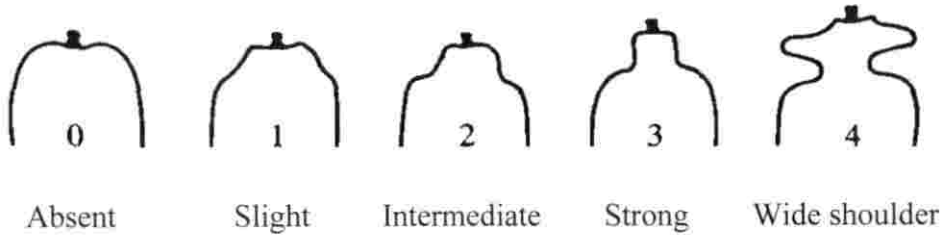


Figure 3.3. Different forms of pod base

3.1.1.3d. Pod rugosity

Rugosity is the measure of smoothness or unevenness of pod surface. Based on the intensity of surface unevenness pods were categorised as given in Table 3.2.

3.1.1.3e. Colour of unripe pod

Colour of ridges and furrows of unripe pods were examined based on presence of anthocyanin pigmentation and classified as the descriptor given in Table 3.2.

3.1.1.3f. Colour of ripe pod

Observations on the colour of ripe pods were recorded based on the intensity of yellow and green colours on the pod ridges and furrows and categorised as given in Table 3.2.

3.1.1.3g. Colour of peeled bean

After removing the outer mucilage, colour of beans were observed and categorised based on the descriptor given in Table 3.2.

3.1.2. Evaluation of quantitative characters

All the 20 hybrids were quantitatively evaluated based on the characters of flower, pod and bean. Five flowers from each hybrid were observed for quantitative evaluation of flower and in case of cocoa pods, ten pods were collected from each hybrid and different observations were recorded.

3.1.2.1. Flower characters

Five flowers were collected from each hybrid during early morning. Each flower was evaluated for twelve characters using laboratory microscope and measuring scale. Characters considered are listed below:

1. Length of pedicel (cm)
2. Diameter of flower (cm)
3. Length of sepal (cm)
4. Width of sepal (cm)
5. Length of petal (cm)
6. Width of petal (cm)
7. Number of stamens (cm)
8. Number of staminodes (cm)
9. Length of ovary (cm)
10. Width of ovary (cm)
11. Length of style (cm)

3.1.2.2. Pod characters

Each pod was examined for below mentioned eight characters under laboratory conditions.

1. Pod weight (g)
2. Pod length (cm)
3. Pod width (cm)
4. Number of ridges/ pod
5. Number of furrows/ pod
6. Rind thickness (cm)
7. Number of beans per pod
8. Number of flat beans per pod

3.1.2.3. Bean characters

From each pod twenty beans were selected randomly and following measurements were taken using vernier caliper or weighing balance.

1. Wet weight of beans/ pod (g)
2. Length of bean (cm)
3. Width of bean (cm)
4. Thickness of bean (cm)
5. Dry weight of single bean (g)
6. Wet weight of single bean (g)

3.1.3. Evaluation of economic characters

In order to get an idea about the amount of pod and bean obtained from a genotype seven different economic characters are usually considered in cocoa. Those seven economic characters include number of pods harvested from a genotype in a year (pod yield), and other six derived parameters like pod value, pod index, efficiency index, conversion index, dry matter recovery and peeling ratio. The description of each economic characters is detailed in Table 3.3.

3.1.4. Genetic parameters

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 in order to assess the extent of variability available in the population.

Table 3.3. Description of economic parameters of cocoa

Sl. No.	Economic characters	Description	Formula	Reference
1.	Pod Yield	No. of pods harvested from each hybrid in a year	-	-
2.	Pod Value (g)	Average dry weight of total beans	Average dry weight of bean x no. of beans per pod	Toxopeus and Jacob (1970)
3.	Pod Index	No. of pods required to get 1 Kg dried beans	1000 g ÷ Pod value	Morera *et al.* (1991)
4.	Efficiency Index	Pod weight required to give 1 Kg dry beans	Pod weight (g) ÷ Pod value	Jacob and Atanda (1971)
5.	Conversion Index	Amount of dry beans obtained from a given amount of wet beans	Pod value ÷ Wet bean weight per pod	Francies (1998)
6.	Dry Matter Recovery (%)	Ratio of dry bean weight obtained after drying to wet bean weight	$\frac{\text{Dry bean weight (g)}}{\text{Wet bean weight (g)}} \times 100$	Francies (1998)
7.	Peeling Ratio (%)	Bean weight obtained after peeling	$\frac{\text{Peeled wet bean weight (g)}}{\text{Unpeeled wet bean weight (g)}} \times 100$	Ajmal (2016)

3.1.4.1. Genotypic Coefficient of Variation (GCV)

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

Where, σg = genotypic standard deviation

3.1.4.2. Phenotypic Coefficient of Variation (PCV)

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

Where, σp = phenotypic standard deviation

The PCV and GCV values were ranked according to Sivasubramaniam and Madhavamenon (1973).

0 – 10% – Low
10.1 – 20% – Moderate
> 20% – High

3.1.4.3. Heritability (H^2)

$$\text{Heritability} = \frac{Vg}{Vp} \times 100$$

Where, Vg is the genotypic variance and Vp the phenotypic variance

Robinson et al., 1949 classified range of heritability as:

0 – 30% – Low
31 – 60% – Moderate
> 61% – High

3.1.4.4. Genetic advance (GA)

$GA = k\sigma pH^2$

Where, k is a constant having value 2.06

3.1.4.5. Genetic gain (GG)

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

0 – 10% – Low

10.1 – 20% – Moderate

> 20% – High

3.2. Evaluation of biochemical characters

Hybrids were evaluated based on biochemical parameters by estimating fat content (%), total polyphenol content (%), major mineral (Na, K, and Ca) content, total antioxidant content, pod husk polyphenol and pod husk wax content by following standard procedure.

Three ripened pods were harvested from each replication and beans were taken out and peeled to remove the mucilaginous covering of each bean. Twenty beans were selected from each pod randomly. Selected beans of each hybrid per replication were pooled and dried to have moisture content below 8 per cent. Under normal sunny weather conditions drying could be completed within seven to eight days. The dry beans were then ground to fine powder using laboratory grinder and the powder was stored for biochemical analysis.

3.2.1. Estimation of fat content

Soxhlet apparatus method proposed by Sadasivam and Manickam (1996) was used for estimating fat content of cocoa beans. Fat present in cocoa beans get dissolved in organic solvents like petroleum ether and can be collected by evaporating the solvent. The procedure is explained in detail below.

Materials required for estimation:

- Cocoa beans powder – 10 g
- Petroleum ether (40-60°C)
- Blotting paper

Procedure:

In soxhlet apparatus cocoa beans powder was defatted using petroleum ether as solvent. Cocoa beans powder of 10g was wrapped in blotting paper and tied using a twine. It was placed inside the extraction tube of the apparatus and sufficient amount of solvent was added. Fat got settled at the bottom of round bottom flask due to the siphoning of petroleum ether in soxhlet apparatus. It took almost 6 hours to complete whole extraction process. When extraction processes were complete, the fat settled was transferred to a pre-weighed beaker and kept open, which allows the remaining solvent to get evaporated. After that weight of fat was taken and expressed as per cent.

$$\text{Fat (\%)} = \frac{\text{Weight of beaker with fat} - \text{Empty weight of beaker}}{\text{Weight of sample}} \times 100$$

3.2.2. Estimation of total phenol content

Folin – Ciocalteau (FC) reagent method proposed by Malik and Singh (1980) was used to estimate total phenol content and it is given in detail below:

Materials required for estimation:

- Cocoa beans powder (defatted) – 500 mg
- 80 percent ethanol
- 20 percent Na_2CO_3
- FC reagent
- Catechol – 100mg

Procedure:

Defatted cocoa powder of weight 500 mg was grinded with 80 percent ethanol in a mortar and pestle. This was transferred into a centrifuge tube and centrifuged at 10,000 rpm for 20 minutes. After that supernatant were transferred to an evaporating dish. This procedure was repeated 2-3 times in order to collect all the phenol present in the sample. To remove the excess ethanol, evaporating dishes were kept over a hot water bath for one hour. To the left-over residue 40 mL of distilled water was added. From that 0.2 mL aliquot was taken to a test tube and 13 mL distilled water was added followed by, addition of 0.5 mL FC reagent. The test tubes with reaction mixture were kept for 3 minutes incubation. After that 2 mL, 20 per cent Na_2CO_3 solution was added. These test tubes were kept for one minute over boiling water bath and incubated at room temperature for 60 minutes. Using spectrophotometer absorbance was read at 650 nm against reagent blank.

For quantification of total phenols, detector was calibrated using the following procedure. Catechin was taken as reference for estimating total phenols in cocoa powder.100 mg catechol in 100 mL distilled water served as the stock

solution. From the stock solution one mL of aliquot was pipetted out into ten mL standard flask and volume was made up. From this, 0.2 mL was pipetted out into a test tube and 13 mL of distilled water was added, followed by addition of FC reagent. After 3 minutes incubation, 2 mL of 20 percent Na_2CO_3 solution was added and mixed well. Absorbance of this solution was read at 650 nm.

Concentration of phenol present in sample was worked out by substituting. The absorbance value thus obtained in the below equation:

$$\text{Total phenol} = \frac{\text{OD of sample}}{\text{OD of standard}} \times \frac{\text{Concentration of standard}}{\text{Volume of sample}} \times 100$$

3.2.3. Estimation of Minerals

Calcium, potassium and sodium mineral content present in cocoa beans were estimated using standard procedure given below.

Materials required for estimation:

- Cocoa bean powder (defatted) – 500mg
- Nitric acid – 25 mL
- Perchloric acid (10%) – 2 mL
- Distilled water – 100 mL

Procedure:

500 mg of defatted cocoa powder was taken in 250 mL beaker. To the sample, 25 mL of concentrated nitric acid was added and this mixture was kept for pre-digestion overnight. After pre-digestion, samples were kept in digestor. When the yellow colour development was seen in the sample, they were taken out and allowed to cool. To this two mL of 10 per cent perchloric acid was added. This was kept for second digestion until the solution turns colourless. At this point 100

mL of distilled water was added, after cooling the mixture. This solution was used for estimating minerals like calcium, sodium and potassium. Mineral concentration was estimated with the help of flame photometer. From the flame photometer concentration in ppm, the mineral content was calculated using below formula:

$$\text{Mineral content (mg/ 100g):} \quad \frac{\text{Concentration of sample (ppm)}}{1000} \times \frac{100}{0.5} \times 100$$

3.2.4. Total antioxidant activity

Total antioxidant activity of defatted cocoa powder was estimated using the procedure given by Schinnella *et al.* (2002) with slight modifications. Detailed procedure is given below:

Materials required for estimation:

- Cocoa bean powder (defatted) – 100mg
- 80 per cent ethanol
- DppH (0.06 Mm) – 3mL

Procedure:

To 100 mg of defatted cocoa bean sample, 10 mL of 80 per cent ethanol was added. This mixture was centrifuged at 10,000 rpm for 20 minutes.20 µL of sample aliquot was pipetted out from the supernatant got after centrifugation. To this 800 µL of 80 per cent ethanol was added. After that 3 mL of DppH radical solution of 0.06 Mm was added and incubated in dark for 30 minutes. Free radical scavenging capacity was then evaluated by measuring the absorbance of reaction mixture at 517 nm in spectrophotometer. The blank was prepared in the same

manner except distilled water was added instead of sample. Reaction mixture without DppH solution served as control. Per cent radical scavenging activity was found out using the given equation:

$$\text{Radical scavenging activity (\%)} = \left[1 - \frac{\text{Abs. of sample} - \text{Abs. of control}}{\text{Abs. of blank}} \right] \text{x } 100$$

3.2.5. Pod husk phenol content estimation

Phenol content of pod husk was estimated by following the Folin – Ciocalteau (FC) reagent method given by Malick and Singh (1980). 0.5g dewaxed cocoa pod husk was taken as the sample. The procedure is same as that of cocoa bean total phenol estimation (*refer 3.2.2.*).

3.2.6. Pod wax estimation

Three medium matured fresh pods were collected from each hybrid. 15 mL of chloroform was poured around the surface of each pod. The chloroform extract thus obtained was boiled over water bath until the smell of chloroform was removed completely. About 5 mL of wax reagent was added to this and boiled for 30 minutes over water bath. Boiled sample was cooled and 12 mL of deionised water was added. After cooling, the extract was filtered using filter paper and filtrate was collected. The intensity of colour formed was determined using spectrophotometer at 590 nm.

3.3. *In vitro* screening for *Phytophthora* pod rot resistance

3.3.1. Isolation of pathogen

During the peak period of infestation *Phytophthora palmivora* infected pods were collected from the field. Surface contamination was removed by washing with water and detergent. Pods were air dried and surface bits of pod husk were taken from infected part of the pod. Bits were surface sterilized with 0.1 per cent $HgCl_2$ for two minutes followed by washing in sterile water three times. These bits, after surface sterilization were placed on carrot agar medium and incubated at 22°C. Plates were observed daily for growth of pathogen. Pattern of fungal growth and shape of sporangiophores were observed. Isolate was purified using hyphal tip culture method and was maintained on PDA plates for further experiments.

3.3.2. Artificial inoculation of pathogen on cocoa pods

The method suggested by Iwaro *et al.* (2000) and followed by Veeresh (2017) was used for artificial inoculation of pathogen. From all the 20 hybrids, immature healthy pods of similar age and size were collected freshly. Highly susceptible genotypes to *Phytophthora* pod rot served as the control. Measurements on length and breadth of each pod were taken.

The pods were washed with water and detergent. 70 per cent ethanol was used as disinfectant. Seven days old *Phytophthora* culture disc of about 10 mm diameter, grown on PDA were placed over the pod of each hybrid. Inoculation was done without pricking on pod surface. The inoculated pods were incubated in polythene bags with a pad of cotton wetted with sterile water in order to provide humidity. Two replications were maintained for each cocoa hybrid. Observations on the length and breadth of lesion developed were recorded in two days interval until the control gets completely infected. Grouping of the genotypes was done as

per the score chart given by Rubiyo and Rivaie (2013) as given in Table 3.4. The per cent of infection was calculated by using the formula (Bhavani, 2007).

Per cent pod area infection =
[Length x Breadth of lesion/ Length x Breadth of pod] x 100

Table 3.4. Score chart for *Phytophthora* pod rot screening

Sl. No.	Score	Pod area infection (%)
1.	Highly resistant	0 – 15
2.	Resistant	15.1 to 25
3.	Moderately resistant	25.1 to 50
4.	Moderately susceptible	50.1 to 75
5.	Susceptible	More than 75

3.4. Screening for tea mosquito bug resistance

Tea mosquito bug (*Helopeltis theivora* Waterhouse) is a major sucking pest of cocoa causing damage to young shoots, cherelles and mature pods. Artificial screening was carried out on budded plant and detached pods to study the reaction of different hybrids against *Helopeltis theivora*. Budded plants of all the hybrids and medium matured freshly collected pods of selected hybrids were screened.

3.4.1. Screening on seedlings

The culture of *Helopeltis theivora* Waterhouse was maintained on cashew seedlings under laboratory condition in insect rearing cages. Three replications having five budded plants of each hybrids were screened once in insect net house. Freshly reared *Helopeltis theivora* at the rate of 100 adults (50 male and 50 female each) per screening test was released into the insect net house. Intensity of

infestation on shoots/ leaves were noted by counting number of feeding punctures at 12 hour interval until 72 hours and was scored (Srikumar and Bhat, 2013).

3.4.2. Screening on pods

Artificial screening was conducted on detached pods of five selected hybrids in insect rearing cages. Three pods of each hybrid was screened once. *Helopeltis theivora* Waterhouse was released to freshly collected pods at the rate of 4 per cage (2 adult male and 2 adult female. Observations on infestation was noted by counting number of feeding punctures at 12 hour interval until 72 hours and it is scored (Srikumar and Bhat, 2013).

3.5. Statistical analysis

3.5.1. Experimental design and Analysis of variance

Randomised Block Design was used to conduct experiment with twenty treatments and three replications. For data obtained from each experiment, analysis of variance was conducted using WASP 2.0 (Web Agri Stat Package) software and Fischer's method of analysis of variance was done. To interpret the data, method proposed by Panse and Sukhatme (1967) was followed. In F'test the level of significance at P = 0.01 was considered. Critical difference and coefficient of variation values were noted when 'F' was significant.

3.5.2. Cluster analysis

Using NTSYS pc version 2.1 software (Rohlf, 1992), the genetic associations among the genotypes were evaluated and estimated by Jaccard's similarity coefficient (Jaccard, 1908). Based on the similarity matrix, cluster analysis was done and a dendrogram was constructed by Unweighted Pair-Group Method (UPGMA) (Sneath and Sokal, 1973).

3.5.3. Correlation analysis

Correlation coefficient represents the magnitude of association between traits. Karl Pearson's Correlation Coefficient was used to carry out correlation analysis. The coefficient of correlation r (x,y) between two variables x and y, for the bivariate dataset (xi,yi) is given below:

$$r(x,y)=cov(x,y)/\sigma x\sigma y$$

where,

cov(x,y): is the covariance between x and y

σx= standard deviation of variable x

σy = standard deviation of variable y

3.5.4. Path coefficient analysis

The correlation coefficients obtained were further analysed by path coefficient analysis. This involves partitioning of the correlation coefficients into direct and indirect effects which helps to identify important component traits useful in indirect selection for complex traits like yield and yield related traits. This analysis was done using the method suggested by Dewey and Lu (1959) following equation:

$$rij = Pij+\Sigma rikpkj$$

The residual effect was estimated by the equation: $(1-R^2)^{-1}$

Where, $R^2 = \Sigma p_{ij} \times r_{ij}$

Where:

- p_{ij} = Component of direct effects of the independent character (i) and dependent character (j) as measured by the path coefficient

- rij = Mutual association between the independent character (i) and dependent character (j) as measured by the correlation coefficient

- Σrikpkj = Summation of components of indirect effect of a given independent character (i) on the given dependent character (j) via all other independent character (k)

3.5.5. Binary logistic regression model

Binary logistic regression model (Logic model) is a uni/ multivariate technique that is used to estimate the probability that a character is present by predicting abinary dependent outcome from a set of explanatory variables and it is used for model binary response data. When response is binary, it takes the value 0 and 1which is indicating success/failure or resistant/susceptible depending upon the type of study was conduct. In this model the dependent variable is categorical. A logistic model is used to predict the effect of change in the independent variable on the probability of belonging to a group when the dependent variable is dichotomous (Mafini and Omoruyi, 2013). Logistic regression indicated that morphological phenes mainly influence on disease susceptibility or resistance.

Results and Discussion

4. RESULTS AND DISCUSSION

Twenty cocoa hybrids which are in the steady bearing stage, evaluated in the Comparative Yield Trial (CYT), CRC farm, is used as the material in the present study entitled'Genetic analysis of cocoa (*Theobroma cacao* L.) hybrids and screening superior hybrids for major biotic stress'.These hybrids were evaluated for general vigour, yield, morphological and biochemical characters along with their resistance reaction to *Phytopthora* and tea mosquito bug. The results of the present study is presented and discussed below.

4.1. Evaluation of morphological characters

Evaluation of morphological characters is having role in providing economic and breeding gain from selected genotypes (Iwaro *et al.*, 2003; Bekele *et al.*, 2006). Variability is essential for selection; Malhotra (2017) stated that expression of crop diversity is estimated from different indicators of variability and among them morphological traits are especially important for cataloguing and characterisation. In the present study morphological characterisation was performed based on qualitative and quantitative floral, pod and bean characters. Flush colour of leaf was also considered. Design employed is Randomised Block Design (RBD) with three replications and in each replication five flowers and three pods were evaluated.

4.1.1. Evaluation of qualitative characters

Qualitative characters were scored using the descriptor given by Bekele and Butler (2000). The observations recorded on qualitative characters are presented in Table 4.1., Table 4.2., Plate 4.1., Plate 4.2. and Plate 4.3. Qualitative characterization was carried out by evaluating twelve characters; flush colour, colour of pedicel, colour of sepal, colour of petal, colour of unripe pod, colour of ripe pod, pod shape, pod apex form, pod basal constriction, pod rugosity and

colour of peeled bean are the characters considered. Most of these characters showed variation among the hybrids. Petal colour was found to be uniform with cream coloured petals in all the hybrids.

4.1.1.1. Qualitative characters of cocoa leaf flush and flower

The observations on qualitative characters of leaf flush and flower are presented in Table 4.1 and Plate 1.Out of twenty hybrids, eight (40%) showed green flush colour.PIV 59.8, SIV 10.11, SIV 1.10, PIV 60.9, PII 12.11, SIV 1.6, PIV 19.9 and VSDI 29.9 are the hybrids with green flush colour. Greenish red (PIV 45.4, PIII 2.3, VSDI 10.13,VSDI 30.8 and PIV 26.8) and reddish green (VSDI 33.4, PIV 58.6, PIV 56.9, VSDI 11.11and PIV 31.9) coloured flush were shown by five hybrids each and together accounts fifty per cent of the hybrids *i.e.* 25 per cent each. Two hybrids (SIV 5.15 and VSDI 23.21) were having red coloured flush which represent ten per cent of the total hybrids. Kochhar (1998) studied on flush colour and revealed that it ranged from light green to red and the present study is in tune with his findings. All the four (green, greenish red, reddish green and red) types of flush leaf colour given in the descriptor of Bekele and Butler (2000) were observed among the hybrids. Bartley (2005) reported that young leaf colour is a distinct trait in cocoa.

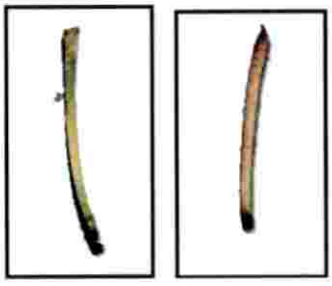
Studies of Enriquez and Soria (1967); Lachenaud *et al.* (1999) and Efombagn *et al.* (2009) revealed that floral characters allow detection of variability in different cocoa cultivars. Three qualitative characters of flower were considered in the study including colour of pedicel, colour of sepal and colour of petal. Except colour of petal, other two showed variation among the hybrids. Green and reddish coloured pedicle was observed among the hybrids. Twelve hybrids (60%) showed green coloured and eight (40%) showed reddish pedicle. Veeresh (2017) conducted study on cocoa flower pedicle colour and reported that green coloured pedicel is most common in cocoa.

Table 4.1. Qualitative characters of leaf and flower of cocoa hybrids

Hybrids	Flush colour	Pedicel colour	Sepal colour	Petal colour
PIV 45.4	Greenish red	Green	Greenish cream	Cream
PIII 2.3	Greenish red	Green	Cream	Cream
PIV 59.8	Green	Green	Cream	Cream
SIV 10.11	Green	Green	Cream	Cream
VSDI 10.13	Greenish red	Green	Cream	Cream
SIV 1.10	Green	Reddish	Greenish cream	Cream
PIV 60.9	Green	Reddish	Cream	Cream
PII 12.11	Green	Green	Cream	Cream
SIV 5.15	Red	Reddish	Cream	Cream
VSDI 33.4	Reddish green	Reddish	Cream	Cream
VSDI 23.21	Red	Reddish	Cream	Cream
PIV 58.6	Reddish green	Reddish	Cream	Cream
PIV 56.9	Reddish green	Reddish	Cream	Cream
VSDI 30.8	Greenish red	Green	Cream	Cream
VSDI 11.11	Reddish green	Green	Cream	Cream
SIV 1.6	Green	Green	Cream	Cream
PIV 19.9	Green	Green	Greenish cream	Cream
PIV 26.8	Greenish red	Reddish	Cream	Cream
PIV 31.9	Reddish green	Green	Cream	Cream
VSDI 29.9	Green	Green	Cream	Cream

Green Greenish red Reddish green Reddish

Plate 4.1. Variability in flush colour



Green Reddish

Plate 4.2. Variability in pedicel colour

Cundeamor Angoleta Amelonado

Plate 4.3. Variability in pod shape

Dark green Intermediate green Purplish

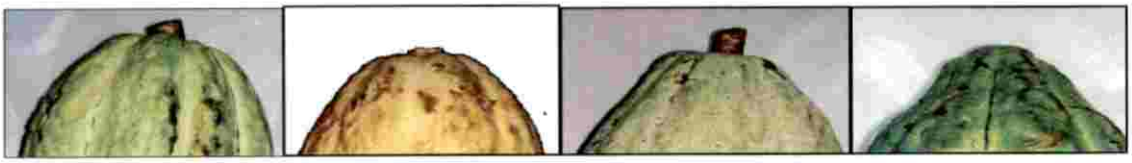
Plate 4.4. Variability in unripe pod colour

Greenish yellow Yellowish green Yellow

Plate 4.5. Variability in ripe pod colour

Attenuate Acute Obtuse Mammalate

Plate 4.6. Variability in pod apex

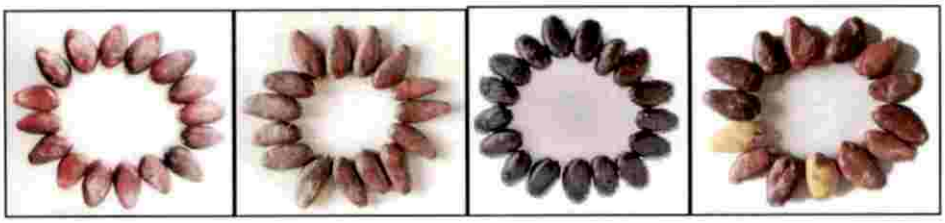


Absent **Slight** **Intermediate** **Strong**

Plate 4.7. Varilability in pod base

Slight **Intermediate** **Intense**

Plate 4.8. Variability in pod rugosity



Light purple **Medium purple** **Dark purple** **Mixed**

Plate 4.9. Variability in bean colour

Bekele and Butler (2000) described four types of sepal colour in their descriptor. Hybrids included in the present study exhibited two types of sepal colour and they are cream and greenish cream. Hybrids PIV 45.4, SIV 1.10 and PIV 19.9 were having greenish cream sepal and the rest seventeen exhibited cream coloured sepals. Veeresh (2017) also reported similar results based on his study on sepal colour of thirty cocoa accessions.

4.1.1.2. Qualitative characters of cocoa pod and bean

Fruits (pods) of *Theobroma* species, especially *Theobroma cacao* show high variability on shape, colour and size (Cuatreasas, 1964). Santos *et al.* (2012) confirmed this result based on their studies in Brazilian *Theobroma* species. Qualitative characters of pod considered in this study includes, pod shape, colour of unripe and ripe pod, pod apex form, pod basal constriction, pod rugosity and bean colour. The observations recorded for these characters are presented in Table 4.2., Plate 4.2 and Plate 4.3.

Wood and Lass (1985) gave detailed description to identify different types of pod shapes in cocoa. As per the descriptor of Bekele and Butler (2000) five different pod shapes are observed naturally in cocoa. Hybrids included in the present study exhibited three pod shapes which are Cundeamor (40%), Amelonado (40%) and Angoleta (20%). Calabacillo and Criollo shaped fruits were not observed. Hybrids PIV 45.4, PIV 59.8, VSDI 10.13, PIV 60.9, VSDI 23.21, PIV 58.6, VSDI 11.11 and PIV 31.9 are the eight hybrids which have Cundeamor shaped fruits. Hybrids with Amelonado shaped fruits are PIII 2.3, SIV 10.11, SIV 1.10, PII 12.11, VSDI 33.4, PIV 56.9, VSDI 30.8 and PIV 26.8. Out of twenty hybrids only four showed Angoleta type fruits, they are SIV 5.15, SIV 1.6, PIV 19.9 and VSDI 29.9.Cundeamor typepods are characterized with deeply ridged and warty surface and bottle neck. Angoleta pods are square shaped at the stalk end and bottleneck is absent. Amelonado types are smooth melon shaped pods with blunt end, shallow furrows and slight bottle neck. Rubeena (2015)

evaluated pod shape of twenty five cocoa hybrids and reported that Cundeamor and Angoleta type pods were present in nine hybrids each and seven hybrids had Amelonado shaped pods.

According to Thi *et al.* (2016), in cocoa, pod colour and shape are the most significant characters which distinguish genotypes. Cocoa hybrids used in the present study found to be variable in unripe pod colour; dark green (35%), intermediate green (55%), light green (5%) and purplish green (5%) colours were observed. All the four types of unripe pod colours given in the descriptor of Bekele and Butler (2000) were observed among the hybrids. The hybrid VSDI 30.8 showed light green coloured unripe pod and the hybrid PIV 56.9 exhibited purplish green coloured pod. Similar to colour of unripe pod, ripe pod colour also showed variability. It was determined by analysing the colour of ridges and furrows. Three variants of colour were observed in case of ripe pod which are yellow (50%), yellowish green (35%) and greenish yellow (15%).

Pod apex form was obtuse in majority (45%) of the hybrids. Acute (30%) and attenuate (20%) types of apex were also observed. Five hybrids with Cundeamor pod shape were having acute pod apex which reveals that pod shape is influenced by pod apex form. Minimol *et al.* (2011) also conducted a study to identify the influence of pod apex on pod shape and concluded that fruit shape is influenced by fruit apex. Pod basal constriction was also noted for all the twenty hybrids and they were grouped into four classes which are absent, slight, intermediate and strong. No pods were observed with wide shoulder. Majority of hybrids had slight constriction (50%). Five hybrids (25%) showed intermediate and three (15%) were having strong basal constriction. Basal constriction was found to be absent in hybrids PIV 56.9 and PIV 26.8. Similar result was reported by Rubeena (2015), in her study45 per cent of the hybrids were having acute pod apex and 75 per cent of hybrids were of slight basal constriction.

Table 4.2. Qualitative characters of cocoa pod and bean of hybrids

Hybrids	Pod shape	Unripe pod colour	Ripe pod colour	Pod apex	Pod base	Pod rugosity	Bean colour
PIV 45.4	Cundeamor	Dark green	Greenish yellow	Acute	Slight	Intense	Medium purple
PIII 2.3	Amelonado	Dark green	Greenish yellow	Obtuse	Slight	Intermediate	Dark purple
PIV 59.8	Cundeamor	Intermediate	Yellow	Acute	Strong	Intense	Medium purple
SIV 10.11	Amelonado	Intermediate	Yellowish green	Mammelate	Slight	Slight	Light purple
VSDI 10.13	Cundeamor	Intermediate	Yellow	Acute	Intermediate	Intermediate	Light purple
SIV 1.10	Amelonado	Intermediate	Yellowish green	Obtuse	Slight	Slight	Medium purple
PIV 60.9	Cundeamor	Intermediate	Yellow	Acute	Intermediate	Slight	Medium purple
PII 12.11	Amelonado	Dark green	Greenish yellow	Obtuse	Slight	Intermediate	Medium purple
SIV 5.15	Angoleta	Intermediate	Yellow	Acute	Slight	Intermediate	Dark purple
VSDI 33.4	Amelonado	Dark green	Yellowish green	Obtuse	Slight	Slight	Dark purple
VSDI 23.21	Cundeamor	Dark green	Yellowish green	Acute	Intermediate	Intermediate	Medium purple

Table 4.2. contd.

Hybrids	Pod shape	Unripe pod colour	Ripe pod colour	Pod apex	Pod base	Pod rugosity	Bean colour
PIV 58.6	Cundeamor	Intermediate	Yellowish green	Obtuse	Strong	Slight	Dark purple
PIV 56.9	Amelonado	Purplish green	Yellow	Obtuse	Absent	Slight	Medium purple
VSDI 30.8	Amelonado	Light	Yellow	Obtuse	Slight	Intermediate	Dark purple
VSDI 11.11	Cundeamor	Dark green	Yellow	Attenuate	Intermediate	Intermediate	Dark purple
SIV 1.6	Angoleta	Dark green	Yellow	Attenuate	Strong	Intense	Mixed
PIV 19.9	Angoleta	Intermediate	Yellowish green	Obtuse	Slight	Slight	Dark purple
PIV 26.8	Amelonado	Intermediate	Yellowish green	Attenuate	Absent	Slight	Light purple
PIV 31.9	Cundeamor	Intermediate	Yellow	Attenuate	Intermediate	Slight	Medium purple
VSDI 29.9	Angoleta	Intermediate	Yellow	Obtuse	Slight	Intermediate	Dark purple

Pod rugosity is the measure of roughness of the pod surface and it can be absent, slight, intermediate or intense as per the descriptor of Bekele and Butler (2000). Hybrids devoid of pod rugosity were not observed. Nine hybrids were having pods with slight rugosity. Intermediate rugosity was observed for eight hybrids and the rest three showed intense rugosity. According to Kochhar (1986) and Malhotra and Apshara (2017) smooth and slight rugous pod surface is the feature of Forastero genotypes.

Cocoa genotypes showed difference in colour of peeled bean or cotyledon. Bekele and Butler (2000) categorised colour of cotyledons as white, grey, light purple, medium purple, dark purple, mottled and mixed. Light purple (15%), medium purple (40%), dark purple (40%) and mixed (5%) beans were observed among the hybrids used for the present study. This result is on par with the findings of Veeresh (2017).

4.1.2. Evaluation of quantitative characters

The experiment was carried out in Randomised Block Design (RBD) with three replications. From each replication five flowers and three pods were considered for evaluation. Observations on eleven floral characters and eleven pod and bean characters were recorded. The mean values obtained for all the quantitative characters shown by different hybrids are presented in Table 4.3a., 4.3b., 4.4 and 4.5.

4.1.2.1. Quantitative characters of cocoa flower

Floral characters such as diameter of the flower (mm), pedicel length (mm), sepal length (mm), sepal breadth (mm), petal length (mm), petal breadth (mm), length of staminode (mm), length of stamen (mm), ovary length (mm), ovary breadth (mm) and length of style (mm) showed significant variation among the hybrids at both five per cent and one per cent level of significance. Mean

values obtained for all the quantitative characters of flower are presented in Table 4.3a. and 4.3b.

Flower diameter measurement varied from 10.19 mm to 15.14 mm among the twenty hybrids. Hybrid SIV 5.15 had the smallest flower while hybrid SIV 10.11 was the largest. Pedicel length varied from 8.32 mm to 19.80 mm and it was shown by the hybrid VSDI 23.21 and PIII 2.3 respectively. Chaidamsari *et al.* (2006) reported that flowers of cocoa are having about 15 mm diameter with long pedicels. Sepal length was the highest in hybrid SIV 10.11 (7.52 mm) and lowest in hybrid PIV 45.4 (5.34 mm). Sepal width ranged from1.38 mm in PIV 45.4 to 2.78 mm in PIII 2.3. Highest petal length was observed in VSDI 23.21 with 5.76 mm and hybrid SIV 5.15 has a measurement of 2.35 mm which was the lowest. Petal breadth varied from 2.64 mm to 1.29 mm in hybrids PIII 2.3 and VSDI 23.21 respectively. Stamen length was the maximum in the hybrid SIV 1.6 with a measurement of 3.37 mm and hybrid VSDI 10.13 showed minimum length which was 1.61 mm. Staminoid length ranged from 6.66 mm in SIV 1.6 to 4.16 mm in PIV 19.9. Ovary length, ovary breadth and style length also showed significant variation among the hybrids and it ranged from 1.36 mm (PIV 45.4 and VSDI 33.4) to 2.54 mm (VSDI 11.11), 0.66 mm (VSDI 23.21) to 1.48 mm (PIV 56.9) and 1.08 mm (PIV 45.4) to 2.67 mm (VSDI 11.11) respectively. The result obtained for all the eleven floral characters were found to be similar with the findings of earlier researches who worked on cocoa flower.

Asna (2013) carried out a study on cocoa floral measurements in 50 cocoa accessions and reported that only pedicel length, sepal length, petal length, stamen length and style length showed significant differences among genotypes. But in the present study all the quantitative characters of cocoa flowers showed significant variation among hybrids. According to her findings pedicel length, sepal length, petal length, stamen length and style length ranged from 6.5 mm to 18.5 mm, 4.2 mm to 8.3 mm, 5.1 mm to 9.6 mm, 1.5 mm to 2.3 mm and 1 mm to 3.2 mm respectively and these results are on par with the present findings.

Table 4.3a. Mean values of quantitative floral characters of cocoa hybrids

Hybrids	Flower diameter (mm)	Pedicel length (mm)	Sepal length (mm)	Sepal width (mm)	Petal length (mm)
PIV 45.4	10.22	10.83	5.34	1.38	2.88
PIII 2.3	13.20	19.80	7.36	2.78	4.36
PIV 59.8	14.84	17.08	7.10	2.52	4.62
SIV 10.11	15.14	16.48	7.52	2.41	4.67
VSDI 10.13	13.49	14.56	6.28	2.74	4.20
SIV 1.10	12.99	9.06	6.71	1.47	5.39
PIV 60.9	13.56	15.08	6.20	1.91	4.06
PII 12.11	12.10	14.20	5.39	1.73	3.20
SIV 5.15	10.19	9.92	6.18	1.55	2.35
VSDI 33.4	13.50	13.53	7.04	2.25	3.36
VSDI 23.21	12.72	8.32	6.31	1.97	5.76
PIV 58.6	12.76	15.12	6.68	2.20	4.56
PIV 56.9	10.37	11.62	6.05	2.01	2.59

Table 4.3a. contd.

Hybrids	Flower diameter (mm)	Pedicel length (mm)	Sepal length (mm)	Sepal width (mm)	Petal length (mm)
VSDI 30.8	13.21	14.05	6.37	1.73	3.55
VSDI 11.11	14.58	17.18	7.28	2.24	4.20
SIV 1.6	13.81	11.28	7.46	1.57	4.60
PIV 19.9	12.08	15.80	5.87	2.18	4.36
PIV 26.8	12.26	17.20	6.08	1.94	3.16
PIV 31.9	10.71	9.56	5.93	1.96	2.74
VSDI 29.9	12.24	12.84	5.56	1.57	3.50
CV (%)	**3.91**	**3.86**	**4.87**	**9.24**	**5.80**
CD (1%)	**0.83**	**0.88**	**0.52**	**0.23**	**0.28**

Table 4.3b. Mean values of quantitative floral characters of cocoa hybrids

Hybrids	Petal breadth (mm)	Stamen length (mm)	Staminode length (mm)	Ovary length (mm)	Ovary breadth (mm)	Style length (mm)
PIV 45.4	1.44	2.62	4.84	1.36	1.00	1.08
PIII 2.3	2.64	1.66	4.82	1.92	1.38	2.32
PIV 59.8	2.02	3.11	6.28	1.53	1.04	2.07
SIV 10.11	2.23	1.62	6.54	1.38	0.84	2.39
VSDI 10.13	2.50	1.61	6.24	2.21	1.37	1.61
SIV 1.10	1.52	2.57	5.54	1.46	0.98	2.28
PIV 60.9	1.65	2.08	5.92	2.06	1.27	2.17
PII 12.11	1.71	1.79	4.59	1.51	1.09	1.67
SIV 5.15	1.69	2.19	4.57	1.75	1.36	1.75
VSDI 33.4	2.30	2.27	4.31	1.36	1.42	1.85
VSDI 23.21	1.29	2.40	5.97	1.72	0.66	1.83
PIV 58.6	2.28	2.29	6.12	1.60	1.18	2.26
PIV 56.9	1.64	2.66	4.67	2.17	1.48	1.86
VSDI 30.8	1.68	2.21	4.69	2.52	1.78	1.54

Table 4.3b. contd.

Hybrids	Petal breadth (mm)	Stamen length (mm)	Staminode length (mm)	Ovary length (mm)	Ovary breadth (mm)	Style length (mm)
VSDI 11.11	2.51	3.15	6.34	2.54	1.46	2.67
SIV 1.6	1.56	3.37	6.66	1.44	1.09	2.44
PIV 19.9	1.76	2.80	4.16	2.00	1.00	2.11
PIV 26.8	1.68	2.60	6.40	1.52	0.78	1.56
PIV 31.9	1.68	2.22	4.65	1.96	1.30	2.09
VSDI 29.9	1.45	2.28	5.46	1.61	1.20	2.32
CV (%)	**9.70**	**15.56**	**4.48**	**9.25**	**11.52**	**8.13**
CD (1%)	**0.30**	**0.62**	**0.41**	**0.21**	**0.23**	**0.27**

Veeresh (2017) conducted similar study in 30 exotic cocoa accessions and concluded that floral characters showed significant variation among the genotypes considered. He also reported that, diameter of the flower, pedicel length, sepal length, sepal breadth, petal length, petal breath, length of staminode, length of stamen, ovary length, ovary breadth and length of style ranged from 8.9 mm to 14.7 mm, 8.2 mm to 18.3 mm, 5.2 mm to 7.4 mm, 1.0 mm to 2.1 mm, 6.5mm to 8.9 mm, 1.2 mm to 2.0 mm, 4.7 mm to 6.8 mm, 1.6 mm to 2.8 mm, 1.1 mm to 2.2 mm, 0.9 mm to 1.3 mm and 1.5mm to 2.5 mm respectively. These results are on consonance with the present findings.

4.1.1.2. Quantitative characters of cocoa pod and bean

Quantitative characters of pod includes seven characters which are pod weight (g), pod length (cm), pod breadth (cm), rind thickness (cm), number of beans per pod, number of flat beans per pod and total weight of wet beans per pod (g). Six bean characters considered are weight of single wet bean (g), weight of single peeled bean (g), weight of single dry bean (g), dry bean length (cm), dry bean width (cm) and dry bean thickness (cm). Mean values for quantitative characters of pod and beans are presented in Table 4.4 and Table 4.5 respectively.

Among the twenty hybrids PIII 2.3 exhibited highest pod weight of 679g followed by hybrid PIV 59.8, which showed a pod weight of 652.5g. Pod weight was the lowest in the hybrid SIV 1.6 (292.9g). Most of the hybrids included in the study satisfied the selection criteria recommended by Francies *et al.* (2002) *i.e.* pod weight more than 350 g. Five hybrids were having pod weight more than 500g. Ajmal (2016) also conducted similar study on cocoa hybrids and found that pod weight of thirty hybrids ranged from 249.64 g to 685 g.

Pod length was the maximum in hybrid PIV 59.8 with 19.21 cm and hybrid PIV 26.8 has the shortest pods with a length of 12.04 cm. Hybrid PIII 2.3 exhibited pods with maximum mean breadth which is 9.59 cm and VSDI 10.13

Table 4.4. Mean values of quantitative characters of pod of cocoa hybrids

Hybrids	Pod weight (g)	Pod length (cm)	Pod breadth (cm)	Rind thickness (cm)
PIV 45.4	498.00	15.54	6.98	1.19
PIII 2.3	629.00	16.45	9.59	1.82
PIV 59.8	652.50	19.21	7.81	1.06
SIV 10.11	492.00	14.12	7.56	0.89
VSDI 10.13	379.70	15.92	6.12	0.75
SIV 1.10	341.00	13.54	7.04	0.78
PIV 60.9	493.20	17.26	7.14	0.97
PII 12.11	626.00	15.61	8.28	1.21
SIV 5.15	663.00	17.08	8.60	1.21
VSDI 33.4	618.50	15.32	8.31	1.32
VSDI 23.21	450.77	15.02	7.74	0.91
PIV 58.6	464.10	15.89	6.82	0.88
PIV 56.9	450.60	15.33	7.91	0.79
VSDI 30.8	354.00	12.78	7.13	1.22
VSDI 11.11	324.50	14.74	6.44	0.65
SIV 1.6	292.90	12.71	5.79	0.67
PIV 19.9	417.60	15.69	9.09	1.36
PIV 26.8	312.00	12.04	6.49	0.77
PIV 31.9	378.00	15.24	6.54	0.80
VSDI 29.9	493.20	15.20	7.23	0.88
CV (%)	**6.79**	**5.41**	**6.23**	**10.28**
CD (%)	**32.75**	**0.95**	**0.54**	**0.12**

Table 4.5. Mean values of quantitative characters of bean

Hybrids	No. of beans/pod	No. of flat beans/ pod	Total weight of wet beans/ pod (g)	Weight of single wet bean (g)	Single peeled bean weight (g)	Single dry bean weight (g)	Dry bean length (cm)	Dry bean width (cm)	Dry bean thickness (cm)
PIV 45.4	36.30	1.55	121.16	2.59	1.32	0.95	1.72	0.96	0.71
PIII 2.3	48.60	0.81	135.07	2.24	1.17	0.93	1.78	0.98	0.62
PIV 59.8	45.10	1.12	176.62	3.72	1.69	1.19	2.05	1.12	0.67
SIV 10.11	49.40	1.17	147.24	2.74	1.21	0.80	1.96	1.09	0.44
VSDI 10.13	47.90	1.23	108.89	1.66	0.66	0.49	1.42	0.68	0.51
SIV 1.10	46.50	0.86	122.63	2.24	0.86	0.63	1.82	0.84	0.45
PIV 60.9	43.80	0.86	153.96	2.66	1.25	0.95	1.97	0.98	0.63
PII 12.11	51.30	0.81	152.98	3.02	1.45	1.30	2.12	1.24	0.58
SIV 5.15	42.20	0.97	185.78	3.64	1.50	1.05	1.86	1.06	0.65
VSDI 33.4	52.00	0.91	129.96	2.71	1.55	1.07	2.01	1.01	0.52
VSDI 23.21	48.90	0.91	147.64	2.24	1.24	0.78	1.87	0.92	0.66
PIV 58.6	46.10	1.02	133.55	2.43	1.16	0.82	1.83	0.92	0.59
PIV 56.9	39.30	1.12	122.33	2.37	1.28	0.95	2.23	0.96	0.55

Table 4.5. contd.

Hybrids	No. of beans/pod	No. of flat beans/ pod	Total weight of wet beans/ pod (g)	Weight of single wet bean (g)	Single peeled bean weight (g)	Single dry bean weight (g)	Dry bean length (cm)	Dry bean width (cm)	Dry bean thickness (cm)
VSDI 30.8	44.50	0.97	115.99	2.38	1.19	0.77	1.78	0.98	0.51
VSDI 11.11	50.00	0.91	109.69	1.82	0.89	0.78	1.64	0.84	0.55
SIV 1.6	39.40	0.97	109.42	2.25	1.15	0.87	1.76	0.93	0.45
PIV 19.9	47.50	0.97	161.27	2.90	1.27	1.06	1.84	0.94	0.68
PIV 26.8	44.10	1.17	130.02	2.30	1.26	0.83	1.72	0.99	0.46
PIV 31.9	43.60	1.87	120.02	2.36	1.08	0.84	1.87	1.02	0.59
VSDI 29.9	49.80	0.81	160.34	2.52	1.41	1.00	1.88	0.91	0.51
CV (%)	**5.82**	**22.17**	**5.93**	**8.30**	**8.67**	**10.32**	**5.14**	**6.75**	**11.77**
CD (1%)	**3.06**	**0.26**	**7.05**	**0.24**	**0.12**	**0.11**	**1.10**	**0.76**	**0.77**

have minimum pod breadth which is 6.12 cm. The maximum rind thickness was also observed in hybrid PIII 2.3 (1.82 cm) which showed highest pod weight. Rind thickness was the lowest in SIV 1.6 (0.67 cm) and the same hybrid showed lowest pod weight. This shows, rind thickness has role in deciding pod weight Rubeena (2015).

Enriquez and Soria (1966) reported that rind thickness of one or less than one is most desirable in cocoa. Out of the twenty hybrids included in the study twelve hybrids have rind thickness less than one.

Quantitative characters of bean includes number of beans per pod, total wet weight of beans per pod (g), wet weight of single bean (g), peeled weight of single bean (g), dry weight of single bean (g), single dry bean length (cm), width (cm) and thickness (cm). All these characters showed significant variation among the twenty hybrids. Hybrid PIV 45.4 has thirty six beans per pod which is the lowest and highest number of beans per pod was fifty two, in hybrid VSDI 33.4. Hybrid SIV 1.6 recorded the lowest total wet bean weight of 109.42 g. Mean value of total wet bean weight was the maximum in hybrid SIV 5.15 and it is 185.78 g. Hybrid PIV 59.8 showed second highest total wet bean weight and highest single wet bean weight and it is 176.62 g and 3.72 g respectively. Single wet bean weight was the lowest in hybrid VSDI 10.13 (1.66g). Weight of single peeled wet bean ranged from 1.69 g in hybrid PIV 59.8 and 0.66 g in hybrid VSDI 10.13. Weight of single peeled dry bean varied from 1.25 g in PII 12.11 to 0.49 g in VSDI 10.13.The study of Pound (1932) and Enriquez and Soria (1966) revealed that wet weight and peeled dry weight of beanrangedfrom0.5 g to2.5g and 0.58 g to 1.72 g respectively and the present study is on par with their findings. In the opinion of Monteiro *et al.* (2009) and Minimol *et al.*(2015) superior genotypes have single peeled dry bean weight more than 0.8 gram. Sumitha *et al.* (2018) and Deepa *et al.* (2019) also employed same criteria (single dry bean weight more than 0.8 gram) for selecting superior genotypes. Fifteen hybrids included in the study have more than 0.8 g single dry bean weight and among the fifteen hybrids,

five exhibited more than one gram single dry bean weight. Dry bean thickness ranged from 0.44 cm (SIV 10.11) to 0.71 cm (PIV 45.4). Hybrid PIV 56.9 excelled other hybrids in dry bean length (2.23 cm). Dry bean breadth (2.12 cm) and single dry bean weight (1.30 g) was the highest in hybrid PII 12.11. Hybrid VSDI 10.13 was found to be inferior for single wet bean weight, single peeled bean weight, single dry bean weight, dry bean length and breadth.

Apart from the number of ridges and number of furrows per pod all other characters showed significant difference among the twenty hybrids at both five per cent and one per cent level of significance. Number of ridges and furrows were ten each in all the hybrids included in the study.

4.1.2. Evaluation of economic characters of cocoa hybrids

Plant breeding approaches usually have three objectives like the end product must be economic, biologically reasonable and environmentally sound (Djocgoue *et al.*, 2006). The main criteria for a variety to become popular among farmers is decided by its economic performance. Hence a plant breeder has to make varieties with high economic value along with other desirable characters like pest and disease resistance, quality etc. However it is generally a difficult task to combine these characters.

In cocoa the economic part is pod and particularly its dried beans. In order to get an idea about the amount of pod and bean obtained from a genotype seven different economic characters are usually considered. Those seven economic characters include number of pods harvested from a genotype in a year (pod yield)and other six derived parameters like pod value (g), pod index, efficiency index, conversion index, dry matter recovery (%) and peeling ratio (%).

Mean value of economic characters calculated for each hybrid is given in Table 4.6.Pooled yield obtained for last five years (2014-2015 to 2018-2019) is

represented as pod yield. Pod yield ranged from 86.20 pods per tree per year in hybrid SIV 10.11 to 135.80 pods per tree per year in hybrid PIV 59.8. Hybrid PIV 60.9 and PIV 19.9 are also high yielders with 126.40 and 118.20 pods per tree per year respectively. The mean pod yield of twenty hybrids included in the study is about 99.31 pods per tree per year. Hybrids with more than 80 pods per tree per year is considered desirable (Francies *et al.*, 2002) and all hybrids met this requirement. Ajmal (2016) evaluated cocoa hybrids based on economic characters and observed pod yield of hybrids varies from 63 pods/ tree/ year to 111 pods/ tree/ year.

Toxopeus and Jacob (1970) introduced the concept of pod value as an economic character of cocoa and it gives an idea about the total weight of dried beans obtained per pod. Mean pod value of the twenty hybrids is 41.4 g. Pod value was the maximum for hybrid PII 12.11 (66.69g) and hybrid VSDI 10.13 exhibited minimum pod value of 23.47 g. This results are in consonance with the result of Velayutham *et al.* (2013) were he reported pod value ranged from15.29 to 52.88 in a study conducted to identify high yielding cocoa trees in Tamil Nadu. Based on the study on cocoa hybrids Ajmal (2016) reported that pod value ranges from 17.71 g to 65.20 g.

Morera *et al.* (1991) used pod index for the first time to measure number of pods required to get 1 Kg beans. Genotype with low pod index is considered superior than others. Pod index ranged from 14.99 (PII 12.11) to 42.61 (VSDI 10.13) among the hybrids. Pound (1932) suggested that, hybrids with pod index 15 or less than that is good for breeding. Among the hybrids included in the study only PII 12.11 has pod index less than 15. Hybrid PII 12.11is not the one having highest number of beans per pod, so we can conclude that dry bean weight has major role in deciding the pod index. Similar results were recorded by early workers (Rubeena, 2015; Ajmal, 2016 and Veeresh, 2017).

Table 4.6. Mean values of economic characters of cocoa hybrids

Hybrids	Yield*	Pod value (g)	Pod index	Efficiency index	Conversion index	Peeling ratio (%)	Dry matter recovery (%)
PIV 45.4	95.40	34.49	29.00	14.44	0.28	50.97	71.97
PIII 2.3	89.80	45.20	22.12	16.13	0.33	52.23	79.49
PIV 59.8	135.80	53.67	18.63	14.02	0.30	45.43	70.41
SIV 10.11	86.20	39.52	25.30	12.45	0.27	44.16	66.12
VSDI 10.13	90.40	23.47	42.61	16.18	0.22	39.76	74.24
SIV 1.10	96.20	29.30	34.14	11.64	0.24	38.39	73.26
PIV 60.9	126.40	41.61	24.03	11.85	0.27	46.99	76.00
PII 12.11	95.80	66.69	14.99	9.39	0.44	48.01	89.66
SIV 5.15	91.40	44.31	22.57	14.96	0.24	41.21	70.00
VSDI 33.4	102.40	55.64	17.97	11.12	0.43	57.20	69.03
VSDI 23.21	97.60	38.14	26.22	11.82	0.26	55.36	62.90
PIV 58.6	96.60	37.80	26.45	12.28	0.28	47.74	70.69
PIV 56.9	91.40	37.34	26.78	12.07	0.31	54.01	74.22
VSDI 30.8	89.20	34.27	29.18	10.33	0.30	50.00	64.71
VSDI 11.11	101.20	39.00	25.64	8.32	0.36	48.90	87.64

Hybrids	Yield*	Pod value (g)	Pod index	Efficiency index	Conversion index	Peeling ratio (%)	Dry matter recovery (%)
SIV 1.6	101.40	34.28	29.17	8.54	0.34	51.11	75.65
PIV 19.9	118.20	50.35	19.86	8.29	0.33	43.79	83.46
PIV 26.8	92.4	36.60	27.32	8.52	0.30	54.78	65.87
PIV 31.9	92.4	36.62	27.30	10.32	0.33	45.76	77.78
VSDI 29.9	96.00	49.80	20.08	9.90	0.33	55.95	70.92
CV (%)	**11.29**	**11.34**	**11.59**	**11.43**	**11.25**	**10.30**	**8.65**
CD (1%)	**13.33**	**5.37**	**3.45**	**1.36**	**0.05**	**5.80**	**7.34**

*** Yield is expressed as no. of pods/tree/ year**

Efficiency index (Jacob and Atanda, 1971) is the pod weight required to get 1 Kg dry beans. Similar to pod index low value of efficiency index is a preferred character. PIV 19.9has the lowest value of efficiency index 8.29 and hybrid VSDI 10.13 with an efficiency index of 16.18 is the highest. This result is on consonance with the reported result of Ajmal (2016) *i.e.* efficiency index ranges from 7.72 to 15.11.

Conversion index is the amount of dry beans obtained from given amount of wet beans (Francies, 1998). Genotypes with high conversion index are desirable. Hybrid PII 12.11 has the highest conversion index of 0.44 and SIV 5.15 has the lowest (0.24) conversion index. Similar result on cocoa hybrids was reported by Ajmal (2016) that conversion index ranges from 0.23 to 0.45.

Vasudevan *et al.* (2011) reported that genotypes with average value of efficiency index low and conversion index high can be considered as superior. Among the twenty hybrids evaluated PII 12.11 showed low efficiency index (9.39) and high conversion index (0.44), which indicates it is a superior genotype.

Francies (1998) introduced the concept of dry matter recovery and it is the ratio of dry bean weight obtained after drying to the wet bean weight. Dry matter recovery is expressed in per cent. VSDI 23.21 has the lowest dry matter recovery of 62.90 per cent and VSDI 11.11 with dry matter recovery 87.64 per cent is the highest. The reason for low dry matter recovery is due to high moisture content in the beans (Rubeena, 2015).

Peeling ratio is the bean weight obtained after peeling and expressed as per cent. Hybrid VSDI 33.4 has the maximum peeling ratio of 57.20 per cent. 38.39 per cent peeling ratio in hybrid SIV 1.10 is the lowest. Low peeling ratio indicates that there is a thick testa covering around the beans of that hybrid and it is an undesirable trait since it affects single peeled wet bean weight.

When seven economic characters of cocoa were considered in twenty hybrids PIV 59.8 is the high yielder. Hybrid PII 12.11 was having high pod value, low pod index and high conversion index which are superior characters. Peeling ratio was the highest in hybrid VSDI 33.4. Hybrid PIV 19.9 exhibited lowest efficiency index. Highest dry matter recovery per cent was observed in VSDI 11.11. Hybrid VSDI 10.13 is found to be inferior for three economic characters like pod value, pod index and efficiency index.

4.2. Evaluation of Biochemical Characters

Biochemical characters have major role in providing quality attributes and there are reports of their role in resistance reactions against biotic and abiotic stresses. In the present study biochemical evaluation was conducted to analyse the biochemical properties of cocoa beans. Data obtained after estimating biochemical characters were analysed using Completely Randomised Design with three replications. Beans are evaluated for fat, total polyphenol content, mineral content and antioxidant activity and the mean values obtained for these characters are presented in Table 4.7. and graphically represented in Figure 4.1., 4.2. and 4.3.

4.2.1. Biochemical characters of cocoa beans

Rossini *et al.* (2011) reported that fat content of beans varies between cocoa genotypes. In the present study fat content varied from 48.2 per cent in the hybrid VSDI 23.21 to 62.533 per cent in the hybrid PIV 31.9. Hybrid SIV 1.10 has the second largest (61.2 %) fat content. Studies of Mossu (1992) revealed the role of fat content in enhancing flavour and aroma of chocolates. Hybrids with high fat content can act as a genetic stock for improving quality in future breeding purposes (Monteiro *et al.*, 2009).Genotypes having fat content more than 45per cent is considered superior (Afoakwa *et al.*, 2013).All the hybrids included in the present study exhibited more than forty five per cent fat content in their beans.

Eighty five per cent of the hybrids used in the study recorded more than fifty per cent fat content.

Polyphenols has role in imparting flavor and colour to chocolate (Kim and Keeney, 1984). Total polyphenol content in the twenty cocoa hybrids included in the study ranged from 3.71 per cent in PIV 31.9 to 9.36 per cent in PII 12.11. Second highest polyphenol content (9.14 %) was recorded in hybrid PIV 45.4. Zumbe (1998) reported that polyphenol content in cocoa beans is about 6-8 per cent and it can reach up to a value of 10 per cent.

Studies of Asna (2013) revealed that total polyphenol content in the unfermented cocoa beans ranged from 2.25 per cent to 9.09 per cent. Rubeena (2015) estimated total polyphenol content in unfermented samples of cocoa hybrids and reported that it ranged from 1.51 per cent to 5.64 per cent. Veeresh (2017) studied total polyphenol content of thirty cocoa accessions and reported that the highest total phenol content was observed in genotype ICS41 (11.81%). The lowest total phenol content was observed in genotype PA303 (6.34%). The minimum level for polyphenol considered desirable in cocoa is 4.5 per cent Veeresh (2017). In the present study nineteen hybrids exhibited more than 4.5 per cent polyphenol content.

Free radicals produced inside the body have the capacity to destroy cells and antioxidants are substances used to scavenge these radicals. Radical scavenging activity is an indirect measure of antioxidant property of a substance. Radical scavenging activity ranged from 49.95 per cent in the hybrid PIV 31.9 to 90.75 per cent in the hybrid PII 12.11. Hybrid PIV 45.4 is also having high radical scavenging activity of 87.01 per cent. Out of the twenty hybrids, ten exhibited more than 80 per cent radical scavenging activity. Othman *et al.* (2007) conducted a study to estimate antioxidant capacity of cocoa beans from different countries and reported that DppH radical scavenging activity of those beans ranged from 20

per cent to 90 per cent. Central Plantation Crops Research Institute (CPCRI) evaluated antioxidant capacity of beans harvested from different cocoa varieties and found out that they differ significantly and it varied from 77 per cent to 98 per cent (Malhotra and Apshara, 2017).The present study is on par with their results.

In mineral estimation, sodium, potassium and calcium content of defatted cocoa powder was estimated using standard procedure and expressed as mg/ 100 g sample. Sodium content of hybrids ranged from 1.06 mg/ 100 g in SIV 1.6 to 2.86 mg/ 100 g in PIV 59.8. Potassium content of cocoa beans varied from 915.15 mg/ 100 g in the hybrid PIV 19.9 to 2021.61 mg/ 100 g in the hybrid SIV 1.10. Afoakwa *et al.* (2012) reported that potassium is the most abundant mineral in cocoa beans. Calcium content was the highest in hybrid PIV 58.6 (213.39 mg/ 100 g) and the lowest in hybrid SIV 1.6 (118.58 mg/ 100 g).

Olaofe *et al.* (1987) reported that cocoa beans have average calcium content of 25.5 µg/ g, 21µg/ g of sodium and potassium content of about 2480 µg/ g. Afoakwa *et al.* (2013) also conducted similar study to quantify the mineral content of cocoa beans collected from different genotypes grown in Ghana. The result revealed that sodium content (2.5 to 3.5mg/ 100g) was high and calcium content was low (140.2 to 170.8mg/ 100g) in those accessions when compared with the values of present study. However, the result of potassium was in tune with the present study. When compared with the findings of present study those genotypes exhibited high sodium and low calcium content, but potassium content is found to be similar in both studies. Veeresh (2017) estimated mineral contents of different cocoa accessions and reported that sodium content varied from1.31mg/ 100g to 1.98mg/ 100g, calcium 201.60 mg/ 100g to 307.20mg/ 100g and potassium content 848mg/ 100g to 2002.07 mg/ 100g. The hybrids evaluated in the present study also expressed similar tendency.

Table 4.7. Mean biochemical characters of bean of cocoa hybrids

Hybrids	Fat (%)	Polyphenol* (%)	Radical scavenging activity* (%)	Sodium (mg/100g)	Potassium (mg/100g)	Calcium (mg/100g)
PIV 45.4	55.66	9.14 (17.59)	87.01 (63.78)	1.89	1766.61	171.75
PIII 2.3	52.66	7.35 (15.73)	77.13 (61.42)	1.28	1865.11	203.92
PIV 59.8	53.00	6.33 (14.56)	75.62 (60.42)	2.86	1756.49	164.38
SIV 10.11	52.56	6.48 (14.74)	80.48 (72.32)	1.65	1578.98	132.62
VSDI 10.13	52.40	7.01 (15.34)	85.06 (67.26)	2.70	1651.16	180.19
SIV 1.10	61.20	6.41 (14.66)	77.60 (68.87)	1.75	2021.61	201.22
PIV 60.9	55.43	6.53 (14.81)	84.81 (65.35)	1.79	1829.21	185.86
PII 12.11	51.63	9.36 (17.78)	90.75 (67.11)	1.37	1425.30	204.84
SIV 5.15	50.90	4.74 (12.55)	72.50 (58.39)	1.78	1814.80	187.07
VSDI 33.4	54.73	6.34 (14.56)	68.86 (56.08)	1.77	1631.69	196.30
VSDI 23.21	48.20	6.16 (14.36)	78.20 (62.16)	1.56	1989.22	185.29

Table 4.7. contd.

Hybrids	Fat (%)	Polyphenol* (%)	Radical scavenging activity* (%)	Sodium (mg/100g)	Potassium (mg/100g)	Calcium (mg/100g)
PIV 58.6	55.73	6.48 (14.74)	70.83 (57.31)	1.57	1537.39	213.39
PIV 56.9	53.76	7.61 (16.00)	83.89 (66.34)	1.66	1663.12	181.63
VSDI 30.8	49.93	7.21 (15.54)	82.98 (65.64)	1.58	1614.72	194.69
VSDI 11.11	55.26	7.86 (16.28)	84.68 (66.96)	2.14	1508.89	183.38
SIV 1.6	49.33	7.73 (16.13)	82.61 (61.76)	1.06	1628.70	118.58
PIV 19.9	56.80	7.98 (16.40)	84.51 (59.20)	1.32	915.15	151.97
PIV 26.8	53.56	5.72 (13.82)	73.79 (44.97)	2.44	1977.28	183.53
PIV 31.9	62.53	3.71(11.09)	49.95 (66.83)	1.59	1371.46	194.25
VSDI 29.9	56.53	5.93(12.23)	74.25 (59.52)	1.74	1214.29	171.26
CV (%)	**2.74**	**4.30**	**0.96**	**7.54**	**0.78**	**4.68**
CD(1%)	**3.272**	**0.316**	**0.187**	**0.381**	**36.694**	**24.043**

* Values in parenthesis are transformed values by using angular transformation

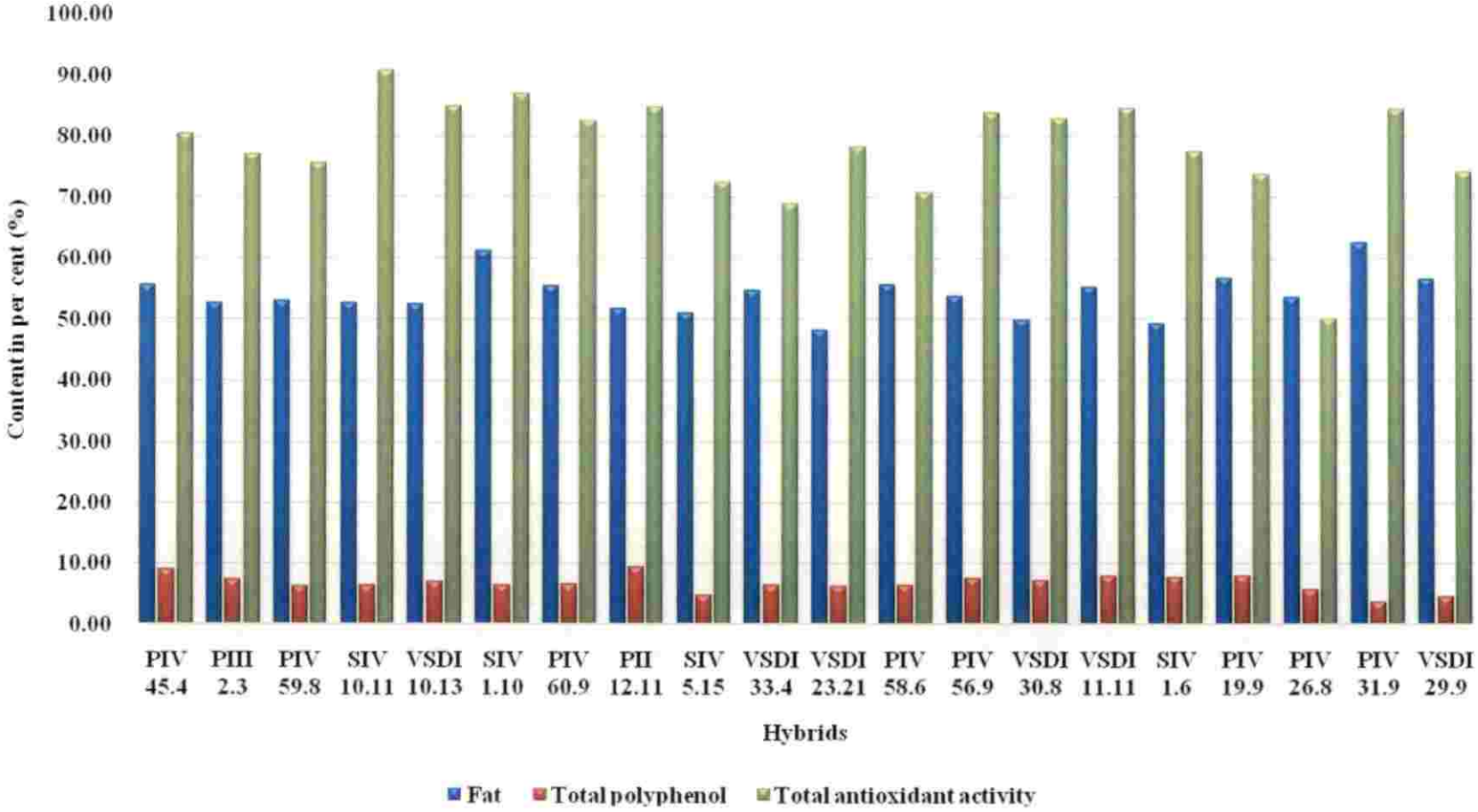


Figure 4.1. Variation in fat, total polyphenol and antioxidant content among the hybrids

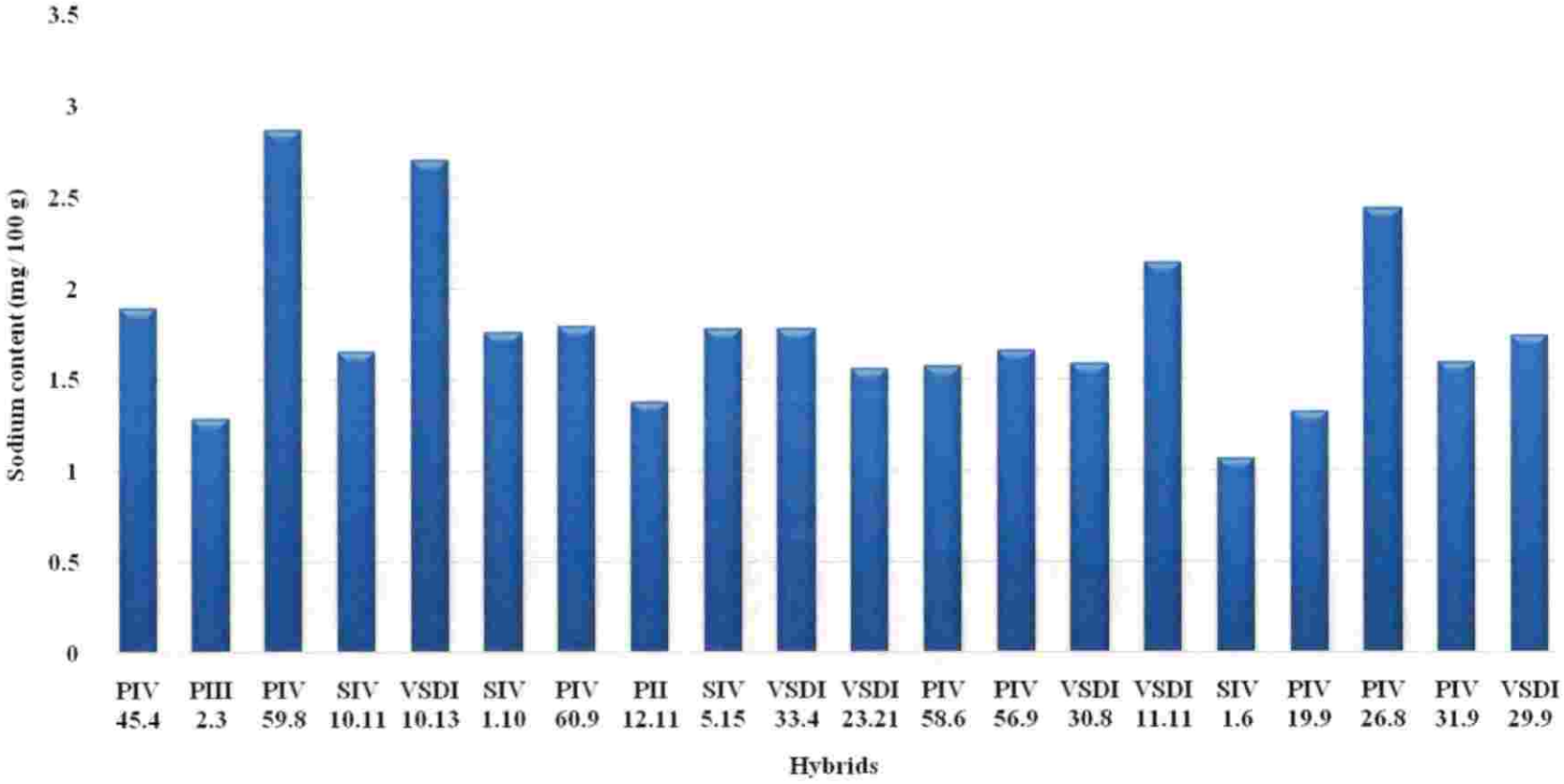


Figure 4.2. Variation in sodium content among the cocoa hybrids

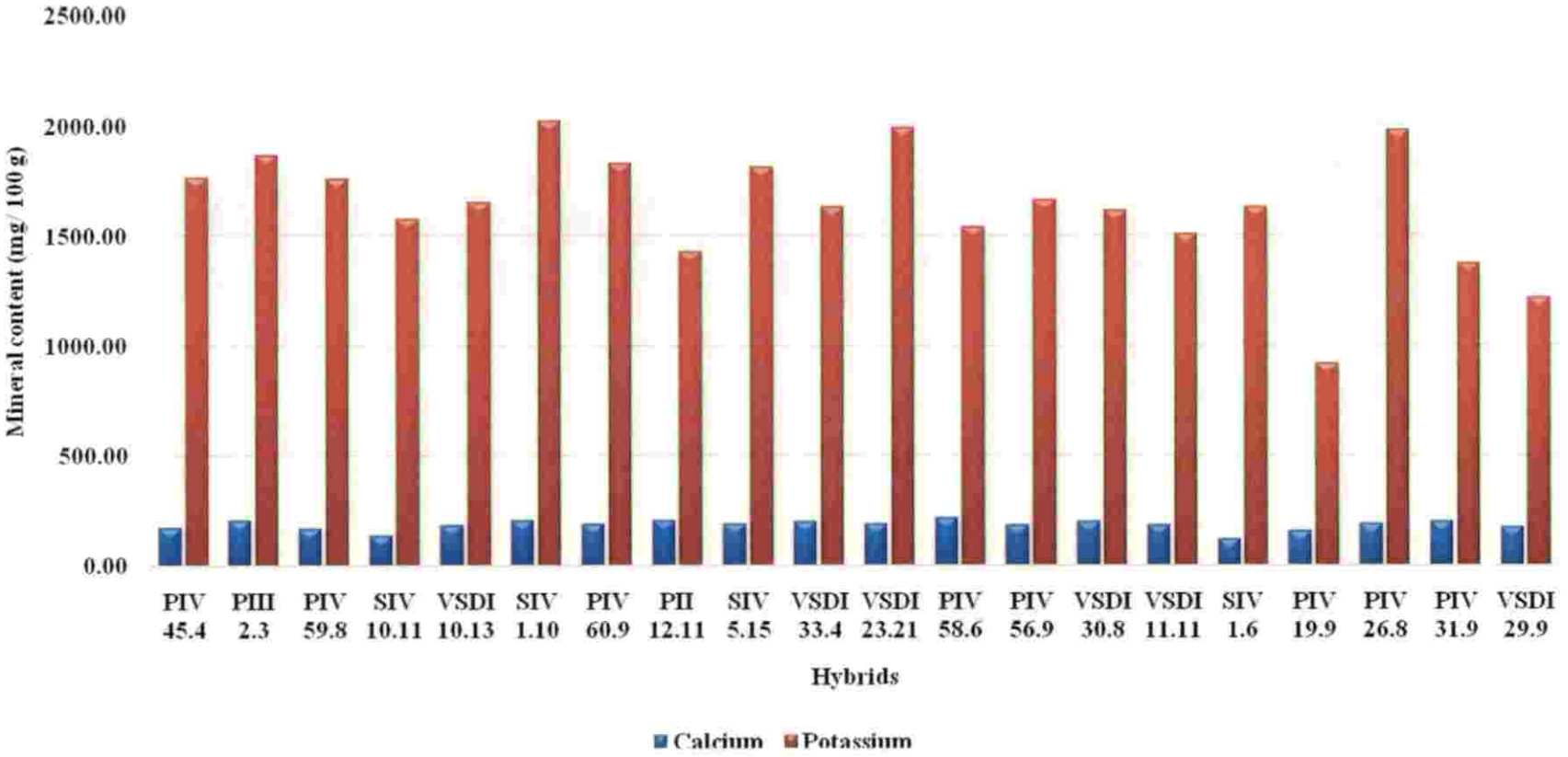


Figure 4.3. Variation in calcium and potassium content among cocoa hybrids

4.3. Correlation studies on biochemical characters of cocoa hybrids

Correlation among biochemical characters of cocoa hybrids were worked out and the result obtained is depicted in Table 4.8. Fat content, total polyphenol content, radical scavenging activity and mineral content are the characters considered for correlation study.

Total polyphenol content and radical scavenging activity of cocoa bean powder obtained from cocoa hybrids showed significant positive correlation (0.825). There are reports that phenols are having antioxidant capacity (Rice-Evans *et al.*, 1997). The phenols present in the cocoa beans, especially the flavanoids may be responsible for the antioxidant activity of cocoa beans. Similar observations are reported by many scientists like Steinberg *et al.* (2002), Sun and Ho, (2005), Aikpokpodion and Dongo, (2010) and Martinez *et al.* (2012). Present study is a confirmation to their findings.

Othman *et al.* (2007) investigated antioxidant capacity and total phenol content of cocoa beans from different countries and concluded that they are positively correlated (r = 0.78). Thus, selecting cocoa genotypes having high polyphenol content in cocoa beans results in indirect selection for high antioxidant activity.

Fat content and radical scavenging activity showed a significant negative correlation (-0.474). Hence, the cocoa beans having high fat content will be low in antioxidant activity.

Table 4.8. Correlation among biochemical characters

Characters	Fat	Bean polyphenol	Radical scavenging activity	Sodium	Potassium	Calcium
Fat	1					
Bean polyphenol	-0.305	1				
Radical scavenging activity	-0.474*	**0.825****	1			
Sodium	0.049	-0.177	-0.019	1		
Potassium	-0.268	-0.064	0.022	0.281	1	
Calcium	0.251	-0.114	-0.248	0.062	0.247	1

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

4.4. Screening of hybrids based on economic and biochemical characters

Based on the performance of twenty hybrids for economic and biochemical characters they were scored and ranked. The score and rank obtained by each hybrid is presented in Table 4.9.

Table 4.9. Score obtained for biochemical and economic characters

Hybrids	Biochemical characters score	Economic characters score	Total score	Rank
PIV 45.4	3	22	25	6
PIII 2.3	5	20	25	6
PIV 59.8	6	16	22	4
SIV 10.11	6	24	30	10
VSDI 10.13	5	28	33	11
SIV 1.10	4	25	29	9
PIV 60.9	5	19	24	5
PII 12.11	4	12	16	1
SIV 5.15	8	25	33	11
VSDI 33.4	6	13	19	3
VSDI 23.21	7	21	28	8
PIV 58.6	5	21	26	7
PIV 56.9	5	19	24	5
VSDI 30.8	6	22	28	8
VSDI 11.11	4	15	19	3
SIV 1.6	6	19	25	6
PIV 19.9	4	14	18	2
PIV 26.8	5	19	24	5
PIV 31.9	5	20	25	6
VSDI 29.9	6	16	22	4

Based on the rank score obtained, six hybrids were selected. Hybrid PII 12.11 (rank 1), PIV 19.9 (rank 2), VSDI 33.4 (rank 3), VSDI 11.11 (rank 3), VSDI 29.9 (rank 4) and PIV 59.8 (rank 4) were the hybrids having top ranks and they were selected as superior genotypes. List of the selected hybrids are given in Table 4.10.

Table 4.10. List of selected hybrids based on rank score

Selected hybrids	Rank obtained
PII 12.11	1
PIV 19.9	2
VSDI 33.4	3
VSDI 11.11	3
VSDI 29.9	4
PIV 59.8	4

4.5. Per cent improvement of selected hybrids over check and selection criteria

Superiority of those selected hybrids over standard check CCRP 13 and CCRP 15 for economic characters were worked out and presented in Table 4.11. and Table 4.12. Superiority of selected hybrids over selection criteria of economic characters was also observed and presented in Table 4.13. Superiority over check and selection criteria is expressed as per cent improvement. Per cent improvement of hybrid PII 12.11, PIV 19.9, VSDI 33.4, VSDI 11.11, VSDI 29.9 and PIV 59.8 over CCRP 13, CCRP 15 are graphically represented in figure 4.4, 4.5, 4.6, 4.7, 4.8, 4.9 respectively. Superiority of selected hybrids over selection criteria is graphically presented in figure 4.10.

Table 4.11. Per cent superiority of selected plants over CCRP 13

Hybrids	Yield	Pod value (g)	Pod index	Efficiency index	Conversion index	Dry matter recovery (%)
PII 12.11	-3.69	78.36	-43.94	1.51	29.41	22.47
PIV 19.9	18.83	34.66	-25.73	-10.38	-2.94	14.00
VSDI 33.4	2.95	48.81	-32.80	20.22	26.47	-5.71
VSDI 11.11	1.74	4.31	-4.11	-10.05	5.88	19.71
VSDI 29.9	-3.49	33.19	-24.91	7.03	-2.94	-3.13
PIV 59.8	36.52	43.54	-30.33	51.57	-11.76	-3.82

Table 4.12.Per cent superiority of selected plants over CCRP 15

Hybrids	Yield	Pod value (g)	Pod index	Efficiency index	Conversion index	Dry matter recovery (%)
PII 12.11	8.70	10.65	-9.64	-11.08	51.72	20.53
PIV 19.9	34.12	-16.46	19.71	-21.50	13.79	12.19
VSDI 33.4	16.19	-7.68	8.32	5.30	48.28	-7.21
VSDI 11.11	14.83	-35.29	54.55	-21.21	24.14	17.81
VSDI 29.9	8.93	-17.37	21.04	-6.25	13.79	-4.66
PIV 59.8	54.09	-10.95	12.30	32.77	3.45	-5.35

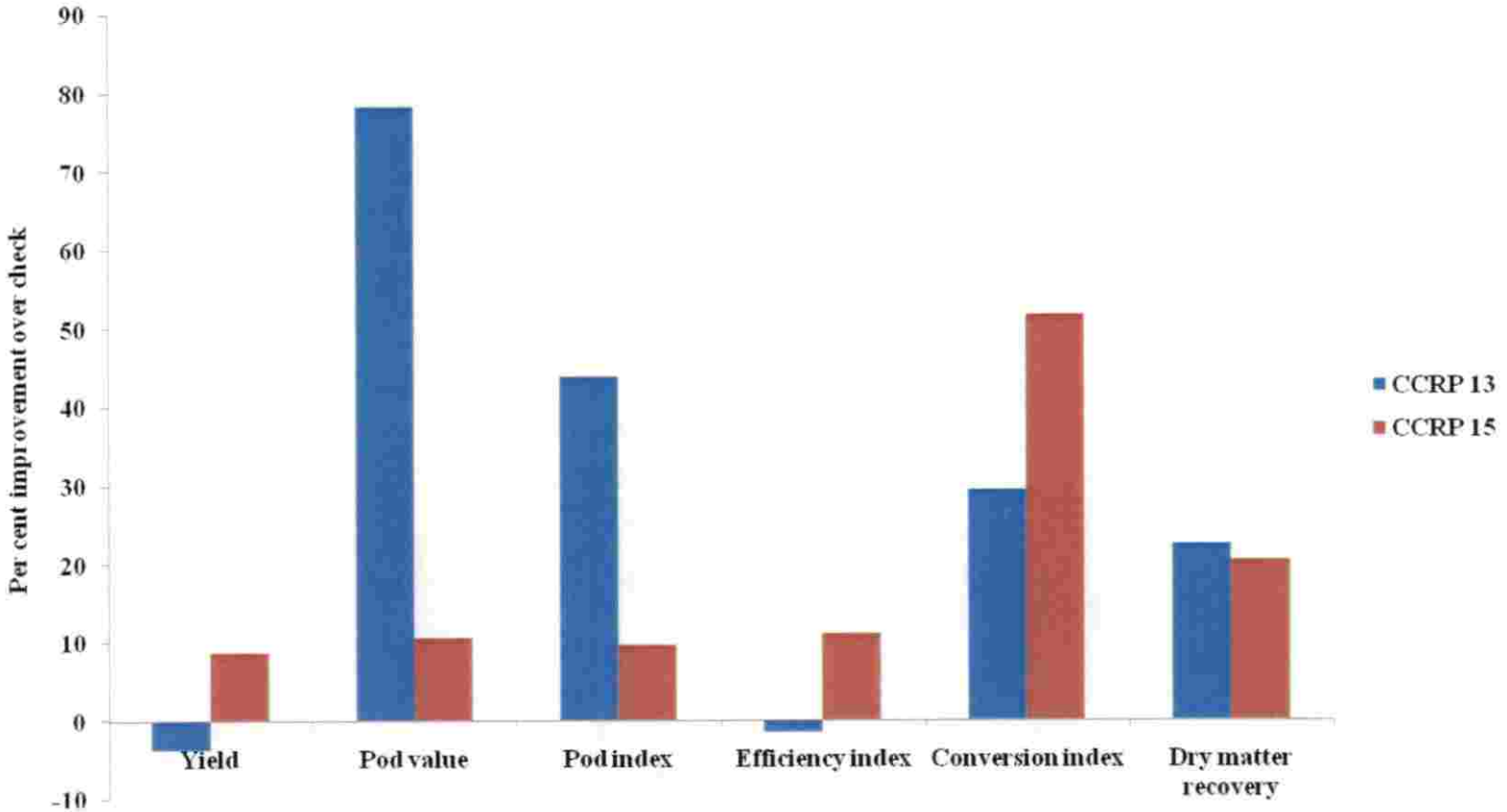


Figure 4.4 Superiority of hybrid PII 12.11 over CCRP 13 and CCRP 15

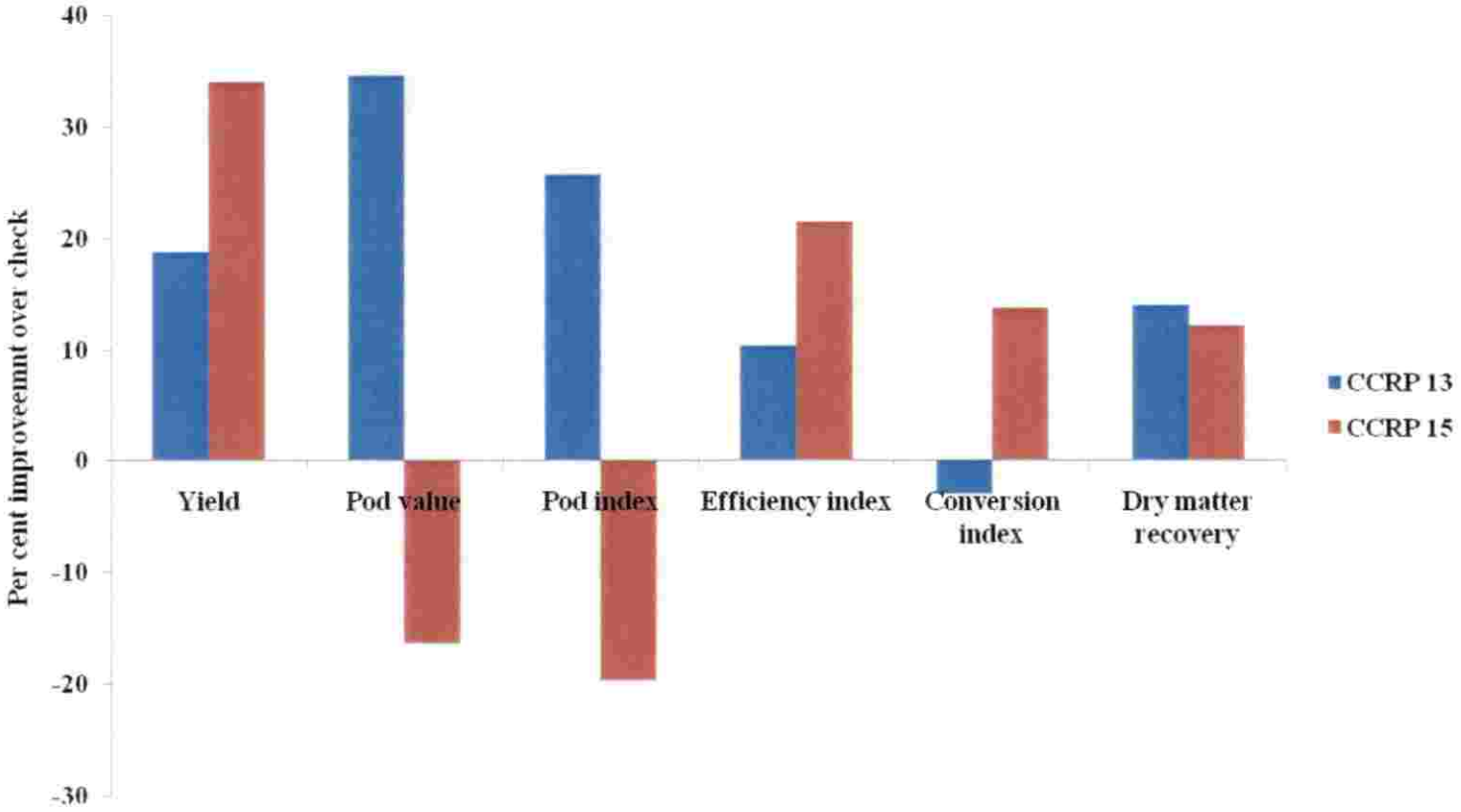


Figure 4.5 Superiority of hybrid PIV 19.9 over CCRP 13 and CCRP 15

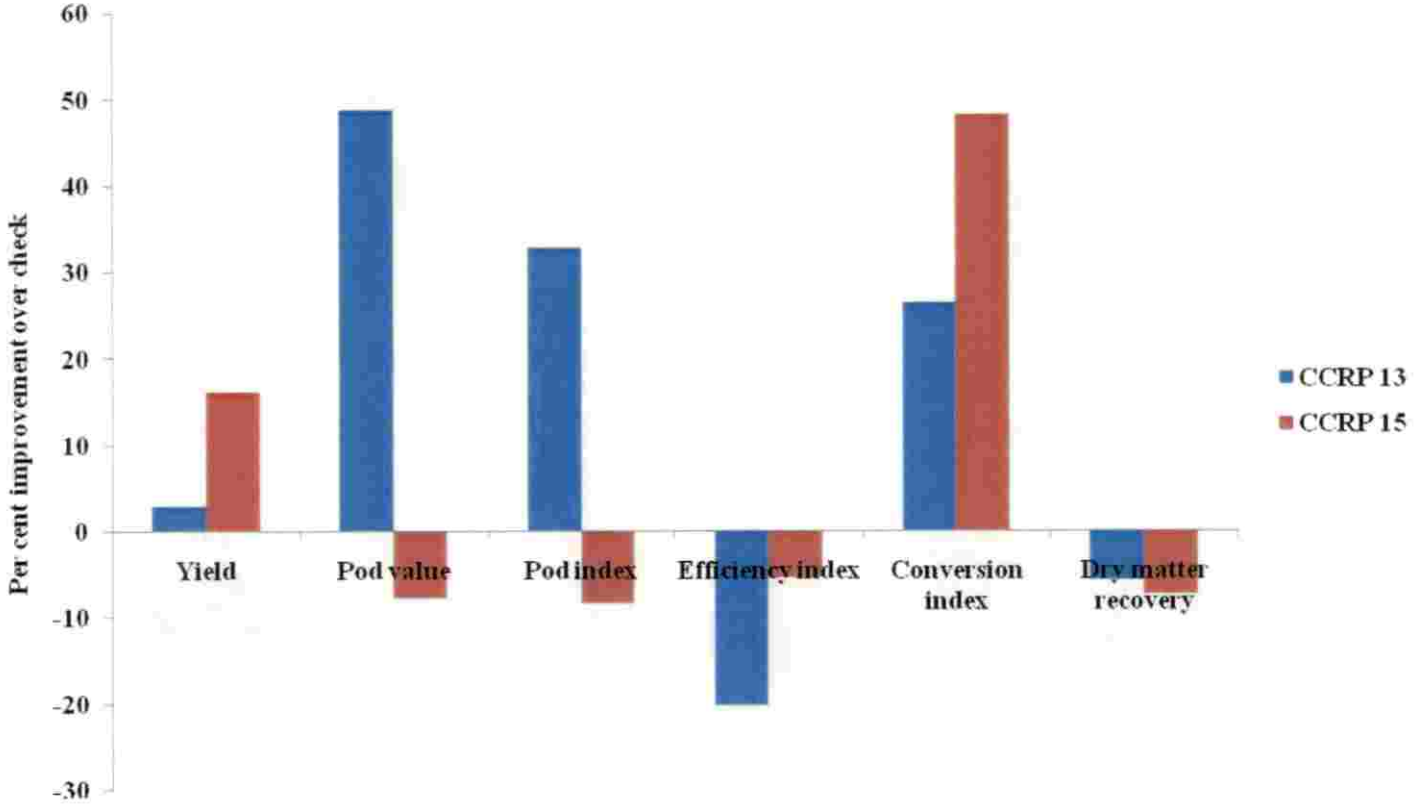


Figure 4.6. Superiority of hybrid VSDI 33.4 over CCRP 13 and CCRP 15

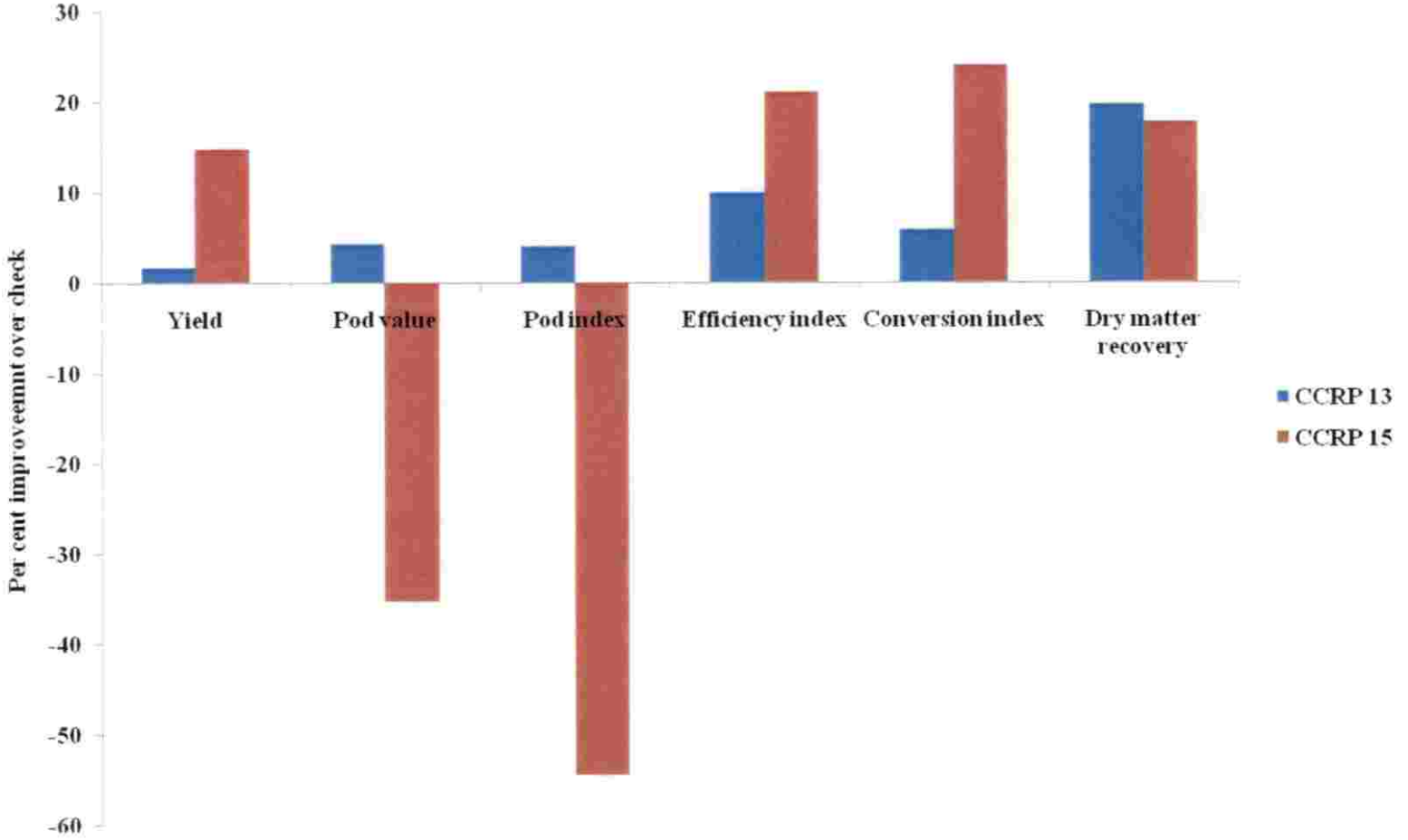


Figure 4.7. Superiority of hybrid VSDI 11.11 over CCRP 13 and CCRP 15

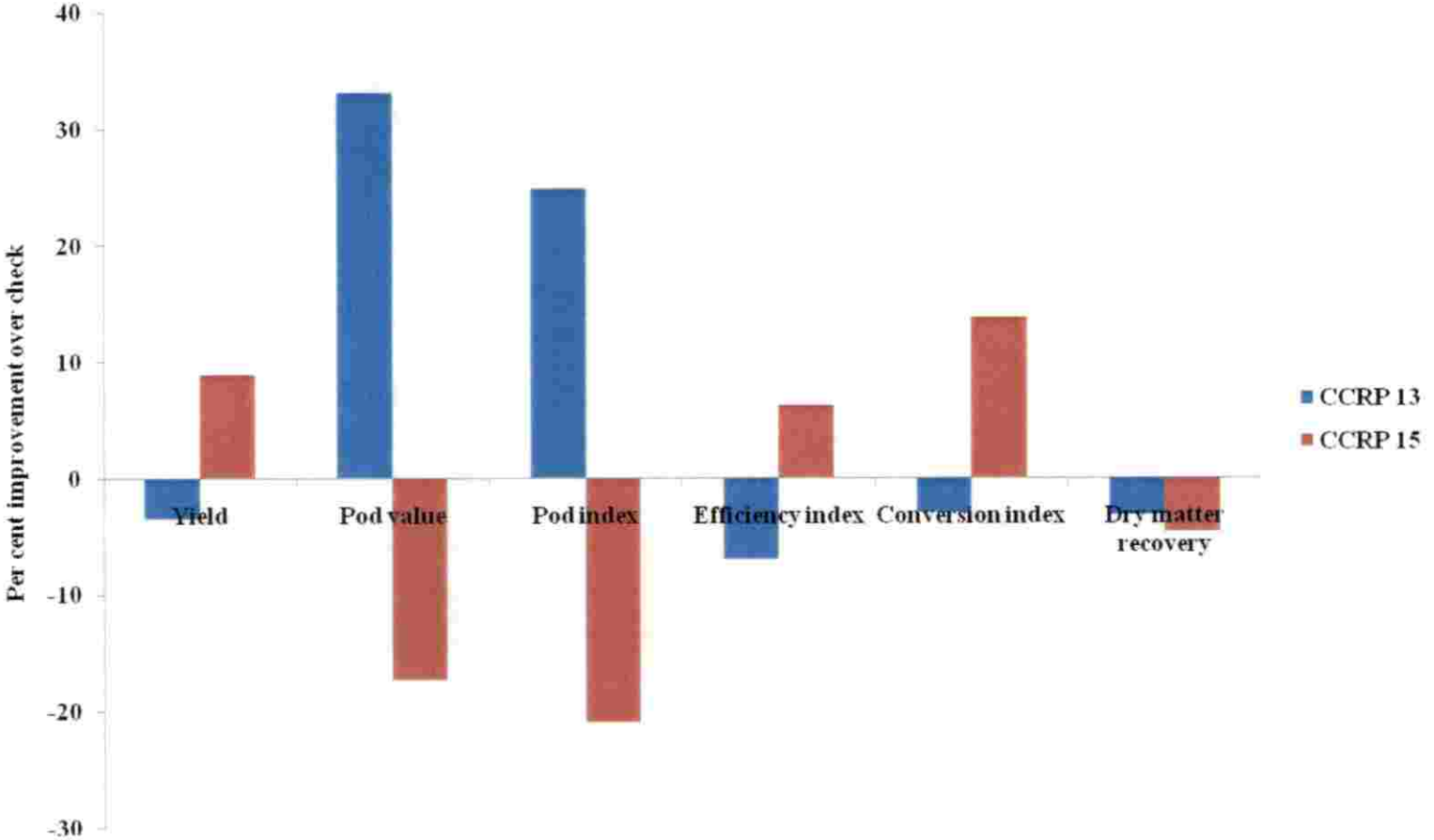


Figure 4.8. Superiority of hybrid VSDI 29.9 over CCRP 13 and CCRP 15

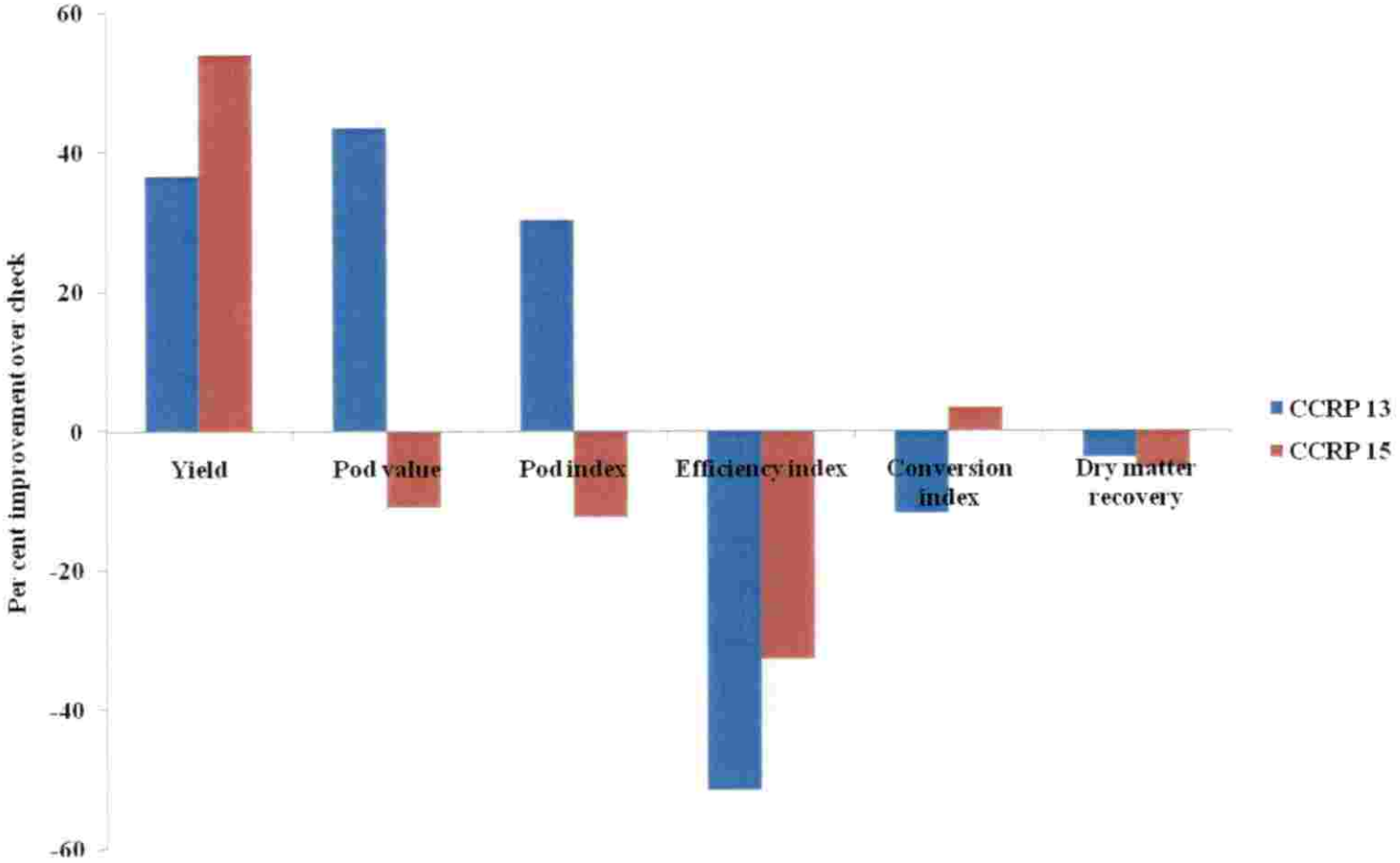


Figure 4.9. Superiority of hybrid PIV 59.8 over CCRP 13 and CCRP 15

Table 4.13. Per cent superiority of selected hybrids over selection criteria

Hybrids	Yield	Pod value (g)	Pod index	Efficiency index	Conversion index
PII 12.11	16.49	47.51	-90.59	-6.49	25
PIV 19.9	32.31	30.48	-43.85	-20.62	0
VSDI 33.4	21.87	37.09	-58.98	10.07	23.25
VSDI 11.11	20.94	10.25	-10.25	-20.19	8.3
VSDI 29.9	16.66	29.7	-42.20	-1.01	0
PIV 59.8	41.08	34.78	-53.35	28.67	10

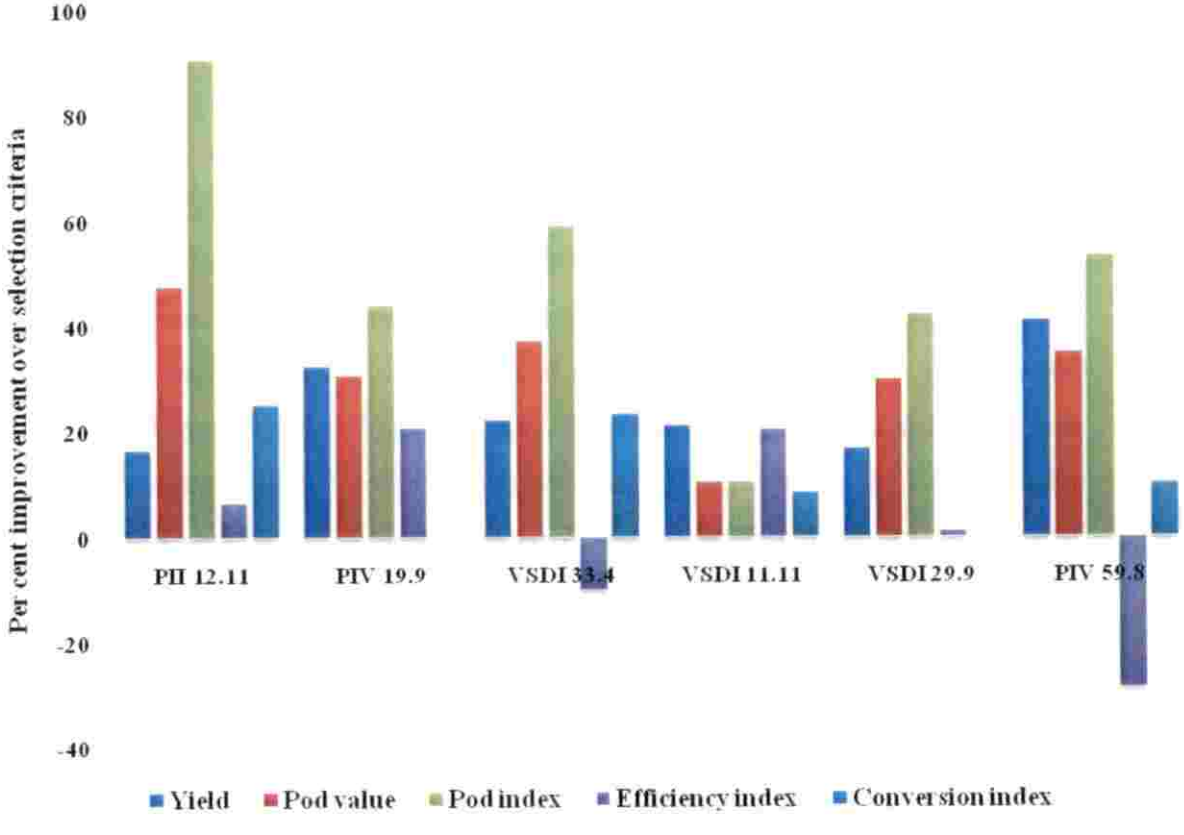


Figure 4.10. Per cent improvement of selected hybrids over selection criteria

4.6. *In vitro* screening for *Phytophthora* pod rot resistance

Incidence of black pod rot caused by *Phytophthora palmivora*is common in cocoa plantations during monsoon season.The most effective method to control black pod rot in cocoa is the use of resistant genotypes (Iwaro, 2000; Nyasse *et al.*, 2007). Genotypes which are completely resistant to black pod rot are not observed in cocoa but it has been observed that different genotypes exhibit difference in susceptibility (Nyadanu *et al.*, 2012; Veeresh, 2017). The twenty cocoa hybrids included in the study were screened for *Phytophthora* resistance by artificially inoculating *Phytophthora* culture on detached cocoa pods (Iwaro *et al.*, 2000).

4.6.1. Artificial inoculation on detached pods

To confirm the pathogenicity of *Phytophthora palmivora* the pure culture of pathogen was inoculated on healthy cocoa pods collected from hybrids included in the study. Three pods of each hybrid were inoculated to confirm the reaction of each genotype to *P. palmivora.* One pod from highly susceptible genotype was kept as control. The pathogen produced circular water soaked lesions on cocoa pods within 48 hours of inoculation. Later, lesions got enlarged and turned into chocolate brown colour. The artificially inoculated detached pods were observed until the control got completely infected or up to 10 days after inoculation and further disease resistance was calculated based on the area of infection by *P. palmivora.* Based on the disease resistance the hybrids were classified using the score chart given by Rubiyo and Rivaie (2013). The different hybrids exhibited differential response towards *Phytophthora* pod rot resistance screening and observations noted are presented in Table 4.14. and Plate 4.4.

Genotypes having per cent infection 0 to 15 per cent were considered as highly resistant. Four hybrids (PIV 59.8, VSDI 10.13, VSDI 11.11 and PIV 31.9) included in the study were found to be highly resistant towards *Phytophthora* pod

rot infection. VSDI 10.13 exhibited maximum resistance reaction towards *Phytophthora* with a per cent pod infection of about 9.52. Pods with per cent infection between 15.1 and 25 per cent were classified as resistant. Hybrids, PII 12.11, SIV 5.15, SIV 1.6, PIV 26.8 and VSDI 29.9 exhibited resistance reaction towards *Phytophthora* pod rot infection. Slightly resistant genotypes will have per cent pod area infection in the range of 25.1 to 50 per cent. In the slightly resistant group ten hybrids were included. Hybrid PIV 56.9 was with 25.58 per cent pod area infection and hybrid PIV 60.9 was having 46.54 per cent infection under the slightly resistant class. Only one hybrid (SIV 10.11) was included in the moderately susceptible class. None of the hybrids considered in the present study was susceptible to *Phytophthora* pod rot infection because pods with pod area infection more than 75 per cent were not observed.

4.6.2. Biochemical characters of cocoa pod husk

Cocoa pods are always affected by different pests and diseases and in most of the cases pod husk is damaged. To study the role of biochemical components in pod husk for determining *Phytophthora* pod rot tolerance, total polyphenol content and wax content of cocoa pod husk were estimated by using standard procedure. The results obtained are presented in Table 4.15.

The major biochemical substances which impart resistance to *Phytophthora* spp. in cocoa are phenols (Omokolo *et al.*, 2002).Total pod polyphenol content of hybrids were estimated and was found to be maximum in hybrid SIV 5.15 (3.67 %) and minimum in hybrid VSDI 23.21 (0.051 %). All the genotypes exhibited significant difference in pod husk phenol content. Eleven hybrids out of twenty hybrids included in the study exhibited less than one per cent pod polyphenol content. Average soluble phenols in cocoa pod husk is about 4.6 per cent. However, in the present study the estimated value of pod phenol was less than 4.6 per cent in all the genotypes.

Table 4.14. Classification based on per cent pod area infection

Genotypes	Pod parameters (cm)		Lesion size (cm)		Classification based on percent pod area infection (%)				
	L	B	L	B	HR	R	SR	MS	S
PIV 45.4	17.60	23.40	12.65	13.00			39.93		
PIII 2.3	18.20	29.60	13.55	16.90			42.50		
PIV 59.8	22.50	27.50	9.25	7.50	11.21				
SIV 10.11	18.00	24.00	17.00	14.25				56.07	
VSDI 10.13	17.20	21.80	5.90	6.05	9.52				
SIV 1.10	16.45	22.25	9.85	13.00			34.98		
PIV 60.9	17.50	23.50	11.60	16.50			46.54		
PII 12.11	18.10	26.85	10.35	10.90		23.21			
SIV 5.15	16.85	21.80	7.50	9.85		20.11			
VSDI 33.4	19.00	29.00	11.60	15.40			32.42		
VSDI 23.21	18.40	21.85	7.80	13.25			25.70		
PIV 58.6	18.90	24.60	9.90	13.40			28.53		
PIV 56.9	14.40	25.50	8.70	10.80			25.58		
VSDI 30.8	13.00	23.50	7.80	14.30			36.51		
VSDI 11.11	18.25	22.50	5.10	9.25	11.48				
SIV 1.6	17.45	22.25	6.85	10.35		18.26			
PIV 19.9	18.25	24.55	10.45	16.55			38.6		
PIV 26.8	15.35	23.65	8.45	12.00		27.93			
PIV 31.9	19.65	22.95	5.55	9.35	11.51				
VSDI 29.9	16.30	24.70	7.85	10.05		19.59			

HR	-	Highly resistant	(0-15 %)
R	-	Resistant	(15.1-25 %)
SR	-	Slightly resistant	(25.1-50 %)
MS	-	Moderately susceptible	(50.1-75 %)
S	-	Susceptible	(>75 %)

L - Length (cm)
B - Breadth (cm)

Table 4.15. Biochemical characters of pod husk

Hybrids	Podpolyphenol content * (%)	Pod wax (Carnauba wax equivalent mg/ g sample)
PIV 45.4	0.503 (4.068)	6.131
PIII 2.3	0.296 (3.119)	6.140
PIV 59.8	1.259 (6.442)	6.250
SIV 10.11	0.518 (4.087)	6.160
VSDI 10.13	0.470 (3.930)	6.287
SIV 1.10	0.884 (5.394)	6.134
PIV 60.9	1.440 (6.889)	6.141
PII 12.11	0.427 (3.748)	6.141
SIV 5.15	3.670 (11.043)	6.242
VSDI 33.4	1.563 (7.182)	6.116
VSDI 23.21	0.051 (1.285)	6.155
PIV 58.6	0.163 (2.310)	6.152
PIV 56.9	1.518 (7.072)	6.147
VSDI 30.8	1.041 (5.847)	6.126
VSDI 11.11	1.197 (6.273)	6.112
SIV 1.6	1.385 (6.759)	6.113
PIV 19.9	0.697 (4.778)	6.127
PIV 26.8	0.280 (3.032)	6.191
PIV 31.9	0.670 (4.696)	6.177
VSDI 29.9	0.657 (4.646)	6.112
CV(%)	**9.658**	**0.351**
CD(1%)	**0.199**	**0.048**

* Values in parenthesis are transformed using angular transformation

Presence of wax on plant parts is a component of preformed defence system in plants towards biotic stresses (Marcell *et al.*, 2002). Epicuticular wax on pod surface of cocoa have role in providing resistance to *Phytophthora* pod infection (Nyadanu *et al.*, 2012). Hybrids included in the present study were analysed to estimate the amount of wax present on the pod surface and it was observed that pods of different hybrids differ significantly with respect to pod wax. Maximum amount of wax on pod husk was exhibited by the hybrid VSDI 10.13 (6.287 mg/ g Carnauba wax equivalent) and lowest in two hybrids VSDI 29.9 and VSDI 11.11 (6.112 mg/ g Carnauba wax equivalent). Pod wax content showed only a slight variation between hybrids, with a range of 0.175 mg/ gCarnauba wax equivalent. Nyandanu *et al.* (2012) estimated epicuticular wax content of cocoa pods and reported that averagea mount of wax on pod husk is about 37.58 µg/ cm^2.

4.7.2. Correlation studies on *Phytophthora* pod infection and pod husk biochemical characters

Correlation analysis was carried out between *Phytophthora* pod infection and pod husk biochemical properties. Biochemical characters considered for correlation study were pod husk phenol and pod wax content. The result of correlation analysis is presented in Table4.16.

Pod wax showed significant negative correlation (-0.293) with *Phytophthora* pod infection at five per cent level of significance. Hence we can conclude that the amount of wax present on pod surface plays a key role in *Phytophthora* resistance. Sporangia of *Phytophthora* species always requires the contact of water to get germinated (Duniway, 1979). If wax is present on pod surface it will repel water, which leads to the formation of droplets and they evaporates easily than a film of water (Grammatikopoulos and Manetas, 1994). Thus cocoa genotypes with high content of wax on pod husk will be having dry

pod surface which make them unsuitable for *Phytophthora* (Nyandanu *et al.*, 2012).

Fungicidal action of epicuticular wax in plants had been reported in many crops (Alcerito *et al.*, 2002). Analysing the nature and composition of wax present on cocoa pods in future will help in confirming such properties of cocoa pod wax.

Phenolic compounds present in plants are having role in plant defence reponse against biotic stresses (Tan *et al.*, 2004; Omokolo *et al.*, 2002) and it was confirmed in different crops including rice, apple, cucumber *etc*. To study the role of cocoa pod husk phenol on *Phytophthora* resistance reaction correlation between pod phenol and pod infection was worked out. Polyphenol content in pod husk was found to be having significant negative correlation (-0.612) with *Phytophthora* pod infection. Hence we can conclude that resistant genotypes will be having high polyphenol content in their pod husk.

The correlation study also revealed that there is a positive significant relation (0.470) between pod husk wax content and pod polyphenol content.

Table 4.16. Correlation among biochemical characters of pod husk and *Phytophthora*pod area infection

Characters	Pod wax	Pod phenol	Pod area infection
Pod wax	1		
Pod phenol	**0.470****	1	
Pod area infection	**-0.293***	**-0.612****	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

4.7.3. Correlation among *Phytophthora* pod infection and pod morphology

Studies of Veeresh (2017) on *Phytophthora* pod rot infection revealed that there is significant correlation between *Phytophthora* infection and pod morphology. Hence, in the present study correlation analysis was carried out among *Phytophthora* pod infection and three pod morphological characters (pod rugosity, pod basal constriction and rind thickness). The result obtained for correlation analysis is presented in Table 4.17.

Phytophthora pod infection and pod basal constriction showed significant negative correlation with each other. *Phytophthora* pod infection will be high in genotypes with prominent basal constriction. Pod rugosity and rind thickness expressed negative correlation with *Phytophthora* pod infection but the values were not significant. Nyadanu *et al.* (2011) observed that the resistant genotypes had thick pod husk and susceptible genotypes had thin pod husk. However in the present study role of rind thickness in imparting *Phytophthora* resistance is not significant.

Table 4.17. Correlation among *Phytophthora* pod infection and pod morphology

Characters	Pod area infection	Basal constriction	Pod rugosity	Rind thickness
Pod area infection	1			
Basal constriction	**-0.497***	1		
Pod rugosity	-0.400	0.123	1	
Rind thickness	-0.216	0.130	-0.229	1

*. Correlation is significant at the 0.05 level (2-tailed).

4.7.4. Phenes influencing *Phytopthora* pod rotresistance

Binomial logistic regression model was used to find out the phenes contributing towards *Phytophthora* pod rot resistance. Nyadanu *et al.* (2012) conducted similar study on regression analysis to confirm influence of physiological characters on disease resistance in cocoa.

In the present study regression analysis was conducted to confirm the influence of both biochemical and morphological characters of cocoa towards *Phytophthora* pod rot resistance. The results are presented in Table4.18 and Table 4.19.Pod husk phenol and pod wax are the two biochemical characters considered. Morphological characters included in regression analysis are pod basal constriction, pod rugosity and rind thickness. The positive coefficient and high value of odds ratio Exp (B) indicates a positive relation between dependent and independent variable. Among the biochemical characters considered only pod husk phenol showed a positive relation with *Phytophthora* pod rot resistance. Pod husk phenol content expressed a significance value less than 0.127 which is the value of constant. Nazaruddin *et al.* (2006) studied the fungal disease resistance by analysing the accumulation of phenol content in infected area and explained that phenols are responsible for disease resistance.

When regression analysis of morphological characters towards *Phytophthora* pod rot resistance was studied only pod basal constriction showed significant relation towards dependent variable. Pod basal constriction showed a significant negative relation. Hence, it is preferred to select genotypes with less basal constriction in order to get more resistance. Nyadanu *et al.* (2011) reported that the rind thickness has role in deciding resistance reaction towards *Phytophthora*. However in the current study no significant relation was identified between *Phytophthora* pod rot resistance and rind thickness.

Table 4.18. Logistic estimate of biochemical phenes influencing *Phytophthora* resistance

Variable	Coefficient	Standard error	Wald	Significance	Exp (B)	Expected per cent of improvement over population
Pod phenol**	24.311	16.061	2.291	0.123	3.613	78.32
Pod wax	0.236	0.294	0.644	0.422	1.266	
Constant	-150.974	99.045	2.324	0.127	0.000	
**Significance value less than 0.127						

Table 4.19. Logistic estimate of morphological phenes influencing *Phytophthora* resistance

Variable	Coefficient	Standard error	Wald	Significance	Exp (B)	Expected per cent of improvement over population
Pod base**	-0.812	0.474	2.932	0.087	.444	30.74
Rind thickness	1.363	1.069	1.625	0.207	3.906	
Pod rugosity	0.084	.655	.016	0.898	0.898	
Constant	2.997	1.848	2.630	0.205	20.022	
**Significance value less than 0.205						

112

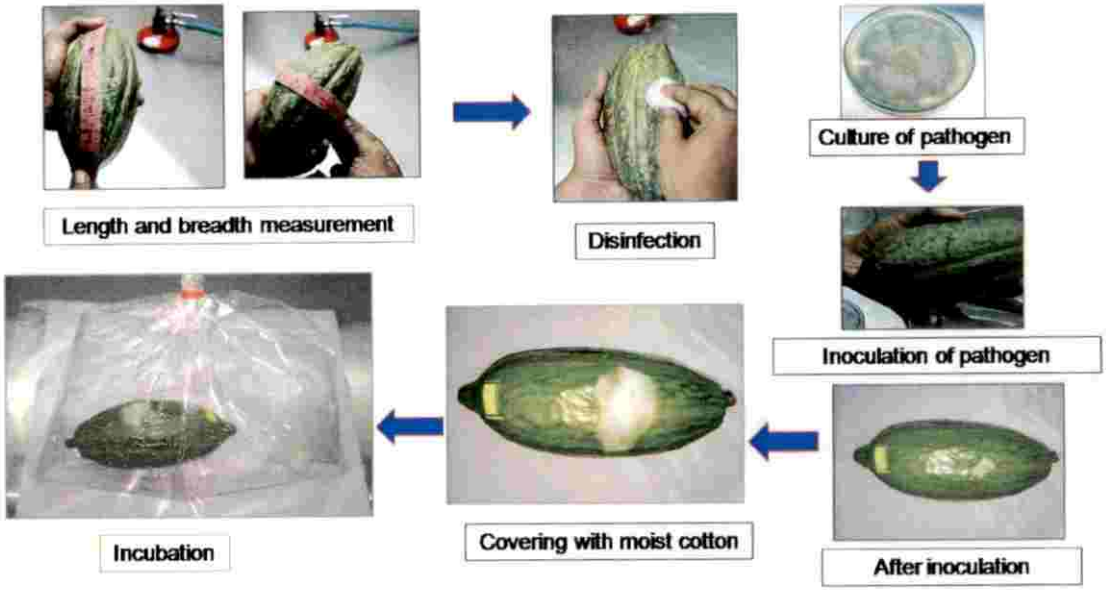


Plate 4.10. Artificial inoculation of pathogen on detached pods

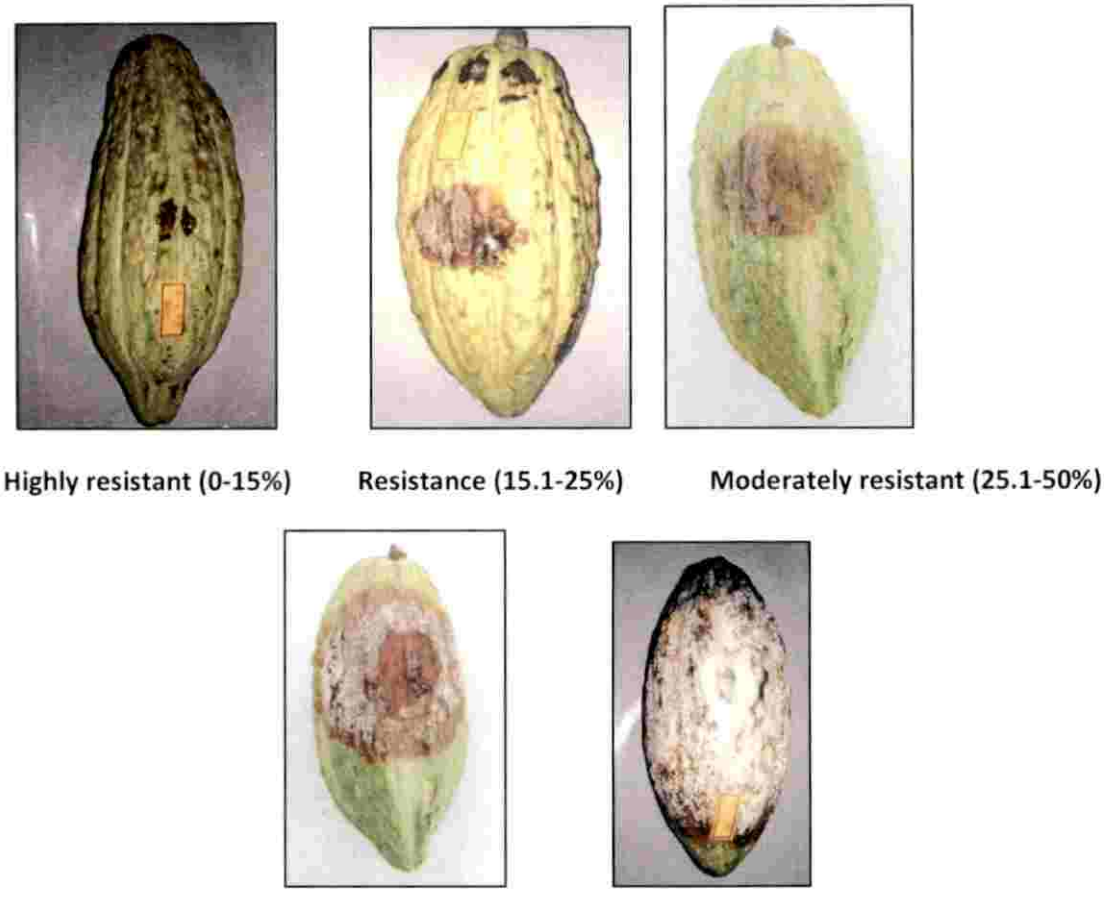


Plate 4.11. Classification based on per cent pod area

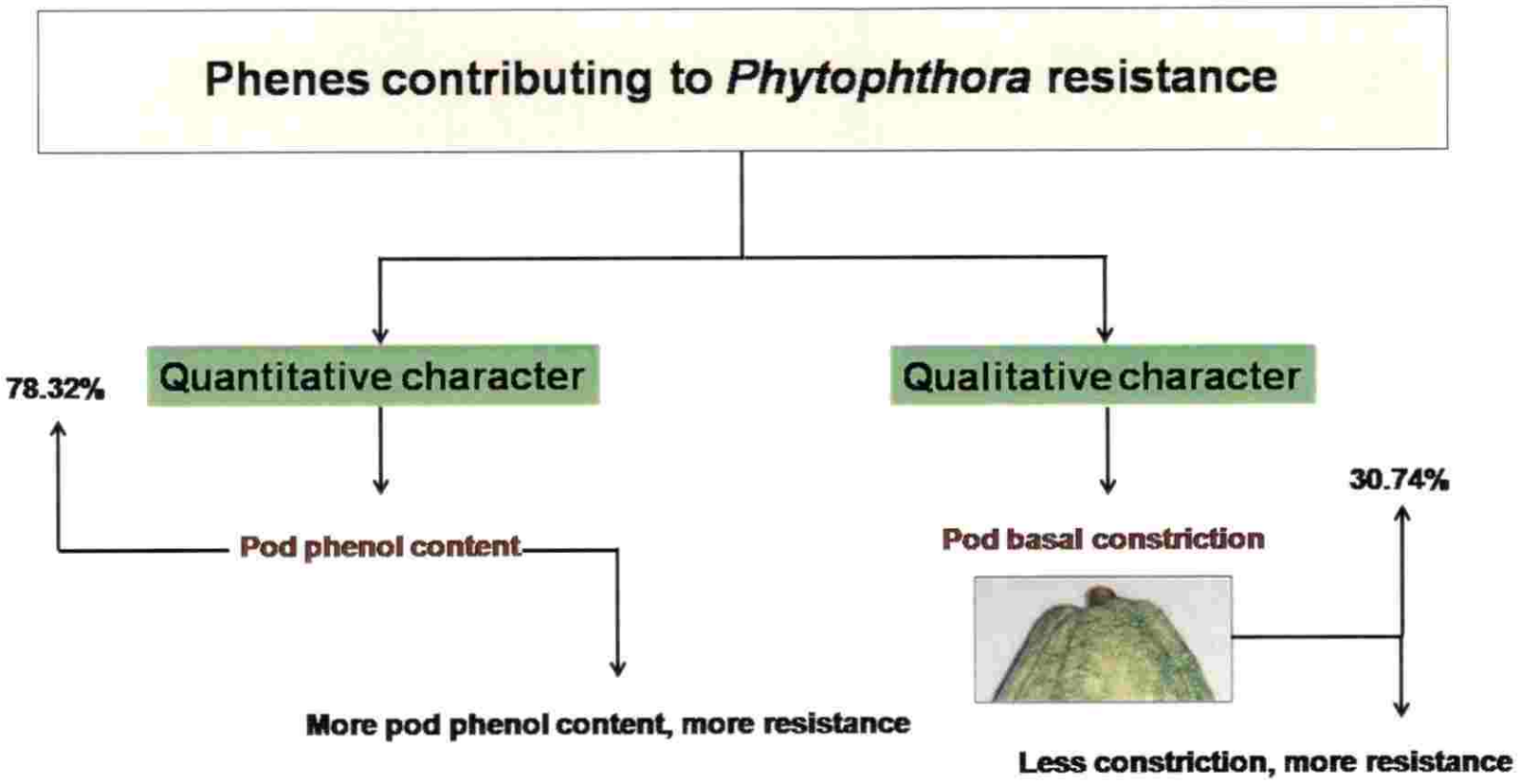


Figure 4.11. Phenes contributing to Phytophthora resistance

Based on Exp (B) value from the regression model, expected percentage of improvement for disease resistance over the base population was calculated and it was found that if selection is based on pod husk phenol, new population formed from the base population will express 78.32 per cent of improvement regarding the resistance. Similarly if pod basal constriction is considered as selection parameter it leads to 30.74 per cent improvement in the newly formed population when compared to the base population with respect to resistance response (Figure 4.11.). There are reports that selection based on pod basal constriction can contribute up to 82 per cent improvement in a population (Veeresh, 2017).

4.8. Screening for tea mosquito bug resistance

Tea mosquito bug (*Helopeltis theivora*) incidence became severe in cocoa plantations in the last few years in South India. Chemical control is difficult against tea mosquito bug in cocoa and hence, resistant varieties are the only alternative (N' Guessan *et al.*, 2004). Artificial screening for tea mosquito bug resistance was carried out on budded plant and detached pods to study the reaction of different hybrids against *Helopeltis theivora*. Budded plants of all the hybrids and medium matured freshly collected pods of selected hybrids were screened based on the method suggested by Srikumar and Bhat (2013).

4.8.1. Screening on budded plants

Three replications having five budded plants of each hybrid were screened in insect net house. Freshly reared *Helopeltis theivora* at the rate of 100 adults (50 male and 50 female each) were released into the insect net house. Intensity of infestation on shoots/ leaves was noted by counting number of feeding punctures at 12 hour interval until 72 hours and was scored. Based on the score obtained the hybrids were classified into different classes and it is depicted in Table 4.20.

Table 4.20.Classification based on number of feeding punctures on budded plants

Hybrids	Classification based on number of feeding punctures				
	HR	R	MS	S	HS
PIV 45.4				11.22	
PIII 2.3					12.44
PIV 59.8	1.99				
SIV 10.11					12.88
VSDI 10.13				10.66	
SIV 1.10			8.33		
PIV 60.9	0.66				
PII 12.11	0.88				
SIV 5.15		4.44			
VSDI 33.4	2.33				
VSDI 23.21		3.44			
PIV 58.6				11.55	
PIV 56.9	1.44				
VSDI 30.8			6.22		
VSDI 11.11		3.33			
SIV 1.6			8.10		
PIV 19.9					11.77
PIV 26.8			6.11		
PIV 31.9		3.88			
VSDI 29.9		5.33			

HR	-	Highly resistant	(0 – 3 punctures)
R	-	Resistant	(3.01 – 6 punctures)
MS	-	Moderately susceptible	(6.01 – 9 punctures)
S	-	Susceptible	(9.01 – 12 punctures)
HS	-	Highly susceptible	(> 12.01 punctures)

Tea mosquito bug feeds on young shoots and tender parts of plants by injecting its toxic saliva into the host and this result in the degradation of cells around the puncture. Within 24 hours of feeding the area becomes dark brown and dries up (Roy *et al.*, 2015). These dark coloured feeding punctures are an indication of tea mosquito bug attack and in the present study the difference in number of feeding punctures in different hybrids are used to screen the genotypes for tea mosquito bug resistance. It is assumed that number of feeding punctures in the susceptible genotypes will be more when compared to resistant ones.

Budded plants having less than three punctures per plant were considered as highly resistant. Five hybrids included in the study showed a highly resistant reaction towards tea mosquito bug attack and they are PIV 59.8, PIV 60.9, PII 12.11, VSDI 33.4 and PIV 56.9. All these five hybrids exhibited less than three punctures per plant after 72 hours of screening. Plants in which number of feeding punctures after 72 hours of screening, between 3.01 and six were classified as resistant genotypes. Among the hybrids considered in the present study five were included in resistant group and they are SIV 5.15, VSDI 23.21, VSDI 11.11, PIV 31.9 and VSDI 29.9. Four hybrids exhibited moderately susceptible reaction towards tea mosquito bug attack with 6.01 to nine punctures at the end of screening. Hybrids SIV 1.10, VSDI 30.8, SIV 1.6 and PIV 26.8 were included in the class moderately susceptible. In the susceptible and highly susceptible class three hybrids each were present and they were having 9.01 to twelve and more than twelve number of punctures respectively. Hybrid PIV 45.4, VSDI 10.13 and PIV 58.6 were grouped as susceptible and PIII 2.3, SIV 10.11 and PIV 19.9 were showing highly susceptible reaction towards tea mosquito bug.

Apart from number of feeding punctures, number of eggs laid by tea mosquito bug on budded plants was also observed. However, no eggs were found on the plants. There is report that longevity and fecundity of tea mosquito bug varies based on rearing conditions (Tan, 1974). From this we can conclude that even if male and females are released in equal proportion tea mosquito bug lays

egg only if favourable conditions are available. The findings have to be confirmed in future studies.

4.8.2. Screening on cocoa pods

Method proposed by Srikumar and Bhat (2013) was followed to screen the cocoa pods against tea mosquito bug attack. Artificial screening was conducted on detached pods of selected hybrids in insect rearing cages. Six superior hybrids selected based on morphological and biochemical performance was screened for tea mosquito bug resistance on pods. The observations recorded are presented in Table 4.21. The hybrids included for the screening were PII 12.11, PIV 19.9, VSDI 33.4, VSDI 11.11, VSDI 29.9 and PIV 59.8.Three pods of each hybrid were screened per replication. Observations on infestation were noted by counting number of feeding punctures at 12 hour interval until 72 hours. Based on the number of feeding punctures on the pod surface hybrids were scored as given below:

Highly resistant	– < 33
Resistant	– 33.01-66
Moderately susceptible	– 66.01-99
Susceptible	– 99.01-132
Highly susceptible	– > 132.01

Hybrids with average number of feeding punctures less than 33 were considered as highly resistant and two hybrids out of five were included in this range. Those hybrids are PII 12.11 and VSDI 33.4. Hybrid PIV 59.8 and VSDI 29.9 were found to be resistant towards tea mosquito bug attack on pods with number of punctures in the range of 33.01 to 66. VSDI 11.11 was grouped under susceptible class with average number of punctures about 103. PIV 19.9 is highly susceptible toward tea mosquito bug attack.

Table 4.21.Classification based on number of feeding punctures on cocoa pods

Hybrids	Average length of the pod	Average breadth of the pod	Average number of feeding lesions					Average no. of hours insect survived
			HR	R	MS	S	HS	
PII 12.11	15.3	21.2	19.1					26
PIV 19.9	13.9	15.3					175.7	65
VSDI 33.4	20.6	24.6	22.4					28
VSDI 29.9	17.3	22.1		45.7				39
VSDI 11.11	16.8	23.4				102.7		52
PIV 59.8	18.4	20.6		56.3				41

HR	-	Highly resistant	(< 33 punctures)
R	-	Resistant	(33.01 – 66 punctures)
MS	-	Moderately susceptible	(66.01 – 99 punctures)
S	-	Susceptible	(99.01 – 132 punctures)
HS	-	Highly susceptible	(> 132.01 punctures)

4.9. Performance of selected hybrids in relation to biotic stress

The superior hybrids selected based on morphological and biochemical characters are presented in Table 4.22. along with their resistance reaction towards *Phytophthora* and tea mosquito bug attack.

Hybrids PII 12.11, VSDI 29.9 and PIV 59.8 were found to be resistant towards both *Phytophthora* and tea mosquito bug attack. PII 12.11 which has the highest score for morpho-biochemical character is having good resistance towards major biotic stresses also. Hence, we can conclude that it is superior over all the twenty hybrids. Hybrid PIV 19.9 which scored second rank was found to be

Plate 4.12. Tea mosquito bug screening on budded plants of cocoa

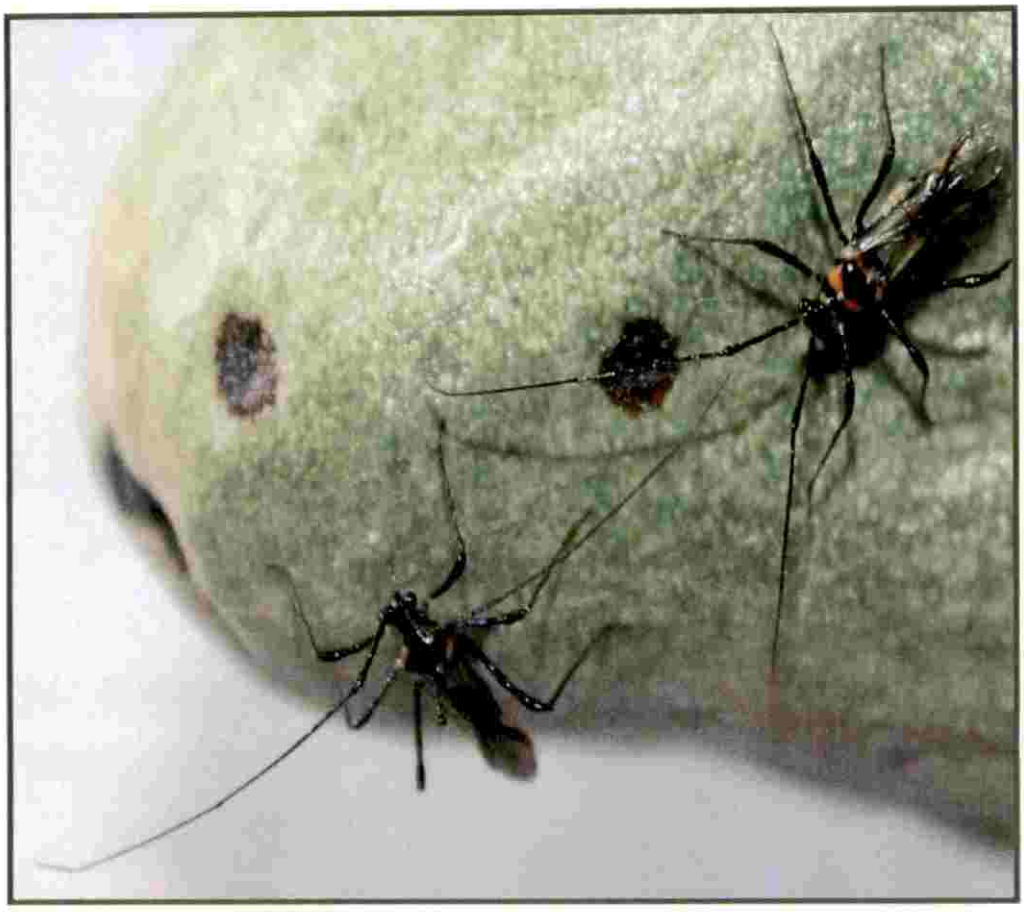


Plate 4.13. Tea mosquito bug screening on cocoa pods

Table 4.22. Reaction of selected hybrids towards major biotic stresses

Selected hybrids	Rank based on morpho-biochemical characters	Reaction against *Phytophthora*	Reaction against tea mosquito bug on shoot	Reaction against tea mosquito bug on pod
PII 12.11	1	Resistant	Highly resistant	Highly resistant
PIV 19.9	2	Slightly resistant	Susceptible	Highly susceptible
VSDI 33.4	3	Slightly resistant	Highly resistant	Highly resistant
VSDI 11.11	3	Highly resistant	Resistant	Susceptible
VSDI 29.9	4	Resistant	Resistant	Resistant
PIV 59.8	4	Highly resistant	Highly resistant	Resistant

slightly resistant towards *Phytophthora* and susceptible to tea mosquito bug attack. Hybrid VSDI 11.11 having resistant reaction towards *Phytophthora* and tea mosquito bug on shoots was found to be susceptible against tea mosquito bug when screened on pods.

4.9. Diversity analysis

Analysis of relation between genotypes is one of the important components of plant breeding programs. Diversity analysis can be used to determine the variability among the genotypes in a population or between populations and helps in stratified selection from a breeding population (Ram and Panwar, 1970; Engels, 1986; Asna, 2013; Ajmal, 2016; Veeresh, 2017).

Hybrids produced by crossing most diverse parents show more heterosis. In cocoa there are reports that double cross hybrids are more superior over single cross hybrids in yield. Hence, the single cross hybrids evaluated in the present study will be a good genetic stock for future breeding programme to produce double cross hybrids. The hybrids which are most diverse can be crossed with each other to produce promising double cross hybrids.

A multivariate analytical method is one among the methods used in diversity analysis which helps to analyse multiple characters of each genotype simultaneously and are useful to analyse morphological, biochemical and molecular data. Cluster analysis is one of the most commonly used multivariate methods (Hair *et al.*, 1995). It is a distance based hierarchical clustering method which is also known as agglomerative hierarchical method (Johnson and Wichern, 1992). Agglomerative hierarchical method helps to represent the result graphically in the form of dendrogram which helps to identify clusters visually (Mohammadi and Prasanna, 2003).

4.9.1. Clustering based on qualitative characters

Based on Jaccard's similarity coefficient, Agglomerative hierarchical clustering was done using UPGMA method (Sneath and Sokal, 1973). Ten qualitative characters including that of cocoa leaf, flower, pod and bean were considered and dendrogram was constructed and presented in Fig. 4.12.

To find the relationship between different accessions, Aikpokpodian (2010) conducted cluster analysis based on 17 agro morphological traits of cocoa. In the present study, based on ten qualitative characters the twenty hybrids were grouped into thirteen clusters at 60 per cent similarity coefficient. The clusters along with the hybrids are presented in Table 4.23. Cluster VI is the largest one with four hybrids; VSDI 10.13, VSDI 11.11, PIV 60.9 and PIV 31.9. All the four hybrids included in cluster VI are having five similar characters; they are colour of pedicel (green), colour of sepal (cream), pod shape (Cundeamor), ripe pod colour (yellow) and pod apex shape (obtuse). In cluster II three hybrids and in cluster III and XII two hybrids are present. Hybrids PIII 2.3, PII 12.11 and VSDI 30.8 are included in cluster two and they show similar colour of pedicel (green), colour of sepal (cream), pod shape (Amelonado), shape of pod apex (obtuse) and pod basal constriction (slight). Cluster III includes SIV 5.15 and VSDI 29.9 having Angoleta shaped pods with intermediate unripe pod colour, yellow ripe pod colour, slight basal constriction and intermediate rugosity. Beans of these hybrids are dark purple. Nine hybrids showed no similarity with other hybrids for these ten characters and they includes genotypes PIV 45.4, PIV 59.8, SIV 1.6, VSDI 23.21, SIV 10.11, SIV 1.10, PIV 19.9, PIV 26.8 and PIV 56.9 and they belongs to the clusters I, IV, V, VII, VIII, IX, X, XI and XIII respectively.

4.9.2. Clustering based on quantitative characters

Clustering was done based on seventeen quantitative characters including six characters of flower, four characters of pod and seven bean characters. The

Table 4.23. Clustering based on qualitative characters in cocoa

Cluster No.	No. of hybrids	Genotypes included in the cluster	Similar characters shared between hybrids
Cluster I	1	PIV 45.4	-
Cluster II	3	PIII 2.3, PII 12.11, VSDI 30.8	Green pedicel colour, cream sepal colour, Amelando pod shape, obtuse pod apex, slight basal constriction.
Cluster III	2	SIV 5.15, VSDI 29.9	Cream colour sepal, Angoleta pod shape, intermediate colour of unripe pod, yellow coloured ripe pod, slight basal constriction, intermediate rugosity, dark purple coloured beans
Cluster IV	1	PIV 59.8	-
Cluster V	1	SIV 1.6	-
Cluster VI	4	VSDI 10.13, VSDI 11.11, PIV 60.9, PIV 31.9	Green pedicel, cream colour sepal, Cundeamor shaped pods, yellow coloured ripe pods, attenuate pod apex

Table 4.23. contd.

Cluster No.	No. of hybrids	Genotypes included in the cluster	Similar characters shared between hybrids
Cluster VII	1	VSDI 23.21	-
Cluster VIII	1	SIV 10.11	-
Cluster IX	1	SIV 1.10	-
Cluster X	1	PIV 19.9	-
Cluster XI	1	PIV 26.8	-
Cluster XII	2	VSDI 33.4, PIV 58.6	Yellowish green ripe pods, obtuse pod apex, slight rugosity, dark purple beans
Cluster XIII	1	PIV 56.9	-

126

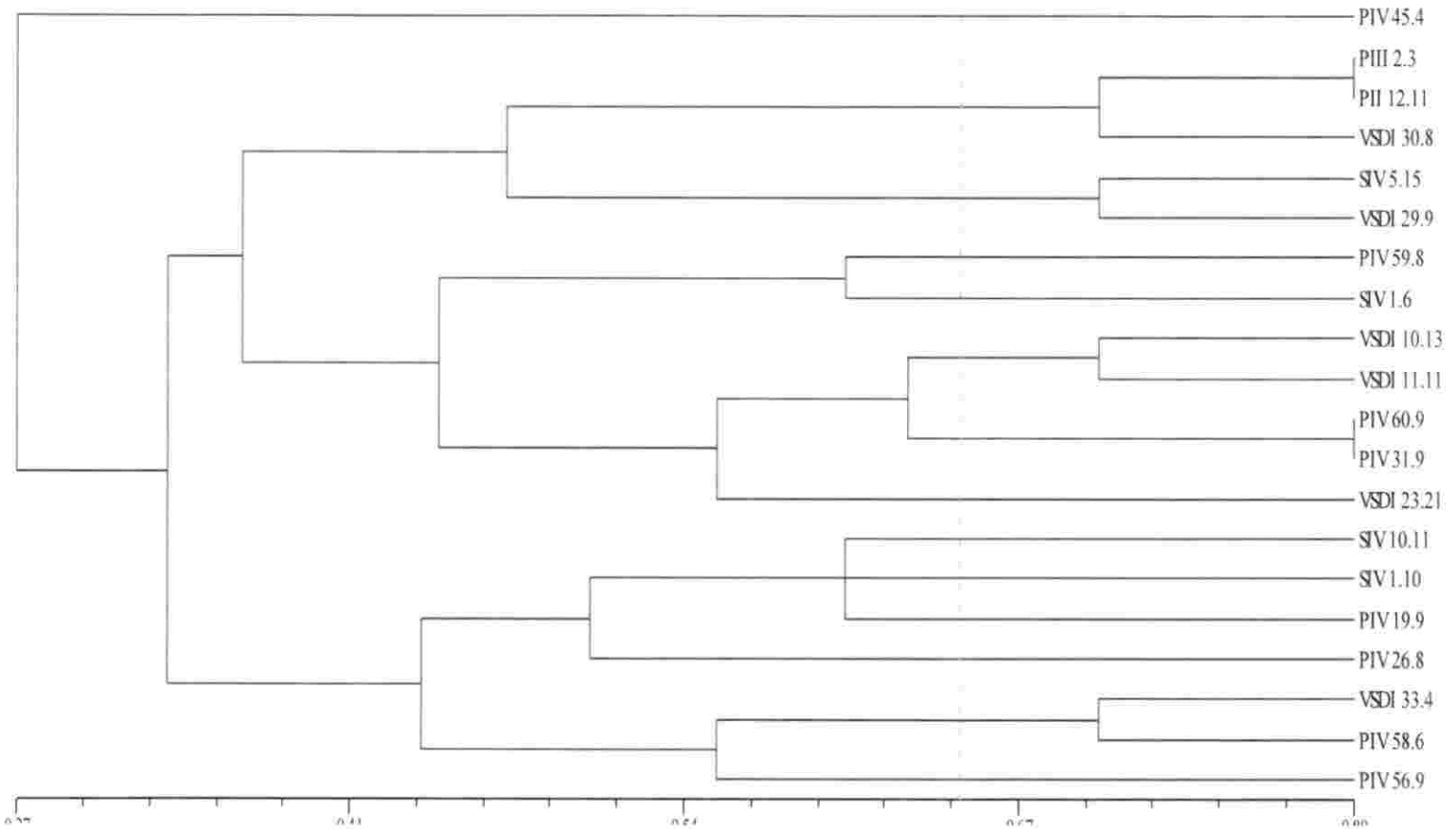


Figure 4.12. Dendrogram based on qualitative characters of cocoa

fourteen clusters obtained (Figure 4.13.) and the hybrids included in each cluster are presented in Table 4.24. The hybrids showed wide variability with respect to quantitative characters and were grouped into fourteen clusters at 50 per cent similarity coefficient. Ten clusters contained only one hybrid because none of them showed similarity with other hybrids included in the study at 50 per cent similarity coefficient.

Cluster two and nine has three hybrids each and they were the largest clusters. SIV 1.10, PIV 58.6 and PIV 26.8 are the hybrids included in cluster two and they showed similarity for ovary length, pod breadth, total wet bean weight per pod, weight of single wet bean and single bean breadth. Three hybrids contained in cluster nine are PIV 60.9, PIV 56.9 and VSDI 33.4 and they have similar values for ovary breadth, pod length, single dry bean weight and single bean breadth. Two hybrids included in cluster IV (VSDI 11.11 and SIV 1.6) were similar for staminode length, stamen length, style length, pod weight, pod length, total bean weight, single dry bean weight, length, breadth and thickness. VSDI 23.21 and VSDI 29.9 are the hybrids present in cluster XI with eleven similar characters.

4.9.3. Homology between qualitative and quantitative clustering pattern

To analyse the homology between the qualitative and quantitative clustering patterns, the percent of distribution of hybrids belonging to each qualitative cluster with respect to different quantitative clusters was worked out.

The grouping based on qualitative characters resulted into 13 clusters whereas; quantitative characters resulted in 14 clusters. The parallelism between qualitative and quantitative clustering pattern is presented in Table 4.25.

Table 4.24. Clustering based on quantitative characters in cocoa hybrids

Cluster No.	No. of hybrids	Genotypes included in the cluster	Similar characters shared between hybrids
Cluster I	1	PIV 45.4	
Cluster II	3	SIV 1.10, PIV 58.6, PIV 26.8	Ovary length, pod breadth, total wet bean weight per pod, weight of single wet bean, single bean width
Cluster III	1	SIV 10.11	-
Cluster IV	2	VSDI 11.11,SIV 1.6	Ovary breadth, pod length, single dry bean weight, single bean breadth.
Cluster V	1	VSDI 10.13	-
Cluster VI	1	PIV 31.9	-
Cluster VII	1	VSDI 30.8	-
Cluster VIII	1	PIII 2.3	-

Table 4.24. contd.

Cluster No.	No. of hybrids	Genotypes included in the cluster	Similar characters shared between hybrids
Cluster IX	3	PIV 60.9,PIV 56.9, VSDI 33.4	Staminode length, stamen length, style length, pod weight, pod length, total bean weight, single dry bean weight, single bean length, single bean breadth, single bean thickness
Cluster X	1	PIV 19.9	-
Cluster XI	2	VSDI 23.21, VSDI 29.9	Stamen length, ovary length, style length, pod weight, pod breadth, rind thickness, number of beans per pod, single bean weight, single bean length, single bean breadth, single bean thickness
Cluster XII	1	SIV 5.15	-
Cluster XIII	1	PIV 59.8	-
Cluster XIV	1	PII 12.11	-

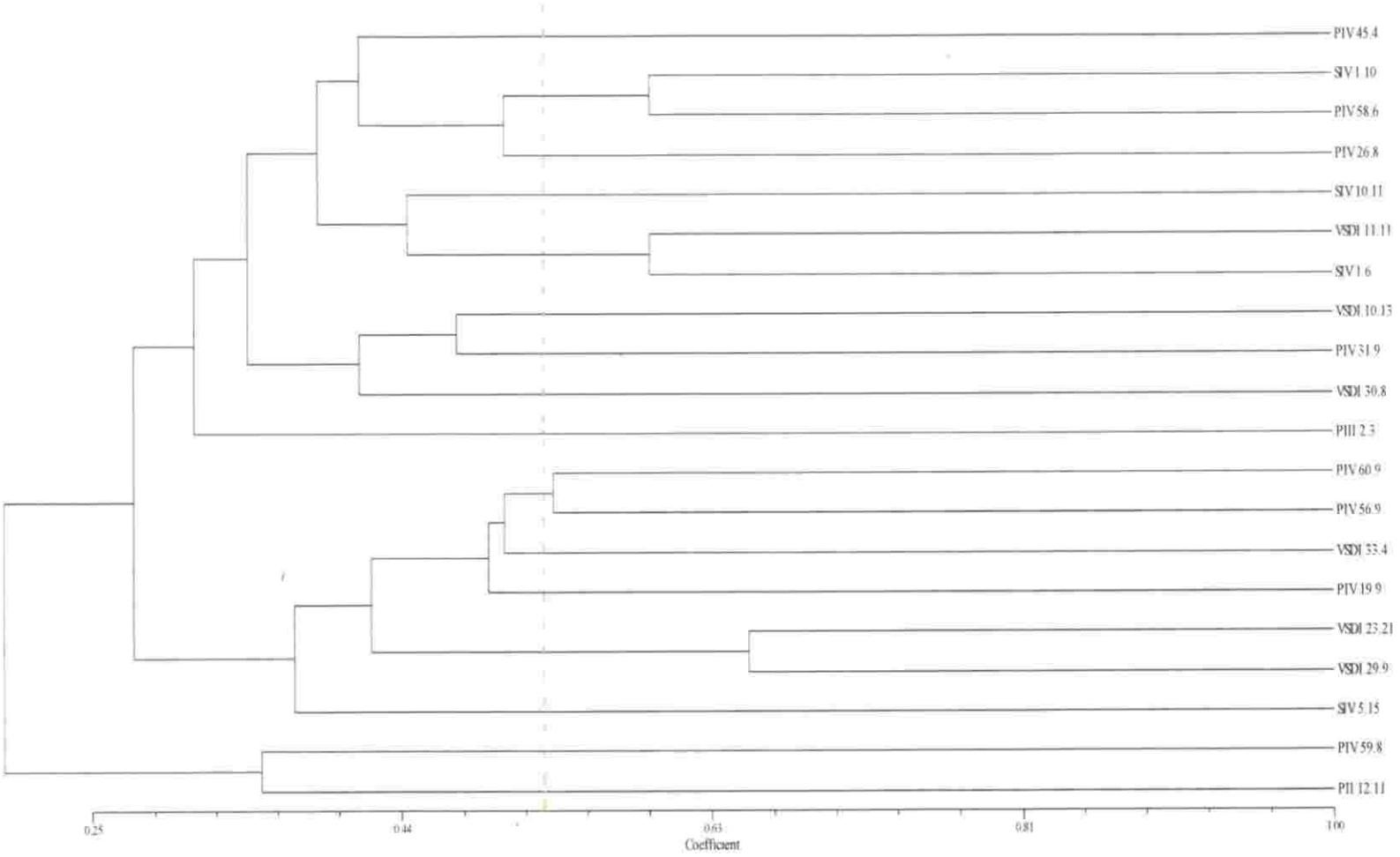


Figure 4.13. Dendrogram based on quantitative characters

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Table 4.25. Homology between qualitative and quantitative data of cocoa hybrids

Qualitative cluster data		Quantitative cluster data													
No. of genotypes ⬇		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
I	1	100	-	-	-	-	-	-	-	-	-	-	-	-	-
II	3	-	-	-	-	-	-	33.3	33.3	-	-	-	-	-	33.3
III	2	-	-	-	-	-	-	-	-	-	-	50	50	-	-
IV	1	-	-	-	-	-	-	-	-	-	-	-	-	100	-
V	1	-	-	-	100	-	-	-	-	-	-	-	-	-	-
VI	4	-	-	-	25	25	25	-	-	25	-	-	-	-	-
VII	1	-	-	-	-	-	-	-	-	-	-	100	-	-	-
VIII	1	-	-	100	-	-	-	-	-	-	-	-	50	50	-
IX	1	-	100	-	-	-	-	-	-	-	-	-	-	-	-
X	1	-	100	-	-	-	-	-	-	-	-	-	-	-	-
XI	1	-	100	-	-	-	-	-	-	-	-	-	-	-	-
XII	2	-	50	-	-	-	-	-	-	50	-	-	-	-	-
XIII	1	-	-	-	-	-	-	-	-	100	-	-	-	-	-

Table4.26. Clustering based on combined quantitative and qualitative characters

Cluster No.	No. of hybrids	Genotypes included in the cluster	Similar characters shared between hybrids
Cluster I	1	PIV 45.4	-
Cluster II	1	PIII 2.3	-
Cluster III	2	VSDI 10.13, VSDI 11.11	Green pedicel, cream sepal, cundeamor pod, yellow coloured ripe pod, intermediate basal constriction, intermediate rugosity, ovary breadth, pod weight, pod breadth, rind thickness, total wet bean weight, single wet bean weight, single bean thickness
Cluster IV	1	VSDI 30.8	-
Cluster V	1	SIV 1.6	-
Cluster VI	1	SIV 10.11	-
Cluster VII	2	SIV 1.10, PIV 58.6	Reddish pedicel, unripe pod colour intermediate, yellowish green ripe pod, obtuse base, slight rugosity, pod breadth, rind thickness, number of beans per pod, total wet bean weight, single wet bean weight, single bean breadth, single bean thickness
Cluster VIII	1	PIV 26.8	-

Table4.26. contd.

Cluster No.	No. of hybrids	Genotypes included in the cluster	Similar characters shared between hybrids
Cluster IX	2	PIV 60.9,PIV 31.9	Cream sepal, cundeamor pod shape, intermediate unripe pod, yellow ripe pod, intermediate base, slight rugosity, medium purple bean, stamen length, ovary length, breadth, style length, pod length, rind thickness, number of beans per pod,
Cluster X	2	VSDI 23.21, VSDI 29.9	Cream sepal, stamen length, intermediate rugosity, ovary length, style length, pod weight, pod breadth, rind thickness, number of beans per pod, single wet bean weight, single bean length, single bean breadth and thickness
Cluster XI	2	PIV 56.9,VSDI 33.4	Reddish green flush colour, reddish pedicel, cream sepal, amelanado pod shape, obtuse pod apex, intermediate rugosity, pedicel length, stamenoid length, ovary breadth, style length, pod length, total wet bean weight,single dry bean weight, bean breadth and thickness
Cluster XII	1	SIV 5.15	-
Cluster XIII	1	PIV 19.9	-
Cluster XIV	1	PIV 59.8	-
Cluster XV	1	PII 12.11	-

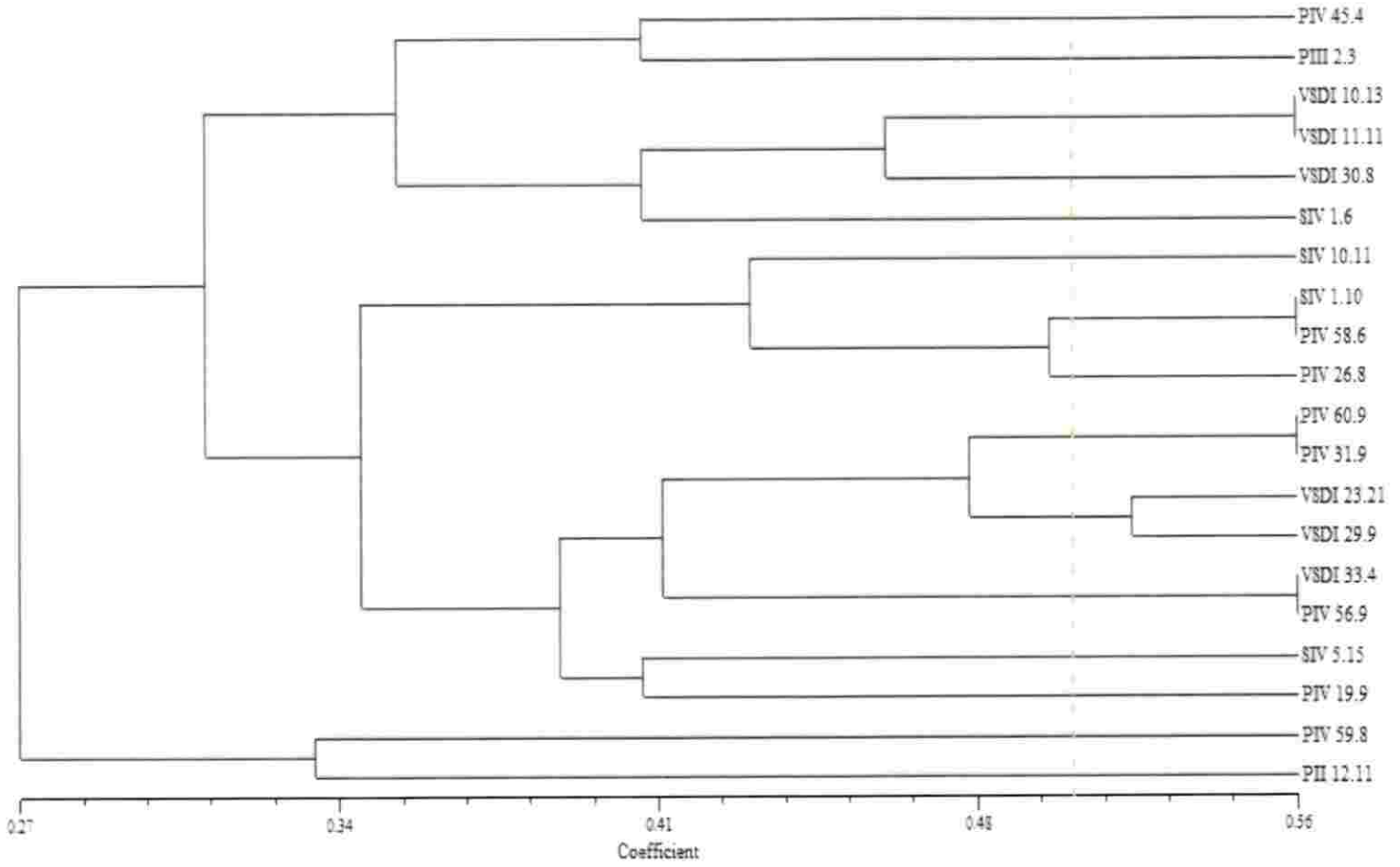

Figure 4.14. Dendrogram based on combined quantitative and qualitative characters

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The number of clusters formed based on quantitative and qualitative characters was different. In all cases hybrid belonging to a single qualitative cluster was found to be distributed in different quantitative clusters. This indicated that even though these hybrids appear to be similar at qualitative level they were different at quantitative level. The similar observations were made by Asna (2013), Ajmal (2016) and Veeresh (2017).By combining both qualitative and quantitative characters a combined cluster analysis was carried out and the resulted dendrogram is depicted in Figure 4.14. and different cluster formed are included in Table 4.26.

4.9.4. Recommended cross combinations for double cross hybrid

Based on diversity analysis recommended cross combinations for double cross hybrid production was worked out and presented in Table 4.27. The hybrids which are included in different clusters in diversity analysis based on combined quantitative and qualitative characters was recommended as parents for double cross hybrid production. Since twenty hybrids were distributed in 15 different clusters in diversity analysis, most of the hybrids were recommended for double cross production. Out of possible 380 crosses, 371 can be recommended if parents of double cross hybrids are selected only based on their diversity in quantitative and qualitative characters.

4.9.5. Clustering based on biochemical characters

Based on eight biochemical characters diversity analysis was carried out and the resulted dendrogram is depicted in Figure 4.15. and clusters formed is arranged in Table 4.28. At 60 per cent similarity coefficient fourteen clusters were formed. Five clusters contained two hybrids each and rest of the nine clusters remained as single independent units. This shows that hybrids are showing high variability with respect to biochemical characters. Similar findings are reported by Rubeena, (2015) and Ajmal (2016) who has also worked in cocoa hybrids.

Table 4.27. Recommended cross combinations for double cross hybrid

	PIV 45.4	PIII 2.3	PIV 59.8	SIV 10.11	VSDI 10.13	SIV 1.10	PIV 60.9	PII 12.11	SIV 5.15	VSDI 33.4	VSDI 23.21	PIV 58.6	PIV 56.9	VSDI 30.8	VSDI 11.11	SIV 1.6	PIV 19.9	PIV 26.8	PIV 31.9	VSDI 29.9
PIV 45.4																				
PIII 2.3																				
PIV 59.8																				
SIV 10.11																				
VSDI 10.13																				
SIV 1.10																				
PIV 60.9																				
PII 12.11																				
SIV 5.15																				
VSDI 33.4																				
VSDI 23.21																				
PIV 58.6																				
PIV 56.9																				

Table 4.27. contd.

	PIV 45.4	PIII 2.3	PIV 59.8	SIV 10.11	VSDI 10.13	SIV 1.10	PIV 60.9	PII 12.11	SIV 5.15	VSDI 33.4	VSDI 23.21	PIV 58.6	PIV 56.9	VSDI 30.8	VSDI 11.11	SIV 1.6	PIV 19.9	PIV 26.8	PIV 31.9	VSDI 29.9
VSDI 30.8																				
VSDI 11.11																				
SIV 1.6																				
PIV 19.9																				
PIV 26.8																				
PIV 31.9																				
VSDI 29.9																				

138

Table 4.28. Clustering based on biochemical characters in cocoa hybrids

Cluster No.	No. of hybrids	Genotypes included in the cluster	Similar characters shared between hybrids
Cluster I	2	PIV 45.4, PIV 60.9	Fat content, antioxidant activity, calcium, sodium and potassium content
Cluster II	2	SIV 5.15, VSDI 23.21	Fat content, antioxidant activity, calcium, sodium and potassium content
Cluster III	2	PIII 2.3, SIV 1.10	Antioxidant activity, calcium and potassium content
Cluster IV	1	PIV 26.8	-
Cluster V	1	PII 12.11	-
Cluster VI	1	PIV 31.9	-
Cluster VII	1	VSDI 29.9	-

139

Table 4.28. contd.

Cluster No.	No. of hybrids	Genotypes included in the cluster	Similar characters shared between hybrids
Cluster VIII	2	SIV 10.11, PIV 58.6	Total polyphenol content, antioxidant activity, sodium and potassium content
Cluster IX	1	VSDI 33.4	-
Cluster X	1	PIV 59.8	-
Cluster XI	2	VSDI 10.13, PIV 56.9	Fat content, total polyphenol content, antioxidant activity, calcium and potassium content
Cluster XII	1	VSDI 11.11	-
Cluster XIII	2	VSDI 30.8,SIV 1.6	Fat content, total polyphenol content, antioxidant activity and potassium content
Cluster XIV	1	PIV 19.9	-

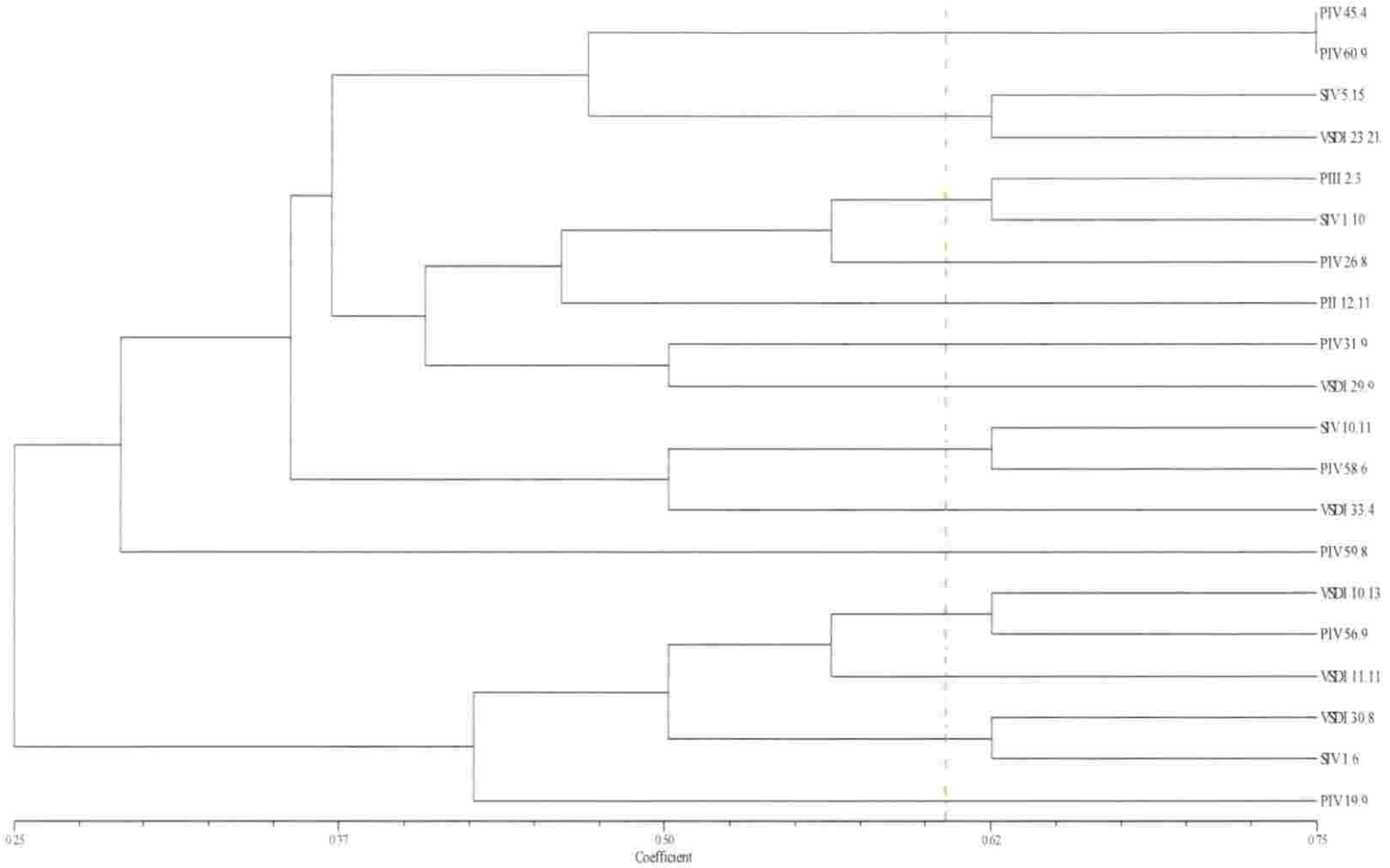

Figure 4.15. Dendrogram based on biochemical characters of cocoa hybrids

4.10. Variability analysis of cocoa hybrids

The true potential of novel genotypes can be determined from genetic parameter studies because; it gives an idea about the magnitude or nature of genetic variability and its association with environmental factors. According to Falconer and Maccay, (1996) to have effective response to selection there must be genetic variation and high heritability for the character. Bisne *et al.* (2009) suggested that heritability and genetic advance are the most important genetic parameters, which help to predict genetic gain under selection. In breeding programs, knowledge on gene action and components of genetic variance are essential for effective selection.

Genetic parameters of twelve different quantitative pod and bean characters were calculated through phenotypic coefficient of variation, genotypic coefficient of variation, heritability, genetic advance and genetic gain. The results obtained are depicted in Table 4.29.

Sivasubramanian and Madhavamenon (1973) classified phenotypic coefficient and genotypic coefficient of variation into three classes; low (0-10%), moderate (10.1-20%) and high (>20%). In the present study high phenotypic coefficient of variation and genotypic coefficient of variation was exhibited by three characters; pod weight (30.35% and 29.59%), rind thickness (29.35%and 28.31%) and total wet bean weight (22.19% and 23.01%).High phenotypic coefficient of variation and moderate genotypic coefficient of variation were showed single wet bean weight (21.35%and 19.72%), single peeled bean weight (21.25%and 19.40%) and single dry bean weight (22.15%and 19.72%). Phenotypic coefficient of variation and genotypic coefficient of variation was low for remaining characters like pod length (12.13%and 10.84%), pod breadth (14.60% and 13.23%), number of beans per pod (10.48% and 8.70%), single dry bean length (10.58% and 9.25%), single dry bean breadth (13.16% and

11.73%)and single dry bean thickness (18.22%and 15.26%). Selection of characters showing high genotypic coefficient of variation will be rewarding.

Heritability is the ratio of genotypic variance to phenotypic variance expressed in percentage. Robinson *et al.* (1949) classified heritability into low (0-30%), moderate (30.1-60%) and high (> 60.1%). Among the twelve characters considered for evaluating genetic parameters all the characters showed high heritability. Pod weight (95.03%) and rind thickness (93.04%) showed highest heritability with more than 90 per cent value. Number of beans per pod exhibited least heritability of 68.87 per cent. High heritability of a character indicates that the role of environment is less in its phenotypic expression and the variability shown by that trait is due to its genotype (Maniee *et al.*, 2009).

Genetic gain was classified into low (0-10%), moderate (10.1-20%) and high (>20%) by Johnson *et al.* (1955). High genetic gain with high heritability indicates that character is controlled by additive genes and selection for such traits will be effective (Johnson *et al.*, 1955 and Kashif *et al.*, 2003). If genetic gain is low it indicates that such traits are controlled by non-additive genes and heterosis breeding helps in improving them. High genetic gain was exhibited by nine characters including pod weight (59.42%), pod breadth (24.68%), rind thickness (56.27%), total wet bean weight (44.09%), single bean weight (37.54%), single peeled bean weight (36.49%), single dry bean weight (36.17%), single bean breadth (21.54%) and single bean thickness (26.34%). Pod length, number of beans per pod and single dry bean length showed moderate genetic gain and none of the characters has low genetic gain.

Pod weight, pod breadth, rind thickness, total wet bean weight, single wet bean weight, single peeled bean weight, single dry bean weight, dry bean breadth and dry bean thickness exhibited high heritability and high genetic gain; hence we can conclude that those characters are controlled by additive genes.

Table 4.29. Variability analysis of cocoa hybrids

Characters	PCV (%)	GCV (%)	H^2 (%)	Genetic Gain (%)
Pod weight (g)	30.35	29.59	95.03	59.42
Pod length (cm)	12.13	10.84	79.82	19.95
Pod breadth (cm)	14.60	13.23	82.03	24.68
Rind thickness (cm)	29.35	28.31	93.04	56.27
No. of beans per pod	10.48	8.70	68.87	14.87
Total wet bean weight (g)	23.01	22.19	93.02	44.09
Single wet bean weight (g)	21.35	19.72	85.34	37.54
Single peeled wet bean weight (g)	21.25	19.40	83.35	36.49
Single dry bean weight (g)	22.15	19.72	79.24	36.17
Single dry bean length (cm)	10.58	9.25	76.46	16.66
Single dry bean breadth (cm)	13.16	11.73	79.43	21.54
Single dry bean thickness (cm)	18.22	15.26	70.19	26.34

4.11. Correlation studies on cocoa hybrids

Mary and Gopalan (2006) suggested that, analysis of variability among the characters used for the study along with knowledge on association of a trait with respect to other trait contributing to yield is important for an effective breeding programme. Magnitude of association between two traits is represented by correlation coefficient. The reason for association between two characters can be due to pleiotrophy or tight linkage between genes controlling those characters.

4.11.1. Correlation studies on quantitative pod and bean characters of cocoa hybrids

Correlation analysis was carried out by Pearson coefficient method among the twelve quantitative pod and bean characters in twenty hybrids used in the study and result obtained is depicted in Table 4.30.

Pod weight showed positive significant correlation with nine characters including pod length (0.728), pod breadth (0.763), rind thickness (0.693), total wet weight of beans per pod (0.682), weight of single wet bean (0.680), weight of single peeled bean (0.677), single dry bean weight (0.681), single dry bean length (0.501) and single dry bean thickness (0.467). Adewale *et al.* (2013) and Thondaiman and Rajamani (2014) reported positive correlation between pod weight and other important pod characters.

Pod length exhibited positive significant correlation with characters like pod weight (0.728), pod breadth (0.453), total wet weight of beans per pod (0.583), weight of single wet bean (0.525), single dry bean weight (0.480) and single dry bean thickness (0.722). Rubeena (2015) also observed that pod length and breadth was positively correlated with total wet bean weight. Correlation study conducted by Veeresh (2017) among different pod and bean characters of cocoa revealed that pod length showed a significant and positive relation with pod

breadth, total wet weight of beans per pod, wet weight of single bean and single dry bean weight.

Pod breadth is found to be correlated with pod weight (0.763), pod length (0.453), rind thickness (0.837), total wet weight of beans per pod (0.595), weight of single wet bean (0.525), weight of single peeled bean (0.591), single dry bean weight (0.587), single dry bean length (0.473), single dry bean width (0.459) and single dry bean thickness (0.474). Glendinning (1963) and Kumaran and Amma (1982) reported that when the pod breadth increased, number, weight and size of the bean also increased. However in present study, number of beans is not found to be correlated with pod breadth. Rubeena (2015) reported that pod length and pod breadth was positively correlated with total wet bean weight and dry bean length.

Rind thickness showed positive significant correlation with pod weight (0.693), pod breadth (0.837), single dry bean weight (0.493) and single dry bean thickness (0.483). Pod weight and rind thickness were showing positive correlation but rind thickness and total wet bean weight is not correlated. From this result it is clear that increase in rind thickness results in increase in pod weight but total wet bean weight may not to be affected. Total wet bean weight can be high even if pod weight is less (Ajmal, 2015).

Ruinard (1961); Thondaiman and Rajamani (2014); Asna *et al.* (2014) and Veeresh (2017) reported positive correlation between number of beans per pod and total wet bean weight. However in the present study number of beans per pod is not correlated with any other character. Maharaj *et al.* (2011) revealed a negative relation between single bean thickness and number of beans per pod. In the present study also it was found that single bean thickness and number of beans per pod is negatively correlated (-0.213) but it is not significant.

Total wet weight of beans per pod exhibited significant positive

correlation with pod weight (0.682), pod length (0.583), pod breadth (0.595), weight of single wet bean (0.845), single dry bean weight (0.627), single dry bean length (0.468) and single dry bean width (0.564).Positive correlation between total wet bean weight and single bean weight, length and width was observed by Kumaran and Amma (1982) and Aikpokpodion (2010).

Weight of single wet bean showed positive significant association with pod weight (0.680), pod length (0.551), pod breadth (0.525), total wet weight of beans per pod (0.845), weight of single peeled bean (0.688), single dry bean weight (0.790), single dry bean length (0.580), single dry bean width (0.751) and single dry bean thickness (0.446). Weight of single peeled bean is having positive significant correlation with pod weight (0.677), pod breadth (0.519), weight of single wet bean (0.688), single dry bean weight (0.877), single dry bean length (0.688) and single dry bean width (0.772). Single dry bean is obtained by drying peeled bean and hence that are positively correlated. Single dry bean weight exhibited positive significant association with pod weight (0.681), pod length (0.480), pod breadth (0.587), rind thickness (0.493), total wet weight of beans per pod (0.627), weight of single wet bean (0.790), weight of single peeled bean (0.877), single dry bean length (0.689), single dry bean width (0.791) and single dry bean thickness (0.471). As proposed by Thondaiman and Rajamani (2014) and Veeresh (2017) the weight of single bean depends upon the size of the bean *i.e.* bean length, bean width and bean thickness.

Dry bean length exhibited positive significant correlation with pod weight (0.501), pod breadth (0.473), total wet weight of beans per pod (0.468), weight of single wet bean (0.580), weight of single peeled bean (0.688), single dry bean weight (0.689) and single dry bean width (0.718). Positive correlation between single bean length, bean width and bean weight were reported by Veeresh (2017).

Table 4.30. Correlation among pod and bean characters of cocoa

Characters	Pod weight (g)	Pod length (cm)	Pod breadth (cm)	Rind thickness (cm)	No. of beans/ pod	Total wet weight of beans/ pod (g)	Wet weight of single bean (g)	Single peeled bean weight (g)	Single dry bean weight (g)	Dry bean length (cm)	Dry bean width (cm)	Dry bean thickness (cm)
Pod weight (g)	1											
Pod length (cm)	**0.728****	1										
Pod breadth (cm)	**0.763****	0.453*	1									
Rind thickness (cm)	**0.693****	0.363	**0.837****	1								
No. of beans/ pod	0.221	0.052	0.269	0.155	1							
Total wet weight of beans/ pod (g)	**0.682****	**0.583****	**0.595****	0.347	0.166	1						
Wet weight of single bean (g)	**0.680****	0.551*	0.525*	0.391	-0.137	**0.845****	1					

148

Table 4.30. contd.

Characters	Pod weight (g)	Pod length (cm)	Pod breadth (cm)	Rind thickness (cm)	No. of beans/ pod	Total wet weight of beans/ pod (g)	Wet weight of single bean (g)	Single peeled bean weight (g)	Single dry bean weight (g)	Dry bean length (cm)	Dry bean width (cm)	Dry bean thickness (cm)
Single peeled bean weight (g)	**0.677****	0.397	0.519*	0.432	-0.040	-0.139	**0.688****	1				
Single dry bean weight (g)	**0.681****	0.480*	**0.587****	0.493*	-0.072	**0.627****	**0.790****	**0.877****	1			
Dry bean length (cm)	0.501*	0.287	0.473*	0.171	-0.058	0.468*	**0.580****	**0.688****	**0.689****	1		
Dry bean width (cm)	**0.591****	0.226	0.459*	0.390	-0.084	**0.564****	**0.751****	**0.772****	**0.791****	**0.718****	1	
Dry bean thickness (cm)	0.467*	**0.722****	0.474*	0.483*	-0.213	0.434	0.446*	0.389	0.471*	0.146	0.220	1

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

Single dry bean width have positive significant correlation with pod weight (0.591), pod breadth (0.459), total wet weight of beans per pod (0.564), weight of single wet bean (0.751), weight of single peeled bean (0.772), single dry bean weight (0.791) and single dry bean length (0.718).Single dry bean thickness expressed positive significant correlation with pod weight (0.467), pod length (0.722), pod breadth (0.474), rind thickness (0.483), weight of single wet bean (0.446) and single dry bean weight (0.471). These observations also confirm that bean size and bean weight is positively related.

4.12. Path coefficient analysis

The concept of path analysis was introduced by Wright (1921). This method helps to quantify direct effects and indirect effects of independent variables on a dependent variable (Li, 1975; Cruz and Regazzi, 2006).

Lenka and Misra (1973) classified the direct and indirect effects of independent variable on dependent variable as:

0.00 to 0.09 – Negligible
0.10 to 0.19 – Low
0.20 to 0.29 – Moderate
0.30 to 0.99 – High
More than 1.00 – Very high

4.12.1. Path analysis of pod and bean characters on total wet bean weight

Result of path coefficient analysis with direct and indirect effects on total wet bean weight is presented in Table 3.31. and Figure 4.16.Contribution of residual effect on total wet bean weight was 0.091 which indicated that 90.9 per cent of traits which contribute to total wet bean weight were considered in this study.

4.6.2.1. Direct effect on total wet bean weight

Single wet bean weight exhibited very high (1.428) positive direct effect on total wet bean weight. Pod weight (0.941) and single bean thickness (0.650) expressed high positive direct effect. Direct effect of pod width (0.171), number of beans per pod (0.199) and single dry bean weight (0.120) on total wet bean weight was low and positive. Single bean length (0.055) showed a negligible positive direct effect. These traits help in indirect selection for genetic improvement of total wet bean weight of hybrids. Similar findings were reported by Almeida *et al.* (1994); Thondaiman and Rajamani (2014); Ajmal (2016) and Veeresh (2017).

High negative direct effect was expressed by pod length (0.966), rind thickness (-0.632), single peeled bean weight (0.603) and single bean width (-0.476). Maharaj *et al.* (2011) reported that rind thickness and total wet bean weight are negatively correlated. Number of flat beans per pod exhibited negligible and negative direct effect. Traits showing negative direct effect will leads in reduction of total wet bean weight.

4.6.2.2. Indirect effects on total wet bean weight

4.4.1.2.a. Pod weight

Pod weight showed high positive indirect effect on total wet bean weight (0.981) through very high positive direct effect (1.428) of single bean weight and high positive indirect effect (0.305) through the high positive direct effect (0.650) of single bean thickness. Pod weight exhibited low positive indirect effect (0.131) through the low direct effect of pod width (0.171).Pod weight also expressed low positive indirect effect on total wet bean weight through low negative direct effect

Table 4.31. Path coefficient analysis of pod and bean characters on total wet bean weight

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
C1	**0.941**	-0.712	0.131	-0.442	0.045	0.017	0.981	-0.412	0.083	0.027	-0.279	0.305
C2	0.694	**-0.966**	0.078	-0.233	0.010	0.001	0.801	-0.241	0.059	0.016	-0.098	0.470
C3	0.724	-0.440	**0.171**	-0.547	0.055	0.024	0.755	-0.318	0.072	0.026	-0.228	0.306
C4	0.658	-0.356	0.148	**-0.632**	0.029	0.015	0.558	-0.268	0.061	0.011	-0.197	0.318
C5	0.212	-0.050	0.048	-0.093	**0.199**	0.029	-0.201	0.053	-0.008	-0.004	0.064	-0.078
C6	-0.241	0.006	-0.061	0.148	-0.088	**-0.066**	-0.109	0.083	-0.019	-0.008	-0.009	0.086
C7	0.646	-0.542	0.090	-0.247	-0.028	0.005	**1.428**	-0.509	0.096	0.032	-0.359	0.239
C8	0.643	-0.387	0.090	-0.281	-0.017	0.009	1.207	**-0.603**	0.108	0.038	-0.369	0.256
C9	0.649	-0.471	0.102	-0.322	-0.014	0.011	1.142	-0.540	**0.120**	0.038	-0.378	0.299
C10	0.467	-0.274	0.080	-0.124	-0.015	0.009	0.839	-0.415	0.083	**0.055**	-0.352	0.119
C11	0.552	-0.199	0.082	-0.261	-0.027	-0.001	1.078	-0.467	0.096	0.041	**-0.476**	0.125
C12	0.441	-0.698	0.080	-0.309	-0.024	-0.009	0.526	-0.237	0.055	0.010	-0.092	**0.650**

Residual – 0.091

C1 Pod Weight (g)
C2 Pod Length (cm)
C3 Pod Width (cm)
C4 Rind thickness (cm)
C5 No. of beans/ pod
C6 No. of flat beans/ pod
C7 Single bean weight (g)
C8 Total dry bean weight (g)
C9 Single dry bean weight (g)
C10 Single bean length (mm)
C11 Single bean width (mm)
C12 Single bean thickness (mm)

of number of flat beans per pod, low positive direct effect of single dry bean weight and low positive direct effect of single bean length. Pod weight exhibited high negative indirect effect (-0.712, -0.442 and -0.412) on total wet bean weight through high negative direct effect of pod length (-0.966), rind thickness (-0.632) and single peeled bean weight (-0.603).

4.4.1.2.b. Pod length

Pod length exhibited high positive indirect effect on total wet bean weight (0.801) through very high positive direct effect (1.428) of single bean weight. Pod length showed high positive indirect effect (0.694) through high positive direct effect (0.941) of pod weight. It also showed high positive indirect effect (0.470) through high positive direct effect of single bean thickness. Negligible positive indirect effects (0.078, 0.059, 0.016, 0.010 and 0.001) were expressed through low positive direct effect of pod width (0.171), single dry bean weight (0.120), single bean length (0.055), number of beans per pod (0.199) and negative direct effect (-0.066) of number of flat beans per pod.

Pod length exhibited moderate negative indirect effect (-0.233 and -0.241) on total wet bean weight through high negative direct effect of rind thickness (-0.632) and single peeled bean weight (-0.603).

4.4.1.2.c. Pod width

Pod width exhibited high positive indirect effect on total wet bean weight (0.755, 0.724 and 0.306) through very high positive direct effect (1.428) of single bean weight, high positive direct effect (0.941) of pod weight and single bean thickness (0.650). Pod width showed negligible indirect effect (0.055, 0.024, 0.072, and 0.026) through low direct effect of number of beans per pod (0.199), negligible negative direct effect (-0.066) of number of flat beans per pod, negligible positive direct effect single bean length and high negative direct effect

single bean width. Pod width expressed a high negative direct effect (-0.440, -0.547 and -0.318) on total wet bean weight through high negative direct effect of pod length (-0.966), rind thickness (-0.632) and single peeled bean weight (-0.603).

4.4.1.2.d. Rind thickness

Rind thickness exhibited high positive indirect effect on total wet bean weight (0.658, 0.558 and 0.318) through high positive direct effect (0.941) of pod weight, very high positive direct effect (1.428) of single bean weight and positive high direct effect of single bean thickness (0.650).Rind thickness showed low positive indirect effect (0.148) on total wet bean weight through pod width (0.171). Rind thickness showed negligible indirect effect (0.029, 0.015, 0.061, and 0.011) through low direct effect of number of beans per pod (0.199), negligible negative direct effect (-0.066) of number of flat beans per pod, low positive direct effect of single dry bean weight and negligible direct effect of single bean length.

Rind thickness exhibited high negative indirect effect (-0.356) on total wet bean weight through high negative direct effect of pod length (-0.966).

4.4.1.2.e. Number of beans per pod

Number of beans per pod exhibited moderate positive indirect effect on total wet bean weight (0.212) through high positive direct effect (0.941) of pod weight. Negligible positive indirect effect (0.048, 0.029, 0.053 and 0.064) was expressed through low positive direct effect of pod width (0.171), negligible negative direct effect (-0.066) of number of flat beans per pod, high negative direct effect of single peeled bean weight (-0.603) and single bean width (-0.476).

4.4.1.2.f. Number of flat beans per pod

Number of flat beans per pod expressed low positive indirect effect (0.148) through high negative direct effect (-0.632) of rind thickness. Negligible positive direct effect (0.006, 0.083 and 0.086) was expressed through high negative direct effect of pod length (-0.966), high negative direct effect (-0.603) of single peeled bean weight and high positive direct effect of single bean thickness (0.650).

4.4.1.2.g. Single wet bean weight

Single wet bean weight expressed high positive indirect effect (0.646) through the high positive direct effect (0.941) of pod weight. Moderate positive indirect effect (0.239) was expressed through high positive direct effect of single bean thickness (0.650). Negligible positive indirect effect (0.090, 0.005, 0.096 and 0.032) was exhibited through low positive direct effect of pod width (0.171), negligible negative direct effect (-0.066) of number of flat beans per pod, low positive direct effect of single dry bean weight (0.120) and negligible direct effect of single bean length (0.055).

Single wet bean weight expressed high negative direct effect (-0.542, -0.509 and -0.359) through the high negative direct effect of pod length (-0.966), single peeled bean weight (-0.603) and single bean width (-0.476).

4.4.1.2.h. Single peeled bean weight

Single peeled bean weight exhibited very high positive indirect effect (1.207) through the very high positive direct effect (1.428) of single bean weight. Single peeled bean weight expressed high positive indirect effect (0.643) through the high positive direct effect (0.941) of pod weight. Moderate positive indirect effect (0.256) was exhibited on total bean weight through positive high direct

effect of single bean thickness (0.650). Negligible positive indirect effect (0.090, 0.009 and 0.038) on total bean weight was expressed through low positive direct effect of pod width (0.171), negligible negative direct effect (-0.066) of number of flat beans per pod and negligible direct effect of single bean length (0.055).

Single peeled bean weight exhibited very high negative indirect effect (-0.387 and -0.369) through the high negative direct effect of pod length (-0.966) and single bean width (-0.476).

4.4.1.2.i. Single dry bean weight

Single dry bean weight showed very high positive indirect effect (1.142) through the very high positive direct effect (1.428) of single bean weight. High positive indirect effect (0.649) was expressed through high positive direct effect (0.941) of pod weight. Moderate positive indirect effect (0.299) was expressed through positive high direct effect of single bean thickness (0.650). Low positive indirect effect (0.102) was exhibited through low direct effect of pod width (0.171). Negligible positive indirect effect (0.011 and 0.038) was expressed through negligible negative direct effect (-0.066) of number of flat beans per pod and negligible direct effect of single bean length (0.055).

Single dry bean weight expressed high negative indirect effect on total wet bean weight through the high negative direct effect of pod length (-0.966), rind thickness (-0.632), single peeled bean weight (-0.603) and single bean width (-0.476).

4.4.1.2.j. Single bean length

Single bean length exhibited high positive indirect effect (0.467 and 0.839) through the high positive direct effect (0.941) of pod weight and very high positive direct effect (1.428) of single bean weight. Low positive indirect effect

(0.119) was exhibited through positive high direct effect of single bean thickness (0.650). Negligible positive indirect effect (0.080, 0.009 and 0.083) was expressed through low direct effect of pod width (0.171), negligible negative direct effect (-0.066) of number of flat beans per pod and low positive direct effect of single dry bean weight (0.120).

Single bean length exhibited high negative indirect effect (-0.415 and -0.352) through the high negative direct effect of single peeled bean weight (-0.603) and single bean width (-0.476).

4.4.1.2.k. Single bean breadth

Single bean breadth showed very high indirect effect (1.078) through very high positive direct effect (1.428) of single bean weight. High positive indirect effect (0.552) was expressed through the high positive direct effect (0.941) of pod weight. Low positive indirect effect was exhibited through positive high direct effect of single bean thickness (0.650). Negligible positive indirect effect (0.082, 0.096 and 0.041) was expressed through low direct effect of pod width (0.171), low positive direct effect of single dry bean weight (0.120) and negligible direct effect of single bean length (0.055).

Single bean breadth showed high negative indirect effect (-0.415) through high negative direct effect (-0.467) of single peeled bean weight.

4.4.1.2.l. Single bean thickness

Single bean thickness expressed high positive indirect effect (0.526 and 0.441) through the very high positive direct effect (1.428) of single bean weight and high positive direct effect (0.941) of pod weight. Negligible positive indirect effect (0.080, 0.055 and 0.010) was expressed through low direct effect of pod width (0.171), low positive direct effect of single dry bean weight (0.120) and

negligible direct effect of single bean length (0.055).Single bean thickness expressed high negative indirect effect (-0.698 and -0.309) through the high negative direct effect of pod length (-0.966) and rind thickness (-0.632).

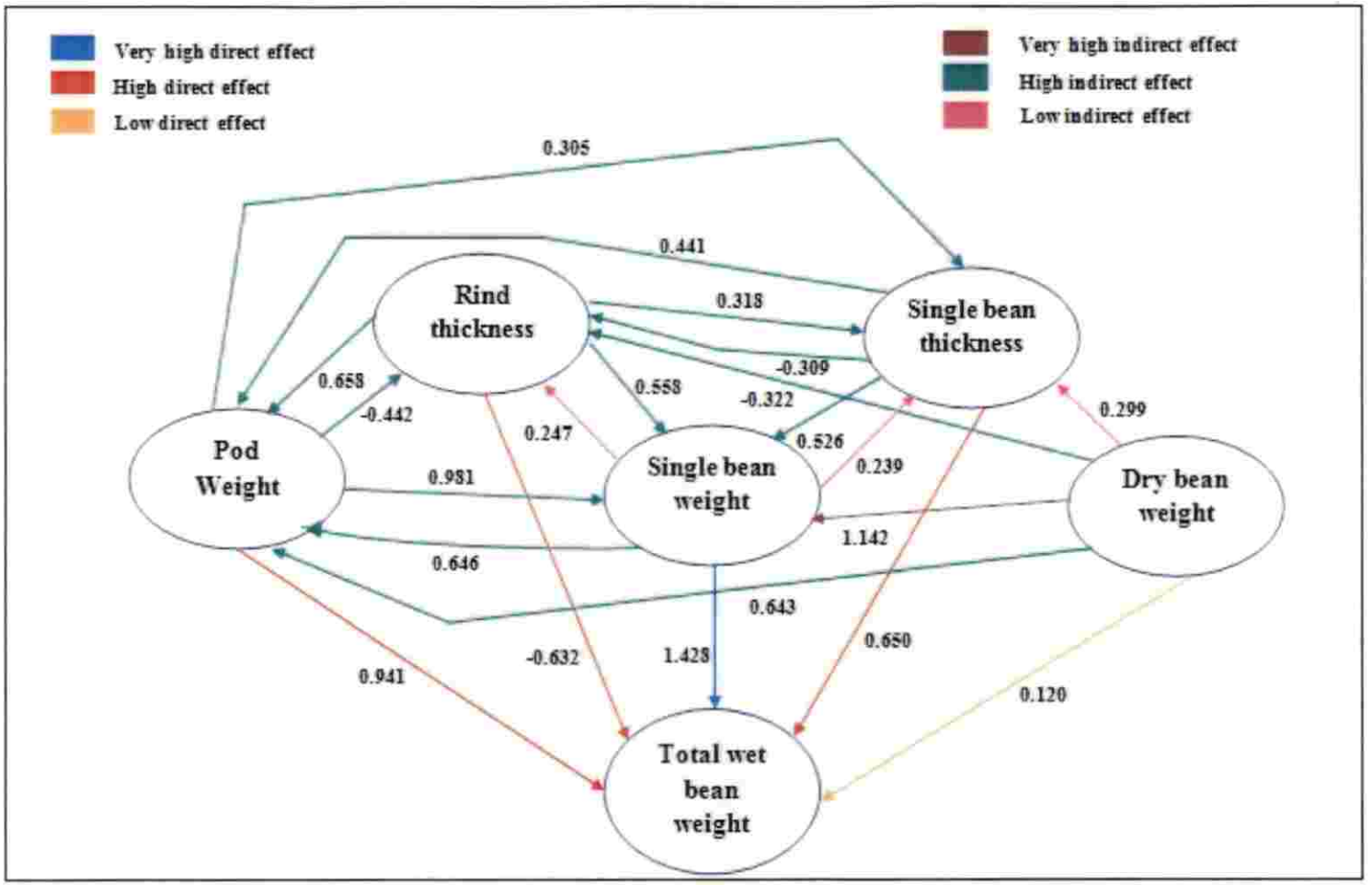

Figure 4.16. Path analysis based on selected characters

Summary

5. SUMMARY

The present study entitled 'Genetic analysis of cocoa (*Theobroma cacao* L.) hybrids and screening superior hybrids for major biotic stress' was carried out in the Department of Plant Breeding and Genetics, College of Horticulture and the Cocoa Research Centre (CRC), Vellanikkara during the period 2017-2019. The objective of the study wasto assess genetic potential of cocoa hybrids and to evaluate the reaction of identified ones against *Phytophthora* pod rot and tea mosquito bug (*H. theivora* Waterhouse).Twenty cocoa hybrids which are in the steady bearing stage, selected from the comparative yield trail (CYT), planted during 2008 at CRC farm served as the material for the study.

The salient findings are summarized below:

- Morphological characterization was carried out based on qualitative and quantitative characters of flower, pod and bean
- Thirteen pod characters, twelve floral characters, six bean characters and flush colour of leaves were studied
- Significant variability was observed among the hybrids for various qualitative and quantitative characters, except colour of petal and number of ridges and furrows
- Hybrids were evaluated based on biochemical properties of beans including fat, polyphenol, antioxidant activity and mineral content and exhibited significant difference
- Polyphenol content showed significant correlation with antioxidant activity
- Economic characters of cocoa hybrids were estimated and based on biochemical and economic characters hybrids were scored and ranked.
- Six hybrids having top rank were selected as superior genotypes and they are PII 12.11, PIV 19.9, VSDI 33.4, VSDI 11.11, VSDI 29.9 and PIV 59.8.

- Twenty hybrids included in the study were screened for *Phytophthora* resistance by artificially inoculating *Phytophthora* culture on cocoa pods
- Hybrids exhibited differential response towards *Phytophthora* pod rot resistance screening
- Hybrids PIV 59.8, VSDI 10.13, VSDI 11.11 and PIV 31.9 were found to be highly resistant against *Phytophthora*
- Correlation studies between *Phytophthora* resistance and pod biochemical and morphological characters were carried out and the result revealed that pod husk phenol and pod wax content are positively correlated to *Phytophthora* resistance
- Artificial screening for Tea Mosquito Bug resistance was carried out on budded plants and detached pods
- PIV 59.8, PIV 60.9, PII 12.11, VSDI 33.4 and PIV 56.9 were found to be highly resistant towards TMB on budded plants
- Among 6 hybrids screened for TMB on pods hybrids PII 12.11 and VSDI 33.4 has least number of punctures
- Hybrids PII 12.11, VSDI 33.4, VSDI 29.9, VSDI 11.11 and PIV 59.8 were resistant towards both biotic stresses
- Based on diversity analysis possible cross combination for double cross hybrids production was recommended

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GENETIC ANALYSIS OF COCOA (*Theobroma cacao* L.) HYBRIDS AND SCREENING SUPERIOR HYBRIDS FOR MAJOR BIOTIC STRESS

By

SHILPA K.S.

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ABSTRACT OF THE THESIS

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Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF PLANT BREEDING AND GENETICS

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

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ABSTRACT

Cocoa is a crop highly influenced by climate change and growing environment, which make it necessary to have long term and dynamic breeding programme. Yield improvement was the prime objective of most of the earlier breeding programmes. However, with the emergence and spread of many diseases and pests, more emphasis is given for evolving disease and pest tolerant cocoa varieties, without sacrificing yield. At present, one of the main challenges faced by cocoa growers is *Phytophthora* pod rot caused by *Phytophthora palmivora*. Since this disease is prevalent during rainy season it is very difficult to control using fungicides. Cultivation of resistant varieties is the most effective and ecofriendly method of control. Tea mosquito bug (TMB) (*Helopeltis theivora*) is a major sucking pest of cocoa causing damage to young shoots, cherelles and pods. The development and use of mirid resistant cocoa varieties is the only effective alternatives to chemical control against TMB.

Twenty cocoa hybrids evaluated in the comparative yield trail (CYT) were considered for the present study. Morphological characterization of the hybrids were carried out based on quantitative and qualitative characters. Thirteen pod characters, twelve floral characters, six bean characters and flush colour of leaves were studied. Except colour of petal and number of ridges and furrows, all other characters expressed high variability among the hybrids. Hybrids were also evaluated based on biochemical properties of beans including fat content, total polyphenol content and total antioxidant activity. Hybrids exhibited significant difference for biochemical characters. More over phenol content showed significant correlation with antioxidant activity.

Based on biochemical and economic characters hybrids were scored and ranked. Six hybrids having top rank were selected as superior genotypes and they are PII 12.11, PIV 19.9, VSDI 33.4, VSDI 11.11, VSDI 29.9 and PIV 59.8.

The twenty cocoa hybrids included in the study were screened for *Phytophthora* resistance by artificially inoculating *Phytophthora* culture on detached cocoa pods. Based on the disease resistance reaction, the hybrids were classified using the score chart. Hybrids exhibited differential response towards *Phytophthora* pod rot resistance screening. Hybrids PIV 59.8, VSDI 10.13, VSDI 11.11 and PIV 31.9 were found to be highly resistant whereas, hybrids PII 12.11, SIV 5.15, SIV 1.6, PIV 26.8 and VSDI 29.9 exhibited resistance towards *Phytophthora*. Correlation studies between *Phytophthora* pod rot resistance and pod husk biochemical properties revealed that pod husk phenol and pod wax content are positively correlated to *Phytophthora* resistance.

Artificial screening for TMB resistance was carried out on budded plant and detached pods to study the reaction of different hybrids against *Helopeltis theivora*. Budded plants of all the hybrids and medium matured freshly collected pods of selected six hybrids were screened. Based on the number of feeding punctures, hybrids were grouped into different classes following score chart. Hybrids PIV 59.8, PIV 60.9, PII 12.11, VSDI 33.4 and PIV 56.9 with average number of feeding punctures less than three on plants were included in highly resistant class. When cocoa pods of six hybrids were screened for TMB resistance, PII 12.11 and VSDI 33.4 had the least number of feeding punctures after 72 hours of feeding by TMB.

Among the six hybrids selected based on economic and biochemical performance, five hybrids *i.e.* PII 12.11, VSDI 33.4, VSDI 29.9, VSDI 11.11 and PIV 59.8 exhibited highly resistant to slightly resistant reaction towards both the biotic stress considered.