GENETIC ANALYSIS OF INBREDS, INBRED CROSSES AND HYBRIDS OF COCOA (*Theobroma cacao* L.)

By

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(2015-22-002)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Genetic analysis of inbred, inbred crosses and hybrids of cocoa (*Theobroma cacao* L.)"** is a bona-fide record of research work done by me during the course of research and that 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.

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Vijayakumar B. Narayanapur

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Symbols / Notations and Abbreviations

USD	-	U.S. Dollars
CRC	-	Cocoa Research Centre
CPBMB	-	Centre for Plant Biotechnology and Molecular Biology
kg	-	Kilogram
g	-	Gram
CD	-	Critical difference
CRD	-	Completely Randomized Design
>	-	More than
DAS	-	Days after sowing
Mg/g	-	Milli gram per gram
µg/ g	-	Micro gram per gram
cm	-	Centimeter
mm	-	Millimeter
et al.	-	Co-workers
Fig.	-	Figure
%	-	Per cent
˚C	-	Degree Celsius
PV	-	Pod Value
PI	-	Pod Index
EI	-	Efficiency Index
CI	-	Conversion Index
Na_2 CO3	-	Sodium Carbonate
rpm	-	Rotation per minute
ml	-	Millilitre
nm	-	Nanometer
FC	-	Folin-Ciocalteau
MSL	-	Mean sea level
OD	-	Optical Density
ppm	-	Parts per million

DMR	-	Dry matter recovery
kDa	-	Kilo Dalton
M	-	Molar
MALTI -TOF	-	Matrix Assisted Laser Desorption Ionization- Time of Flight Spectrophotometry
mM	-	Millimole
pH	-	Hydrogen ion concentration
SDS	-	Sodium Dodecyl Sulphate
2DE	-	Two Dimensional Gel Electrophoresis
LCMS	-	Liquid chromatography-mass spectrometry
LC Q TOF	-	Liquid chromatography-Quadrupole-Time of flight
CV	-	Coefficient of variation
CD	-	Critical Difference

Affectionately dedicated to

My beloved brother

Shri Sayabanna B. Narayanapur

and

Shri Shivayogishwararu

Shri Prabhudevar Betta, Jambagi (A)

Introduction

1. INTRODUCTION

Cocoa (*Theobroma cacao* L.) is the third important beverage from neo-tropical rain forest Amazon basin and Guyana Plateu of South America. It is a diploid species with diploid chromosomes in somatic cells (2n=20). Primarily, the processed seeds of which are used for the production of chocolate, cocoa powder and cocoa butter. Cocoa butter is of great importance because of its use in the chocolate and cosmetic industry. Various byproducts of cocoa are used in preparation of cosmetics, confectionaries, perfumeries, pharmaceuticals etc., It is native species of tropical humid forests on the lower eastern equatorial slopes between 10 and 20 degrees' latitude north and south of equator in the Andes, in South America (Cheesman, 1944, Motamayor *et al.,* 2002). In Central America, the domestication of cocoa was started approximately 3000 years ago. It spread all over the tropical regions of the world in the 18^{th} and 19^{th} centuries.

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

Cocoa was originally placed under the family Sterculiaceae. Based on the recent phylogenetic studies on combined analysis of plastid atpB and rbcL DNA sequences, morphological, anatomical, palynological, and chemical characteristics included it into broadly defined Malvaceae family (Judd and Manchester 1997; Alverson *et al*., 1999 and Bayer *et al*., 1999).

Among the 22 species under the genus, Theobroma, the species cacao is of economic importance (Bartley, 2005). The most important economic part of cocoa is properly fermented and dried beans, which are the only source of chocolate which impart aroma and flavor to the chocolate (Amma, *et al.,* 2011). There is no substitute to cocoa butter in the world, due to its pleasant aroma, flavor and melting point. Cocoa butter is the only butter which melts at human body temperature.

Cocoa is an introduced crop in India during early 1960's and the major cultivating states are Kerala, Andhra Pradesh, Karnataka and Tamil Nadu. In India, cocoa is grown in an area of about 82,940 ha with an annual production of 18,920 tonnes. Tamil Nadu leads in area of cocoa cultivation (22,389 ha) with a production of 1040 tonnes annually. Kerala leads first in production with an annual production of 7,105 tonnes (DCCD, 2017).

The productivity of cocoa in India, is 580 kg/ha (DCCD, 2017). This is far lower than the world productivity. Cocoa farming will be profitable if the productivity of cocoa is high.

Cocoa is predominantly grown in mixed stands in rubber and backyards of the humid tropics of Kerala, in Tamil Nadu, it is grown as an intercrop in the coconut and in Karnataka it is grown in arecanut and coconut plantations of tropical region.

Hence, there is scope for improving the productivity of cocoa in India. The productivity of cocoa can be improved by following good management practices, inclusion of high yielding varieties and hybrids and by proper management of pests and diseases. Cocoa is predominantly out breeding with highly complex genetic structure. The cross pollinating nature coupled with existence of self/cross incompatibility poses much difficulty to the cocoa breeders. Though, synthesis of dynamic population of hybrids has become a reality, very high variability is exhibited by these hybrids. All the hybrids in a cross do not show the same level of superiority due to the use of heterozygous parents in the breeding programme (Rosemery, 1998).

Production of very good heterotic hybrids thus became an important thrust of cocoa breeding. The only alternative to secure maximum heterotic vigour in hybrid

population is by production of inbreds with homozygosity and taking up crosses between two distant inbreds. But factors like the genetic complexity of cocoa, difficulty in pollination, long breeding cycle and high percentage of self-incompatibility in advanced generations of inbreeds forced most of institutes to drop inbreeding programme by second or third generation.

Inbreeding programme was started at Kerala Agricultural University by utilizing self-compatible plants located through controlled self-pollination since 1989. The aim was to produce fully homozygous inbreds by selfing 7-8 generations and subsequent production of heterotic hybrids by using divergent inbreds.

For developing high yielding hybrid, highly homozygous inbred lines are the pre requisite. Cocoa Research Centre (CRC) has started the development of inbred lines, and achieved till fifth generation inbred.

As part of the project, inbreds of various genotypes belonging to different generations were field established. By the year 2006, the centre succeeded in producing first ever fifth generation inbred of cocoa reported in the world. Upon flowering, these S5 inbred was continuously selfed during 2009 to 2014 and resulted in no pod set and it was concluded to be self-incompatible (SIC) and therefore further advancement of generation was not possible (Mallika *et al.,* 2002). To understand the actual incompatibility mechanism, it is very much necessary to study the protein profile at critical stages of pollination and different standard methods will be used to overcome the self-incompatibility. The study of various selfed generations of various cocoa genotypes will yield the effect of inbreeding on growth and yield attributes of inbreds. Evaluation of inbreds, inbred cross and hybrids during early stage of growth at physiological level will help to establish a relationship between factors leading to heterosis or inbreeding depression.

In this background the present study entitled “Genetic analysis of inbred lines and inbred crosses of cocoa (*Theobroma cacao* L.), which forms a part of ongoing project at Cocoa Research Centre (CRC) was taken up with the following objectives

i. To evaluate the inbreds to quantify the magnitude of inbreeding depression in yield and yield attributes in various self-generations.
ii. To establish a physiological relationship between the vigour of inbreds, inbred crosses and hybrids.
iii. To study the protein profiling at critical stages of pollination using two dimensional gel electrophoresis protein profile analysis.
iv. To standardise pollination techniques to break self-incompatibility in fifth generation self-incompatible inbred

Review of Literature

2. REVIEW OF LITERATURE

2.1 Cocoa origin and cultivation

Cocoa (*Theobroma cacao* L.) tree is the source of one of the world's most delicious and familiar products chocolate. It is the third most important beverage crop, on which the chocolate industry is very much dependent. There is no substitute for cocoa for best chocolate making. Amazon basin and tropical areas of South and Central America are the centres of cocoa origin. *Theobroma cacao* is the only species, having beverage property and is under commercial cultivation out of other 22 species in the genus *Theobroma* organized in six sections (Cuatrecasas, 1964). The species *T cacao* is characterized by large genetic diversity (Bartley, 2005; Motamayor *et al*., 2008).

Earlier, it was grouped under the family Sterculiaceae (Purseglove, 1974), but now reclassified under Malvaceae family based on the morphological, anatomical and biotechnological studies (Sailaja *et al.,* 2015). Cocoa was cultivated by Mayas over 1500 years ago in the rainforest of Northern America and later it was distributed to Central America (Mirinda, 1962; Motamayor *et al.,* 2002,) Optimal fermented and dried cocoa beans are used to produce chocolate and several intermediate products such as cocoa liquor, cocoa butter, cocoa cake and raw cocoa powder. Cocoa powder can be used for flavouring biscuits, other dairy products, cakes and drinks (Frost *et al*., 2011).

The scientific name *Theobroma cacao* was given to the species by the Swedish botanist Carl Linnaeus in 1753, when he published it in his famous book *Species Plantarum*. *Theobroma* means 'food of the gods' in Latin, and *cacao* is derived from the Nahuatl (Aztec language) word xocolatl, from xococ (bitter) and atl (water) (Cuatrecasas, 1964). Cocoa was a valuable crop played an important role in many ancient South American cultures. In its earliest forms, the Mayans used cocoa to create a ritual beverage that was shared during betrothal and marriage ceremonies, providing one of the first known links between chocolate and romance.

For nearly 100 years after the Spaniards were introduced to Chocolatl the coveted drink of New World inhabitants, they kept the secret of its production to themselves. In the same years as Shakespeare wrote his final plays, the missionary and theologian Jose de Acosta wrote about cocoa from Lima, Peru, saying "it is so much esteemed among the Indian that it is one of the richest and the greatest traffickes of New Spain.

After a century, Spain lost its monopoly on the European chocolate market. By the mid-1600s, the drink made from the little brown beans had gained widespread popularity in France. It was praised as a delicious, health-giving food enjoyed by the wealthy. One enterprising Frenchman opened the first hot chocolate shop in London and by the 1700s, these "chocolate houses" were a common sight in England. By the 18th century, every country, from England to Austria, was producing confections from the fruit of the cocoa tree. During this period, the introduction of the steam engine mechanized cocoa bean grinding, reducing production costs and making chocolate affordable to all.

Chocolate is more than just a delicacy; evidence suggests that eating between 46 and 105g chocolate a day can have a moderate effect on lowering blood pressure. Cocoa has been used for an array of medicinal purposes. Unfermented cocoa seeds and the seed coat are used to treat a variety of ailments, including diabetes, digestive and chest complaints. Cocoa powder, prepared from fermented cocoa seeds, is used to prevent heart disease. Cocoa butter is taken to lower cholesterol levels, although its efficacy is unclear.

2.2. Importance and status of cocoa

The natural habitat of the genus "*Theobroma*" are evergreen forest, it is mainly cultivated in agroforestry ecosystem with other commercial crops like coconut, arecanut rubber, oil palm etc., Cocoa is now being a major export commodity from West African countries (Guiltinan *et al*., 2008). FAOSTAT (2015) reported that the exchange of bean

worths about US$ of 9.2 billion. Recently, the yearly world production of cocoa was estimated around 4 million tonnes (ICCO, 2016).

In India, Cocoa is widely cultivated in Kerala, Karnataka, Tamil Nadu and Andra Pradesh with an acreage of 78,000 hectares, which contributes around 18,920 million tonnes of cocoa beans annually. The productivity of cocoa beans is reported to be around 580 kg per hectare per year (DCCD, 2017).

2.3 Biodiversity in Theobroma

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

On the basis of morphology, genetics, geographical origin and flavor quality attributes, cocoa has been classified into three types, *viz*., Forastero, Criollo, and Trinitario (Cheesman, 1944). Cocoa pods vary with varieties in different qualitative aspects like size, colour, appearance and shape. The typical characters of Criollo types are small and elongated pod, intense rugosity, red or yellow coloured pod, deeply furrowed pod surface with ten ridges and furrows, slight pod basal constriction, attenuate pod apex form, white cotyledon colour, large bean size, increased dry bean weight and low husk thickness. Forastero type pods are generally thick walled, moderately sized, smooth textured, green coloured with bulbous or round shaped (Wood and Lass, 1985). Trinitario types are natural hybrids developed by crossing between Criollo and Forastero types and are indigenous to Trinidad and Tobago (Cheesman 1944). Trinitario types have red or yellow coloured pod and sometimes it can be orange or purple coloured with warty or smooth skin and elongated pods (Wood and Lass, 1985).

Among the three cocoa types, Forastero is considered as one with low quality, Trinitario with intermediate quality and the Criollo having high quality (Ciferri and Ciferri, 1957). The selection procedure for Criollo type is based on phenotypic traits like sweet pulp, white beans, elongated pods and high quality based on sensory attributes

(Engels, 1983). The Criollo beans are white to ivory or have a very pale purple colour, due to the presence of an anthocyanin inhibitor gene (Fowler, 1999).

The 'fine or flavour' cocoa based on quality are the Criollo and Trinitario. They have a very high demand among the chocolate manufacturers because of its high quality which fetch premium prices in the world market and they are used for the production of fine chocolates (Mooledhar, 1995). Fine cocoas are characterized as aromatic and smoother (Luna *et al*., 2002). Criollo beans are nutty and floral in flavour, Trinitario are acidic and fruity in flavour and Forastero is generally known as bulk cocoa with bitter and astringent flavour (Afoakwa, et al, 2008).

Criollo cocoa was cultivated during the pre-Columbian and colonial period in Latin America and it is characterized by premium quality when compared to Forastero types, but low performance in yield and vigour (Cheesman, 1944). At present, red pigmented fruits, a characteristic trait of Criollo and Trinitario types are controlled by a single dominant gene. However, they are not popular in Nigeria. This could be due to limited use of Criollo and Trinitario clones in Nigerian cocoa breeding programme (Bartley, 2005). Even though West African Amelonado cocoa types shows less vigour, it possesses attractive flavours to chocolate manufacturers. However, they were replaced by Upper Amazon Forastero types because of high vigour, so there is a need to retain the characteristic flavour quality profile of Criollo through breeding programmes (Aikpokpodion, 2010).

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

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

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

2.4 Studies on performance evaluation of cocoa

Ahnert, (2006) proposed ideotype breeding in cocoa and stressed that the characters like medium to fast-growing plants, early yield, plants with erect-growing branches, low vigour, resistance to pests and diseases, low pod index, seeds with more than 55 per cent fat content *etc*. are to be considered for selecting trees as elite materials for crop improvement. However, he also added that the cocoa trees in farmer’s fields possess much of the diversity to exploit them for developing elite cocoa varieties.

2.4.1 Tree morphology

Elain Apshara *et al.* (2009) evaluated six year old 44 trees clones of Nigerian cocoa germplasm for biometrical observation on yield and yield contributing characters at CPCRI- RS, Vittal. They revealed that plant height differed significantly among trees of all the clones. The trees of Cocoa NC-12 showed short stature with a mean height of 1.31m while, the cocoa trees of NC-23 have grown to a height of 4.55 m as the tallest one.

2.4.1.1 First branching height

The first branching height (Jorquette height) showed significant difference among the clones at CPCRI, Kasaragod which were introduced from Nigeria. The clone NC-36and NC-49 branched at a height of 0.42 m and the clone NC-26 had the first branching at1.10 m from ground level (Elain Apshara *et al*., 2009).

The variability for jorquette height was studied in a group of 135 cocoa trees of Forastero type in cocoa plantation of Alpara, district. The jorquette height ranged from 0.53 to 2.90m (Gregory, 1983)

The performances of elite progenies obtained from three progeny trials in their initial years of growth were studied by Elain Apshara *et al.* (2008) at CPCRI, Vittal. The results showed that the hybrids had their first jorquette at a height ranging from 0.26 m to 1.12 m and there was no significant difference in the first branching height.

Aikpokpodion *et al.* (2011) studied the performance of twenty-four hybrids of cocoa in Nigeria and reported that the maximum jorquette height in PA 13x P 19 (134.3 cm), followed by SNK 12 x PA 150 (128.7 cm), and lowest in T 85/799 x T79/501 (92.0 cm).

Thondaiman *et al.* (2013) assessed the variability in the plus trees of cocoa surveyed in farmers plantations of Tamil Nadu and reported that the jorquette height showed variation ranging from a minimum of 0.36 m to a maximum of 2.25 m with the

mean of 1.22 m. He also added that the jorquetting height was the minimum (0.36 m) in SEB 18, followed by 0.45 m in SEB 17 and was the maximum in KUL 2(2.25 m) and in SMJ 46 (2.10 m). The coefficient of variation for the jorquetting height was 26.90 per cent.

2.4.1.2 Stem girth

The girth of 135 cocoa trees of Forastero type measured at 15 cm above the ground level in plantations of Alpara, Thrissur district. The girth was ranged between 14.1 cm and 49.3 cm with a mean of 30.57 cm (Gregory, 1983).

Elain Apshara *et al.* (2008) assessed the performances of elite progenies obtained from three progeny trials in their initial years of growth at CPCRI, Vittal. The girth of the main trunk at 15 cm above ground level differed significantly with the highest value 43.00 cm in the hybrid in PII-5 and the lowest value of 25.23 cm in the hybrid P IIII-400.

The girth of the stem differed significantly among the Nigerian cocoa clones evaluated by Elain Apshara *et al.* (2009). The lowest tree girth of 10.13 cm was recorded in the clone NC-36 and the highest value was observed in the clone NC-63 with 36.25 cm. It was also observed that the stem girth increased correspondingly with plant height which indicated the vigour of the plant.

Opoku *et al.* (2011) evaluated the initial growth of 98 cocoa clones in local clone observation trial and reported that the girth of the main trunk measured 22 months after transplanting showed significant differences among the clones. The maximum girth of 44.4 cm was recorded in T 90/1383 clone. The stem girth ranged between 31.2 cm and 44.4 cm in the clones studied.

At Nigeria, Aikpokpodion *et al.* (2011) studied the performance evaluation of twenty four hybrids of cocoa and found that the stem girth varied from 7.8 cm to 12.8 cm and the hybrid T 85/799 x T 79/501 recorded the lowest stem girth. while, PA 13 x P 19 registered the maximum stem girth among the hybrids.

Thondaiman *et al.* (2013) studied the variability for stem girth by the plus trees of cocoa in farmer's plantations and the stem girth varied from a minimum of 22.30cm ` (SEB 10) to the maximum of 51.00 cm (SME 24). The mean value for tree girth was 35.22 cm and the coefficient of variation was 16.13 per cent.

2.4.1.3 Flowering

Flowering in cocoa is not uniform throughout the year and there are peaks during some months of the year in flowering. These periods of peak flowering are often different for different regions indicating its strong association with climatic factors. In a crop like cocoa, flowering is conditioned by many factors such as effect of sunlight, distribution of rainfall and presence of larger quantities of pod having strong inhibitory effect on flowering (Alvim, 1984).

Rajamony and Mohankumaran (1995) studied the flowering behaviour in eight-year-old cocoa trees of Forastero type planted at the Instructional farm of College of Horticulture, Kerala Agricultural University Thrissur and found a significant difference in flower production during different months of the year. The highest flowering was observed during December (17.31 per cent) followed by March (13.08 per cent). However, the peak period of flowering varies from place to place. In Ghana, the peak period was from March to July (Hewison and Ababio, 1930), in Bahia from October to May (Alvim, 1966) and in Cuba from June to September (Delpinalrivero and Acunagale,1967).

It was also observed that there was significant and negative correlation between rainfall of the preceding month and flowering of the trees. The minimum temperature and relative humidity one month proceeding the months of flowering had significant negative correlation with flowering, while, sunshine hours during the same period had significant and positive correlation (Rajamony and Mohankumaran, 1995).

The peak flower production during the cooler winter months *viz*., December to February was observed in the germplasm maintained at USDA-ARS, Tropical

Agriculture Research Station in Mayaguez (TARS), Puerto ico (Irish and Goenaga, 2012).

Flowering in cocoa trees is also influenced by the type of pruning. According to Govindaraj (2012). The highest number of flower cushions, flowers per cushion and number of flower per cushion and number of flowers per tree were seen in medium pruning. An adult cocoa plant can produce thousands of flowers per year, sometimes more than 50,000 of which only a small proportion (usually less than a percent) are pollinated and an even smaller proportion (0.5- 2.0 per cent) (Alvim, 1984) produce fruit set.

High temperature and increased soil moisture promote flushing and flower initiation and flowering intensity in cocoa (Omolaja *et al.* 2009).

2.4.1.4 Flower number per cushion

A significant difference between cocoa trees of eight-year-old in number of flowers per cushion was observed at KAU by Rajamony and Mohankumaran (1995). The average number of flowers per cushion was 9.3. The range was between one and twelve flowers per cushion.

2.4.1.5 Flower cushion number per tree

Among the flower characters, flower cushion number per tree is considered as the important trait for consideration of bean yield in cocoa. Variation in number of flower cushions per tree in cocoa was noticed. In cocoa, the number of cushions on trunk of 50 cm length varied from 5 to 32 with a mean value of 17.38 (Gregory, 1983). The number of cushions per unit length of 50 cm on the main trunk as well as in the fan branches was in the range of 237.50 to 281.60 (Rajamony and Mohanakumaran, 1995).

2.4.1.6 Pod set per cent

Cocoa flowers profusely with low per cent of pod set. At KAU, Rajamony and Mohanakumaran (1995) studied the pod set and development in Forastero types of cocoa

and reported that the pod set was 28.29 per cent. Efron *et al.*(2006) observed a range of eight to twenty-nine per cent in SG 2 hybrids widely grown in Papua New Guinea.

Amores *et al.* (2011) assessed the differences among the local clones of Ecuador for flowering, fruit set, cherelle wilt and reported that among the clones of study, only 54 flowers per tree (*i.e.* 19 per cent of all the flowers produced) on an average were able to set fruits. He also added that around 45 per cent of the fruits were affected by cherelle wilt and only 32 per cent of the fruits developed and ripened normally up to harvest while20 per cent of them reached harvest time as diseased pods.

Cherelle wilting is a mechanism by which the cocoa tree adjusts its production. They found that wilting of young cherelles occurred during the first 70 to 90 days of growth with a peak about 40 to 50 days after pollination. This period corresponded to the exponential phase of pod growth which is the time when the cacao tree eliminates pods exceeding its load capacity (Valle *et al.* 1990).Some workers (Pound 1933, Voelcker 1938, Humphries 1943 and Alvim 1954) have shown that the incidence of cherelle wilt is correlated with vegetative growth and is particularly severe during or shortly after a period of intense flushing.

Govindaraj (2012) observed that the incidence of cherelle wilt is common in neglected cocoa gardens and with less shade. He also reported that severe pruning resulted in increased sunlight and temperature inside the crop canopy that lead to the incidence of more cherelle wilt.

Aneja *et al*. (1999) studied the flowering and pod set per cent of cocoa clones and reported that the percentage of flowers that set into pods was very low, *i.e.* 0.5-5 per cent. They also added that it was partly due to the fact that the effective rate of auto pollination in auto incompatible trees was low. while, in auto compatible trees it can reach up to 43 per cent.

Hasenstein and Zavada (2001) deliberate the endogenous hormone levels in cocoa flowers during flowering and reported that there was a strong negative correlation

between number of flowers and endogenous auxin levels. It was also reported that high auxin levels also override the abscission signal, affecting or controlling the auto incompatibility response in cocoa.

The main pollinators, biting midges (Ceratopogonidae), and gall midges (Cecidomyiidae), which are moisture-loving dipterans, are known to increase in population during the rainy months (May to July) and much less in the dry seasons (Brew, 1988).

Pound (1933), Voelcker (1938), Humphries (1943) and Alvim (1984) have reported that the incidence of cherelle wilt is correlated with vegetative growth and is particularly severe during or shortly after a period of intense flushing.

2.4.1.7 Pod characters

In cocoa, pod and bean are the economically important traits to be considered for improvement. The number of pods per tree, number of beans per pod, dry bean weight and dry bean yield per tree are to be enhanced for improving the yield of cocoa.

Variability in length, width, thickness of shell, pulp percentage and number of beans per pod in fresh samples of cocoa from different genetic origin were also observed. Interclonal differences in these characters were found to be highly significant indicating their usefulness for clonal classification.

2.4.1.8 Pod length

A wide variation in the pod length of 135 cocoa trees of Forastero type was observed by Gregory (1983). The pod length varied from 10.3 cm to 18.3 cm with a mean value of 14.3 cm. In yet another study conducted at Kerala Agricultural University, Homey (1993) reported variability in pod length ranging from 9.4 cm to18.35 cm with the mean pod length of 15.6 cm.

Bekele *et al.* (2006) studied the patterns of morphological variation in 600 cocoa accessions maintained in germplasm of the International Cocoa Genebank, Trinidad and

reported that the pod length varied from 11.7 to 22.2 cm in the wild cocoa trees while the same was in the range of 11.2 to 22.6 cm in the cultivated cocoa types. The mean pod length in the same population differentiated as Forastero, Refractarios and Trinitarios was15.9 cm, 16.0 cm and 16.3 cm respectively.

Efombagan *et al.* (2009) studied the phenotypic variation of cocoa trees in farms and gene bank of Cameroon and found that the average pods length of 14.8 cm among the 300 accessions in farms and 18.3 cm among the 77 accessions maintained in the gene bank.

Twenty-one elite progenies from progeny trials were evaluated for a period of four years by Elain Apshara *et al.* (2008) at CPCRI-RS, Vittal. The results showed significant variability for length of pod and the hybrids PI-II-400 (19.95 cm) and PI-I-38 (19.71 cm) registered the highest value. In yet another study, Elain Apshara *et al*. (2009) observed smaller pods with 13.20 cm length and 6.7 cm breadth among the clonal population of cocoa.

At Guyana, observation on pod length in 21 selected organic farms of cocoa showed that the mean pod length of sixty five accessions studied was 165.9 mm (Chesney, 2007).

Thondaiman *et al.* (2013) reported that the plus trees of cocoa in Tamil Nadu showed variability for pod length and the values varied from a minimum of 10.20 cm (SEB 10) to a maximum of 20.10 cm (VPS 8) with a mean value of 15.11 cm.

2.4.1.9 Pod girth

Gregory (1983) evaluated the Forastero cocoa types at Alpara, Kerala, and registered a wide variation of pod girth with a range of 5.2 cm to 10.1 cm The pod girth of the wild cocoa trees maintained at the International Gene Bank, Trinidad varied from 6.0 cm to 11.1 cm. while, the cultivated types showed a pod girth of 6.7cm to10.5cm.The mean girth of the pods in wild and cultivated types was 7.83cm and 8.18cm respectively. The same population was divided as Forasteros, Refractarios and

Trinitarios and analyzed for mean girth of pods. The results showed that the pod girth was 7.82, 8.29 and 8.09 cm respectively in trees of Forastero, Refractarios and Trinitarios type (Bekele *et al.,* 2006).

The hybrids PII-5 and P-I-38 were recorded bold pods with pod girth of 8.29 cm and 8.13 cm respectively among the twentyone elite progenies of cocoa (Elain Apshara *et al.,* 2008).

The cocoa trees in gene banks and farms were evaluated for their performance at Cameroon and observed a significant variation in pod girth of 8.3cm and 7.0cm respectively. (Efombagan *et al.,* 2009).

Thondaiman *et al.*(2013) recorded an average pod girth in plus trees of cocoa in Tamil Nadu and it varied widely from 21.09 cm (SME 21) to 32.50 cm (VPS 21) with a mean pod girth of 25.89 cm.

2.4.1.10 Pod weight

Gregory (1983) reported a wide variability in cocoa of Forastero type and revealed that the pod weight ranged from 162 g to 804 g with a mean value of 483 g. A wide variability in pod weight with the range of 138.75 to 248.75g was reported by Homey (1993).

In a study conducted at CPCRI-RS Vittal, it was noticed that the total pod weight per tree per year ranged from a minimum of 2.5kg to a maximum of 29.20 kg between the clones. Among the 44 clones tested, the weight of the individual pods ranged from 240.70g to 745.40 g. Heavier pods measuring more than 500g were harvested from 16 other clones that showed significant variability for all the pod characters of the study (Elain Apshara *et al*., 2008).

A significant variability for pod weight between the cocoa trees in farms and gene bank of Cameroon was reported by Efombagan *et al., (*2009). The mean weight of

pods of cocoa trees in gene bank was 622.5 g. while, the mean weight of pods in the farms was 510.6g.

Efron *et al.*(2006) conducted a study on the variability for yield and yield components in ten SG2 hybrids of Papua New Guinea and reported that the average pod weight ranged from 292 g to 588 g in the crosses *viz.,* KEE 12 x K82 and KEE 5 x KA2 -106 respectively.

Elain Apshara *et al.* (2008) evaluated the performance of 21 progenies of cocoa hybrids at CPCRI-RS, Vittal and reported that the hybrids PII-5, PIII-I-14, PI-I-38 and PI- II-400 yielded pods with weight more than 400 g and marked them as heavy pod yielders in terms of pod weight.

Marfu *et al.*(2011) assessed the performance of cocoa clones of four sub groups *viz.,* big (625plants/ha), intermediate (714plants/ha), small (833plants/ha) and very small (1000plants/ha) in Papua New Guinea. The results showed that the pod weight of the clones differed significantly and were in the range from 330 to 900 g in big clones, from 325 to 635 g in intermediate clones, 305 to 615 g in small clones and 300to 655 g in very small clones.

Thondaiman *et al.* (2013) studied the variability for plus trees in cocoa and reported that the pod weight of all trees studied varied from the lowest value of 438.11g in SME21 to the highest value of 815.00g in VPS8. The mean value of the pod weight of all trees was 427.48g.

2.4.1.11 Bean characters

Bean is the economically important produce in cocoa. The quantitative and qualitative characters of cocoa should be given much importance in any cocoa improvement programme. Enriquez and Soria (1966) studied the variability of biometric characters in cocoa in Costa Rica. They indicated that yield expressed in terms of dry or wet weight of the cocoa beans was a highly variable character and of quantitative nature. There existed a high variability in weight of seed even within a single pod.

2.4.1.12 Bean length

Homey (1993) evaluated the performance of 19 hybrids of cocoa at KAU and he reported that among the bean characters studied, the bean length ranged from 14.6 mm to 33.8 mm.

Lachenaud and Oliver (2005) studied the variability for morphological bean traits in 96 wild cocoa trees in French Guiana and reported that the average bean length varied from 20.43 mm to 25.98 mm with a CV of 5.27 percent.

The bean length of wild and cultivated types of cocoa in the International Cocoa Gene Bank, Trinidad was in the range of 1.47 to 2.69 cm and 1.73 to 2.72cm respectively. The mean length of bean was 2.08 cm and 2.21 cm respectively in the wild and cultivated type of cocoa (Bekele *et al.,* 2006). He also reported that the average bean length of Forasteros, Refractarios and Trinitarios type in the germplasm collection was 2.08, 2.17 and 2.31 cm respectively.

Efombagan *et al.,* (2009) reported significant variation for seed length between the accessions of cocoa maintained in farms and gene bank. The maximum value for seed length was registered in gene bank accessions as 26.6 cm while the farm accessions registered a value of 23.8 cm.

2.4.1.13 Bean girth

The bean girth of 19 cocoa hybrids developed at Kerala Agricultural University was found to be in the range from 8.4 mm to 13.0 mm (Homey, 1993). In French Guiana, the wild trees of Forastero type cocoa was evaluated for bean characters and it was found that the average bean width varied from 10.09 to 13.92mm with a CV of 7.14 percent (Lachenaud and Oliver, 2005).

Bekele *et al.,*(2006) assessed the variability for bean characters of 600 accessions of wild and cultivated cocoa types of Trinidad and found that the average bean girth was maximum in the wild type (1.58cm). while, the cultivated type registered the mean bean

girth of 1.23 cm. He also reported that the mean bean girth in the Forateros, Refractarios and Trinitarios was 1.16, 1.20 and 1.29 cm respectively.

Efombagan *et al.* (2009) studied the variability of farm accessions and gene bank accessions of cocoa and reported that the mean seed girth of 13.1cm was observed in farm accessions while the gene bank accessions had a higher mean seed girth of 14.5cm

2.4.1.14 Bean number per pod

Gregory (1983) reported a significant variability for number of beans per pod in a population of 135 trees of Forastero type and the mean value was 41.00. In Ghana, Adomako and Adu-Ampomah (2003) studied the variability in cocoa and observed that the number of beans per pod ranged from 30.70 to 37.90 with the mean value of 34.6 and the coefficient of variation of 5.09 per cent. Naturalized cocoa population originating from the Oyapok and Tanpok basins in French Guiana was studied for their bean characters by Assemat *et al.* (2005). They reported that the bean count ranged from 83 to 110 per pod.

Elain Apshara *et al*. (2009) evaluated the elite progenies of cocoa and found that the number of beans per pod had a large variability ranging from 26.30 to 49.70. In this trial, pods with more than 40 beans were observed in 34 cocoa clones.

Bekele *et al.* (2006) studied the morphological variation of wild and cultivated types of cocoa in Trinidad. They reported that the mean number of beans per pod was 40.6 and 38.8 in the wild and cultivated types respectively. The cultivated cocoa types showed a range of 17 to 54 while wild types exhibited a range of 21 to 58 for bean number. The number of beans per pod was noted as 40.5, 38.5 and 39.2 in Forastero, Refractarios and Trinitarios type trees respectively among the 600 accessions.

At International Cocoa Gene Bank, Trinidad, Iwaro *et al*. (2003) evaluated 581 genotypes of cocoa and found that the bean number was normally distributed and it varied from 17 to 58 with a mean of 40 per pod. There were significant differences ($p \leq 0.001$) among the three main groups (Forastero, Refractario and Trinitario) and

among the six population (B, ICS, IMC, JA, NA and PA) evaluated for bean number. Ninety-nine (17.0 per cent) of the 581 genotypes had a large bean number (>45). Among the three main groups of cocoa evaluated, Forastero had a relatively higher percentage (22.6per cent) of genotypes with large bean number than Trinitario (14.0 per cent) or Refractario (6.1 per cent). The greatest proportion of genotypes with an intermediate (36-45) or a small (<36) bean number was observed in the Refractario group (60.0 percent and 33.9 per cent, respectively). The IMC population had the highest percentage of genotypes with large bean number (68.6 per cent), while the greatest proportion of genotypes with intermediate or small bean numbers was observed in the JA (72.0 per cent) and PA (43.2 per cent)population.

The number of beans per pod varied significantly between the cocoa trees of gene bank and those maintained in the farms. The farm accessions had a mean seed number of 40.5. while, the gene bank accessions had a value of 38.5 (Efombagan *et al.,*2009).

Elain Apshara *et al.,* (2008), evaluated twenty one elite progenies of cocoa at CPCRI-RS, Vittal. The number of beans per pod showed variability ranging from 34.13 to 47.60

Chesney (2007) studied the 65 cocoa trees selected from 21 organic farms of Guyana and reported that the mean number of beans per pod was 34.8 with a S.D of 9.1.

Lamin *et al.* (2011) assessed the performance of cocoa clones at Madai and Jengka regions of Malaysia comprising of sixteen and sixty two clones respectively. The average number of bean per pod was found to be 34 and 37respectively.

Maharaj *et al.* (2011) evaluated the phenotypic characters of thirty cocoa clones selected in farmers' fields of Trindad and Tobago and reported that an average of 46 beans per pod was observed in the trees studied.

Morphological characterization of germplasm containing 2300 accessions in the International Cocoa Gene Bank,Trinidad revealed that the number of beans per pod

ranged from 17(B5/11) to 59 (IMC39),with a mean value of 39.6. It was also observed that four hundred and seventy-six accessions had bean numbers equal to or greater than 40 per pod and one hundred and seventyeight accessions had ≥ 45 beans per pod and fifty accessions had ≥50 beans per pod (Bekele and Bidaisee, 2006).

Lambert *et al.* (2009) analyzed sixty eight accessions of cocoa selected by the South Sulawesi Extension Service, Sulawesi for number of beans per pod and reported that the farm accessions varied significantly for bean count from 52 to 170 numbers per pod.

The variability among the cocoa trees in farmers' plantations of Tamil Nadu was studied by Thondaiman *et al.* (2013) and the results showed that the number of beans per pod varied from a minimum of 25.50 in KUL 15 to a maximum of 50.50 in SMJ 7. The mean value of the number of beans per pod of all trees was 9.45.

Flat and shriveled beans contain very little nib and hence reduce the yield of edible material. Flat beans are the result of aborted embryos (Martinson, 1966) under unfavorable conditions and their percentage in pods depends on genotype-environment interactions (Lachenaud *et al.,*1994).

Abundance of flat beans (6.9 per cent), were more in the population of the Cam 3, Cam 9 and Cam 13 and they indicated the greater susceptibility of these accessions to unfavourable environmental conditions, such as competition between trees and certain nutrient deficiencies. (Lachenaud and Oliver, 2005)

Assemat *et al.* (2005) found flat bean percentages less than one percent in their fermented dried bean samples of wild Guianian population.

Cope (1962) studied the floral biology of cocoa trees and reported that the number of fertilized ovules was limited by the number of pollen grains applied.

2.4.1.15 Single fresh bean weight

The cocoa trees of Forastero type in Kerala showed variability for single bean wet weight with a range from1.7g to 4.7g (Gregory, 1983). The mean weight per bean in wet stage was 2.55 g. Variability in 96 wild cocoa trees belonging to ten populations from the Camopi and Tanpok rivers of French Guiana were characterized for their average fresh bean weight by Lachenaud and Oliver (2005). Substantial variability was noticed for average fresh bean weight, with the coefficient of variation of 10.5 per cent and a range of 2.20 to 3.82g.

2.4.1.16 Single dry bean weight

Single dry bean weight of cocoa is the prime important bean character to be considered for crop improvement programme. Pound (1932) reported a wide range of single bean weight from 0.8 to 2.5g. The average weight of a dry bean (seed index) in cocoa trees from plantations of Alpara, Kerala was observed as 0.97g. The weight of single bean was in the range from 0.60g to 1.20 g (Gregory, 1983). Adomako and Adu-Ampomah (2003) studied the variability in cocoa trees of forastero type and reported that the dry bean weight was found to be ranged from 1.1g – 1.2g with the mean value of 1.1g and coefficient of variation as 4.6 percent.

A study was conducted by Cilas *et al*. (1989) in cocoa with twenty clones of cocoa belonging to upper Amazon, Amelonado and Trinitario types which resulted in extreme variability on bean size and tended to be the greatest in Trinitario types. It was also noticed that the average bean weight per 100 fermented and dried beans was found to range from 212.60 g for clone UF 66F (Trinitario) to 67.50 g for SCA 6 (Upper Amazon).

The variability for single bean dry weight in elite clones evaluated by Elain Apshara *et al.* (2009) at CPCRI-RS, Vittal was in the range from 0.7 to1.29g. Among the clones evaluated, seventeen clones recorded more than one gram of single bean dry weight.

Among the cultivated and wild cocoa trees maintained in the germplasm of International Cocoa Gene bank, Trinidad, a significant variation of single dry bean weight was observed and it ranged from 0.51 to 1.39g and 0.55 to 1.88g in the wild and cultivated types respectively. The mean weight of dry bean was 0.94 g and 1.09g respectively. The germplasm was divided into trees of Forasteros, Refractarios and Trinitarios type and analyzed for variation in single dry bean weight. The results showed an average bean weight of 0.94, 1.08 and 1.15 g respectively (Bekele *et al.,* 2006).

Iwaro *et al.* (2003) evaluated the performance of 581cocoa genotypes in the germplasm of ICG, Trinidad and reported that the bean weight varied from 0.44 g to1.84 g with a normal distribution and a mean of 0.96g. Sixty-four genotypes (11.0 per cent) had a large bean weight (>1.2g) among the 581 accessions assessed. The Trinitario group possessed the highest percentage (36.9 per cent) of genotypes with large bean weight, while the Forastero group had the highest proportion of genotypes (27.4 per cent) with small bean weight (<0.81g). Among the populations, ICS had the highest percentage of genotypes (44.1percent) with large bean weight, while the greatest proportions of genotypes with intermediate and small bean weight were recorded in the B (83.4 per cent) and NA(25.6 per cent) populations, respectively.

The single bean dry weight of farm and gene bank accessions of cocoa in Cameroon varied and was found that the gene bank accessions had the maximum value of 0.93g while the farm accessions had a value of 0.92 g for individual bean dry weight (Efombagan *et al.,* 2009).

Elain Apshara *et al.* (2008) assessed the performance of twenty one hybrid progenies and reported that the single dry bean weight ranged from 0.673 to 1.017 g and the hybrids PIII-I-8, PIII-III-20, PIII-I-23, PI-I-38 and PII-4 showed considerably higher single dry bean weight nearing unity.

The average of single dry bean weight of 65 cocoa trees selected from 21organic farms in Guyana was 1.0 g (Chesney, 2007).

Lamin *et al.* (2011) assessed sixteen individual trees in clonal trials at Malaysian Cocoa Board, Research and Development centre in Madai and reported that the mean dry bean weight of the clones was 1.07g with more than eight clones yielding a single dry bean weight >1.0g.

In yet another study conducted by Lamin *et al.* (2011) at Research and Development centre of MCB, Malyasia, the average bean weight of sixty two clones was1.12 g with seven clones yielding a single dry bean weight more than 1.10g.

The average single bean dry weight of cocoa clones selected in farmers' fields of Trinidad and Tobago was 1.15 g (Maharaj *et al.,*2011).

Gonzalez *et al.* (2011) evaluated twenty-five clones for their yield performance in the International Clonal trial of Venezuela. The results showed that the mean single dry bean weight of two clones *viz.,* PA 150 and EET 59 was 3.1 and 3.2g respectively. In yet another study conducted by Gonzalez *et al.* (2011) for evaluating the two population of 15 families derived from crosses between Trinitario clones in Venezuela, the single dry weight of the bean was in the range from 1.4 to 1.8 g and 1.3 to 1.6 g in population I and II respectively.

Bekele and Bidaisee (2006) evaluated 2300 accessions of cocoa maintained in the International Cocoa Gene bank, Trinidad and observed that the single bean dry weight ranged from 0.44g (B10/28 and PA46) to 1.84g (UF11), with a mean value of 0.97g. They also reported that one hundred and twenty-eight accessions had single bean dry weight equal to or greater than1.2g while sixty-seven accessions had bean weights of 1.29 g or greater and ten had bean weights ≥1.5g.

Lambert *et al.* (2009) analyzed the performance of sixty eight accessions of cocoa selected in farmers' fields of Sulawesi and found that the average single bean dry weight was 1.07 g with a minimum dry weight of 0.59 g and maximum weight of 1.92 g. Single bean dry weight of cocoa trees studied in farmers plantations of Tamil Nadu

varied from a minimum of 0.59 g in tree SMJ43 to a maximum of 1.71g in tree SMJ 36 with an overall mean of 1.00 g (Thondaiman *et al.,* 2013).

2.4.1.17 Fresh bean weight per pod

A significant variability for wet weight of beans per pod in cocoa plantations of Alpara, Kerala was reported by Gregory (1983) and it ranged from 51.4 g to 263.2 g with a mean value of 157.3 g per pod.

The evaluation of elite progenies of cocoa by Elain Apshara *et al*. (2009) revealed the variability in wet weight of the beans per pod ranging from 69.83 to 145g.

Dias and Kageyama (1997) assessed the five cacao cultivars (selfs) and 20 hybrids for five years and reported that the wet bean weight per pod varied from 80.90 to 129.99g while the wet bean weight per tree ranged from 1.66 kg to 5.61 kg. The wet bean weight per tree was observed to be more in hybrids (4.23 kg) than in the cultivars (2.72 kg).

The wet bean weight per pod from cocoa trees of SG 2 hybrids were studied by Efron *et al.* (2006) in Papua New Guinea. The results showed that the percent of wet bean in the pod was found to be ranged from 25.6 per cent to 33.6 percent in KEE47x KA2-106 and KEE 5 x KA2-106 respectively.

In Kerala, the performance of 19 cocoa hybrids was evaluated by Homey (1993) and he reported that the wet bean weight of 20 beans was observed to range from 7.5g to 24.3g.

The wet bean weight per pod of cocoa trees studied in farmers plantations of Tamil Nadu varied from a minimum of 73.79g in SME 21 to a maximum of 210.50 g in VPS 7 with a mean value of 121.42g (Thondaiman *et al*., 2013).

2.4.1.17 Dry bean weight per pod

In Karnataka, the elite clonal progenies of cocoa were evaluated and the study revealed that the dry weight of beans per pod ranged from 27.8 to 56.5 g with a mean of 42.15 g (Elain Apshara *et al*., 2009).

The comparative performance of elite progenies of cocoa at CPCRI-RS, Vittal showed that the weight of 50 beans ranged from 16.76 to 55.80 g in the hybrids tested (Elain Apshara *et al.,* 2008).

2.1.5 Yield characters

Yield is considered as the prime criterion in any crop improvement programme. Existence of considerable variability is essential to formulate any breeding programme. A considerable quantity of variation in cocoa pod and bean yield traits was observed by many cocoa workers both in India and in many cocoa growing countries.

2.5.1 Number of pods harvested per tree

The yield performance of 19 cocoa hybrids was studied by Homey (1993) and reported that among the pod characters of study, the number of pods varied from 1 to 91 with the coefficient of variation of 58.85 per cent. The variability in the yield of pods per tree per year was studied by Gregory (1983). He reported that the number of pods produced per tree per year ranged from 2 to 134 with a mean of 33.51. Pound (1932) had also reported that Forastero type produced 100-200 pods per tree per year.

At Central Plantation Crops Research Institute, Regional Station, Vittal, Elain Apshara *et al.,* (2008) studied the variability in pod characters in clonal populations of cocoa and observed the number of pods obtained per tree during each harvest was accounted from fifth year of bearing up to twelfth year along with pooled mean yield over eight years. A gradual increase in pod yield was observed during 2000-2007 among the clones of the study. Initial number of pod yield per tree at fifth year was observed to be ranged from a minimum of 2.80 to a maximum of 43.60 while at the age of twelve

years, the pod yield ranged from 14.5 to 63.8 and some of the clones registered good pod yield with an average of more than 45 pods per tree per year.

Dias and Kageyama (1997) assessed the five cacao cultivars (selfs) and 20 hybrids for five years and reported a wide range of pod yield per tree. The range was observed from 17.49 to 56.39 pods per tree. The mean number of pods harvested per tree was higher in the hybrids (39.07) than cultivars (28.44).

Efron *et al.* (2006) studied the yield performance of ten SG2 hybrids in Papua New Guinea and reported that the average number of pods per tree ranged between 49.0 and 74.8.

The comparative performance of elite progenies of Cocoa was studied by Elain Apshara *et al.* (2009) at CPCRI-RS, Vittal and the number of pods per tree per year analyzed over a period of four years was more than 50 in the hybrids PI-IV-478, PI-I-38,PIII-I-23, PIII-II-54 and PI-I-18 and termed them as potential high yielders.

Observations on number of pods per tree in 151 cocoa trees studied in farmers plantations of Tamil Nadu varied from a minimum of 32 in SEB 15 to a maximum of 108 in SMJ 11, with an overall mean of 60.49 (Thondaiman *et al.,*2013).

Adjaloo *et al*. (2012) studied the floral phenology and fruiting characters of cocoa trees maintained in farmer fields in Ghana and reported that the number of diseased pods exceeded total fruit production during high rainy season *i.e.,* between August and October.

2.5.2 Dry bean yield per tree

A study conducted at Kerala Agricultural University, Thrissur, Kerala by Homey (1993) revealed that among the 19 cocoa hybrids studied, the yield per tree in terms of dry cocoa bean ranged from 1319.90g to 4897.02 g and the coefficient of variation was 57.61 per cent.

The variability in yield of Nigerian cocoa clonal populations was studied by Elain Apshara *et al*. (2009) and reported significant variation in yield of clonal populations which indicated a range of 0.24 to 2.53 kg of dry beans of cocoa per tree and 22 clones yielded more than one kg of dry bean yield per tree. Irish and Goenaga (2012) reported that the dry bean yield per tree ranged from 0.13 kg per tree for the least productive to 1.94 kg/tree for a local hybrid selection'TARS27'.They also added that under existing TARS cacao germplasm cultural and management practices, some of the most productive clones yielded approximately 3000 kg/hectare.

Goenaga *et al.* (2009) evaluated the TARS series of cacao germplasm selections and reported that in the initial four years of evaluation of the 1,320 trees observed in all population and locations, only nine trees were superior yielders with a mean production of 2,170 kg per ha per year of dry beans.

Elain Apshara *et al*. (2009) evaluated the146 accessions of cocoa maintained in the germplasm of CPCRI-RS, Vittal for yield characters and reported that the tree number 1-56 yielded the maximum dry bean weight of 1.20g while the lowest value was registered byNC42/94 (1.08g.). They also reported that the four hybrids and one clone selected in the crop improvement programme of cocoa had an yield potential of 1.13 to 1.48 and1.15 kg per tree per year respectively.

Elain Apshara *et al*. (2008) assessed the performance of twenty one progenies of cocoa at CPCRI-RS, Vittal and the results showed that the annual dry bean weight per tree ranged from the lowest of 0.22 kg to the highest of 2.64 kg in the hybrids tested. Twelve hybrids out of 21 showed high field efficiencies of more than 1kg of dry bean per tree per year.

Monteiro *et al.* (2011) evaluated 45 cocoa clones selected in farm surveys in six distinct farms in Bahia, Brazil. They reported that the productivity mean of the clones was 975 kg per ha in the main harvest season and 420 kg per ha in the secondary harvest season, during the period from 2004 to2007.

In multilocation on-farm variety trial of 30 cocoa clones conducted at farms of Joventina, NovaTranquilidade, Santa Helena and Monte Alegre, Brazil, Monteiro *et al.* (2011) observed significant difference in dry bean weight per plant among the clones. In the Santa Helena farm, the mean dry bean weight per plant and per clone ranged from 173.7 to 975.0 g and the coefficient of variation ranged from 75.7 to159.9 per cent. In the Joventina farm, the ranges observed were 85.0 to 1662.3 g for dry bean weight and 48 to 192 percent for the coefficient of variation and for the Monte Alegre farm, the values were 50.2 to 1120.7 g and 45 to 156 per cent for dry bean yield per plant and coefficient of variation respectively.

In yet another multi location trial of cocoa clones conducted by Monteiro *et al.* (2011) at four locations of Brazil, the dry bean yield per tree differed significantly. The mean dry bean yield in BeloHorizonte ranged from 47.3 to 241.0g per plant and the coefficient of variation ranged from 79.6 to 181.2 per cent, while the mean values for the control varieties SCA 6, SIAL 169, SIC 23 and TSH 1188 were 97.8, 74.2, 41.9and 117.6g per plant, respectively. Similarly, in Lagoa Grande, the dry bean yield per plant ranged from 179.0 to 920.7 g and the coefficient of variation ranged from 48.0 to120.4 percent. The control varieties SCA6, SIAL169, SIC23 and TSH1188 registered a mean dry bean yield of 298.0, 179.0, 200.9 and 747.8 g per plant, respectively.

The trial conducted at Nova Vitória, revealed that the dry bean yield per tree ranged from 128.1 to 750.2g among the cocoa clones studied and the coefficient of variation ranged from 54.1 to 120.4 per cent. The mean dry bean yield per tree of control varieties SCA 6, SIAL 169, SIC 23 and TSH 1188 were 411.0, 128.0, 137.0 and 375.0 g per plant, respectively. In Porto Seguro, the mean values for dry bean yield per tree ranged from105.0 to 289.0 g per plant, with coefficients of variation ranging from 92.3to 140.1 per cent and the mean dry bean yield of the control varieties SCA 6, SIAL 169, SIC 23 and TSH 1188 were 286.0, 148.0, 105.9 and 283.5 g per plant, respectively.

Aikpokpodion *et al.*(2011) assessed the performance of 23 bi-parental hybrids along with F_3 Amazon as control in Nigeria and reported that the mean dry yield

increased from 338.46 kg per ha during second year of planting to 1560 kg per ha at eight year after planting.

The performance of thirty eight high yielding clones was assessed by Marfu *et al.* (2011) in the experimental farms of East New Britain and Madang of Papua New Guinea during 2003-09. The clones were divided in to four subgroups *viz.,* big (625plants/ha), intermediate (714 plants/ha), small (833 plants/ha) and very small (1000 plants/ha).The results showed that the total yield variations among the four sub-groups tested varied between 5891 kg/ha (very small) and 5156 kg/ha (intermediate). The highest yields came from the very small clones, followed by small clones (5353 kg/ha), big clones (5248 kg/ha) and intermediate clones (5156 kg/ha).

One hundred and fifty one cocoa trees were studied for their variability in farmers' plantations of Tamil Nadu by Thondaiman *et al*. (2013) and the results showed that the yield per tree varied from a minimum of 0.85kg in SMJ 40 to a maximum of 3.96 kg in SME 24. The mean value of the dry bean yield per tree of all trees was 2.39kg.

2.5.3 Pod value

In cocoa, pod value or index is defined as the number of pods required to produce one kg of dry beans. Pound (1932) observed that the pod value was found to vary from 6 to 22 pods per pound of dry cocoa beans. The pod value of 135 cocoa trees observed in the cocoa plantations of Alpara, Kerala was found to be varied and ranged from 18 to57 with a mean value of 37.5.The cocoa trees evaluated in the germplasm collection of USDA-ARS, Tropical Agriculture Research Station in Mayaguez (TARS), Puerto Rico for five years showed the pod value ranging from 51.6 to 14.0 (Irish and Goenaga,2012). The pod value of wild and cultivated cocoa types maintained in the germplasm of International Cocoa Genebank, Trinidad varied significantly with the cultivated cocoa types having pod value of 25.0 and wild cocoa trees having 27.6 as pod value. The mean pod value of Forastero, Refractarios and Trinitarios in the same population was 27.8, 25.6 and 23.3 respectively (Bekele *et al.,*2006).

Iwaro *et al.* (2003) studied the germplasm collections maintained in ICG, Trinidad and reported that the Pod index ranged from 13.9 to 66.1 with a mean of 27.9. He added that only 56 of the 581genotypes evaluated (9.6 per cent) had low pod index (<20.1).Genotypes with low pod index were more prominent in the Trinitario group (28.1 per cent) than in the Refractario (10.9 per cent) and Forastero groups (4.5 percent). The greatest proportions of genotypes with high and moderate pod indices were observed in the PA (23.0 per cent) and B (83.3 per cent) populations, respectively.

Efombagan *et al.* (2009) studied the farm and gene bank accessions of Cameroon for their pod characters and reported that the gene bank accessions had the low pod value (24.2) than the farm accessions (26.3).

The mean pod value of 65 trees surveyed in 21 organic farms of cocoa in Guyana was 36.3 with range from 10.2 to 124.5 (Chesney, 2007)

The mean pod value of cocoa clones evaluated at Research and Development centers of Malaysian Cocoa board in clonal evaluation trials consisting of sixteen and sixty-two clones was observed to be 30 and 25 respectively (Lamin *et al.,*2011).

Maharaj *et al.* (2011) assessed the performance of thirty cocoa clones in Trinidad and Tobago and reported that the clones exhibited an average pod value of 22.1.

The performance of twentyfive international clones was assessed in experimental farms of Venezuela by Gonzalez *et al.* (2011). The results showed that six of the total clones studied *viz.,*MAN15-2, PA107, EET59, Mocorongo, IMC47 and PA150 had pod values between 22 and 30, with EET59 (22.0) and MAN15-2 (22.1) having the lowest average values within that range.

In yet another study conducted by Gonzalez *et al.* (2011) for evaluating two populations of the 15 families derived from crosses between Trinitario clones in Venezuela, the pod values of the hybrids were in the range from 19 to 23 and 17 to 22 in population I and II respectively.

Bekele and Bidaisee (2006) evaluated 2300 accessions of cocoa maintained in the International Cocoa Gene bank, Trinidad and reported that the pod value ranged from 13.9 (UF11) to 92.8 (B9/10/35), with a mean value of 27.8

Pod value of 151 cocoa trees observed in farmer's plantations of Tamil Nadu varied from a minimum of 15.29 in tree SMJ 36 to a maximum of 52.88 in tree KUL 26, with an overall mean value of 26.54 (Thondaiman *et al.,*2013).

2.6 Pests and Diseases

Cacao black pod, an economically serious problem throughout the world, where cocoa is grown cause significant pod losses of up to 30 percent and results in the death of 10 percent of the trees annually (Matos *et al.,* 1998).

Cocoa black pod, is a devastating diseases in cocoa growing areas of the world, which causes significant pod losses up to 30 per cent and 10 percent death of trees annually (Matos et al., 1998)

Black pod disease generally called as black cocoa was first reported from Guyana and West Indies (Jenman and Harrison, 1897). In India it was first reported in 1965 (Ramakrishnan and Thankappan, 1965) and the causal organism for black pod disease was reported as *Phytophthora palmivora* (Chandramohanan, 1979). Chandramohanan (1982) observed certain level of tolerance in Nigerian cocoa collections against black pod disease.

Pods or cherelles can be infected at any location, infection mostly occurs at the tip or stem end of the pod and more frequently on pods close to the soil. Firm, spreading, chocolate-brown lesion affects the whole pod. When husk become infected, Phytophthora sp. enter inside the pod and results in discoloration and shrivelling of the cocoa beans. Later infected pods became black and mummified (Deberdt et al., 2008). Prabha and Chandramohanan (2011) conducted a survey in Southern states of India to find the occurrence of major diseases of cocoa revealed that Phytophthora diseases were the most important one which causes great economic loss. Among the Phytophthora

diseases black pod disease caused by *Phytophthora palmivora* was mostly noticed. In Kerala black pod incidence was reported as 90.75 per cent of the gardens surveyed. Vascular streak die back disease incidence was reported as 17.8 per cent of the gardens surveyed in Kerala. Cherelle wilt, Colletotrichum pod rot, chupon blight, twig dieback, white thread blight, horse hair blight and pink disease were also reported but not arised as a serious problem.

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

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

Rodents, another important group of major pests reported from almost all cocoa growing countries (Taylor, 1972; Williams, 1973; Gratz and Arata, 1975). Abraham and Padmanabhan (1967) reported rat damage in the cocoa pod from India as early in 1967. In cocoa plantations, a heavy damage by rodents of about 75 per cent has been reported (Advani, 1982). Black rat (*Rattus rattus*), the Western Ghat squirrel (*Funambulus*

tristriatus) and the South Indian palm squirrel (*F. palmarum*) are the major rodent pests which causes damage to cocoa pods and beans. Timely harvest of mature pods reduced squirrel attack from 52 to 25 percent just through increased pod harvest from 12 to 21 per year (Bhat, 1978; Abraham and Remamony, 1979; Advani, 1984: Abraham and Remaony, 1979). He also suggested covering of cocoa pods with gunny bags or bitumen smeared polythene cover, which will be very effective. Bhat, (1978) noticed that squirrels attack the central part of the pod and rat attacks near the stalk end of the pod. He suggested poison bait trap for the control of rats and single catch live traps for the control of squirrels.

Peter *et al.*(2018) tested the local cocoa selections of Sulawesi, Indonesia for resistance to Vascular streak dieback (VSD). In participatory trials located in their provinces in Sulawesi 2.5 year-old trees. Which had been clonally propagated from local genotypes or the hybrid progeny of resistant parents, were evaluated for diseases severity from 2010 to 2012. The consistent resistance rankings were obtained for clones common to the trials, there were confirmed by re-evaluation in 2014. From plot averages of disease severity, broad-sense heritability was estimated as 0.67 -0.92. Two progeny clones, KW617 and ICCR103, from East Java, had similar levels of resistance in the trials as their respective (resistant) parental clones, PBC 123 and Scavina 6. Among four clones monitored for 3 months in West Sulawesi, PBC 123 had a higher proportion of healthy leaves on the branch tips and a more restricted spread of infection within the xylem. Individual branches of KW617, monitored from an early stage of symptom development, had a significantly lower number of diseased leaves and higher ratio of new to infected leaves after 9-16 weeks than that in our four other clones. Other cocoa clones with a relatively high number of diseased leaves during this period overcame infections with the addition of new flushes, Resistance in farm selections did not usually co-exist with yield and bean quality. Deriving new genotypes from crosses between parents with VSD-resistance and high-yield and/or quality traits is required for the production of promising clones with good resistance, yield and quality.

Atta Ofori *et al.,* (2016) investigated genetic variation and associations of vigour (estimated as stem diameter increments) and yield and its component traits (bean weight, number of beans per pod, pod value and yield efficiency) in 116 cocoa clones introduced into Ghana over different time periods. After eight-months of grafting, stem diameter increments (SDI) in both juvenile and productive stage and yield component traits were evaluated Clone effects were highly significant($P < 0.01$) for all the traits except SDI in the productive stage. Pod value and bean yield varied from14 to 57 and 183 to 952 kg/ha, respectively. Heritability was generally low for all the traits and the highest observed (0.27 ± 0.06) was for bean weight. A positive genetic correlation ($r = 0.47$, $P \leq 0.001$) was observed between SDI in the juvenile stage and bean yield. Some of the best performing clonesT65/238, ICS 40, T16/613, SGU 50 and T63/961 combined high yields with high bean weight and high yield efficiency. Overall they found considerable genetic variation for yield in the available germplasm clones.

2.7. Proteome profiling

The phenotype of an organism is the result of complex regulation and expression of different proteins. Environmental conditions alter the proteome of an organism that modifies the expression pattern of a particular character to counteract the adverse conditions. In every organism, genome codes for protein sets. The entire protein complement of a genome is known as proteome (Wasinger *et al*., 1995). Hence a comprehensive study of proteome of an organism under defined conditions will provide accurate information about proteins encoded by the genome and details of differentially expressed proteins compared to the proteome profile under control conditions (Anderson and Anderson, 1998). Giorgianni *et al.* (2003) stated that proteomics is an essential aspect in biological science to thoroughly realize varied biological systems.

The lack of genomic information in cocoa with respect to self incompatibility is a challenge in cocoa breeding research. Advances in proteomic technologies have capability to identify protein candidates playing role in each physiological condition.

2.7.1. Protein sample preparation

Cocoa is a crop rich in polyphenols, fats, polysaccharides and other metabolites. The protein isolation and sample preparation from such crops with higher fractions of secondary metabolites is a great challenge. In quantitative proteomics, desire for well resolved proteins and reproducible data, demands effective and accurate sample preparation protocol. Carpentier *et al*. (2005) found that, occurrence of proteases and interfering compounds such as phenols, carbohydrates and lipids, affect the protein yield and reproducibility of results. In tropical plant species, the greater part of cell mass is occupied by cell wall and vacuoles and least part from cytosol. Islam *et al*. (2004) studied that, bacterial and animal cells contain higher protein content compared to plant tissues as cytosolic part is more. Hence, standardization of an effective protein isolation protocol in plant specimens is essential, as plant species and its tissues vary from one another in amount and types of non-protein interfering compounds (Shaw and Riederer, 2003; Gorg *et al*., 2004; Carpentier *et al*., 2005).

Umadevi and Anandaraj (2015) isolated total leaf protein from *P. nigrum* using five different extraction methods (Method I - Modified TCA/Acetone method (Damerval *et al*., 1986) with some modifications; Method II - Dense SDS/Phenol method (Wang *et al*., 2003); Method III - PVP/ TCA acetone method (Shen *et al*., 2002) with some modifications; Method IV - Phenol method (Hurkman and Tanaka, 1986) with slight modification; Method V-Lysis buffer extraction (O'Farrell, 1975) with some modifications). Of the different methods evaluated, modified lysis buffer method and phenol method yielded good quality protein required for SDS-PAGE and Two-dimensional gel electrophoretic analysis. Among the two methods, the phenol method was time consuming and hazardous as it involved use of toxic phenol. They concluded that, modified lysis buffer method of protein isolation is superior, quick and yielding protein with negligible polyphenol contamination due to denaturing extraction in presence of sodium chloride and magnesium chloride at elevated temperature allowing the solubilization of membrane proteins in addition to recovery of soluble protein and allowing the direct precipitation of total proteins.

Li *et al.,* (2014) analyzed the proteome of green cotton fiber (21 days post-anthesis) using two-dimensional gel electrophoresis, to yield the first reference proteome map. Of 220 individual spots that were excised and analyzed by MALDI-TOF/TOF MS, 156 were identified and cataloged according to their functions. Many of the proteins were related to carbohydrate metabolism and energy production, oxidoreductase, cell wall related and cytoskeleton proteins.

Márquez *et al*. (2017) compared and evaluated two methods (A - described by Wang *et al*., 2003 and B-described by Sellés *et al*., 2008) of tissue preparation for total protein extract by phenol/ SDS extraction protocol form pod husk. The difference in the application of the two methods was that extensively washed dry powder of pod tissue were made in Method A, whereas that crude extract were prepared in Method B. Extracted proteins were examined using one-dimensional electrophoresis (1-D). They found that in each extraction method isolated a unique subset of cocoa pod proteome. Principal component analysis showed little variation in the data obtained using Method A, while that in Methods B showed no low reproducibility, thus demonstrating that Method A is a reliable for preparing cocoa pod proteins.

The lysis buffer converts all proteins into individual conformations, prevent formation of protein aggregates, prevent protein oxidation, dissolve hydrophobic proteins in the solution, inactivate proteases and cleave disulphide and hydrogen bonds. The buffer is composed of a concentrated urea medium (urea and a stronger denaturing chaotrope like thiourea) that facilitates conversion of proteins into single conformation, dissolve hydrophobic proteins and avoid protein-protein interactions. The use of zwitterionic or non-ionic detergents (CHAPS, Triton X-100 or NP-40) increases the solubility of hydrophobic proteins. The use of DTT/DTE prevents protein oxidation and related oxidative damages. The use of IPG buffers and carrier ampholytes enhance solubility of differentially charged proteins from the mixture into the solution. Use of protease inhibitors while tissue homogenization such as phenyl methyl sulphonyl fluoride (PMSF) or broad-range protease inhibitor cocktail prevents protein degradation by the proteases and enhance recovery of proteins (Zhang *et al*., 2004).

Zhao *et al*. (2013) compared five kinds of protein extraction methods from flower buds of *Solanum lycopersicum* for Two-dimensional gel electrophoresis. They found that TCA/ acetone method is most suitable for protein extraction from tomato flower buds, and got the ideal 2-DE map.

Singh *et al.* (2015) compared methods of protein extraction for 2-D gel electrophoresis from different tissues of pigeon pea, representing vegetative (young leaves), and reproductive (flowers and seeds) organs. Their study explicitly demonstrated that the efficacy of a particular protein extraction protocol is dependent on the different tissues, such as leaves, flowers and seeds that differ in their structure and metabolic constituents. Phenol-based protocol showed an efficacy toward higher protein yield, better spot resolution and a minimal streaking on 2-DE gel for both leaves and flowers. Protein extraction from seeds was best by employing phosphate-TCA-acetone protocol. Addition of phosphate buffer to TCA-Acetone helped in maintaining pH of the solution, which resulted in extraction of high quality protein.

Wang *et al.* (2003) developed a protocol for isolating proteins suitable for two-dimensional electrophoresis (2-DE) from olive leaf. Olive leaf tissue is notoriously recalcitrant to common protein extraction methods due to high levels of interfering compounds. The additional steps involved in the procedure was further grinding dry acetone powder of leaf tissue to a finer extent, extensive organic solvent washes to remove pigments, lipids *etc.*, aqueous trichloroacetic acid washes to remove water-soluble contaminants, and phenol extraction of proteins in the presence of sodium dodecyl sulfate. The final protein preparation is free of interfering compounds based on its well-resolved 2-DE patterns. The total time required for completing the procedure was within 3 h, and protein yield obtained was 2.49 mg per g of aged leaf.

2.7.2. Separation of proteins by SDS-PAGE

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is the normally used analytical method to resolve different components of a protein mixture and allows qualitative estimation of a particular protein sample. This technique

developed by Laemmli (1970) is a authoritative tool to estimate different molecular weight proteins in a protein mixture (Weber *et al*., 1971; Chambach and Rodbard, 1971) based upon their difference in electrophoretic mobility through a resolving polyacrylamide gel matrix (Scopes, 1994). The use of discontinuous buffer systems (Tris-HCL/ Tris-glycine and pH 6.8/ 8.3, respectively) enabled loading of larger volumes of treated protein samples while maintaining good resolution of sample components because proteins are fixed as thin bands before entering the resolving gel (Ornstein, 1964; Davis, 1964). Sodium dodecyl sulphate (SDS) and reducing agents cause protein denaturation that facilitates better separation of proteins (Shapiro and Maizel, 1969). SDS being an anionic detergent binds strongly with amino acids of the protein at an approximate ratio of one dodecyl sulphate molecule per two amino acid residues thus imparting net negative charge to the proteins (Reynolds and Tanford, 1970) and enabling electrophoretic separation in the resolving gel. The use of tris-glycine-SDS (Laemmli, 1970), tris-borate (Neville, 1974) and tris-tricine (Shagger, 1987) electrode buffer systems have improved the resolving power.

The use of two types of polyacrylamide gel system, upper one-third stacking gel (pH 6.8) and the lower two-third resolving gel (pH 8.8) in SDS-PAGE provides possibility for efficient resolution of protein bands. The initial electrophoresis of proteins through the stacking gel allows passing of all the protein fractions from the mixture through the gel and linearize the protein movement before they enter into the resolving gel where the actual separation of proteins take place.

Bertolde *et al.* (2014) conducted a study to find an **e**fficient method of protein extraction from *Theobroma cacao* L. roots for two-dimensional gel electrophoresis and mass spectrometry analysis. This included precipitation with trichloroacetic acid /acetone overnight to prepare the acetone dry powder (ADP), several additional steps of sonication in the ADP preparation and extractions with dense sodium dodecyl sulfate and phenol, and adding two stages of phenol extractions. Proteins were extracted from roots using this new protocol (Method B: modification of method A) and a protocol described in the literature for *T. cacao* leaves and meristems (Method A: described by

Pirovani *et al.,* 2008). Using these methods, they obtained 0.7 and 2.5 mg proteins per 1.0 g lyophilized root, and a total of 60 and 400 spots were separated, respectively. Through Method B, good amount of high-quality protein suited for 2-DE gels was isolated from the roots of *T. cacao*. To demonstrate the quality of the extracted proteins from roots of *T. cacao* using Method B, several protein spots were cut from the 2-DE gels, analyzed by tandem mass spectrometry, and identified. In another study by Yan *et al.,* (2000) further tested Method B on *Citrus* leaves, with a protein yield of about 2.7 mg per 1.0 g lyophilized leaves and 800 detected spots.

Pirovani *et al*. (2008) developed three new protocols; one for apoplastic washing fluid (AWF) extraction, and two for protein extraction under denaturing and nondenaturing conditions. The first described method allows a quick and easy collection of AWF – using infiltration–centrifugation procedure – that is representative of its composition in intact leaves according to the smaller symplastic contamination detected by the use of the hexose phosphate isomerase marker. Protein extraction under denaturing conditions for 2-DE was remarkably improved by the combination of chemically and physically modified processes including phenol, SDS dense buffer and sonication steps. With this protocol, high-quality proteins from cacao leaves and meristems were isolated, and for the first time well-resolved 1-DE and 2-DE protein patterns of cacao vegetative organs are shown. They also reported that sonication associated with polysaccharide precipitation using *tert*-butanol was a crucial step for the nondenaturing protein extraction and subsequent enzymatic activity detection.

Yan *et al*. (2000) have conducted a study to systematically compare the physiological mechanisms underlying somatic and zygotic embryogenesis in *T. cacao* on the proteome level. About 1000 protein spots per fraction were separated by two-dimensional isoelectric focusing/ SDS PAGE and more than 50 of the protein spots clearly differed in abundance between zygotic and somatic embryos:

2.7.3. Proteome profiling by two-dimensional gel electrophoresis

The proteome of plant cells is extremely complex, comprising of thousands of proteins expressed at a time. Hence, separation of protein fractions only based upon

molecular weight might not give a relevant protein profile. In an SDS-PAGE resolving gel, a single protein band might correspond to numerous proteins of similar molecular weight. With the introduction of recent proteome profiling methodology of two-dimensional gel electrophoresis (2DE or 2D gel electrophoresis) by O'Farrell (1975), has revolutionized the proteomic research. 2D gel electrophoresis has been agreed by many proteomic researchers around the globe because of its enhanced resolution, advantage of storing proteins in the gel until further analyses and is unrivalled by any other alternative technique.

Two-dimensional gel electrophoresis individualistically separates proteins based upon two factors *i.e* isoelectric point (pI) in first dimension gel electrophoresis or isoelectric focusing and molecular mass (M_r) in the second dimensional gel electrophoresis or SDS-PAGE (O'Farrell, 1975). Up to 10,000 proteins could be resolved instantaneously with an average of 2000 proteins routinely. Protein spots of one nanogram and above can be detected and quantified.

2.7.3.1. First dimensional gel electrophoresis (Isoelectric focusing)

Proteins in the mixture have amphoteric actions containing varied proportions of acidic and basic groups that make each protein different from others based on net charge on them. Net charge of a protein is sum total of all negative or positive charges of the amino acid side chains. These proteins can become protonated or deprotonated depending upon pH atmosphere. The acidic groups become negatively charged in basic atmosphere and the basic groups become positively charged in the acidic atmosphere. In the presence of electric field, the proteins migrate towards respective electrodes of opposite sign of its net charge. At a point, when the proteins reach their respective isoelectric points, the net charge on them turns into zero and they become immobilized. This principle is used to separate proteins from mixtures based on isoelectric point in first dimensional gel electrophoresis.

Earlier, first dimensional gel electrophoresis was done in thin polyacrylamide gel rods in glass or plastic tubes containing urea, detergent, reducing agent and carrier

ampholytes to form pH gradient in the electric field. There were disadvantages using gel rods for isoelectric focusing as they required great experimental skills to handle and the focusing patterns were not reproducible enough. Hence, modern methods have replaced gel rods with immobilized pH gradient (IPG) strips with a broad pH gradient (3-10, 4-7) that can focus both acidic and basic proteins in a single gel. The advantages of using IPG strips are that it offers greater reproducibility and allows loading of greater amount of protein sample.

Length of the IPG strip used, purity of the protein sample loaded, IEF voltage parameters applied and the temperature used determine the successful IEF run.

2.7.3.2. Second dimensional gel electrophoresis (SDS-PAGE)

The second dimensional electrophoretic run is similar to basic SDS-PAGE protocol, with the difference that only resolving gel is used for protein separation and the IPG strip containing the focused proteins is directly placed in contact at top of the resolving gel. Due to presence of SDS, all the proteins separated by isoelectric focusing get masked with net negative charge imparted by SDS, hence the proteins get separated in the electric field purely based on the molecular weight. Proteins with higher molecular weights migrate to lesser distances in the resolving gel whereas the low molecular weight proteins migrate to longer distances. The critical difference that exists here is that, here in the resolving gel all the proteins appear as individual distinct spots whereas in basic SDS-PAGE protocol, the separated proteins appear as thin protein bands with corresponding molecular weight.

2.7.4. Visualization of protein profile in the resolving gel

Visualization of the resolved proteins in the gel is by three methods: Coomassie Brilliant Blue (CBB) staining, silver staining and Fluorescent staining with SYPRO dyes.

Coomassie Brilliant Blue staining is the widely adopted practice to visualize protein profile as it is reproducible, gives clear background, rational sensitivity (30 ng

per band), cheaper and has excellent compatibility with mass spectrometry (Candiano *et al*., 2004). The principle of this staining is the strong affinity of CBB dye with the proteins. So when the entire gel is destained, the background becomes transparent except for the protein bands or spots that remain stained purple or violet.

Silver staining is the most sensitive, non-radioactive protein visualization method that can detect proteins at nanogram level (Yan *et al*., 2000; Candiano *et al*., 2004). Adoption of this staining procedure is quite limited as it involves laborious multiple steps, high background and not compatible with mass spectrometry (Candiano *et al*., 2004).

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled "Genetic analysis of inbreds, inbred crosses and hybrids of cocoa (*Theobroma cacao* L.) was carried out in the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during the period 2015-2018.

3.1 Materials

Inbreds of various genotypes belonging to different generations, maintained at Cocoa Research Centre (CRC), College of Horticulture was used as base material for evaluation of inbreds and to study the different methods to overcome the self-incompatibility. For comparative evaluation, 100 hybrids, 36 inbred crosses and 170 inbreds planted in the year 2015 at CRC farm, College of Horticulture, Vellanikkara were used.

3.2 Observations recorded

3.2.1. Evaluation of inbreds

Cocoa inbreds evaluated in the present study are presented in Annexure I.

3.2.1.1 Morphological characterization

The biometric observations were recorded in cocoa inbreds, inbred crosses and hybrids studied. The morphological descriptors are useful in selecting superior genotypes for further crop improvement programme (Engles, 1980). The morphological observations were taken as per the descriptors developed by Bekele and Butler (2000). Both quantitative and qualitative characters of pod and bean were observed. For morphological characterization of pods and beans, five pods were collected from each inbred line to record the observation. The pods were collected as and when they ripe. Husk was split opened to evaluate bean characters and the outer mucilage was peeled using forceps to record the bean observation.

Plate 1 Inbreds plants of cocoa

3.2.1.1.1 Growth observation

3.2.1.1.1.1 Plant height (cm)

The height of the tree trunk was measured from the ground level to the tip of the main chupan or the top most node which has just unfurled its leaves.

3.2.1.1.1.2 Girth (cm)

The girth of tree trunk was recorded 15 cm above the ground level.

3.2.1.1.1.3. Canopy spread (cm)

The diameter of canopy spread of each plant was recorded in the East-West and North- South directions and average canopy spread value was computed.

3.2.1.1.1.4. Leaf area (cm^2)

The leaf area was computed by taking the length of leaf along the midrib and the width at top, middle and bottom portion of leaf.

3.2.1.1.2 Yield and yield attributes

3.2.1.1.2.1 Number of pods

The total number of mature pods including damaged pods (due to pest and disease attack) harvested from each tree was recorded throughout the year.

3.2.1.1.2.2 Pod weight (g)

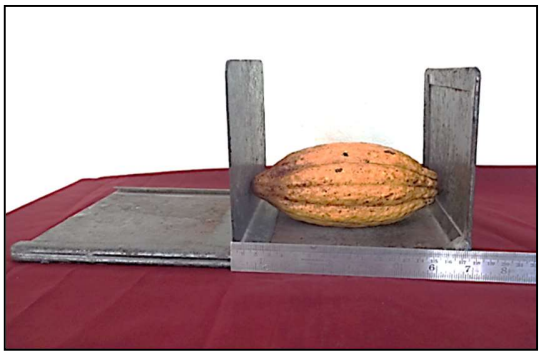
The average weight of five pods produced by each tree was computed from the weight of each harvested pod and expressed in gram.

3.2.1.1.2.3 Pod length(cm)

The distance from the base of the pod to its apex was measured and expressed in centimetre. The average pod length for individual tree was computed from five representative pods.

3.2.1.1.2.4 Pod width (cm)

The average width of pod on individual tree basis was arrived at, from the measure of five representative pods harvested and expressed in centimetre.



a. Length of the pod (cm)

b. Breadth of the pod (cm)

c. Thickness of the pod (cm)

d. Weight of the pod (g)

Plate 2. Pod characters

3.2.1.1.2.5 Wet bean weight/ pod (g)

The weight of wet beans (with mucilage) from five pod was recorded, the average computed and expressed in gram on individual tree basis.

3.2.1.1.2.7. Number of beans /pod

The number of beans per pod was recorded on individual tree basis from five pods and the average value was computed.

3.2.1.1.2.8 Flat bean (per cent)

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

For the bean observations, beans from five pods per tree were pooled and twenty beans were selected randomly, peeled using forceps and used for further observations.

3.2.1.1.2.9 Bean length (mm)

The average length of a bean for each tree was computed from the measure of five randomly selected fresh peeled beans using Vernier callipers and expressed in millimetre.

3.2.1.1.2.10 Bean width (mm)

The average width of bean for each tree was computed from the measure of randomly selected fresh peeled beans and was expressed in millimetre.

3.2.1.1.2.11 Bean thickness (mm)

The average thickness of beans for each tree was computed from the measure of five randomly selected fresh peeled beans and is expressed in millimetre.

3.2.1.1.2.12 Pericarp thickness (mm)

The average thickness of the pericarp was calculated from the mean pod husk thickness at the ridges and furrows measured using Vernier callipers and expressed in millimetre.

a. Breadth of bean (mm)

b. Thickness of bean (mm)

c. Total wet bean weight (g)

d. Single wet bean weight (g)

e. Single dry bean weight (g)

Plate 3. Bean characters

3.2.1.1.2.13 Dry weight of bean (g)

The dry weight of a single bean was computed as an average value of twenty dried beans and expressed in gram.

3.2.1.1.3 Economic characters

3.2.1.1.3.1 Pod value (g)

Pod value refers to the dry bean weight obtained per pod (Toxopeus and Jacob, 1968) and computed as the product of dry weight/bean and number of beans/pod.

3.2.1.1.3.2 Pod index

Pod index indicates the number of pods required to produce one kilogram of dried cocoa beans (Morera *et. al.,* 1991)

Pod Index = 1000g/ Pod value (g)

3.2.1.1.3.3 Efficiency index

Efficiency index is an indication of the pod weight required to produce one gram dry bean (Jacob and Atanda, 1971)

Efficiency index = Pod weight / Pod value

3.2.1.1.3.4 Conversion Index (CI)

The amount of dry bean obtained from a given amount of wet bean is the Conversion Index (C.I.). It was computed by using the following formula (Francies, 1998).

C. I. =Pod value(g)/ wet bean weight per pod(g)

3.2.1.1.3.5 Dry Matter Recovery (%)

The ratio of dry bean weight to wet bean weight was calculated and expressed in per cent (Francies, 1998).

Dry Matter Recovery=Dry bean weight(g)/wet bean weight(g)*100

3.2.1.1.3.6 Pod Yield (kg)

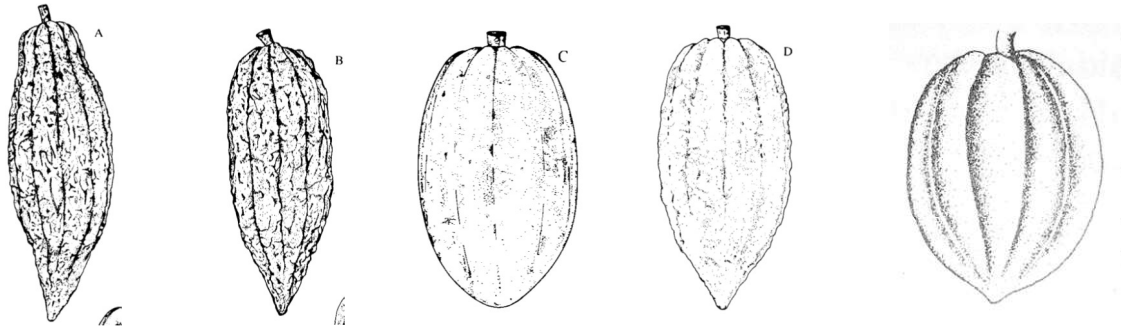
The yield on individual tree basis was estimated in terms of total dry bean weight produced in a given period and expressed in kilogram.

Yield (kg) = Total number of pods X Pod value (g) / 1000

3.2.1.2 Qualitative evaluation of pod and beans

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

3.2.1.2.1 Pod shape

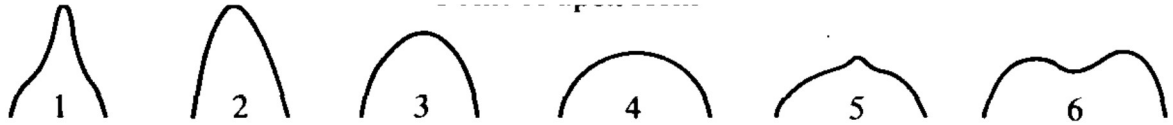


Cundeamo Criollo Amelonado Angoleta Calabacillo

Descriptor states and description

1 Cundeamor - characterized by bottle neck
2 Angoleta - deeply ridged, warty and square at the stalk end
3 Amelonado - characterized by slight bottle neck, smooth and shallow furrows and melon shaped with blunt end
4 Calabacillo - spherical and small in shape
5 Criollo - intense surface with acute apex

3.2.1.2.2 Pod apex form



1 Attenuate

2 Acute

3 Obtuse

4 Rounded

5 Mammelate

6 Indented

3.2.1.2.3 Pod basal constriction

0 Absent

1 Slight

2 Intermediate

3 Strong

4 Wide shoulder

3.2.1.2.4 Pod rugosity

0 Absent

3 Slight

5 Intermediate

7 Intense

3.2.1.2.5 Colour of ripe pod (Ridge and furrow colour)

0 Absent (Green)

3 = Slight (Greenish yellow)

6 = Intermediate (Yellowish green)

7 = Intense (Yellow)

3.2.1.2.6 Colour of unripe pod

3 = Light

5 = Intermediate

7 = Purplish green

9 = Dark green

3.2.1.2.7 Bean colour

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

1 = White

2 = Grey

3 = Light purple

4 = Medium purple

5 = Dark purple

6 = Mottled

7 = Mixed

3.2.1.3 Biochemical characterization

One hundred and forty inbred plants were evaluated for biochemical characterization. Fat content and total phenol content were estimated following standard procedures.

Sample preparation

Five ripened pods were harvested from each of the inbreds based on ripeness and maturity indices. Pod husk was split opened and beans were scooped out. Beans

from all the pods were pooled for analysis. From this 20 beans were selected randomly. They were peeled using forceps and dried under sun or by using an oven until the moisture reached below 8 percent. The drying was completed under sun within six to seven days. The dried beans were then ground to fine powder using laboratory grinder and the powder was tightly packed, labelled and stored for further biochemical analysis.

3.2.1.3.1 Fat estimation

Method: Soxhlet apparatus method

Materials required: Cocoa bean powder: 10 g

Petroleum ether (40-60°C)

Blotting paper

Procedure: Cocoa nibs were defatted to extract the fat with petroleum ether (40-60°C) in a soxhlet apparatus (Sadasivam and Manickam, 2008). Ten grams of cocoa bean powder was wrapped in a blotting paper and tied with twine. The sample was placed in the extraction tube of soxhlet apparatus. The fat present in the cocoa powder was extracted through siphoning of petroleum ether through the apparatus and fat got settled at the bottom of the flask along with a little amount of petroleum ether. This was transferred to a pre-weighed beaker and kept open for the petroleum ether to evaporate. The cream coloured substances left behind after the evaporation of solvent was the fat and it was weighed and expressed as percentage.

3.2.1.3.2 Total phenol estimation

Method: Folin- Ciocalteu (FC) reagent method

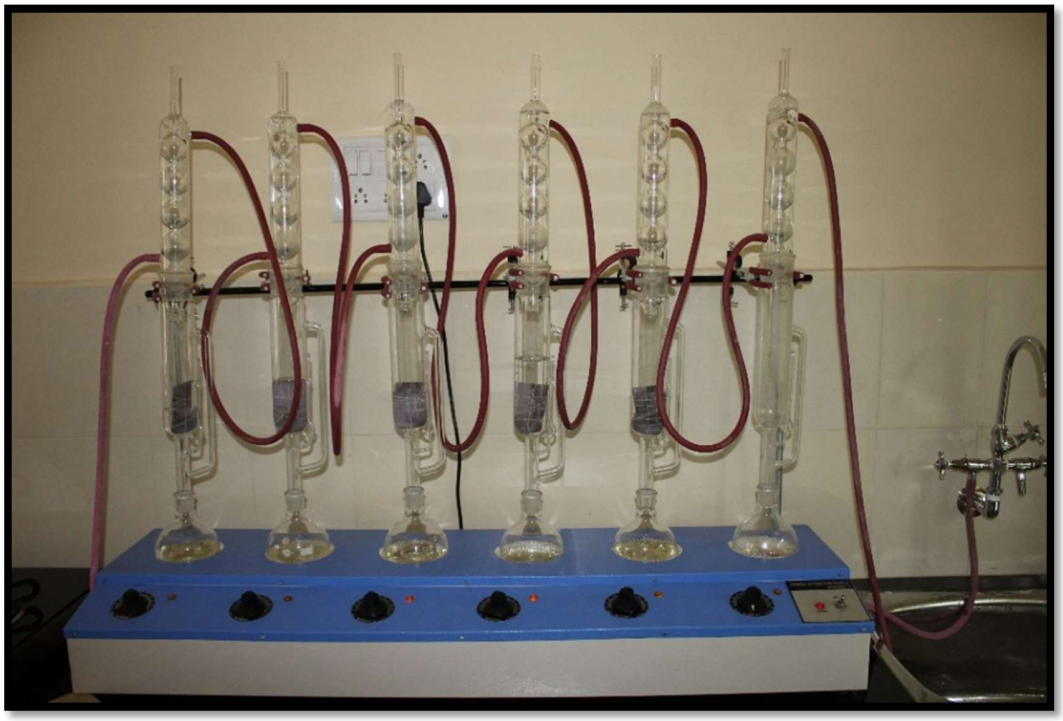
Required: Powdered sample- 500 mg

Ethanol (80 percent)

Na_2CO_3 (20 percent)

FC reagent

Catechol – 100 mg



a. Soxhlet apparatus for fat extraction

b. Extracted fat

Plate 4. Fat extractionfrom cocoa beans

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

Exactly 500 mg of powdered defatted sample was taken and ground it with 5 ml of 80 percent ethanol using mortar and pestle and the extract was centrifuged at 10,000 rpm for 20 minutes. The supernatant was collected in evaporation dish and the remaining residue settled down was re-extracted with 2.5 ml of 80 percent ethanol. Again centrifuged and the supernatant was collected in evaporation dish. Then supernatant was allowed to evaporate on the hot water bath. Forty millilitres water was used to dissolve the phenols in the residue. An aliquot of 0.2 ml was taken into a test tube and then the volume was made up to 10 ml using distilled water followed by the addition of 0.5 ml of Folin-Ciocalteu reagent. Kept it for three minutes and added 2 ml of 20 percent Na_2CO_3 solution and mixed well. The test tubes were kept in a boiling water bath for one minute. The test tubes were cooled at room temperature and incubated at room temperature for 60 minutes for colour development. A blue coloured complex, molybdenum blue was formed as the phenol undergoes a complex redox reaction with phosphomolibdic acid present in Folin-Ciocalteu reagent in alkaline medium. Absorbance was read at 650 nm.

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

Concentration of phenols present in the extract was worked out by substituting the absorbance value, thus obtained in the calibration equation. The total

phenol content was calculated as mg catechol equivalent of phenol per gram sample and expressed it as percent.

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

Where, OD sample = absorbance value of sample

OD standard = absorbance value of standard

3.2.1.4 Pests and disease scoring

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

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

3.2.1.5. Inbreeding depression

Inbreeding depression in various selfed generations was computed and the significance was tested as per usual procedure

$$\text{Inbreeding depression (\%)} = \frac{S0-S1}{S1} \text{ x } 100$$

S_0 = Average Performance of the inbreds in the preceding generation

S1 = Average performance of the inbreds in the succeeding generation

3.2.2 Comparative evaluation of inbreds, inbred crosses and hybrids

The inbreds, inbred crosses and hybrids planted during 2015 at CRC farm, CoH, Vellanikkara were used for comparative evaluation in the present study

3.2.2.1 Growth observation

3.2.2.1.1 Plant height (cm)

The height of the tree trunk was measured from the ground level to the tip of the main chupon or the top most node which has just unfurled its leaves.

3.2.2.1.2 Girth (cm)

The girth of tree trunk was recorded 15 cm above the ground level.

3.2.2.1.3 Canopy spread (cm)

The diameter of canopy spread of each plant was recorded in the East-West and North- South directions and average canopy spread value computed

3.2.2.1.4 Leaf area (cm^2)

The leaf area was measured by taking the length of leaf along the midrib and the width at top, middle and bottom portion of leaf.

3.2.2.2 Physiological observations

3.2.2.2.1 Chlorophyll content (mg/g)

The chlorophyll a, chlorophyll b and total chlorophyll were estimated as per the method suggested by Hiscox and Israelstam (1979). For chlorophyll estimation, 10 ml of DMSO (Dimethylsulphoxide) was added to 100 mg leaf sample and incubated in dark overnight. The final volume made up to 25ml after filtering in the next day. The chlorophyll content was estimated in spectromphotometer at two wavelengths 645 nm and 663 nm and expressed as milligram g^{-1} fresh weight of plant tissue. The amount of chlorophyll was arrived at using formulae.

Chlorophyll ‘a’ = [(12.7 X A 663) – (2.69 X A 645)] X V/1000 X W

Chlorophyll ‘b’ = [(22.9 X A 645) – (4.68 X A 663)] X V/1000 X W

Total Chlorophyll = [(20.2 X A 645) – (8.02 X A 663)] X V/1000 X W

Where,

A = Absorbance at given wavelength

V = Total volume of sample in extraction medium

W = Weight of sample in milligrams

3.2.2.2.2 Plant nutrient status of index leaf

The index leaf, fully matured third leaf from the top of fan branch were collected from each plant, washed, air dried and oven dried for 24 hours at 70^0 C to get a constant weight. Then the samples were powdered and analyzed for different nutrients. The standard methods used for the analysis of different nutrients are given in Table 1.

Table1. Analytical methods followed in plant analysis

S. No.	Nutrient	Method
1.	N	Microkjeldhal digestion and distillation method (Jackson, 1958)
2.	P	Diacid digestion of sample followed by filtration. Vanadomolybdate phosphoric yellow colour in nitric acid system (Piper, 1966)
3.	K	Diacid digestion of plant sample followed by filtration (Piper, 1966), Flame photometry determination.

3.2.2.2.3 Relative water content

The relative water content was estimated as per Barrs and Weatherly (1962) by measuring the fresh weight, turgid weight and dry weight of twenty leaf discs from the index leaf at 10 am on the day. After measuring the fresh weight of sample, it was submerged in distilled water for 3 hours and then the turgid weight was taken. The dry weight of the sample was measured after keeping the samples in oven at 80 ° C for 3 consecutive days. The relative water content was calculated using the formula.

$$RWC\ (\%) = FW - DW/TW - DW \times 100$$

Where,

FW = fresh weight of twenty leaf discs taken immediately after excision.

TW = fully turgid weight determined upon re-hydration of leaf discs by immersing them in a petridish containing distilled water for three hours.

DW = dry weight obtained after drying at 80 ^{0} C for 3 days until no further weight
change occurred.

3.2.2.3 Plant phenology

3.2.2.3.1 Bud break

Number of days taken from initiation of bud in the cushion to complete appearance of bud was recorded

3.2.2.3.2 Flushing

Year round leaf production was recorded

3.2.2.3.3 Cushion formation

Number of cushions formed on the main stem and fan branches were recorded

3.2.2.3.4 Year round flowering

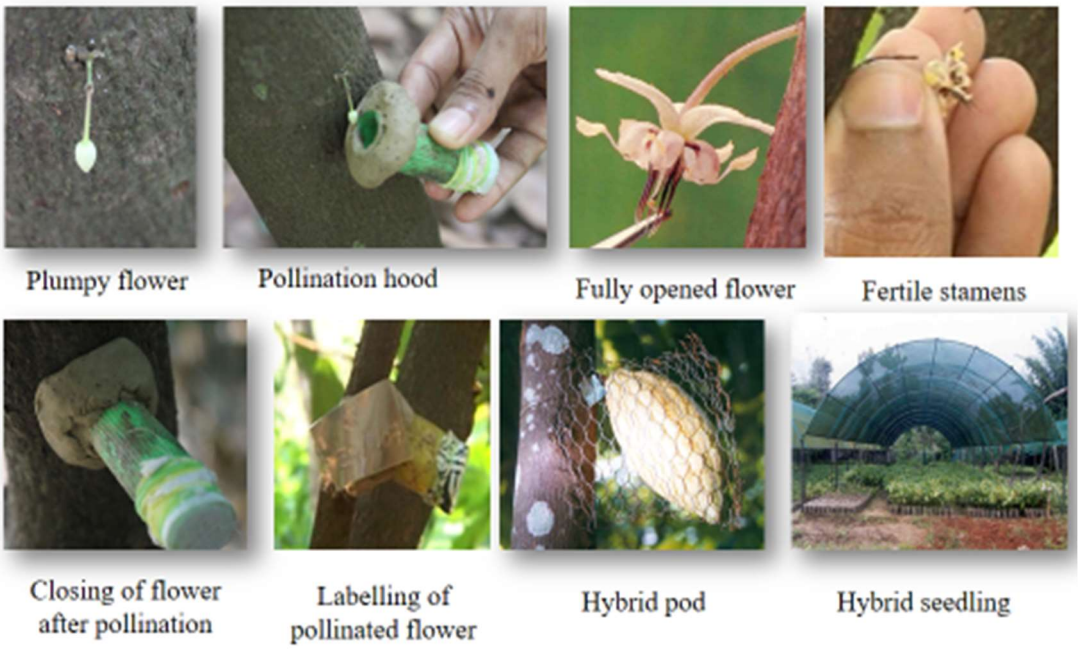
Month wise number of flowers produced on the main stem were recorded

3.2.2.4 Self and cross incompatibility of hybrids

3.2.3 Study of methods to overcome self incompatibility

3.2.3. Selfing

Mallika *et al.* (2002) standardized the artificial pollination in cocoa. In artificial pollination, a flower bud that will open the following day, recognized by whitish colour and swollen appearance, is selected. The bud is covered with a pollination hood made of plastic tube/hose pipe piece of 5 x 1.5-2 cm, which is sealed to the bark using materials like plasticine/glaze putty. The tube is covered with muslin cloth at the top, and kept in place with rubber band. This ensures circulation of air and exclusion of insects.



Opened flowers are collected from the desired male parent and stamens are carefully taken out by pushing the corresponding petal. One entire stamen with a part of filament is deposited on the stigma. One or two staminodes may be pinched off to give access to the stigma. Emasculation is not necessary due to the presence of self-incompatibility. For selfing also, hand pollination is done using stamens from the same flower. The pollinated flowers are labelled using tin foil pieces fixed in the cushion using ball pins. The hoods are removed 24 hours after pollination and in three to five days, fertilization is confirmed by the visual swelling of the ovary. In order to prevent undue shedding and wilting of fruits from hand pollinations, it is usual to remove all the developing fruits on the tree produced by open pollination. Developing pods are covered with wire mesh after six to eight weeks to protect them from mammalian pests. The pods are collected at maturity, beans are extracted and sown in the nursery.

3.2.4 Methods to overcome the self-incompatibility

The following techniques and methods were tried to overcome the self-incompatibility in the various generation inbreds, among the different methods and techniques followed to overcome the self-incompatibility, the best method was tried in the S_5 generation.

a. Bud pollination

b. Intra ovarian method of pollination

Plate 5. Bud and Intra ovarian pollination methods

3.2.4.1 Bud pollination

In bud pollination, pollen grains are applied to immature non receptive stigma, generally one day before the opening of the flower (Plate 5a).

3.2.4.2 Surgical method

Removal of stigmatic surface and half a portion of style and then placing the pollen grains on the cut portion of style (Plate 6).

3.2.4.3. Intra-ovarian pollination

The pollen grains are directly applied to the ovary by removing the whole style (Plate 5b).

3.2.4.4. High temperature or heat treatment

Days before flower opening flowers are covered with pollination hood. The distal end of the pollination hood was covered with the small polythene bag and air is blown inside. The flowers are then covered with the hood, on the next day, the flowers inside the hood opened. Normal selfing was followed.

3.2.4.5. High humidity

As in the case of high temperature treatment, the flowers are covered in the same way a day before pollination. The water was sprayed inside the pollination hood so that the humidity got increased. On the next day, the opened flowers are pollinated by normal selfing.

3.2.4.6. Salt spray

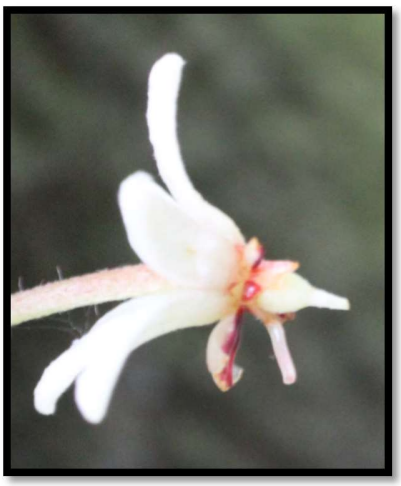
Ten percent sodium chloride solution spray was done a day before flower opening at the time covering the flower bud with the pollination hood.

3.2.4.7. Gamma irradiation

The pollen grains collected from the flowers are taken to gamma irradiation chamber and they are exposed to 10 and 20 gray. After irradiation, the pollen grains were used for selfing in various generations of inbreds.

a. Flower at peak receptivity

b. . Removal of staminodes



c. Portion of style removed for pollination

Plate 6. Surgical method of pollination

3.2.4.8. Application of flower organ extract

The flower of the same plants is crushed along with pollen grains of the same plant and is applied on the stigmatic surface to carry out the normal selfing.

3.2.4.9. Application of plant hormones

The flowers are applied with the Naphthalene Acetic Acid (NAA) @ 100 and 200 ppm at the time of covering the flower bud.

In all of the above methods, the covering of flower bud was done a day previous to opening of flower as in case of selfing.

3.2.5 Studies on germination of pollen grains and pollen tube using fluorescent microscope

The self pollinated flowers after six to twelve hours of pollination were fixed and used for fluorescent microscopic study. The self pollinated flowers were fixed in FAA fixing solution (formaldehyde: acetic acid: alcohol = 1:1:8 by volume). The sections of flowers were prepared according to the procedure suggested by Martin (1959) with some modifications. After keeping the flowers in fixative solution for 24 hours, the stigma was washed by deionized water followed by maceration in 1N NaOH for 45 minutes, then thoroughly washed in deionized water for 3 times. Finally, the stigma was dyed in solution of 0.1% alinile blue dissolved in 0.1 N K_3PO_4 for 12 h. Subsequently, the stigma was placed in a drop of glycerine on a microscope slide and squashed under a cover slip to make the material spread out evenly. The Trinocular research microscope Leica fluorescent microscope DM2000LED was used for microscopy observations and photographs.

3.4. Study of floral proteome in relation to self-incompatibility

An intensive study on the standardization for extraction method and single dimensional electrophoresis in SDS PAGE was done at Cocoa Research Centre, Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture (CoH), Vellanikkara, and Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, during March 2016 to June 2018.

3.4.1. Materials

3.4.1.1. Plant materials

Fifty four self-incompatible cocoa plants showing self-incompatibility as reported by Minimol *et al.,* (2015) formed the experimental material for the study which included the different generation inbreds from S1 to S5 of the genotypes M 18.7, G VI 251.2, S IV 1.5, G VI 282, S IV H 10.27, P II 7.18.

3.4.1.2. Chemicals, glass wares and plastic wares

The chemicals (Molecular Biology grade) were procured from Sigma Aldrich, USA; Sisco Research laboratories (SRL), India; HiMedia, India and Merck, Germany. Plasticware and glassware were from Borosil and Tarsons India Ltd.

3.4.1.3. Equipment

Proteome profiling, one dimensional gel electrophoresis, imaging and documentation of protein were done using facility at CPBMB. Procedures and protocols followed are elaborated in this chapter.

3.3.2. Methods

3.4.2.1. Collection and preparation of Gynoecia for protein extraction

Flowers from the self-incompatible plants have been collected in ice box and gynoecia were separated in CRC under air conditioned room. The separated gynoecia were placed in Petri dishes containing solution of Polyvinyl pyrolidine (PVP). To reduce further browning of gynoecia before grinding, the flowers were immersed in one per cent ascorbic acid. The cocoa flowers were used to turn brown soon after separation from the plant and the gynoecia used to turn dark brown after separation from the flowers. To check browning and oxidation of phenols the flowers were collected directly in the liquid nitrogen in the field itself. The frozen flowers were ground in liquid nitrogen with pinch of PVP in pre-chilled pestle and mortar. The fine powder obtained after grinding was utilized for extraction of flower protein.

3.4.2.2. Collection of leaves

Because of very low concentration of protein in the cocoa flower, the bands did not appeared. Hence, it was decided to extract the protein from leaves and run in SDS PAGE. The index leaf (fully matured green leaf from the tip of fan branches) from the self-incompatible plants were collected and placed in test tubes containing liquid nitrogen kept in thermos flask. The frozen leaves were ground in liquid nitrogen with pinch of PVP in pre-chilled pestle and mortar for further extraction of protein. The fine powder obtained after grinding was utilized for extraction of leaf protein

3.4.2.3. Collection of pods

Four months old pods were collected and beans along with the pulp were used for extraction of protein.

3.4.2.4. Challenge in collection and use of gynoecia for protein isolation

The gynaecium were initially collected from cocoa flowers and place in icebox. Soon after removal of flower from the ice box, the flowers turned brown. Later, flowers are kept in 1 per cent PVP followed by 0.3 per cent ascorbic acid. The browning of flowers and gynaecium did not stop. Finally, the flowers are collected directly in the liquid nitrogen in the field and then the treated with 1 per cent PVP followed by 0.3 per cent ascorbic acid and used for protein extraction.

3.4.3 Protein extraction

About 200 µg of lyophilized plant tissue was extracted in 300 µL of lysis buffer for 15 min. under ice by intermittent vertexing. After this, the extract was centrifuged at 20,000 g for 20 min. at 4 $^{\circ}$C. The supernatant was collected and further subjected for protein precipitation.

3.4.3.1. Precipitation of total protein

Total proteins from the flower homogenate were precipitated by trichloroacetic acid (TCA) method of protein precipitation. About 1.5 volume of freshly prepared 10 per cent TCA containing 0.07 % β-mercapto ethanol in ice cold acetone was added to the protein extract.

Initially, five standard methods were followed.

Leaf and flower sample
↓
Grinding in liquid nitrogen
↓
Powdered tissue stored at -80 0C

Method I	Method II	Method III	Method IV	Method V
Modified TAC/Acetone method	**Dense SDS/Phenol Method**	**PVP/TCA acetone method**	**Phenol method with slight modification**	**Lysis buffer extraction**
Precipitation 10 % TCA + 0.07%2ME in cold acetone ↓ **Washing** X 2 0.07 % 2 ME in cold acetone ↓ **Air Drying** ↓ **Storing at -80^{0}C**	**Homogenization and Extraction** 0.1 M Tris Hcl pH 8.65 30 % sucrose, 2 % SDS, 1mM PFSF, 2 % 2 ME Equal volume of Tris buffered phenol pH 8.0 ↓ **Precipitation** **A**. 1.5 volume of 0.1 ammonium acetate in cold methanol stored at -20^{0}C for 2 hours ↓ stored at -20^{0}C for overnight **B.** 5 volumes of 10% TCA+ 0.07% ME in cold acetone Stored at -20^{0}C for 2 hours Stored at -20^{0}C for overnight ↓ **Washing** ↓ **Air Drying** ↓ **Stored at -80^{0}C**	(Leaf and flower sample added with PVP and powdered in liquid nitrogen) ↓ **Homogenization and Extraction** 40mM of Tris Hcl pH 7.5, 250mM sucrose, 10mM EDTA, 1% Tritonx-100 , 1 mM PMSF, 1mM DTT, 2 % v/v 2 ME ↓ **Precipitation** a.1.5 volumes of 0.1 M ammonium acetate in cold methanol stored at -20^0 C for 2 hours stored at -20^{0}C for overnight b.1.5 volumes of 10 % TCA + 0.07 % ME in cold acetone stored at -20^{0}C for 2 hours stored at -20^{0}C for overnight ↓ **Washing** ↓ **Air Drying** ↓ **Stored at -80^{0}C**	**Homogenization and Extraction** 0.1M Tris Hcl pH 8.8, 10mM EDTA, 0.4 % 2 ME 0.9M Sucrose Equal volume of Tris buffered phenol pH8. 2 micro litre protease inhibitor cocktail ↓ **Re extraction and pooling of phenol phase** **Precipitation** **A**.1.5 volumes 0.1M ammonium acetate in cold methanol, Stored at -20^{0}C for 2 hours Stored at -20^{0}C for overnight **B**.1.5 volumes of 10 % TCA + 0.07% ME in cold acetone Stored at -20^{0}C for 2 hours Stored at -20^{0}C for overnight ↓ **Washing** ↓ **Air Drying** ↓ **Stored at -80^{0}C**	**Homogenization and Extraction** 7M Urea, 7M thiourea, 2 % CHAPS, 40mM DTT, 2 microlitre protease inhibitor cocktail ↓ **Precipitation** a.1.5 volumes 0.1 M ammonium acetate in cold methanol Stored at -20^{0}C fo2 hours Stored at -20^{0}C for overnight b. 1.5 volumes of 10% TCA +0.07% ME in cold acetone stored at -20^{0}C for 2 hours stored at – 20^{0}C for overnight ↓ **Washing** ↓ **Air Drying** ↓ **Storing at -80^{0}C**

The mixture was assorted gently by inversion and then incubated overnight undisturbed at -20 ºC. On the next day, white precipitated proteins at the bottom was

recovered by centrifugation at 15,000 g for 20 minutes at 4 °C. Supernatant was discarded and the white pellet at the bottom was air dried over blotting paper followed by dissolution of pellet in 60 µL rehydration buffer

3.4.3.2. Recovery of precipitated protein and protein purification

After overnight precipitation, a white precipitate of protein was seen at the bottom which was recovered by centrifugation at 12000 g for 20 minutes at 4°C. The supernatant was then discarded and the protein pellet was purified by giving two washes with ice cold acetone containing 0.07 per cent 2-mercaptoethanol. For each washing step, the pellet was first completely resuspended in acetone by vortexing, then incubated at -20 ºC for 15 min. and finally centrifuged at 12000 g for 15 min. at 4 °C to recover the full protein pellet. The protein pellet was air dried to remove traces of acetone. The recovered protein pellet was directly used in 2DE assays.

3.4.3.3. Protein solubilization

The protein pellet was first dissolved in minimum quantity of rehydration buffer by vortexing. The undissolved impurities were removed by carrying out centrifugation at 10,000 rpm for 15 min. at 4ºC. The impurities settled at the bottom of tube and the supernatant was collected in fresh pre-chilled microfuge tube. This was further advanced for protein quantification protocol by Bradford's method.

3.4.3.4. Protein quantification by Bradford's method

Principle of this quantification assay is that Coomassie Blue G250 dye in Bradford reagent binds with the protein and more is the bound dye with the protein, more blue the solution and more is the protein content (Bradford, 1976). Intensity of blue coloured solution is measured spectrophotometrically to quantify the protein content.

3.4.3.4.1. Chemicals required

1. Bovine serum albumin (BSA) stock solution (1 mg ml^{-1})
2. Bradford reagent
3. 0.1 N NaOH

The composition and preparation reagents are given in Annexure –.

3.4.3.4.2. Procedure

Several BSA protein standards (0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg ml^{-1}) of about 1 ml final volume were prepared by pipetting required volume in test tubes separately and diluting it with required volume of 0.1 *N* NaOH. Test tube with 1 ml of 0.1 *N* NaOH was taken as blank. About 5 ml of Bradford reagent was added in each tube and the contents were mixed properly. It was left undisturbed for 30 minutes. After 30 minutes, the absorbance of the coloured solutions were measured using spectrophotometer set at 595 nm and zero blank. The OD values obtained for all the protein standards were plotted on Y-axis with their respective protein concentration on X-axis to obtain a standard plot (straight line plot). Similar reaction was progressed with the protein test samples and the standard plot was used to extrapolate protein concentration (mg/ ml) for unknown OD values of the protein test sample.

3.4.4. Protein profiling by SDS-PAGE

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) protocol standardized by Laemmli (1970) was attempted in current research to generate a protein profile containing different molecular weight proteins resolved in a polyacrylamide gel, to study the number and quality of resolved protein bands and to validate the presence or absence of different proteins in the total protein profile. The principle of SDS-PAGE is that the SDS being an anionic detergent imparts net negative charge to all the proteins in the loaded sample, hence, the charge on proteins is negative, the separation of proteins is purely based on molecular weight. Lower molecular weight proteins resolve towards the bottom of the resolving gel and the larger molecular weight proteins resolve at the top of the resolving gel.

3.4.4.1. Chemicals required

1. Monomer solution (30 per cent acrylamide, 27 per cent bis-acrylamide)
2. 4X resolving gel buffer (1.5 *M* Tris-Cl, pH 8.8)
3. 4X stacking gel buffer (0.5 *M* Tris-Cl, pH 6.8)
4. Electrode buffer (0.025 *M* Tris, 0.192 *M* glycine, pH 8.3)
5. 2X treatment buffer (0.125 *M* Tris-Cl)
6. Ammonium persulphate (APS) (10 per cent)

7. Sodium dodecyl sulphate (SDS) (10 per cent)
8. Tetramethylethylenediamine (TEMED)
9. Fixer solution
10. Coomassie brilliant blue staining solution
11. Destaining solution
12. Prestained protein molecular weight ladder

3.4.4.2. SDS-PAGE protocol

3.4.2.1. Treatment of protein samples

The amount of proteins to be taken was decided based on Bradford was assay. To the microfuge tubes containing 15-20 µg of total protein, treatment buffer of about one-third the volume of protein was added and mixed by vortexing. The mixture was boiled in water bath at 100 °C for 4-5 minutes for complete denaturation of proteins. The heat denatured protein in the treatment buffer was loaded in the gel.

3.4.4.2.2. Components of resolving gel

Component	7.5 % gel	10 % gel	15 % gel
Monomer solution	2.49 ml	3.33 ml	4.99 ml
4X resolving gel buffer	2.5 ml	2.5 ml	2.5 ml
SDS (10 per cent)	0.1 ml	0.1 ml	0.1 ml
Distilled water	4.85 ml	4 ml	2.36 ml
APS (10 per cent)	50 µl	50 µl	50 µl
TEMED	10 µl	10 µl	10 µl
Total	10 ml	10 ml	10 ml

3.4.4.2.3. Components of stacking gel (5 per cent)

Components	5 per cent gel
Monomer solution	1.0 ml
4X stacking gel buffer	0.75 ml
SDS (10 per cent)	60 µl
Distilled water	4.1 ml
APS (10 per cent)	60 µl
TEMED	20 µl

3.4.4.2.4. Procedure for SDS-PAGE

The base plate and the bind plate were cleaned thoroughly in running water and then air dried. Both the plates were swiped with cotton soaked in 70 per cent ethanol. The plates were then assembled in the gel casting apparatus. First in a small 25 ml beaker, all the components of resolving gel buffer were added and mixed properly except the TEMED which was added at the last. Then with the help of pipette, the resolving gel was poured into the gap of base plate and bind plate assembly leaving one-third of the head space. The head space was filled with isopropanol to prevent air contact with the gel and dissolve air bubbles if any. The resolving gel was kept undisturbed to set and solidify for 30 minutes. After solidification was complete, the isopropanol over the resolving gel was discarded by tilting the stand and getting isopropanol absorbed by tissue paper. Then the components of stacking gel were mixed properly in a small 25 ml beaker except the TEMED, which was added at last. The stacking gel was then poured in the head space completely till the top and comb was placed immediately such that there was 1 cm gap between the bottom of wells formed by the comb and top of the resolving gel. The assembly was kept undisturbed for 30 minutes for the stacking gel to solidify.

After the complete polymerization of the gels, the plate assembly with solidified gel was removed from the casting apparatus and fixed in the gel running gasket vertically. A dummy plate was fixed on the other side of the running gasket. This assembly was tightened properly to eliminate any gaps for preventing the leakage of inner electrode buffer. Then the gasket was placed inside the electrophoresis tank. The electrode buffer was loaded till to the top of the inner tank for checking the leakage. Then the comb was gently removed, clearly demarcating distinct wells formed. The electrode buffer was poured outside the inner tank up to the half level marked in the electrophoresis tank.

The protein samples in treatment buffer were loaded into the respective wells with the help of pipette. Also 3 μL of BAS was loaded into one of the wells to check the movement of proteins while electrophoresis. The lid was placed over the electrophoresis tank and the chords were connected to electrophoresis power pack.

The initial running voltage was set at 80 V and the electrophoresis was allowed to run. After first 15 minutes of the electrophoretic run when the samples have travelled through stacking gel completely and reached just above the level of resolving gel, then the voltage was changed to 100 V and kept the same voltage condition until the tracking dye reaches the bottom of the gel plate. To prevent the protein samples from degradation due to heat generation while electrophoresis, ice packs were kept on both sides of the tank or the electrophoresis was carried out in air-conditioned room maintained at 20 °C.

When the tracking dye reached the bottom end of the plate, then the electrophoresis was stopped by switching off the power pack. The chords were unplugged from the power pack and the lid of the electrophoresis tank was opened. The gel running gaskets containing the plates were removed out from the tank and the locks were relaxed. The dummy plate was removed first followed by the base plate bind plate assembly. With the help of gel releasers, the base plate and the bind plate was separated and with the same tool the resolving gel was cut separated from the stacking gel. Care was taken while detaching the gel from the glass plate as there may be chance of breakage of resolving gel while mishandling. Hence, if there was fragile gel sticking to one of the plates then it was detached patiently by squeezing with distilled water. The resolving gel was transferred to a staining dish containing distilled water. With mild shaking, the gel was washed in distilled water to remove the adhering electrode buffer. After two washes with distilled water, fixer solution was added into the staining dish containing the resolving gel to fix the proteins in the gel. The fixing step was carried out for 30 minutes over a shaker.

3.4.4.2.5. Coomassie brilliant blue staining of resolving gel

After fixation of proteins in the resolving gel, the fixer solution was drained off and Coomassie brilliant blue staining solution was added into the staining dish. The staining of the gel was done overnight by constant shaking over a shaker in order to attain uniform and saturated staining of the gel.

3.4.4.2.6. Destaining of the stained resolving gel

After staining was over, the staining solution was transferred into a glass bottle and destaining solution was added into the staining dish. The destaining was carried out in constant shaking over a shaker. Periodic changes with fresh destaining solution were given when the solution turned dark blue. The destaining was carried out until the background of the gel became completely transparent and colourless and the proteins were prominent with dark bluish-purple stained bands. Property of coomassie brilliant blue stain is that only proteins hold up the stain and appear as dark bluish-purple bands in the resolving gel even after complete destaining. The destained gel was preserved in distilled water containing few drops of glacial acetic acid at 4ºC to prevent fungal contamination in the gel.

Results and Discussion

4. Results and Discussion

The present study was conducted in the Department of Plantation Crops and Spices, Department of Plant Biotechnology, College of Horticulture, and Cocoa Research Centre, Vellanikkara during 2015 to 2018. The cocoa inbreds of various selfed generations were evaluated to quantify the magnitude of inbreeding depression in yield and yield attributes. The cocoa inbreds, inbred crosses and hybrids were evaluated to establish the physiological relationship between them. The self incompatibility mechanism, different methods to overcome the self incompatibility and differential expression of protein in the fifth generation inbred was carried out. The results of the research are presented and discussed in this chapter

4.1 Evaluation of cocoa inbreds

A total of 113 cocoa inbreds were evaluated and the details are given in Annexure I. The inbreds were selected on the basis of availability of preceding and succeeding generations in the field maintained at CRC

4.1.1 Morphological characterization

The qualitative and quantitative characters are the corridor for evaluating the genotype morphologically. Morphological characterization was well known in its applicability to derive economic and breeding gain from accession studied (Hawkes, 1983, Brown *et. al.* 1989, and Iwaro *et al.,* 2003). The morphological characterization was done using descriptors developed by Bekele and Butler, (2000). The plant height and girth at collar region was recorded for the inbreds. Five pods were collected from each cocoa inbred for morphological observations on distinguishable quantitative and qualitative characterization.

4.1.2 Qualitative pod and bean characters of inbreds

The observations on qualitative characters are described in Table 2. Qualitative evaluation was carried out by recording six qualitative parameters *viz*., pod shape, ridge colour, pod apex form, pod basal constriction, pod rugosity and colour of bean (cotyledon colour). Bekele and Butler (2000) described five type of pod shapes, which

include Amelanado Angoleta, Cundeamore, Calabacillo, and Criollo. In the present evaluation, 41 inbreds (36%) showed Amelanado, thirty two inbreds (28.31%) showed Angoleta, twenty two inbreds (19.16%) showed cundeamore and 18 inbreds (15.92%) showed Calabacillo. Among the inbred Criollo type pods were not found. Similar studies were conducted by Minimol *et al.,* (2011) and Veeresh (2018). Minimol *et al.,* (2011) reported 12 accessions with Angoleta fruit type, 7 accessions with Calabacillo type and 3 accessions with Cundeamore type and one accession with Amelonado type. Veeresh (2018) reported 47 percent Cundeamore, 30 percent Angoleta, 10 percent each Amelanado and Calabacillo type. Even though high amount of similarity was recorded for qualitative characters among the inbreds of same genotype, there were some amounts of variation. However, the pod attributes such as pod shape, pod colour, pod apex, pod basal constriction, pod rugosity and cotyledon colour showed wide variability among the inbreds. The pod colour showed very less variability compared to other qualitative characters.

The pod colour showed very less variability compared to other qualitative characters of pod and bean presented in table 2.

Table 2. Qualitative pod characters of cocoa inbreds over generations

Plant No.	Genotype	Generation	Pod shape	Pod color (Ridge)	Apex	Base	Rugosity	Bean colour
1.1	G VI 295.4	S_2	Amelanado	Absent	Obtuse	Absent	Slight	Medium
1.2	G VI 295.4	S_2	Amelanado	Absent	Obtuse	Absent	Slight	Medium and light
1.3	G VI 295.4	S_2	Amelanado	Absent	Obtuse	Absent	Slight	Medium
1.4	G VI 295.4	S_2	Amelanado	Absent	Rounded	Absent	Absent	Medium and light
1.5	G VI 295.4	S_2	Amelanado	Absent	Intermediate	Absent	Slight	Medium
1.6	G VI 295.4	S_2	Amelanado	Absent	Acute	Absent	Slight	Medium
1.7	G VI 295.4	S_2	Amelanado	Absent	Mammeleate	Absent	Absent	Medium and light
1.8	G VI 295.4	S_2	Amelanado	Absent	Rounded	Slight	Absent	Medium
1.9	G VI 295.4	S_2	Amelanado	Absent	Obtuse	Absent	Slight	Medium and light
1.1	G VI 295.4	S_2	Amelanado	Absent	Obtuse	Absent	Absent	Medium
1.11	G VI 295.4	S_2	Amelanado	Absent	Obtuse	Slight	Absent	Medium
2.1	M 18.7	S_3	Amelanado	Absent	Obtuse	Absent	Absent	Medium
2.2	M 18.7	S_3	Amelanado	Absent	Acute	Absent	Slight	Medium and light
2.3	M 18.7	S_3	Amelanado	Absent	Rounded	Slight	Absent	Medium
2.4	M 18.7	S_3	Amelanado	Absent	Obtuse	Absent	Absent	Medium
2.5	M 18.7	S_3	Calabacillo	Absent	Acute	Slight	Slight	Medium
3.1	M 18.7	S_4	Angoleta	Absent	Attenuate	Strong	Intermediate	Medium and light
3.2	M 18.7	S_4	Cundeamore	Absent	Obtuse	Slight	Slight	Medium
3.3	M 18.7	S_4	Cundeamore	Absent	Acute	Slight	Slight	Dark
3.4	M 18.7	S_4	Calabacillo	Absent	Attenuate	Intermediate	Medium	Dark

4.1	H 7.3 (86)	S_1	Angoleta	Yellowish	Acute	Slight	Slight	Medium purple
4.2	H 7.3 (86)	S_1	Angoleta	Yellowish	Acute	Slight	Slight	Dark
4.3	H 7.3 (86)	S_1	Calabacillo	Absent	Attenuate	Absent	Slight	Dark
4.4	H 7.3 (86)	S_1	Angoleta	Yellowish	Acute	Slight	Intermediate	Medium
4.5	H 7.3 (86)	S_1	Angoleta	Absent	Attenuate	Slight	Slight	Medium
4.6	H 7.3 (86)	S_1	Angoleta	Yellowish	Acute	Slight	Slight	Medium
4.7	H 7.3 (86)	S_1	Angoleta	Yellowish	Acute	Slight	Slight	Dark
4.8	H 7.3 (86)	S_1	Angoleta	Yellowish	Acute	Slight	Slight	Dark
4.9	H 7.3 (86)	S_1	Cundeamore	Absent	Acute	Slight	Slight	Medium
4.1	H 7.3 (86)	S_1	Angoleta	Absent	Attenuate	Absent	Intermediate	Medium
4.11	H 7.3 (86)	S_1	Amelanado	Absent	Rounded	Slight	Slight	Dark
4.12	H 7.3 (86)	S_1	Angoleta	Yellowish	Acute	Slight	Slight	Medium
4.13	H 7.3 (86)	S_1	Angoleta	Absent	Acute	Slight	Slight	Medium
4.14	H 7.3 (86)	S_1	Calabacillo	Absent	Obtuse	Absent	Slight	Dark
4.15	H 7.3 (86)	S_1	Angoleta	Absent	Acute	Intermediate	Absent	Dark
4.16	H 7.3 (86)	S_1	Angoleta	Yellowish	Acute	Slight	Slight	Dark
4.17	H 7.3 (86)	S_1	Angoleta	Yellowish	Obtuse	Absent	Slight	Medium
4.18	H 7.3 (86)	S_1	Calabacillo	Absent	Acute	Absent	Absent	Medium
5.1	H 7.3 (86)	S_2	Amelanado	Absent	Obtuse	Slight	Absent	Medium
5.2	H 7.3 (86)	S_2	Angoleta	Absent	Acute	Slight	Slight	Medium
5.3	H 7.3	S_2	Calabasilo	Absent	Acute	Absent	Slight	Medium
6.1	H1 1.2	S_1	Amelanado	Absent	Obtuse	Absent	Slight	Light
6.2	H1 1.2	S_1	Angoleta	Absent	Acute	Intermediate	Slight	Light
6.3	H1 1.2	S_1	Calabacillo	Yellow	Mammelleate	Intense	Intermediate	Light

6.4	H1 1.2	S_1	Angoleta	Yellow	Acute	Slight	Absent	Medium and light
7.1	H1 1.2	S_2	Amelanado	Yellow	Obtuse	Slight	Slight	Medium
8.1	GII 7.4	S_3	Angoleta	Yellow	Obtuse	Slight	Slight	Medium
9.1	GII 7.4	S_4	Angoleta	Absent	Acute	Slight	Absent	Light
10.1	GII 7.4	S_5	Cundeamore	Absent	Acute	Slight	Slight	Light
11.1	G IV 35.7	S_4	Calabacillo	Absent	Obtuse	Intermediate	Slight	Light
12.1	G VI 135	S_1	Cundeamore	Absent	Obtuse	Absent	Absent	Medium
12.2	G VI 135	S_1	Cundeamore	Absent	Obtuse	Absent	Absent	Medium
12.3	G VI 135	S_1	Calabacillo	Absent	Obtuse	Slight	Slight	Medium
12.4	G VI 135	S_1	Amelanado	Absent	Slight	Slight	Absent	Light and medium
12.5	G VI 135	S_1	Calabacillo	Absent	Obtuse	Slight	Slight	Light and medium
12.6	G VI 135	S_1	Cundeamore	Absent	Slight	Slight	Absent	Light and medium
12.7	G VI 135	S_1	Cundeamore	Absent	Mammeleate	Slight	Absent	Light and medium
12.8	G VI 135	S_1	Amelanado	Absent	Rounded	Absent	Slight	Light and medium
13.1	G VI 135	S_2	Amelanado	Absent	Mammeleate	Absent	Intermediate	Light and medium
14.1	G VI 141	S_1	Angoleta	Yellowish	Obtuse	Slight	Slight	Dark and light
14.2	G VI 141	S_1	Cundeamore	Yellowish	Acute	Slight	Intermediate	Dark and light
15.1	G VI 141	S_2	Cundeamore	Absent	Acute	Slight	Absent	Dark and light
15.2	G VI 141	S_2	Angoleta	Yellowish	Obtuse	Intermediate	Slight	Dark and light
15.3	G VI 141	S_2	Angoleta	Absent	Obtuse	Intermediate	Slight	Dark and light
15.4	G VI 141	S_2	Cundeamore	Absent	Obtuse	Slight	Slight	Dark and light
15.5	G VI 141	S_2	Cundeamore	Absent	Acute	Slight	Absent	Dark and light
15.6	G VI 141	S_2	Angoleta	Yellowish	Acute	Slight	Slight	Dark and light
15.7	G VI 141	S_2	Cundeamore	Absent	Acute	Slight	Slight	Dark and light

15.8	G VI 141	S_2	Amelanado	Absent	Obtuse	Slight	Slight	Dark and light
15.9	G VI 141	S_2	Amelanado	Absent	Obtuse	Slight	Absent	Dark and light
16.1	P II 13.12	S_1	Calabacillo	Absent	Slight	Obtuse	Absent	Dark and light
16.2	P II 13.12	S_1	Amelanado	Absent	Obtuse	Absent	Absent	Dark and light
16.3	P II 13.12	S_1	Amelanado	Absent	Rounded	Absent	Absent	Light
17.1	PII 13.12	S_2	Amelanado	Absent	Intermediate	Slight	Absent	Dark
17.2	PII 13.12	S_2	Amelanado	Absent	Obtuse	Absent	Absent	Light
17.3	PII 13.12	S_2	Calabacillo	Absent	Acute	Slight	Slight	Dark
17.4	PII 13.12	S_2	Calabacillo	Absent	Obtuse	Slight	Medium	Dark
17.5	PII 13.12	S_2	Angoleta	Absent	Acute	Intermediate	Slight	Light
17.6	PII 13.12	S_2	Cundeamore	Absent	Acute	Intermediate	Slight	Dark
17.7	PII 13.12	S_2	Cundeamore	Absent	Obtuse	Strong	Slight	Light
17.8	PII 13.12	S_2	Amelanado	Yellowish	Rounded	Absent	Absent	Light
18.1	G VI 256.5	S_1	Amelanado	Absent	Rounded	Slight	Slight	Dark
18.2	G VI 256.5	S_1	Angoleta	Yellowish	Acute	Slight	Slight	Medium
18.3	G VI 256.5	S_1	Cundeamore	Yellow	Acute	Intermediate	Slight	Medium
18.4	G VI 256.5	S_1	Angoleta	Absent	Attenuate	Strong	Absent	Medium
18.5	G VI 256.5	S_1	Cundeamore	Absent	Acute	Strong	Slight	Light
18.6	G VI 256.5	S_1	Angoleta	Absent	Slight	Acute	Absent	Light
19.1	P II 4.8	S_1	Amelanado	Absent	Rounded	Absent	Absent	Medium
19.2	P II 4.8	S_1	Angoleta	Absent	Attenuate	Slight	Slight	Light
19.3	P II 4.8	S_1	Cundeamore	Absent	Acute	Slight	Slight	Medium
19.4	P II 4.8	S_1	Amelanado	Absent	Acute	Slight	Absent	Light
20.1	P II 4.8	S_2	Cundeamore	Absent	Attenuate	Slight	Slight	Light

20.2	P II 4.8	S_2	Angoleta	Absent	Acute	Slight	Slight	Medium
20.3	P II 4.8	S_2	Cundeamore	Absent	Attenuate	Slight	Slight	Medium
20.4	P II 4.8	S_2	Cundeamore	Absent	Acute	Intermediate	Slight	Light
20.5	P II 4.8	S_2	Calabacillo	Absent	Acute	Slight	Slight	Light
20.6	P II 4.8	S_2	Cundeamore	Yellowish	Attenuate	Slight	Slight	Light
20.7	P II 4.8	S_2	Angoleta	Yellowish	Attenuate	Slight	Slight	Medium
21.1	P II 13.8	S_1	Calabacillo	Absent	Acute	Absent	Slight	Light
21.2	P II 13.8	S_1	Amelanado	Absent	Obtuse	Absent	Absent	Medium
21.3	P II 13.8	S_1	Amelanado	Absent	Obtuse	Absent	Absent	Medium
22.1	P II 13.8	S_2	Amelanado	Absent	Rounded	Absent	Slight	Dark
22.2	P II 13.8	S_2	Amelanado	Absent	Obtuse	Absent	Absent	Dark
22.3	P II 13.8	S_2	Amelanado	Absent	Rounded	Absent	Slight	Medium
22.4	P II 13.8	S_2	Amelanado	Absent	Rounded	Absent	Slight	Light
22.5	P II 13.8	S_2	Amelanado	Absent	Rounded	Absent	Absent	Light
23.1	P II 12.9	S_1	Angoleta	Yellowish	Acute	Slight	Slight	Light
23.2	P II 12.9	S_1	Calabacillo	Absent	Obtuse	Absent	Intermediate	Light
23.3	P II 12.9	S_1	Calabacillo	Slight	Obtuse	Absent	Slight	Medium
23.4	P II 12.9	S_1	Angoleta	Slight	Obtuse	Absent	Slight	Medium
23.5	P II 12.9	S_1	Calabacillo	Yellowish	Acute	Absent	Slight	Light
24.1	P II 12.9	S_2	Amelanado	Absent	Obtuse	Absent	Slight	Light
24.2	P II 12.9	S_2	Amelanado	Absent	Acute	Slight	Slight	Medium

According to Kochhar (1986) pods of Criollo types were morphologically pointed with warty surface area, Forastero group exhibited smooth surface of pods and obtuse apex. In the present study, pod apex showed wide variation among the inbreds and the result is presented in table 3. Thirty seven per cent of the inbreds showed acute type, 31.85 per cent obtuse, 11.50 per cent rounded, 10.6 per cent attenuate, 3.5 per cent each mammaleate and 2.65 per cent intermediate types among the cocoa inbred over generations.

4.1.3 Quantitative pod and bean characters of inbreds

4.1.3.1 Evaluation of S_1 inbreds of cocoa

4.1.3.1.1 Growth parameters

4.1.3 .1.1.1 Plant height (cm)

The details of plant height in S_1 inbreds of cocoa are presented in table 3.

The plant height varied among the S_1 inbreds. The plant height during 2016 ranged between 100 cm and 720 cm. The maximum plant height of 720 cm was recorded in inbred P II 4.8 (Plant number 19.1) followed by 685 cm in S_1 inbred G VI 256.5 (Plant number 18.3) and the least plant height of 100 was observed in S_1 inbred H 1.2 (Plant number 6.4)

The plant height varied among the S_1 inbreds during 2017. The plant height ranged between 108 cm and 750 cm. The maximum plant height of 750 cm was recorded in inbred P II 4.8 (Plant number 19.1) followed by 700 cm in S_1 inbred G VI 256.5 (Plant number 18.3) and the least plant height of 108 was observed in S_1 inbred H 1.2 (Plant number 6.4)

The plant height varied among the S_1 inbreds during 2018. The plant height ranged between 110 cm and 780 cm. The maximum plant height of 780 cm was recorded in inbred P II 4.8 (Plant number 19.1) and P II 13.12 (Plant number 16.2) followed by 750 cm in S_1 inbred P II 12.9 (Plant number 23.3) and the least plant height of 110 was observed in S_1 inbred H 1.2 (Plant number 6.4).

Table 3. Plant height and collar girth of S_1 inbreds

Plant No.	Genotype	Plant height (cm)			Girth (cm)		
		2016	2017	2018	2016	2017	2018
4.1	H 7.3	180	190	200	52	54	53
4.2	H 7.3	195	202	218	31	28	32
4.3	H 7.3	180	190	210	50	54	61
4.4	H 7.3	185	192	197	42	46	51
4.5	H 7.3	480	490	520	55	58	58
4.6	H 7.3	380	390	410	49	50	51
4.7	H 7.3	215	222	229	47	45	48
4.8	H 7.3	190	205	220	34	36	36
4.9	H 7.3	390	405	420	43	37	45
1.1	H 7.3	410	415	426	42	46	52
1.11	H 7.3	390	405	410	67	63	87
1.12	H 7.3	201	209	220	68	72	78
1.13	H 7.3	220	226	235	53	56	58
1.14	H 7.3	210	220	232	52	56	62
1.15	H 7.3	480	495	515	47	48	53
1.16	H 7.3	180	186	205	48	49	49
1.17	H 7.3	190	196	205	50	52	60
1.18	H 7.3	185	195	206	42	44	44
6.1	H1 1.2	210	235	250	47	48	48
6.2	H1 1.2	220	226	236	55	56	52
6.3	H1 1.2	120	430	145	45	46	47
6.4	H1 1.2	100	108	110	58	60	56
12.1	G VI 135	340	350	360	67	69	72
12.2	G VI 135	480	495	510	41	44	68
12.3	G VI 135	280	325	385	56	60	73
12.4	G VI 135	320	365	415	52	64	74
12.5	G VI 135	450	470	480	59	62	69
12.6	G VI 135	280	315	345	71	72	75
12.7	G VI 135	480	490	510	71	74	77
12.8	G VI 135	280	305	330	73	73	74
14.1	G VI 141	120	130	150	31	32	36
14.2	G VI 141	400	420	440	37	39	44
16.1	P II 13.12	580	640	720	47	48	52
16.2	P II 13.12	650	680	780	39	40	46
16.3	P II 13.12	480	510	550	36	37	39
18.1	G VI 256.5	580	605	620	35	36	38
18.2	G VI 256.5	650	690	720	39	40	42
18.3	G VI 256.5	685	700	715	33	36	37

18.4	G VI 256.5	480	495	550	34	38	42
18.5	G VI 256.5	580	610	650	36	38	39
18.6	G VI 256.5	380	410	470	35	36	37
19.1	P II 4.8	720	750	780	63	64	65
19.2	P II 4.8	675	690	720	43	44	45
19.3	P II 4.8	540	580	610	47	48	49
19.4	P II 4.8	480	505	540	53	56	57
21.1	P II 13.8	420	475	530	29	30	34
21.2	P II 13.8	480	520	555	35	36	36
21.3	P II 13.8	320	242	360	24	26	30
23.1	P II 12.9	680	695	730	52	54	55
23.2	P II 12.9	570	590	610	46	47	48
23.3	P II 12.9	650	680	740	45	48	48
23.4	P II 12.9	620	690	740	49	50	51
23.5	P II 12.9	480	490	530	26	35	36

4.1.3 .1.1.2 Collar girth (cm)

The collar girth in S_1 inbreds is presented in table in 3. During 2016, the maximum collar girth (73 cm) was observed in G VI 135 (Plant number 12.8) followed by 72 cm collar girth in G VI 135 (Plant number 12.6). The least collar girth of 24 cm was observed in P II 13.8 (Plant number 21.3).

The collar girth varied among the inbreds of S_1 during 2017. The maximum collar girth of 73 cm was recorded in G VI 135 (Plant number 12.8) followed by 72 cm girth in G VI 135 (Plant number 12.7) and H 7.3 (Plant number 1.12), and the least collar girth of 26 cm was observed in P II 13.8 (Plant number 21.3).

During 2018, the maximum collar girth of 78 cm was observed in S_1 inbred H 7.3 (Plant number 1.12) followed by 74 cm girth in G VI 135 (Plant number 12.6) and the least collar girth of 30 cm was observed in P II 13.8 (Plant number 21.3).

4.1.3 .1.2 Pod characters of S_1 inbreds of cocoa

4.1.3 .1.2.1 Pod weight (g)

The details of pod characters of S_1 inbred of cocoa are presented in table 4. The mean pod weight varied between 104 g and 532 g among the S_1 generation of different genotypes. The mean pod weight recorded among the S_1 inbreds was 303.02 g (Table 3). The maximum pod weight of 532 g was recorded in PII 13.8 inbred and the least pod weight of 104g was observed in H 7.3(86) inbred plant. The wide variation in pod weight was observed among the S_1 progeny of same parent indicating the high amount of segregation and heterozygous nature of the parent (Minimol *et. al.,* 2015).

In S_1 generation of H 7.3 significant difference for pod weight was observed, the pod weight in H 7.3 ranged from 104 g in plant number 4.9 to 488 g in plant number 4.12. The pod weight in H 1 1.2 did not vary significantly (Fig 1). The pod weight in G VI 135 ranged from 218 g in plant number 23.6 to 362 g in plant number 23.14. The pod weight varied significantly in G VI 141 genotype and it ranged between 318 g to 444 g in plant number 14.2 and 14.1 respectively. The pod weight varied significantly in inbred P II 13.12 from 254 g in plant number 16.2 to 402 g in plant number 16.1.The pod weight in G VI 256.5 varied significantly and it ranged from 149 g in plant number

Table 4 Pod characters of S_1 inbreds of cocoa

Plant No.	Genotype	Pod weight (g)	Pod length (cm)	Pod breadth (cm)	Ridge thickness (cm)	Furrow thickness (cm)	No. of beans/pod	Flat bean
4.2	H 7.3 (86)	390.00	14.20	7.22	1.54	1.18	36.40	0.80
4.3	H 7.3 (86)	272.00	12.14	7.02	1.62	0.72	39.60	1.80
4.4	H 7.3 (86)	346.00	15.30	7.54	1.14	0.74	39.60	1.40
4.5	H 7.3 (86)	262.00	13.50	7.18	0.82	0.62	40.20	1.00
4.6	H 7.3 (86)	388.00	15.20	8.12	1.22	0.98	40.40	1.40
4.7	H 7.3 (86)	378.00	15.00	6.92	1.04	0.82	43.80	1.00
4.8	H 7.3 (86)	304.00	12.82	6.80	1.22	0.66	23.60	0.60
4.9	H 7.3 (86)	104.00	8.10	6.30	1.14	0.80	22.80	1.40
4.10	H 7.3 (86)	300.00	13.90	7.10	1.42	1.00	47.40	1.20
4.11	H 7.3 (86)	336.00	12.04	7.86	0.98	0.72	40.00	6.20
4.12	H 7.3 (86)	488.00	15.46	8.28	1.02	0.86	43.40	0.40
4.13	H 7.3 (86)	215.00	13.80	7.44	1.20	0.80	30.00	0.60
4.14	H 7.3 (86)	296.00	12.20	7.30	1.50	0.90	35.60	0.20
4.15	H 7.3 (86)	278.00	13.12	6.72	1.34	0.76	35.60	0.80
4.16	H 7.3 (86)	268.00	12.72	7.44	0.88	0.68	36.20	2.20
4.17	H 7.3 (86)	356.00	14.98	7.20	1.06	0.88	43.40	0.40
4.18	H 7.3 (86)	164.00	10.28	5.76	1.24	1.08	27.60	1.60
6.1	H1 1.2 (86)	256.00	10.74	6.80	1.00	0.68	42.20	0.20
6.2	H1 1.2 (86)	296.00	14.00	7.20	0.78	0.34	35.80	0.20
6.3	H1 1.2 (86)	246.00	11.74	6.06	0.72	0.48	36.00	0.40
6.4	H1 1.2 (86)	320.00	12.94	7.14	1.02	0.72	42.20	0.80
12.1	G VI 135	288.00	13.20	7.00	1.72	0.94	38.60	0.40
122	G VI 135	218.00	10.84	6.66	1.50	0.90	31.80	1.20
12.3	G VI 135	300.00	12.12	7.44	1.72	0.84	32.00	1.20
12.4	G VI 135	362.00	13.90	8.10	1.64	1.16	42.60	1.80
12.5	G VI 135	280.00	12.50	6.80	1.18	0.80	40.20	1.20
12.6	G VI 135	264.00	12.60	5.90	1.20	0.82	34.00	0.60
12.7	G VI 135	220.00	10.30	7.10	1.28	0.80	34.80	0.80
12.8	G VI 135	332.00	11.50	7.36	1.28	0.82	44.20	1.20
14.1	G VI 141	444.00	14.20	7.00	1.42	1.14	35.60	1.00
14.2	G VI 141	318.00	15.66	6.96	1.42	0.96	26.60	2.00
16.1	P II 13.12	402.00	16.30	8.36	1.56	0.66	45.60	1.00
16.2	P II 13.12	254.00	11.12	7.74	1.32	1.16	31.80	5.00
16.3	P II 13.12	256.00	10.70	7.26	1.14	0.76	35.00	1.20
18.1	G VI 256.5	149.00	10.74	5.76	1.00	0.84	18.40	0.20
18.2	G VI 256.5	268.00	15.30	7.20	0.98	0.76	39.00	0.80
18.3	G VI 256.5	342.00	16.50	7.50	1.04	0.66	44.20	0.60

18.4	G VI 256.5	272.00	16.50	6.90	1.24	0.76	33.80	1.20
18.5	G VI 256.5	246.00	14.76	7.30	1.34	0.82	32.40	0.60
18.6	G VI 256.5	198.00	13.08	6.30	2.54	0.62	37.20	2.80
19.1	P II 4.8	254.00	10.90	7.30	1.12	0.86	29.60	1.40
19.2	P II 4.8	314.00	15.60	6.96	1.82	1.46	31.40	1.80
19.3	P II 4.8	414.00	17.34	7.94	1.22	0.82	40.60	1.60
19.4	P II 4.8	318.00	15.50	7.56	1.80	0.88	36.60	1.20
21.1	P II 13.8	428.00	12.76	8.76	2.06	1.82	34.40	2.80
21.2	P II 13.8	532.00	14.80	7.10	2.30	1.44	34.00	1.40
21.3	P II 13.8	316.00	13.90	8.90	1.98	1.48	30.00	1.40
22.1	P II 13.8	252.00	12.58	6.32	1.26	0.92	27.20	1.40
23.1	P II 12.9	518.00	14.80	7.70	1.16	0.84	38.60	3.00
23.2	P II 12.9	190.00	9.72	6.14	2.26	1.94	19.00	1.60
23.3	P II 12.9	316.00	14.12	7.86	1.62	0.74	32.60	1.80
23.4	P II 12.9	292.00	13.30	7.34	1.80	0.76	30.40	1.40
23.5	P II 12.9	240.00	10.46	6.10	1.56	0.92	15.20	5.20
	CV(%)	19.46	8.93	8.74	39	15.92	13.73	10.11
	CD(0.05)	77.09	1.467	0.78	0.66	0.18	5.97	1.79

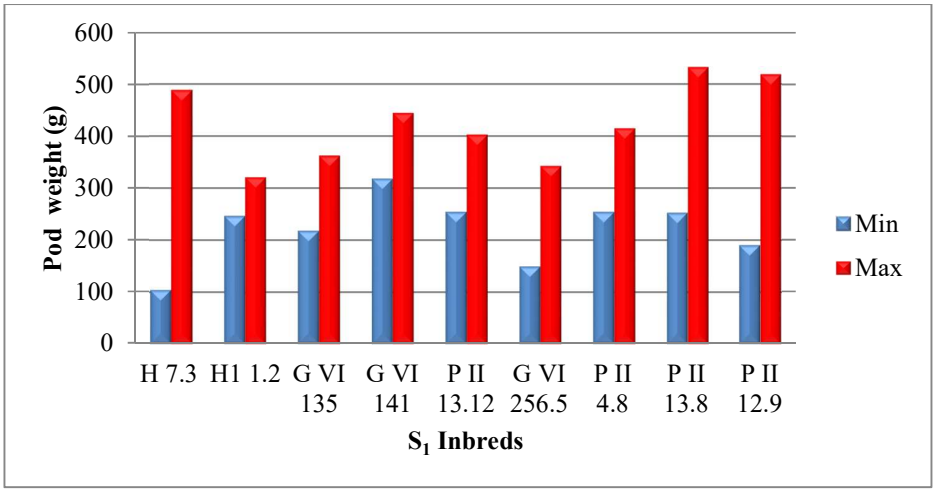


Fig. 1 Pod weight of S_1 inbreds

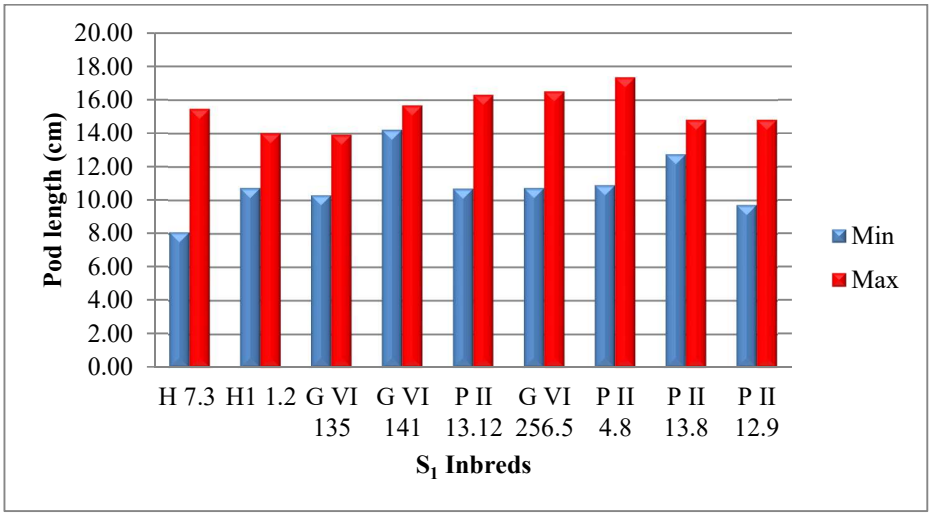


Fig. 2 Pod length of S_1 inbreds

18.1 to 342 g in plant number 18.3. The pod weight in P II 4.8 ranged from 254 g in plant number 19.1 to 414 g in plan number 19.3. Whereas, it varied from 252 g in plant number 22.1 to 532 g in plant number 21.2 in genotype PII 13.8. The pod weight ranged significantly in P II 12.9 genotype, the pod weight ranged from 190 g in plant number 23.2 to 518 g in plant number 23.1.

Only one genotype did not show significant variation among the progeny with respect to pod weight, where as all other expressed wide variability indicating the segregation and heterozygote nature of the parent material (Minimol *et al.,* 2015)

4.1.3 .1.2.2 Pod length (cm)

The details of pod characters of S_1 inbred of cocoa are presented in table 4.

The mean pod length varied between 8.1 cm and 17.34 cm among the S_1 generation of different genotypes. The mean pod length recorded among the S_1 inbreds was 13.24 cm (Table 4). The maximum pod length of 17.34 cm was recorded in plant number 19.3 and the minimum pod length of 8.10 cm was observed in H 7.3 (86) inbred plant (Fig 2) The wide variation in pod length was observed among the S_1 progeny of same parent indicating the high amount of segregation and heterozygous nature of the parent.

The pod length in H 7.3 ranged from 8.10 cm in plant number 4.9 to 15.46 cm in plant number 4.12. Even though pod weight did not vary significantly, a significant difference was observed for pod length in H 1 1.2 genotypes among individual is significant. The pod length in H 1 1.2 ranged between 10.74 cm and 14 cm in plant number 6.1 and 6.2 respectively. The pod length in G VI 135 ranged from 10.30 cm in plant number 12.7 to 13.9 cm in plant number 12.4 Wide variations was observed with respect to pod weight, however, no much difference was observed for pod length in G VI 141inbred. The pod length varied significantly in genotype P II 13.12. The pod length varied from 10.70 cm in plant number 16.3 to 16.30 cm in plant number 16.1. Significant difference for pod length was observed in G VI 256.5 and it ranged from 10.74 cm in plant number 18.1 to 16.5 cm in plant number 18.3. The maximum and minimum pod length observed in inbred P II 4.8 was 10.90 cm in plant number 19.1 and

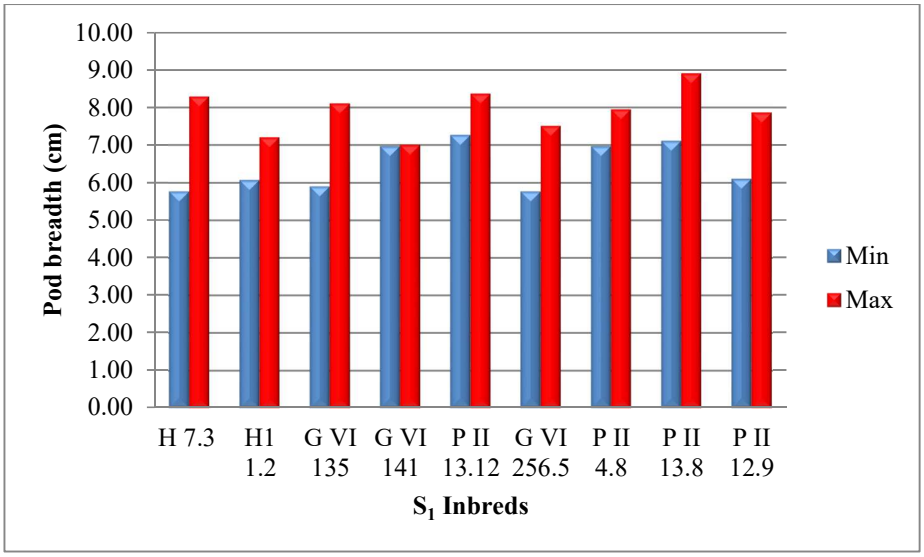


Fig. 3 Pod breadth of S_1 inbreds

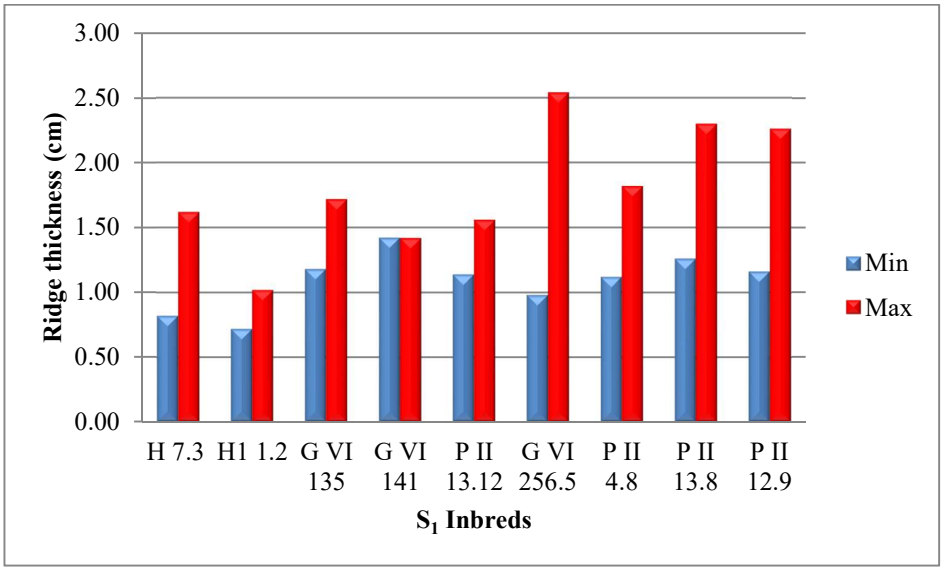


Fig. 4 Ridge thickness of S_1 inbreds

17.34 cm in plant number 19.3. The pod length varied significantly in PII 13.8 inbred and it was 12.76 cm in plant number 22.1 to 14.80 cm in plant number 21.2. The pod length ranged in P II 12.9 ranged from 9.72 cm in plant number 23.2 to 14.80 cm in plant number 23.1.

4.1.3.1.2.3 Pod breadth (cm)

The pod breadth significantly varied among the S_1 generation inbreds. The pod breadth ranged from 5.76 cm in plant number 4.18 to 8.90 cm in plant number 21.3 (Fig. 3)

The pod breadth among the plants of S_1 generation of H 7.3 genotype varied significantly, and it ranged from 5.76 cm in plant number 4.18 to 8.28 cm in plant number 4.12. The pod breadth in H 1 1.2 ranged between 6.06 cm and 7.20 cm in plant number 6.3 and 6.2 respectively. The pod breadth in G VI 135 ranged from 5.90 cm in plant number 12.6 to 13.90 cm in plant number 12.4. Like pod length individuals in S_1 generation of G VI 141 also did not exhibited significant difference in pod breadth. The pod breadth varied significantly within genotypes of P II 13.12 inbred. The pod breadth varied from 7.76 cm in plant number 16.3 to 8.36 cm in plant number 16.1. Significant difference in pod length was observed in G VI 256.5. The pod breadth ranged from 5.76 cm in plant number 18.1 to 7.5 cm in plant number 18.3. The pod breadth varied significantly in P II 4.8. The pod breadth ranged from 6.96 cm in plant number 19.2 to 7.94 cm in plant number 19.3. The pod breadth varied from 7.10 cm in plant number 22.2 to 8.9 cm in plant number 21.3 in inbred P II 13.8. Significant variation was observed for pod breadth in inbred P II 12.9 and it ranged from 6.10 cm in plant number 23.5 to 7.86 cm in plant number 23.3

4.1.3.1.2.4 Ridge thickness (cm)

Ridge thickness varied significantly among the genotypes in S_1 generation. The ridge thickness ranged from 0.72 cm in plant number 6.3 to 2.54 cm in plant number 18.6 (Fig.4).

The ridge thickness among the plants of S_1 generation of H 7.3 genotype varied significantly, the ridge thickness ranged from 0.82 cm in plant number 4.5 to 1.62 cm in plant number 4.3. The ridge thickness did not differed significantly among the inbreds H1 1.2, G VI 135 and G VI 141 indicating less amount segregation with respect to this character. The ridge thickness varied significantly in P II 13.12. The ridge thickness varied from 1.14 cm in plant number 16.3 to 1.56 cm in plant number 16.1. Significant difference in ridge thickness was observed in G VI 256.5. The ridge thickness ranged from 0.98 cm in plant number 18.2 to 2.54 cm in plant number 18.6. The ridge thickness varied significantly in P II 4.8. The ridge thickness ranged from 1.12 cm in plant number 19.1 to 1.82 cm in plant number 19.2. The ridge thickness varied significantly in PII 13.8 and it ranged from 1.26 cm in plant number 22.1 to 2.3 cm in plant number 21.2. The ridge thickness in P II 12.9 ranged from 1.16 cm in plant number 23.1 to 2.26 cm in plant number 23.2

4.1.3.1.2.5 Furrow thickness (cm)

Furrow thickness among the S_1 generation inbred varied significantly from 0.34 in plant number 6.2 to 1.94 cm in plant number 23.2. The variation in furrow thickness of S_1 inbreds is presented in fig 5.

The furrow thickness among the plants of S_1 generation of H 7.3 genotype varied significantly, the furrow thickness ranged from 0.62 cm in plant number 4.5 to 1.18 cm in plant number 4.2. Even though ridge thickness did not show much variation, the furrow thickness ranged from 0.34 cm in plant number 6.2 to 0.72 cm in plant number 6.4 of H 1 1.2. The furrow thickness ranged from 0.80 cm in plant number 12.5 to 1.16 cm in plant number 12.4 in inbred G VI 135.

The same trend followed in ridge thickness also did not expressed any significant variation in furrow thickness in G VI 141 The furrow thickness in H 1 1.2 did not differed significantly

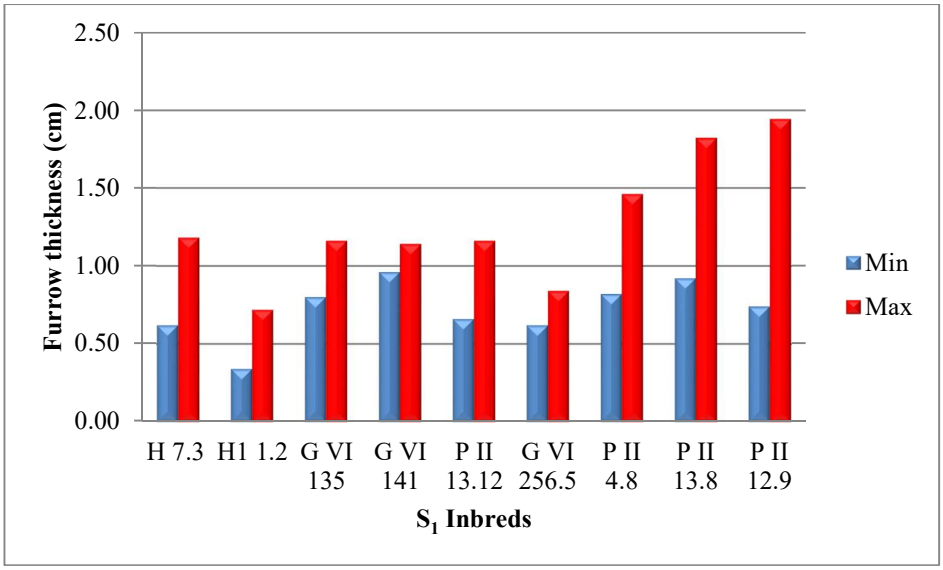


Fig. 5 Furrow thickness of S_1 inbreds

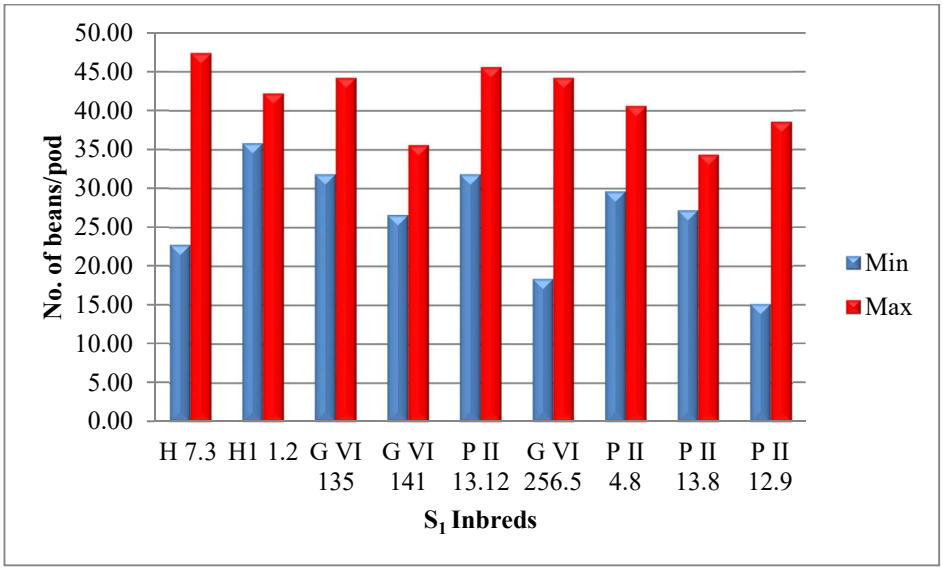


Fig. 6 No. of beans per pod of S_1 inbreds

The furrow thickness varied significantly in P II 13.12 and it varied from 0.66 cm in plant number 16.1 to 1.16 cm in plant number 16.2. Significant difference in furrow thickness was observed in G VI 256.5. The furrow thickness ranged from 0.62 cm in plant number 18.6 to 0.84 cm in plant number 18.1. The furrow thickness varied significantly in P II 4.8. The furrow thickness ranged from 0.82 cm in plant number 19.3 to 1.46 cm in plant number 19.2 The furrow thickness varied significantly in P II 13.8 and it ranged from 0.92 cm in plant number 22.1 to 1.82 cm in plant number 21.1.The furrow thickness in P II 12.9 ranged from 0.74 cm in plant number 23.3 to 1.94 cm in plant number 23.2

4.1.3.1.2.6 Number of beans per pod

The number of beans per pod varied significantly among genotypes of S_1 generation. The number of beans per pod ranged from 15.2 in plant number 23.5 to 47.4 in plant number 4.10. The variation in number of beans of S_1 inbreds is presented in fig 6

The number of beans per pod among the plants of S_1 generation of H 7.3 genotype varied significantly, and it ranged from 22.80 in plant number 4.9 to 47.40 in plant number 4.10

The number of beans per pod ranged from 35.80 to 42.20, 31.80 to 44.20 and 26.60 to 35.6 in inbred H1 1.2, G VI 135 and G VI 141 respectively.

The number of beans per pod varied significantly in P II 13.12. The number of beans per pod varied from 31.80 in plant number 16.2 to 45.6 in plant number 16.1 Significant difference in number of beans per pod was observed in G VI 256.5. The number of beans per pod ranged from 18.40 in plant number 18.1 to 44.20 in plant number 18.3. The number of beans per pod varied significantly in P II 4.8. The number of beans per pod ranged from 29.60 in plant number 19.1 to 40.6 in plant number 19.1. Significant variation was observed for number of beans per pod in P II 12.9 inbred and

the number of beans per pod ranged from 15.2 in plant number 23.5 to 38.60 in plant number 23.1

Generally the average number of beans per pod is 45 to 50, whereas, there is wide variation in the present study ranging from 15.2 to 47.4 among the different genotype of same generation and also among the population of same genotype and generation.

4.1.3.1.2.7 Number of flat beans per pod

The number of flat bean per pod varied significantly, and it ranged from 0.2 in plant number 4.14 to 6.2 in plant number 4.11. The difference in the number of flat beans per pod is presented in fig 7.

The number of flat beans per pod among the plants of S_1 generation of H 7.3 genotype varied significantly, the number of flat beans per pod ranged from 0.2 in plant number 4.14 to 6.2 in plant number 4.11.

The number of flat beans per pod did not differed significantly among the inbreds of H 1 1.2, G VI 135 and G VI 141

The number of flat beans per pod varied significantly in inbred P II 13.12. The number of flat beans per pod varied from 1 in plant number 16.1 to 5 in plant number 16.2, where as there was no significant difference in the inbreds of G VI 256.5, P II 4.8, and PII 13.8

Significant variation was observed for number of flat beans per pod in P II 12.9. The number of flat beans per pod ranged from 1.40 in plant number 23.4 to 5.20 in plant number 23.5

Similar studies were conducted by Adewale *et al.,* (2010) and reported that the bean characters exhibited maximum diversity in exotic germplasm. Enriquez and Soria (1966) and Pound (1932) revealed that dry or wet weight of bean is considered to be yield expressing characters and similar finding were reported with respect to number of

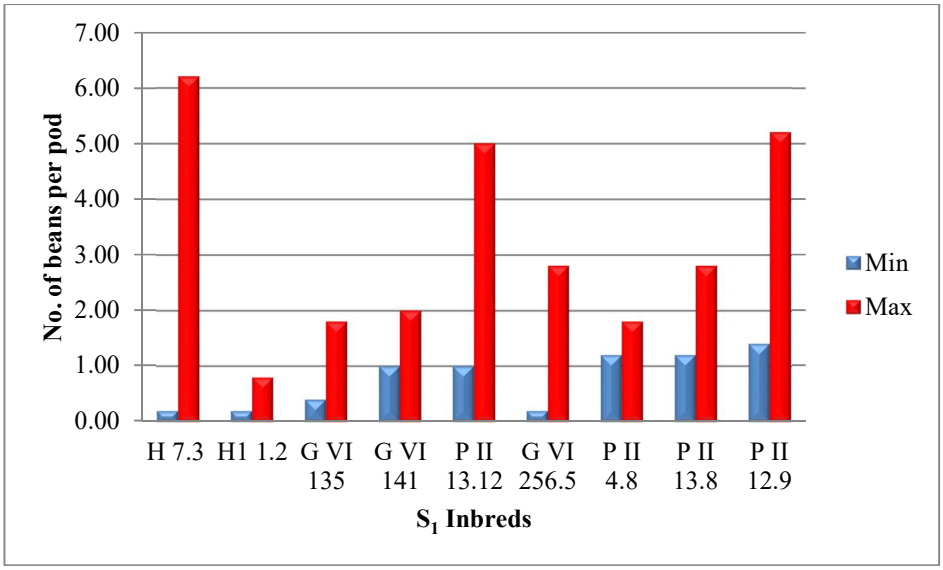


Fig. 7 No. of flat bean per pod of S_1 inbreds

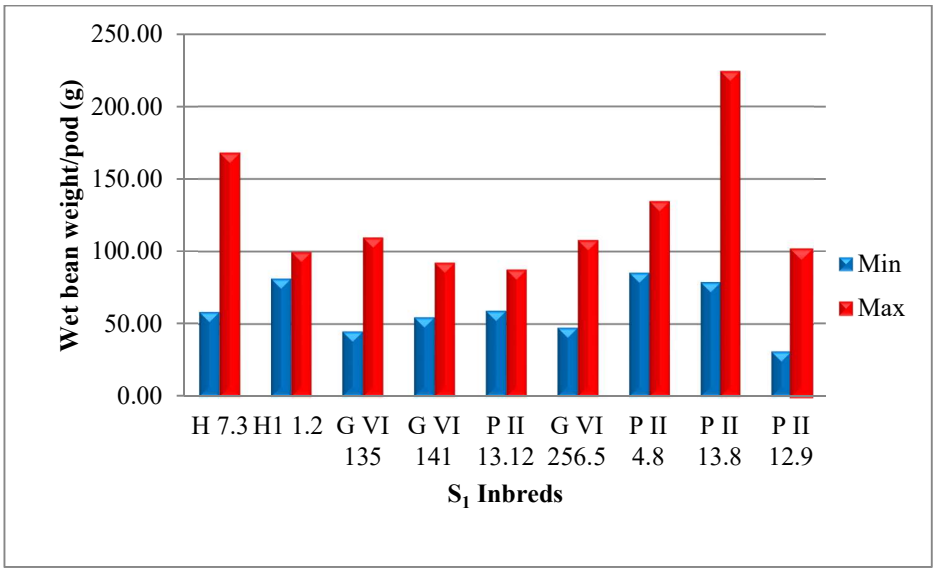


Fig. 8 Wet bean weight per pod of S_1 inbreds

flat beans per pod in the inbreds of cocoa which ranged between 0 and 9 in cocoa inbred studies (CCRP, Report. 2013).

4.1.3.1.3 Bean characters of S_1 inbreds of cocoa

The details of bean characters of S_1 inbred of cocoa are presented in table 5. Important bean characters such as wet bean weight per pod, dry bean weight per pod, single dry bean weight (SDBW), bean length, bean breadth, and bean thickness ranged from 31.26 to 224 g, 9.58 to 51.62 g, 0.57 to 1.19 g, 11.22 to 22.28 mm, 9.25 to 13.31 mm, 5.48 to 8.55 mm respectively. The maximum wet bean weight per pod was recorded in plant number 21.2 and the minimum wet bean weight was observed in plant number 23.5. The maximum (1.19 g) and minimum (0.57 g) SDBW was observed in plant number 4.12 and 18.6. The bold bean size is an important criterion for selection of superior plant. The maximum bean length (22.28 mm) and bean breadth (13.31 mm) was recorded in plant number 4.5 and 4.9 respectively. The thick bean (8.55 mm) was observed in plant number 16.1, whereas the thinnest was observed in plant number 6.1.

4.1.3.1.3.1 Wet bean weight per pod (g)

The bean characters of S_1 inbreds are presented in table 5. The variation in wet bean weight per pod is presented in fig. 8. The wet bean weight per pod among the plants of S_1 generation of H 7.3 genotype varied significantly, the wet bean weight per pod ranged from 58.40 g in plant number 4.8 to 168 g in plant number 4.10. The wet bean weight per pod varied significantly and it ranged from 81.24 g in plant number 6.1 to 100g in 6.2 in H1 1.2. The wet bean weight per pod ranged significantly in G VI 135 and it from 45.10 g to 110 g in plant number 12.2 and 12.5 respectively.

The wet bean weight per pod ranged between 54.84 g to 92.74 g in plant number 14.2 and 14.1 respectively in G VI 141. The wet bean weight per pod varied significantly in P II 13.12. The wet bean weight per pod varied from 59.30 g in plant number 16.2 to 88 g in plant number 16.3. Significant variation for wet bean weight per pod was observed in G VI 256.5. The maximum (108.22 g) and minimum (47.74 g) wet bean weight per pod was observed in plant number 18.3 and 18.6 respectively.

Table 5 Bean characters of S_1 inbreds of cocoa

Plant No.	Genotype	Wet bean weight per pod (g)	Dry bean weight per pod (g)	Single bean weight (g)	Bean length (mm)	Bean breadth (mm)	Bean thickness (mm)
4.2	H 7.3	91.48	28.63	0.79	17.14	11.45	7.50
4.3	H 7.3	64.42	29.05	0.73	16.57	11.41	7.54
4.4	H 7.3	166.36	42.96	1.09	22.04	12.50	6.52
4.5	H 7.3	84.16	31.01	0.78	22.28	13.31	7.05
4.6	H 7.3	97.58	41.33	1.02	19.67	11.38	6.77
4.7	H 7.3	89.80	37.63	0.86	20.23	11.07	6.47
4.8	H 7.3	58.40	22.79	0.97	13.46	10.49	7.45
4.9	H 7.3	63.00	17.14	0.75	11.22	9.25	6.07
4.10	H 7.3	168.00	37.35	0.79	13.26	10.18	7.68
4.11	H 7.3	96.92	37.99	0.93	18.47	12.64	5.67
4.12	H 7.3	115.82	51.62	1.19	18.58	11.48	6.64
4.13	H 7.3	61.34	23.12	0.77	18.50	10.25	6.49
4.14	H 7.3	75.08	24.72	0.71	16.44	10.69	7.62
4.15	H 7.3	80.48	31.76	0.89	18.55	12.09	6.55
4.16	H 7.3	69.18	34.51	0.95	19.82	10.57	7.10
4.17	H 7.3	90.56	37.06	0.86	17.66	11.14	6.73
4.18	H 7.3	111.80	20.89	0.76	18.99	10.35	7.26
6.1	H1 1.2	81.24	32.05	0.76	17.19	11.42	5.48
6.2	H1 1.2	100.00	29.15	0.82	17.40	10.39	7.19
6.3	H1 1.2	87.76	24.43	0.68	15.12	10.61	6.59
6.4	H1 1.2	81.32	29.41	0.68	17.34	11.45	6.67
12.1	G VI 135	67.44	29.02	0.75	17.37	10.45	6.84
122	G VI 135	45.10	20.89	0.64	16.97	10.94	6.69
12.3	G VI 135	64.48	24.37	0.76	16.07	10.99	6.35
12.4	G VI 135	86.46	32.02	0.75	16.61	11.01	7.61
12.5	G VI 135	110.00	28.98	0.72	16.20	10.48	7.11
12.6	G VI 135	106.00	26.21	0.77	16.38	10.54	6.89
12.7	G VI 135	81.40	25.63	0.74	17.19	10.35	6.14
12.8	G VI 135	87.10	30.99	0.70	16.99	11.32	6.31
14.1	G VI 141	92.74	27.11	0.76	17.60	11.27	7.20
14.2	G VI 141	54.84	19.84	0.75	16.96	10.71	7.57
16.1	P II 13.12	85.22	36.20	0.79	18.77	12.23	8.55
16.2	P II 13.12	59.30	21.03	0.66	17.64	11.64	6.40
16.3	P II 13.12	88.00	26.47	0.76	17.18	11.23	6.39
18.1	G VI 256.5	81.00	12.23	0.67	13.37	9.28	6.41

18.2	G VI 256.5	64.24	26.72	0.69	21.22	10.49	8.26
18.3	G VI 256.5	108.22	32.90	0.74	19.35	11.24	6.31
18.4	G VI 256.5	106.00	27.22	0.81	19.39	11.31	6.27
18.5	G VI 256.5	90.00	24.99	0.77	16.35	9.26	6.37
18.6	G VI 256.5	47.74	21.10	0.57	18.82	9.42	5.88
19.1	P II 4.8	85.40	20.28	0.69	17.17	11.71	6.48
19.2	P II 4.8	135.00	24.55	0.78	15.31	12.34	6.22
19.3	P II 4.8	94.84	32.99	0.82	13.50	9.50	6.50
19.4	P II 4.8	97.00	28.01	0.76	18.35	10.91	8.51
21.1	P II 13.8	158.00	29.58	0.86	16.76	11.33	6.79
21.2	P II 13.8	224.00	29.59	0.87	19.52	11.46	6.90
21.3	P II 13.8	144.00	28.95	0.97	20.86	12.74	7.38
22.1	P II 13.8	78.80	22.09	0.81	17.92	12.51	7.50
23.1	P II 12.9	101.08	44.07	1.14	21.23	13.12	6.72
23.2	P II 12.9	38.80	14.48	0.76	20.84	11.42	8.06
23.3	P II 12.9	51.20	25.96	0.80	19.17	10.58	6.53
23.4	P II 12.9	50.80	25.16	0.83	19.99	11.56	5.90
23.5	P II 12.9	31.26	9.58	0.63	14.87	9.96	6.44
	CV (%)	19.36	16.70	8.58	3.70	4.74	6.39
	CD (0.05)	23.502	5.836	0.085	0.81	0.652	0.542

Significant variation for wet bean weight per pod was observed in P II 4.8. The maximum (135 g) and minimum (85.40 g) wet bean weight per pod was observed in plant number 19.2 and 19.1 respectively. The wet bean weight per pod was ranged from 78.8 g in plant number 22.1 to 224 g in plant number 21.2 of PII 13.8 inbred. Significant variation was observed for wet bean weight per pod in P II 12.9. The wet bean weight per pod ranged from 31.26 g in plant number 23.5 to 101.80 g in plant number 23.1

4.1.3.1.3.2 Dry bean weight per pod (g)

The dry bean weight per pod varied significantly among the S_1 generation inbreds is presented in table 5**.**

The variation in dry bean weight per pod is presented in fig 9. The dry bean weight per pod among the plants of S_1 generation of H 7.3 genotype varied significantly, the dry bean weight per pod ranged from 17.14 to 51.62 in plant number 4.9 and 4.12 respectively. The dry bean weight per pod varied significantly in H1 1.2 and it ranged from 24.43 in plant number 6.3 to 32.05 in plant number 6.1.The dry bean weight per pod varied significantly in G VI 135 and it ranged from 20.89 to 32.02 in plant number 12.2 and 12.4, respectively. The dry bean weight per pod was maximum (27.11) in plant number 14.1 and minimum (19.84) in plant number 14.4 in GVI 141 inbred. Significant variation for dry bean weight per pod was observed in P II 13.12 and the maximum (36.20) and minimum (21.03) was observed in plant number 16.1 and 16.2, respectively.

Significant variation for dry bean weight per pod was observed in G VI 256.5 and the maximum (27.22) and minimum (12.23) dry matter recovery was observed in plant number 18.4 and 18.1, respectively. The dry bean weight per pod in P II 4.8 ranged from 20.28 in plant number 19.1 to 32.99 in plant number 19.4. The dry bean weight per pod varied significantly among the plants of P II 13.8. The minimum dry bean weight per pod (22.09) was observed in plant number 21.1 and maximum dry bean weight per pod (29.59) in plant number 22.1.

The dry bean weight per pod ranged from 9.58 in plant number 23.5 to 44.07 in plant number 23.1 respectively in P II 12.9.

a. Hardened mucilage and seed germination inside the pods

b. Vivipary in inbreds of cocoa

c. Mucilage hardened

Plate 7. Pods of selfed generation showing vivipary and hardened mucilage

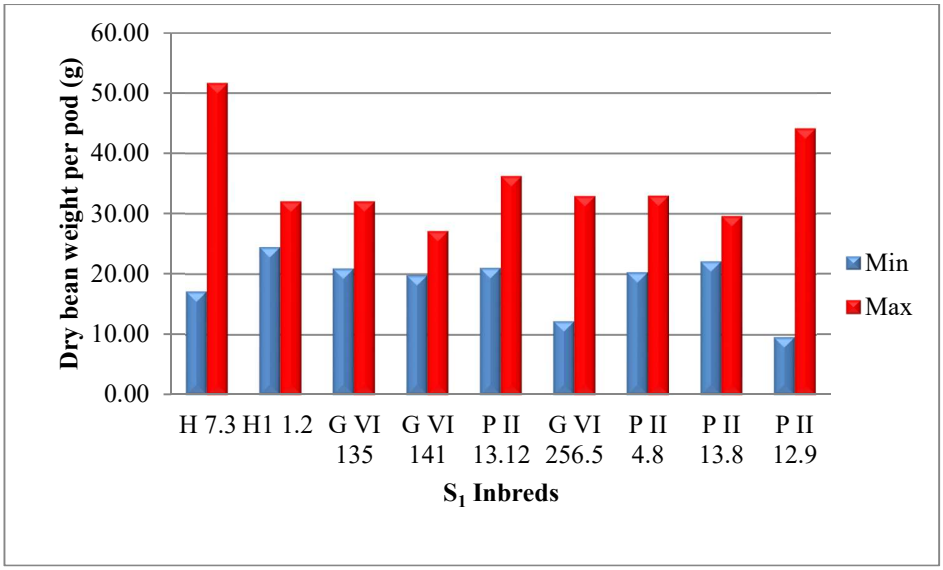


Fig. 9 Dry bean weight per pod of S_1 inbreds

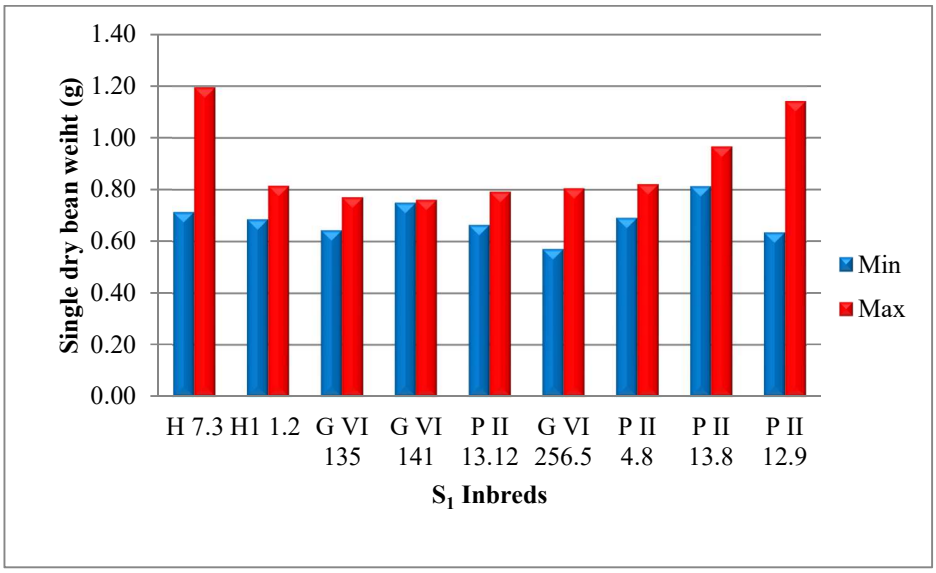


Fig. 10 Single dry bean weight of S_1 inbreds

4.1.3.1.3.3 Single dry bean weight (g)

The data pertaining to single bean weight is presented in table 5.Single bean weight varied significantly among the S_1 generation inbreds**.**

The single bean weight among the plants of S_1 generation of H 7.3 genotype varied significantly, the single bean weight ranged from 0.71 g in plant number 4.14 to 1.09 g in plant number 4.4.The single bean weight varied significantly and it ranged from 0.68 g in plant number 6.4 to 0.82 g in plant number 6.2 in H11.2 (Fig. 10).

The single bean weight ranged from 0.64 g to 0.77 g in plant number 12.2 and 12.6 respectively in G VI 135. The single bean weight in G VI 141 ranged from 0.75 g to 0.76 g in plant number 14.2 and 14.1 respectively. Significant variation for single bean weight was observed in P II 13.12 and the maximum (0.79 g) and minimum (0.66 g) was observed in plant number 16.1 and 16.2 respectively. Significant variation for single bean weight was observed in G VI 25.5 and the maximum (0.81 g) and minimum (0.57 g) single bean weight was observed in plant number 18.4 and 18.6 respectively.

No **s**ignificant variation for single bean weight was observed in P II 4.8. The maximum (0.97g) and minimum (0.81g) single bean weight was observed in plant number 21.3 and 22 in P II 13.8. Significant variation was observed for single bean weight in P II 12.9 and it ranged from 0.63 g in plant number 23.5 to 1.14 g in plant number 23.1

Similar studies were conducted by Adewale *et al.* (2010) and reported that the bean characters exhibited maximum diversity. Enriquez and Soria (1966) and Pound (1932) revealed that dry or wet weight of bean is considered to be yield expressing characters.

4.1.3.1.3.4 Bean length (mm)

Bean length varied significantly among the S_1 generation inbreds**.**

The variation in bean length is presented in fig 11. The bean length among the plants of S_1 generation of H 7.3 genotype varied significantly, the bean length ranged

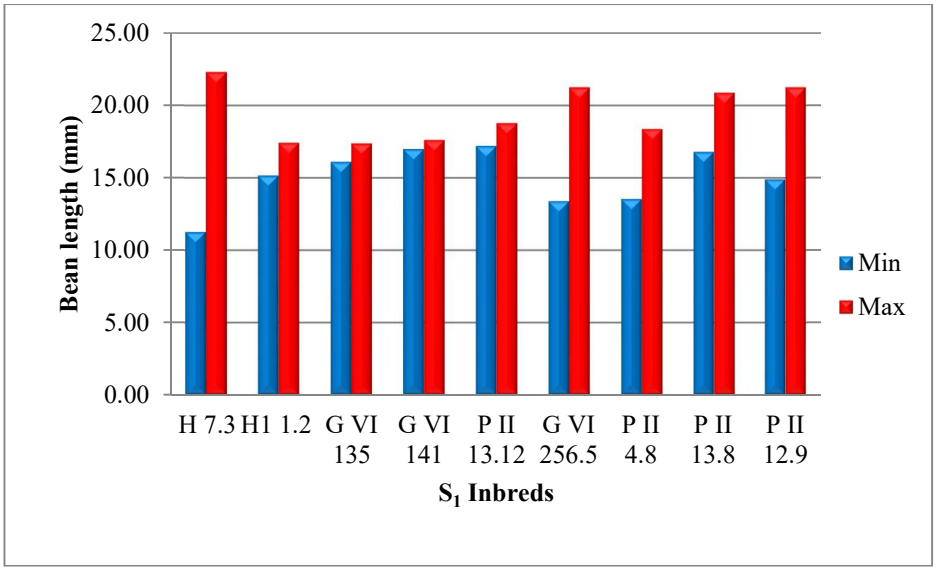


Fig. 11 Bean length of S_1 inbreds

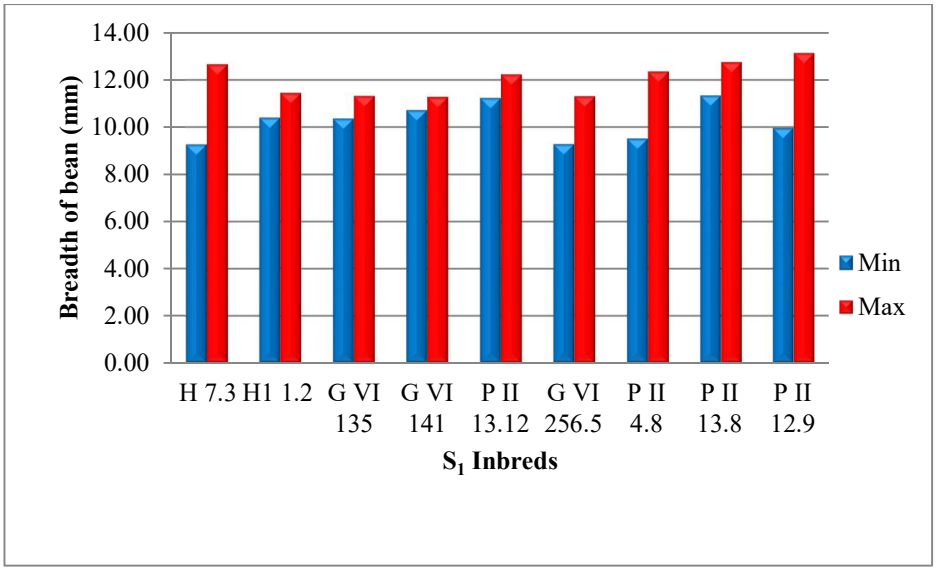


Fig.12 Bean breadth of S_1 inbreds

from 11.22 mm in plant number 4.9 to 22.28 mm in plant number 4.5. The bean length varied significantly in H1 1.2 and it ranged from 15.12 mm in plant number 6.3 to 17.40 mm in plant number 6.2.The bean length ranged from 16.07 mm to 17.37 mm in plant number 12.3 and 12.1 respectively in G VI 135.

The bean length did not varied significantly in G VI 141. Significant variation for bean length was observed in P II 13.12 and the maximum (18.77 mm) and minimum (17.18 mm) was observed in plant number 16.1 and 16.3 respectively. Significant variation for bean length was observed in G VI 256.5 and the maximum (21.22 mm) and minimum (13.37 mm) bean length was observed in plant number 18.2 and 18.1 respectively. Significant variation for bean length was observed in P II 4.8 and the maximum (18.35 mm) and minimum (13.50 mm) bean length was observed in plant number 19.4 and 19.3 respectively. Significant variation for bean length was observed in P II 13.8 and the maximum (20.86 mm) and minimum (16.76 mm) bean length was observed in plant number 21.3 and 22.1. The bean length ranged from 14.87 mm in plant number 23.5 to 21.23 mm in plant number 23.1in P II 12.9.

4.1.3.1.3.5 Bean breadth (mm)

Bean breadth varied significantly among the S_1 generation inbreds (Table 5).

The bean breadth among the plants of S_1 generation of H 7.3 genotype varied significantly and it ranged from 9.25 mm in plant number 4.9 to 13.31mm in plant number 4.5. The bean breadth varied significantly in H1 1.2 and it ranged from 10.39 mm in plant number 6.2 to 11.45mm in plant number 6.4. The bean breadth ranged from 9.25 mm to 13.31 mm in plant number 4.9 and 4.5, respectively in G VI 135 (Fig 12).

Significant variation for bean breadth was observed in P II 13.12. The maximum (12.23 mm) and minimum (11.23 mm) was observed in plant number 16.1 and 16.3 respectively in P II 13.12. Significant variation for bean breadth was observed in G VI 256.5 and the maximum (11.31 mm) and minimum (9.26 mm) bean breadth was observed in plant number 18.2 and 18.5 respectively. The maximum (12.34 mm) and

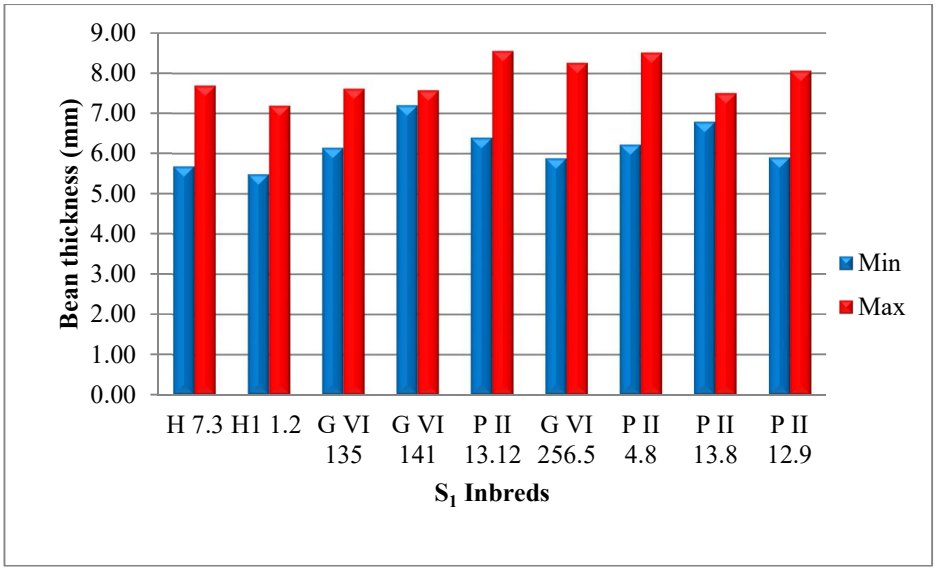


Fig.13 Bean thickness of S_1 inbreds

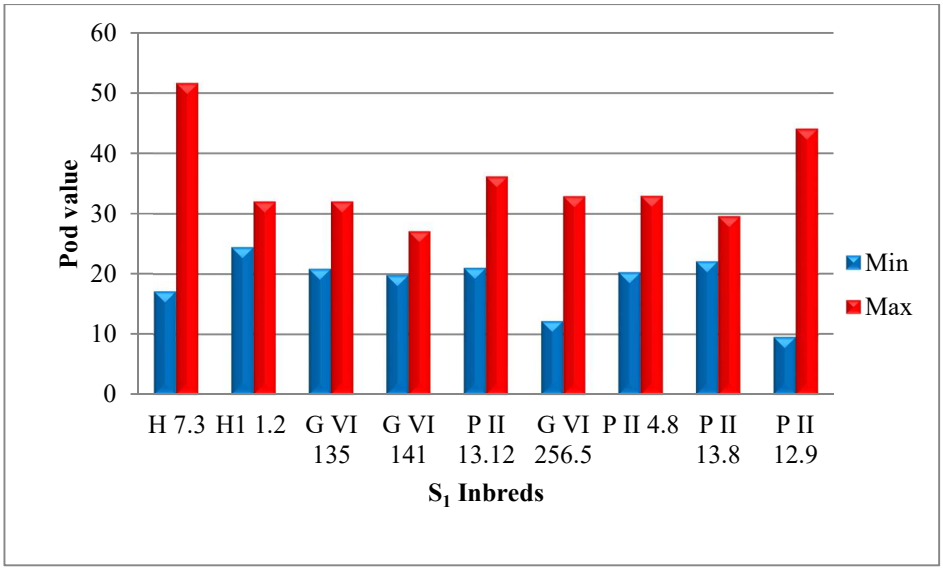


Fig. 14 Pod value of S_1 inbreds

minimum (9.50 mm) was observed in plant number 19.3 and 19.2 respectively in P II 4.8. Significant variation for bean length was observed in P II 13.8 and the maximum (12.74 mm) and minimum (11.33 mm) bean breadth was observed in plant number 21.3 and 22.1respectively. Significant variation was observed for bean length in P II 12.9 and it ranged from 9.96 mm in plant number 23.5 to 13.12 mm in plant number 23.1 respectively.

4.1.3.1.3.6. Bean thickness (mm)

Bean thickness varied significantly among the S_1 generation inbreds (table 5)**.**

The bean thickness among the plants of S_1 generation of H 7.3 genotype varied significantly, the bean thickness ranged from 5.67 mm in plant number 4.11 to 7.68 mm in plant number 4.10 (Fig.13)

The bean thickness varied significantly in H1 1.2 and it ranged from 5.48 mm in plant number 6.1 to 7.19 mm in plant number 6.2. The bean thickness ranged from 6.31 mm to 7.61 mm in plant number 12.8 and 12.4 respectively in G VI 135. Significant variation for bean breadth was observed in P II 13.12 and the maximum (8.55 mm) and minimum (6.39 mm) was observed in plant number 16.1 and 16.3 respectively. Significant variation for bean thickness was observed in G VI 256.5 and the maximum (8.26 mm) and minimum (5.88 mm) bean thickness was observed in plant number 18.6 and 18.2 respectively. The maximum (8.51 mm) and minimum (6.22 mm) bean thickness in P II 4.8 was observed in plant number 19.4 and 19.2 respectively. Significant variation for bean thickness was observed in P II 13.8 and the maximum (7.50 mm) and minimum (6.90 mm) bean thickness was observed in plant number 22.1 and 22.2 respectively. The bean thickness ranged from 5.90 mm in plant number 23.4 to 8.06 mm in plant number 23.2 respectively in P II 12.9.

4.1.3.1.4 Economic characters of S_1 inbreds of cocoa

The economic characters such as pod yield, pod value, efficiency index, conversion index, dry bean weight per pod and dry matter recovery showed significant difference among the inbreds and are summarized in table 6.

4.1.3.1.4.1 Pod value

The pod value varied significantly among the S_1 generation inbreds (Fig.14).

Pod value is the dry bean obtained per pod. The pod value ranged from 9.58 to 51.61. The maximum pod value was obtained in H 7.3 (Plant number 4.12) and the least in P II12.9 (Plant number 23.5).

The pod value among the plants of S_1 generation of H 7.3 genotype varied significantly and ranged from 17.14 to 51.62 in plant number 4.9 and 4.12 respectively. The pod value varied significantly in H 1 1.2 and it ranged from 24.43 in plant number 6.3 to 32.05 in plant number 6.1. The pod value in G VI 135 ranged from 20.89 to 32.02 in plant number 12.2 and 12.4 respectively. The pod value ranged significantly in G VI 141, and it ranged from 19.84 in plant number 19.84 to 27.11 in plant number 14.1. Significant variation for value was observed in P II 13.12 and the maximum (36.20) and minimum (21.03) was observed in plant number 16.1 and 16.2 respectively.

Significant variation for pod value was observed in G VI 256.5 and the maximum (32.90) and minimum (12.23) pod value was observed in plant number 18.3 and 18.1 respectively. Significant variation for pod value was observed in P II 4.8 and the maximum (32.99) and minimum (20.28) was observed in plant number 19.3 and 19.1 respectively. Significant variation for bean thickness was observed in P II 13.8 and the maximum (29.59) and minimum (22.09) pod value was observed in plant number 21.2 and 22.1 respectively. Significant variation was observed for pod value in P II 12.9 and it ranged from 14.48 in plant number 23.2 to 44.07 in plant number 23.1 respectively.

Table 6. Economic characters of S_1 inbreds of cocoa

S. No.	Genotype	Pod Value	Pod Index	Efficiency Index	Conversion Index	Dry Matter Recovery (%)
4.2	H 7.3	28.63	28.92	13.69	0.32	31.63
4.3	H 7.3	29.05	33.15	9.56	0.46	45.59
4.4	H 7.3	42.96	26.51	8.07	0.30	29.87
4.5	H 7.3	31.01	32.66	8.87	0.36	36.70
4.6	H 7.3	41.33	26.51	9.49	0.42	42.42
4.7	H 7.3	37.63	25.35	10.05	0.42	42.19
4.8	H 7.3	22.79	39.16	13.56	0.39	39.20
4.9	H 7.3	17.14	63.05	6.24	0.27	27.21
4.10	H 7.3	37.35	30.57	8.06	0.23	22.82
4.11	H 7.3	37.99	25.78	8.32	0.42	42.16
4.12	H 7.3	51.62	23.94	9.78	0.45	44.85
4.13	H 7.3	23.12	39.04	9.44	0.38	37.66
4.14	H 7.3	24.72	52.34	12.52	0.34	34.00
4.15	H 7.3	31.76	35.61	9.44	0.40	40.19
4.16	H 7.3	34.51	30.80	7.73	0.50	49.94
4.17	H 7.3	37.06	26.69	9.66	0.41	41.25
4.18	H 7.3	20.89	42.30	8.15	0.19	18.84
6.1	H1 1.2	32.05	38.06	8.01	0.40	39.74
6.2	H1 1.2	29.15	33.26	10.32	0.30	29.32
6.3	H1 1.2	24.43	41.00	10.08	0.28	27.85
6.4	H1 1.2	29.41	41.44	12.34	0.39	38.69
12.1	G VI 135	29.02	33.14	9.95	0.43	43.22
122	G VI 135	20.89	55.41	12.84	0.45	45.15
12.3	G VI 135	24.37	42.81	12.46	0.38	38.02
12.4	G VI 135	32.02	33.41	11.31	0.37	37.10
12.5	G VI 135	28.98	33.60	9.76	0.27	26.42
12.6	G VI 135	26.21	38.01	10.03	0.25	24.77
12.7	G VI 135	25.63	38.85	8.60	0.32	31.69
12.8	G VI 135	30.99	33.42	10.88	0.36	35.87
14.1	G VI 141	27.11	35.63	16.53	0.30	29.69
14.2	G VI 141	19.84	49.31	16.36	0.36	36.66
16.1	P II 13.12	36.20	32.30	11.21	0.42	42.44
16.2	P II 13.12	21.03	44.90	12.00	0.36	35.49
16.3	P II 13.12	26.47	39.20	9.71	0.30	30.16
18.1	G VI 256.5	12.23	81.35	13.06	0.15	15.20
18.2	G VI 256.5	26.72	43.17	10.07	0.43	42.95
18.3	G VI 256.5	32.90	30.71	10.39	0.31	30.99

18.4	G VI 256.5	27.22	37.60	10.12	0.26	25.92
18.5	G VI 256.5	24.99	38.96	9.91	0.28	27.81
18.6	G VI 256.5	21.10	47.28	9.37	0.45	44.60
19.1	P II 4.8	20.28	48.83	12.55	0.24	23.76
19.2	P II 4.8	24.55	43.51	12.74	0.20	19.57
19.3	P II 4.8	32.99	31.44	12.66	0.36	36.11
19.4	P II 4.8	28.01	35.83	11.48	0.29	28.85
21.1	P II 13.8	29.58	33.99	14.55	0.19	18.91
21.2	P II 13.8	29.59	33.49	18.01	0.13	13.41
21.3	P II 13.8	28.95	40.32	11.79	0.21	21.00
22.1	P II 13.8	22.09	42.34	11.34	0.28	28.12
23.1	P II 12.9	44.07	26.72	11.76	0.44	43.88
23.2	P II 12.9	14.48	64.37	13.59	0.37	37.23
23.3	P II 12.9	25.96	43.69	12.28	0.51	50.71
23.4	P II 12.9	25.16	40.11	11.67	0.50	49.62
23.5	P II 12.9	9.58	95.14	25.97	0.31	31.25
	CV (%)	16.70	31.39	26.54	17.89	17.84
	CD (0.05)	5.836	15.458	3.716	0.076	7.547

4.1.3.1.4.2 Pod index

The pod index varied significantly among the S_1 generation inbreds (Table 6).

Pod index is the number of pods required to produce kg dried beans. The pod index should be minimum as per the selection criterion. It was observed minimum (23.94) in H7.3 (86) (Plant number 4.12).

The difference in the pod index of S_1 inbreds is presented in fig. 15. The pod index among the plants of S1 generation of H 7.3 genotype varied significantly, and it ranged from 23.94 to 63.05 in plant number 4.12 and 4.9 respectively. The pod index varied significantly in H 1 1.2 and it ranged from 33.26 in plant number 6.2 to 41.44 in plant number 6.4.The pod index in G VI 135 ranged from 33.14 to 55.41 in plant number 12.1 and 12.2 respectively. The pod index did not varied significantly among the plants of GVI 141 genotypes. Pod index varied significantly, in P II 13.12 and the maximum (44.90) and minimum (32.30) was observed in plant number 16.2 and 16.1 respectively. Significant variation for pod index was observed in G VI 256.5 and the maximum (81.35) and minimum (30.71) pod index was observed in plant number 18.1 and 18.3 respectively.

The maximum (48.83) and minimum (31.44) pod index in P II 4.8 was observed in plant number 19.1 and 19.3 respectively. No significant variation for pod index was observed in P II 13.8. The pod index varied significantly in P II 12.9 and it ranged from 26.72 in plant number 23.1 to 64.37 in plant number 23.2 respectively.

The least pod index of 39.08 in the in fifth generation was reported by Minimol *et al.* 2015. The maximum pod index (95.14) was observed in P II 12.9 indicating it non suitable for future selection of this inbred for this particular criterion.

4.1.3.1.4.3 Efficiency Index

Efficiency index is an indication of the pod weight required to produce one gram dry bean. Efficiency Index should be minimum for selection from genotype. The

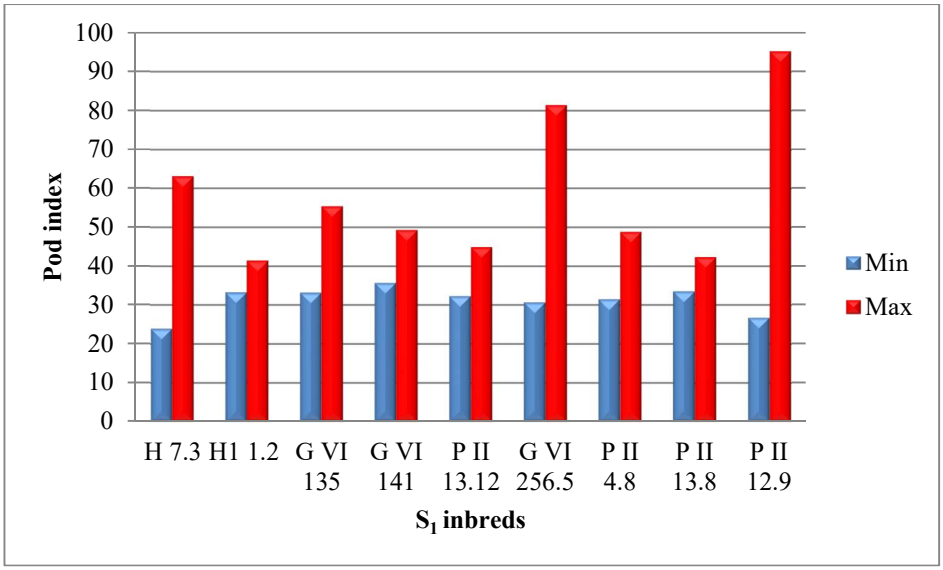


Fig. 15 Pod Index of S_1 inbreds

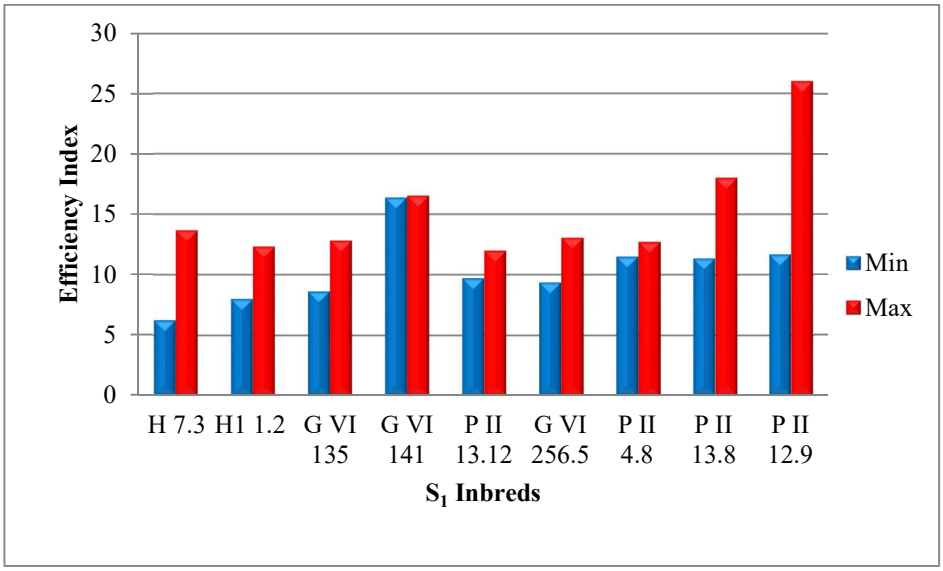


Fig. 16 Efficiency Index of S_1 inbreds

minimum efficiency index (6.24) was observed in genotype H7.3 (86) (Plant number 4.9) followed by H7.3 (86) (Plant number 4.16), H1 1.2 (86) (Plant number 6.1).

The efficiency index varied significantly among the S_1 generation inbreds (Fig. 16)

The efficiency index among the plants of S_1 generation of H 7.3 genotype varied significantly, the efficiency index ranged from 6.24 to 13.69 in plant number 4.9 and 4.2 respectively. The efficiency index in H1 1.2 varied significantly in H1 1.2 and it ranged from 8.01 in plant number 6.1 to 12.34 in plant number 6.4.

The efficiency index in G VI 135 ranged from 9.76 to 12.84 in plant number 12.5 and 12.2 respectively in G VI 135.

The efficiency index did not varied significantly among the plants of GVI 141 genotypes. Significant variation for efficiency index in P II 13.12 was observed in P II 13.12 and the maximum (12.00) and minimum (9.71) was observed in plant number 16.2 and 16.3 respectively.

Significant variation for efficiency index in G VI 256.5 was observed in G VI 256.5 and the maximum (13.06) and minimum (9.37) efficiency index was observed in plant number 18.1 and 18.6 respectively. Significant variation was observed for efficiency index in P II 12.9 and it ranged from 11.67 in plant number 23.4 to 25.97 in plant number 23.5 respectively. Non significant variation for efficiency index was observed in P II 4.8 and P II 13.8.

Minimol *et al.* (2015) reported lowest self efficiency index (6.55) in S_3 generation of GII 7.4.

4.1.3.1.4.4 Conversion Index

Conversion is the amount of dry bean obtained from a given amount of wet bean. Maximum conversion index of 0.5 was recorded in P II 12.9 (Plant number 23.5) followed by H 7.3 (86) (Plant number 4.3).

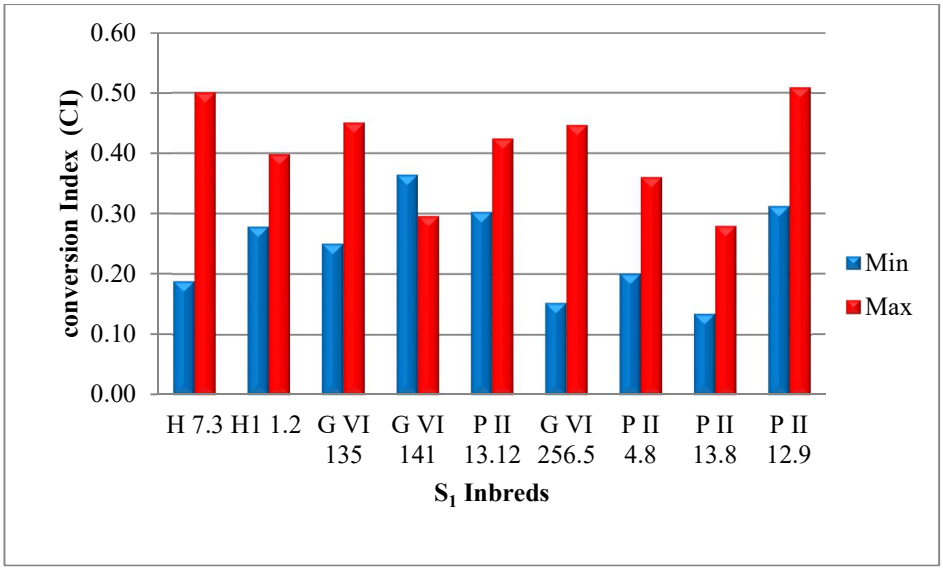


Fig. 17 Conversion Index of S_1 inbreds

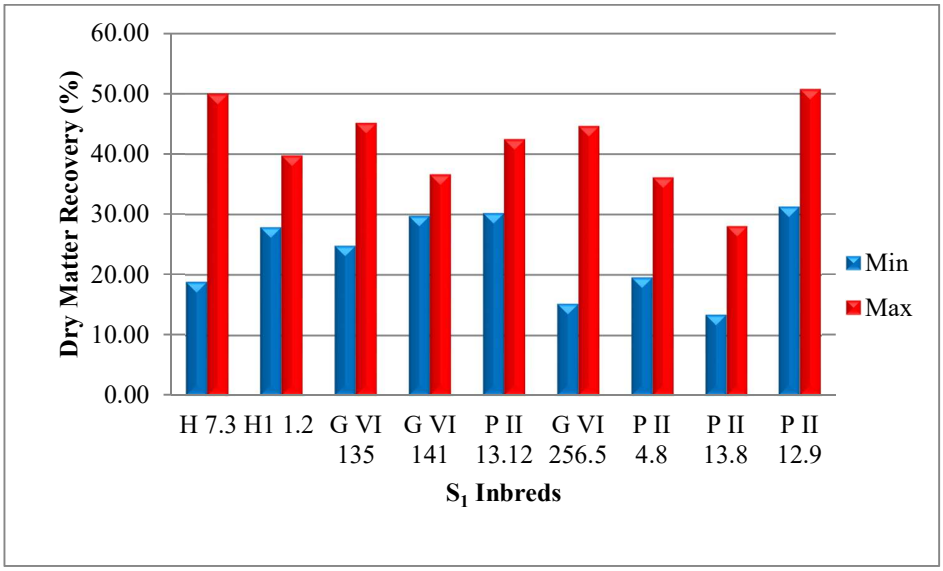


Fig. 18 Dry matter recovery of S_1 inbreds

The conversion index varied significantly among the S_1 generation inbreds**.**

The conversion index among the plants of S_1 generation of H 7.3 genotype varied significantly, the efficiency index ranged from 0.13 to 0.50 in plant number 21.2 and 4.16 respectively (Fig. 17).

The conversion index varied significantly in H1 1.2 and it ranged from 0.28 in plant number 6.3 to 0.40 in plant number 6.1.

The conversion index varied significantly in G VI 135 and the conversion index ranged from 0.25 to 0.45 in plant number 12.6 and 12.2 respectively.

The conversion index was maximum (0.36) in plant number 14.3 and minimum (0.30) in plant number 14.1 in G VI 141.The maximum (0.42) and minimum (0.30) conversion index in P II 13.12 was observed in plant number 16.1 and 16.3 respectively.

Significant variation for conversion index was observed in G VI 256.5 and the maximum (0.45) and minimum (0.15) conversion index was observed in plant number 18.6 and 18.1 respectively. Significant variation for conversion index was not observed in P II 4.8. The conversion index varied significantly among the plants of PII 13.8 and the minimum conversion index (0.13) was observed in plant number 21.2 and maximum conversion index (0.28) in plant number 22.1.

Significant variation was observed for conversion index in P II 12.9. The conversion index ranged from 0.31 in plant number 23.5 to 0.50 in plant number 23.4 respectively in P II 12.9. The least conversion index (0.13) was observed in P II 13.8(Plant number 21.2).

Minimol *et al.* (2015) calculated conversion index and the values in the present study are coming in the range observed by them.

4.1.3.1.4.5 Dry Matter Recovery (%)

Dry Matter Recovery (%) is the ratio of dry bean weight to wet bean weight. High dry matter recovery is a good criterion for selection from the germplasm. The

maximum values (50.71 %) were observed for the P II 12.9 (Plant number 23.2), minimum values (13.41 %) in P II 13.8 (Plant number 21.2).

The dry matter recovery varied significantly among the S_1 generation inbreds**.** The dry matter recovery among the plants of S_1 generation of H 7.3 genotype varied significantly and it ranged from 18.84 to 49.94 in plant number 4.18 and 4.16 respectively.

The dry matter recovery varied significantly in H1 1.2 and it ranged from 27.85 in plant number 6.3 to 39.74 in plant number 6.1. The dry matter recovery varied significantly. The dry matter recovery in G VI 135 ranged from 24.77 to 45.15 in plant number 12.6 and 12.2 respectively.

The dry matter recovery varied significantly among the plants of GVI 141 genotypes and the maximum (36.66) in plant number 14.2 and minimum (29.69) in plant number 14.1. Significant variation for dry matter recovery was observed in P II 13.12 and the maximum (42.44) and minimum (30.16) was observed in plant number 16.1 and 16.3 respectively. Significant variation for dry matter recovery was observed in G VI 256.5 and the maximum (42.95) and minimum (15.20) dry matter recovery was observed in plant number 18.2 and 18.1 respectively. Significant variation for dry matter recovery was not observed in P II 4.8. The dry matter recovery varied significantly among the plants of P II 13.8. The minimum dry matter recovery (13.41) was observed in plant number 21.2 and maximum dry matter recovery (28.12) in plant number 22.1. Significant variation was observed for dry matter recovery in P II 12.9 and it ranged from 31.25 in plant number 23.5 to 50.71 in plant number 23.3 respectively.

4.1.3.1.4. Biochemical characters of S_1 inbreds of cocoa

The details of biochemical variation in S_1 inbreds is presented in table 7. Cocoa beans are the major economic parts which contain the cocoa butter used for making chocolate. The quality of chocolate mainly depends upon the biochemical constituents present in the bean. Fat is responsible for softness, aroma and flavor and polyphenols for

Table 7. Biochemical characters of S_1 inbreds of cocoa

Plant No.	Genotype	Fat content (%)	Phenol (%)
4.2	H 7.3	54.33	2.52
4.3	H 7.3	52.33	2.51
4.4	H 7.3	52.13	2.46
4.5	H 7.3	51.83	2.16
4.6	H 7.3	40.87	2.18
4.7	H 7.3	53.23	2.34
4.8	H 7.3	53.30	2.24
4.9	H 7.3	60.87	2.37
4.10	H 7.3	57.50	3.47
4.11	H 7.3	54.83	3.25
4.12	H 7.3	50.87	2.86
4.13	H 7.3	50.07	2.94
4.14	H 7.3	48.40	2.28
4.15	H 7.3	46.07	2.59
4.16	H 7.3	41.97	2.26
4.17	H 7.3	53.13	2.89
4.18	H 7.3	56.20	3.37
6.1	H1 1.2	55.33	2.94
6.2	H1 1.2	54.70	2.96
6.3	H1 1.2	55.17	2.19
6.4	H1 1.2	53.33	2.20
12.1	G VI 135	41.63	1.85
12.2	G VI 135	38.63	1.84
12.3	G VI 135	48.60	1.89
12.4	G VI 135	49.87	2.77
12.5	G VI 135	55.30	2.70
12.6	G VI 135	55.40	2.68
12.7	G VI 135	55.23	2.68
12.8	G VI 135	55.20	2.68
14.1	G VI 141	48.73	2.47
14.2	G VI 141	58.23	3.60
16.1	P II 13.12	34.43	3.35
16.2	P II 13.12	56.80	3.57
16.3	P II 13.12	54.67	3.48
18.1	G VI 256.5	55.47	3.35
18.2	G VI 256.5	58.50	3.28
18.3	G VI 256.5	58.30	3.28
18.4	G VI 256.5	58.50	3.29
18.5	G VI 256.5	58.53	3.23

18.6	G VI 256.5	58.67	3.06
19.1	P II 4.8	54.33	2.04
19.2	P II 4.8	45.17	2.01
19.3	P II 4.8	45.90	2.02
19.4	P II 4.8	61.23	2.92
21.1	P II 13.8	61.43	1.47
21.2	P II 13.8	60.10	1.72
21.3	P II 13.8	62.93	2.41
23.1	P II 12.9	45.20	2.13
23.2	P II 12.9	64.93	2.16
23.3	P II 12.9	61.57	2.13
23.4	P II 12.9	54.53	2.23
23.5	P II 12.9	36.70	2.13
	CV (%)	1.254	0.646
	CD(0.05)	1.077	0.027

colour of chocolate. In the present study the fat and poly phenols estimated are summarized in table 7.

4.1.3.4.1 Fat content (%)

The fat content varied significantly among the S_1 generation inbreds. The fat content ranged from 38.63 in genotype P II 13.12 (plant number12.3) to maximum of 64.93 per cent in genotype PII 12.9 (Plant number 16.1).

The fat content among the plants of S_1 generation of H 7.3 genotype varied significantly, the fat content ranged from 40.87 to 60.87 in plant number 4.6 and 4.9 respectively. The fat content varied significantly in H1 1.2 and it ranged from 53.33 in plant number 6.4 to 55.33 in plant number 6.1.

The fat content varied significantly in G VI 135 and it ranged from 38.63 to 55.23 in plant number 12.2 and 12.7, respectively. The fat content varied significantly among the plants of GVI 141 genotypes and it was maximum (58.23) in plant number 14.2 and minimum (48.73) in plant number 14.1.

Significant variation for fat content was observed in P II 13.12 and the maximum (56.80) and minimum (34.43) was observed in plant number 16.2 and 16.1, respectively. The maximum (58.67) and minimum (55.47) fat content in G VI 256.5 was observed in plant number 18.6 and 18.1, respectively.

The fat content ranged from 54.33 in plant number 19.1 to 45.17 in plant number 19.4 in P II 4.8. The fat content varied significantly among the plants of P II 13.8 and the minimum fat content (60.10) was observed in plant number 21.2 and maximum fat content (62.93) in plant number 22.3. Significant variation was observed fat content of P II 12.9 and it ranged from 36.70 in plant number 23.5 to 54.53 in plant number 23.4 respectively.

Fat estimation in cocoa was also estimated previously by Ajmal (2016) in cocoa hybrids and Veeresh (2018) in 30 exotic germplasm. In the present study, 73 per cent of

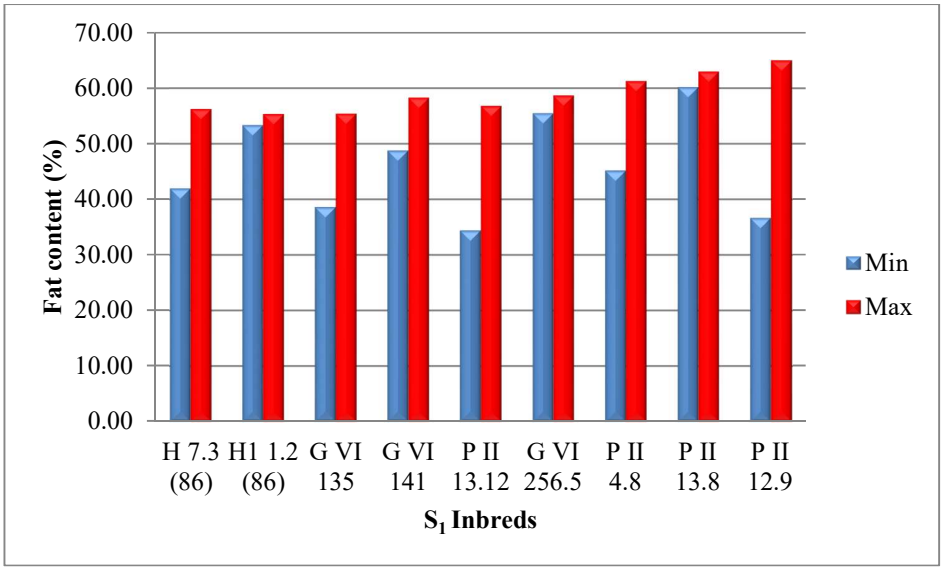


Fig. 19 Fat content of S_1 inbreds

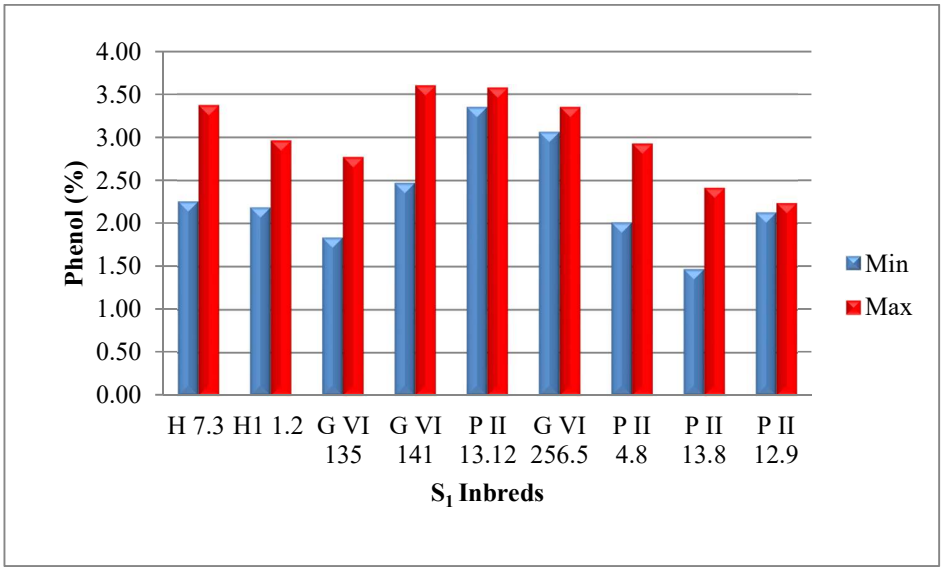


Fig. 20 Phenol content of S_1 inbreds

S_1 inbreds recorded more than 50 per cent fat. High fat content of cocoa beans is major attribute responsible for flavor and aroma of chocolate (Mossu, 1992). So the inbreds showing high fat content can be selected for further breeding programme.

4.1.3.1.4.2 Polyphenol content (%)

The variation in Polyphenol content is presented in fig. 20. Total poly phenol content in cocoa beans extracts of inbreds were determined by following Folin-Ciocalteau procedure. According to Kim and Keeny (1984) poly phenols comprise 12 - 18 percent of the total bean weight is responsible for colour of the chocolate. In the present study poly phenols ranged between 1.47 to 3.6 percent among the inbreds. The maximum poly phenols (3.6 per cent) are observed in G VI 141(Plant number 14.2) followed by P II 13.12 (3.57 per cent). The least poly phenol content estimated in P II 13.8 inbred (Plant number 21.1).

The phenol content among the plants of S_1 generation of H 7.3 genotype varied significantly and it ranged from 2.16 to 3.47 in plant number 4.5 and 4.10, respectively. The polyphenol content varied significantly in H1 1.2 and it ranged from 2.96 in plant number 6.2 to 2.19 in plant number 6.3. The polyphenol content varied significantly in G VI 135 and it ranged from 1.84 to 2.77 in plant number 12.2 and 12.4, respectively. The polyphenol content varied significantly among the plants of GVI 141 and the maximum (3.6) in plant number 14.2 and minimum (2.47) in plant number 14.1.

Significant variation for polyphenol content was observed in P II 13.12 and the maximum (3.57) and minimum (3.35) was observed in plant number 16.2 and 16.1, respectively. Significant variation for polyphenol content was observed in G VI 256.5 and the maximum (3.35) and minimum (3.06) polyphenol content was observed in plant number 18.1 and 18.6, respectively. Significant variation for polyphenol content in PII 4.8 was observed and it ranged from 2.01 in plant number 19.2 to 2.92 in plant number 2.92.

The polyphenol content varied significantly among the plants of P II 13.8 and the minimum polyphenol content (1.47) was observed in plant number 21.1 and maximum fat content (2.41) in plant number 21.3. Significant variation was observed polyphenol content in P II 12.9 and it ranged from 2.13 in plant number 23.1 to 2.23 in plant number 23.4 respectively.

Polyphenol estimation in cocoa was also estimated previously by Ajmal (2016) in selected cocoa hybrids and Veeresh (2018) in 30 exotic germplasm. In the present study, the Polyphenol content varied significantly mainly because of additive gene effect.

4.1.3.2 Evaluation of S_2 inbreds of cocoa

4.1.3.2.1. Growth observation

The growth parameters such as plant height and collar girth of S_2 inbreds are presented in table 8.

4.1.3.2.1.1 Plant height (cm)

During 2016, the maximum plant height of 345 cm was observed in S_2 inbred G VI 141 (Plant number 15.9), whereas the shortest plant height of 95 cm was observed in P II 13.8 (Plant number 22.3)

In 2017, the plant height ranged between 115 cm to 360 cm in S_2 genotype G VI 141 (Plant number 15.9) and PII 13.12 (Plant number 17.8) respectively.

The maximum plant height of 375 cm was recorded in S_2 inbred G VI 141 (Plant number 15.9), where as the least plant height of 135 cm was recorded in S_2 inbred P II 4.8 (Plant number 20.4) in 2018

4.1.3.2.1.2 Collar girth (cm)

The collar girth during 2016, ranged from 14 cm to 56 cm in P II 13.2 (Plant number 17.6) and H 7.3 (Plant number 5.1) respectively. The collar girth during 2017, ranged from 15 cm to 57 cm in P II 13.2 (Plant number 17.6) and H 7.3 (Plant number

Table 8. Plant height and collar girth of S_2 inbreds of cocoa

Plant No.	Genotype	Plant height (cm)			Girth (cm)		
		2016	2017	2018	2016	2017	2018
1.1	G VI 295.4	127	140	160	37	38	39
1.2	G VI 295.4	175	178	180	42	43	44
1.3	G VI 295.4	115	150	235	31	31	38
1.4	G VI 295.4	185	195	215	44	45	48
1.5	G VI 295.4	189	194	208	38	38	42
1.6	G VI 295.4	210	220	230	38	39	40
1.7	G VI 295.4	190	220	245	33	34	34
1.8	G VI 295.4	170	250	375	38	39	40
1.9	G VI 295.4	165	210	350	37	39	42
1.1	G VI 295.4	155	200	285	32	35	38
1.11	G VI 295.4	180	240	365	38	35	41
5.1	H 7.3 (86)	180	195	225	56	57	58
5.2	H 7.3 (86)	295	320	335	42	43	45
5.3	H 7.3 (86)	280	310	350	48	50	55
7.1	H1 1.2 (86)	280	300	340	38	38	40
13.1	G VI 135	115	120	150	34	35	38
15.1	G VI 141	240	260	295	32	34	35
15.2	G VI 141	210	310	350	32	33	37
15.3	G VI 141	180	205	225	19	26	31
15.4	G VI 141	195	202	205	19	22	26
15.5	G VI 141	230	280	305	36	43	46
15.6	G VI 141	120	128	135	42	43	47
15.7	G VI 141	240	280	335	43	46	46
15.8	G VI 141	185	192	195	35	38	39
15.9	G VI 141	345	360	375	44	45	49
17.1	PII 13.12	290	320	365	25	27	28
17.2	PII 13.12	250	258	265	21	22	27

17.3	PII 13.12	140	145	150	23	24	28
17.4	PII 13.12	160	165	175	29	31	34
17.5	PII 13.12	225	295	340	24	25	28
17.6	PII 13.12	225	130	240	14	15	19
17.8	PII 13.12	100	115	135	23	24	28
17.9	PII 13.12	100	180	280	23	24	25
20.1	P II 4.8	115	200	285	30	31	31
20.2	P II 4.8	155	162	170	35	38	42
20.3	P II 4.8	162	168	173	38	41	44
20.4	P II 4.8	120	125	135	29	31	33
20.5	P II 4.8	105	145	360	35	37	38
20.6	P II 4.8	145	147	150	29	37	41
20.7	P II 4.8	165	170	175	38	39	42
22.1	P II 13.8	170	181	185	21	22	23
22.2	P II 13.8	160	163	165	15	16	19
22.3	P II 13.8	95	130	170	25	29	32
22.4	P II 13.8	215	345	365	27	27	28
22.5	P II 13.8	115	170	235	15	16	19
24.1	P II 12.9	245	280	340	25	27	27
24.2	P II 12.9	120	130	145	23	25	27

5.1) respectively. The collar girth during 2018, ranged from 19 cm to 58 cm in P II 13.2 (Plant number 17.6) and H 7.3 (Plant number 5.1) respectively.

4.1.3.2.2 Pod characters of S_2 inbreds of cocoa

The details of pod characters of S_2 inbred of cocoa are presented in table 9.

4.1.3.2.2.1 Pod weight (g)

The variation in pod weight of S_2 inbred is presented in fig. 21.The pod weight varied between 106 g and 464 g. The maximum pod weight of 464 g was recorded in PII 13.12 (Plant number 17.4) inbred and the least pod weight of 106g was observed in P II 4.8 (Plant number 20.1) inbred plant.

The pod weight varied significantly in G VI 295.4 and the maximum pod weight of 412 g was observed in plant number 1.10 and the least pod weight (144 g) was observed in plant number 1.7.

The pod weight ranged from 324 g to 442 g. in H1 7.3, the maximum pod weight (442 g) was recorded in plant number 5.1 and the minimum pod weight (324 g) was recorded in plant number 5.3. The pod weight of 310g and 178 g was observed in genotype H 1.2 and G VI 135

The pod weight ranged between 180 g to 300 g. in G VI 141 and the maximum (300 g) and minimum (180g) was recorded in plant number 15.4 and 15.2 respectively. The maximum (464 g) and minimum (116 g) pod weight was observed in plant number 17.4 and 17.3 respectively in P II 13.12. The pod weight varied significantly in P II 4.8 and the maximum (456 g) and minimum (106 g) pod weight was observed in plant number 20.2 and 20.1 respectively.

The pod weight varied significantly in P II 13.8 and the maximum (364 g) and minimum (202 g) pod weight was observed in plant number 22.2 and 22.3. The pod

Table 9. Pod characters of S_2 inbreds of cocoa

S. No	Genotype	Pod weight (g)	Pod length (cm)	Pod breadth (cm)	Ridge thickness (cm)	Furrow thickness (cm)	No of beans/pod	Flat beans/pod
1.1	G VI 295.4	352.00	12.50	6.40	1.20	0.66	40.80	0.80
1.2	G VI 295.4	310.00	11.60	6.60	1.12	0.72	36.80	0.80
1.3	G VI 295.4	292.00	9.90	6.20	1.04	0.78	35.40	0.80
1.4	G VI 295.4	158.00	9.66	6.60	0.82	0.44	23.40	2.80
1.5	G VI 295.4	344.00	13.20	7.34	1.60	1.10	44.20	0.20
1.6	G VI 295.4	202.00	10.90	6.60	1.22	0.54	42.60	0.40
1.7	G VI 295.4	144.00	11.60	5.64	1.12	0.66	28.60	4.20
1.8	G VI 295.4	334.00	14.00	7.20	1.14	0.54	40.60	0.40
1.9	G VI 295.4	328.00	12.90	8.20	1.18	1.00	36.20	1.20
1.10	G VI 295.4	412.00	14.80	8.40	1.62	1.08	43.00	0.20
1.11	G VI 295.4	360.00	14.10	7.70	0.92	0.72	42.00	1.80
5.1	H 7.3	442.00	13.90	7.20	0.80	0.60	22.20	0.60
5.2	H 7.3	391.00	13.80	7.20	1.76	0.76	37.60	2.20
5.3	H 7.3	324.00	11.04	6.72	1.84	1.50	34.00	2.20
7.1	H1 1.2	310.00	12.46	7.20	1.72	0.56	30.40	0.40
13.1	G VI 135	178.00	9.82	6.50	1.24	0.66	31.60	1.80
15.1	G VI 141	296.00	14.40	6.80	1.16	1.46	41.80	1.40
15.2	G VI 141	300.00	12.84	6.14	1.72	1.14	28.80	0.80
15.3	G VI 141	256.00	11.90	5.88	1.64	1.18	30.40	0.60
15.4	G VI 141	180.00	10.74	5.90	1.16	0.76	36.40	0.60
15.5	G VI 141	344.00	14.56	7.20	1.80	1.16	39.80	0.00
15.6	G VI 141	220.00	12.62	5.70	1.26	1.00	40.40	0.20
15.7	G VI 141	260.00	14.50	6.80	1.36	1.16	37.80	1.20
15.8	G VI 141	184.00	11.90	6.22	1.58	0.88	44.60	2.00
15.9	G VI 141	286.00	12.00	5.90	1.26	0.80	40.00	1.40
17.1	PII 13.12	248.00	11.70	7.30	1.42	0.50	24.20	2.40
17.2	PII 13.12	256.00	11.20	8.10	0.84	0.58	41.20	8.00
17.3	PII 13.12	116.00	9.60	4.70	1.00	0.86	20.80	1.20
17.4	PII 13.12	464.00	16.70	10.40	1.46	0.80	32.00	0.60
17.5	PII 13.12	144.00	10.40	5.62	1.02	0.76	26.00	1.40
17.6	PII 13.12	268.00	14.40	6.52	1.40	0.82	36.40	0.80
17.7	PII 13.12	310.00	14.10	7.10	1.18	0.62	34.00	2.00
17.8	PII 13.12	260.00	10.06	6.98	1.24	1.02	22.20	6.60
20.1	P II 4.8	106.00	10.66	4.68	1.44	1.16	14.20	1.40
20.2	P II 4.8	456.00	18.30	8.60	1.32	0.94	33.80	1.20
20.3	P II 4.8	246.00	17.94	6.36	0.92	0.48	35.80	2.20

20.4	P II 4.8	250.00	15.50	6.64	1.70	0.84	26.80	1.60
20.5	P II 4.8	412.00	16.90	7.40	1.18	0.94	37.00	4.80
20.6	P II 4.8	432.00	19.16	7.50	1.46	1.10	36.40	7.00
20.7	P II 4.8	435.00	18.90	8.00	1.16	0.72	35.40	1.80
22.1	P II 13.8	252.00	12.58	6.32	1.26	0.92	27.20	1.40
22.2	P II 13.8	364.00	12.80	8.50	1.12	0.64	29.80	5.40
22.3	P II 13.8	202.00	9.38	6.30	0.88	0.62	29.80	4.00
22.4	P II 13.8	316.00	11.30	7.80	1.62	0.90	28.60	0.60
22.5	P II 13.8	326.00	11.30	6.90	0.94	0.80	33.60	1.80
24.1	P II 12.9	256.00	10.70	7.20	1.08	0.48	28.40	1.60
24.2	P II 12.9	144.00	9.20	5.66	1.00	0.86	23.20	1.60
	CV (%)	15.292	7.955	8.545	12.596	16.191	12.16	26.23
	CD(0.05)	54.328	1.268	0.728	0.199	0.167	5.024	1.77

weight ranged from 144 g to 256 g in plant number 24.2 and 24.1 respectively in PII 12.9.

Minimol *et al.* (2015) revealed the breeding cycle of fifth generation inbred in cocoa, the pod weight range between 234 and 308g among the S_0 to S_5 generations of GII 7.4 inbred. In the present study the maximum pod weight observed was 464g. The range and average pod weight of S_2 generation inbreds is lesser than S_1 generation inbreds in the present study.

4.1.3.2.2.2 Pod length (cm)

The pod length ranged between 9.2 and 19.16 cm. The maximum pod length of 19.16 cm was observed in PII 4.8(Plant number 20.6) inbred and the minimum pod length (9.20cm) was recorded in P II 12.9 (Plant number 24.2) inbred (Fig. 22).

In inbred G VI 295.4, the pod length varied significantly, the maximum pod length of 14.80 cm was observed in plant number 1.10 and the least pod length (9.66 cm) was observed in plant number 1.4.

The pod length ranged from 11.04 cm to 13.90 cm in H1 7.3 and the maximum pod length (13.90 cm) was recorded in plant number 5.1 and the minimum pod length (11.04cm) was recorded in plant number 5.3.

The pod length of 12.46 cm and 9.82 cm was observed in genotype H 1.2 and G VI 135. The pod length ranged between 10.74 cm to 14.56 cm in G VI 141 and the maximum (14.56 cm) and minimum (10.74 cm) was recorded in plant number 15.5 and 15.4 respectively. The maximum (16.70 cm) and minimum (9.60 cm) pod length in P II 13.12 was observed in plant number 17.4 and 17.3 respectively.

The pod length varied significantly in P II 4.8 and the maximum (19.16 cm) and minimum (10.66 cm) pod length was observed in plant number 20.6 and 20.1 respectively. The maximum (12.80 cm) and minimum (9.38 cm) pod length in P II 13.8 was observed in plant number 22.2 and 22.3.

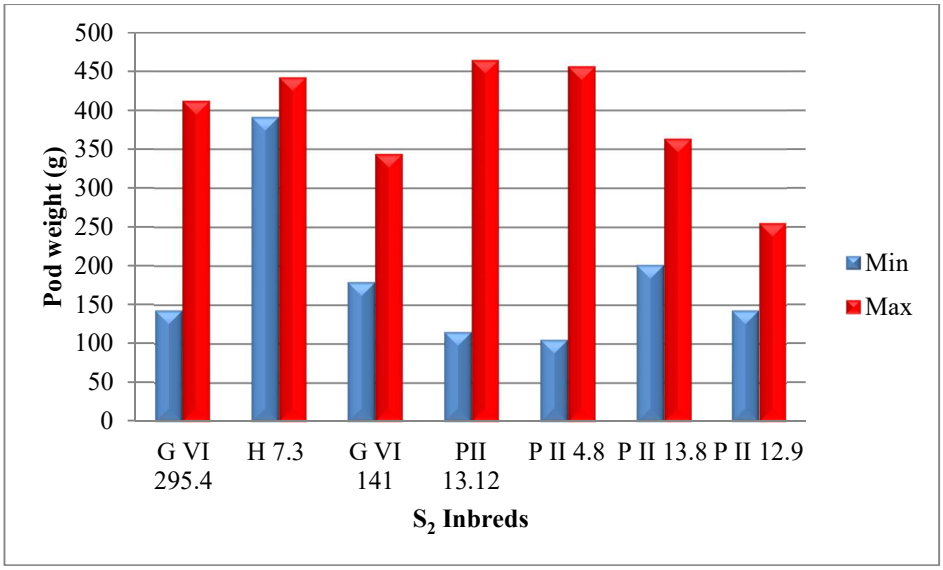


Fig.21 Pod weight of S_2 inbreds

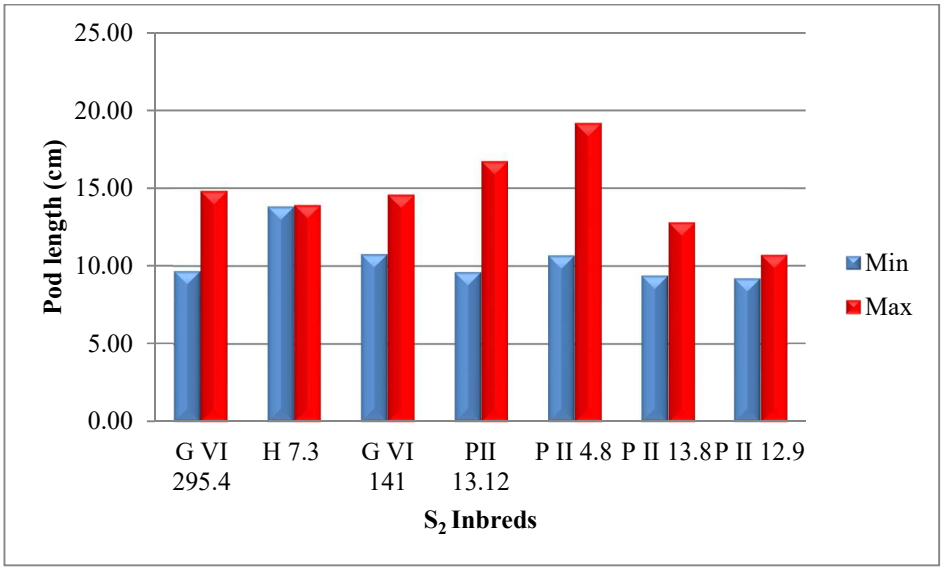


Fig.22 Pod length of S_2 inbreds

The pod length in P II 12.9 ranged from 9.20 cm to 10.70 cm in plant number 24.2 and 24.1 respectively.

Similar finding are reported in cocoa inbred (CCRP Annual Report, 2013).

4.1.3.2.2.3 Pod breadth (cm)

The difference in the pod breadth of S_2 inbreds are presented in fig. 23. The pod breadth ranged between 4.68 and 10.40 cm. The maximum pod breadth of 10.40 cm was observed in PII 13.12 (Plant number 17.4) inbred and the minimum pod breadth (4.80cm) was recorded in P II 4.8 (Plant number 20.1) inbred.

The pod breadth varied significantly in G VI 295.4 and the maximum pod breadth of 8.40 cm was observed in plant number 1.10 and the least pod breadth (5.64 cm) was observed in plant number 1.7. The pod breadth in H1 7.3 ranged from 6.72 cm to 7.20 cm and the maximum pod breadth (7.20 cm) was recorded in plant number 5.1, 5.2 and the minimum pod breadth (6.72 cm) was recorded in plant number 5.3. The pod breadth in G VI 141 ranged between 5.70 cm to 6.80 cm and the maximum (6.80 cm) and minimum (5.70 cm) pod breadth was recorded in plant number 15.1 and 15.6 respectively. The maximum (10.40 cm) and minimum (4.70 cm) pod breadth in P II 13.12 was observed in plant number 17.4 and 17.3 respectively.

The pod breadth varied significantly in P II 4.8 and the maximum (8.60 cm) and minimum (4.68 cm) pod breadth was observed in plant number 20.2 and 20.1 respectively. The maximum (8.50 cm) and minimum (6.30 cm) pod breadth in PII 13.8 was recorded in plant number 22.2 and 22.3. The pod breadth in P II 12.9 ranged from 5.66 cm to 7.20 cm in plant number 24.2 and 24.1 respectively.

4.1.3.2.2.4 Ridge thickness (cm)

The variation in ridge thickness of S_2 inbred is presented in fig. 24. Significant variation was observed for ridge thickness among the S_2 generation inbred. The ridge thickness ranged between 0.80 and 1.84 cm. The maximum (1.84cm) and minimum

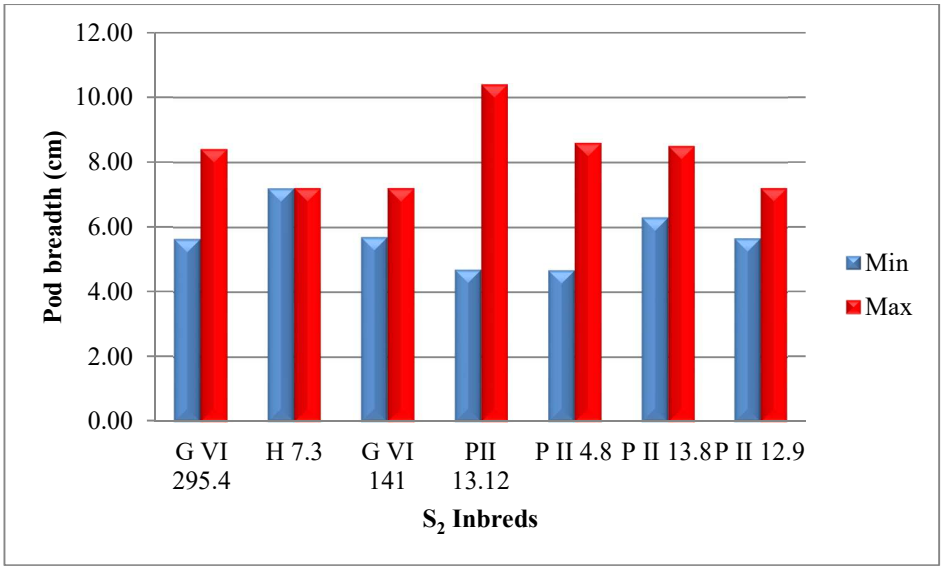


Fig. 23 Pod breadth of S_2 inbreds

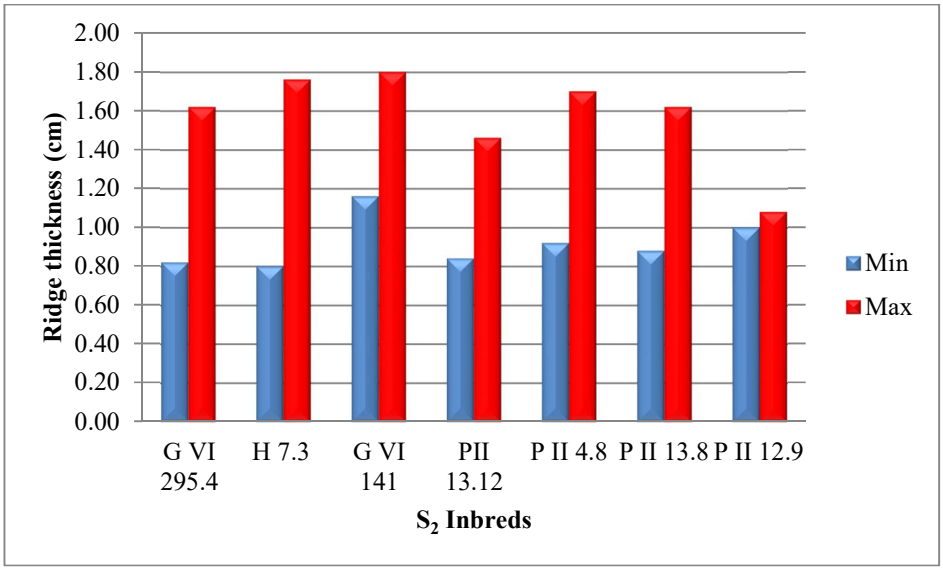


Fig.24. Ridge thickness of S_2 inbreds

(0.80cm) were observed in H 7.3 (86) (Plant number 5.1) and H 7.3 (86) (Plant number 5.3) inbreds respectively.

The ridge thickness varied significantly in G VI 295.4, the maximum ridge thickness of 1.62 cm was observed in plant number 1.10 and the least ridge thickness (0.82 cm) was observed in plant number 1.4. The ridge thickness in H1 7.3 ranged from 0.80 cm to 1.84 cm. The maximum ridge thickness (1.84 cm) was recorded in plant number 5.3 and the minimum ridge thickness (0.80 cm) was recorded in plant number 5.3.

The ridge thickness of 1.72 cm and 1.24 cm was observed in genotype H 1.2 and G VI 135. The ridge thickness in G VI 141, ranged from 1.16 cm to 1.80 cm. The maximum (1.80 cm) and minimum (1.16 cm) ridge thickness was recorded in plant number 15.5 and 15.4 respectively. The maximum (1.46 cm) and minimum (0.84cm) ridge thickness in P II 13.12 was observed in plant number 17.4 and 17.2 respectively.

The ridge thickness varied significantly in P II 4.8, the maximum (1.70 cm) and minimum (0.92 cm) ridge thickness was observed in plant number 20.4 and 20.3 respectively. The maximum (1.62 cm) and minimum (0.88 cm) ridge thickness in PII 13.8 was observed in plant number 22.4 and 22.3.No significant difference was observed for ridge thickness in P II 12.9

The variation in the ridge thickness among the same generation inbreds was also reported (CCRP Annual Report, 2013).

4.1.3.2.2.5. Furrow thickness (cm)

Significant variation was observed for furrow thickness among the S_2 generation inbred. The furrow thickness ranged between 0.44 and 1.50 cm. The inbred H 7.3 (86) (Plant number 5.3) and G VI 295.4 has shown the maximum (1.50cm) and minimum (0.44cm) furrow thickness among the S_2 inbreds. The furrow thickness in G VI 295.4 varied significantly, the maximum furrow thickness of 1.10 cm was observed in plant

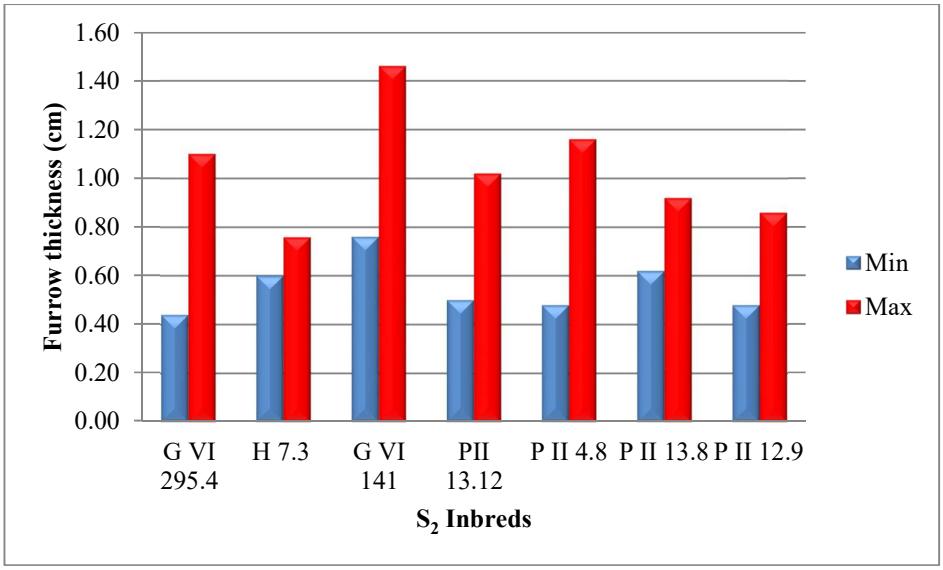


Fig.25 Furrow thickness of S_2 inbreds

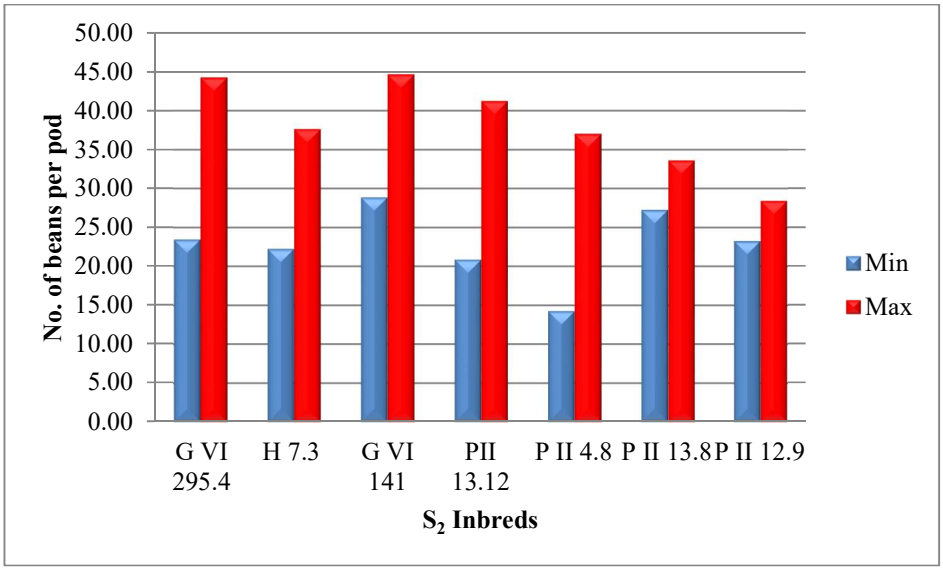


Fig. 26 Number of beans pod of S_2 inbreds

number 1.5 and the least furrow thickness (0.44 cm) was observed in plant number 1.4.(Fig. 25)

The furrow thickness in H1 7.3 ranged from 0.60 cm to 1.50 cm. The maximum furrow thickness (1.50 cm) was recorded in plant number 5.3 and the minimum furrow thickness (0.60 cm) was recorded in plant number 5.1. The furrow thickness of 0.56 cm and 0.66 cm was observed in genotype H 1.2 and G VI 135. The furrow thickness in G VI 141 ranged from 0.76 cm to 1.46 cm. The maximum (1.46 cm) and minimum (0.76 cm) furrow thickness was recorded in plant number 15.1 and 15.4 respectively.

The maximum (1.02 cm) and minimum (0.50 cm) furrow thickness was observed in plant number 17.8 and 17.1 respectively in P II 13.12. The furrow thickness in P II 4.8 varied significantly, the maximum (1.16 cm) and minimum (0.48 cm) furrow thickness was observed in plant number 20.1 and 20.3 respectively.

The maximum (0.92 cm) and minimum (0.62 cm) furrow thickness in PII 13.8 was observed in plant number 22.1 and 22.3. The furrow thickness in P II 12.9 ranged from 0.48 cm to 0.86 cm in plant number 24.1 and 24.2 respectively.

The variation in the furrow thickness among the same generation inbreds was also reported (CCRP, Annual Report, 2013).

4.1.3.2.2.6 Number of beans per pod

The number of beans per pod varied significantly among the inbreds. Maximum of 44.6 beans per pod was observed in G VI 141(Plant number 15.8) inbred. P II 4.8(Plant number 20.1) recorded the minimum number of bean per pod

The number of beans per pod varied significantly in G VI 295.4 and the maximum number of beans per pod of 44.20 was observed in plant number 1.5 and the least number of beans per pod (23.40) was observed in plant number 1.4. The number of beans per pod in H1 7.3 ranged from 22.20 to 37.60. The maximum number of beans per pod (37.60) was recorded in plant number 5.2 and the minimum number of beans per

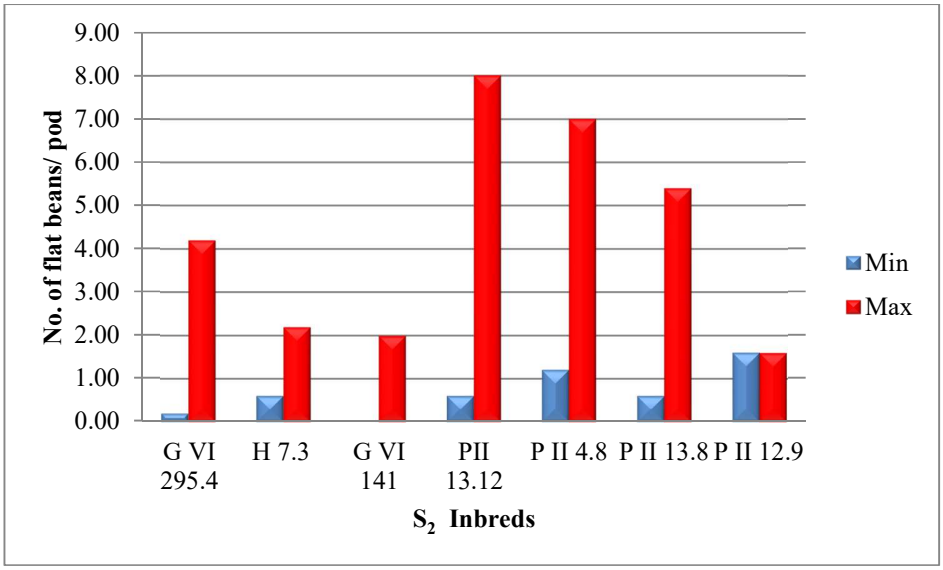


Fig. 27 Number of flat beans per pod of S_2 inbreds

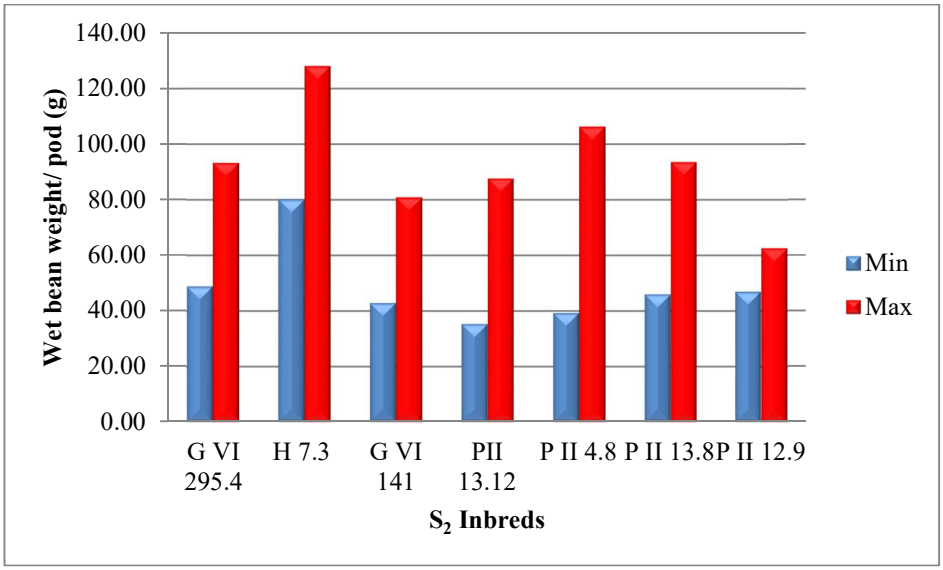


Fig. 28 Wet bean weight per pod of S_2 inbreds

pod (22.20) was recorded in plant number 5.1. The number of beans per pod of 30.40 and 31.60 was observed in genotype H 1.2 and G VI 135(Fig. 26).

The number of beans per pod in G VI 141 ranged from 28.80 to 44.60. The maximum (44.60) and minimum (28.80) number of beans per pod was recorded in plant number 15.8 and 15.2 respectively. The maximum (41.20) and minimum (20.80) number of beans per pod in P II 13.12 was observed in plant number 17.2 and 17.3, respectively. The number of beans per pod in P II 4.8 varied significantly, the maximum (37.00) and minimum (14.20) number of beans per pod was observed in plant number 20.1 and 20.5, respectively.

The maximum (33.60) and minimum (27.20) number of beans per pod in P II 13.8 was observed in plant number 22.5 and 22.1. The number of beans per pod in PII 12.9 ranged from 23.20 to 28.40 in plant number 24.2 and 24.1, respectively.

The variation in the number of beans per pod among the same generation inbreds was also reported (CCRP Annual Report, 2013).

4.1.3.2.2.7 Number of flat beans per pod

The number of flat beans is an indication of improper or no fertilization. Flat beans per pod varied significantly among the inbreds. The variation in number of flat bean per pod presented in fig. 27. Maximum of 8 beans per pod was observed in PII 13.12 (Plant number 17.2). Flat beans were not found in G VI 141 (Plant number 15.5) inbred.

The number of flat beans per pod in G VI 295.4 varied significantly, the maximum number of flat beans per pod (4.20) was observed in plant number 1.7 and the minimum number of flat beans per pod (0.20) was observed in plant number 1.4 and 1.10. The number of flat beans per pod in H1 7.3 ranged from 0.60 to 2.20. The maximum number of flat beans per pod (2.20) was recorded in plant number 5.2 and 5.3 and the minimum number of beans per pod (0.60) was recorded in plant number 5.1.

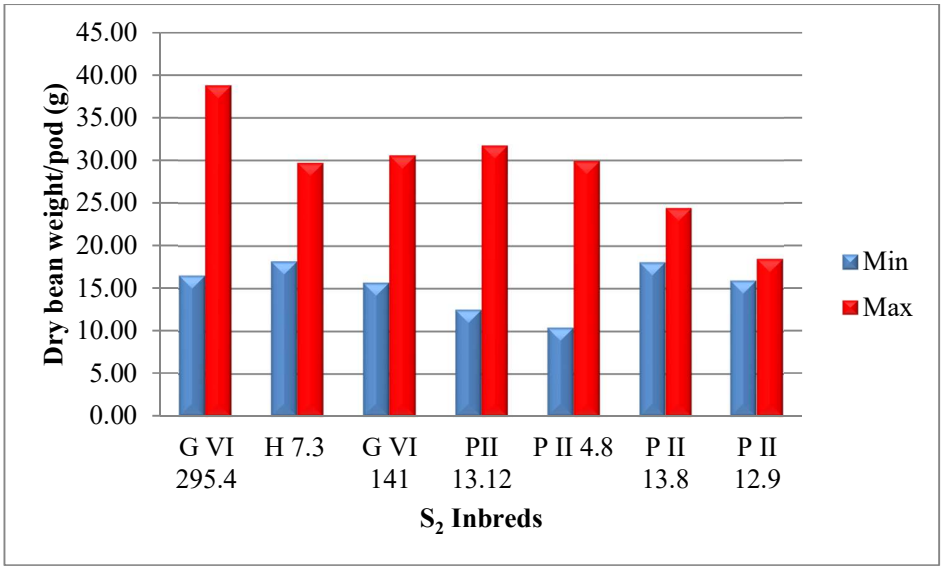


Fig. 29 Dry bean weight per pod of S_2 inbreds

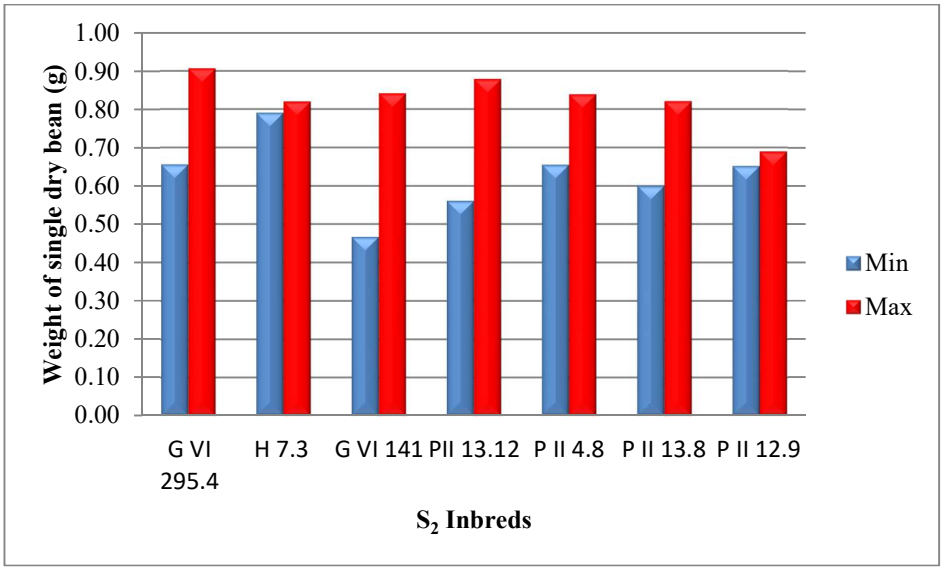


Fig 30 Weight of single dry bean weight of S_2 inbreds

The number of flat beans per pod of 0.40 and 1.80 was observed in genotype H 1.2 and G VI 135. The number of flat beans per pod in G VI 141 ranged from 0 to 2.0. There were no flat beans in stand number 15.5. The highest (2.0) number of flat beans per pod was recorded in plant number 15.8. The maximum (8.0) and minimum (0.60) number of flat beans per pod in P II 13.12 was observed in plant number 17.2 and 17.4, respectively.

The number of flat beans per pod in P II 4.8 varied significantly, the maximum (7.00) and minimum (1.20) number of flat beans per pod was observed in plant number 20.6 and 20.2, respectively.

The number of flat beans per pod in P II 13.8 varied significantly and the maximum (5.40) and minimum (0.60) number of flat beans per pod was observed in plant number 22.2 and 22.4. There was no significant difference between the numbers of flat beans per pod in P II 12.9.

4.1.3.2.3. Bean characters of S_2 inbreds of cocoa

4.1.3.2.3.1 Wet bean weight per pod (g)

The details of bean characters of S_2 inbred are presented in table 10. The wet bean weight per pod ranged between 35.28g in inbred P II 13.8 (Plant number 17.8) and 128g in H 7.3 (86) (Plant number 5.1). The average wet bean weight of S_2 generation inbred was 67.63g. (Fig. 28)

The wet bean weight per pod in G VI 295.4 varied significantly and it ranged from 48.70 g to 93.10 g and the maximum (93.10 g) and minimum (48.70 g) wet bean weight per pod was recorded in plant number 1.8 and 1.4 respectively.

The wet bean weight per pod in H 7.3 ranged from 79.98 g in plant number 5.2 to 128.00 g in plant number 5.1.The wet bean weight per pod was 68.60 g and 43.96 g in H1 1.2 and G VI 135. The wet bean weight per pod in G VI 141 ranged between 42.82 g and 80.80 g in plant number 15.8 and 15.4 respectively.

Table 10. Bean characters of S_2 inbreds of cocoa

S. No	Genotype	Wet bean weight /pod (g)	Dry bean weight/pod (g)	Single bean weight (g)	Bean length (mm)	Bean breadth (mm)	Bean thickness (mm)
1.1	G VI 295.4	89.10	32.96	0.81	20.55	11.38	5.98
1.2	G VI 295.4	68.48	30.52	0.83	18.42	11.60	7.02
1.3	G VI 295.4	65.82	25.12	0.71	16.62	10.52	5.33
1.4	G VI 295.4	48.70	16.52	0.71	15.66	9.54	6.68
1.5	G VI 295.4	77.58	34.75	0.79	16.79	10.52	6.86
1.6	G VI 295.4	60.82	28.54	0.67	17.58	11.47	5.50
1.7	G VI 295.4	68.80	21.54	0.76	13.15	10.53	5.45
1.8	G VI 295.4	93.10	32.30	0.80	20.39	11.39	7.56
1.9	G VI 295.4	74.84	28.57	0.79	16.61	12.41	7.71
1.10	G VI 295.4	91.26	38.77	0.91	21.50	12.68	6.46
1.11	G VI 295.4	69.96	27.50	0.66	16.37	10.51	6.48
5.1	H 7.3	128.00	18.17	0.82	17.59	9.66	7.15
5.2	H 7.3	79.98	29.71	0.79	18.45	10.49	7.32
5.3	H 7.3	87.20	25.90	0.76	17.24	10.20	6.82
7.1	H1 1.2	68.60	19.18	0.63	15.37	8.18	6.37
13.1	G VI 135	43.96	21.36	0.68	16.18	10.23	5.36
15.1	G VI 141	57.78	27.64	0.66	17.22	9.21	7.19
15.2	G VI 141	66.16	15.67	0.55	15.44	11.14	7.14
15.3	G VI 141	79.40	23.08	0.76	16.27	10.23	6.07
15.4	G VI 141	80.80	30.59	0.84	15.36	10.38	7.06
15.5	G VI 141	59.06	28.66	0.72	17.67	10.42	7.70
15.6	G VI 141	62.90	22.21	0.55	15.34	9.57	5.38
15.7	G VI 141	65.94	27.09	0.72	15.33	9.19	5.22
15.8	G VI 141	42.82	20.84	0.47	13.37	9.32	5.51
15.9	G VI 141	57.02	27.61	0.69	15.17	11.31	6.53
17.1	PII 13.12	41.78	20.81	0.87	16.06	11.07	7.20
17.2	PII 13.12	67.30	30.65	0.74	17.65	11.63	5.77
17.3	PII 13.12	49.00	17.71	0.85	17.32	11.90	6.46
17.4	PII 13.12	87.52	28.18	0.88	21.98	12.69	7.42
17.5	PII 13.12	42.80	22.86	0.88	21.22	10.49	8.26
17.6	PII 13.12	56.58	31.74	0.87	19.28	9.35	7.25
17.7	PII 13.12	57.52	24.26	0.71	17.13	9.45	5.57
17.8	PII 13.12	35.28	12.53	0.56	21.41	10.47	8.26
20.1	P II 4.8	39.20	10.44	0.74	16.66	10.76	7.60
20.2	P II 4.8	96.00	27.22	0.81	18.36	11.55	7.46
20.3	P II 4.8	58.28	23.39	0.65	18.39	9.97	8.23

20.4	P II 4.8	48.96	18.46	0.69	18.47	10.55	8.46
20.5	P II 4.8	76.00	24.46	0.66	17.26	10.27	6.87
20.6	P II 4.8	106.12	26.05	0.72	16.30	9.98	6.93
20.7	P II 4.8	69.44	29.96	0.84	18.39	10.36	7.49
22.1	P II 13.8	78.80	22.09	0.81	17.92	12.51	7.50
22.2	P II 13.8	63.12	18.06	0.60	16.21	11.03	6.44
22.3	P II 13.8	68.00	24.45	0.82	14.30	9.97	6.43
22.4	P II 13.8	45.92	23.02	0.80	17.31	11.82	7.22
22.5	P II 13.8	93.44	22.30	0.66	19.24	11.45	7.52
24.1	P II 12.9	46.84	18.52	0.65	18.91	11.97	7.09
24.2	P II 12.9	62.40	15.94	0.69	15.12	10.65	6.93
	CV (%)	12.341	15.508	8.098	3.527	4.133	5.826
	CD(0.05)	10.346	6.171	0.074	0.758	0.547	0.492

The wet bean weight per pod in P II 13.12 varied significantly, the maximum wet bean weight per pod (87.52 g) was observed in plant number 17.4 and minimum wet bean weight per pod (35.28 g) was observed in plant number 17.8. The wet bean weight per pod in P II 4.8 ranged from 39.20 g in plant number 20.1 to 106.12 g in plant number 20.6. The wet bean weight per pod in P II 13.8 varied significantly, and it ranged from 45.92 g to 93.44 g in plant number 22.4 and 22.5 respectively.

The wet bean weight per pod in P II 12.9 varied between 46.84 g and 62.40 g.

4.1.3.2.3.2 Dry bean weight per pod (DBWP) (g)

Dry bean per pod is the quantity of bean obtained from each pod. The dry bean weight per pod ranged from 10.44 to 38.77 g. The maximum dry bean weight per pod is useful criterion for selection of inbred. The maximum DBWP was observed in G VI 295.4 indicating its suitability for selection in breeding programme. The least value was observed in PII 13.12.

The DBWP in G VI 295.4 varied significantly and it ranged from 16.52 g to 38.77 g. The maximum (38.77 g) and minimum (16.52 g) DBWP was recorded in plant number 1.10 and 1.4, respectively. The DBWP in H 7.3 ranged from 18.17 g in plant number 5.1 to 29.71 g in plant number 5.2. The DBWP was 19.18 g and 21.36 g in H1 1.2 and G VI 135.

The DBWP in G VI 141 ranged between 15.67 g and 30.59 g in plant number 15.2 and 15.4, respectively. The DBWP in P II 13.12 varied significantly, the maximum DBWP (30.65 g) was observed in plant number 17.2, and minimum DBWP (12.53 g) was observed in plant number 17.8. The DBWP in P II 4.8 ranged from 10.44 g in plant number 20.1 to 29.96 g in plant number 20.7.

The DBWP in P II 13.8 varied significantly, and it ranged from 18.06 g to 24.45 g in plant number 22.3 and 22.2, respectively. The DBWP in P II 12.9 varied significantly, and it ranged from 15.94 g to 18.52 g in plant number 24.2 and 24.1, respectively.

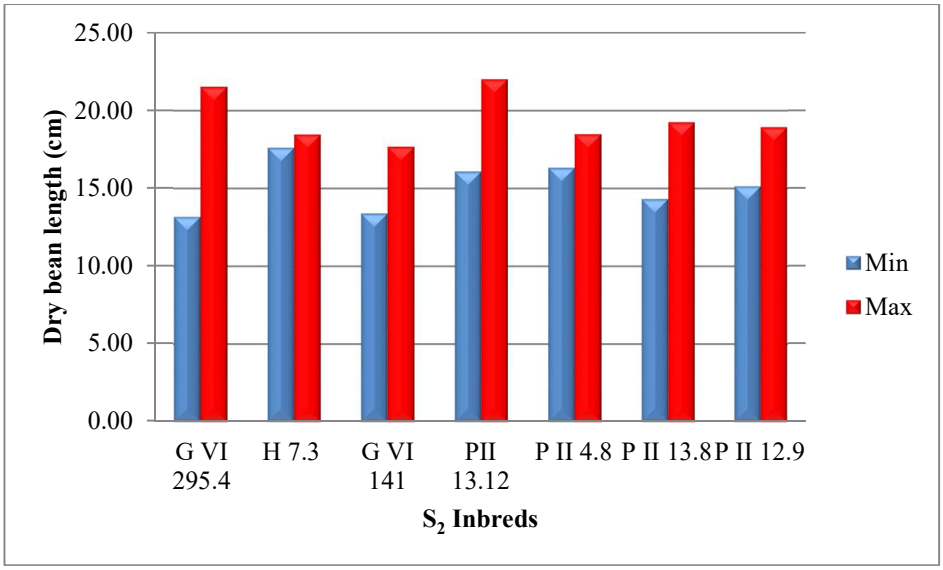


Fig. 31 Dry bean length of S_2 inbreds

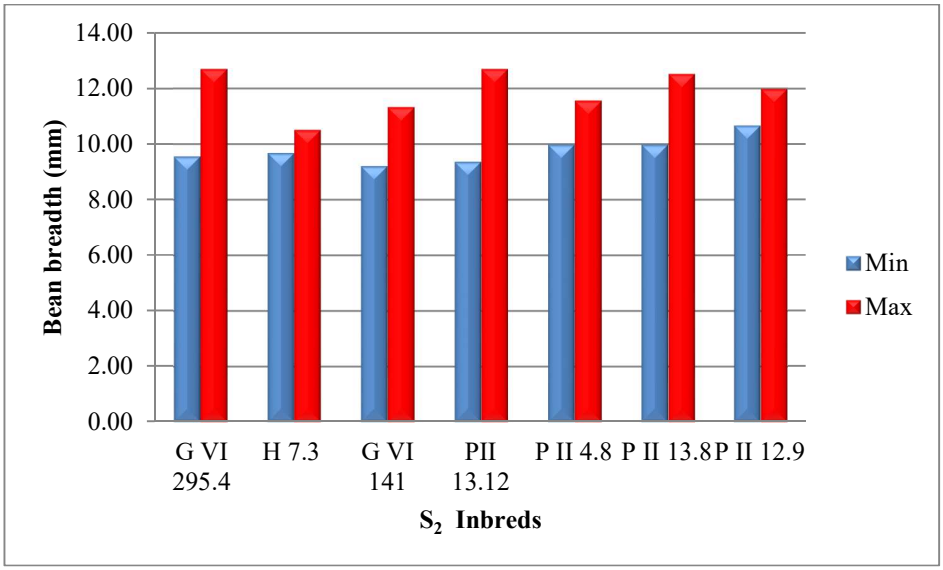


Fig. 32 Dry bean breadth of S_2 inbreds

4.1.3.2.3.3 Single Dry Bean Weight (g)

The variation in single dry bean weight is presented in fig. 30. The single dry bean weight ranged between 0.47 and 0.91 g in inbred G VI 141 (Plant number 15.8) and G VI 295.4 (Plant number 1.9).

The single dry bean weight in G VI 295.4 varied significantly and it ranged from 0.66 g to 0.91 g. The maximum (0.91 g) and minimum (0.66 g) single dry bean weight in G VI 295.4 was recorded in plant number 1.11 and 1.10 respectively.

The single dry bean weight in H 7.3 ranged from 0.76 g in plant number 5.3 to 0.82 g in plant number 5.1. The single dry bean weight was 0.63 g and 0.68 g in H1 1.2 and G VI 135. The single dry bean weight in G VI 141 ranged between 0.47 g and 0.84 g in plant number 15.8 and 15.4, respectively.

The single dry bean weight in P II 13.12 varied significantly, the maximum single dry bean weight (0.88 g) was observed in plant number 17.4, and 17.5, and minimum single dry bean weight (0.56 g) was observed in plant number 17.8. The single dry bean weight in P II 4.8 ranged from 0.65 g in plant number 20.3 to 0.84 g in plant number 20.7. The single dry bean weight in P II 13.8 varied significantly, and it ranged from 0.60 g to 0.82 g in plant number 22.2 and 22.3, respectively.

The single dry bean weight in P II 12.9 varied significantly, and it ranged from 0.65 g to 0.69 g in plant number 24.1 and 24.2, respectively.

4.1.3.2.3.4. Dry bean length (mm)

The dry bean length varied significantly and it ranged from 13.15mm in genotype G VI 295.4 (Plant number 1.7) to 21.98 mm in genotype P II 13.12 (Plant number 17.4) (Fig. 31).

The dry bean length in G VI 295.4 varied significantly and it ranged from 13.15 mm to 21.50 mm. The maximum (21.50 mm) and minimum (13.15 mm) dry bean length was recorded in plant number 1.10 and 1.7 respectively. The dry bean length in H 7.3 ranged from 17.24 mm in plant number 5.3 to 18.45 mm in plant number 5.2.

The dry bean length was 15.37 mm and 16.18 mm in H1 1.2 and G VI 135. The dry bean length in G VI 141 ranged between 13.37 mm and 17.22 mm in plant number 15.8 and 15.1, respectively. The dry bean length in P II 13.12 varied significantly, the maximum dry bean length (21.98 mm) was observed in plant number 17.4, and minimum dry bean length (16.06 mm) was observed in plant number 17.1.

The dry bean length in P II 4.8 ranged from 16.30 mm in plant number 20.6 to 18.47 mm in plant number 20.4. The dry bean length in P II 13.8 varied significantly, and it ranged from 14.30 mm to 19.24 mm in plant number 22.3 and 22.5, respectively.

The dry bean length in PII 12.9 varied significantly, and it ranged from 15.12 mm to 18.91 mm in plant number 24.2 and 24.1, respectively

4.1.3.2.3.5 Dry bean breadth (mm)

The dry bean breadth varied significantly and it ranged from 8.18 mm in genotype H 1 1.2 (86) (Plant number 7.1) to 12.69 mm in genotype P II 13.12 (Plant number 17.4)

The dry bean breadth in G VI 295.4 varied significantly and it ranged from 9.54 mm to 12.68 mm. The maximum (12.68 mm) and minimum (9.54 mm) dry bean breadth was recorded in plant number 1.10 and 1.4 respectively.

The dry bean breadth in H 7.3 ranged from 9.66 mm in plant number 5.1 to 10.49 mm in plant number 5.2.(Fig. 31)

The dry bean breadth was 8.18 mm and 10.23 mm in H1 1.2 and G VI 135. The dry bean breadth in G VI 141 ranged between 9.19 mm and 11.31 mm in plant number 15.7 and 15.9, respectively. The dry bean breadth in P II 13.12 varied significantly, the maximum dry bean breadth (12.69 mm) was observed in plant number 17.4, and minimum dry bean breadth (9.35 mm) was observed in plant number 17.6.

The dry bean breadth in P II 4.8 ranged from 9.97 mm in plant number 20.3 to 10.76 mm in plant number 20.1. The dry bean breadth in P II 13.8 varied significantly,

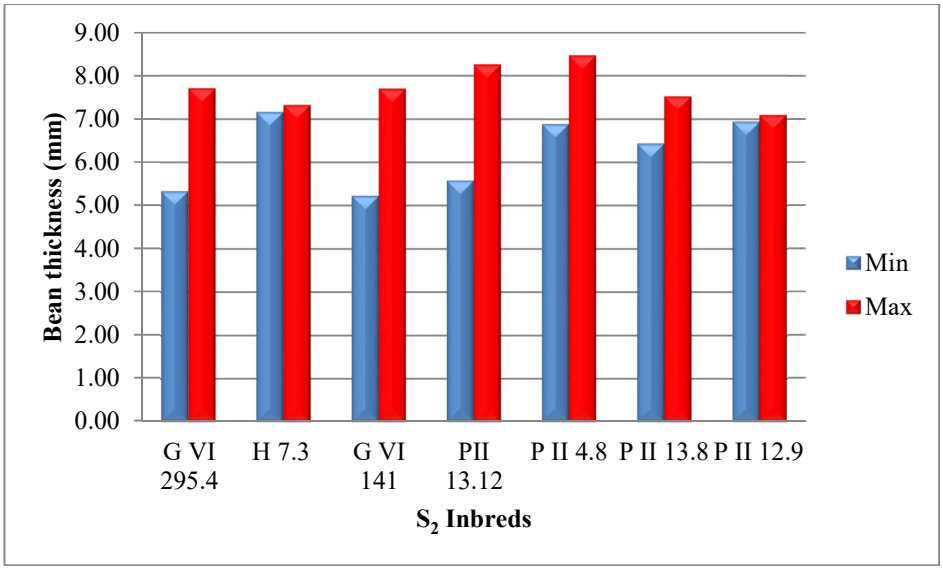


Fig. 33 Dry bean thickness of S_2 inbreds

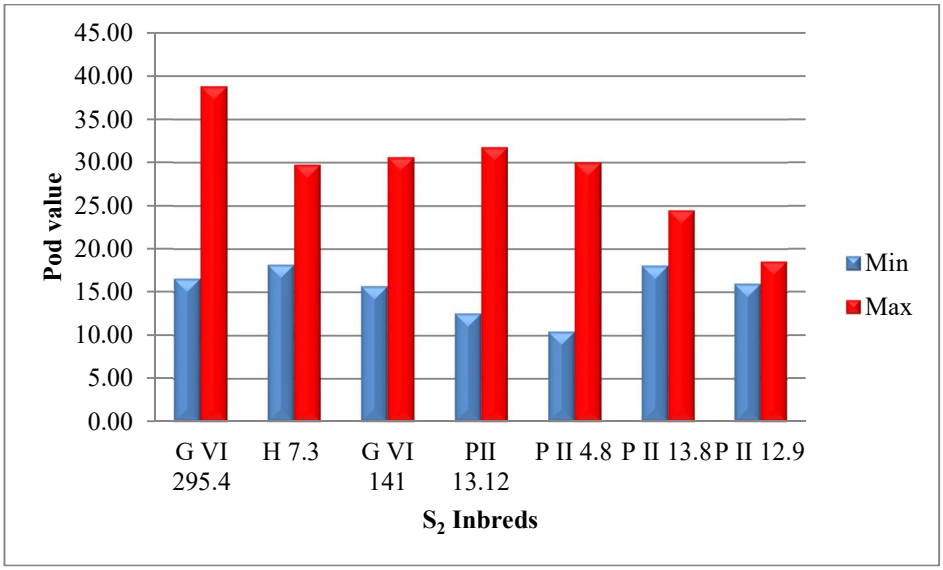


Fig. 34 Pod value of S_2 inbreds

and it ranged from 9.97 mm to 11.82 mm in plant number 22.3 and 22.4, respectively. The dry bean breadth in P II 12.9 varied significantly, and it ranged from 10.65 mm to 11.97 mm in plant number 24.2 and 24.1, respectively

4.1.3.2.3.6 Dry bean thickness (mm)

The variation dry bean thickness is presented in fig 33.The dry bean thickness varied significantly and it ranged from 5.22 mm in genotype G VI 141 (Plant number 15.7) to 8.46 mm in genotype P II 4.8 (Plant number 20.4)

The dry bean thickness in G VI 295.4 varied significantly and it ranged from 5.33 mm to 7.71 mm. The maximum (7.71 mm) and minimum (5.33 mm) dry bean thickness in G VI 295.4 was recorded in plant number 1.9 and 1.3, respectively.

The dry bean thickness in H 7.3 ranged from 6.82 mm in plant number 5.3 to 7.32 mm in plant number 5.2.The dry bean thickness was 6.37 mm and 5.36 mm in H1 1.2 and G VI 135. The dry bean thickness in G VI 141 ranged between 5.22 mm and 7.70 mm in plant number 15.7 and 15.5, respectively. The dry bean thickness in P II 13.12varied significantly and the maximum dry bean thickness (8.26 mm) was observed in plant number 17.8, and minimum dry bean thickness (5.57 mm) was observed in plant number 17.7. The dry bean thickness in P II 4.8 ranged from 6.93 mm in plant number 20.6 to 8.46 mm in plant number 20.4.

The dry bean thickness in P II 13.8 varied significantly, and it ranged from 6.43 mm to 7.52 mm in plant number 22.3 and 22.5, respectively. The dry bean thickness in P II 12.9 varied significantly, and it ranged from 6.93 mm to 7.09 mm in plant number 24.2 and 24.1, respectively.

4.1.3.2.4 Economic characters of S_2 inbreds of cocoa

The difference in economic characters of S_2 inbreds are presented in table 11.

4.1.3.2.4.1 Pod value

The variation in pod value of S_2 inbreds is presented in fig.34. The pod value ranged from 10.44 to 38.77 among the S_2 generation inbreds. The lowest pod value was

Table 11. Economic characters of S_2 inbreds of cocoa

S. No	Genotype	Pod Value	Pod Index	Efficiency Index	Conversion Index	Dry matter Recovery (%)
1.1	G VI 295.4	32.96	31.07	11.16	0.37	37.09
1.2	G VI 295.4	30.52	32.86	10.19	0.44	44.65
1.3	G VI 295.4	25.12	39.98	11.69	0.38	38.30
1.4	G VI 295.4	16.52	61.21	9.68	0.34	33.92
1.5	G VI 295.4	34.75	29.19	10.07	0.45	45.21
1.6	G VI 295.4	28.54	35.28	7.09	0.47	46.85
1.7	G VI 295.4	21.54	47.22	6.83	0.31	31.49
1.8	G VI 295.4	32.30	31.14	10.37	0.35	34.72
1.9	G VI 295.4	28.57	35.56	11.59	0.38	38.35
1.10	G VI 295.4	38.77	26.16	10.67	0.43	42.72
1.11	G VI 295.4	27.50	36.42	13.09	0.39	39.31
5.1	H 7.3	18.17	57.83	25.49	0.15	14.63
5.2	H 7.3	29.71	33.76	13.19	0.37	37.26
5.3	H 7.3	25.90	38.88	12.67	0.30	29.75
7.1	H1 1.2	19.18	52.75	16.42	0.28	28.05
13.1	G VI 135	21.36	49.98	8.77	0.48	48.37
15.1	G VI 141	27.64	37.24	11.08	0.48	48.20
15.2	G VI 141	15.67	65.48	18.57	0.24	23.61
15.3	G VI 141	23.08	43.43	11.05	0.29	29.13
15.4	G VI 141	30.59	32.89	5.94	0.38	37.90
15.5	G VI 141	28.66	35.50	12.15	0.49	48.43
15.6	G VI 141	22.21	45.27	9.96	0.35	35.27
15.7	G VI 141	27.09	37.30	9.84	0.41	41.14
15.8	G VI 141	20.84	49.13	9.15	0.49	49.46
15.9	G VI 141	27.61	36.41	10.50	0.50	50.42
17.1	PII 13.12	20.81	48.77	12.25	0.51	51.35
17.2	PII 13.12	30.65	32.64	8.39	0.45	45.60
17.3	PII 13.12	17.71	57.26	6.69	0.39	38.63
17.4	PII 13.12	28.18	36.92	17.23	0.34	33.51
17.5	PII 13.12	22.86	44.06	6.32	0.54	53.47
17.6	PII 13.12	31.74	32.29	8.88	0.61	61.40
17.7	PII 13.12	24.26	42.33	13.21	0.43	42.95
17.8	PII 13.12	12.53	84.87	21.75	0.37	36.69
20.1	P II 4.8	10.44	96.85	10.20	0.27	26.61
20.2	P II 4.8	27.22	36.93	16.78	0.29	28.47
20.3	P II 4.8	23.39	47.78	11.95	0.40	40.33
20.4	P II 4.8	18.46	60.39	15.71	0.37	37.42

20.5	P II 4.8	24.46	40.95	16.87	0.32	32.42
20.6	P II 4.8	26.05	39.37	16.76	0.25	24.97
20.7	P II 4.8	29.96	34.60	15.18	0.43	42.86
22.1	P II 13.8	22.09	45.56	11.34	0.28	28.12
22.2	P II 13.8	18.06	58.34	21.12	0.30	29.64
22.3	P II 13.8	24.45	41.63	8.41	0.36	36.02
22.4	P II 13.8	23.02	45.45	14.34	0.51	50.75
22.5	P II 13.8	22.30	44.90	14.65	0.24	23.91
24.1	P II 12.9	18.52	54.63	13.93	0.40	39.72
24.2	P II 12.9	15.94	63.21	9.11	0.25	25.55
	CV (%)	15.508	19.12	25.085	21.247	21.155
	CD(0.05)	4.695	10.649	3.826	0.1	9.957

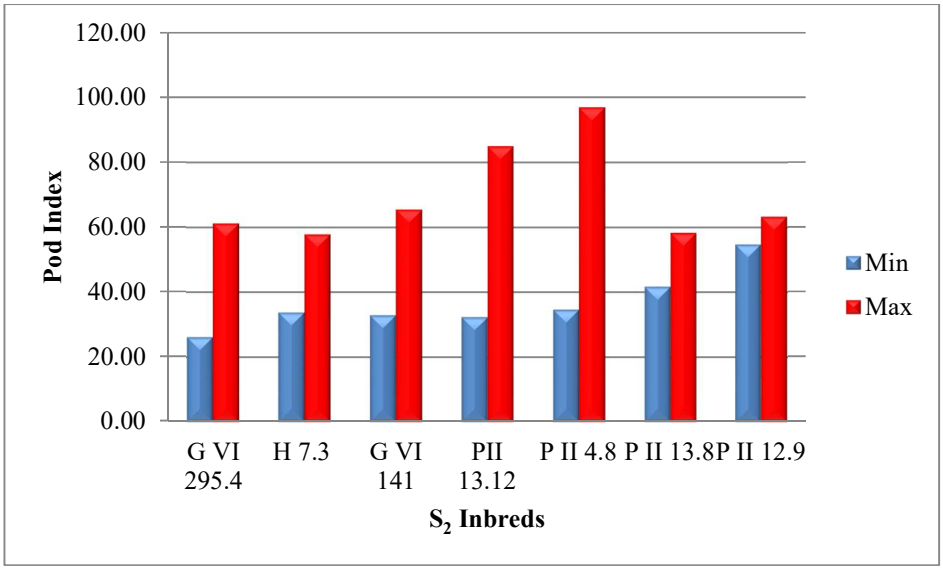


Fig.35 Pod Index of S_2 inbreds

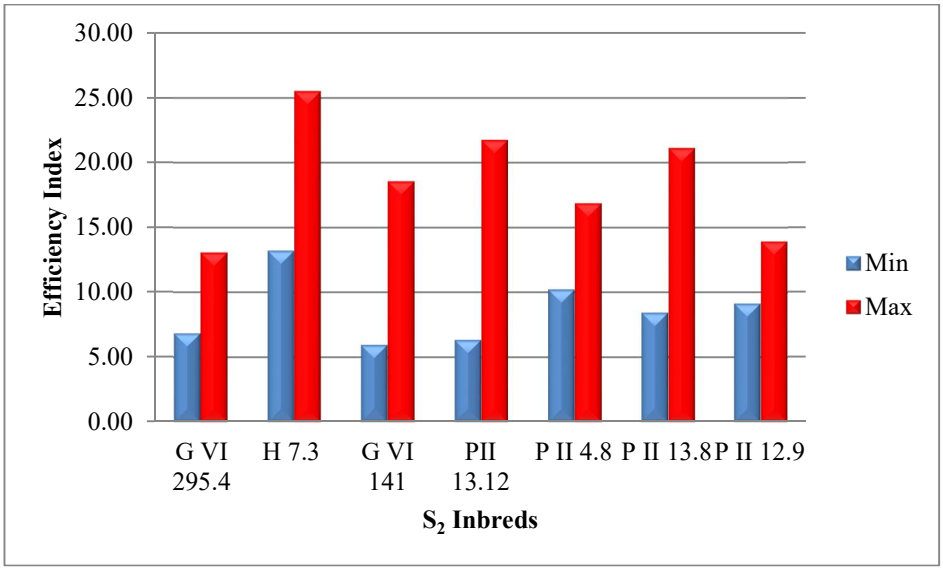


Fig. 36 Efficiency Index of S_2 inbreds

recorded in P II 4.8 (Plant number 20.1) and the maximum pod value in G VI 295.4 (Plant number 1.10).

The pod value in G VI 295.4 varied significantly and it ranged from 16.52 to 38.77. The maximum (38.77) and minimum (16.52) pod value was recorded in plant number 1.10 and 1.4, respectively.

The pod value in H 7.3 ranged from 18.17 in plant number 5.1 to 29.71 in plant number 5.2. The pod value was 19.18 and 21.36 in H1 1.2 and G VI 135. The pod value in G VI 141 ranged between 15.67 and 30.59 in plant number 15.2 and 15.4, respectively.

The pod value in P II 13.12 varied significantly, the maximum pod value (31.74) was observed in plant number 17.6, and minimum pod value (12.53) was observed in plant number 17.8. The pod value in P II 4.8 ranged from 10.44 in plant number 20.1 to 29.96 in plant number 20.7. The pod value in P II 13.8 varied significantly, and it ranged from 18.06 to 24.45 in plant number 22.3 and 22.2, respectively. The pod value in P II 12.9 varied significantly, and it ranged from 15.94 to 18.52 in plant number 24.2 and 24.1, respectively.

4.1.3.2.4.2 Pod index

The variation in pod index of S_2 inbreds is presented in fig.35. The pod index is the number of pods required to produce one kilogram of dried cocoa beans and the formula is 1000g/pod value (g). The pod index ranged from 26.16 to 96.85. The minimum pod index was observed in G VI 295.4 inbred (Plant number 1.10). The maximum pod index was observed in P II 4.8 (Plant number 20.1).

The pod index in G VI 295.4 varied significantly and it ranged from 26.16 to 61.21. The maximum (61.21) and minimum (26.16) pod index was recorded in plant number 1.10 and 1.4, respectively.

The pod index in H 7.3 ranged from 33.76 in plant number 5.2 to 57.83 in plant number 5.1. The pod index was 52.75 and 49.98 in H1 1.2 and G VI 135. The pod index in G VI 141 ranged between 32.89 and 65.48 in plant number 15.4 and 15.2, respectively. The pod index P II 13.12 varied significantly, the maximum pod index (84.87) was observed in plant number 17.8, and minimum pod index (32.29) was observed in plant number 17.6.

The pod index in P II 4.8 ranged from 34.60 in plant number 20.7 to 96.85 in plant number 20.1. The pod index in P II 13.8 varied significantly, and it ranged from 41.63 to 58.34 in plant number 22.3 and 22.2, respectively. The pod index in P II 12.9 varied significantly, and it ranged from 54.63 to 63.21 in plant number 24.2 and 24.1, respectively.

Asna *et al.,* (2014) estimated pod index for exotic and indigenous accessions. Results of her experiment revealed that pod index ranged from 12 to 49. So the inbred having low pod index can be selected for further breeding programme. Pound (1932) found that the hybrids exhibited pod index value less than or almost equal to 15.

4.1.3.2.4 .3 Efficiency Index

Minimum efficiency index is the criterion for selection of inbred. The variation in efficiency index of S_2 inbreds is presented in fig.36. Minimum efficiency index of 5.94 was observed in G VI 141 and maximum efficiency index of 25.49 was observed in H 7.3 (86) (Plant number 5.1).

The efficiency index in G VI 295.4 varied significantly and it ranged from 6.83 to 13.09. The maximum (13.09) and minimum (6.83) efficiency index was recorded in plant number 1.10 and 1.4, respectively.

The efficiency index in H 7.3 ranged from 12.67 in plant number 5.3 to 25.49 in plant number 5.1. The efficiency index was 16.42 and 8.77 in H1 1.2 and G VI 135. The

efficiency index in G VI 141 ranged between 5.94 and 18.57 in plant number 15.4 and 15.2, respectively.

The efficiency index in P II 13.12 varied significantly, the maximum efficiency index (21.75) was observed in plant number 17.8, and minimum efficiency index (6.69) was observed in plant number 17.3. The efficiency index in P II 4.8 ranged from 10.20 in plant number 20.1 to 16.87 in plant number 20.5.

The efficiency index in P II 13.8 varied significantly, and it ranged from 8.41 to 21.12 in plant number 22.3 and 22.2, respectively. The efficiency index in P II 12.9 varied significantly, and it ranged from 9.11 to 13.93 in plant number 24.2 and 24.1, respectively.

4.1.3.2.4.4 Conversion Index

Conversion index indicates the amount of dry bean weight obtained from given amount wet bean weight. The variation in conversion index of S_2 inbreds is presented in fig.37. Maximum conversion index (0.61) was in P II 13.12. Minimum conversion index was observed in H 7.3 (86). Efficiency index and conversion index were estimated by Minimol *et al.,* 2015. The values in the present study are coming under the range observed by them.

The conversion index in G VI 295.4 varied significantly and it ranged from 0.31 to 0.47. The maximum (0.47) and minimum (0.31) conversion index was recorded in plant number 1.7 and 1.6, respectively.

The conversion index in H 7.3 ranged from 0.15 in plant number 5.1 to 0.37 in plant number 5.2. The conversion index was 0.28 and 0.48 in H1 1.2 and G VI 135. The conversion index in G VI 141 ranged between 0.24 and 0.50 in plant number 15.2 and 15.9, respectively. The conversion index in P II 13.12 varied significantly, the maximum conversion index (0.61) was observed in plant number 17.6, and minimum conversion index (0.34) was observed in plant number 17.4.

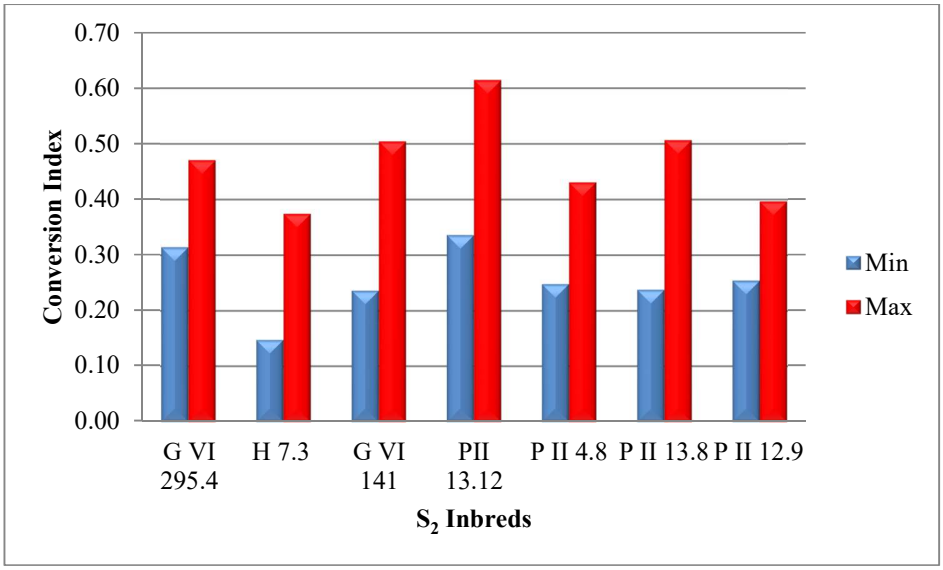


Fig.37 Conversion Index of S_2 inbreds

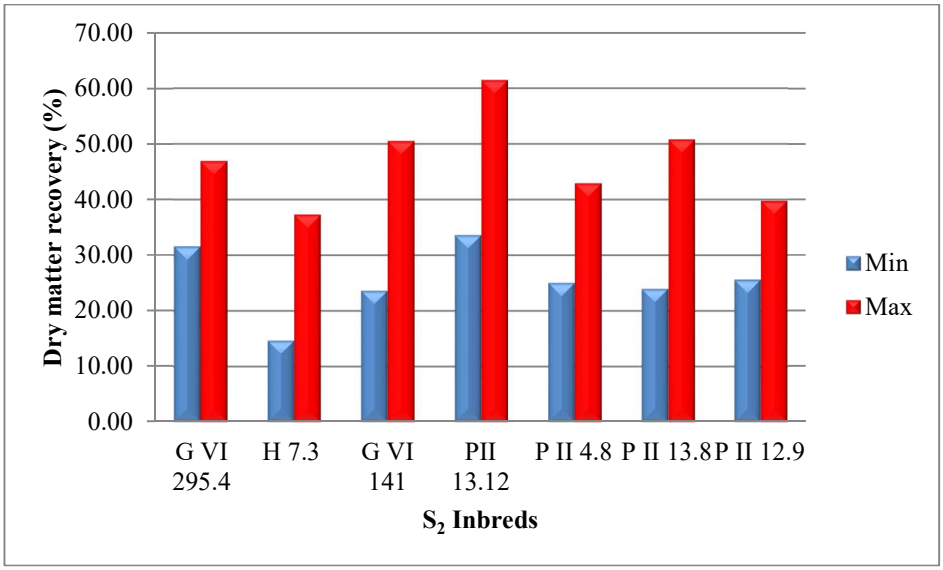


Fig.38 Dry matter recovery of S_2 inbreds

The conversion index in P II 4.8 ranged from 0.25 in plant number 20.6 to 0.43 in plant number 20.7. The conversion index in P II 13.8 varied significantly, and it ranged from 0.24 to 0.51 in plant number 22.5 and 22.4, respectively. The conversion index in P II 12.9 varied significantly, and it ranged from 0.25 to 0.40 in plant number 24.2 and 24.1, respectively.

4.1.3.2.4.6 Dry matter recovery (%)

The dry matter recovery ranged from 14.63 to 61.40 per cent among the inbreds. The maximum dry bean recovery of 61.40 per cent was observed in PII 13.12 followed by PII 13.12(Plant number 17.1). Minimum dry matter recovery was observed in H 7.3 (86)

The dry matter recovery in G VI 295.4 varied significantly and it ranged from 31.49 to 46.85. The maximum (46.85) and minimum (31.49) dry matter recovery was recorded in plant number 1.7 and 1.6, respectively. The dry matter recovery in H 7.3 ranged from 14.63 in plant number 5.1 to 37.26 in plant number 5.2.

The dry matter recovery was 28.05 and 48.37 in H1 1.2 and G VI 135. The dry matter recovery in G VI 141 ranged between 23.61 and 50.42 in plant number 15.2 and 15.9, respectively. The dry matter recovery in P II 13.12 varied significantly, the maximum dry matter recovery (61.40) was observed in plant number 17.6, and minimum dry matter recovery (33.51) was observed in plant number 17.4.

The dry matter recovery in P II 4.8 ranged from 24.97 in plant number 20.6 to 42.86 in plant number 20.7. The dry matter recovery in P II 13.8 varied significantly, and it ranged from 23.91 to 50.75 in plant number 22.5 and 22.4, respectively. The dry matter recovery in P II 12.9 varied significantly, and it ranged from 25.55 to 39.72 in plant number 24.2 and 24.1, respectively.

4.1.3.2.5 Biochemical characters of S_2 inbreds of cocoa

Cocoa beans are the major economic parts which contain the cocoa butter used for making chocolate. The quality of chocolate mainly depends upon the biochemical constituents present in the bean. Fat is responsible for softness, aroma and flavor and poly phenols for colour of chocolate. In the present study the fat and poly phenols estimated are summarized in table 12.

4.1.3.2.5.1 Fat content (%)

The maximum fat content of 63.10 per cent was observed in G VI 295.4 (Plant number 1.3) followed by G VI 295.4 (62.93 per cent) (Plant number 1.2). The minimum fat content of 32.97 per cent was estimated in PII 12.9 inbred (Plant number 24.1). Fat estimation in cocoa was also estimated previously by Ajmal (2016) in cocoa hybrids and Veeresh (2018) in 30 exotic germplasm. In the present study, 53 per cent of S_1 inbreds recorded more than 50 per cent fat. High fat content of cocoa beans is major attribute responsible for flavor and aroma of chocolate (Mossu, 1992). So the inbreds showing high fat content can be selected for further breeding programme. The variation in fat content of S_2 inbreds is presented in fig. 39.

The fat content in G VI 295.4 varied significantly and it ranged from 37.87 to 63.10 per cent. The maximum (63.10 per cent) and minimum (37.87 per cent) fat content was recorded in plant number 1.3 and 1.9, respectively.

The fat content did not vary significantly in H 7.3. The fat content was 43.17 and 51.43 per cent in H1 1.2 and G VI 135. The fat content in G VI 141 ranged between 47.17 and 54.53 per cent in plant number 15.8 and 15.9, respectively. The fat content in PII 13.12 varied significantly, the maximum fat content (61.80 %) was observed in plant number 17.3, and minimum fat content (38.23%) was observed in plant number 17.6.

The fat content in P II 4.8 ranged from 52.17 per cent in plant number 20.2 to 53.27 per cent in plant number 20.7. The fat content in P II 13.8 varied significantly,

Table 12. Biochemical characters of S_2 inbreds of cocoa

S. No.	Genotype	Fat content (%)	Phenol (%)
1.1	G VI 295.4	48.27	3.47
1.2	G VI 295.4	62.93	2.91
1.3	G VI 295.4	63.10	2.90
1.4	G VI 295.4	45.20	2.94
1.5	G VI 295.4	55.13	2.79
1.6	G VI 295.4	44.20	2.82
1.7	G VI 295.4	42.63	3.00
1.8	G VI 295.4	46.97	2.98
1.9	G VI 295.4	37.87	2.78
1.10	G VI 295.4	37.97	2.46
1.11	G VI 295.4	49.50	2.68
5.1	H 7.3	54.10	2.15
5.2	H 7.3	54.50	2.16
5.3	H 7.3	54.20	2.17
7.1	H1 1.2	43.17	2.27
13.1	G VI 135	51.43	2.49
15.1	G VI 141	52.33	3.61
15.2	G VI 141	52.33	3.45
15.3	G VI 141	52.63	3.47
15.4	G VI 141	52.53	3.55
15.5	G VI 141	52.80	3.43
15.6	G VI 141	49.70	3.74
15.7	G VI 141	59.70	3.73
15.8	G VI 141	47.17	2.91
15.9	G VI 141	54.53	3.03
17.1	PII 13.12	47.90	2.47
17.2	PII 13.12	54.70	2.49
17.3	PII 13.12	61.80	2.47
17.4	PII 13.12	56.77	2.49
17.5	PII 13.12	46.73	2.43
17.6	PII 13.12	38.23	2.48
17.7	PII 13.12	39.97	3.67
17.8	PII 13.12	39.90	3.68
20.1	P II 4.8	52.33	2.43
20.2	P II 4.8	52.17	2.41
20.3	P II 4.8	52.53	2.41
20.4	P II 4.8	52.60	2.16
20.5	P II 4.8	52.50	2.35

20.6	P II 4.8	53.17	2.41
20.7	P II 4.8	53.27	2.34
22.1	P II 13.8	34.00	2.51
22.2	P II 13.8	47.07	2.48
22.3	P II 13.8	46.73	2.50
22.4	P II 13.8	63.10	2.51
22.5	P II 13.8	58.47	2.58
24.1	P II 12.9	32.97	2.54
24.2	P II 12.9	61.73	2.54
	CV (%)	1.003	0.869
	CD(0.05)	0.827	0.039

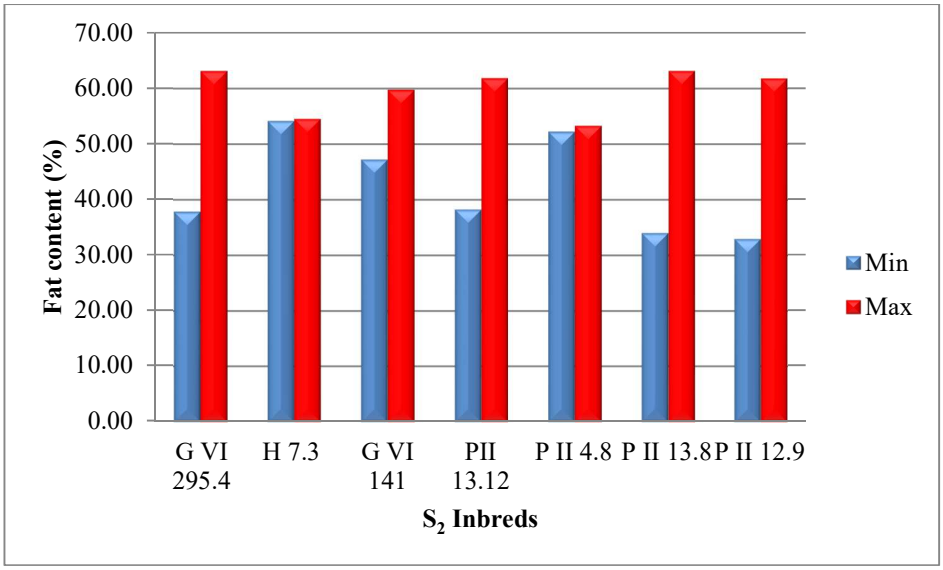


Fig. 39 Fat content of S_2 inbreds

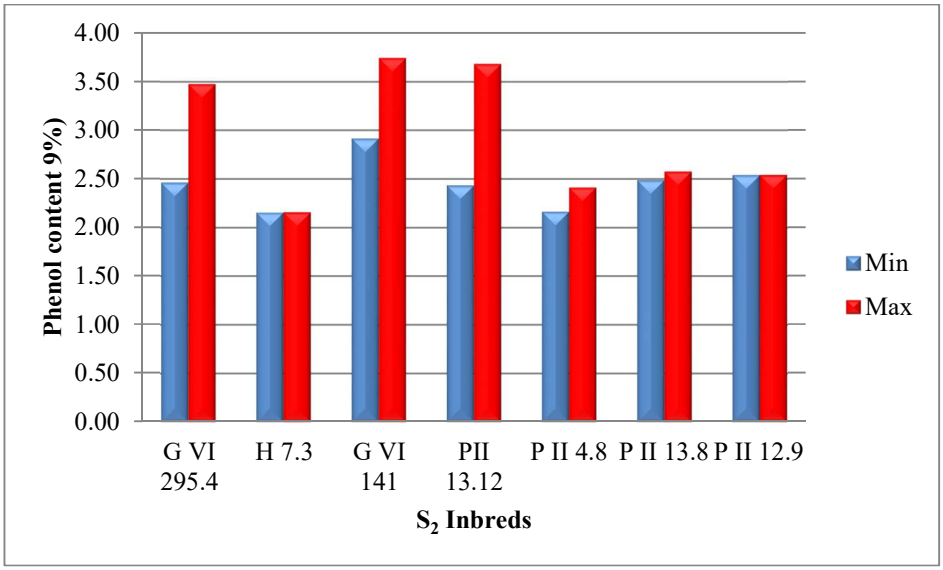


Fig. 40 Phenol content of S_2 inbreds

and it ranged from 34.00 to 63.10 per cent in plant number 22.1 and 22.4, respectively. The fat content in P II 12.9 varied significantly, and it ranged from 32.97 to 61.73 per cent in plant number 24.1 and 24.2, respectively.

.1.3.2.5.2 Polyphenol content (%)

Total poly phenol content in cocoa beans extracts of inbreds were determined by following Folin-Ciocalteau procedure. According to Kim and Keeny 1984, poly phenols comprise 12 -18 percent of the total bean weight is responsible for colour of the chocolate. In the present study poly phenols ranged between 2.15 to 3.74 percent among the inbreds (fig. 40). The maximum poly phenols (3.74 per cent) are observed in G VI 141(Plant number 15.6) followed by P II 13.12 (3.68 per cent) (Plant number 17.8). The least poly phenol content (2.15 per cent) estimated in H 7.3 (86) inbred (Plant number 5.2).

The phenol content in G VI 295.4 varied significantly and it ranged from 2.46 to 3.47 per cent. The maximum (3.47 per cent) and minimum (2.46 per cent) phenol content was recorded in plant number 1.1 and 1.10, respectively. The phenol content in H 7.3 ranged from 2.15 per cent to 2.17 per cent in plant number 5.1 and 5.3. The phenol content was 2.27 and 2.49 per cent in H1 1.2 and G VI 135.

The phenol content in G VI 141 ranged between 2.91 and 3.74 per cent in plant number 15.8 and 15.6, respectively. The phenol content in P II 13.12 varied significantly, the maximum phenol content (3.68 per cent) was observed in plant number 17.8 and minimum phenol content (2.49 per cent) was observed in plant number 17.4.

The phenol content in P II 4.8 ranged from 2.34 per cent in plant number 20.7 to 2.43 per cent in plant number 20.1.

The phenol content in P II 13.8 varied significantly, and it ranged from 2.48 to 2.58 in plant number 22.2 and 22.5, respectively. The phenol content in P II 12.9 did not vary significantly.

4.1.3.3 Evaluation of S_3 inbreds of cocoa

4.1.3.3.1 Growth observation of S_3 inbreds of cocoa

The details of growth observation of S_3 inbreds of cocoa are presented in table 12.

4.1.3.3.1.1 Plant height (cm)

The maximum plant height of 550 cm was observed in M 18.7 (Plant number 2.4) and the minimum plant height of 180 cm was observed in M 18.7 (Plant number 2.1) during 2016.

The maximum plant height of 580 cm was observed in M 18.7 (Plant number 2.4) and the minimum plant height of 195 cm was observed in M 18.7 (Plant number 2.1) during 2017.

The maximum plant height of 620 cm was observed in M 18.7 (Plant number 2.4) and the minimum plant height of 225 cm was observed in M 18.7 (Plant number 2.5) during 2018.

4.1.3.3.1.2 Collar girth (cm)

The maximum collar girth of 54 cm was observed in M 18.7 (Plant number 2.5) and the minimum collar girth of 31 cm was observed in M 18.7 (Plant number 2.4) during 2016.

The maximum collar girth of 55 cm was observed in M 18.7 (Plant number 2.5) and the minimum collar girth of 32 cm was observed in M 18.7 (Plant number 2.4) during 2017.

The maximum collar girth of 60 cm was observed in M 18.7 (Plant number 2.5) and the minimum collar girth of 34 cm was observed in M 18.7 (Plant number 2.4) during 2018.

Table 13. Plant height and collar girth of S_3 inbreds of cocoa

Plant No.	Genotype	Plant height (cm)			Girth (cm)		
		2016	2017	2018	2016	2017	2018
2.1	M 18.7	180	195	230	53	54	57
2.2	M 18.7	290	305	345	49	52	58
2.3	M 18.7	290	305	320	45	46	47
2.4	M 18.7	550	580	620	31	32	34
2.5	M 18.7	185	205	225	54	55	60
8.1	GII 7.4	420	450	470	49	51	58

Table 14. Pod characters of S_3 inbreds of cocoa

Plant No.	Genotype	Pod weight (g)	Pod length (cm)	Pod breadth (cm)	Ridge thickness (cm)	Furrow thickness (cm)	No of beans/pod	Flat bean
2.1	M 18.7	336.00	14.10	7.90	1.82	1.46	38.40	0.80
2.2	M 18.7	320.00	12.36	8.34	1.50	0.72	37.20	0.40
2.3	M 18.7	232.00	10.70	7.44	1.80	1.22	26.40	0.40
2.4	M 18.7	339.60	11.10	7.90	1.70	1.12	37.00	0.40
2.5	M 18.7	258.00	13.26	7.38	1.22	0.98	37.20	0.40
8.1	GII 7.4	278.00	13.50	7.92	1.06	0.80	38.40	2.20
CV (%)		23.08	8.05	8.601	7.469	14.339	15.026	131.517
CD (0.05)			1.315		0.148	0.197	7.016	

4.1.3.3.1 Pod characters of S_3 inbreds of cocoa

4.1.3.3.1 Pod weight (g)

The details of pod characters of S_3 inbred of cocoa are presented in table 14. No significant difference was observed among the inbreds for this trait

4.1.3.3.1.2 Pod length (cm)

The pod length ranged between 10.70 and 14.10 cm. The maximum pod length of 14.10 cm was observed in M 18.7 (Plant number 2.1) inbred and the minimum pod length (10.70cm) was recorded in M 18.7 (Plant number 2.3) inbred. Pod length of 13.50 cm was observed in GII 7.4. Similar finding are reported in cocoa inbred (CCRP Annual Report, 2013).

4.1.3.3.1.3 Pod breadth (cm)

No significant difference was observed among the inbreds for this trait

4.1.3.3.1.4 Ridge thickness (cm)

The ridge thickness in M 18.7 ranged between 1.22 and 1.82 cm. The maximum (1.82cm) and minimum (1.22 cm) were observed in plant number 2.1 and 2.5 respectively. In G II 7.4 inbreds, the ridge thickness was 1.06 cm. The variation in the ridge thickness among the same generation inbreds was also reported (CCRP Annual Report, 2013).

4.1.3.3.1 Furrow thickness (cm)

The furrow thickness in M 18.7, ranged between 0.72 and 1.46 cm. The maximum (1.46 cm) and minimum (0.72 cm) was observed in plant number 2.1 and 2.2 respectively. Furrow thickness of inbred G II 7.4 was 0.80cm. The variation in the furrow thickness among the same generation inbreds was also reported (CCRP, Annual Report, 2013).

4.1.3.3.1.6 Number of beans per pod

No significant difference was observed for this trait among the inbreds

4.1.3.3.1.7 Number of flat beans per pod

No significant difference was observed for this trait among the inbreds

4.1.3.3.2 Bean characters of S_3 inbreds of cocoa

4.1.3.3.2.1 Wet bean weight per pod (g)

The details of bean characters of S_3 inbred are presented in table 15. The wet bean weight per pod ranged between 66.3g in plant number 2.5 and 103.20 g plant number 2.1. The wet bean weight per pod in G II 7.4 was 83.52 g.

4.1.3.3.2.2 Single Dry Bean Weight (g)

The single dry bean weight ranged between 0.79 and 0.96g in M 18.7 in plant number 2.5 and 2.2 respectively.

The bean characters such as dry bean length, breadth and thickness in M 18.7 ranged from 17.48 to 19.91mm, 11.46 to 13.73mm, and 6.66 to 7.90mm respectively. The bean length, breadth and thickness in G II 7.4 were 18.23cm, 13.73 cm and 6.60 mm respectively.

4.1.3.3.3 Economic characters of S_3 inbreds of cocoa

The economic and biochemical characters of S_3 inbreds of cocoa are presented in table 16.

4.1.3.3.3.1 Pod value

The pod value ranged from 20.86 to 35.77 among the S_3 generation M 18.7 inbreds. The lowest pod value of 20.86 was recorded in plant number 2.3 and the maximum pod value was recorded plant number 2.2

Table 15. Bean characters of S_3 inbreds of cocoa

Plant No.	Genotype	Wet bean weight /pod (g)	Single Bean weight (g)	Bean length (mm)	Bean breadth (mm)	Bean thickness (mm)
2.1	M 18.7	103.20	0.89	19.73	11.56	6.66
2.2	M 18.7	69.06	0.96	19.91	13.49	6.73
2.3	M 18.7	86.60	0.79	17.48	11.46	7.90
2.4	M 18.7	99.00	0.95	19.86	13.65	7.38
2.5	M 18.7	66.30	0.79	17.53	11.55	6.73
8.1	GII 7.4	83.52	0.91	18.23	13.73	6.60
	CV (%)	13.211	8.358	2.05	2.492	4.636
	CD (0.05)	14.592	0.096	0.503	0.409	0.424

Table 16. Economic and biochemical characters of S_3 inbreds of cocoa

Plant No	Genotype	Pod value	Pod Index	Efficiency Index	Conversion Index	Dry bean weight/pod (g)	Dry matter recovery (%)	Fat content (%)	Phenol (%)
2.1	M 18.7	34.23	29.72	9.98	0.33	34.23	33.23	52.13	2.01
2.2	M 18.7	35.77	28.45	9.12	0.53	35.77	53.01	56.10	2.05
2.3	M 18.7	20.86	49.51	11.39	0.24	20.86	24.21	57.93	2.12
2.4	M 18.7	35.10	28.77	9.80	0.36	35.10	35.57	42.33	2.12
2.5	M 18.7	29.43	34.34	8.77	0.44	29.43	44.42	47.43	3.42
8.1	GII 7.4	34.90	30.61	7.67	0.42	34.90	42.26	63.77	2.38
CV (%)		17.98	19.10	18.55	17.603	17.981	17.531	1.089	0.019
CD (0.05)		7.444	8.371	7.21	0.089	7.444	8.876	1.136	0.449

4.1.3.3.3.2 Pod index

The pod index is the number of pods required to produce one kilogram of dried cocoa beans and the formula is 1000g/pod value (g). The pod index ranged from 28.45 to 49.51. The minimum pod index was observed in M 18.7 (Plant number 2.2) inbred. The maximum pod index was observed in M 18.7(Plant number 2.3). Asna *et al.* (2014) estimated pod index for exotic and indigenous accessions. Results of her experiment revealed that pod index ranged from 12 to 49. So the inbred having low pod index can be selected for further breeding programme. Pound (1932) found that the hybrids exhibited pod index value less than or almost equal to 15.

4.1.3.3.3.3 Efficiency Index

No significant difference was observed efficiency index among the S_3 inbreds in the study.

4.1.3.3.3.4 Conversion Index

Conversion index indicates the amount of dry bean weight obtained from given amount wet bean weight. Conversion index was maximum (0.53) in M 18.7(Plant number 2.2) and minimum (0.24) conversion index was observed in M 18.7(Plant number 2.3). Efficiency index and conversion index were also estimated by Minimol *et al.,* 2015. The values in the present study are coming under the range observed by them.

4.1.3.3.3.5 Dry bean weight per pod (DBWP) (g)

Dry bean per pod is the quantity of bean obtained from each pod. The dry bean weight per pod ranged from 20.86 to 35.77 g. The maximum dry bean weight per pod is useful criterion for selection of inbred. The maximum DBWP was observed in M 18.7(Plant number 2.2) indicating its suitability for selection in breeding programme. The least value was observed in M 18.7(Plant number 2.3)

4.1.3.3.3.6 Dry matter recovery (%)

The dry matter recovery ranged from 24.21 to 53.01 per cent among the inbreds. The maximum dry bean recovery of 53.01 per cent was observed in M 18.7 (Plant number 2.2). Minimum dry matter recovery (24.21 per cent) was observed in M 18.7(Plant number 2.3)

4.1.3.3.4 Biochemical characters of S_3 inbreds of cocoa

Cocoa beans are the major economic parts which contain the cocoa butter used for making chocolate. The quality of chocolate mainly depends upon the biochemical constituents present in the bean. Fat is responsible for softness, aroma and flavor and poly phenols for colour of chocolate. In the present study the fat and poly phenols estimated are summarized in table 16.

4.1.3.3.4.1 Fat content (%)

The maximum fat content of 63.77 per cent was observed in G II 7.4 (Plant number 8.1). The minimum fat content of 42.33 was estimated in M 18.7 inbred (Plant number 2.4). Fat estimation in cocoa was also estimated previously by Ajmal (2016) in cocoa hybrids and Veeresh (2018) in 30 exotic germplasm. In the present study, 53 per cent of S_1 inbreds recorded more than 50 per cent fat. High fat content of cocoa beans is major attribute responsible for flavor and aroma of chocolate (Mossu, 1992). So the inbreds showing high fat content can be selected for further breeding programme.

4.1.3.3.4.2 Poly phenol content (%)

Total poly phenol content in cocoa beans extracts of inbreds were determined by following Folin-Ciocalteau procedure. According to Kim and Keeny (1984) poly phenols comprise 12 -18 percent of the total bean weight is responsible for colour of the chocolate. In the present study poly phenols ranged between 2.01 to 3.42 percent among the inbreds. The maximum poly phenols (3.42 per cent) are observed in M 18.7. The least poly phenol content estimated in M 18.7 inbred.

4.1.3.4 Evaluation of S_4 inbreds

4.1.3.4.1 Growth observation

The details on growth observation of S_4 inbreds are presented in table 17.

4.1.3.4.1.1Plant height (cm)

The maximum plant height in S_4 inbreds of cocoa varied from 120 cm in M 18.7 (Plant number 3.1) to 365 cm in M 18.7 (Plant number 3.4) during 2016. The same trend in plant height was observed during 2017 and 2018.

4.1.3.4.1.1 Collar girth (cm)

The maximum and minimum plant girth of 49, 51, 52 and 29, 30 31 cm was observed in M 18.7 (Plant number 3.3) and G IV 35.7 (Plant number 11.1) respectively.

4.1.3.4.1 Pod characters of S_4 inbreds of cocoa

4.1.3.4.1.1 Pod weight (g)

The details of pod characters of S_4 inbred of cocoa are presented in table 18. The pod weight varied between 146 g and 364 g in M 18.7 inbred. The pod weight of 266 and 520 g was observed in genotype G II 7.4 and G IV 35.7 inbreds. The mean pod weight recorded among the S_4 inbreds was 291.33 g (Table 16). Minimol *et al.,* (2015) revealed the breeding cycle of fifth generation inbred in cocoa, the pod weight range between 234 and 308g among the S_0 to S_5 generations of GII 7.4 inbred. In the present study the maximum pod weight observed was 464 g. The range and average pod weight of S_2 generation inbreds is lesser than S_1 generation inbreds in the present study.

4.1.3.4.1.2 Pod length (cm)

The pod length varied between 12 and 15.80 cm in M 18.7 inbred. The pod length of 13 cm and 18.4 cm was observed in genotype G II 7.4 and G IV 35.7 inbreds. Similar finding are reported in cocoa inbred (CCRP Annual Report, 2013).

Table 17. Plant height and collar girth of S_4 inbreds of cocoa

Plant No.	Genotype	Plant height (cm)			Girth (cm)		
		2016	2017	2018	2016	2017	2018
3.1	M 18.7	120	135	140	43	45	46
3.2	M 18.7	190	195	200	31	32	33
3.3	M 18.7	150	155	160	49	51	52
3.4	M 18.7	365	400	435	37	42	47
9.1	G II 7.4	145	240	265	46	49	52
11.1	G IV 35.7	130	140	145	29	30	31

Table 18. Pod characters of S_4 inbreds of cocoa

Plant No.	Genotype	Pod weight (g)	Pod length (cm)	Pod breadth (cm)	Ridge thickness (cm)	Furrow thickness (cm)	No of beans/pod	Flat bean
3.1	M 18.7	146	12.0	5.96	1.36	1.20	32.2	0.4
3.2	M 18.7	250	12.6	7.26	1.24	0.62	40.8	0.4
3.3	M 18.7	202	12.0	6.32	1.08	0.94	37.0	0.2
3.4	M 18.7	364	15.8	7.40	1.10	0.80	38.2	1.2
9.1	GII 7.4	266	13.0	7.10	2.04	1.72	31.2	1.8
11.1	G IV 35.7	520	18.4	8.80	1.72	1.02	55.0	1.4
	CV (%)	13.985	6.662	7.856	8.011	19.595	7.411	78.567
	CD(0.05)	53.186	1.215	0.732	0.149	0.269	3.78	0.923

4.1.3.4.1.3 Pod breadth (cm)

The pod breadth varied between 5.96 cm and 7.4 cm in M 18.7 inbred. The pod breadth of 7.1 and 8.8 mm was observed in genotype G II 7.4 and G IV 35.7 inbreds.

4.1.3.4.1.4 Ridge thickness (cm)

The ridge thickness varied between 1.08 cm and 1.36 cm in M 18.7 inbred. The ridge thickness of 2.04 and 1.72 cm was observed in genotype G II 7.4 and G IV 35.7 inbreds. The variation in the ridge thickness among the same generation inbreds was also reported (CCRP Annual Report, 2013).

4.1.3.4.1.5 Furrow thickness (cm)

The furrow varied between 0.62 cm and 1.20 cm in M 18.7 inbred. The furrow thickness of 1.72 and 1.02 cm was observed in genotype G II 7.4 and G IV 35.7 inbreds. The variation in the furrow thickness among the same generation inbreds was also reported (CCRP Annual Report, 2013).

4.1.3.4.1.6 Number of beans per pod

The number of beans per pod varied between 32.2 and 40.8 in M 18.7 inbred. The number of beans per pod of 31.2 and 55 was observed in genotype G II 7.4 and G IV 35.7 inbreds.

4.1.3.4.1.7 Number of flat beans per pod

The number of flat bean per pod varied between 0.2 and 1.2 in M 18.7 inbred. The number of flat bean per pod of 1.8 and 1.4 was observed in genotype G II 7.4 and G IV 35.7 inbreds.

4.1.3.4.2 Bean characters of S_4 inbreds of cocoa

The details of bean characters of S_4 inbreds of cocoa are presented in table 19

Table 19. Bean characters of S_4 inbreds of cocoa

Plant No.	Genotype	Wet bean weight /pod (g)	Dry bean weight/pod (g)	Single Bean weight (g)	Bean length (mm)	Bean breadth (mm)	Bean thickness (mm)
3.1	M 18.7	35.40	21.72	0.67	18.41	11.44	8.69
3.2	M 18.7	48.40	24.86	0.60	15.21	10.73	5.24
3.3	M 18.7	65.00	28.40	0.76	17.65	10.57	7.31
3.4	M 18.7	94.94	34.31	0.89	16.58	9.85	6.74
9.1	GII 7.4	92.00	22.71	0.73	18.22	11.89	6.47
11.1	G IV 35.7	130.96	52.06	0.95	19.27	12.45	5.26
	CV (%)	10.757	11.283	5.911	1.465	2.296	4.207
	CD (5%)	10.923	4.518	0.059	0.336	0.334	0.364

Table 20. Economic and biochemical characters of S_4 inbreds of cocoa

Plant No.	Genotype	Pod value	Pod Index	Efficiency Index	Conversion Index	Dry matter recovery (%)	Fat content (%)	Phenol (%)
3.1	M 18.7	21.72	47.39	7.18	0.62	61.56	58.17	2.07
3.2	M 18.7	24.86	40.52	10.04	0.52	51.79	57.37	2.05
3.3	M 18.7	28.40	35.30	7.13	0.44	43.82	57.13	2.05
3.4	M 18.7	34.31	29.68	10.74	0.37	36.48	57.30	2.06
9.1	GII 7.4	22.71	44.09	11.72	0.25	24.72	53.07	2.03
11.1	G IV 35.7	52.06	19.33	10.06	0.40	40.03	53.23	2.57
	CV (%)	11.283	12.729	21.39	13.865	13.739	0.318	0.54
	CD (5%)	4.518	5.99	2.647	0.078	7.724	0.329	0.021

4.1.3.4.2.1 Wet bean weight per pod (g)

The wet bean weight ranged from 35.4 to 94.94 g among the S_4 generation M 18.7 inbred. The single dry bean weight of 92.00 and 130.96g was observed in inbred G II 7.4 and G IV 35.7 respectively.

4.1.3.4.2.2 Dry bean weight per pod (g)

The wet bean weight per pod varied from 21.72 g in M 18.7 (Plant number 3.1) to 52.06 g in G IV 35.7 (Plant number 11.1)

4.1.3.4.2.3 Single Dry Bean Weight (g)

The single dry bean weight ranged from 0.60 to 0.95g among the S_4 generation inbred. The maximum single dry bean weight of was observed in G IV 35.7 and the least was observed in M 18.7 (Plant number 3.2).

The dry bean length ranged from 15.21 to 18.41mm among the S_4 generation M 18.7 inbred. The single dry bean length of 18.22 and 19.27 mm was observed in inbred G II 7.4 and G IV 35.7 respectively.

The dry bean breadth ranged from 9.85 to 11.44mm among the S_4 generation M 18.7 inbred. The dry bean breadth of 11.89 and 12.45 mm was observed in inbred G II 7.4 and G IV 35.7 respectively.

The dry bean thickness ranged from 5.24 to 8.69 mm among the S_4 generation M 18.7 inbred. The dry bean thickness of 6.47 and 5.26 mm was observed in inbred G II 7.4 and G IV 35.7 respectively.

4.1.3.4.3 Economic characters of S_4 inbreds of cocoa

The details of economic characters of S_3 inbreds of cocoa are presented in table 20.

4.1.3.4.3 Pod value

The pod value ranged from 21.72 to 34.31 among the S_4 generation M 18.7 inbred. The pod value of 22.71 and 52.06 was observed in inbred G II 7.4 and G IV 35.7 respectively.

4.1.3.4.3.2 Pod index

The pod index is the number of pods required to produce one kilogram of dried cocoa beans and the formula is 1000g/pod value (g). The pod index ranged from 35.30 to 47.39 in M 18.7 genotype. The pod index of 44.09 and 19.33 was observed in inbred G II 7.4 and G IV 35.7 respectively. Asna *et al.* (2014) estimated pod index for exotic and indigenous accessions. Results of her experiment revealed that pod index ranged from 12 to 49. So the inbred having low pod index can be selected for further breeding programme. Pound (1932) found that the hybrids exhibited pod index value less than or almost equal to 15.

4.1.3.4.3.3 Efficiency Index

The efficiency index ranged from 7.13 to 10.74 in M 18.7 inbred. An efficiency index of 11.72 and 10.06 was observed in inbred G II 7.4 and G IV 35.7 respectively.

4.1.3.4.3.4 Conversion Index

Conversion index indicates the amount of dry bean weight obtained from given amount wet bean weight. Conversion index ranged from 0.37 to 0.62 in M 18.7. The conversion index of 0.25 and 0.40 was observed in G II 7.4 and G IV 35.7.

Efficiency index and conversion index were also estimated by Minimol *et a*l. (2015). The values in the present study are coming under the range observed by them.

4.1.3.4.3.5 Dry bean weight per pod (DBWP) (g)

Dry bean per pod is the quantity of bean obtained from each pod. The dry bean weight per pod among the plants of S_4 generation of M 18.7 ranged from 21.72 to

34.31g. The dry bean weight per pod in G II 7.4 and G IV 35.7 observed was 22.71 and 52.06 g. respectively.

4.1.3.4.3.6 Dry matter recovery (Percentage)

The dry matter recovery ranged from 36.48 to 61.54 per cent among the S_4 generation M 18.7 inbreds. The dry matter recovery of 24.72 and 40.03 per was observed in G II 7.4 and G IV 35.7.

4.1.3.4.4 Biochemical characters of S_4 inbreds of cocoa

The quality of chocolate mainly depends upon the butter content in the cocoa beans. The aroma and flavor of cocoa is mainly depend on the fat and poly phenol content in the beans. In the present study the fat and poly phenols estimated by following standard procedure are summarized in table 20.

4.1.3.4.4.1 Fat content (%)

The fat content in S_4 generation of inbreds of M 18.7 ranged from 57.13 to 58.17 per cent. Fat content of 53.07 and 53.23 per cent was observed in S_4 generation of G II 7.4 and G IV 35.7 respectively. Fat estimation in cocoa was also estimated previously by Ajmal (2016) in cocoa hybrids and Veeresh (2018) in 30 exotic germplasm. In the present study, 53 per cent of S_4 inbreds recorded more than 50 per cent fat. High fat content of cocoa beans is major attribute responsible for flavor and aroma of chocolate (Mossu, 1992). So the inbreds showing high fat content can be selected for further breeding programme.

4.1.3.4.4.2 Poly phenol content (%)

Poly phenols comprise 12 -18 percent of the total bean weight is responsible for colour of the chocolate. In the present study, poly phenols did not vary significantly among the inbreds of M 18.7. The poly phenols content in G II 7.4 and G IV 35.7 observed was 2.03 and 2.57 percent.

4.1.3.5 Evaluation of S_5 inbreds of cocoa

A plant height of 230, 250 and 295 cm and collar girth of 37, 42 and 48 cm was observed in S_5 inbred of G II 7.4 during 2016, 2017 and 2018 respectively (table 21)

4.1.3.5.1 Pod and bean characters of S_5 inbreds of cocoa

The pod, bean, economic and biochemical characters of S_5 inbred G II 7.2 are presented in table 22, 23 and 24 respectively.

In S_5 generation of G II 7.4, the pod weight, pod length, pod breadth, ridge thickness, furrow thickness, number of beans per pod and flat bean per pod observed were 202g, 11.68cm, 6.32cm, 1.04cm, 0.62cm, 34.80 and 2.0 respectively.

Wet bean weight per pod, dry bean weight per pod, single dry bean weight, bean length, bean breadth, and bean thickness observed are 96.87g, 25.89 g, 0.75 g, 19.62 mm, 12.76 mm, and 5.57 mm respectively in the S_5 generation of G II 7.4

The pod value, pod index, efficiency index, conversion index, dry bean weight per pod, dry matter recovery, fat content, and phenol content observed were 25.89, 39.53, 7.56, 0.27, 25.89g., 26.67, 58.8 per cent and 2.85 per cent in S_5 generation of G II 7.4

4.1.4 Incidence of major pests and diseases

The details of incidence of pests and diseases in inbreds are presented in table 25. Mealy bug is the major pest and black pod was the major disease affected. Besides, rodents like rats and squirrels also caused the damage to the pods. The percentage of infestation by each pest, disease and rodents are presented in Table 2.

Among the inbreds evaluated, the mealy bug infestation was the major, High infestation of 33.5 per cent was observed in H 7.3 (Plant number 4.6) followed by 25 per cent infestation in G VI 141(Plant number 14.2). The least pest infestation of 2.5 per cent was observed in G VI 256.5 (Plant number 18.4). Tea mosquito bug infestation was

Table 21. Plant height and collar girth of S_5 inbreds of cocoa

Plant No	Genotype	Plant height (cm)			Girth (cm)		
		2016	2017	2018	2016	2017	2018
10.1	GII 7.4	230	250	295	37	42	48

Table 22. Pod characters of S_5 inbreds of cocoa

Plant No.	Genotype	Pod weight (g)	Pod length (cm)	Pod breadth (cm)	Ridge thickness (cm)	Furrow thickness (cm)	No of beans/pod	Flat bean
10.1	GII 7.4	202.00	11.68	6.32	1.04	0.62	34.80	2.00

Table 23. Bean characters of S_5 inbreds of cocoa

Plant No.	Genotype	Pod weight (g)	Wet bean weight /pod (g)	Dry bean weight/pod (g)	Single Bean weight (g)	Bean length (mm)	Bean breadth (mm)	Bean thickness (mm)
10.1	GII 7.4	202.00	96.87	25.89	0.75	19.62	12.76	5.57

Table 24. Economic and biochemical characters of S_5 inbreds of cocoa

Plant No.	Genotype	Pod value	Pod Index	Efficiency Index	Conversion Index	Dry matter recovery (%)	Fat (%)	Phenol (%)
10.1	GII 7.4	25.89	39.53	7.56	0.27	26.67	58.8	2.85

Table 25. Percentage of infestation and infection of pests and diseases in cocoa inbreds

Plant No.	Genotype	Generation	Mealy bug (%)	Tea mosquito bug (%)	Black pod (%)	Rats (%)	Squirrels (%)
1.1	G VI 295.4	S_2	-	25	-	-	-
1.2	G VI 295.4	S_2	-	-	-	-	-
1.3	G VI 295.4	S_2	-	-	68	-	-
1.4	G VI 295.4	S_2	-	-	-	-	15
1.5	G VI 295.4	S_2	-	-	-	-	-
1.6	G VI 295.4	S_2	3	10	-	5	-
1.7	G VI 295.4	S_2	-	-	-	-	-
1.8	G VI 295.4	S_2	-	-	25	-	-
1.9	G VI 295.4	S_2	-	-	-	-	-
1.1	G VI 295.4	S_2	-	7.5	-	-	10
1.11	G VI 295.4	S_2	-	-	-	-	-
2.1	M 18.7	S_3	-	-	-	-	-
2.2	M 18.7	S_3	-	-	-	-	-
2.3	M 18.7	S_3	5	-		-	20
2.4	M 18.7	S_3	-	-	15	-	-
2.5	M 18.7	S_3	-	2.5	-	-	-
3.1	M 18.7	S_4	-	-	-	-	-
3.2	M 18.7	S_4	-	-	-	-	15
3.3	M 18.7	S_4	-	-	-	-	-
3.4	M 18.7	S_4	-	-	12.5	-	-
4.1	H 7.3 (86)	S_1	-	5	-	10	-
4.2	H 7.3 (86)	S_1	-	-	-	-	10
4.3	H 7.3 (86)	S_1	-	-	15	-	-
4.4	H 7.3 (86)	S_1	-	-	-	-	-
4.5	H 7.3 (86)	S_1	-	-	-	-	-
4.6	H 7.3 (86)	S_1	33.5	-	-	-	25
4.7	H 7.3 (86)	S_1	-	7.5	22.5	-	-
4.8	H 7.3 (86)	S_1	-	-	-	-	-
4.9	H 7.3 (86)	S_1	-	-	-	-	-
4.1	H 7.3 (86)	S_1	25	-	-	-	-
4.11	H 7.3 (86)	S_1	-	-	-	-	-
4.12	H 7.3 (86)	S_1	-	-	-	-	-
4.13	H 7.3 (86)	S_1	-	-	42.5	-	22
4.14	H 7.3 (86)	S_1	-	-		-	-
4.15	H 7.3 (86)	S_1	-	-		-	-

4.16	H 7.3 (86)	S_1	-	-		-	-
4.17	H 7.3 (86)	S_1	-	-	35	-	-
4.18	H 7.3 (86)	S_1	-	-	-	-	-
5.1	H 7.3 (86)	S_2	-	-	-	-	15
5.2	H 7.3 (86)	S_2	-	-	-	-	-
5.3	H 7.3	S_2	-	57.5	25	12.5	-
6.1	H1 1.2	S_1	-	-	-	-	-
6.2	H1 1.2	S_1	-	-	-	-	-
6.3	H1 1.2	S_1	3	-	30	-	-
6.4	H1 1.2	S_1	-	-	-	-	10
7.1	H1 1.2	S_2	-	-	-	-	-
8.1	GII 7.4	S_3	-	-	-	-	-
9.1	GII 7.4	S_4	-	-	-	-	-
10.1	GII 7.4	S_5	-	-	-	-	-
11.1	G IV 35.7	S_4	-	2.5	-	-	-
12.1	G VI 135	S_1	-	-	-	-	-
12.2	G VI 135	S_1	-	-	55	-	-
12.3	G VI 135	S_1	-	-	-	-	22.5
12.4	G VI 135	S_1	-	-	-	10	-
12.5	G VI 135	S_1	-	-	-	-	-
12.6	G VI 135	S_1	-	7.5	-	-	-
12.7	G VI 135	S_1	-	-	50	-	20
12.8	G VI 135	S_1	-	-	-	-	-
13.1	G VI 135	S_2	-	-	-	-	-
14.1	G VI 141	S_1	-	-	-	-	-
14.2	G VI 141	S_1	25	-	10	-	-
15.1	G VI 141	S_2	-	10	-	-	15
15.2	G VI 141	S_2	-	-	-	-	-
15.3	G VI 141	S_2	20	-	-	-	-
15.4	G VI 141	S_2	-	-	-	15	10
15.5	G VI 141	S_2	-	-	-	-	-
15.6	G VI 141	S_2	-	-	25	-	-
15.7	G VI 141	S_2	-	15	-	-	-
15.8	G VI 141	S_2	20	-	-	-	-
15.9	G VI 141	S_2	-	-	-	-	-
16.1	P II 13.12	S_1	-	-	-	-	-
16.2	P II 13.12	S_1	-	-	-	-	-
16.3	P II 13.12	S_1	-	-	-	-	-
17.1	PII 13.12	S_2	-	-		-	

17.2	PII 13.12	S_2	-	12.5	-	-	15
17.3	PII 13.12	S_2	25	-	-	-	-
17.4	PII 13.12	S_2		-	-	-	-
17.5	PII 13.12	S_2		-	-	-	-
17.6	PII 13.12	S_2	10	-	-	-	-
17.7	PII 13.12	S_2	-	-	-	25	-
17.8	PII 13.12	S_2	-	-	-	-	35
18.1	G VI 256.5	S_1	-	-	-	-	-
18.2	G VI 256.5	S_1	-	-	25.5	-	-
18.3	G VI 256.5	S_1	-	2.5	-	-	-
18.4	G VI 256.5	S_1	2.5	-	-	-	15
18.5	G VI 256.5	S_1	-	-	-	-	-
18.6	G VI 256.5	S_1	-	-	-	-	-
19.1	P II 4.8	S_1	-		-	-	-
19.2	P II 4.8	S_1	-	10	10	-	-
19.3	P II 4.8	S_1	-	-	-	-	5
19.4	P II 4.8	S_1	12.5	-	-	-	-
20.1	P II 4.8	S_2	-	-	5	-	-
20.2	P II 4.8	S_2	-	2.5	-	-	-
20.3	P II 4.8	S_2	24	-	-	-	-
20.4	P II 4.8	S_2	-	-	-	-	7.5
20.5	P II 4.8	S_2	-	-	-	-	-
20.6	P II 4.8	S_2	-	-	-	-	-
20.7	P II 4.8	S_2	-	15	5	-	-
21.1	P II 13.8	S_1	-	-		-	-
21.2	P II 13.8	S_1	10	-	-	-	-
21.3	P II 13.8	S_1	-	-	-	-	-
22.1	P II 13.8	S_2	-	-	-	-	-
22.2	P II 13.8	S_2	15	-	-	-	-
22.3	P II 13.8	S_2		-	-	-	22.5
22.4	P II 13.8	S_2	-	-	-	20	-
22.5	P II 13.8	S_2	-	22.5	-	-	-
23.1	P II 12.9	S_1	-	-	-	-	-
23.2	P II 12.9	S_1	-	-	-	-	15
23.3	P II 12.9	S_1	-	-	-	-	-
23.4	P II 12.9	S_1	-	-	-	-	-
23.5	P II 12.9	S_1	-	10	-	-	-
24.1	P II 12.9	S_2	-	-	-	-	5
24.2	P II 12.9	S_2	-	-	-	-	-

also observed. The maximum tea mosquito bug infestation of 57.5 per cent was observed in H 7.3 (Plant No.5.3) followed by G IV 295.4 (Plant No.1.1) (25%) whereas, the least infestation was observed in G IV 35.7 (Plant No.11.1) (2.5%)

Black pod was the major disease affected the inbreds, the highest infection of 68 per cent was observed in G VI295.4 (Plant No. 1.3) followed by 55 per cent in G VI 135 (Plant No. 12.2) and the least infection of 10 per cent was observed in G VI 141(Plant No.15.3). Apart from these, rat damage was an observed in G VI 141(Plant No. 15.4) (15%) followed by H 7.3 (Plant No. 5.3) (12.5%) and the least damage was observed in G VI 295.4(5 %). The maximum squirrel damage of 35 per cent was observed in P II 13.12(Plant No.17.8) followed by H 7.3 (Plant No. 4.6) (25%) and the least damage was observed in P II 12.9 (Plant No.24.1).

4.1.3.6 Inbreeding Depression

Genetic variation is a key factor in competition among individuals in ecological communities which provides an opportunity for plant breeder to develop new and improved cultivars with the desirable characteristics. The use of variability is the ultimate objective of the activities of breeding programme in selection as well as in crop improvement programme. Variation within the population is the basis for the selection and adaptation which makes it possible to continue and advance the adaptive process on which evolutionary success depends. The macro evolutionary concept of conversion was from out crossing to self-fertilization. The self fertilization in out crossing plants would benefit about 50 percent of transmission because of the contribution of the pollens to their own ovules. As the crossing shift to selfing, the associated changes occur mainly in floral morphology and the reproductive investment and mating pattern (Loveless and Hamrick, 1984) which in turn changes the population size and their stability to survive. The possibility of shifting mating system may promote to speciation which can affect the inferences about speciation and extinction (Magnuson and Otto, 2012). With the increased selfing will lead to inbreeding depression indicates the reduced fitness. As a result, becomes homozygous which may be unfavorable or favourable recessive genes.

The unfavorable recessive genes will be eliminated whereas the favourable genes with no injurious effects will be utilized.

Inbreeding depression is the lowered fitness of inbred individuals compared to out crossed individual that can directly affect the intrinsic selective advantage of increased selfing. The magnitude of inbreeding depression in natural populations is expected to evolve with the mating system. Darwin (1876) reported that progeny obtained from self fertilization were weaker than those obtained from out crossing which published in his book *Cross and Self Fertilization in Vegetable Kingdom*. The genetic basis beyond the self fertilization will reveal how the increased homozygosity will lead to the inbreeding depression which is due to non-additive gene action. The rate at which deleterious alleles are eliminated from population will depend on the genetic parameters. The effect of deleterious recessive allele or partially recessive allele will result in phenotype appearance.

As the cocoa is a perennial and cross pollinated crop, limited availability of inbreds and self pollinated cocoa varieties is the basic reason for its insufficient exploitation in cocoa breeding at present. The hybrid developed now in cross pollinated crops doesn't give the hybrid vigour to the full potentials of the crop, because the selected type is cross pollinated one and hence it's a hybrid by itself. These crosses with open pollinated ecotypes will yield segregation for different characters in first generation itself and give only weak hybrids. The cross between two diverse inbreds is only useful in developing hybrids to its full potential with high vigour, early flowering, high yield and other beneficial traits. This also segregates faster to lesser valued individuals. Hence, inbreeding depression study was undertaken to evaluate inbreds in cocoa and then identify the diverse inbreds for developing good hybrids in cocoa. On inbreeding open pollinated cocoa, there will be possibility of obtaining variable forms by crossing two outcrossing cocoa plants. The variability will be useful for selection of parents for developing hybrid with low segregation and high vigour, high yield and beneficial economic and biochemical characters of commercial importance.

The present investigation on inbreeding depression was carried out for yield, pod, bean characters, economic and biochemical characters in 13 genotypes, of which, 2 genotypes belong to S_1 generation, 8 genotypes to S_2 generations in, two genotypes to S_4 generations and one genotype to S_5 generation.

4.1.3.6.1 Inbreeding depression in M 18.7 genotype over generations

The details of inbreeding depression for yield, pod, bean, economic and biochemical characters of M18.7 genotype are presented in table 26

4.1.3.6.1.1 S_1 generation

Positive inbreeding depression was observed for 17 characters out of 21 characters. The pod weight, ridge thickness, furrow thickness, pod length, pod breadth and number of beans per pod are interdependent and are directly associated with dry bean yield per plant (Veeresh, 2018). The husk furrow thickness and number of flat beans expressed negative inbreeding depression of -12% and -7.5 respectively. The increased husk thickness and number of flat beans per pod is negatively associated with dry bean yield per pod. The average yield per tree per year has shown a very meager inbreeding depression of 4.21 per cent over the preceding generation indicating the decrease in yield over the preceding generation of S0. There is no inbreeding depression for husk ridge thickness. Pod weight, pod length, number of beans per pod, weight of wet bean per pod, bean length, breadth, and thickness, fat and phenols content have expressed an inbreeding depression of less than 5 percent. Pod breadth, single bean weight, conversion index and dry matter recovery have shown an inbreeding depression of less than 10 percent. Negative inbreeding depression was expressed for pod index and efficiency index This clearly confirm that dry weight of beans per pod and pod weight required to produce one gram of dry bean is high. Similar results were reported by Minimol *et al.* (2015) in breeding cycle of GII 7.4 genotype of cocoa. Results of their study revealed an inbreeding depression of 40.44 for wet bean yield per pod, in contrast to the present study only 4.21 per cent inbreeding was observed.

Tables 26. Inbreeding depression of M 18.7 genotype over generation

S. No.	Characters	S_1	S_2	S_3	S_4
1	Av. Yield (No. of pods/tree/year)	4.21	3.93	5.70	43.58
2	Pod weight (g)	2.50	2.24	2.58	19.06
3	Husk Ridge thickness (cm)	0.00	-1.23	1.95	25.68
4	Husk furrow thickness (cm)	-12.00	0.00	1.79	19.09
5	Pod length (mm)	0.08	0.15	6.86	-6.47
6	Pod breadth (mm	5.80	0.12	3.92	13.57
7	No. of beans per pod	2.78	4.38	3.32	-5.14
8	No of flat beans per pod	-7.50	-4.65	-6.67	-14.58
9	Wet bean weight per pod (g)	4.59	2.40	10.00	28.17
10	Dry bean weight per pod (g)	12.31	7.50	4.75	11.81
11	Single bean wt. (g)	9.80	3.26	1.48	16.12
12	Bean length (mm)	0.10	0.21	1.55	10.26
13	Bean breadth (mm)	0.70	3.04	0.87	13.69
14	Bean thickness (mm)	0.14	0.00	-0.01	1.15
15	Pod value	12.31	7.50	4.20	12.09
16	Pod index	-14.04	-8.11	-4.39	-13.75
17	Efficiency Index	-11.19	-5.68	-1.69	7.92
18	Conversion Index	8.09	5.22	-6.44	-22.38
19	Dry Matter Recovery (%)	8.09	5.22	-5.83	-22.78
20	Fat content (%)	1.77	-0.02	-3.77	-10.96
21	Phenol content (%)	0.84	0.42	1.71	13.59

Rubino and Wehner (1986) also reported some yield improvement during the inbreeding process of pickling cucumber population, which is also a cross pollinated crop. When average girth (cm) at three years after planting was compared among generations not much differences were observed between inbred population and parental population.

Inbreeding depression was studied for S_2 nut yield and its attributes in coconut. The family IIS_2-2 and VS_2-1 expressed positive inbreeding depression for palm height and inter nodal length, but no inbreeding depression for stem girth. The leaf characteristics also exhibited positive inbreeding depression in S_2 (Chetana, 2016).

4.1.3.6.1.2 S_2 generation

Positive inbreeding depression was observed for 16 characters. For all the 16 characters the inbreeding expression is to a tune of less than five per cent. Pod value, conversion index, dry matter recovery have shown an inbreeding depression between 5 to 10 percent. Negative inbreeding depression was observed for husk ridge thickness, number of flat bean per pod, pod index, efficiency index and fat content. The similar trend was observed for these traits in S_1 generation too. The magnitude of inbreeding depression is quite low compared to S_1 generation.

Chetana (2016) assessed the inbreeding depression in coconut, the germination percentage revealed positive inbreeding depression in all the selfed families of 12 families. High inbreeding depression was noticed in IAS_3-2, $IIIS_3$-2, IVS_3-2, VS_3-1 and IVS_3-1 and the lowest in family $1BS_3$-1 and $1AS_3$-1. Seedling height expressed high inbreeding depression in VS_3-1. Collar girth didn't show inbreeding depression in S_3. The collar girth is an important trait correlated with yield. Both positive and negative inbreeding depression was recorded for total number of leaves produced by the seedlings in S_3 generation.

4.1.3.6.1.3 S_3 generation

Maximum inbreeding depression of 10 per cent was observed for wet bean weight per pod followed by pod length (6.86 per cent), the main yield deciding factors. The negative inbreeding depression for number of flat bean per pod, pod value and efficiency index persist in S_3 generation. The conversion index, dry matter recovery and fat content have also shown negative inbreeding depression. The magnitude of inbreeding depression is less than 10 per cent for most of the characters.

4.1.3.6.1.4 S_4 generation

Over the generations, the maximum inbreeding depression is observed for majority of the characters in the study. Highest inbreeding depression of 43.58 per cent was observed for number of pods/tree/year. The negative inbreeding depression for number of flat bean pod, pod index, conversion index, dry matter recovery and fat estimation persisted and was more than in the preceding generation. The characters like number of flat bean per pod, has negative association with the final yield.

4.1.3.6.2 Inbreeding depression of G II 7.4 genotype over generation

The details of inbreeding depression for yield, pod, bean, economic and biochemical characters of G II 7.4 genotype are presented in table 27.

4.1.3.6.2.1 S_1 generation

Positive inbreeding depression was observed for 10 characters out of 21. The husk ridge and furrow thickness and number of flat beans expressed negative inbreeding depression of -64.52, -44.29 and -9.09 respectively. The negative inbreeding depression for these traits demonstrate the increase in these traits over preceding generation and these are all negatively correlated with the final dry bean yield per plant. Hence, increase in these parameters over the preceding generation is a disadvantage. The expression of negative inbreeding depression for characters like husk ridge thickness, furrow thickness, pod breadth, number of beans per pod, number of flat beans per pod,

Table 27. Inbreeding depression of G II 7.4 genotype over generation

S. No.	Characters	S_1	S_2	S_3	S_4	S_5
1	Av. Yield (No. of pods/tree/year)	21.92	15.42	21.14	-5.37	39.11
2	Pod weight (g)	9.61	5.71	-18.70	4.32	24.06
3	Husk Ridge thickness (mm)	-64.52	-9.8	5.36	-92.45	49.02
4	Husk furrow thickness (mm)	-44.29	-9.11	12.09	-1.15	63.95
5	Pod length (mm)	6.09	-6.01	-0.75	0.04	0.10
6	Pod breadth (mm)	-4.45	0.85	-13.47	10.30	10.90
7	No. of beans per pod	-3.74	5.67	-4.92	18.75	-11.50
8	No of flat beans per pod	-9.09	8.33	-100	18.18	-11.11
9	Wet bean weight per pod (g)	-0.70	18.49	-54.64	-10.15	-5.30
10	Dry bean weight per pod (g)	16.02	-23.46	-7.39	34.89	-8.48
11	Single bean wt. (g)	19.05	-30.88	-2.36	19.87	2.74
12	Bean length (mm)	-7	0.12	-9.47	0.01	-7.64
13	Bean breadth (mm)	0.38	-1.81	-28.68	13.40	-7.33
14	Bean thickness (mm)	-.6.01	31.60	-56.40	1.85	14.08
15	Pod value	16.04	-22.93	-7.62	34.93	-14.00
16	Pod index	-19.11	18.66	7.08	-53.68	12.28
17	Efficiency Index	-7.59	23.17	-10.17	-47.05	33.39
18	Conversion Index	16.62	-50.82	30.41	40.93	-8.27
19	Dry Matter Recovery (%)	16.6	-51.46	30.55	40.89	-3.02
20	Fat content (%)	-18.43	26.56	-32.31	20.16	-9.6
21	Phenol content (%)	0.78	13.24	-4.5	17.36	-29.01

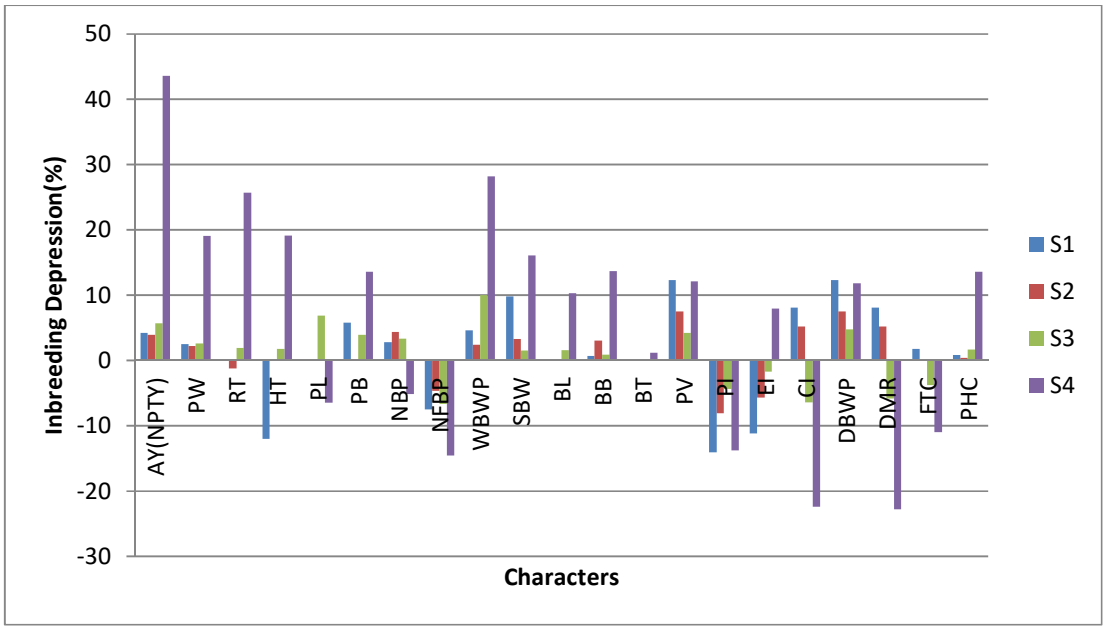


Fig. 41 Inbreeding depression in M 18.7 inbred over generations

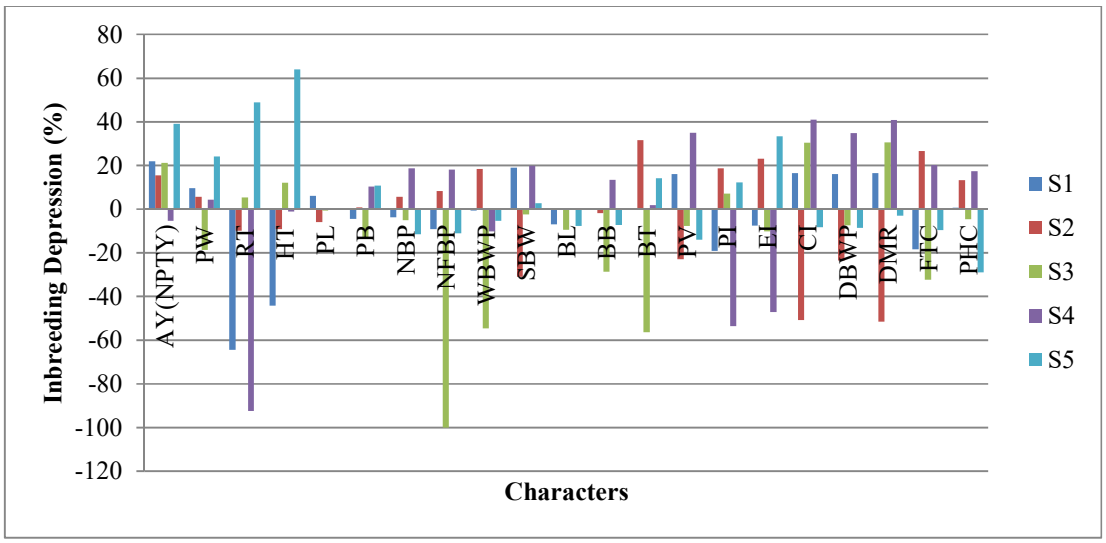


Fig. 42 Inbreeding depression in G II 7.4 inbred over generations

bean thickness, pod index and efficiency indes is attributed to the additive gene control. The average yield per tree per year, single bean weight, pod value, conversion index, dry bean weight per pod, and dry matter recovery, have shown positive inbreeding depression. The reduction in the values for these parameters and increase in inbreeding depression over the past generation results in lower yield. The fat content has shown a negative inbreeding depression of -18.43 per cent over the preceding generation. Less inbreeding depression was observed for wet bean weight per pod, bean breadth and phenol content. A similar result of was reported by Minimol *et al.,* (2015) in breeding cycle of GII 7.4 genotype of cocoa. Results of their study reveal an inbreeding depression of 40.44 for wet bean yield per pod, in contrast in the present study only 4.21 per cent inbreeding was observed. The variation in inbreeding depression among the genotypes was reported by Mallika *et al.,* 2002. Further, the possibility of utilizing homozygous inbreds for production of commercial hybrids cannot be ruled out by analyzing inbreeding generation of only one genotype. The percentage of inbreeding depression in G II 7.4 varied significantly for different traits selected under study.

Although most cross pollinated species have higher or lower levels of inbreeding depression as a consequence of inbreeding, there are some in which self-pollination can happen in a continuous way with no vigor loss. Cucurbits, being cross-pollinated, are an example of a group of species in which certain lines seem to lose little vigor by inbreeding (Allard, 1971; Whitaker & Robinson, 1986; Robinson, 1999). In some aspects, research results are contradictory regarding loss of vigor in inbred of cross pollinated crops like cucurbits. The vigour and reproductive capacity did not affected in *Cucurbita maxima* after self-pollination for 10 generations (Cummings, 1928). Bushnell, (1922), while studying the effect of inbreeding in *C. pepo* opined that the vigor loss during self pollination did not necessarily occur.

4.1.3.6.2.2 S_2 generation

Positive inbreeding depression was observed for 15 characters. The maximum inbreeding depression was observed for bean thickness (31.80%) followed by fat content

(26.46%), and efficiency index (23.17%). An inbreeding depression of 15.42 per cent and 18.49 per cent was registered for average pod yield per tree per year and wet bean weight per pod. Negative inbreeding depression was observed for conversion index, dry matter recovery, pod value, single dry bean weight, husk thickness and pod length. A contrary result was obtained for pod breadth, bean thickness, dry bean weight per pod, dry matter recovery and fat content in S_1 generation.

4.1.3.6.2.3 S_3 generation

Only seven characters expressed positive inbreeding depression in S_3 generation of G II 7.4 genotype. When compared to the preceding generation, contrary results are observed for pod weight(g), husk furrow thickness (mm), pod breadth (mm), wet bean weight per pod (g), bean breadth, bean thickness, efficiency Index, fat content (%). Bushnell (1922) opined that inbreeding depression may necessarily occur in the succeeding generations in his studies on inbreeding in *Cucurbita maxima*. Maximum inbreeding depression of 30.55 per cent was observed for dry matter recovery (%), followed by conversion index (table 25)

4.1.3.6.2.4 S_4 generation

Negative inbreeding depression was found in S_4 generation of G II 7.4. Highest negative inbreeding depression (-92.45 per cent) was observed for ridge thickness *i.e.,* high ridge thickness, which has negative association with economic yield. A contrary result was obtained for average yield, pod weight, husk thickness, bean characters, pod value and phenol content. The expression of inbreeding depression over S_3 generations is unstable when compared to its earlier generation S_2.

4.1.3.6.2.5 S_5 generation

The maximum inbreeding depression (63.95 per cent) was observed for husk furrow thickness followed by husk ridge thickness (49.02), average yield (No. of pods per tree per year, pod weight (g) (39.11 per cent), efficiency index (33.39 per cent). Negative inbreeding depression was observed for number of beans per pod (-11.50),

number of flat per pod (-11.11). bean length, Bean breadth, pod value, conversion index, dry bean weight per pod (g), dry Matter Recovery (%), fat content (%), phenol content (%) have also shown negative inbreeding depression(Table 26).

4.1.6.3 Inbreeding depression of H1 1.2 genotype over generation

The inbreeding depression of H1 1.2 is presented in table 28.

4.1.6.3.1 S_1 generation

Positive inbreeding was observed in 16 characters out of 21 characters indicating the reduction in their values over the preceding generation. Chekalina (1976) had already reported a reduction in fruit weight of different cultivars of *C. maxima and C. pepo,* two cross pollinated crops as a result of inbreeding after three generations of self pollination. Number of flat bean per pod recorded -33.33 percent inbreeding indicating more number of flat beans over preceding generation. Several scientists assumed the hypothesis of reduced inbreeding depression in *Cucurbita*, some researchers have shown inbreeding depression for several characters of *C. pepo* and *C. maxima* (Borghi et al., 1973; Chekalina, 1976).

4.1.6.3.2 S_2 generation

Inbreeding depression was observed for 16 characters in S_2 generation of H1 1.2. Husk thickness increased in S_2 generation.

4.3.6.4 Inbreeding depression of G IV 35.7 genotype over generation

The details of inbreeding depression in of G IV 35.7 genotype over generation is presented in table 29.

4.3.6.4.1 S_1 generation

Positive inbreeding depression was observed for 13 characters. Av. Yield (No. of pods per tree per year, pod weight (g), husk ridge thickness(mm), husk furrow thickness (mm), wet bean weight per pod (g), single bean weight(g), efficiency index have shown

Table 28. Inbreeding depression of H1 1.2 genotype over generation

S. No.	Characters	S_1	S_2
1	Av. Yield (No. of pods/tree/year)	16.32	9.38
2	Pod weight (g)	12.66	-10.91
3	Husk Ridge thickness (mm)	4.35	-95.45
4	Husk furrow thickness (mm)	4.31	-0.90
5	Pod length (mm)	6.68	0.01
6	Pod breadth (mm)	5.69	-5.90
7	No. of beans per pod	7.02	22.10
8	No of flat beans per pod	-33.33	0.00
9	Wet bean weight per pod (g)	8.99	21.67
10	Dry bean weight per pod (g)	25.67	33.32
11	Single bean wt. (g)	20.05	14.34
12	Bean length (mm)	0.70	8.31
13	Bean breadth (mm)	0.21	25.41
14	Bean thickness (mm)	1.63	1.67
15	Pod value	25.57	33.31
16	Pod index	-34.36	-49.94
17	Efficiency Index	-17.36	-66.30
18	Conversion Index	18.22	14.85
19	Dry Matter Recovery (%)	18.33	14.87
20	Fat content (%)	2.66	4.50
21	Phenol content (%)	1.57	8.55

Table 29. Inbreeding depression of G IV 35.7 genotype over generation

S. No.	Characters	S_1	S_2	S_3	S_4
1	Av. Yield (No. of pods/tree/year)	18.12	10.46	25.14	16.19
2	Pod weight (g)	15.22	34.12	-44.09	-51.94
3	Husk Ridge thickness (mm)	2.04	2.08	5.33	3.37
4	Husk furrow thickness (mm)	1.63	15.70	0.00	0.00
5	Pod length (mm)	-28.05	0.16	-0.05	-.0.88
6	Pod breadth (mm)	0.10	2.60	2.56	3.51
7	No. of beans per pod	-6.67	-6.25	-.1.96	-5.77
8	No of flat beans per pod	-20.79	-8.20	-9.09	
9	Wet bean weight per pod (g)	82.20	6.17	-79.70	-8.93
10	Dry bean weight per pod (g)	2.22	3.41	0.08	-2.10
11	Single bean wt. (g)	8.33	9.09	2.00	3.47
12	Bean length (mm)	-0.21	0.31	-0.10	0.01
13	Bean breadth (mm)	0.24	1.26	0.32	0.24
14	Bean thickness (mm)	1.47	0.93	0.56	0.30
15	Pod value	2.22	3.41	0.08	-2.10
16	Pod index	-2.27	-3.53	-0.08	2.06
17	Efficiency Index	13.29	31.79	-44.21	-48.81
18	Conversion Index	-39.72	-2.94	44.40	
19	Dry Matter Recovery (%)	-449.37	-2.94	44.40	6.27
20	Fat content (%)	-59.25	1.99	0.04	-7.46
21	Phenol content (%)	1.21	-0.40	1.63	-4.67

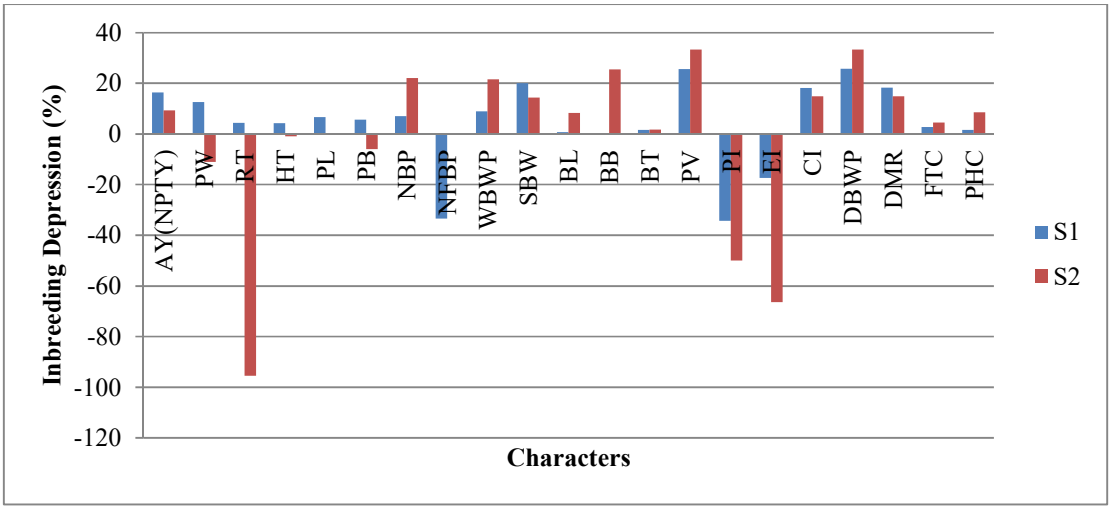


Fig. 43 Inbreeding depression in H1 1.2 inbred over generations

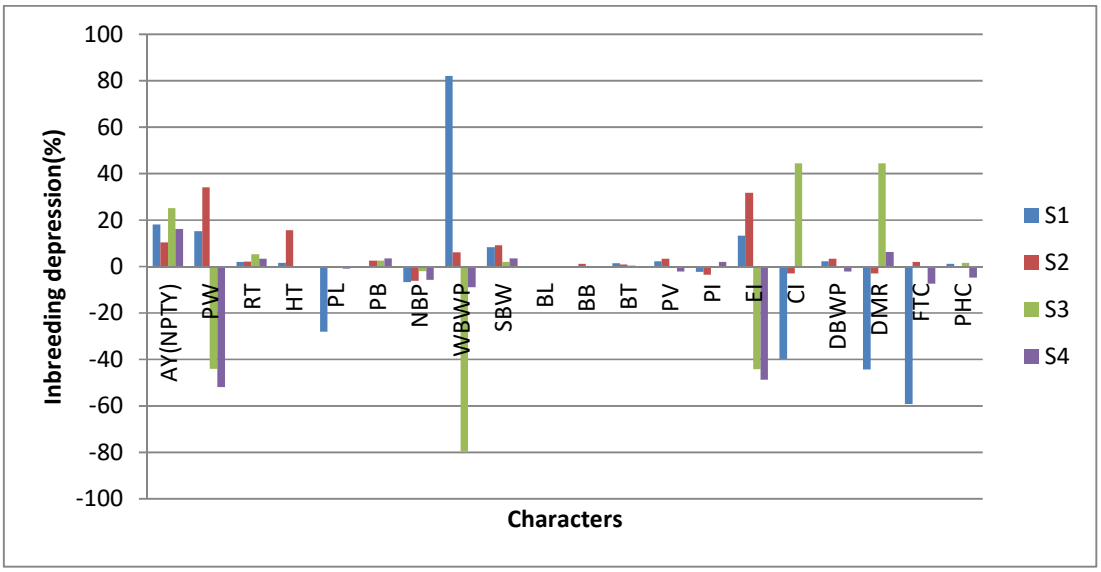


Fig. 44 Inbreeding depression in GIV 35.7 inbred over generations

the inbreeding depression over S_0 generation. These characters lost some vigour due to inbreeding depression. Cucurbits, being cross pollinated, are the other examples in which certain lines loose little vigour by inbreeding (Allard, 1971; Whitaker & Robinson, 1986; Robinson, 1999).

4.3.6.4.2 S_2 generation

Positive inbreeding depression was observed for fifteen characters out of 21. Maximum inbreeding was observed for pod weight (34.12), followed by efficiency index (31.79), furrow thickness (15.70), 20 bean weight (11.59) and average pod yield(10.46)

4.3.6.4.3 S_3 generation

The maximum negative inbreeding depression was observed for wet bean weight per pod (-79.70) followed by average pod weight (-44.09) and efficiency index (-44.21). These observations point out the increase in their values over the S_2 generation. Whereas the average pod yield, conversion index (44.40) and dry matter recovery (44.40) have shown maximum inbreeding depression.

4.3.6.4.4 S_4 generation

The inbreeding depression in S_4 generation is almost similar to that of S_3 generation except the fat content and poly phenol content expressed negative inbreeding depression.

4.3.6.5 Inbreeding depression of G VI 135 genotype over generation

The details of inbreeding depression in of G VI 135 genotype over generations are presented in table 30.

Positive inbreeding depression over the preceding generation was observed for majority of the characters except wet bean weight per pod (-5.44) and pod index (-2.33) in S_1 generation and number of flat beans per pod (-71.43), conversion index (-44.37)

Table 30. Inbreeding depression of G VI 135 genotype over generation

S. No.	Character	S_1	S_2
1	Av. Yield (No. of pods/tree/year)	23.95	45.81
2	Pod weight (g)	3.02	37.10
3	Husk Ridge thickness (mm)	2.7	13.89
4	Husk furrow thickness (mm)	2.75	25.00
5	Pod length (mm)	2.88	0.19
6	Pod breadth (mm)	6.32	7.80
7	No. of beans per pod	2.57	15.20
8	No of flat beans per pod	0.94	-71.43
9	Wet bean weight per pod (g)	-5.44	45.73
10	Dry bean weight per pod (g)	6.50	21.54
11	Single bean wt. (g)	4.03	7.46
12	Bean length (mm)	1.34	3.24
13	Bean breadth (mm)	0.83	4.92
14	Bean thickness (mm)	0.400	20.48
15	Pod value	2.28	21.64
16	Pod index	-2.33	-27.62
17	Efficiency Index	0.76	19.73
18	Conversion Index	7.32	-44.37
19	Dry Matter Recovery (%)	11.32	-44.56
20	Fat content (%)	0.24	-1.42
21	Phenol content (%)	2.40	0.40

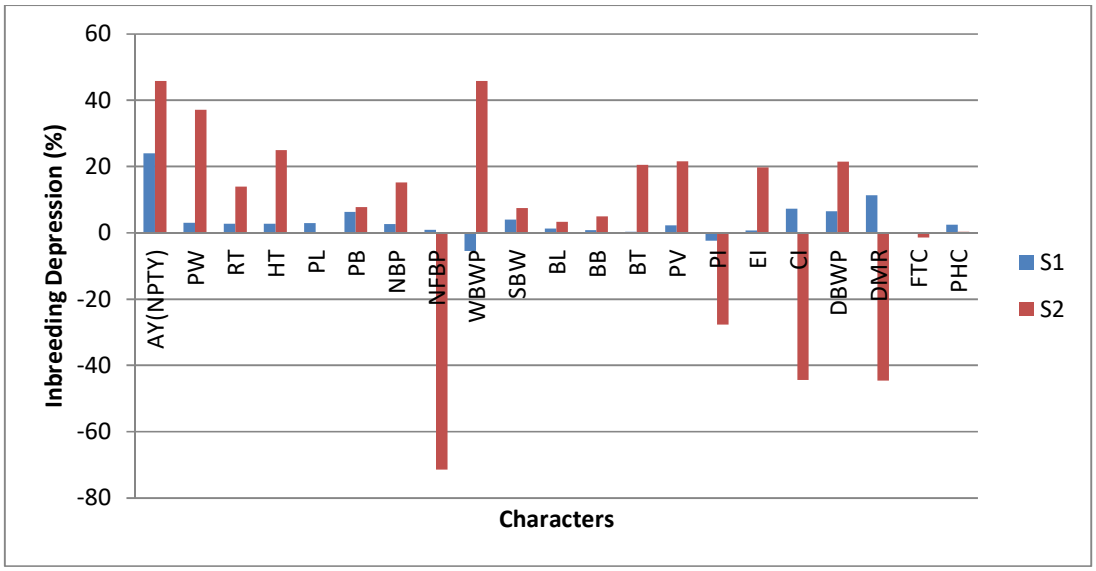


Fig. 45 Inbreeding depression in G VI 135 inbred over generations

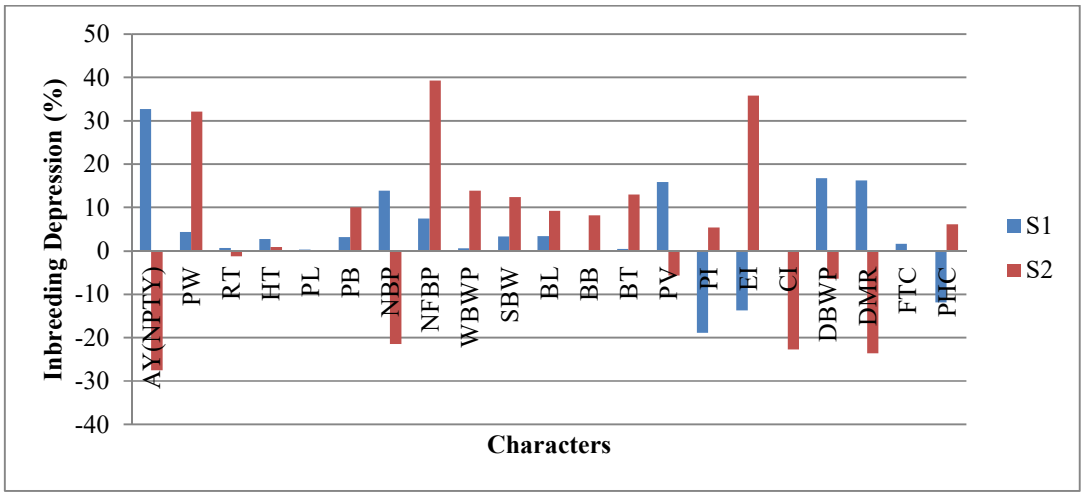


Fig. 46 Inbreeding depression in G VI 141 inbred over generations

Table 31. Inbreeding depression of G VI 141 genotype over generation

S. No.	Character	S_1	S_2
1	Av. Yield (No. of pods/tree/year)	32.78	-27.59
2	Pod weight (g)	4.32	32.20
3	Husk Ridge thickness (mm)	0.70	-1.25
4	Husk furrow thickness (mm)	2.78	0.90
5	Pod length (mm)	0.33	0.14
6	Pod breadth (mm)	3.19	10.00
7	No. of beans per pod	13.9	-21.47
8	No of flat beans per pod	7.41	39.26
9	Wet bean weight per pod (g)	0.62	13.89
10	Dry bean weight per pod (g)	16.77	-6.42
11	Single bean wt. (g)	3.33	12.39
12	Bean length (mm)	3.42	9.22
13	Bean breadth (mm)	0.09	8.23
14	Bean thickness (mm)	0.50	13.00
15	Pod value	15.85	-5.72
16	Pod index	-18.83	5.41
17	Efficiency Index	-13.70	35.84
18	Conversion Index		-22.77
19	Dry Matter Recovery (%)	16.25	-23.58
20	Fat content (%)	1.60	0.19
21	Phenol content (%)	-11.92	6.17

and dry matter recovery(-44.56) and pod index (-27.62) in S_2 generations. This trend in increase and decrease in the vigour was similar to that of results obtained by Allard, 1971; Whitaker & Robinson, 1986; Robinson, 1999 in their study of inbreeding depression in cucurbits

4.3.6.6 Inbreeding depression of G VI 141 genotype over generation

The details of inbreeding depression in of G VI 141 genotype over generations is presented in table 31

The positive inbreeding depression was observed in majority of characters in S_1 generations. Negative inbreeding depression was expressed for index (-18.83) followed by efficiency index (-13.70) and phenol content (-11.92). The negative inbreeding depression was observed for average yield per tree per year (-27.59) followed by dry matter recovery (-23.58), conversion index (-22.77) and number of beans per pod (-21.47). These characters were at par or even superior when compared to preceding generation indicating little inbreeding depression. This is supported by the study of Luiz *et al.,* (1997). The reason for non expression of inbreeding depression can be attributed to additive gene control for these traits.

4.3.6.7 Inbreeding depression of P II 13.12 genotype over generation

The details of inbreeding depression in of P II 13.12 genotype over generation is presented in table 32.

The positive inbreeding depression was expressed for majority of the characters in S_1 generation over S_0 generation. In case of S_2 generation, positive inbreeding depression was expressed for 16 characters and negative inbreeding depression for five characters.

Table 32. Inbreeding depression of P II 13.12 genotype over generation

S. No.	Character	S_1	S_2
1	Av. Yield (No. of pods/tree/year)	21.05	27.46
2	Pod weight (g)	6.75	15.05
3	Husk Ridge thickness (mm)	1.47	10.82
4	Husk furrow thickness (mm)	6.52	13.37
5	Pod length (mm)	1.04	0.03
6	Pod breadth (mm)	3.87	8.90
7	No. of beans per pod	1.71	21.00
8	No of flat beans per pod	0.41	-19.79
9	Wet bean weight per pod (g)	0.96	29.40
10	Dry bean weight per pod (g)	3.50	14.75
11	Single bean wt. (g)	1.82	-7.91
12	Bean length (mm)	2.18	-6.41
13	Bean breadth (mm)	1.53	6.99
14	Bean thickness (mm)	2.01	1.27
15	Pod value	0.00	15.43
16	Pod index	0	-18.24
17	Efficiency Index	6.75	-0.45
18	Conversion Index		-19.79
19	Dry Matter Recovery (%)	2.56	-20.75
20	Fat content (%)	1.30	0.79
21	Phenol content (%)	2.02	25.27

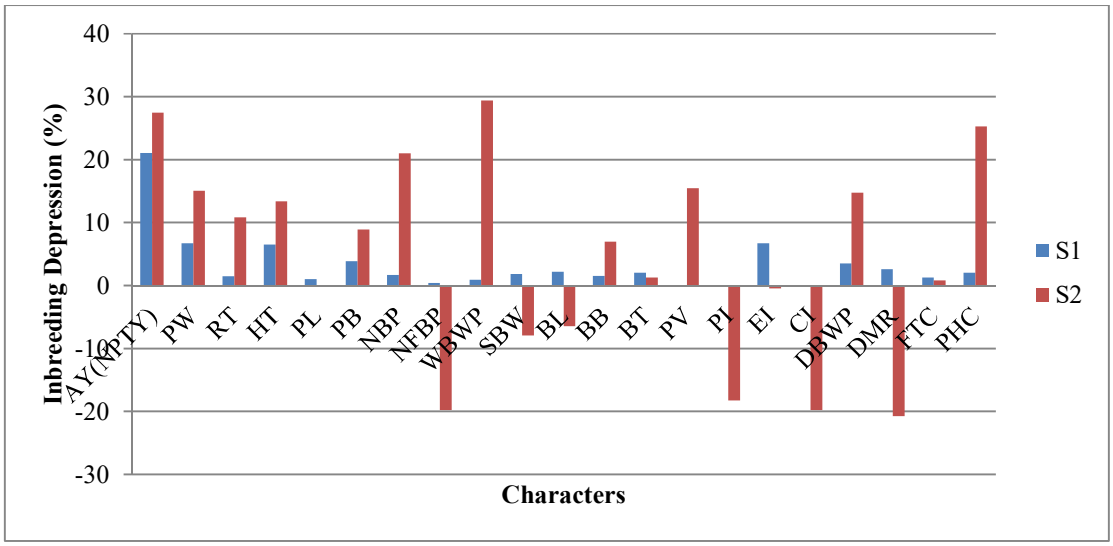


Fig. 47 Inbreeding depression in P II 13.12 inbred over generations

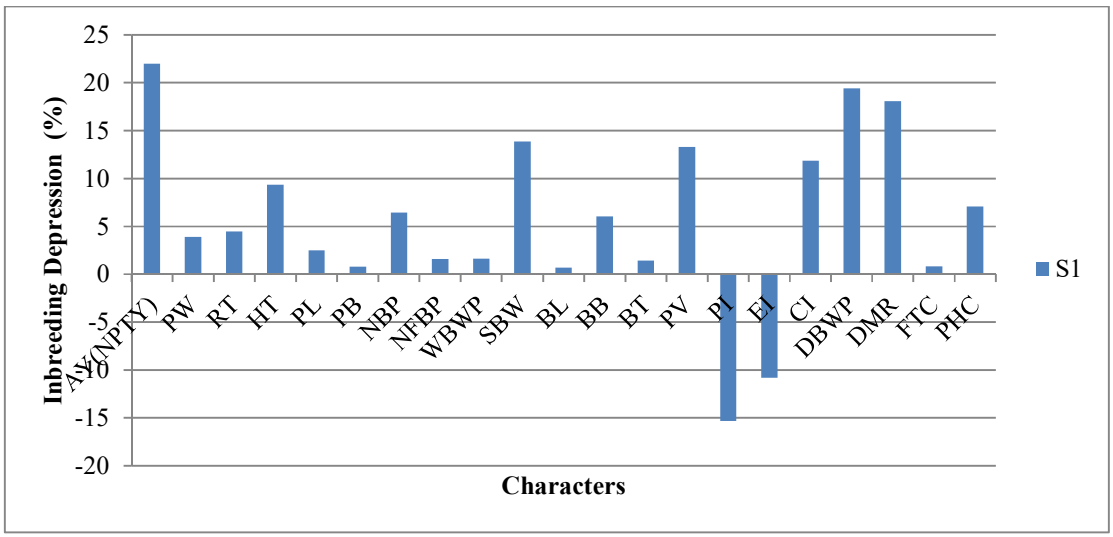


Fig. 48 Inbreeding depression in G VI 256.5 inbred over generations

Table 33 Inbreeding depression of G VI 256.5 genotype over generation

S. No.	Character	S_1
1	Av. Yield (No. of pods/tree/year)	21.95
2	Pod weight (g)	3.92
3	Husk Ridge thickness (mm)	4.46
4	Husk furrow thickness (mm)	9.35
5	Pod length (mm)	2.49
6	Pod breadth (mm)	0.78
7	No. of beans per pod	6.44
8	No of flat beans per pod	1.59
9	Wet bean weight per pod (g)	1.64
10	Dry bean weight per pod (g)	19.41
11	Single bean wt. (g)	13.86
12	Bean length (mm)	0.69
13	Bean breadth (mm)	6.03
14	Bean thickness (mm)	1.44
15	Pod value	13.28
16	Pod index	-15.32
17	Efficiency Index	-10.80
18	Conversion Index	11.84
19	Dry Matter Recovery (%)	18.07
20	Fat content (%)	0.81
21	Phenol content (%)	7.08

Table 34 Inbreeding depression of P II 4.8 genotype over generation

S. No.	Character	S_1	S_2
1	Av. Yield (No. of pods/tree/year)	17.82	-13.04
2	Pod weight (g)	3.51	-2.73
3	Husk Ridge thickness (mm)	2.61	11.98
4	Husk furrow thickness (mm)	1.47	12.15
5	Pod length (mm)	8.60	-0.13
6	Pod breadth (mm)	0.78	1.85
7	No. of beans per pod	6.44	2.01
8	No of flat beans per pod	1.59	1.32
9	Wet bean weight per pod (g)	8.41	31.52
10	Dry bean weight per pod (g)	4.40	13.15
11	Single bean wt. (g)	2.44	4.26
12	Bean length (mm)	1.08	-9.99
13	Bean breadth (mm)	.13	5.62
14	Bean thickness (mm)	0.74	-9.37
15	Pod value	5.17	13.62
16	Pod index	-5.45	-15.77
17	Efficiency Index	-1.75	-18.92
18	Conversion Index	-3.54	-26.15
19	Dry Matter Recovery (%)	-4.37	-26.84
20	Fat content (%)	1.55	-1.88
21	Phenol content (%)	12.89	-4.26

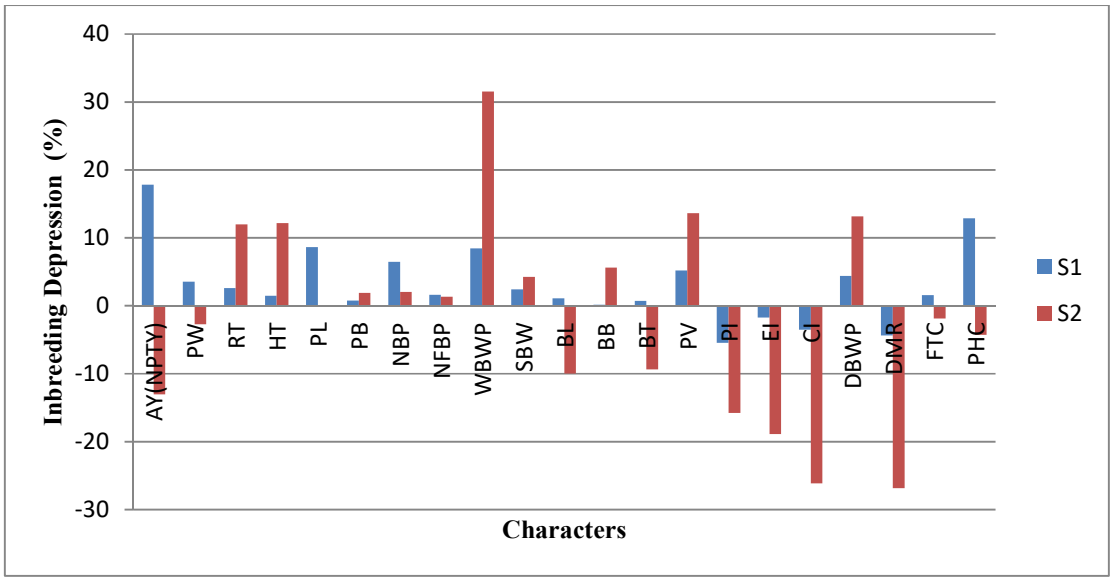


Fig. 49 Inbreeding depression in P II 4.8 inbred over generations

Fig. 50 Inbreeding depression in P II 13.8 inbred over generations

Table 35. Inbreeding depression of P II 13.8 genotype over generation

S. No.	Character	S_1	S_2
1	Av. Yield (No. of pods/tree/year)	8.70	59.88
2	Pod weight (g)	1.54	31.35
3	Husk Ridge thickness (mm)	0.31	44.92
4	Husk furrow thickness (mm)	1.25	50.89
5	Pod length (mm)	2.12	0.17
6	Pod breadth (mm)	0.80	13.2
7	No. of beans per pod	0.97	9.15
8	No of flat beans per pod	2.78	-41.43
9	Wet bean weight per pod (g)	7.49	60.16
10	Dry bean weight per pod (g)	20.39	25.39
11	Single bean wt. (g)	19.61	17.87
12	Bean length (mm)	10.30	10.75
13	Bean breadth (mm)	5.72	4.10
14	Bean thickness (mm)	0.28	-0.03
15	Pod value	-5.30	25.16
16	Pod index	5.03	-33.63
17	Efficiency Index	6.50	8.26
18	Conversion Index	-13.82	-87.83
19	Dry Matter Recovery (%)	13.94	-87.27
20	Fat content (%)	-15.21	23.30
21	Phenol content (%)	34.76	-26.79

4.3.6.8 Inbreeding depression of G VI 256.5 genotype over generation

The details of inbreeding depression in of G VI 256.5 genotype in S_1 generation is presented in table 33. All the characters under study showed a positive inbreeding depression except pod index and efficiency index.

4.3.6.9 Inbreeding depression of P II 4.8 genotype over generation

The details of inbreeding depression of P II 4.8 genotype over generations is presented in table 34. Significant inbreeding depression was observed for average yield per tree per year and phenol content. In S_2 generation the majority of the characters have shown little or no inbreeding depression. These kinds of results in cocoa are supported by Luiz *et al.,* (1997). Wet bean weight has shown positive inbreeding depression in S_2 generation.

4.3.6.10 Inbreeding depression of P II 13.8 genotype over generation

The details of inbreeding depression in P II 13.8 genotype over generations is presented in table 35. Significant inbreeding depression was observed for average yield per tree per year, single bean weight, bean length, bean breadth, dry bean weight per pod and dry matter recovery. Negative and less inbreeding depression was observed for conversion index (-13.82) and fat content (-15.21), the non expression of inbreeding depression for these characters are controlled by additive gene. In S_2 generation the majority of the characters have shown little or no inbreeding depression except number of flat beans per pod (-41.43), pod index (-33.63), conversion index (-87.83), dry matter recovery (-87.27) and phenol content (-26.79).

4.3.6.11 Inbreeding depression of H 1.2 genotype over generation

The details of inbreeding depression in H 1.2 genotype over S_1 generation is presented in table 36. The husk ridge thickness (-16.35) and bean thickness (-12.23) have shown negative inbreeding depression indicating that these characters are controlled by additive genes.

Table 36. Inbreeding depression of H 1.2 genotype over generation

S. No.	Character	S_1
1	Av. Yield (No. of pods/tree/year)	17.36
2	Pod weight (g)	13.62
3	Husk Ridge thickness (mm)	-8.14
4	Husk furrow thickness (mm)	-16.35
5	Pod length (mm)	19.00
6	Pod breadth (mm)	13.07
7	No. of beans per pod	6.25
8	No of flat beans per pod	9.43
9	Wet bean weight per pod (g)	24.00
10	Dry bean weight per pod (g)	30.81
11	Single bean wt. (g)	26.19
12	Bean length (mm)	12.38
13	Bean breadth (mm)	1.15
14	Bean thickness (mm)	-12.23
15	Pod value	0.00
16	Pod index	0.00
17	Efficiency Index	13.62
18	Conversion Index	
19	Dry Matter Recovery (%)	8.95
20	Fat content (%)	2.14
21	Phenol content (%)	8.16

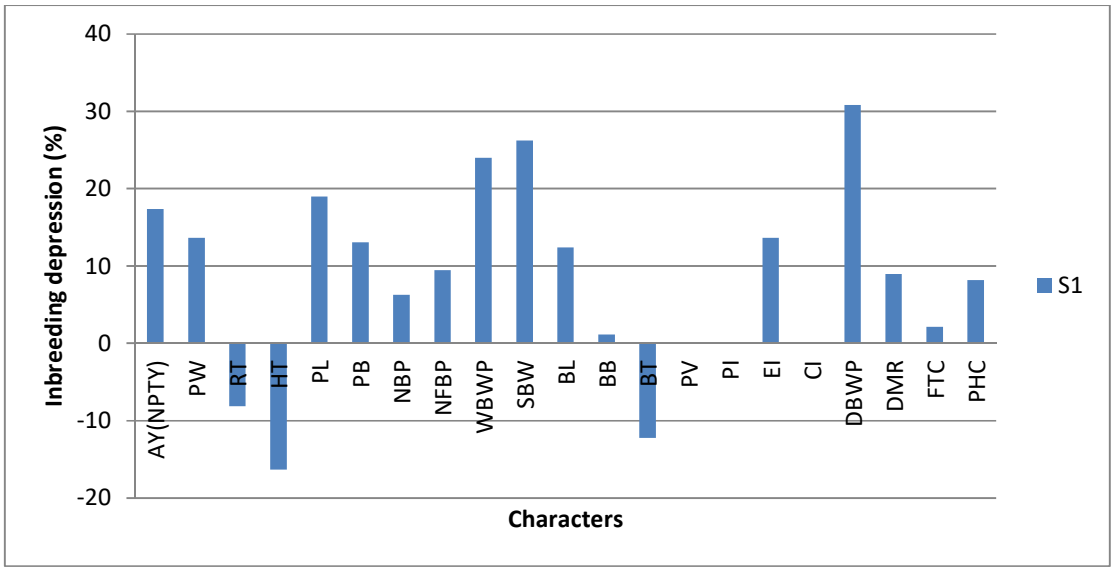


Fig. 51 Inbreeding depression in H 1.2 inbred over generations

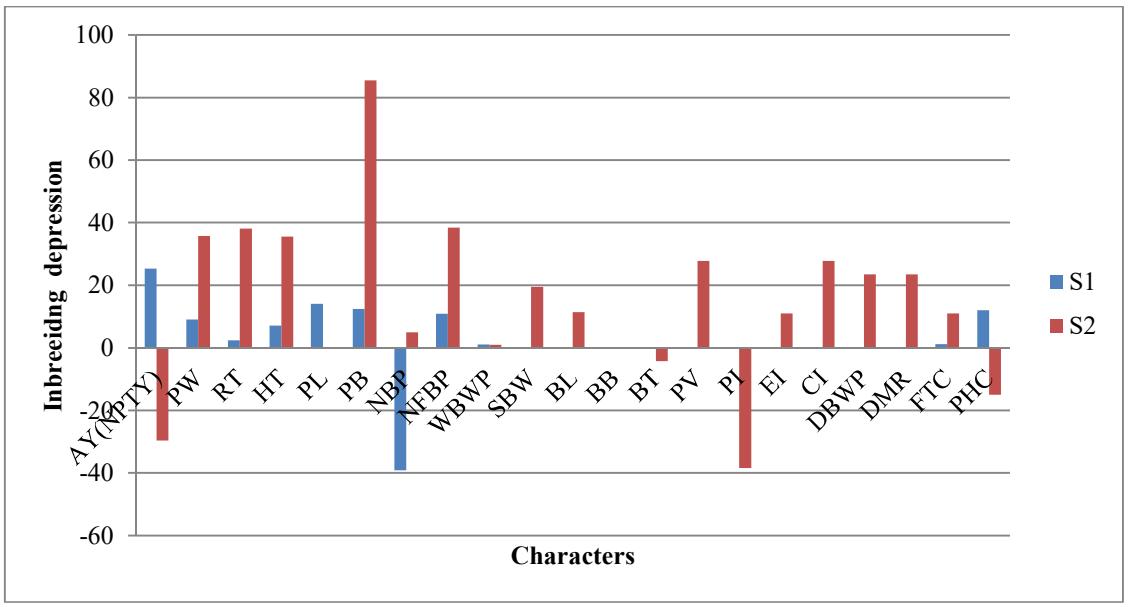


Fig. 52 Inbreeding depression in P II 12.9 inbred over generations

Table 37. Inbreeding depression of PII 12.9 genotype over generations

S. No.	Character	S_1	S_2
1	Av. Yield (No. of pods/tree/year)	25.28	-29.56
2	Pod weight (g)	9.01	35.73
3	Husk Ridge thickness (mm)	2.33	38.10
4	Husk furrow thickness (mm)	7.14	35.50
5	Pod length (mm)	14.05	0.20
6	Pod breadth (mm)	12.48	85.50
7	No. of beans per pod	-39.14	5.01
8	No of flat beans per pod	10.96	38.46
9	Wet bean weight per pod (g)	1.10	1.01
10	Dry bean weight per pod (g)		23.54
11	Single bean wt. (g)		19.51
12	Bean length (mm)		11.46
13	Bean breadth (mm)		0.15
14	Bean thickness (mm)		-4.22
15	Pod value		27.76
16	Pod index		-38.43
17	Efficiency Index		11.04
18	Conversion Index		27.75
19	Dry Matter Recovery (%)		23.53
20	Fat content (%)	1.18	11.07
21	Phenol content (%)	12.04	-14.96

4.3.6.12 Inbreeding depression of PII 12.9 genotype over generation

The details of inbreeding depression of PII 12.9 genotype over S_1 generation is presented in table 37.

4.3.6.12.1 S_1 generation

The number of flat beans per pod (-39.14) expressed negative inbreeding depression indicating that these characters are controlled by additive genes in the S_1 generation.

4.3.6.12.2 S_2 generation

In the S_2 generation, majority of the characters expressed positive inbreeding depression expect average yield per pod per tree (-29.56), pod index (-38.43) and phenol content (-14.96) indicating these characters are controlled by additive gene action.

4.3.6.13 Inbreeding depression of H 7.3 genotype over generation

The details of inbreeding depression in H 7.3(86) genotype over generations are presented in table 38.

4.3.6.13.1 S_1 generation

The positive and significant inbreeding depression was observed for average yield per tree per year (21.74) husk thickness furrow thickness (10.99), pod value (13.12), conversion index (8.99), dry bean weight per pod (13.96) and dry matter recovery(9.56) whereas the negative inbreeding depression was observed for pod index (-30.59) and efficiency index(-11.59) in S_1 generation.

4.3.6.13.2 S_2 generation

Positive inbreeding depression was observed for 15 characters. Pod weight (-24.22), husk ridge thickness (-22), husk furrow thickness (-13.94), number of flat beans per pod (-28.21) pod index (-26.32), efficiency index (-66.48) have expressed negative

Table 38. Inbreeding depression of H 7.3 genotype over generation

S. No.	Character	S_1	S_2
1	Av. Yield (No. of pods/tree/year)	21.74	-8.34
2	Pod weight (g)	3.39	-24.65
3	Husk Ridge thickness (mm)	3.05	-22.00
4	Husk furrow thickness (mm)	10.99	-13.94
5	Pod length (mm)	5.25	10.52
6	Pod breadth (mm)	0.31	2.73
7	No. of beans per pod	10.02	15.72
8	No of flat beans per pod	-3.17	-28.21
9	Wet bean weight per pod (g)	4.86	-5.26
10	Dry bean weight per pod (g)	13.96	24.32
11	Single bean wt. (g)	4.38	10.20
12	Bean length (mm)	1.51	0.91
13	Bean breadth (mm)	0.46	9.75
14	Bean thickness (mm)	3.19	-1.68
15	Pod value	13.42	25.12
16	Pod index	-30.59	-26.32
17	Efficiency Index	-11.59	-66.48
18	Conversion Index	8.99	28.86
19	Dry Matter Recovery (%)	9.56	28.10
20	Fat content (%)	-3.18	-4.39
21	Phenol content (%)	0.75	23.15

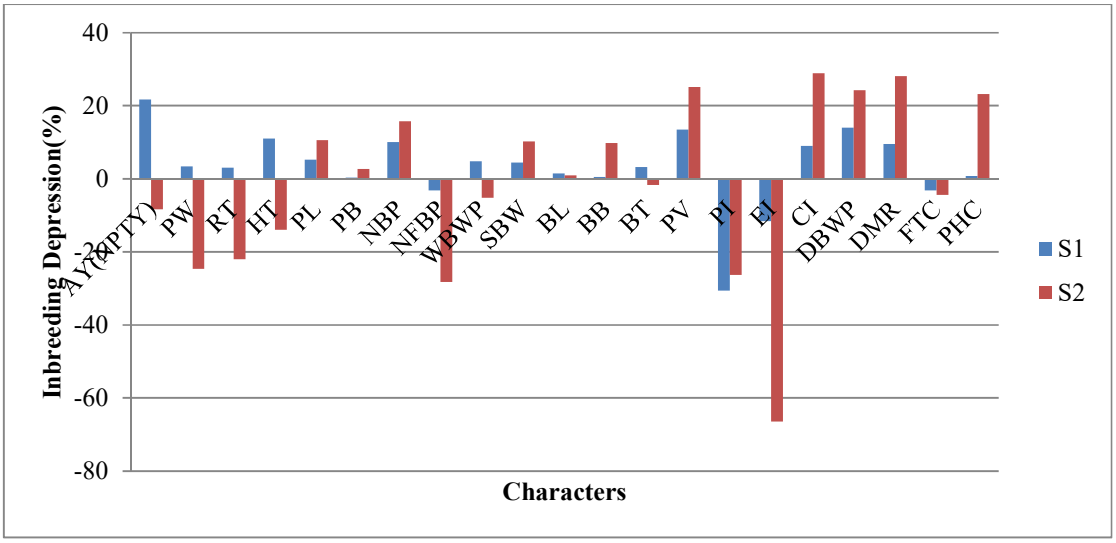


Fig. 53 Inbreeding depression in H 7.3 inbred over generations

inbreeding depression. The general tendency of cocoa population is exhibited in inbreeding population also (Nair, 2010).

S_2 generation showed inferiority in maximum number of traits when compared to its preceding generation S_1. Generally most of the characters were at par or even superior when compared to preceding generation indicating little inbreeding depression. This is supported by the study of Luiz *et al*. (1997). Similar reports were also recorded in a highly cross pollinated family cucurbitaceous (Allard, 1990., Nurgul and Rana, 2003. and Oviedo *et al.,*2008). Non significant role played by dominance and dominance x dominance form of epistasis may be the reason for lack of inbreeding depression. It was reported in INGENIC (1994) that, most of the economic traits of cocoa is controlled by additive genes.

4.2 Comparative evaluation of inbreds, inbred crosses and hybrids

The comparative evaluation of inbreds, inbred crosses and hybrids planted during 2015 was conducted at Cocoa Research Centre, Vellanikkara to establish the physiological relationship in the vigour between them. The results thus obtained through evaluation of inbreds, inbred crosses and hybrids based on biometric, physiological observation and plant phenology are presented below

4.2.1 Morphological characterization

The growth parameters such as plant height, collar girth, plant spread are recorded for individual plants in inbreds, inbred crosses and hybrids for three years from 2015 to 2018. The details of growth parameters such as plant height, girth and plant spread for inbreds, inbred crosses and hybrids in the year 2016 are presented in table 39, 40 and 41 respectively. The data in table 42, 43 and 44 represents the details of growth observation in 2017 for inbreds, inbred crosses and hybrids. The growth parameters of inbreds, inbred crosses and hybrids in 2018 are presented in table 45 to 50.

4.2.1.1 Evaluation of inbred self

The biometric observations of inbreds during 2018 are presented in table 45.

4.2.1.1.1 Plant height

The plant height in inbred self plants ranged between 70 and 310 cm in S_4 generation of M 18.7 (Stand number 2.8) and S_4 generation of G 4 35.7(Stand number 10.3) respectively. The average plant height of inbred population in the present study was 146.09 cm, whereas the jorquetting occurred in few plants, the height of chupan up to jorquetting ranged from 10 to 195 cm in S4 generation of G4 35.7 (Stand number 9.8) to S_4 generation of M 18.7 (Stand number 4.8) respectively. The average height of chupan was 116.23cm.

Table 39. Plant height, girth and plant spread of cocoa inbreds during 2016

S. No.	Generation	Genotype	Stand No	Plant height (cm)	E W (cm)	N S (cm)	Girth (cm)
1	S_4	M 18.7	1.1	75	85	95	8
2	S_4	M 18.7	1.2	95			6
3	S_4	M 18.7	1.4	85			7
4	S_4	M 18.7	1.5	80			6.5
5	S_4	M 18.7	1.6	85			6.5
6	S_4	M 18.7	1.9	80			8
7	S_4	M 18.7	1.10	90			5
8	S_4	M 18.7	1.11	75			6
9	S_4	M 18.7	1.12	95			8
10	S_4	M 18.7	1.13	95			6.5
11	S_4	M 18.7	1.14	105	70	60	8
12	S_4	M 18.7	1.15	115			6
13	S_4	M 18.7	2.1	90	85	80	7
14	S_4	M 18.7	2.4	105	50	55	10.5
15	S_4	M 18.7	2.5	50			6.5
16	S_4	M 18.7	2.6	95	70	60	8
17	S_4	M 18.7	2.7	110	60	45	8
18	S_4	M 18.7	2.8	95			7
19	S_4	M 18.7	2.9	55			5
20	S_4	M 18.7	2.10	105			7
21	S_4	M 18.7	2.11	105			7
22	S_4	M 18.7	2.12	45			5
23	S_4	M 18.7	2.13	110	100	95	9
24	S_4	M 18.7	2.14	125	80	65	9
25	S_4	M 18.7	2.15	50			7
26	S_4	M 18.7	3.1	60			4
27	S_4	M 18.7	3.2	70	85	80	7
28	S_4	M 18.7	3.3	65			6
29	S_4	M 18.7	3.4	75			8
30	S_4	M 18.7	3.5	75			5
31	S_4	M 18.7	3.8	90			6
32	S_4	M 18.7	3.9	110			8
33	S_4	M 18.7	3.10	125			8
34	S_4	M 18.7	3.11	100			7.5
35	S_4	M 18.7	3.12	105	100	110	9.5
36	S_4	M 18.7	3.13	130	130	115	9

37	S_4	M 18.7	3.14	120			8
38	S_4	M 18.7	3.15	90	95	70	9.5
39	S_4	M 18.7	4.1	65			6
40	S_4	M 18.7	4.2	85			8
41	S_4	M 18.7	4.3	110	95	70	8
42	S_4	M 18.7	4.4	150			9
43	S_4	M 18.7	4.6	115			8
44	S_4	M 18.7	4.7	70			6
45	S_4	M 18.7	4.8	145	100	80	9
46	S_4	M 18.7	4.9	130			8
47	S_4	M 18.7	4.10	80			6
48	S_4	M 18.7	4.12	95			7
49	S_4	M 18.7	4.13	105	100	80	9
50	S_4	M 18.7	4.14	105			12
51	S_4	M 18.7	4.15	65			6
52	S_4	M 18.7	5.1	70	75	80	10
53	S_4	M 18.7	5.2	60			8
54	S_4	M 18.7	5.3	75	65	70	10
55	S_4	M 18.7	5.4	65	110	75	11
56	S_4	M 18.7	5.5	85			8
57	S_4	M 18.7	5.6	50			8
58	S_4	M 18.7	5.7	65			10
59	S_4	M 18.7	5.8	95			9
60	S_4	M 18.7	5.9	65	65	90	9
61	S_4	M 18.7	5.10	70			8
62	S_4	M 18.7	5.11	75			10
63	S_4	M 18.7	5.12	65			10
64	S_4	M 18.7	5.13	95			8
65	S_4	M 18.7	5.14	100			10
66	S_4	M 18.7	5.15	130	100		10
67	S_4	M 18.7	6.1	70	130	120	8
68	S_4	M 18.7	6.2	125	70		10
69	S_4	M 18.7	6.3	70			9
70	S_4	M 18.7	6.4	100			11
71	S_4	M 18.7	6.6	55			9
72	S_4	M 18.7	6.7	95			8
73	S_4	M 18.7	6.8	60			8
74	S_4	M 18.7	6.9	110			8
75	S_4	M 18.7	6.10	100			8
76	S_4	M 18.7	6.11	60			9

77	S_1	G 4 35.7	1.16	120	120	90	9
78	S_1	G 4 35.7	1.17	130			8
79	S_1	G 4 35.7	3.16	75			8
80	S_1	G 4 35.7	3.17	110			9
81	S_1	G 4 35.7	4.16	90			9
82	S_1	G 4 35.7	4.17	120			9
83	S_1	G 4 35.7	5.16	100	70		11
84	S_1	G 4 35.7	5.17	115			9
85	S_1	G 4 35.7	6.16	90			10
86	S_1	G 4 35.7	6.17	120			8
87	S_1	G 4 35.7	7.17	150			10
88	S_1	G 4 35.7	8.16	110			9
89	S_1	G 4 35.7	8.17	75			12
90	S_1	G 4 35.7	9.17	120			9
91	S_1	G 4 35.7	10.16	100			14
92	S_1	G 4 35.7	10.17	115			9
93	S_4	G 4 35.7	6.13	95	90	95	11
94	S_4	G 4 35.7	6.14	145			11
95	S_4	G 4 35.7	6.15	100			8
96	S_4	G 4 35.7	7.1	100	110	105	11
97	S_4	G 4 35.7	7.4	80			12
98	S_4	G 4 35.7	7.6	105			9
99	S_4	G 4 35.7	7.7	120			9
100	S_4	G 4 35.7	7.9	85			8
101	S_4	G 4 35.7	7.10	65			8
102	S_4	G 4 35.7	7.11	135			10
103	S_4	G 4 35.7	7.12	105			9
104	S_4	G 4 35.7	7.13	100			10
105	S_4	G 4 35.7	8.1	110			9
106	S_4	G 4 35.7	8.2	115	95	105	11
107	S_4	G 4 35.7	8.3	110			12
108	S_4	G 4 35.7	8.4	80			8
109	S_4	G 4 35.7	8.5	85			9
110	S_4	G 4 35.7	8.6	115			12
111	S_4	G 4 35.7	8.7	90			9
112	S_4	G 4 35.7	8.8	110			10
113	S_4	G 4 35.7	8.9	100			6
114	S_4	G 4 35.7	8.10	55			9
115	S_4	G 4 35.7	8.11	85			9
116	S_4	G 4 35.7	8.14	90			9

117	S_4	G 4 35.7	8.15	150	115	0	11
118	S_4	G 4 35.7	9.1	70	0	0	6
119	S_4	G 4 35.7	9.2	130	0	0	9
120	S_4	G 4 35.7	9.5	155	0	0	9
121	S_4	G 4 35.7	9.7	110	0	0	6
122	S_4	G 4 35.7	9.8	85	0	0	6
123	S_4	G 4 35.7	9.10	80	0	0	7
124	S_4	G 4 35.7	9.11	90	0	0	5
125	S_4	G 4 35.7	9.12	105	0	0	7
126	S_4	G 4 35.7	9.13	95	0	0	6
127	S_4	G 4 35.7	9.14	115	0	0	8
128	S_4	G 4 35.7	9.15	110	0	0	9
129	S_4	G 4 35.7	10.1	100	0	0	8
130	S_4	G 4 35.7	10.3	100	0	0	8
131	S_4	G 4 35.7	10.8	90	0	0	8
132	S_4	G 4 35.7	10.9	110	0	0	8
133	S_4	G 4 35.7	10.10	85	0	0	7
134	S_4	G 4 35.7	10.11	75	0	0	6
135	S_4	G 4 35.7	10.12	130	0	0	9
136	S_4	G 4 35.7	10.14	65	0	0	8
137	S_4	G 4 35.7	10.15	140	0	0	10

Table 40. Plant height, girth and plant spread of cocoa inbred crosses during 2016

S. No.	Genotype	Stand No.	Plant height (cm)	E-W	N-S	Stem girth (cm)
1	S_3H_7 3(86) X S_3G_4 35.7	2.1	165	50	45	10
2	S_3H_7 3(86) X S_3G_4 35.7	2.2	120	60	50	9
3	S_3H_7 3(86) X S_3G_4 35.7	2.3	80	15	20	9
4	S_3H_7 3(86) X S_3G_4 35.7	2.4	85	55	45	9
5	S_3H_7 3(86) X S_3G_4 35.7	2.6	125	80	85	9
6	S_3H_7 3(86) X S_3G_4 35.7	2.7	110	40	35	8
7	S_3H_7 3(86) X S_3G_4 35.7	4.2	85	55	45	7
8	S_3H_7 3(86) X S_3G_4 35.7	4.3	85	55	50	9
9	S_3H_7 3(86) X S_3G_4 35.7	4.4	100	55	60	8
10	S_3H_7 3(86) X S_3G_4 35.7	4.5	130	45	45	8
11	S_3H_7 3(86) X S_3G_4 35.7	4.6	140	50	45	9
12	S_3H_7 3(86) X S_3G_4 35.7	4.7	95	40	40	7
13	S_3H_7 3(86) X S_3G_4 35.7	5.2	50			8
14	S_3H_7 3(86) X S_3G_4 35.7	5.3	115			7
15	S_3H_7 3(86) X S_3G_4 35.7	6.1	110	20	40	8
16	S_3H_7 3(86) X S_3G_4 35.7	6.2	100			9
17	S_3H_7 3(86) X S_3G_4 35.7	6.3	65			7
18	S_3H_7 3(86) X S_3G_4 35.7	7.1	65			7
19	S_3H_7 3(86) X S_3G_4 35.7	7.2	95			7
20	S_3H_7 3(86) X S_3G_4 35.7	7.3	100			8
21	S_3H_7 3(86) X S_3G_4 35.7	7.4	100			10
22	S_3H_7 3(86) X S_3G_4 35.7	7.5	100	35		8
23	$S_3G_2$7.4X$S_3G_4$35.7	1.1	110	55	40	9
24	$S_3G_2$7.4X$S_3G_4$35.7	1.3	120	55	50	8
25	$S_3G_2$7.4 X$S_3G_4$35.7	3.1	120	65	70	9
26	$S_3G_2$7.4 X$S_3G_4$35.7	3.2	115	45	40	8
27	$S_3G_2$7.4 X$S_3G_4$35.7	3.3	100	45	45	7
28	$S_3G_2$7.4 X$S_3G_4$35.7	3.4	120	40	40	8
29	$S_3G_2$7.4 X$S_3G_4$35.7	3.8	90	45	60	7
30	$S_3G_2$7.4 X$S_3G_4$35.7	4.1	105	95	85	10
31	$S_3G_2$7.4 X$S_3G_4$35.7	4.8	85	50	60	8

32	$S_3G_2$7.4 $XS_3G_4$35.7	5.4	105			8
33	$S_3G_2$7.4 $XS_3G_4$35.7	5.6	105			7
34	$S_3G_2$7.4 $XS_3G_4$35.7	5.7	105			7
35	$S_3G_2$7.4 $XS_3G_4$35.7	5.8	70			9
36	$S_3G_2$7.4 $XS_3G_4$35.7	6.4	50			7
37	$S_3G_2$7.4 $XS_3G_4$35.7	6.5	110			9
38	$S_3G_2$7.4 $XS_3G_4$35.7	6.6	125			10
39	$S_3G_2$7.4 $XS_3G_4$35.7	6.8	95			9
40	$S_5G_1$7.4 x S_3 G_4 35.7	3.5	55			8
41	$S_5G_1$7.4 x S_3 G_4 35.7	3.7	85	60	45	9

Table 41. Plant height, girth and plant spread of cocoa hybrids during 2016

S. No.	Hybrid	Stand No.	Plant height (cm)	E W (cm)	N S (cm)	Girth (cm)
1.	CCRP 8	8.1	60			6.5
2.	CCRP 8	8.2	100			8.5
3.	CCRP 8	8.4	70			6.5
4.	CCRP 8	23.6	80	60		7.5
5.	CCRP 8	23.7	105			9.0
6.	CCRP 8	8.5L	95			9.0
7.	CCRP 8	8.6L	55			6.0
8.	CCRP 8	23.3L	55			6.0
9.	CCRP 9	9.2	60	85	60	8.0
10.	CCRP 9	9.3	45			5.5
11.	CCRP 9	24.6	75			6.5
12.	CCRP 9	24.7	90			8.0
13.	CCRP 9	9.5L	85			7.0
14.	CCRP 9	9.6L	70			7.0
15.	CCRP 9	24.3L	115			8.5
16.	CCRP 10	10.2	100	65	30	8.5
17.	CCRP 10	25.6	40			5.0
18.	CCRP 10	25.7	90	80		8.0
19.	CCRP 10	10.5L	85			7.5
20.	CCRP 10	10.6L	60			6.0
21.	CCRP 10	25.1L	65			6.0
22.	CCRP 10	25.2L	92			8.0
23.	CCRP 10	25.3L	75			6.5
24.	CCRP 11	11.3	50			5.0
25.	CCRP 11	11.4	60			6.0
26.	CCRP 11	11.5	90			8.0
27.	CCRP 11	26.6	50			4.5
28.	CCRP 11	26.7	60			6.0
29.	CCRP 11	11.6L	65			6.0
30.	CCRP 11	26.1L	60			6.0
31.	CCRP 11	26.2L	80			7.0
32.	CCRP 11	26.3L	100			8.0
33.	CCRP 12	12.3	75			7.5
34.	CCRP 12	12.4	95			8.5
35.	CCRP 12	12.5	50			5.0
36.	CCRP 12	12.6	65			6.5
37.	CCRP 12	27.6	50			6.0

38.	CCRP 12	27.7	60			6.0
39.	CCRP 12	12.6L	75			6.5
40.	CCRP 12	27.1L	100			8.0
41.	CCRP 12	27.2L	65			6.0
42.	CCRP 12	27.3L	85			8.0
43.	CCRP 13	13.4	75			6.5
44.	CCRP 13	13.6	75			6.0
45.	CCRP 13	28.6	95			8.5
46.	CCRP 13	28.7	55			6.0
47.	CCRP 13	13.6L	75			6.5
48.	CCRP 13	28.2L	65			6.0
49.	CCRP 13	28.3L	25			3.0
50.	CCRP 14	14.3	55			5.5
51.	CCRP 14	14.4	50			5.0
52.	CCRP 14	14.5	90			8.0
53.	CCRP 14	14.6	70			6.5
54.	CCRP 14	29.6	75			6.5
55.	CCRP 14	29.7	55			4.5
56.	CCRP 14	14.6L	70			6.5
57.	CCRP 14	29.1L	85			8.0
58.	CCRP 14	29.2L	65			6.0
59.	CCRP 15	15.3	90			8.0
60.	CCRP 15	15.4	100			8.5
61.	CCRP 15	15.5	70			6.0
62.	CCRP 15	15.6	90			8.5
63.	CCRP 15	31.6	105	95	85	9.0
64.	CCRP 15	15.6L	70			6.5
65.	CCRP 15	30.1L	65			6.0
66.	CCRP 15	30.2L	75			6.5
67.	CCRP 15	30.3L	110			8.5

Table 42. Plant height, plant spread and collar girth of inbreds during 2017

S. No.	Generation	Genotype	Stand No	Plant height (cm)	E W (cm)	N S (cm)	Girth (cm)
1	S_4	M 18.7	1.1	130	95	115	10
2	S_4	M 18.7	1.2	110	55		8
3	S_4	M 18.7	1.4	140	55		7
4	S_4	M 18.7	1.5	130	55		10
5	S_4	M 18.7	1.6	125	70		10
6	S_4	M 18.7	1.8	65			12
7	S_4	M 18.7	1.9	110	55	115	16
8	S_4	M 18.7	1.10	125			9
9	S_4	M 18.7	1.12	115			10
10	S_4	M 18.7	1.13	100			8
11	S_4	M 18.7	1.14	130	100	85	9
12	S_4	M 18.7	2.1	160	95	105	12
13	S_4	M 18.7	2.4	135	80	75	16
14	S_4	M 18.7	2.5	100	55		8
15	S_4	M 18.7	2.6	145	140	110	13
16	S_4	M 18.7	2.7	125	70	75	10
17	S_4	M 18.7	2.8	120	80	75	10
18	S_4	M 18.7	2.9	140	55	80	9
19	S_4	M 18.7	2.10	190	30		16
20	S_4	M 18.7	2.11	130			10
21	S_4	M 18.7	2.13	145	100		13
22	S_4	M 18.7	2.14	145	135	90	14
23	S_4	M 18.7	3.1	90			6
24	S_4	M 18.7	3.2	140	70	85	12
25	S_4	M 18.7	3.3	100	70		9
26	S_4	M 18.7	3.4	120	75	70	10
27	S_4	M 18.7	3.5	85			6
28	S_4	M 18.7	3.8	130	85	105	11
29	S_4	M 18.7	3.9	125	120	130	13
30	S_4	M 18.7	3.10	135	65		12
31	S_4	M 18.7	3.11	125	55		9
32	S_4	M 18.7	3.12	175	120	115	15
33	S_4	M 18.7	3.13	195	145	195	14
34	S_4	M 18.7	3.14	180	110	70	12
35	S_4	M 18.7	3.15	100	140	100	16
36	S_4	M 18.7	4.3	195	130	115	14

37	S_4	M 18.7	4.4	195	180	75	13
38	S_4	M 18.7	4.6	140	65		10
39	S_4	M 18.7	4.7	110			9
40	S_4	M 18.7	4.8	195	150	150	15
41	S_4	M 18.7	4.9	145	80	75	16
42	S_4	M 18.7	4.10	145	70		12
43	S_4	M 18.7	4.12	140	95		8
44	S_4	M 18.7	4.13	160	85	65	12
45	S_4	M 18.7	4.14	140	55	55	16
46	S_4	M 18.7	5.1	155	140	110	12
47	S_4	M 18.7	5.2	120			12
48	S_4	M 18.7	5.3	120			13
49	S_4	M 18.7	5.4	80			12.5
50	S_4	M 18.7	5.6	65			10
51	S_4	M 18.7	5.7	130	30		12
52	S_4	M 18.7	5.8	135	55		10
53	S_4	M 18.7	5.9	135			13
54	S_4	M 18.7	5.10	110	65	70	10
55	S_4	M 18.7	5.12	115	50		11
56	S_4	M 18.7	5.13	115	50		10
57	S_4	M 18.7	5.14	175			12
58	S_4	M 18.7	5.15	175	160	115	15
59	S_4	M 18.7	6.1	90	195	215	19
60	S_4	M 18.7	6.2	160	120	60	11
61	S_4	M 18.7	6.6	70	30		12
62	S_4	M 18.7	6.7	100			11
63	S_4	M 18.7	6.8	65			10
64	S_4	M 18.7	6.9	165	85		11
65	S_4	M 18.7	6.10	120			9
66	S_4	M 18.7	6.11	65	30		12
67	S_1	G 4 35.7	1.16	135	125	135	18
68	S_1	G 4 35.7	1.17	150	145		12
69	S_1	G 4 35.7	2.16	135	130	125	10.5
70	S_1	G 4 35.7	2.17	130	180	135	13
71	S_1	G 4 35.7	3.16				
72	S_1	G 4 35.7	3.17	130	195	170	12
73	S_1	G 4 35.7	4.17	125	195	85	11
74	S_1	G 4 35.7	5.16				
75	S_1	G 4 35.7	5.17	140	65	45	11
76	S_1	G 4 35.7	6.16	100	45	60	13

77	S_1	G 4 35.7	6.17				
78	S_1	G 4 35.7	7.17	175	130	85	12
79	S_1	G 4 35.7	8.16	120	85	65	13
80	S_1	G 4 35.7	8.17	100			13.5
81	S_1	G 4 35.7	9.16	120	170	95	15
82	S_1	G 4 35.7	10.16	120	130	150	15
83	S_1	G 4 35.7	10.17				
84	S_4	G 4 35.7	6.13	110			12.5
85	S_4	G 4 35.7	6.14	170	60	65	15
86	S_4	G 4 35.7	6.15	135			10
87	S_4	G 4 35.7	7.1	120	160	195	16
88	S_4	G 4 35.7	7.3	100	85	70	9
89	S_4	G 4 35.7	7.4	110	105	120	14
90	S_4	G 4 35.7	7.6	155	90	85	11
91	S_4	G 4 35.7	7.9	145	35		10
92	S_4	G 4 35.7	7.10	160	80	85	12
93	S_4	G 4 35.7	7.11	145	50		12
94	S_4	G 4 35.7	7.16	150	160	120	14
95	S_4	G 4 35.7	8.2	125	150	100	15
96	S_4	G 4 35.7	8.3	120	145	100	13
97	S_4	G 4 35.7	8.5	160	120	90	16
98	S_4	G 4 35.7	8.6	160	75	45	13
99	S_4	G 4 35.7	8.7	155	70	85	15
100	S_4	G 4 35.7	8.8	115			11
101	S_4	G 4 35.7	8.10	90	40	30	10
102	S_4	G 4 35.7	8.14	180	60	180	13
103	S_4	G 4 35.7	8.15	160			13
104	S_4	G 4 35.7	9.1				
105	S_4	G 4 35.7	9.2	170	75	110	15
106	S_4	G 4 35.7	9.5	165	130	85	13
107	S_4	G 4 35.7	9.7	130	75	55	9
108	S_4	G 4 35.7	9.8	100			8
109	S_4	G 4 35.7	9.9	80	65	55	9
110	S_4	G 4 35.7	9.10	115			9
111	S_4	G 4 35.7	9.12	160			10
112	S_4	G 4 35.7	9.13	105			10
113	S_4	G 4 35.7	9.14	135			11
114	S_4	G 4 35.7	10.1	145	90	80	13
115	S_4	G 4 35.7	10.3	195	195	180	16
116	S_4	G 4 35.7	10.4	115			5

117	S_4	G 4 35.7	10.7	100	80	120	13
118	S_4	G 4 35.7	10.8	105	85	100	10
119	S_4	G 4 35.7	10.10	135			10
120	S_4	G 4 35.7	10.11	150	85	90	13
121	S_4	G 4 35.7	10.14	195	70	65	15
122	S_4	G 4 35.7	10.15	225	125	130	17

Table 43. Plant height, plant spread and collar girth of inbred crosses during 2017

S. No.	Inbred cross	Stand No	Plant height (cm)	E W (cm)	N S (cm)	Girth (cm)
1	$S_3G_27XS_3G_435.7$	1.1	185			13
2	$S_3G_27XS_3G_435.7$	3.1	145	40		15
3	$S_3G_27XS_3G_435.7$	3.2	135			10
4	$S_3G_27XS_3G_435.7$	3.3	125			9
5	$S_3G_27.4XS_3G_435.7$	3.4	135			10
6	$S_3G_27XS_3G_435.7$	3.8	110			10
7	$S_3G_27XS_3G_435.7$	4.1	115	55		14
8	$S_3G_27XS_3G_435.7$	4.8	135			9
9	$S_3G_27XS_3G_435.7$	5.4	125			9
10	$S_3G_27XS_3G_435.7$	5.6	130			12
11	$S_3G_27XS_3G_435.7$	5.7	145			10
12	$S_3G_27XS_3G_435.7$	6.5	130			12
13	$S_3G_27XS_3G_435.7$	6.8	155			10
14	S_3H_7 3(86) X S_3G_4 35.7	2.1	180			12
15	S_3H_7 3(86) X S_3G_4 35.7	2.2	125			12
16	S_3H_7 3(86) X S_3G_4 35.7	2.4	105			10
17	S_3H_7 3(86) X S_3G_4 35.7	2.6	55	160		13
18	S_3H_7 3(86) X S_3G_4 35.7	4.4	140			10
19	S_3H_7 3(86) X S_3G_4 35.7	4.5	150			10
20	S_3H_7 3(86) X S_3G_4 35.7	4.6	170			14
21	S_3H_7 3(86) X S_3G_4 35.7	4.7	170			9
22	S_3H_7 3(86) X S_3G_4 35.7	5.3	150			14
23	S_3H_7 3(86) X S_3G_4 35.7	6.1	135			12
24	S_3H_7 3(86) X S_3G_4 35.7	7.3	120			10
25	S_3H_7 3(86) X S_3G_4 35.7	7.4	120			16
26	S_3H_7 3(86) X S_3G_4 35.7	7.5	130			12

Table 44. Plant height, plant spread and collar girth of hybrids during 2017

S. No	Hybrid	Stand No	Plant height (cm)	E W (cm)	N S (cm)	Girth (cm)
1	CCRP 8	8.1	95			8.5
2	CCRP 8	8.2	150			8.5
4	CCRP 8	8.4	80			12
5	CCRP 8	23.6	80	70		9.5
6	CCRP 8	23.7	180			9.5
9	CCRP 8	23.3L	100			9
10	CCRP 9	9.3	135			8
11	CCRP 9	9.4	105			10
12	CCRP 9	9.5	75			9
13	CCRP 9	24.6	140			11
14	CCRP 9	24.7	92			
16	CCRP 9	24.3L	120			12
17	CCRP 10	10.2	50	75	50	12
18	CCRP 10	10.3	105			9
19	CCRP 10	10.4	35	90		11
20	CCRP 10	25.3 L	150	140	135	18
21	CCRP 10	10.6L	60			9
22	CCRP 10	25.1L	65			8
23	CCRP 10	25.2L	92			10
24	CCRP 10	25.3L	75			10
25	CCRP 11	11.3	50			10
26	CCRP 11	11.4	60			12
27	CCRP 11	11.5	90			12
28	CCRP 11	26.6	95			9
29	CCRP 11	26.7	95			9
30	CCRP 11	11.6L	110			9
31	CCRP 11	26.1L	60			9
32	CCRP 11	26.2L	80			11
33	CCRP 11	26.3L	100			12
34	CCRP 12	12.3	75			12
35	CCRP 12	12.4	95			12
36	CCRP 12	12.5	95			11
37	CCRP 12	12.6	65			11
38	CCRP 12	27.6	120			12

39	CCRP 12	27.7	125			12
40	CCRP 12	12.6L	75			11
41	CCRP 12	27.1L	100			13
42	CCRP 12	27.2L	65			10
43	CCRP 12	27.3L	85			11
44	CCRP 13	13.4	75			9
45	CCRP 13	13.6	75			9.5
46	CCRP 13	28.6	95			12.5
47	CCRP 13	28.7	55			12.5
48	CCRP 13	13.6L	75			11
49	CCRP 13	28.2L	65			9.5
50	CCRP 13	28.3L	95			4.5
51	CCRP 14	14.3	85			5.5
52	CCRP 14	14.4	50			5.5
53	CCRP 14	14.5	110			9
54	CCRP 14	14.6	105			7.5
55	CCRP 14	29.6	75			8.5
56	CCRP 14	29.7	80			5.5
57	CCRP 14	14.6L	70			9.5
58	CCRP 14	29.1L	85			9.5
59	CCRP 14	29.2L	105			10
61	CCRP 15	15.3	90			11
62	CCRP 15	15.4	155			12
63	CCRP 15	15.5	70			9
64	CCRP 15	15.6	90			12.5
65	CCRP 15	31.6	125	120	105	13
66	CCRP 15	15.6	70			11
67	CCRP 15	30.1L	65			10
68	CCRP 15	30.2L	75			11
69	CCRP 15	30.3L	110			12

Table 45. Plant height, plant spread and collar girth in inbreds during 2018

S. No.	Generation	Inbred self	Stand No	Plant height (cm)	E W (cm)	N S (cm)	Girth (cm)	Chupan height (cm)
1	S_4	M 18.7	1.1		240	250	18	65
2	S_4	M 18.7	1.2	175	125	150	13	
3	S_4	M 18.7	1.4	110	60	80	9	
4	S_4	M 18.7	1.5	210	165	180	15	
5	S_4	M 18.7	1.6	180	200	200	18	
6	S_4	M 18.7	1.8	135	120	165	13	
7	S_4	M 18.7	1.9	235	250	200	22	
8	S_4	M 18.7	1.10	165	150	175	12	
9	S_4	M 18.7	1.12	120	65	110	11	
10	S_4	M 18.7	1.13	135	140	120	13	
11	S_4	M 18.7	1.14	100	135	140	13	
12	S_4	M 18.7	2.1		225	200	16	95
13	S_4	M 18.7	2.4	85	200	200	22	
14	S_4	M 18.7	2.5	95	55	55	12	
15	S_4	M 18.7	2.6	70	220	230	19	
16	S_4	M 18.7	2.7	110	165	170	14	
17	S_4	M 18.7	2.8	70	220	200	140	
18	S_4	M 18.7	2.11	130	30	30	9	
19	S_4	M 18.7	2.13	90	200	175	15	
20	S_4	M 18.7	2.14	105	175	150	14	
21	S_4	M 18.7	3.1	145	70	75	9	
22	S_4	M 18.7	3.2	200	170	165	14	
23	S_4	M 18.7	3.3		140	80	11	70
24	S_4	M 18.7	3.4		160	125	13	75
25	S_4	M 18.7	3.8		220	290	17	65
26	S_4	M 18.7	3.9		360	390	21	
27	S_4	M 18.7	3.10	125	145	175	17	
28	S_4	M 18.7	3.11	135	125	100	11	
29	S_4	M 18.7	3.12	110	315	310	22	
30	S_4	M 18.7	3.13	245	325	400	24	
31	S_4	M 18.7	3.14	220	250	130	17	
32	S_4	M 18.7	3.15	215	115	105	19	
33	S_4	M 18.7	4.3	220	290	340	28	
34	S_4	M 18.7	4.4		295	245	20	150
35	S_4	M 18.7	4.6		140	180	17	90
36	S_4	M 18.7	4.7		105	85	10	85

37	S_4	M 18.7	4.8		315	350	24	195
38	S_4	M 18.7	4.9		315	360	23	120
39	S_4	M 18.7	4.10		135	160	12	80
40	S_4	M 18.7	4.12		155	140	12	70
41	S_4	M 18.7	4.13	200	200	200	16	
42	S_4	M 18.7	4.14	155	120	120	16	
43	S_4	M 18.7	5.1		200	220	16	75
44	S_4	M 18.7	5.2		60	55	7	70
45	S_4	M 18.7	5.4		70	90	11	100
46	S_4	M 18.7	5.7	105	90	115	10	
47	S_4	M 18.7	5.8	120	140	120	12	
48	S_4	M 18.7	5.9	120	95	75	11	
49	S_4	M 18.7	5.10		175	160	13	160
50	S_4	M 18.7	5.13		150	140	15	135
51	S_4	M 18.7	5.14	145	105	125	9	
52	S_4	M 18.7	5.15	235	260	235	23	
53	S_4	M 18.7	6.2	120	180	200	20	
54	S_4	M 18.7	6.8		95	125	9	85
55	S_4	M 18.7	6.9	115	105	130	9	
56	S_4	M 18.7	6.10		85	90	11	65
57	S_4	G 4 35.7	6.13		135	130	11	150
58	S_4	G 4 35.7	6.14		200	165	10	125
59	S_4	G 4 35.7	7.1		220	280	17	110
60	S_4	G 4 35.7	7.3	120	70	50	9	
61	S_4	G 4 35.7	7.4		220	220	12	115
62	S_4	G 4 35.7	7.6	150	55	40	12	
63	S_4	G 4 35.7	7.8		135	200	12	140
64	S_4	G 4 35.7	7.10		170	170	16	105
65	S_4	G 4 35.7	7.11		50	150	9	115
66	S_4	G 4 35.7	8.2		200	200	17	120
67	S_4	G 4 35.7	8.3		100	100	12	145
68	S_4	G 4 35.7	8.5		250	220	15	160
69	S_4	G 4 35.7	8.6		170	165	14	170
70	S_4	G 4 35.7	8.7		200	250	17	100
71	S_4	G 4 35.7	8.8		180	120	14	125
72	S_4	G 4 35.7	8.10		175	100	12	90
73	S_4	G 4 35.7	8.14		140	220	16	190
74	S_4	G 4 35.7	9.2	200	200	200	14	
75	S_4	G 4 35.7	9.5		230	215	17	145
76	S_4	G 4 35.7	9.7		160	18	12	130

77	S_4	G 4 35.7	9.8	90	40	30	10	10
78	S_4	G 4 35.7	9.9		170	150	11	80
79	S_4	G 4 35.7	9.10	135	100	100	10	
80	S_4	G 4 35.7	9.12	135	40	65	8	
81	S_4	G 4 35.7	9.13		105	105	14	100
82	S_4	G 4 35.7	9.14		90	100	10	120
83	S_4	G 4 35.7	10.1		200	265	20	165
84	S_4	G 4 35.7	10.3	310	365	335	19	
85	S_4	G 4 35.7	10.7		230	260	19	85
86	S_4	G 4 35.7	10.8		220	250	16	110
87	S_4	G 4 35.7	10.10		135	135	14	125
88	S_4	G 4 35.7	10.11		165	200	16	130
89	S_4	G 4 35.7	10.14		100	135	16	160
90	S_4	G 4 35.7	10.15		335	225	18	170
91	S_1	G 4 35.7	1.16	100	325	305	23	
92	S_1	G 4 35.7	1.17	150	385	360	20	
93	S_1	G 4 35.7	2.16		365	400	24	140
94	S_1	G 4 35.7	2.17		235	240	19	130
95	S_1	G 4 35.7	3.17		350	385	23	110
96	S_1	G 4 35.7	5.17		145	130	13	125
97	S_1	G 4 35.7	6.16		200	200	15	165
98	S_1	G 4 35.7	7.16		320	285	23	155
99	S_1	G 4 35.7	7.17		330	250	19	135
100	S_1	G 4 35.7	8.16		170	200	12	90
101	S_1	G 4 35.7	8.17	80	20	20	12	
102	S_1	G 4 35.7	9.16		240	290	23	125
103	S_1	G 4 35.7	10.16		110	200	20	110

Table 46. Leaf area of inbreds of cocoa during 2018.

S. No.	Generation	Genotype	Stand No.	Leaf area (cm^2)
1	S_4	M 18.7	1.1	129.65
2	S_4	M 18.7	1.2	63.36
3	S_4	M 18.7	1.4	162.30
4	S_4	M 18.7	1.5	49.88
5	S_4	M 18.7	1.6	84.91
6	S_4	M 18.7	1.8	348.64
7	S_4	M 18.7	1.9	187.19
8	S_4	M 18.7	1.1	89.81
9	S_4	M 18.7	1.12	151.69
10	S_4	M 18.7	1.13	265.02
11	S_4	M 18.7	1.14	180.26
12	S_4	M 18.7	2.1	156.38
13	S_4	M 18.7	2.4	160.94
14	S_4	M 18.7	2.5	174.08
15	S_4	M 18.7	2.6	79.47
16	S_4	M 18.7	2.7	124.19
17	S_4	M 18.7	2.8	209.73
18	S_4	M 18.7	2.9	365.30
19	S_4	M 18.7	2.1	252.55
20	S_4	M 18.7	2.11	161.09
21	S_4	M 18.7	2.13	83.83
22	S_4	M 18.7	2.14	331.81
23	S_4	M 18.7	3.1	363.73
24	S_4	M 18.7	3.2	212.13
25	S_4	M 18.7	3.3	224.58
26	S_4	M 18.7	3.4	128.75
27	S_4	M 18.7	3.5	270.64
28	S_4	M 18.7	3.8	343.77
29	S_4	M 18.7	3.9	531.04
30	S_4	M 18.7	3.1	130.13
31	S_4	M 18.7	3.11	224.86
32	S_4	M 18.7	3.12	187.90
33	S_4	M 18.7	3.14	200.08
34	S_4	M 18.7	3.15	347.71

35	S_4	M 18.7	4.1	291.22
36	S_4	M 18.7	4.2	157.77
37	S_4	M 18.7	4.3	151.00
38	S_4	M 18.7	4.4	123.36
39	S_4	M 18.7	4.5	114.18
40	S_4	M 18.7	4.6	60.52
41	S_4	M 18.7	4.7	76.14
42	S_4	M 18.7	4.8	219.57
43	S_4	M 18.7	4.9	122.97
44	S_4	M 18.7	4.10	227.49
45	S_4	M 18.7	4.11	202.99
46	S_4	M 18.7	4.12	144.26
47	S_4	M 18.7	4.13	78.49
48	S_4	M 18.7	4.14	355.20
49	S_4	M 18.7	4.15	168.60
50	S_4	M 18.7	5.1	173.08
51	S_4	M 18.7	5.2	196.02
52	S_4	M 18.7	5.4	231.33
53	S_4	M 18.7	5.6	162.73
54	S_4	M 18.7	5.7	482.86
55	S_4	M 18.7	5.8	250.45
56	S_4	M 18.7	5.9	274.08
57	S_4	M 18.7	5.12	125.30
58	S_4	M 18.7	5.1	335.49
59	S_4	M 18.7	5.13	62.17
60	S_4	M 18.7	5.14	221.95
61	S_4	M 18.7	5.15	191.81
62	S_4	M 18.7	6.1	132.39
63	S_4	M 18.7	6.2	44.45
64	S_4	M 18.7	6.6	132.92
65	S_4	M 18.7	6.7	371.42
66	S_4	M 18.7	6.8	151.95
67	S_4	M 18.7	6.9	162.47
68	S_4	M 18.7	6.1	215.56
69	S_4	M 18.7	6.11	161.89
70	S_1	G 4 35.7	1.16	175.17
71	S_1	G 4 35.7	1.17	357.51
72	S_1	G 4 35.7	2.16	151.81

73	S_1	G 4 35.7	2.17	289.72
74	S_1	G 4 35.7	3.17	122.41
75	S_1	G 4 35.7	5.17	133.18
76	S_1	G 4 35.7	6.16	168.17
77	S_1	G 4 35.7	7.16	119.42
78	S_1	G 4 35.7	7.17	260.20
79	S_1	G 4 35.7	8.16	186.52
80	S_1	G 4 35.7	8.17	192.81
81	S_1	G 4 35.7	9.16	133.39
82	S_1	G 4 35.7	10.16	45.45
83	S_4	G 4 35.7	6.13	136.92
84	S_4	G 4 35.7	6.14	371.42
85	S_4	G 4 35.7	6.15	156.95
86	S_4	G 4 35.7	7.1	161.47
87	S_4	G 4 35.7	7.3	225.56
88	S_4	G 4 35.7	7.4	162.89
89	S_4	G 4 35.7	7.6	172.17
90	S_4	G 4 35.7	7.8	347.51
91	S_4	G 4 35.7	7.10	153.81
92	S_4	G 4 35.7	7.11	286.72
93	S_4	G 4 35.7	8.2	132.41
94	S_4	G 4 35.7	8.3	113.18
95	S_4	G 4 35.7	8.5	158.17
96	S_4	G 4 35.7	8.6	139.42
97	S_4	G 4 35.7	8.7	266.20
98	S_4	G 4 35.7	8.8	256.23
99	S_4	G 4 35.7	8.10	139.45
100	S_4	G 4 35.7	8.14	192.56
101	S_4	G 4 35.7	9.2	134.56
102	S_4	G 4 35.7	9.5	49.25
103	S_4	G 4 35.7	9.7	134.56
104	S_4	G 4 35.7	9.8	348.26
105	S_4	G 4 35.7	9.1	153.48
106	S_4	G 4 35.7	9.12	165.89
107	S_4	G 4 35.7	9.13	254.26
108	S_4	G 4 35.7	9.14	163.25
109	S_4	G 4 35.7	10.1	176.45
110	S_4	G 4 35.7	10.3	359.45

111	S_4	G 4 35.7	10.7	162.45
112	S_4	G 4 35.7	10.8	286.89
113	S_4	G 4 35.7	10.1	124.26
114	S_4	G 4 35.7	10.11	134.25
115	S_4	G 4 35.7	10.14	172.46
116	S_4	G 4 35.7	10.15	120.45

Table 47. Plant height, plant spread and collar girth of inbred crosses during 2018

S. No.	Inbred cross	Stand No.	Plant height (cm)	E W (cm)	N S (cm)	Girth (cm)	Chupan height (cm)
1	$S_3G_27.4$ X $S_3G_435.7$	1.1		90	100	11	100
2	$S_3G_27XS_3G_435.7$	3.1	105	40	35	11	
3	$S_3G_27XS_3G_435.7$	3.2		140	85	11	150
4	$S_3G_27XS_3G_435.7$	3.3		35	35	9	135
5	$S_3G_27XS_3G_435.7$	3.4	155	20	40	9	
6	$S_3G_27XS_3G_435.7$	4.8		110	110	12	125
7	$S_3G_27XS_3G_435.7$	5.6		40	45	9	130
8	$S_3G_27XS_3G_435.7$	5.7	155	95	80	12	
9	S_3H_7 3(86) X S_3G_4 35.7	2.6	200	150	170	13	
10	S_3H_7 3(86) X S_3G_4 35.7	4.4	90	25	50	11	
11	S_3H_7 3(86) X S_3G_4 35.7	4.5	195	20	25	7	
12	S_3H_7 3(86) X S_3G_4 35.7	4.6	155	45	35	12	
13	S_3H_7 3(86) X S_3G_4 35.7	4.7	105	20	25	6	
14	S_3H_7 3(86) X S_3G_4 35.7	7.4		75	50	7	130
15	S_3H_7 3(86) X S_3G_4 35.7	7.5	150	110	105	14	

Table 48. Leaf area of inbred crosses of cocoa

S. No.	Inbred cross	Plant No.	Leaf area (cm^2)
1	S_3 $G_2$7.4 X S_3G_4 35.7	1.1	50.50
2	S_3 $G_2$7.4 X S_3G_4 35.7	3.1	195.89
3	S_3 $G_2$7.4 X S_3G_4 35.7	3.2	175.35
4	S_3 $G_2$7.4 X S_3G_4 35.7	3.3	130.86
5	S_3 $G_2$7.4 X S_3G_4 35.7	3.4	88.53
6	S_3 $G_2$7.4 X S_3G_4 35.7	3.8	118.60
7	S_3 $G_2$7.4 X S_3G_4 35.7	4.1	148.65
8	S_3 $G_2$7.4 X S_3G_4 35.7	4.8	123.13
9	S_3 $G_2$7.4 X S_3G_4 35.7	5.7	191.58
10	S_3 $G_2$7.4 X S_3G_4 35.7	5.7	336.96
11	S_3 $G_2$7.4 X S_3G_4 35.7	5.8	136.34
12	S_3 $G_2$7.4 X S_3G_4 35.7	6.6	195.93
13	S_3 $G_2$7.4 X S_3G_4 35.7	6.4	86.34
14	S_3 $G_2$7.4 X S_3G_4 35.7	6.5	93.19
15	S_3 $G_2$7.4 X S_3G_4 35.7	6.8	114.71
16	S_3H_7 3 (86) X S_3G_4 35.7	2.1	97.30
17	S_3H_7 3 (86) X S_3G_4 35.7	2.2	162.99
18	S_3H_7 3 (86) X S_3G_4 35.7	2.4	137.36
19	S_3H_7 3 (86) X S_3G_4 35.7	2.6	120.94
20	S_3H_7 3 (86) X S_3G_4 35.7	2.7	111.68
21	S_3H_7 3 (86) X S_3G_4 35.7	4.4	123.28
22	S_3H_7 3 (86) X S_3G_4 35.7	4.6	158.37
23	S_3H_7 3 (86) X S_3G_4 35.7	4.4	256.33
24	S_3H_7 3 (86) X S_3G_4 35.7	4.5	103.53
25	S_3H_7 3 (86) X S_3G_4 35.7	4.6	301.46
26	S_3H_7 3 (86) X S_3G_4 35.7	4.7	331.66
27	S_3H_7 3 (86) X S_3G_4 35.7	5.3	303.81

28	S_3H_7 3 (86) X S_3G_4 35.7	7.2	263.62
29	S_3H_7 3 (86) X S_3G_4 35.7	7.4	358.29
30	S_3H_7 3 (86) X S_3G_4 35.7	7.5	414.66
31	S_3H_7 3 (86) X S_3G_4 35.7	6.1	233.43
32	S_5G_1 7.4 X S_3G_4 35.7	3.5	142.50
33	S_5G_1 7.4 X S_3G_4 35.7	3.6	122.10
34	S_5G_1 7.4 X S_3G_4 35.7	3.7	172.57

Table 49. Plant height, plant spread and collar girth in hybrids during 2018

S. No.	Hybrid	Stand No	Plant height (cm)	E W (cm)	N S (cm)	Girth (cm)
1	CCRP 8	8.1	130	75	55	18
2	CCRP 8	8.2	150	75	90	15
3	CCRP 8	8.3	65			8
4	CCRP 8	8.4	95	90	50	8
5	CCRP 8	23.6	195	195	145	13
6	CCRP 8	23.7	180	195	165	13
7	CCRP 8	23.3 L	100	50	55	9
8	CCRP 9	9.3	135	55	45	7
9	CCRP 9	9.4	105			6
10	CCRP 9	9.5	75			4
11	CCRP 9	24.6	170	70	95	11
12	CCRP 9	24.7	95	105	70	9
13	CCRP 9	24.3 L	125	130	115	11
14	CCRP 10	10.1	185	135	140	12
15	CCRP 10	10.2	50			4
16	CCRP 10	10.3	105			5
17	CCRP 10	10.4	45			5
18	CCRP 10	25.6	60	45		6
19	CCRP 10	25.3 L	160	145	135	18
20	CCRP 11	11.3	55	45	40	6
21	CCRP 11	11.2	120			8
22	CCRP 11	26.6	125	85	90	8
23	CCRP 11	26.7	110	90		7
24	CCRP 11	26.3 L	100	45		9
25	CCRP 12	12.5	110	65	50	6
26	CCRP 12	12.6	65			6
27	CCRP 12	27.6	155	90	80	9
28	CCRP 12	27.7	150	130	95	9
29	CCRP 12	27.2	170	65	60	9

30	CCRP 12	27.3 L	120	100	80	12
31	CCRP 13	13.3	55	30	45	8
32	CCRP 13	13.4	135	85	70	9
33	CCRP 13	13.6	165	65	100	10
34	CCRP 13	28.6	200	210	185	13
35	CCRP 13	28.7	195	95	70	7
36	CCRP 13	28.1 L	135	85	95	13
37	CCRP 13	28.3 L	110	165	150	12
38	CCRP 14	14.3	95			7
39	CCRP 14	14.5	155	90	70	8
40	CCRP 14	14.6	125			9
41	CCRP 14	29.7	100			6
42	CCRP 14	29.2 L	130	115		12
43	CCRP 15	15.4	195	200	195	20
44	CCRP 15	15.5	145	55	70	11
45	CCRP 15	15.6	140	90	65	9
46	CCRP 15	15.7	175	80	70	9
47	CCRP 15	31.6	200	210	230	15
48	CCRP 15	30.1 L	140	115	50	10

Table 50. Leaf area of hybrids of cocoa during 2018

S. No.	Hybrid	Stand No.	Leaf area (cm^2)
1	CCRP 8	8.1	120.40
2	CCRP 8	8.2	162.72
3	CCRP 8	8.4	156.81
4	CCRP 8	23.3L	109.68
5	CCRP 8	23.7L	144.96
6	CCRP 8	23.6	102.62
7	CCRP 8	8.6L	136.54
8	CCRP 8	8.5L	218.52
9	CCRP 9	9.3	255.22
10	CCRP 9	9.5L	231.32
11	CCRP 9	9.6L	140.14
12	CCRP 9	24.6L	74.60
13	CCRP 9	24.7R	90.92
14	CCRP 9	24.3L	137.59
15	CCRP 10	10.2	299.92
16	CCRP 10	10.5	187.85
17	CCRP 10	25.3L	139.21
18	CCRP 11	11.4	329.05
19	CCRP 11	11.6l	222.38
20	CCRP 11	26.2l	92.26
21	CCRP 11	26.6	301.00
22	CCRP 11	26.7	108.47
23	CCRP 12	12.3	136.29
24	CCRP 12	12.5	261.95
25	CCRP 12	12.6	156.71
26	CCRP 12	12.6L	146.29
27	CCRP 12	27.7R	356.17

28	CCRP 12	27.6	171.54
29	CCRP 12	27.2l	93.02
30	CCRP 12	27.3L	141.06
31	CCRP 13	13.4	207.36
32	CCRP 13	13.6	119.58
33	CCRP 13	13.5L	110.17
34	CCRP 13	13.6L	130.82
35	CCRP 13	28.6R	44.49
36	CCRP 13	28.7R	173.07
37	CCRP 13	28.1L	225.16
38	CCRP 13	28.3L	195.40
39	CCRP 14	14.3	177.93
40	CCRP 14	14.5	130.13
41	CCRP 14	14.6	188.32
42	CCRP 14	14.5L	118.54
43	CCRP 14	14.6L	223.27
44	CCRP 14	29.2L	122.37
45	CCRP 15	15.4	109.91
46	CCRP 15	15.5	414.19
47	CCRP 15	15.6	268.01
48	CCRP 15	15.7	337.70
49	CCRP 15	15.6L	98.47
50	CCRP 15	31.6R	93.31
51	CCRP 15	30.1L	63.29

4.2.1.1.2 Plant spread

The plant spread in EW and NS direction ranged between 20 and 385 and 18 to 400 cm. the maximum plant spread was recorded in S_1 generation of G435.7 (Stand number 1.17 and 2.16). The minimum plant spread was recorded in S_4 generation of M 18.7 (Stand number 9.7) and S_1 generation of G4 35.7(Stand number 8.17).

4.2.1.1.3 Girth

The girth at collar region ranged between 7 cm and 28cm in S_4 generation of M 18.7(Stand number 5.2) and M 18.7(Stand number 4.3) respectively.

4.2.1.1.4 Leaf area

The leaf area was measured using leaf area meter. The leaf area in inbred self ranged from 44.45cm^2 to 531cm^2. The average leaf area was 193.11cm^2 (Table 46).

4.2.1.2 Inbred crosses

Observations recorded on three inbred crosses are presented in table 47.

4.2.1.2.1 Plant height

The plant height among the inbred crosses range between 90 cm and 200cm in S_3H_7 3(86) X S_3G_4 35.7(stand number 2.6) and S_3H_7 3(86) X S_3G_4 35.7(stand number 4.4) respectively. Some inbred crosses produced chupan, the chupan height ranged between 100 and 150 cm in $S_3G_2$7.4 X $S_3G_4$35.7 stand number 3.2 and 1.1 respectively.

4.2.1.2.2 Plant spread

The plant spread in EW and NS direction ranged between 20 and 150 cm and 25 to 170 cm respectively. The maximum plant spread was observed in S_3H_7 3(86) x S_3G_4 35.7(Stand number 2.6). The average plant spread in E-W and N-S direction was 67.67 and 66 cm respectively.

4.2.1.2.3 Girth

The plant girth ranged from 6 to 14 cm in S_3H_7 3(86) x S_3G_4 35.7(Stand number 4.7 and 7.5) respectively. The average girth of plant observed was 10.27cm.

4.2.1.2.4 Leaf area

The leaf area was measured using leaf area meter.

The leaf area in inbred cross ranged between 50.50 and 414.66 cm^2. The mean leaf area observed was 179.48cm^2 (Table 48).

4.2.1.3 Hybrids

Biometric observations in hybrids are presented in table 49.

4.2.1.3.1 Plant height

The plant height in hybrids ranged between 45 and 200 cm. The maximum plant height of 200 cm was observed in CCRP 13 (Stand number 28.6). The minimum plant height was observed in CCRP 10(Stand number 10.4). The average plant height recorded was 127.08cm. Chupan production and jorquetting are not observed in any of the hybrids.

4.2.1.3.2 Plant spread

The maximum plant spread in EW and NS directions were recorded in CCRP 13 (Stand No.28.6) and CCRP 15(Stand No.31.6). The average plant spread in EW and NS direction was 78.54 and 65 cm respectively.

4.2.1.3.3 Girth

The maximum and minimum plant girth in hybrids observed are 4 to 20 cm in CCRP 10 (Stand number 10.2) and CCRP 15 (Stand No.15.4) respectively The average girth observed was 9.5cm.

4.2.1.3.4 Leaf area

The leaf area was measured using leaf area meter.

In hybrids, the leaf area measured from 44.49 to 414.49 cm^2 with an average leaf area of 172.09cm^2 (Table 50).

4.2.2 Chlorophyll content

The photosynthetic activity is highly influenced by the Chlorophyll content Chlorophyll content in plant is considered as a favorite aspect for plant growth (Farquhar and Richards, 1984). Chlorophyll content of leaf is indicator of photosynthetic capability of plant tissues (Nageswararao *et al.,* 2001; Wright *et al.,* 1994). The chlorophyll content in plant forms a large part of light harvesting pigment for photosystem II. In the limiting conditions, higher chlorophyll b content is advantageous to harvest a large percentage of available energy (Lewandowska *et al.,* 1977). The results of chlorophyll b variability study highlight the shade tolerance nature of cocoa genotypes. According to Boardman (1977), Young and Smith (1997) and Satheesan and Ramadasan (1992) higher chlorophyll b and lower a/b ratios are typical of shade ecotypes and may able more efficient absorption of light under shaded conditions due to difference in the absorption spectra of chlorophyll a and b.

The higher contents of chlorophyll had a strong relationship between shade tolerance and yield efficiency in cocoa. The importance of shade in trees species has been discussed by Wilson (1991). He opined that plant growth is more closely related to the incident radiation in the wavelength band 400-700 nm, the photosynthetically active radiation (PAR).

4.2.2.1 Inbred self

The details of chlorophyll content in inbreds are presented in table 51.

Significant variation in chlorophyll content was observed among the inbreds of cocoa. The chlorophyll A content in inbred ranged from 1.746 to 6.352, Chlorophyll B ranged from 0.299 to 3.557 and total chlorophyll content ranged from 5.490 to 9.627. The total maximum chlorophyll content was observed in S1 generation of G4 35.7(stand number 2.6). The variation in chlorophyll content was also reported by Lewandowska *et al.,* 1977. The average chlorophyll A. Chlorophyll B and total chlorophyll content observed in the inbreds was 3.726, 1.787 and 5.492 respectively (Table 10)

4.2.2.2 Inbred cross

The details of chlorophyll content in inbreds are presented in table 52.

Significant variation in the chlorophyll content of inbred cross was observed. The details of chlorophyll content in inbred crosses are presented in table 11. The average chlorophyll A, Chlorophyll B and total Chlorophyll observed was 3.785, .671 and 4.457 mg/g. Chlorophyll A ranged from 1.823mg/g in S_5G_1 7.4 X S_3G_4 35.7 (stand number 3.7) to 5.816 mg/g in S_3H_7 3 (86) X S_3G_4 35.7 (stand number 4.4). The chlorophyll B content ranged from 0.289mg/g in S_3 $G_2$7.4 X S_3G_4 35.7(5.7) to 1.087mg in (S_3H_7 3 (86) X S_3G_4 35.7 (stand number 4.7). The total chlorophyll content ranged between 2.23 in S3H7 3 (86) X S_3G_4 35.7 (stand number 7.5) and 6.805 in S_3H_7 3 (86) X S_3G_4 35.7 (stand number 4.4).

4.2.2.3 Hybrids

The details of chlorophyll content in inbreds are presented in table 53

Significant variation in the chlorophyll content of hybrids was observed. The details of chlorophyll content in inbred crosses are presented in table 12. The average chlorophyll A, Chlorophyll B and total Chlorophyll observed was 2.885, .594 and 3.461 mg/g. Chlorophyll A ranged from 1.055mg/g in CCRP 10 (stand number 25.3) to 4.663

Table 51. Chlorophyll content (mg/g) in inbreds of cocoa

S. No.	Generation	Genotype	Stand No.	Chloro A	Chloro B	Chloro A+B
1	S_4	M 18.7	1.1	4.283	0.747	5.03
2	S_4	M 18.7	1.2	3.778	1.962	5.74
3	S_4	M 18.7	1.4	2.422	3.068	5.49
4	S_4	M 18.7	1.5	4.882	1.202	6.084
5	S_4	M 18.7	1.6	3.546	0.299	3.845
6	S_4	M 18.7	1.8	3.297	1.494	3.916
7	S_4	M 18.7	1.9	3.319	0.835	4.154
8	S_4	M 18.7	1.1	3.338	1.503	4.997
9	S_4	M 18.7	1.12	4.32	0.953	5.273
10	S_4	M 18.7	1.13	4.93	1.694	6.624
11	S_4	M 18.7	1.14	4.036	2.505	6.541
12	S_4	M 18.7	2.1	4.306	1.978	6.284
13	S_4	M 18.7	2.4	3.605	2.065	5.67
14	S_4	M 18.7	2.5	2.993	0.612	2.381
15	S_4	M 18.7	2.6	4.804	2.858	7.662
16	S_4	M 18.7	2.7	4.038	3.557	7.595
17	S_4	M 18.7	2.8	2.3	1.706	4.006
18	S_4	M 18.7	2.9	3.747	1.87	5.617
19	S_4	M 18.7	2.1	3.071	0.863	3.934
20	S_4	M 18.7	2.11	3.646	0.914	4.56
21	S_4	M 18.7	2.13	1.746	1.59	3.336
22	S_4	M 18.7	2.14	3.374	1.215	4.589
23	S_4	M 18.7	3.1	3.886	0.818	4.704
24	S_4	M 18.7	3.2	3.413	0.581	3.994
25	S_4	M 18.7	3.3	4.289	0.861	5.15
26	S_4	M 18.7	3.4	3.003	0.856	3.859
27	S_4	M 18.7	3.5	4.28	1.168	5.448
28	S_4	M 18.7	3.8	2.823	0.743	3.566
29	S_4	M 18.7	3.9	2.547	1.33	3.877
30	S_4	M 18.7	3.1	5.512	1.315	6.828
31	S_4	M 18.7	3.11	3.753	0.422	4.175
32	S_4	M 18.7	3.12	4.474	1.567	6.041
33	S_4	M 18.7	3.14	3.347	2.329	5.677
34	S_4	M 18.7	3.15	3.59	2.039	5.628
35	S_4	M 18.7	5.1	3.51	2.223	5.733
36	S_4	M 18.7	5.2	3.382	2.129	5.511
37	S_4	M 18.7	5.4	3.517	2.079	5.596

38	S_4	M 18.7	5.6	3.407	2.068	5.475
39	S_4	M 18.7	5.7	4.348	1.988	6.336
40	S_4	M 18.7	5.8	3.453	1.605	5.058
41	S_4	M 18.7	5.9	3.453	1.328	4.78
42	S_4	M 18.7	5.12	3.422	1.598	5.019
43	S_4	M 18.7	5.1	3.627	2.167	5.794
44	S_4	M 18.7	5.13	3.515	1.918	5.433
45	S_4	M 18.7	5.14	3.688	2.209	5.896
46	S_4	M 18.7	5.15	3.483	2.091	5.575
47	S_4	M 18.7	6.1	3.634	0.996	4.63
48	S_4	M 18.7	6.2	2.425	1.511	3.935
49	S_4	M 18.7	6.6	3.646	1.592	5.237
50	S_4	M 18.7	6.7	3.633	1.151	4.784
51	S_4	M 18.7	6.8	4.928	0.784	5.712
52	S_4	M 18.7	6.9	3.72	1.964	5.684
53	S_4	M 18.7	6.1	3.389	2.314	5.702
54	S_4	M 18.7	6.11	3.348	2.148	5.496
55	S_1	G 4 35.7	1.16	5.124	2.907	8.03
56	S_1	G 4 35.7	1.17	4.828	1.596	6.424
57	S_1	G 4 35.7	2.16	6.352	3.275	9.627
58	S_1	G 4 35.7	2.17	4.768	0.76	5.528
59	S_1	G 4 35.7	3.17	5.641	2.398	8.038
60	S_1	G 4 35.7	5.17	4.51	2.813	7.322
61	S_1	G 4 35.7	6.16	5.29	2.619	7.909
62	S_1	G 4 35.7	7.16	4.606	2.79	7.396
63	S_1	G 4 35.7	7.17	4.658	2.77	7.428
64	S_1	G 4 35.7	8.16	4.608	2.808	7.416
65	S_1	G 4 35.7	8.17	4.6	2.85	7.45
66	S_1	G 4 35.7	9.16	4.602	3.036	7.639
67	S_1	G 4 35.7	10.16	4.534	2.791	7.324
68	S_4	G 4 35.7	6.13	3.714	0.843	4.557
69	S_4	G 4 35.7	6.14	3.423	1.797	5.22
70	S_4	G 4 35.7	6.15	3.434	1.864	5.298
71	S_4	G 4 35.7	7.1	3.769	1.475	5.244
72	S_4	G 4 35.7	7.3	3.442	1.784	5.226
73	S_4	G 4 35.7	7.4	3.446	1.75	5.196
74	S_4	G 4 35.7	7.6	3.333	1.792	5.125
75	S_4	G 4 35.7	7.8	3.547	1.751	5.298
76	S_4	G 4 35.7	7.1	3.772	1.732	5.504
77	S_4	G 4 35.7	7.11	3.389	1.991	5.38

78	S_4	G 4 35.7	8.2	3.439	1.533	4.972
79	S_4	G 4 35.7	8.3	3.323	1.576	4.899
80	S_4	G 4 35.7	8.5	3.305	1.712	5.017
81	S_4	G 4 35.7	8.6	3.337	1.72	5.057
82	S_4	G 4 35.7	8.7	3.334	1.682	5.016
83	S_4	G 4 35.7	8.8	3.297	1.902	5.199
84	S_4	G 4 35.7	8.1	3.279	2.013	5.291
85	S_4	G 4 35.7	8.14	3.329	2.013	5.342
86	S_4	G 4 35.7	9.2	3.664	1.875	5.539
87	S_4	G 4 35.7	9.5	3.55	1.821	5.371
88	S_4	G 4 35.7	9.7	3.508	1.811	5.319
89	S_4	G 4 35.7	9.8	3.297	1.831	5.128
90	S_4	G 4 35.7	9.1	3.364	1.884	5.248
91	S_4	G 4 35.7	9.12	3.326	1.937	5.263
92	S_4	G 4 35.7	9.13	3.281	1.921	5.202
93	S_4	G 4 35.7	9.14	3.385	1.889	5.274
94	S_4	G 4 35.7	10.1	3.397	1.878	5.275
95	S_4	G 4 35.7	10.3	3.343	1.801	5.144
96	S_4	G 4 35.7	10.7	3.307	1.841	5.147
97	S_4	G 4 35.7	10.8	3.281	2.025	5.306
98	S_4	G 4 35.7	10.1	3.284	1.92	5.204
99	S_4	G 4 35.7	10.11	3.336	2.017	5.353
100	S_4	G 4 35.7	10.14	3.544	1.881	5.425
101	S_4	G 4 35.7	10.15	3.22	2.381	5.236
			CV (%)	4.934	7.428	3.582
			CD (0.05)	0.294	0.212	0.315

Table 52. Chlorophyll content (mg/g) of inbred crosses of cocoa

S. No.	Inbred cross	Stand No.	Chloro A	Choro B	A+B
1	S_3H_7 3 (86) X S_3G_4 35.7	2.6	2.658	0.598	3.255
2	S_3 $G_2$7.4 X S_3G_4 35.7	3.1	4.64	0.608	5.247
3	S_3 $G_2$7.4 X S_3G_4 35.7	3.4	3.518	0.755	4.273
4	S_3 $G_2$7.4 X S_3G_4 35.7	3.8	5.623	0.932	6.555
5	S_3 $G_2$7.4 X S_3G_4 35.7	4.1	5.32	0.793	6.113
6	S_3 $G_2$7.4 X S_3G_4 35.7	4.8	3.769	0.764	4.534
7	S_3 $G_2$7.4 X S_3G_4 35.7	5.7	3.017	0.289	3.306
8	S_3H_7 3 (86) X S_3G_4 35.7	4.4	5.816	0.989	6.805
9	S_3H_7 3 (86) X S_3G_4 35.7	4.5	5.66	0.854	6.514
10	S_3H_7 3 (86) X S_3G_4 35.7	4.6	3.123	0.592	3.715
11	S_3H_7 3 (86) X S_3G_4 35.7	4.7	4.688	1.087	5.775
12	S_3H_7 3 (86) X S_3G_4 35.7	5.3	5.392	0.799	6.19
13	S_3H_7 3 (86) X S_3G_4 35.7	7.5	1.847	0.383	2.23
14	S_5G_1 7.4 X S_3G_4 35.7	3.5	1.829	0.455	2.283
15	S_5G_1 7.4 X S_3G_4 35.7	3.6	1.823	0.437	2.261
16	S_5G_1 7.4 X S_3G_4 35.7	3.7	1.851	0.407	2.259
		CV (%)	0.45	5.669	0.825
		CD (0.05)	0.028	0.063	0.061

Table 53. Chlorophyll content (mg/g) in hybrids of cocoa

S. No.	Hybrid	Stand No	Chlor A	Chlor B	A+B
1	CCRP 8	8.1	2.922	0.370	3.292
2	CCRP 8	8.2	2.924	0.500	3.42
3	CCRP 8	8.4	2.904	0.520	3.42
4	CCRP 8	23.3L	2.943	0.479	3.419
5	CCRP 8	23.7L	2.392	0.730	3.119
6	CCRP 8	23.6	2.889	0.519	3.405
7	CCRP 8	8.6L	2.886	0.514	3.396
8	CCRP 8	8.5L	2.136	0.754	2.888
9	CCRP 9	9.3	4.082	0.664	4.721
10	CCRP 9	9.5L	3.916	0.929	4.811
11	CCRP 9	9.6L	3.492	0.680	4.155
12	CCRP 9	24.6L	3.394	1.096	4.46
13	CCRP 9	24.7R	3.315	0.812	4.107
14	CCRP 9	24.3L	2.854	1.376	4.197
15	CCRP 10	10.2	1.077	0.468	1.567
16	CCRP 10	10.5	1.159	0.235	1.423
17	CCRP 10	25.3L	1.055	0.248	1.333
18	CCRP 11	11.4	1.099	0.637	1.751
19	CCRP 11	11.6l	1.615	0.458	2.088
20	CCRP 11	26.2l	2.35	0.439	2.795
21	CCRP 11	26.6	2.626	0.101	2.74
22	CCRP 11	26.7	2.41	0.214	2.637
23	CCRP 12	12.3	2.587	0.821	3.399
24	CCRP 12	12.5	2.588	0.821	3.399
25	CCRP 12	12.6	2.9	0.744	3.632
26	CCRP 12	12.6L	2.902	0.704	3.596
27	CCRP 12	27.7R	3.359	0.534	3.883
28	CCRP 12	27.6	3.465	0.481	3.936
29	CCRP 12	27.2l	3.552	0.494	4.035
30	CCRP 12	27.3L	3.666	0.419	4.075
31	CCRP 13	13.4	2.277	0.342	2.629
32	CCRP 13	13.6	2.686	0.235	2.93
33	CCRP 13	13.5L	2.05	0.512	2.569
34	CCRP 13	13.6L	2.055	0.490	2.553
35	CCRP 13	28.6R	2.398	0.382	2.787
36	CCRP 13	28.7R	2.262	0.452	2.721

37	CCRP 13	28.1L	2.375	0.547	2.925
38	CCRP 13	28.3L	2.227	0.642	2.869
39	CCRP 14	14.3	2.583	0.234	2.828
40	CCRP 14	14.5	2.876	0.321	3.201
41	CCRP 14	14.6	2.982	0.307	2.699
42	CCRP 14	14.5L	2.985	0.771	3.743
43	CCRP 14	14.6L	2.799	0.939	3.72
44	CCRP 14	29.2L	2.562	0.549	3.111
45	CCRP 15	15.4	4.603	1.044	5.604
46	CCRP 15	15.5	4.612	0.976	5.546
47	CCRP 15	15.6	4.663	0.918	5.54
48	CCRP 15	15.7	4.49	1.107	5.551
49	CCRP 15	15.6L	3.893	0.792	4.66
50	CCRP 15	31.6R	4.134	0.474	4.59
51	CCRP 15	30.1L	4.173	0.492	4.646
		CV (%)	1.191	3.387	0.584
		CD (0.05)	0.055	0.032	0.032

mg/g in CCRP 15 (stand number 15.6). The chlorophyll B content ranged from 0.101mg/g in CCRP 11 (stand number 26.6) to 1.376mg/g in CCRP 9(stand number 24.3L). The total chlorophyll content ranged between 1.333 in CCRP 10 (stand number 25.3) and 5.604 in CCRP 15 (stand number 15.4).

4.2.3 Relative water content (RWC)

The relative water content technique, formerly known as relative turgidity, was described by Weatherley (1951). According to Barrs (1968) relative water content is widely accepted as a reproducible and meaningful index of plant water status. Ritchie *et al.,* 1990 opined high relative water content (RWC) is a resistant mechanism to drought, and that high relative water content is the result of more osmotic regulation or less elasticity of tissue cell wall. Growth and development of cells is highly influenced by the osmotic regulation during stress (Pessarkli, 1999). According to Cornic (2000) decrease in relative water content in the leaves, close the stomata and thereby the photosynthesis rate gets reduced.

In the present study, the RWC was measured separately for inbreds, inbred crosses and hybrids for two years (2016 and 2017) in four seasons *viz.,* Summer (April), Monsoon (June), Most monsoon (September) and Winter season (December)

The results on RWC during 2016 are presented here under

4.2.3.1 Inbred self

The RWC was measured in 99 inbred plants during 2016 for four seasons the results are presented in table 54.

Summer 2016

The average relative water content (RWC) in inbred self was 44.58 per cent. The RWC ranged from 16.62 to 79.05 percent. The maximum RWC (79.05 percent) and was recorded in S_4 generation of M 18.7 (Stand number 1.10) the least RWC (16.62 per cent) was recorded S_4 generation of G4 35.7(Stand number 10.10).

Table 54 Relative water content (RWC) in inbreds during 2016

S. No.	Generation	Inbred self	Stand No	Apr-16	Jun-16	Sep-16	Dec-16
1	S_4	M 18.7	1.1	36.50	49.78	55.94	40.36
2	S_4	M 18.7	1.2	41.39	49.92	65.39	49.05
3	S_4	M 18.7	1.4	36.35	40.68	54.06	38.12
4	S_4	M 18.7	1.5	70.38	67.07	71.87	67.95
5	S_4	M 18.7	1.6	50.25	53.91	62.66	52.90
6	S_4	M 18.7	1.8	42.51	38.59	52.46	42.30
7	S_4	M 18.7	1.9	69.89	71.84	79.67	67.50
8	S_4	M 18.7	1.10	79.05	68.00	81.79	77.66
9	S_4	M 18.7	1.12	51.05	62.09	71.77	49.67
10	S_4	M 18.7	1.13	56.86	62.14	72.79	49.08
11	S_4	M 18.7	1.14	48.09	52.59	63.28	49.43
12	S_4	M 18.7	2.1	41.52	47.94	52.77	45.80
13	S_4	M 18.7	2.5	55.61	62.34	72.36	58.96
14	S_4	M 18.7	2.6	42.03	48.27	57.35	42.08
15	S_4	M 18.7	2.7	57.75	66.86	66.64	67.28
16	S_4	M 18.7	2.8	38.10	36.36	52.26	47.33
17	S_4	M 18.7	2.9	53.18	67.47	63.84	55.46
18	S_4	M 18.7	2.10	52.44	62.49	68.61	55.42
19	S_4	M 18.7	2.11	23.45	32.04	42.37	33.13
20	S_4	M 18.7	2.13	33.39	41.95	53.32	42.87
21	S_4	M 18.7	2.14	77.03	73.86	81.66	73.97
22	S_4	M 18.7	3.1	29.96	31.77	40.31	36.40
23	S_4	M 18.7	3.3	51.88	50.36	59.86	52.46
24	S_4	M 18.7	3.4	41.85	42.68	52.35	52.20
25	S_4	M 18.7	3.5	51.39	52.13	53.29	53.40
26	S_4	M 18.7	3.8	41.96	49.36	62.72	42.72
27	S_4	M 18.7	3.9	63.51	67.11	73.10	63.72
28	S_4	M 18.7	3.10	47.71	52.89	62.90	48.26
29	S_4	M 18.7	3.11	42.37	47.88	62.13	43.19
30	S_4	M 18.7	3.12	36.41	46.53	52.46	38.07
31	S_4	M 18.7	3.14	55.78	63.72	76.35	54.49
32	S_4	M 18.7	3.15	43.26	52.87	62.45	42.43
33	S_4	M 18.7	5.1	62.36	72.42	82.26	62.36
34	S_4	M 18.7	5.2	57.47	62.46	68.21	53.88
35	S_4	M 18.7	5.4	60.43	64.11	72.72	62.96
36	S_4	M 18.7	5.6	52.23	64.21	66.85	52.59

37	S_4	M 18.7	5.7	62.31	62.84	73.19	58.63
38	S_4	M 18.7	5.8	62.27	71.79	75.64	57.91
39	S_4	M 18.7	5.9	64.62	71.72	75.61	68.26
40	S_4	M 18.7	5.12	47.28	52.86	61.85	58.93
41	S_4	M 18.7	5.10	47.07	53.58	54.38	57.43
42	S_4	M 18.7	5.13	43.70	42.44	53.39	53.05
43	S_4	M 18.7	5.14	52.36	62.64	63.34	58.26
44	S_4	M 18.7	5.15	63.48	72.15	77.86	67.53
45	S_4	M 18.7	6.1	42.35	50.93	61.36	48.09
46	S_4	M 18.7	6.2	31.48	39.39	47.41	41.88
47	S_4	M 18.7	6.6	46.35	48.79	54.39	49.11
48	S_4	M 18.7	6.7	51.54	63.06	63.05	58.04
49	S_4	M 18.7	6.8	55.06	58.05	66.41	42.28
50	S_4	M 18.7	6.9	46.59	52.98	63.85	48.00
51	S_4	M 18.7	6.10	41.61	52.61	72.41	47.81
52	S_4	M 18.7	6.11	47.88	42.54	53.20	52.87
53	S_1	G 4 35.7	1.16	42.71	47.93	52.20	45.40
54	S_1	G 4 35.7	1.17	38.53	42.26	42.39	42.74
55	S_1	G 4 35.7	3.17	36.45	44.42	54.42	36.30
56	S_1	G 4 35.7	2.16	78.03	72.25	87.18	87.61
57	S_1	G 4 35.7	2.17	68.70	71.65	85.35	65.79
58	S_1	G 4 35.7	5.17	47.18	42.12	58.29	59.30
59	S_1	G 4 35.7	6.16	42.49	42.23	56.58	44.76
60	S_1	G 4 35.7	7.16	43.36	52.13	62.92	48.09
61	S_1	G 4 35.7	7.17	42.69	52.20	62.53	54.86
62	S_1	G 4 35.7	8.16	32.63	42.13	46.52	38.63
63	S_1	G 4 35.7	8.17	32.81	49.27	55.86	42.82
64	S_1	G 4 35.7	9.16	37.40	52.34	61.21	41.17
65	S_1	G 4 35.7	10.16	33.26	47.97	68.90	44.10
66	S_4	G 4 35.7	6.13	46.70	42.14	56.53	52.09
67	S_4	G 4 35.7	6.14	52.48	52.44	61.60	56.33
68	S_4	G 4 35.7	6.15	42.70	42.23	52.21	45.62
69	S_4	G 4 35.7	7.1	35.57	42.33	56.70	39.43
70	S_4	G 4 35.7	7.3	33.35	36.19	55.74	36.92
71	S_4	G 4 35.7	7.4	33.80	34.19	44.53	36.60
72	S_4	G 4 35.7	7.6	46.36	42.14	51.33	51.42
73	S_4	G 4 35.7	7.8	23.38	32.30	42.70	28.09
74	S_4	G 4 35.7	7.10	30.18	38.12	44.39	38.01
75	S_4	G 4 35.7	7.11	34.47	42.00	53.26	38.63
76	S_4	G 4 35.7	8.2	38.96	47.72	59.23	47.75

77	S_4	G 4 35.7	8.3	23.20	31.72	39.18	36.56
78	S_4	G 4 35.7	8.5	27.01	35.82	42.97	26.32
79	S_4	G 4 35.7	8.6	48.27	57.19	63.64	52.61
80	S_4	G 4 35.7	8.7	37.48	32.79	42.21	41.84
81	S_4	G 4 35.7	8.8	33.22	42.12	48.68	35.11
82	S_4	G 4 35.7	8.10	23.51	31.98	43.96	26.28
83	S_4	G 4 35.7	8.14	29.25	31.83	40.06	36.81
84	S_4	G 4 35.7	9.2	72.77	77.47	83.36	68.82
85	S_4	G 4 35.7	9.5	47.05	52.26	61.56	51.20
86	S_4	G 4 35.7	9.7	28.70	34.91	43.98	38.26
87	S_4	G 4 35.7	9.8	26.46	32.79	42.58	38.99
88	S_4	G 4 35.7	9.10	37.20	46.45	52.66	46.86
89	S_4	G 4 35.7	9.12	58.28	68.92	68.37	68.37
90	S_4	G 4 35.7	9.13	24.08	45.40	49.29	38.92
91	S_4	G 4 35.7	9.14	36.59	46.53	52.79	38.90
92	S_4	G 4 35.7	10.1	37.76	62.07	77.46	64.10
93	S_4	G 4 35.7	10.3	48.60	58.60	62.25	52.28
94	S_4	G 4 35.7	10.7	32.61	45.72	53.52	39.09
95	S_4	G 4 35.7	10.8	33.97	45.53	42.33	46.84
96	S_4	G 4 35.7	10.10	16.62	44.50	38.23	38.83
97	S_4	G 4 35.7	10.11	31.16	37.30	42.98	38.90
98	S_4	G 4 35.7	10.14	31.66	41.06	43.53	48.24
99	S_4	G 4 35.7	10.15	32.56	43.88	52.42	27.73
			CV (%)	2.324	1.94	1.771	1.959
			CD (0.05)	1.67	1.589	1.692	1.549

Table 55. Relative water content (RWC) in inbred crosses during 2016

S. No.	Cross	Stand No	Apr-16	Jun-16	Sep-16	Dec-16
1	S_3 $G_2$7.4 X S_3G_4 35.7	3.1	15.66	27.14	36.06	18.93
2	S_3 $G_2$7.4 X S_3G_4 35.7	3.4	14.64	26.35	37.52	17.25
3	S_3 $G_2$7.4 X S_3G_4 35.7	3.8	14.86	27.78	38.20	16.46
4	S_3 $G_2$7.4 X S_3G_4 35.7	4.1	20.62	32.18	43.15	29.24
5	S_3 $G_2$7.4 X S_3G_4 35.7	4.8	16.05	27.30	37.48	24.56
6	S_3 $G_2$7.4 X S_3G_4 35.7	5.7	18.61	27.90	41.86	26.56
7	S_3H_7 3 (86) X S_3G_4 35.7	2.6	12.58	23.52	39.46	23.07
8	S_3H_7 3 (86) X S_3G_4 35.7	4.4	11.51	23.62	35.90	22.57
9	S_3H_7 3 (86) X S_3G_4 35.7	4.5	12.32	23.47	38.22	22.52
10	S_3H_7 3 (86) X S_3G_4 35.7	4.6	12.68	23.88	39.35	23.48
11	S_3H_7 3 (86) X S_3G_4 35.7	4.7	13.98	27.02	28.26	24.26
12	S_3H_7 3 (86) X S_3G_4 35.7	5.3	9.83	22.85	34.25	21.92
13	S_3H_7 3 (86) X S_3G_4 35.7	7.5	13.25	26.99	38.62	23.85
14	S_5G_1 7.4 X S_3G_4 35.7	3.5	17.90	29.03	37.60	18.94
15	S_5G_1 7.4 X S_3G_4 35.7	3.6	16.53	29.86	39.13	27.14
16	S_5G_1 7.4 X S_3G_4 35.7	3.7	16.74	28.03	39.13	26.90
		CV (%)	1.06	1.08	1.01	1.08
		CD (0.05)	4.26	2.43	1.60	2.81

Monsoon 2016

The average relative water content (RWC) in inbred self was 50.83 per cent. The RWC ranged from 31.72 to 77.47 percent. The maximum RWC (77.47 percent) was recorded in G_4 35.7(Stand number 9.2) and minimum RWC (31.72 per cent) was recorded S_4 generation of G_4 35.7(Stand number 8.3).

Post monsoon 2016

The average relative water content (RWC) in inbred self during post monsoon season was 59.28 per cent. The RWC ranged from 38.23 to 87.18 percent. The maximum RWC (87.18 percent) was recorded in G_4 35.7(Stand number 2.16) and minimum RWC (38.23 per cent) was recorded S_4 generation of G_4 35.7(Stand number 10.10).

Winter 2016

The average relative water content (RWC) in inbred self during winter season was 49.07 per cent. The RWC ranged from 26.28 to 87.61 percent. The maximum RWC (87.61 percent) was recorded in G_4 35.7(Stand number 2.16) and minimum RWC (26.28 per cent) was recorded S_4 generation of G_4 35.7(Stand number 8.10).

4.2.3.2 Inbred cross

The RWC was measured in 16 inbred cross plants during 2016 for four seasons the results are presented in table 55.

Summer 2016

The average relative water content (RWC) in inbred cross was 14.86 per cent. The RWC ranged from 9.83 to 20.62 percent. The maximum RWC (20.62 percent) and was recorded in $G_2$7.4 X S_3G_4 35.7 (Stand number 4.1) the minimum RWC (9.83 per cent) was recorded in S_3H_7 3 (86) X S_3G_4 35.7 (Stand number 5.3).

Monsoon 2016

The average relative water content (RWC) in inbred cross was 26.68 per cent. The RWC ranged from 32.18 to 22.85 percent. The maximum RWC (32.18 percent) was recorded in $G_2$7.4 x S_3G_4 35.7 (Stand number 4.1) and minimum RWC (22.85 per cent) was recorded in S_3H_7 3 (86) x S_3G_4 35.7 (Stand number 5.3).

Post monsoon 2016

The average relative water content (RWC) in inbred cross during post monsoon season was 37.76 per cent. The RWC ranged from 28.26 to 43.15 percent. The maximum RWC (43.15 percent) was recorded in $G_2$7.4 x S_3G_4 35.7 (Stand number 4.1) and minimum RWC (28.26 per cent) was recorded in S_3H_7 3 (86) x S_3G_4 35.7 (Stand number 4.7).

Winter 2016

The average relative water content (RWC) in inbred cross during winter season was 22.98 per cent. The RWC ranged from 16.46 to 29.24 percent. The maximum RWC (29.24 percent) was recorded in $G_2$7.4 x S_3G_4 35.7 (Stand number 4.1) and minimum RWC (16.46 per cent) was recorded in $G_2$7.4 x S_3G_4 35.7(Stand number 3.8).

4.2.3.3 Hybrids

The RWC was measured in 48 hybrids plants during 2016 for four seasons the results are presented in table 56.

Summer 2016

The average relative water content (RWC) in hybrids was 31.48 per cent. The RWC ranged from 14.87 to 82.73 percent. The maximum RWC (82.73 percent) was recorded in CCRP12 (Stand number 12.6) the minimum RWC (14.87 per cent) was recorded in CCRP 14 (Stand number 14.6).

Table 56. Relative water content (RWC) in hybrid during 2016

S. No.	Hybrids	Stand No	Apr-16	Jun-16	Sep-16	Dec-16
1	CCRP 8	8.1	36.89	39.06	59.17	52.87
2	CCRP 8	8.2	45.66	42.57	56.21	49.17
3	CCRP 8	8.4	48.32	42.47	62.42	42.08
4	CCRP 8	23.3L	35.38	33.19	51.96	33.87
5	CCRP 8	23.7L	44.33	40.83	57.63	41.76
6	CCRP 8	23.6	25.73	26.39	45.37	42.08
7	CCRP 8	8.6L	19.21	14.82	35.47	30.13
8	CCRP 8	8.5L	27.38	22.83	45.60	42.02
9	CCRP 9	9.3	35.57	28.35	45.50	41.64
10	CCRP 9	9.5L	45.88	37.21	55.63	51.96
11	CCRP 9	9.6L	32.82	29.08	49.02	41.59
12	CCRP 9	24.6L	33.64	29.08	49.36	52.98
13	CCRP 9	24.7R	31.69	26.40	56.46	41.76
14	CCRP 9	24.3L	27.42	17.59	41.65	34.66
15	CCRP 10	10.2	46.55	40.46	59.47	51.74
16	CCRP 10	10.5	28.72	23.79	47.83	41.61
17	CCRP 10	25.3L	28.31	23.28	48.12	42.22
18	CCRP 11	11.4	24.61	21.83	48.04	41.69
19	CCRP 11	26.6	31.47	30.09	52.36	41.68
20	CCRP 11	26.7	41.32	40.17	52.58	45.12
21	CCRP 12	12.3	26.43	23.57	48.66	41.81
22	CCRP 12	12.5	41.77	39.93	52.72	38.44
23	CCRP 12	12.6	82.73	65.34	68.26	57.99
24	CCRP 12	12.6L	27.46	22.14	48.35	27.99
25	CCRP 12	27.7R	35.65	26.41	46.42	42.34
26	CCRP 12	27.6	27.68	21.67	37.86	34.39
27	CCRP 12	27.3L	22.71	17.25	35.92	36.50
28	CCRP 13	13.4	24.67	15.99	36.96	32.35
29	CCRP 13	13.6	31.65	24.17	47.14	41.34
30	CCRP 13	13.5L	27.80	19.81	36.91	31.32
31	CCRP 13	13.6L	19.47	13.93	32.40	21.56
32	CCRP 13	28.6R	23.85	17.93	32.92	22.34
33	CCRP 13	28.7R	19.07	12.88	29.87	21.28
34	CCRP 13	28.1L	26.02	12.15	31.81	22.22
35	CCRP 13	28.3L	31.47	22.41	39.36	30.08
36	CCRP 14	14.3	24.83	19.18	37.34	34.22
37	CCRP 14	14.5	26.72	19.03	38.24	28.36
38	CCRP 14	14.6	14.87	12.15	29.80	21.98

39	CCRP 14	14.5L	25.04	24.39	32.83	25.51
40	CCRP 14	14.6L	31.82	28.52	46.43	31.71
41	CCRP 14	29.2L	26.81	20.42	47.10	32.91
42	CCRP 15	15.4	29.67	29.28	45.36	34.28
43	CCRP 15	15.5	28.61	32.14	47.46	41.93
44	CCRP 15	15.6	33.80	34.92	47.52	38.61
45	CCRP 15	15.7	28.05	29.37	42.53	33.23
46	CCRP 15	15.6L	23.90	26.40	44.44	35.14
47	CCRP 15	31.6R	29.77	30.61	32.82	22.58
48	CCRP 15	30.1L	27.72	27.27	52.50	42.55
		CV (%)	0.838	1.08	0.783	1.65
		CD (0.05)	1.641	2.459	1.058	2.73

Monsoon 2016

The average relative water content (RWC) in hybrids in monsoon 2016 was 27.06 per cent. The RWC ranged from 12.15 to 65.34 percent. The maximum RWC (65.34 percent) was recorded in CCRP12 (Stand number 12.6) the minimum RWC (12.15 per cent) was recorded in CCRP 13 (Stand number 28.1).

Post monsoon 2016

The average relative water content (RWC) in hybrids in post-monsoon 2016 was 45.62 per cent. The RWC ranged from 29.80 to 68.26 percent. The maximum RWC (68.26 percent) was recorded in CCRP12 (Stand number 12.6) the minimum RWC (29.80 per cent) was recorded in CCRP 14 (stand number 14.6).

Winter 2016

The average relative water content (RWC) in hybrids in post-monsoon 2016 was 37.24 per cent. The RWC ranged from 21.28 to 57.99 percent. The maximum RWC (57.99 percent) was recorded in CCRP12 (Stand number 12.6) the minimum RWC (21.28 per cent) was recorded in CCRP 13 (Stand number 28.7).

Result of relative water content in inbreds crosses and hybrids during 2017 are presented here under.

4.2.3.4 Inbred self

The RWC was measured in 99 inbred plants during 2017 for four seasons the results are presented in table 57.

Summer 2017

The average relative water content (RWC) in inbred self was 45.58 per cent. The RWC ranged from 17.80 to 80.56 percent. The maximum RWC (80.56 percent) and was recorded in S_1 generation of $G_4$35.7 (Stand number 2.16) the minimum RWC (17.80 per cent) was recorded S4 generation of G_4 35.7(Stand number 10.10).

Table 57. Relative water content (RWC) in inbreds during 2017

S. No.	Generation	Inbred	Stand No	Apr-17	Jun-17	Sep-17	Dec-17
1	S_4	M 18.7	1.1	39.491	61.483	66.084	38.491
2	S_4	M 18.7	1.2	43.145	59.389	65.708	42.204
3	S_4	M 18.7	1.4	35.915	47.142	59.92	37.334
4	S_4	M 18.7	1.5	70.972	75.979	79.179	72.089
5	S_4	M 18.7	1.6	49.647	60.882	69.657	51.658
6	S_4	M 18.7	1.8	43.029	43.991	57.524	43.227
7	S_4	M 18.7	1.9	69.358	75.745	88.592	71.964
8	S_4	M 18.7	1.10	80.445	73.495	85.949	81.919
9	S_4	M 18.7	1.12	52.742	65.045	77.496	53.295
10	S_4	M 18.7	1.13	57.759	66.757	76.754	56.611
11	S_4	M 18.7	1.14	50.493	59.029	70.266	51.715
12	S_4	M 18.7	2.1	41.993	51.046	60.576	42.693
13	S_4	M 18.7	2.5	57.529	69.679	74.801	57.869
14	S_4	M 18.7	2.6	41.279	52.031	59.69	41.726
15	S_4	M 18.7	2.7	60.651	69.024	74.124	72.695
16	S_4	M 18.7	2.8	40.069	40.169	59.356	48.997
17	S_4	M 18.7	2.9	55.702	68.524	76.498	57.462
18	S_4	M 18.7	2.10	52.996	63.947	73.218	55.409
19	S_4	M 18.7	2.11	25.202	36.618	50.834	35.226
20	S_4	M 18.7	2.13	35.68	43.939	56.856	47.895
21	S_4	M 18.7	2.14	78.74	87.719	91.956	76.758
22	S_4	M 18.7	3.1	29.214	37.83	48.179	30.165
23	S_4	M 18.7	3.3	53.383	52.761	64.103	53.973
24	S_4	M 18.7	3.4	40.568	49.233	56.023	42.618
25	S_4	M 18.7	3.5	53.265	53.311	56.483	54.362
26	S_4	M 18.7	3.8	42.222	52.688	62.226	43.258
27	S_4	M 18.7	3.9	64.084	70.884	75.452	65.501
28	S_4	M 18.7	3.10	47.345	56.382	67.688	48.003
29	S_4	M 18.7	3.11	44.309	50.923	68.043	42.969
30	S_4	M 18.7	3.12	38.466	50.179	54.158	40.57
31	S_4	M 18.7	3.14	58.362	69.146	78.842	59.405
32	S_4	M 18.7	3.15	46.226	55.889	64.383	47.332
33	S_4	M 18.7	5.1	63.712	73.12	84.542	65.538
34	S_4	M 18.7	5.2	58.902	69.122	72.516	60.197
35	S_4	M 18.7	5.4	60.378	68.427	77.928	62.149
36	S_4	M 18.7	5.6	54.01	67.367	71.529	54.871

37	S_4	M 18.7	5.7	62.103	72.175	79	62.99
38	S_4	M 18.7	5.8	63.043	72.902	79.03	63.866
39	S_4	M 18.7	5.9	57.323	73.114	82.064	65.391
40	S_4	M 18.7	5.12	47.87	57.368	67.072	47.72
41	S_4	M 18.7	5.10	47.073	56.456	60.455	48.66
42	S_4	M 18.7	5.13	43.038	43.27	54.569	43.582
43	S_4	M 18.7	5.14	51.004	61.033	70.767	52.463
44	S_4	M 18.7	5.15	64.017	74.255	85.245	65.406
45	S_4	M 18.7	6.1	43.765	53.951	66.043	45.597
46	S_4	M 18.7	6.2	31.005	41.324	47.893	32.54
47	S_4	M 18.7	6.6	46.263	51.527	57.126	47.329
48	S_4	M 18.7	6.7	51.196	64	67.159	52.682
49	S_4	M 18.7	6.8	56.435	61.499	68.964	58.812
50	S_4	M 18.7	6.9	46.224	60.863	68.823	47.095
51	S_4	M 18.7	6.10	42.775	56.066	77.227	44.204
52	S_4	M 18.7	6.11	49.94	49.449	57.405	52.095
53	S_1	G 4 35.7	1.16	44.053	49.793	58.634	42.912
54	S_1	G 4 35.7	1.17	40.53	49.934	50.468	41.795
55	S_1	G 4 35.7	3.17	39.074	49.081	59.209	40.532
56	S_1	G 4 35.7	2.16	80.563	88.849	93.969	89.788
57	S_1	G 4 35.7	2.17	69.779	77.94	86.164	69.766
58	S_1	G 4 35.7	5.17	49.267	48.92	58.827	50.733
59	S_1	G 4 35.7	6.16	42.734	42.934	59.271	44.042
60	S_1	G 4 35.7	7.16	44.922	57.001	67.378	47.221
61	S_1	G 4 35.7	7.17	42.077	52.87	64.514	42.936
62	S_1	G 4 35.7	8.16	35.002	44.593	49.283	36.788
63	S_1	G 4 35.7	8.17	38.918	48.938	62.381	42.415
64	S_1	G 4 35.7	9.16	38.527	48.916	59.061	40.158
65	S_1	G 4 35.7	10.16	35.081	46.25	57.388	32.292
66	S_4	G 4 35.7	6.13	48.049	48.514	59.829	49.025
67	S_4	G 4 35.7	6.14	53.095	53.122	66.434	54.327
68	S_4	G 4 35.7	6.15	43.06	43.237	57.54	43.957
69	S_4	G 4 35.7	7.1	36.816	48.458	59.19	37.459
70	S_4	G 4 35.7	7.3	34.03	37.298	58.604	35.06
71	S_4	G 4 35.7	7.4	34.066	34.137	50.155	34.908
72	S_4	G 4 35.7	7.6	47.875	48.136	60.192	48.368
73	S_4	G 4 35.7	7.8	23.828	33.204	46.936	25.808
74	S_4	G 4 35.7	7.10	30.716	39.189	49.923	37.113
75	S_4	G 4 35.7	7.11	34.188	47.373	60.557	34.975
76	S_4	G 4 35.7	8.2	39.002	49.429	62.536	39.268

77	S_4	G 4 35.7	8.3	22.997	32.365	45.366	26.79
78	S_4	G 4 35.7	8.5	26.531	36.938	48.955	28.192
79	S_4	G 4 35.7	8.6	49.258	58.871	70.529	52.855
80	S_4	G 4 35.7	8.7	37.1	37.404	50.691	39.978
81	S_4	G 4 35.7	8.8	33.094	42.069	53.882	34.942
82	S_4	G 4 35.7	8.10	23.787	33.007	46.001	26.037
83	S_4	G 4 35.7	8.14	31.885	31.959	44.255	34.992
84	S_4	G 4 35.7	9.2	73.244	81.626	86.673	72.625
85	S_4	G 4 35.7	9.5	45.885	54.618	68.505	48.991
86	S_4	G 4 35.7	9.7	28.94	37.832	49.128	32.66
87	S_4	G 4 35.7	9.8	24.814	33.412	47.144	27.021
88	S_4	G 4 35.7	9.10	38.915	47.407	60.694	42.832
89	S_4	G 4 35.7	9.12	62.092	70.671	74.013	64.793
90	S_4	G 4 35.7	9.13	20.61	32.251	43.814	26.365
91	S_4	G 4 35.7	9.14	34.848	43.926	50.392	37.091
92	S_4	G 4 35.7	10.1	60.432	68.687	80.247	62.967
93	S_4	G 4 35.7	10.3	49.825	61.897	70.469	52.416
94	S_4	G 4 35.7	10.7	35.361	44.23	57.079	37.21
95	S_4	G 4 35.7	10.8	36.518	46.14	47.807	40.832
96	S_4	G 4 35.7	10.10	17.801	29.118	40.692	27.076
97	S_4	G 4 35.7	10.11	30.282	34.194	46.865	27.105
98	S_4	G 4 35.7	10.14	30.45	39.163	49.56	27.003
99	S_4	G 4 35.7	10.15	31.959	42.242	53.881	30.458
			CV (%)	2.269	1.147	3.19	1.14
			CD (0.05)	3.089	1.323	3.105	1.49

Monsoon 2017

The average relative water content (RWC) in inbred self was 53.80 per cent. The RWC ranged from 29.12 to 88.85 percent. The maximum RWC (88.85 percent) and was recorded in S_1 generation of $G_4$35.7 (Stand number 2.16) the minimum RWC (29.12 per cent) was recorded S_4 generation of $G_4$35.7 (Stand number 10.10).

Post monsoon 2017

The average relative water content (RWC) in inbred self during post monsoon season was 63.75 per cent. The RWC ranged from 40.69 to 93.97 percent. The maximum RWC (93.97 percent) was recorded in G4 35.7(Stand number 2.16) and minimum RWC (40.69 per cent) was recorded S4 generation of $G_4$35.7(Stand number 10.10).

Winter 2017

The average relative water content (RWC) in inbred self during winter season was 47.49 per cent. The RWC ranged from 25.81 to 89.79 percent. The maximum RWC (89.71 percent) was recorded in G_4 35.7(Stand number 2.16) and minimum RWC (25.81 per cent) was recorded S4 generation of G_4 35.7(Stand number 7.8).

4.2.3.5 Inbred cross

The RWC was measured in 16 inbred cross plants during 2017 for four seasons the results are presented in table 58.

Summer 2017

The average relative water content (RWC) in inbred cross was 15.49 per cent. The RWC ranged from 12.39 to 21.24 percent. The maximum RWC (21.24 percent) and was recorded in S_3 $G_2$7.4 x S_3G_4 35.7 (Stand number 4.1) the minimum RWC (12.39 per cent) was recorded in S3 $G_2$7.4 x S_3G_4 35.7 (Stand number 34).

Table 58 Relative water content (RWC) in inbred crosses during 2017

S. No.	Cross	Stand No	Apr-17	Jun-17	Sep-17	Dec-17
1	S_3 $G_2$7.4 X S_3G_4 35.7	3.1	15.72	15.99	25.66	17.18
2	S_3 $G_2$7.4 X S_3G_4 35.7	3.4	12.39	14.59	19.43	16.79
3	S_3 $G_2$7.4 X S_3G_4 35.7	3.8	15.45	16.46	22.50	18.46
4	S_3 $G_2$7.4 X S_3G_4 35.7	4.1	21.24	25.41	28.96	23.89
5	S_3 $G_2$7.4 X S_3G_4 35.7	4.8	18.52	22.35	23.36	18.72
6	S_3 $G_2$7.4 X S_3G_4 35.7	5.7	17.32	19.02	18.21	20.60
7	S_3H_7 3 (86) X S_3G_4 35.7	2.6	18.41	19.39	19.37	21.87
8	S_3H_7 3 (86) X S_3G_4 35.7	4.4	15.36	18.85	11.23	18.89
9	S_3H_7 3 (86) X S_3G_4 35.7	4.5	12.56	15.24	12.39	17.80
10	S_3H_7 3 (86) X S_3G_4 35.7	4.6	13.28	12.96	12.48	18.22
11	S_3H_7 3 (86) X S_3G_4 35.7	4.7	13.93	13.65	12.65	18.13
12	S_3H_7 3 (86) X S_3G_4 35.7	5.3	13.88	14.36	11.61	18.38
13	S_3H_7 3 (86) X S_3G_4 35.7	7.5	14.22	15.40	13.46	16.58
14	S_5G_1 7.4 X S_3G_4 35.7	3.5	15.41	17.31	17.67	18.53
15	S_5G_1 7.4 X S_3G_4 35.7	3.6	14.59	18.56	16.39	18.41
16	S_5G_1 7.4 X S_3G_4 35.7	3.7	15.58	18.38	16.65	17.48
		CV (%)	0.935	1.256	0.956	1.123
		CD (0.05)	3.61	4.32	3.24	3.5

Monsoon 2017

The average relative water content (RWC) in inbred cross was 17.34 per cent. The RWC ranged from 12.96 to 25.41 percent. The maximum RWC (25.41 percent) was recorded in S_3 $G_2$7.4 x S_3G_4 35.7 (Stand number 4.1) and minimum RWC (12.41per cent) was recorded in S_3H_7 3 (86) x S_3G_4 35.7 (Stand number 4.6).

Post monsoon 2017

The average relative water content (RWC) in inbred cross during post monsoon season was 17.63 per cent. The RWC ranged from 11.23 to 28.96 percent. The maximum RWC (43.15 percent) was recorded in S_3 $G_2$7.4 x S_3G_4 35.7 (Stand number 4.1) and minimum RWC (11.23 per cent) was recorded in S_3H_7 3 (86) x S_3G_4 35.7 (Stand number 4.4).

Winter 2017

The average relative water content (RWC) in inbred cross during winter season was 18.75 per cent. The RWC ranged from 16.58 to 23.89 percent. The maximum RWC (23.89 percent) was recorded in S3 $G_2$7.4 x S_3G_4 35.7 (Stand number 4.1) and minimum RWC (16.58 per cent) was recorded in S_3H_7 3 (86) x S_3G_4 35.7

4.2.3.6 Hybrids

The RWC was measured in 48 hybrids plants during 2017 for four seasons the results are presented in table 59.

Summer 2017

The average relative water content (RWC) in hybrids was 27.03 per cent. The RWC ranged from 10.61 to 78.78 percent. The maximum RWC (78.78 percent) was recorded in CCRP12 (Stand number 12.6) the minimum RWC (10.61 per cent) was recorded in CCRP 14 (Stand number 14.6).

Table 59 Relative water content (RWC) in hybrid during 2017

S. No.	Hybrids	Stand No	Apr-17	Jun-17	Sep-17	Dec-17
1	CCRP 8	8.1	35.002	33.288	46.58	44.457
2	CCRP 8	8.2	43.815	43.45	46.583	43.185
3	CCRP 8	8.4	48.115	42.701	52.757	48.309
4	CCRP 8	23.3L	35.965	34.154	41.76	36.346
5	CCRP 8	23.7L	40.512	41.804	48.677	43.662
6	CCRP 8	23.6	21.025	26.242	34.673	29.699
7	CCRP 8	8.6L	13.142	15.126	22.717	17.375
8	CCRP 8	8.5L	22.798	23.164	32.806	27.028
9	CCRP 9	9.3	31.549	27.801	38.145	32.364
10	CCRP 9	9.5L	39.27	36.469	42.869	39.285
11	CCRP 9	9.6L	32.104	29.362	36.757	30.883
12	CCRP 9	24.6L	28.019	29.134	38.579	33.357
13	CCRP 9	24.7R	26.244	26.481	44.164	39.544
14	CCRP 9	24.3L	23.293	16.246	26.356	21.5
15	CCRP 10	10.2	40.626	41.805	52.002	47.649
16	CCRP 10	10.5	23.441	25.465	36.687	31.58
17	CCRP 10	25.3L	22.19	23.502	36.886	31.258
18	CCRP 11	11.4	21.75	22.478	35.642	30.24
19	CCRP 11	26.6	27.439	30.398	42.646	37.267
20	CCRP 11	26.7	37.114	39.139	51.505	46.547
21	CCRP 12	12.3	22.662	24.504	36.577	31.5
22	CCRP 12	12.5	37.708	38.58	42.652	37.454
23	CCRP 12	12.6	78.782	64.126	63.172	59.413
24	CCRP 12	12.6L	23.461	24.143	35.429	30.245
25	CCRP 12	27.7R	29.203	26.478	35.701	31.568
26	CCRP 12	27.6	24.45	21.617	28.591	24.541
27	CCRP 12	27.3L	19.466	17.281	24.565	24.474
28	CCRP 13	13.4	20.379	16.498	27.067	22.357
29	CCRP 13	13.6	26.936	23.801	35.719	31.232
30	CCRP 13	13.5L	22.297	19.266	23.585	18.31
31	CCRP 13	13.6L	16.334	13.928	21.585	17.524
32	CCRP 13	28.6R	19.19	17.555	23.508	19.369
33	CCRP 13	28.7R	15.18	13.207	18.578	16.205
34	CCRP 13	28.1L	16.688	12.198	19.818	14.262
35	CCRP 13	28.3L	24.479	22.476	28.492	21.537
36	CCRP 14	14.3	18.597	18.134	28.74	22.222

37	CCRP 14	14.5	19.122	18.876	28.25	23.253
38	CCRP 14	14.6	10.61	11.837	17.918	18.333
39	CCRP 14	14.5L	21.097	23.837	32.814	29.396
40	CCRP 14	14.6L	27.168	27.177	35.585	36.086
41	CCRP 14	29.2L	19.56	23.978	36.485	36.357
42	CCRP 15	15.4	26.303	28.133	35.139	32.358
43	CCRP 15	15.5	24.233	32.502	35.903	35.634
44	CCRP 15	15.6	29.579	27.477	35.368	34.221
45	CCRP 15	15.7	24.876	27.911	34.703	35.171
46	CCRP 15	15.6L	18.504	26.616	32.938	32.143
47	CCRP 15	31.6R	25.786	29.911	16.673	18.39
48	CCRP 15	30.1L	21.563	27.81	26.549	18.292
		CV (%)	3.577	4.772	6.068	1.068
		CD (0.05)	1.57	2.079	3.429	0.536

Monsoon 2017

The average relative water content (RWC) in hybrids in monsoon 2017 was 26.83 per cent. The RWC ranged from 11.84 to 64.13 percent. The maximum RWC (64.13 percent) was recorded in CCRP12 (Stand number 12.6) the minimum RWC (11.84 per cent) was recorded in CCRP 14 (Stand number 11.84).

Post monsoon 2017

The average relative water content (RWC) in hybrids in post-monsoon 2017 was 34.81 per cent. The RWC ranged from 16.67 to 63.17 percent. The maximum RWC (63.17 percent) was recorded in CCRP12 (Stand number 12.6) the minimum RWC (16.67 per cent) was recorded in CCRP 15 (Stand number 31.6).

Winter 2017

The average relative water content (RWC) in hybrids in post-monsoon 2017 was 30.90 per cent. The RWC ranged from 14.26 to 59.41 percent. The maximum RWC (59.41 percent) was recorded in CCRP12 (Stand number 12.6) the minimum RWC (14.26 per cent) was recorded in CCRP 13 (Stand number 28.1).

4.2.4 Leaf nutrient content

The nutrient content in inbred self is presented in table 60.

Nitrogen content in leaf varied significantly among the inbred self of cocoa. The maximum leaf nitrogen content of 2.65 per cent was observed in M 18.7 (stand number 5.7 and 5.12) followed by M 18.7 (Stand number 5.9). The least nitrogen content of 1.05 per cent was observed in M 18.7 (Stand number 4.11). The difference in nutrient content in leaf may be due to difference in nutrient uptake ability of plant and translocation of nitrogen. The height of plant influence the root distribution pattern, there by the uptake of nutrient also differs among the inbreds of cocoa.

The phosphorus content in leaf ranged from 0.04 per cent in S1 generation of G IV 35.7 (Stand number 8.16) to .0.5 per cent in M 18.7 (Stand number 4.7). The

Table 60. Leaf nutrient content in cocoa inbreds of cocoa

S. No.	Generation	Genotype	Stand No.	N content (Nitrogen) (%)	P content (Phosphorus) (%)	K content (Potassium) (%)
1	S_4	M 18.7	1.1	1.52	0.18	1.42
2	S_4	M 18.7	1.2	1.53	0.15	1.44
3	S_4	M 18.7	1.4	1.55	0.21	1.40
4	S_4	M 18.7	1.5	1.53	0.18	1.24
5	S_4	M 18.7	1.6	1.61	0.15	1.37
6	S_4	M 18.7	1.8	1.53	0.13	1.35
7	S_4	M 18.7	1.9	1.53	0.23	1.33
8	S_4	M 18.7	1.1	1.53	0.21	1.35
9	S_4	M 18.7	1.12	1.07	0.21	1.31
10	S_4	M 18.7	1.13	1.06	0.19	1.41
11	S_4	M 18.7	1.14	1.94	0.16	1.06
12	S_4	M 18.7	2.1	1.33	0.16	1.05
13	S_4	M 18.7	2.4	1.32	0.15	1.88
14	S_4	M 18.7	2.5	1.59	0.15	1.45
15	S_4	M 18.7	2.6	2.54	0.19	1.42
16	S_4	M 18.7	2.7	2.41	0.16	1.35
17	S_4	M 18.7	2.8	2.09	0.17	1.38
18	S_4	M 18.7	2.9	2.13	0.17	1.34
19	S_4	M 18.7	2.10	2.16	0.13	1.13
20	S_4	M 18.7	2.11	1.94	0.20	1.37
21	S_4	M 18.7	2.13	1.93	0.12	1.43
22	S_4	M 18.7	2.14	1.94	0.14	1.42
23	S_4	M 18.7	3.1	2.11	0.12	1.43
24	S_4	M 18.7	3.2	2.12	0.13	1.54
25	S_4	M 18.7	3.3	1.75	0.24	1.46
26	S_4	M 18.7	3.4	1.73	0.26	1.56
27	S_4	M 18.7	3.5	1.73	0.23	1.58
28	S_4	M 18.7	3.8	1.75	0.12	1.52
29	S_4	M 18.7	3.9	1.74	0.13	1.55
30	S_4	M 18.7	3.10	1.72	0.13	1.24
31	S_4	M 18.7	3.11	1.76	0.13	1.25
32	S_4	M 18.7	3.12	1.72	0.14	1.28
33	S_4	M 18.7	3.14	1.74	0.14	1.26
34	S_4	M 18.7	3.15	1.73	0.15	1.31
35	S_4	M 18.7	4.1	1.77	0.12	1.26

36	S_4	M 18.7	4.2	1.51	0.45	1.25
37	S_4	M 18.7	4.3	1.93	0.13	1.23
38	S_4	M 18.7	4.4	1.43	0.14	1.24
39	S_4	M 18.7	4.5	1.44	0.14	1.26
40	S_4	M 18.7	4.6	1.45	0.15	1.25
41	S_4	M 18.7	4.7	1.77	0.50	1.26
42	S_4	M 18.7	4.8	1.72	0.14	1.31
43	S_4	M 18.7	4.9	1.76	0.15	1.36
44	S_4	M 18.7	4.10	1.62	0.14	1.31
45	S_4	M 18.7	4.11	1.05	0.14	1.28
46	S_4	M 18.7	4.12	1.14	0.15	1.30
47	S_4	M 18.7	4.13	1.42	0.48	1.26
48	S_4	M 18.7	4.14	1.42	0.16	1.31
49	S_4	M 18.7	4.15	1.48	0.15	1.32
50	S_4	M 18.7	5.1	1.54	0.13	1.35
51	S_4	M 18.7	5.2	1.47	0.14	1.39
52	S_4	M 18.7	5.4	1.77	0.15	1.17
53	S_4	M 18.7	5.6	1.36	0.13	1.19
54	S_4	M 18.7	5.7	1.65	0.14	1.19
55	S_4	M 18.7	5.8	1.24	0.13	1.13
56	S_4	M 18.7	5.9	2.64	0.14	1.15
57	S_4	M 18.7	5.10	2.65	0.19	1.16
58	S_4	M 18.7	5.11	2.64	0.16	1.15
59	S_4	M 18.7	5.13	2.48	0.19	1.18
60	S_4	M 18.7	5.14	1.95	0.16	1.17
61	S_4	M 18.7	5.15	1.74	0.09	1.22
62	S_4	M 18.7	6.1	1.93	0.20	1.28
63	S_4	M 18.7	6.2	1.88	0.16	1.26
64	S_4	M 18.7	6.6	1.24	0.19	1.28
65	S_4	M 18.7	6.7	1.34	0.19	1.28
66	S_4	M 18.7	6.8	1.28	0.21	1.26
67	S_4	M 18.7	6.9	1.56	0.20	1.16
68	S_4	M 18.7	6.10	1.42	0.19	1.28
69	S_4	M 18.7	6.11	2.04	0.21	1.27
70	S_1	G 4 35.7	1.16	1.45	0.13	1.27
71	S_1	G 4 35.7	1.17	1.67	0.12	1.23
72	S_1	G 4 35.7	2.16	1.78	0.14	1.27
73	S_1	G 4 35.7	2.17	1.69	0.21	1.27
74	S_1	G 4 35.7	3.17	1.46	0.16	1.18
75	S_1	G 4 35.7	5.17	1.28	0.08	1.18

76	S_1	G 4 35.7	6.16	1.40	0.12	1.47
77	S_1	G 4 35.7	7.16	1.52	0.11	1.43
78	S_1	G 4 35.7	7.17	1.06	0.26	1.34
79	S_1	G 4 35.7	8.16	1.40	0.04	1.16
80	S_1	G 4 35.7	8.17	1.42	0.26	1.17
81	S_1	G 4 35.7	9.16	2.20	0.24	1.16
82	S_1	G 4 35.7	10.16	1.84	0.25	1.18
83	S_4	G 4 35.7	6.13	1.78	0.12	1.06
84	S_4	G 4 35.7	6.14	1.66	0.12	1.09
85	S_4	G 4 35.7	6.15	1.77	0.11	1.23
86	S_4	G 4 35.7	7.1	1.56	0.22	1.25
87	S_4	G 4 35.7	7.3	1.92	0.25	1.34
88	S_4	G 4 35.7	7.4	1.92	0.21	1.19
89	S_4	G 4 35.7	7.6	1.75	0.09	1.34
90	S_4	G 4 35.7	7.8	1.10	0.15	1.36
91	S_4	G 4 35.7	7.10	2.05	0.15	1.33
92	S_4	G 4 35.7	7.11	1.93	0.15	1.33
93	S_4	G 4 35.7	8.2	1.23	0.22	1.31
94	S_4	G 4 35.7	8.3	1.94	0.22	1.19
95	S_4	G 4 35.7	8.5	1.77	0.14	1.23
96	S_4	G 4 35.7	8.6	1.76	0.24	1.17
97	S_4	G 4 35.7	8.7	1.76	0.16	1.23
98	S_4	G 4 35.7	8.8	1.57	0.18	1.21
99	S_4	G 4 35.7	8.10	1.76	0.17	1.24
100	S_4	G 4 35.7	8.14	1.53	0.18	1.28
101	S_4	G 4 35.7	9.2	1.40	0.16	1.28
102	S_4	G 4 35.7	9.5	1.44	0.16	1.24
103	S_4	G 4 35.7	9.7	1.62	0.15	1.22
104	S_4	G 4 35.7	9.8	1.63	0.16	1.24
105	S_4	G 4 35.7	9.10	1.84	0.13	1.22
106	S_4	G 4 35.7	9.12	1.84	0.15	1.28
107	S_4	G 4 35.7	9.13	1.91	0.18	1.29
108	S_4	G 4 35.7	9.14	1.88	0.24	1.36
109	S_4	G 4 35.7	10.1	1.85	0.27	1.33
110	S_4	G 4 35.7	10.3	1.64	0.24	1.33
111	S_4	G 4 35.7	10.7	1.95	0.28	1.31
112	S_4	G 4 35.7	10.8	1.46	0.24	1.19
113	S_4	G 4 35.7	10.10	1.92	0.25	1.23
114	S_4	G 4 35.7	10.11	1.45	0.25	1.17
115	S_4	G 4 35.7	10.14	1.54	0.27	1.23

116	S_4	G 4 35.7	10.15	1.63	0.25	1.21
			CV (%)	3.694	42.917	2.761
			CD (0.05)	0.101	0.123	0.057

Table 61. Leaf nutrient content of inbred crosses of cocoa

	Inbred cross	Plant No	N (%)	P (%)	K (%)
1	S_3 $G_2$7.4 X S_3G_4 35.7	1.1	1.92	0.187	1.84
2	S_3 $G_2$7.4 X S_3G_4 35.7	3.1	1.38	0.22	1.24
3	S_3 $G_2$7.4 X S_3G_4 35.7	3.2	1.41	0.22	1.24
4	S_3 $G_2$7.4 X S_3G_4 35.7	3.3	2.08	0.273	1.01
5	S_3 $G_2$7.4 X S_3G_4 35.7	3.4	1.73	0.117	1.21
6	S_3 $G_2$7.4 X S_3G_4 35.7	3.8	1.56	0.223	1.33
7	S_3 $G_2$7.4 X S_3G_4 35.7	4.1	1.42	0.143	1.22
8	S_3 $G_2$7.4 X S_3G_4 35.7	4.8	1.07	0.14	1.24
9	S_3 $G_2$7.4 X S_3G_4 35.7	5.6	1.04	0.457	1.39
10	S_3 $G_2$7.4 X S_3G_4 35.7	5.7	1.05	0.173	1.37
11	S_3 $G_2$7.4 X S_3G_4 35.7	5.8	1.23	0.213	1.41
12	S_3 $G_2$7.4 X S_3G_4 35.7	6.6	1.41	0.25	1.31
13	S_3 $G_2$7.4 X S_3G_4 35.7	6.4	1.67	0.42	1.11
14	S_3 $G_2$7.4 X S_3G_4 35.7	6.5	1.22	0.227	1.28
15	S_3 $G_2$7.4 X S_3G_4 35.7	6.8	1.35	0.23	1.22
16	S_3H_7 3 (86) X S_3G_4 35.7	2.1	1.88	0.257	1.24
17	S_3H_7 3 (86) X S_3G_4 35.7	2.2	1.75	0.33	1.43
18	S_3H_7 3 (86) X S_3G_4 35.7	2.4	1.96	0.207	1.31
19	S_3H_7 3 (86) X S_3G_4 35.7	2.6	1.59	0.21	1.29
20	S_3H_7 3 (86) X S_3G_4 35.7	2.7	1.41	0.54	1.37
21	S_3H_7 3 (86) X S_3G_4 35.7	4.4	1.59	0.22	1.34
22	S_3H_7 3 (86) X S_3G_4 35.7	4.6	1.73	0.317	1.34
23	S_3H_7 3 (86) X S_3G_4 35.7	4.4	1.60	0.64	1.79
24	S_3H_7 3 (86) X S_3G_4 35.7	4.5	0.85	0.127	1.33
25	S_3H_7 3 (86) X S_3G_4 35.7	4.6	1.59	0.11	1.34
26	S_3H_7 3 (86) X S_3G_4 35.7	4.7	1.57	0.2	1.35
27	S_3H_7 3 (86) X S_3G_4 35.7	5.3	1.58	0.12	1.42
28	S_3H_7 3 (86) X S_3G_4 35.7	7.2	1.92	0.153	1.19
29	S_3H_7 3 (86) X S_3G_4 35.7	7.4	1.41	0.143	1.12
30	S_3H_7 3 (86) X S_3G_4 35.7	7.5	2.12	0.157	1.22
31	S_3H_7 3 (86) X S_3G_4 35.7	6.1	1.40	0.13	1.32
32	S_5G_1 7.4 X S_3G_4 35.7	3.5	1.41	0.127	1.16

33	S_5G_1 7.4 X S_3G_4 35.7	3.6	1.42	0.12	1.14
34	S_5G_1 7.4 X S_3G_4 35.7	3.7	1.41	0.13	1.15
	CV (%)		6.18	4.44	1.93
	CD (0.05)		0.154	0.016	0.041

Table 62. Leaf nutrient contents in hybrid of cocoa

S. No.	Hybrid	Stand No.	N (%)	P (%)	K (%)
1	CCRP 8	8.1	1.42	0.12	1.43
2	CCRP 8	8.2	1.42	0.13	1.29
3	CCRP 8	8.4	1.06	0.13	1.20
4	CCRP 8	23.3 L	1.56	0.16	1.42
5	CCRP 8	23.7 L	1.41	0.08	1.15
6	CCRP 8	23.6	1.42	0.13	1.14
7	CCRP 8	8.6 L	1.52	0.18	1.18
8	CCRP 8	8.5 L	1.23	0.17	1.18
9	CCRP 9	9.3	1.42	0.11	1.33
10	CCRP 9	9.5 L	1.41	0.81	1.21
11	CCRP 9	9.6 L	1.05	0.81	1.41
12	CCRP 9	24.6 L	1.08	0.71	1.18
13	CCRP 9	24.7 R	1.09	0.62	1.16
14	CCRP 9	24.3 L	1.05	0.09	1.27
15	CCRP 10	10.2	0.83	0.46	1.17
16	CCRP 10	10.5	0.77	0.06	1.24
17	CCRP 10	25.3 L	0.74	0.16	1.17
18	CCRP 11	11.4	0.88	0.11	1.30
19	CCRP 11	11.6 L	0.92	0.18	1.13
20	CCRP 11	26.2 L	1.75	0.19	1.31
21	CCRP 11	26.6	1.11	0.13	1.25
22	CCRP 11	26.7	1.02	0.12	1.14
23	CCRP 12	12.3	0.72	0.15	1.24
24	CCRP 12	12.5	0.84	0.15	1.26
25	CCRP 12	12.6	1.19	0.12	1.22
26	CCRP 12	12.6 L	0.71	0.12	1.26
27	CCRP 12	27.7 R	0.94	0.13	1.28
28	CCRP 12	27.6	0.99	0.19	1.33
29	CCRP 12	27.2 L	1.05	0.32	1.06
30	CCRP 12	27.3 L	1.07	0.18	1.07
31	CCRP 13	13.4	1.06	0.11	1.22
32	CCRP 13	13.6	1.02	0.09	1.14
33	CCRP 13	13.5 L	0.74	0.09	1.19
34	CCRP 13	13.6 L	1.33	0.06	1.18
35	CCRP 13	28.6 R	1.50	0.07	1.17
36	CCRP 13	28.7 R	1.04	0.06	1.18
37	CCRP 13	28.1 L	1.15	0.05	1.11
38	CCRP 13	28.3 L	1.55	0.05	1.12

39	CCRP 14	14.3	0.53	0.17	1.28
40	CCRP 14	14.5	0.36	0.09	1.13
41	CCRP 14	14.6	1.05	0.18	1.20
42	CCRP 14	14.5 L	1.12	0.06	1.18
43	CCRP 14	14.6 L	1.12	0.06	1.15
44	CCRP 14	29.2 L	1.05	0.14	1.11
45	CCRP 15	15.4	0.36	0.12	1.15
46	CCRP 15	15.5	1.35	0.27	1.17
47	CCRP 15	15.6	1.70	0.10	1.18
48	CCRP 15	15.7	1.37	0.06	1.18
49	CCRP 15	15.6 L	1.74	0.04	1.15
50	CCRP 15	31.6 R	1.75	0.06	1.16
51	CCRP 15	30.1 L	1.56	0.10	1.32
		CV (%)	0.06	0.03	0.102
		CD (0.05)	3.226	12.887	5.183

The girth of inbred self was superior over inbred crosses and hybrids with a comparison ratio of 1.59 and 1.70 respectively. Among the inbred crosses and hybrids, inbred crosses were superior with a comparison ratio of 1.07 (Table 74).

The chupan height was higher in inbred crosses when compared with the inbred self. The hybrids did not produce any chupan during 2018 (Table 75).

4.2.6.4 Chlorophyll content (%)

The percent chlorophyll content among the inbreds, inbred crosses and hybrids are presented in table76. The comparison of Chlorophyll A, Chlorophyll B and total chlorophyll are presented in 77, 78, 76 and 79. With respect to chlorophyll A, The inbred self had slight high chlorophyll content than inbred cross with a comparison ratio of 0.98. Among the hybrids and inbred self, the inbred self plants had high chlorophyll content with a comparison ratio of 1.29. When inbred crosses and hybrids are compared, the inbred crosses are superior over hybrids with a comparison value of 1.31(Table 77)

The mean chlorophyll B content in inbred self was higher than inbred cross and hybrid with a comparison ratio of 2.66, 3.00 and 1.12 respectively. Among these the chlorophyll content was less in hybrids (Table 78).

The total chlorophyll content was high in inbred self followed by inbred cross and hybrids. The comparison ratio for inbred self : inbred cross, inbred self : hybrid and inbred cross : hybrid was 1.23, 1.59 and 1.29 respectively(Table 79).

4.2.6.5 Relative water content (%) during 2016

The details on percentage of relative water content in leaf in different seasons during 2016 in inbred self, inbred cross and hybrids of cocoa is presented in table 80, 81, 82, 83 and 84. The relative content during April 2016 was higher in inbred self than inbred cross with a comparison ratio of 3.0. Among the hybrids and inbred self, inbred self had more relative water content than hybrid with a comparison ratio of 1.42. Hybrids had high relative water than inbred cross with a comparison ratio of 0.47. The

variation in the nutrient content in the leaf of cocoa is due to the difference in the nutrient uptake capacity of the plant and translocation of P within the plant. Even the root distribution pattern also influences the nutrient uptake within the plant.

The average potassium content in the leaf of inbred self was 1.29 per cent. Whereas, the maximum potassium content of 1.88 per cent was registered in M 18.7 (Stand number 2.4). The minimum potassium content (1.05 per cent) was registered in M 18.7 (Stand number 2.1)

The nutrient content in inbred cross are presented in table number 61.

The average nitrogen content in inbred cross was 1.52 per cent. The nitrogen content in leaf varied from 0.85 per cent in S_3H_7 3 (86) X S_3G_4 35.7 (Stand number 4.5) to 2.12 per cent in S_3H_7 3 (86) X S_3G_4 35.7 (Stand number 7.5).

The phosphorus content in inbred cross varied significantly, the phosphorus content in inbred cross ranged from 0.11 per cent in S_3H_7 3 (86) X S_3G_4 35.7 (Stand number 4.6) to 0.64 per cent in S_3H_7 3 (86) X S_3G_4 35.7 (Stand number 4.4). The average phosphorus content in inbred cross was 0.23 per cent.

The potassium content in inbred cross registered a significant variation with average potassium content was 1.31 per cent. The maximum potassium content of 1.84 per cent was registered in S_3 $G_2$7.4 X S_3G_4 35.7 9 (Stand number 1.1). The minimum potassium content of 1.01 per cent was registered in S_3 $G_2$7.4 X S_3G_4 35.7 (Stand number 1.1).

The nutrient content in hybrids are presented in table number 62.

The nitrogen content in hybrids varied significantly, the maximum nitrogen content of 1.75 per cent was registered in CCRP 11 (Stand number 26.2). The least nitrogen content of 0.36 per cent was observed in CCRP 15 (Stand number 15.4). The average nitrogen content in hybrids was 1.14 per cent.

The phosphorus content in hybrids ranged between 0.05 per cent in CCRP 13 (Stand number 28.1 and 28.3) to 0.81 per cent in CCRP 9 (Stand number 9.5 and 9.6L). The average phosphorus content in hybrid was 0.18 per cent.

The average potassium content in hybrids was 1.21 per cent. The maximum potassium content of 1.43 per cent was observed in CCRP 8(Stand number 8.1) and the minimum potassium content (1.06 per cent) in hybrids was registered in CCRP 12 (Stand number 27.2).

4.2.5 Plant phenological observation

The plant phenological observations in inbred self, inbred crosses and hybrids of cocoa are presented in table 63, 64 and 65 respectively.

4.2.5.1 Bud break

The bud break is the time from appearance of bud in the cushion till opening of the flower. In inbred-self, the bud break was between 18 and 21 days.

In inbred cross, the flowering and bud break was not observed

In hybrids, the bud break occurred between 18 and 20 days

4.2.5.2 Flushing

The flushing in inbred self took place in the month of July and December months.

In inbred cross, flushing took place in the month of August and September. In hybrids, the flushing took place in the month of July and November.

4.2.5.3 No. of cushions formed

The maximum number of cushions (18 No's) were formed in M 18.7 (stand number 3.8). In inbred cross, cushions did not form.

a. Flushing in inbreds, inbred crosses and hybrids

b. Cushion formation in inbreds and hybrids

Plate 8. Flushing and cushion formation in inbreds, inbred crosses and hybrids

4.2.5.4 Year round flowering

The flowering took place in the month of September to December in inbred self. In inbred cross, flowering did not occur.

In hybrid, the flowering took place between September and December.

Relative water content (%) in inbreds, inbred crosses and hybrids over different seasons during 2016					
		Summer (April)	**Monsoon (June)**	**Post monsoon (Sept)**	**Winter (Dec)**
Inbred self 2016	Range	16.62- 79.05	31.72-77.47	38.23-87.18	26.28-87.61
	Average	44.58	50.83	59.28	49.07
Inbred cross 16	Range	9.83-20.62	22.85-32.18	28.26-43.15	16.46-29.24
	Average	14.86	26.68	37.76	22.98
Hybrids 16	Range	14.87-82.73	12.15-65.34	29.80-68.26	21.28-57.99
	Average	31.48	27.06	45.62	37.24

Relative water content (%) in inbreds, inbred crosses and hybrids over different seasons during 2017					
		Summer (April)	**Monsoon (June)**	**Post monsoon (Sept)**	**Winter (Dec)**
Inbred self 17	Range	17.80-80.56	29.12-88.85	40.69-93.97	25.81-89.79
	Average	45.58	53.80	63.75	47.49
Inbredcross 17	Range	12.39-21.24	12.96-25.41	11.23-28.96	16.58-23.89
	Average	15.49	17.37	17.63	18.75
Hybrids 17	Range	10.61-78.78	11.84-64.13	16.67-63.17	14.26-59.41
	Average	27.03	26.83	34.81	30.90

4.2.6 Comparative evaluation of inbreds, inbred crosses and hybrids of cocoa

4.2.6.1 Growth parameters during 2016

The mean growth parameters among the inbreds, inbred crosses and hybrids are presented in table 66. The comparison of inbreds, inbred crosses and hybrids during 2016 with respect to plant height and girth is presented in table 67 and 68 respectively. With respect to plant height during 2016, the inbred cross was superior over inbred self

Table 63 Phenological observations in inbreds of cocoa

S. No.	Generation	Genotype	Stand No.	Bud break	Flushing	No. of cushions formed	Year round flowering		
							2016	2017	2018
1	S_4	M 18.7	1.1	18	Jul and Dec	4	Nil		Sept
2	S_4	M 18.7	1.2	19	Jul and Dec	1	Nil		Sept
3	S_4	M 18.7	1.4		Jul and Dec	0	Nil		
4	S_4	M 18.7	1.5		Jul and Dec	0	Nil		
5	S_4	M 18.7	1.6	18	Jul and Dec	5	Nil		Sept
6	S_4	M 18.7	1.8		Jul and Dec	0	Nil		
7	S_4	M 18.7	1.9		Jul and Dec	0	Nil		
8	S_4	M 18.7	1.10	18	Jul and Dec	1	Nil	Oct	Sept
9	S_4	M 18.7	1.12		Jul and Dec	0	Nil		
10	S_4	M 18.7	1.13		Jul and Dec	0	Nil		
11	S_4	M 18.7	1.14		Jul and Dec	0	Nil		
12	S_4	M 18.7	2.1	19	Jul and Dec	4	Nil		Sept
13	S_4	M 18.7	2.4		Jul and Dec	0	Nil		
14	S_4	M 18.7	2.6	18	Jul and Dec	3	Nil	Sept	Nov
15	S_4	M 18.7	2.7	19	Jul and Dec	9	Nil		Sept
16	S_4	M 18.7	2.8	18	Jul and Dec	6	Nil		
17	S_4	M 18.7	2.11		Jul and Dec	0	Nil		
18	S_4	M 18.7	2.13	18	Jul and Dec	13	Nil		Sept
19	S_4	M 18.7	2.14	19	Jul and Dec	6	Nil		Sept
20	S_4	M 18.7	3.1		Jul and Dec	0	Nil		
21	S_4	M 18.7	3.2		Jul and Dec	0	Nil		
22	S_4	M 18.7	3.3		Jul and Dec	0	Nil		
23	S_4	M 18.7	3.4	18	Jul and Dec	4	Nil		Oct
24	S_4	M 18.7	3.8	18	Jul and Dec	18	Nil		Oct
25	S_4	M 18.7	3.9	18	Jul and Dec	1	Nil		Oct
26	S_4	M 18.7	3.10		Jul and Dec	0	Nil		
27	S_4	M 18.7	3.11	19	Jul and Dec	6	Nil		Sept
28	S_4	M 18.7	3.12	18	Jul and Dec	3	Nil		Sept
29	S_4	M 18.7	3.13	19	Jul and Dec	11	Nil		Sept
30	S_4	M 18.7	3.14	18	Jul and Dec	8	Nil		Sept
31	S_4	M 18.7	3.15	18	Jul and Dec	2	Nil		Sept
32	S_4	M 18.7	4.3		Jul and Dec	0	Nil		
33	S_4	M 18.7	4.4	19	Jul and Dec	4	Nil		Sept
34	S_4	M 18.7	4.6		Jul and Dec	0	Nil		

35	S_4	M 18.7	4.7		Jul and Dec	0	Nil		
36	S_4	M 18.7	4.8	19	Jul and Dec	4	Nil		Sept
37	S_4	M 18.7	4.9	18	Jul and Dec	9	Nil		Sept
38	S_4	M 18.7	4.10		Jul and Dec	0	Nil		
39	S_4	M 18.7	4.12		Jul and Dec	0	Nil		
40	S_4	M 18.7	4.13		Jul and Dec	0	Nil		
41	S_4	M 18.7	4.14		Jul and Dec	0	Nil		
42	S_4	M 18.7	5.1		Jul and Dec	0	Nil		
43	S_4	M 18.7	5.2		Jul and Dec	0	Nil		
44	S_4	M 18.7	5.4		Jul and Dec	0	Nil		
45	S_4	M 18.7	5.6		Jul and Dec	0	Nil		
46	S_4	M 18.7	5.7		Jul and Dec	0	Nil		
47	S_4	M 18.7	5.8		Jul and Dec	0	Nil		
48	S_4	M 18.7	5.9		Jul and Dec	0	Nil		
49	S_4	M 18.7	5.10		Jul and Dec	0	Nil		
50	S_4	M 18.7	5.13		Jul and Dec	0	Nil		
51	S_4	M 18.7	5.14		Jul and Dec	0	Nil		
52	S_4	M 18.7	5.15	18	Jul and Dec	2	Nil		Sept
53	S_4	M 18.7	6.2		Jul and Dec	0	Nil		
54	S_4	M 18.7	6.7		Jul and Dec	0	Nil		
55	S_4	M 18.7	6.8	18	Jul and Dec	2	Nil		Sept
56	S_4	M 18.7	6.9		Jul and Dec	0	Nil		
57	S_4	M 18.7	6.10		Jul and Dec	0	Nil		
58	S_1	G 4 35.7	1.16	20	Jul and Dec	7	Nil		Sept
59	S_1	G 4 35.7	1.17		Jul and Dec	0	Nil		
60	S_1	G 4 35.7	2.16	19	Jul and Dec	3	Nil		Sept
61	S_1	G 4 35.7	2.17	20	Jul and Dec	3	Nil		Sept
62	S_1	G 4 35.7	3.17	20	Jul and Dec	7	Nil		Sept
63	S_1	G 4 35.7	5.17		Jul and Dec	0	Nil		
64	S_1	G 4 35.7	7.16	21	Jul and Dec	3	Nil		Sept
65	S_1	G 4 35.7	7.17		Jul and Dec	0	Nil		
66	S_1	G 4 35.7	8.16		Jul and Dec	0	Nil		
67	S_1	G 4 35.7	8.17		Jul and Dec	0	Nil		
68	S_1	G 4 35.7	9.16		Jul and Dec	0	Nil		
69	S_1	G 4 35.7	10.16	0	Jul and Dec	0	Nil		
70	S_4	G 4 35.7	6.13		Jul and Dec	0	Nil		
71	S_4	G 4 35.7	6.14		Jul and Dec	0	Nil		
72	S_4	G 4 35.7	7.1		Jul and Dec	0	Nil		
73	S_4	G 4 35.7	7.3		Jul and Dec	0	Nil		
74	S_4	G 4 35.7	7.4		Jul and Dec	0	Nil		

75	S_4	G 4 35.7	7.6		Jul and Dec	0	Nil		
76	S_4	G 4 35.7	7.8	19	Jul and Dec	1	Nil		Sept
77	S_4	G 4 35.7	7.10		Jul and Dec	0	Nil		
78	S_4	G 4 35.7	7.11		Jul and Dec	0	Nil		
79	S_4	G 4 35.7	8.2		Jul and Dec	0	Nil		
80	S_4	G 4 35.7	8.3		Jul and Dec	0	Nil		
81	S_4	G 4 35.7	8.5		Jul and Dec	0	Nil		
82	S_4	G 4 35.7	8.6		Jul and Dec	0	Nil		
83	S_4	G 4 35.7	8.7		Jul and Dec	0	Nil		
84	S_4	G 4 35.7	8.8		Jul and Dec	0	Nil		
85	S_4	G 4 35.7	8.10		Jul and Dec	0	Nil		
86	S_4	G 4 35.7	8.14		Jul and Dec	0	Nil		
87	S_4	G 4 35.7	9.2		Jul and Dec	0	Nil		
88	S_4	G 4 35.7	9.5	19	Jul and Dec	4	Nil		Sept
89	S_4	G 4 35.7	9.7		Jul and Dec	0	Nil		
90	S_4	G 4 35.7	9.8		Jul and Dec	0	Nil		
91	S_4	G 4 35.7	9.9	19	Jul and Dec	3	Nil		Sept
92	S_4	G 4 35.7	9.10		Jul and Dec	0	Nil		
93	S_4	G 4 35.7	9.12		Jul and Dec	0	Nil		
94	S_4	G 4 35.7	9.13		Jul and Dec	0	Nil		
95	S_4	G 4 35.7	9.14		Jul and Dec	0	Nil		
96	S_4	G 4 35.7	10.1	20	Jul and Dec	1	Nil		Sept
97	S_4	G 4 35.7	10.3		Jul and Dec	0	Nil		
98	S_4	G 4 35.7	10.7		Jul and Dec	0	Nil		
99	S_4	G 4 35.7	10.8	19	Jul and Dec	2	Nil		Sept
100	S_4	G 4 35.7	10.10		Jul and Dec	0	Nil		
101	S_4	G 4 35.7	10.11	20	Jul and Dec	2	Nil		Sept
102	S_4	G 4 35.7	10.14		Jul and Dec	0	Nil		
103	S_4	G 4 35.7	10.15		Jul and Dec	0	Nil		

Table 64. Phenological observations in inbred crosses of cocoa

S. No.	Inbred cross	Stand No.	Bud break	Flushing	No. of cushions formed	Year round flowering
1	S_3 $G_2$7.4 X S_3G_4 35.7	1.1	0	Aug	0	Nil
2	S_3 $G_2$7.4 X S_3G_4 35.7	5.6	0	Aug	0	Nil
3	S_3 $G_2$7.4 X S_3G_4 35.7	1.6	0	Aug and Dec	0	Nil
4	S_3 $G_2$7.4 X S_3G_4 35.7	3.1	0	Aug and Dec	0	Nil
5	S_3 $G_2$7.4 X S_3G_4 35.7	3.2	0	Aug and Dec	0	Nil
6	S_3 $G_2$7.4 X S_3G_4 35.7	3.3	0	Aug and Dec	0	Nil
7	S_3 $G_2$7.4 X S_3G_4 35.7	3.4	0	Aug and Dec	0	Nil
8	S_5G_1 7.4 X S_3G_4 35.7	3.5	0	Aug and Dec	0	Nil
9	S_5G_1 7.4 X S_3G_4 35.7	3.6	0	Aug and Dec	0	Nil
10	S_3H_7 3 (86) X S_3G_4 35.7	4.4	0	Aug and Dec	0	Nil
11	S_3H_7 3 (86) X S_3G_4 35.7	4.5	0	Aug and Dec	0	Nil
12	S_3H_7 3 (86) X S_3G_4 35.7	4.6	0	Aug and Dec	0	Nil
13	S_3H_7 3 (86) X S_3G_4 35.7	7.4	0	Aug and Dec	0	Nil
14	S_3H_7 3 (86) X S_3G_4 35.7	7.5	0	Aug and Dec	0	Nil

Table 65. Phenological observations in hybrids of cocoa

S. No.	Hybrid	Stand No.	Bud break	Flushing	No. of cushions formed	Year round flowering
1	CCRP 8	8.1		Jul and Nov	0	
2	CCRP 8	8.2		Jul and Nov	0	
3	CCRP 8	8.3		Jul and Nov	0	
4	CCRP 8	8.4		Jul and Nov	0	
5	CCRP 8	8.5		Jul and Nov	0	
6	CCRP 8	8.6		Jul and Nov	0	
7	CCRP 8	23.3		Jul and Nov	0	
8	CCRP 8	23.6		Jul and Nov	0	
9	CCRP 8	23.7	18	Jul and Nov	2	Aug to Dec
10	CCRP 9	9.3		Jul and Nov	0	
11	CCRP 9	9.4		Jul and Nov	0	
12	CCRP 9	9.5		Jul and Nov	0	
13	CCRP 9	9.5	18	Jul and Nov	2	Aug to Dec
14	CCRP 9	9.6	18	Jul and Nov	3	Aug to Dec
15	CCRP 9	24.3		Jul and Nov	0	
16	CCRP 9	24.6		Jul and Nov	0	
17	CCRP 9	24.7		Jul and Nov	0	
18	CCRP 10	10.2		Jul and Nov	0	
19	CCRP 10	10.3		Jul and Nov	0	
20	CCRP 10	10.4		Jul and Nov	0	
21	CCRP 10	10.5		Jul and Nov	0	
22	CCRP 10	10.5		Jul and Nov	0	
23	CCRP 10	10.6	20	Jul and Nov	1	Sept to Dec
24	CCRP 10	25.3		Jul and Nov	0	

25	CCRP 10	25.6		Jul and Nov	0	
26	CCRP 10	25.7		Jul and Nov	0	
27	CCRP 11	11.3		Jul and Nov	0	
28	CCRP 11	11.4	19	Jul and Nov	2	Sept to Dec
29	CCRP 11	11.5		Jul and Nov	0	
30	CCRP 11	11.5		Jul and Nov	0	
31	CCRP 11	26.3		Jul and Nov	0	
32	CCRP 11	26.6		Jul and Nov	0	
33	CCRP 11	26.7		Jul and Nov	0	
34	CCRP 12	12.4		Jul and Nov	0	
35	CCRP 12	12.5		Jul and Nov	0	
36	CCRP 12	12.6		Jul and Nov	0	
37	CCRP 12	27.6		Jul and Nov	0	
38	CCRP 12	27.7		Jul and Nov	0	
39	CCRP 12	12.5	19	Jul and Nov	2	Aug to Dec
40	CCRP 12	12.6	21	Jul and Nov	1	Sept to Dec
41	CCRP 12	27.2		Jul and Nov	0	
42	CCRP 12	27.3		Jul and Nov	0	
43	CCRP 13	13.3		Jul and Nov		
44	CCRP 13	13.4	19	Jul and Nov	2	Sept to Dec
45	CCRP 13	13.6		Jul and Nov	0	
46	CCRP 13	28.6		Jul and Nov	0	
47	CCRP 13	28.7		Jul and Nov	0	
48	CCRP 13	13.5		Jul and Nov	0	
49	CCRP 13	13.6		Jul and Nov	0	
50	CCRP 13	28.2		Jul and Nov	0	
51	CCRP 13	28.3		Jul and Nov	0	
52	CCRP 13	28.4	19	Jul and Nov	2	Sept to Dec
53	CCRP 14	14.3		Jul and Nov	0	

54	CCRP 14	14.5		Jul and Nov	0	
55	CCRP 14	14.6		Jul and Nov	0	
56	CCRP 14	29.6		Jul and Nov	0	
57	CCRP 14	29.7		Jul and Nov	0	
58	CCRP 14	14.5		Jul and Nov	0	
59	CCRP 14	14.6	20	Jul and Nov	2	Sept to Dec
60	CCRP 14	29.3		Jul and Nov	0	
61	CCRP 15	15.4	20	Jul and Nov	4	Sept to Dec
62	CCRP 15	15.5		Jul and Nov	0	
63	CCRP 15	15.6		Jul and Nov	0	
64	CCRP 15	15.7		Jul and Nov	0	
65	CCRP 15	30.6		Jul and Nov	0	
66	CCRP 15	15.5		Jul and Nov	0	
67	CCRP 15	15.6		Jul and Nov	0	
68	CCRP 15	30.2		Jul and Nov	0	

Table 66. The mean plant height, plant spread and girth in inbreds self, inbred crosses and hybrids of cocoa during 2016

	Plant height(cm)	E W(cm)	N S(cm)	Girth (cm)
Inbred self	95.40	18.53	14.53	8.36
Inbred cross	99.76	50.42	49.57	8.24
Hybrids	13.99	77.00	58.33	6.81

Table 67. Comparison of plant height (cm) among inbreds, inbred crosses and hybrids during 2016

	Inbred self (95.40)	Inbred cross (99.76)
Inbred cross	0.95	
Hybrid(13.99)	6.80	7.13

Table 68. Comparison of girth (cm) among inbreds, inbred crosses and hybrids during 2016

	Inbred self (8.36)	Inbred cross (8.24)
Inbred cross	1.01	
Hybrid(6.81)	1.22	1.21

Table 69. The mean plant height, plant spread and girth in inbreds, inbred crosses and hybrids of cocoa during 2017

	Plant height (cm)	E W(cm)	N S (cm)	Girth (cm)
Inbred self	132.61	96.08	99.85	11.85
Inbred cross	135.35	19.62	00	11.42
Hybrids	90.22	99	96.77	10.20

with a comparison ratio of 0.95. Among the hybrids and inbred self, the inbred self plants are superior with a comparison ratio of 6.80. When inbreds crosses and hybrids are compared, the inbred crosses were superior over hybrids with a comparison value of 7.13.

The girth of inbred self was superior over inbred crosses and hybrids with a comparison ratio of 1.01 and 1.22 respectively. Among the inbred crosses and hybrids, inbred crosses were superior with a comparison ratio of 1.21.

4.2.6.2 Growth parameters during 2017

The growth parameters among the inbreds, inbred crosses and hybrids are presented in table 69. With respect to plant height during 2017, the inbred cross was superior over inbred self with a comparison ratio of 0.97. Among the hybrids and inbred self, the inbred self plants were superior with a comparison ratio of 1.47. When inbred crosses and hybrids are compared, the inbred crosses are superior over hybrids with a comparison value of 1.50 (Table 70).

The girth of inbred self was superior over inbred crosses and hybrids with a comparison ratio of 1.04 and 1.16 respectively. Among the inbred crosses and hybrids, inbred crosses were superior with a comparison ratio of 1.11(Table 71).

4.2.6.3 Growth parameters during 2018

The growth parameters among the inbreds, inbred crosses and hybrids are presented in table 72. With respect to plant height during 2018, the inbred cross and inbred self were on par with a comparison ratio of 1.00. Among the hybrids and inbred self, the inbred self plants were superior with a comparison ratio of 1.15. When inbred crosses and hybrids are compared, the inbred crosses are superior over hybrids with a comparison value of 1.14 (Table 73).

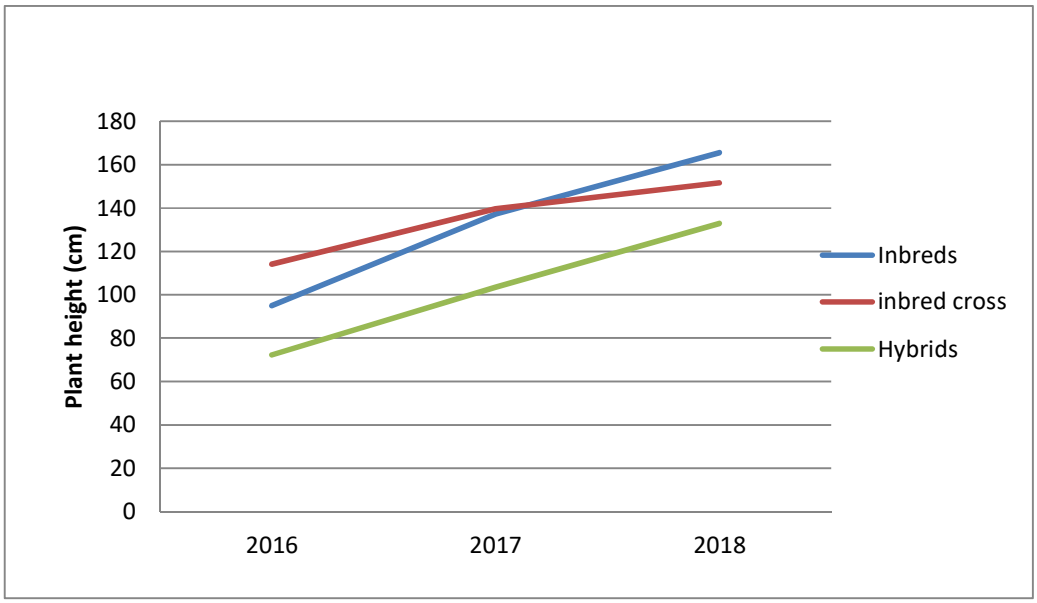


Fig. 54 Plant height of inbreds, inbred crosses and hybrids over the year

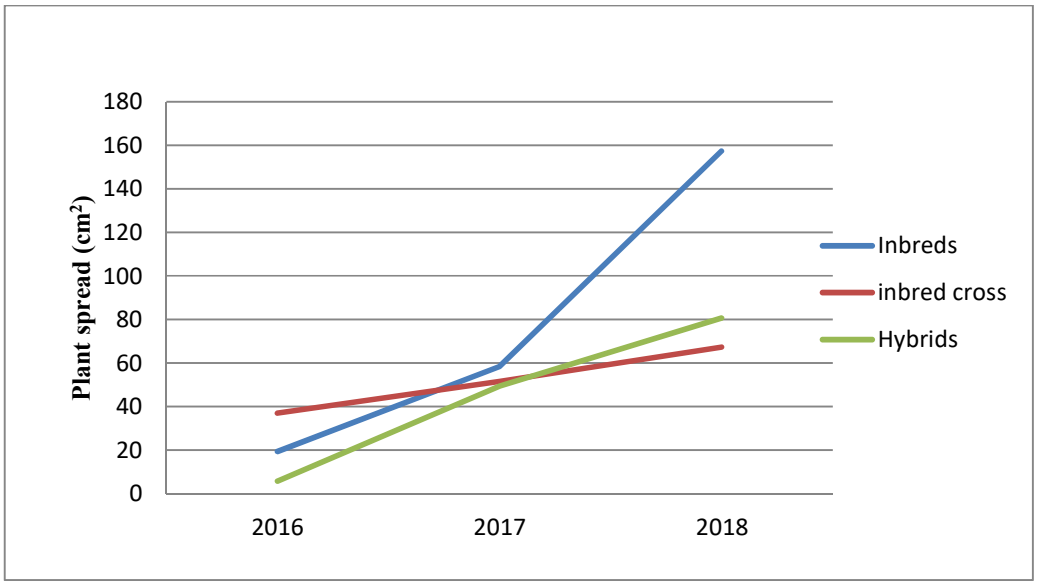


Fig. 55 Plant spread of inbreds, inbred crosses and hybrids over year

Table 70. Comparison of plant height (cm) among inbreds, inbred crosses and hybrids during 2017

	Inbred self (132.61)	Inbred cross (135.55)
Inbred cross	0.97	
Hybrid(90.22)	1.47	1.50

Table 71. Comparison of girth (cm) among inbreds, inbred crosses and hybrids during 2017

	Inbred self (11.85)	Inbred cross (11.42)
Inbred cross	1.04	
Hybrid(10.20)	1.16	1.11

Table 72. The mean plant height, plant spread and girth and chupan height in inbreds, inbred crosses and hybrids of cocoa during 2018

	Plant height	E W	N S	Girth	Chupan
Inbred self	146.09	176.36	178.82	16.33	116.23
Inbred cross	145.56	67.67	66.00	10.27	128.33
Hybrids	127.08	78.54	65.00	9.56	-

Table 73. Comparison of plant height (cm) among inbreds, inbred crosses and hybrids during 2018

	Inbred self (146.09)	Inbred cross (145.56)
Inbred cross	1.00	
Hybrid(127.08)	1.15	1.14

Table 74. Comparison of girth (cm) among inbreds, inbred crosses and hybrids during 2018

	Inbred self (16.33)	Inbred cross (10.27)
Inbred cross	1.59	
Hybrid(9.56)	1.70	1.07

Table 75. Comparison of chupan (cm) among inbreds, inbred crosses and hybrids during 2016

	Inbred self (116.23)	Inbred cross (128.33)
Inbred cross	0.90	
Hybrid(0.00)	0	0

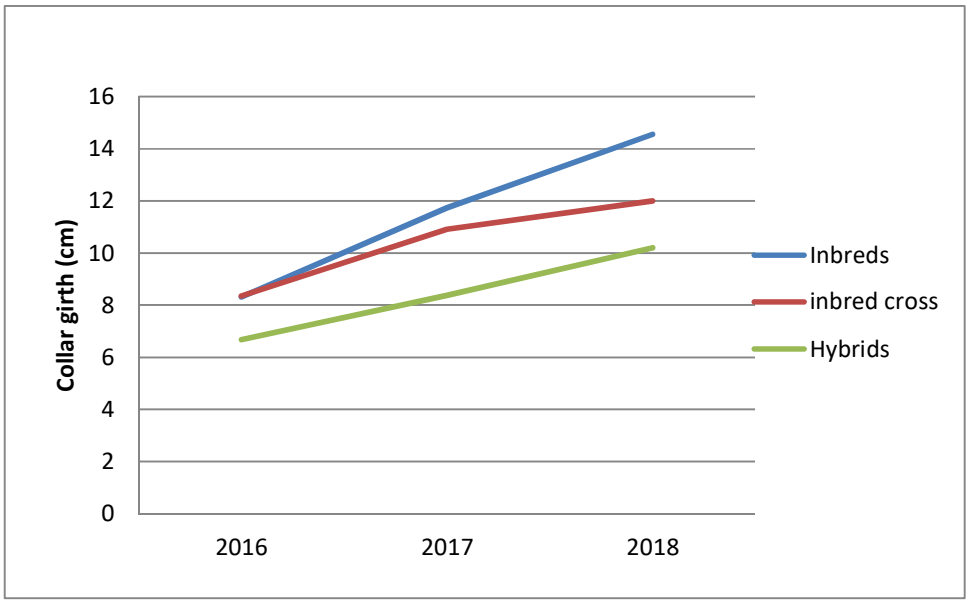


Fig. 56 Collar girth (cm) of inbreds, inbred crosses and hybrids over year

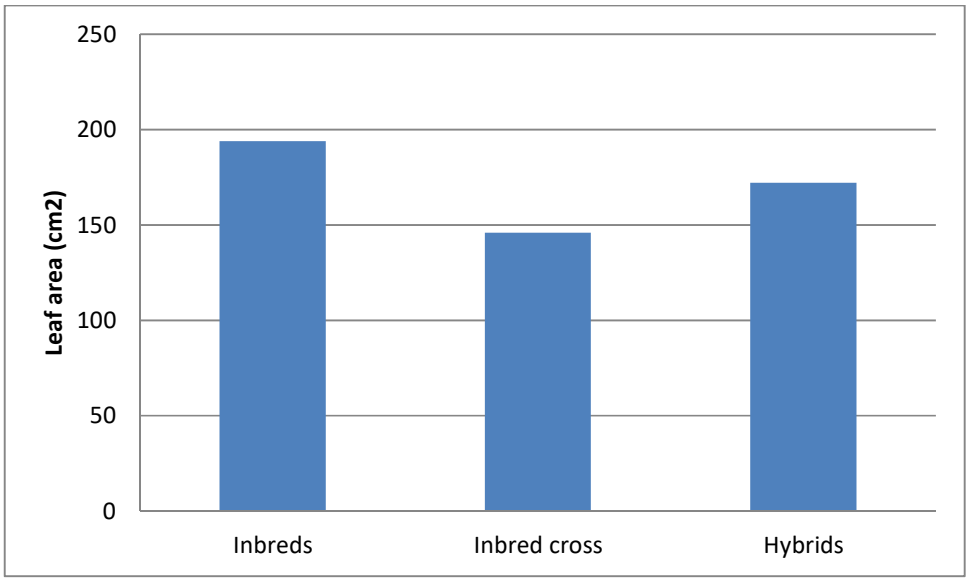


Fig. 57 Leaf area (cm^2) of inbreds, inbred crosses and hybrids

RWC during April 2016 was in the order of inbred self > hybrids > inbred cross. RWC in inbred cross was the lowest.

The relative content during June 2016 was high in inbred self than inbred cross with a comparison ratio of 1.58. Among the hybrids and inbred self, inbred self had more relative water content than hybrid with a comparison ratio of 1.88. Inbred cross had high relative water than hybrids with a comparison ratio of 1.19. The RWC during June 2016 was in the order of inbred self > Inbred cross > hybrids. RWC in hybrids was the lowest.

The relative content during September 2016 is presented in table 81. RWC was higher in inbred self than inbred cross with a comparison ratio of 1.37. Among the hybrids and inbred self, inbred self had more relative water content than hybrid with a comparison ratio of 1.23. Hybrids had higher relative water than inbred cross with a comparison ratio of 0.95. The RWC during September 2016 was in the order of inbred self > hybrids > inbred cross. RWC in inbred self was the highest.

The relative content during December 2016 is presented in table 82. RWC was higher in inbred self than inbred cross with a comparison ratio of 1.67. Among the hybrids and inbred self, inbred self had more relative water content than hybrid with a comparison ratio of 1.31. Hybrids had higher relative water than inbred cross with a comparison ratio of 0.78. The RWC during December 2016 was in the order of inbred self > hybrids > inbred cross. RWC in inbred self was the highest.

4.2.6..6 Relative water content (%) during 2017

The details on percentage of relative water content in leaf in different seasons during 2017 in inbred self, inbred cross and hybrids of cocoa is presented in table 85, 86, 87, 88, and 89. The relative content during April 2017 was high in inbred self than inbred cross with a comparison ratio of 2.94. Among the hybrids and inbred self, inbred self had more relative water content than hybrid with a comparison ratio of 1.68. Hybrids had high relative water than inbred cross with a comparison ratio of 0.57. The

Table 76. The mean chlorophyll content in inbreds, inbred crosses and hybrids

	Chlorophyll A	Chlorophyll B	Chlorophyll A+B
Inbred self	3.726	1.787	5.492
Inbred cross	3.785	0.671	4.457
Hybrids	2.885	0.594	3.461

Table 77 Comparison of chlorophyll A content among inbreds, inbred crosses and hybrids

	Inbred self (3.726)	Inbred cross (3.785)
Inbred cross	3.726/3.785=0.98	-
Hybrid(2.885)	3.726/2.885=1.29	3.785/2.885=1.31

Table 78 Comparison of chlorophyll B content among inbreds, inbred crosses and hybrids

	Inbred self (1.787)	Inbred cross (0.671)
Inbred cross	1.787/0.671=2.663	-
Hybrid(0.594)	1.787/0.594=3.00	0.671/0.594=1.12

Table 79 Comparison of chlorophyll A+B content among inbreds, inbred crosses and hybrids

	Inbred self (5.492)	Inbred cross (4.457)
Inbred cross	5.492/4.457= 1.23	-
Hybrid(3.461)	5.492/3.461= 1.59	4.457/3.461= 1.29

Table 80 The mean relative water content in inbreds, inbred crosses and hybrids of cocoa during 2016

	April 16	June 16	Sept 16	Dec 16
Inbred self	44.58	50.83	59.28	49.07
Inbred cross	14.86	32.18	43.15	29.24
Hybrids	31.48	27.06	45.62	37.24

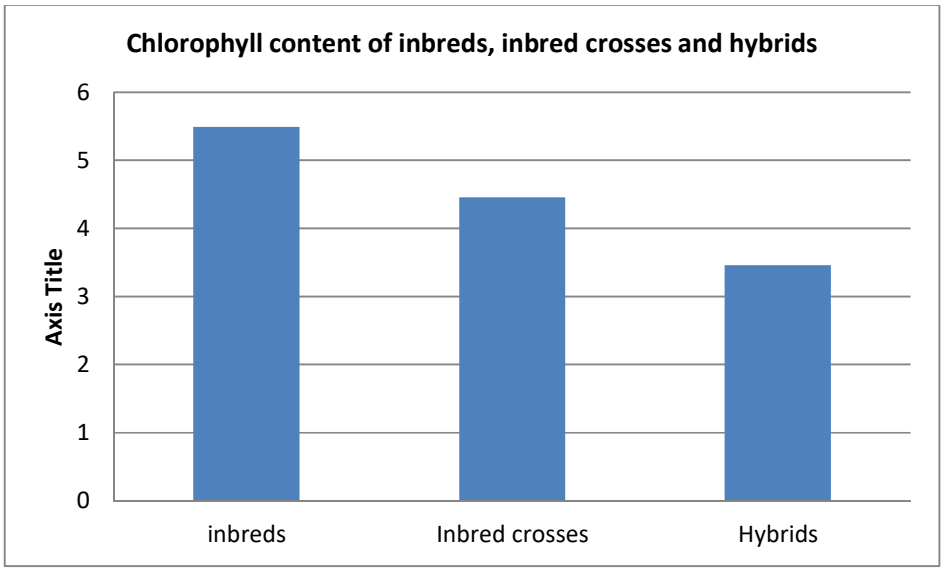


Fig. 58 Chlorophyll content (%) of inbreds, inbred crosses and hybrids

Table 81 Comparison of RWC during April 2016 content among inbreds, inbred crosses and hybrids

	Inbred self (44.58)	**Inbred cross (14.86)**
Inbred cross	44.58/14.86 = 3.00	-
Hybrid(31.48)	44.58/31.48 = 1.42	14.86 / 31.48 = 0.47

Table 82 Comparison of RWC during June 2016 content among inbreds, inbred crosses and hybrids

	Inbred self (50.83)	**Inbred cross (32.18)**
Inbred cross	50.83 /32.18 = 1.58	-
Hybrid(27.06)	50.83 /27.06 = 1.88	32.18/27.06 = 1.19

Table 83. Comparison of RWC during September 2016 content among inbreds, inbred crosses and hybrids

	Inbred self (59.28)	**Inbred cross (43.15)**
Inbred cross	59.28/43.15 = 1.37	
Hybrid(45.62)	59.28 /45.62 = 1.23	43.15/ 45.62 = 0.95

Table 84. Comparison of RWC in December 2016 content among inbreds, inbred crosses and hybrids

	Inbred self (49.07)	**Inbred cross (29.24)**
Inbred cross	49.07 /29.24 = 1.67	
Hybrid(37.24)	49.07 /37.24 = 1.31	29.24 / 37.24 = 0.78

Table 85 The mean relative water content in inbreds, inbred crosses and hybrids of cocoa during 2017

	April 17	**June 17**	**Sept 17**	**Dec 17**
Inbred self	45.58	53.80	63.75	47.79
Inbred cross	15.49	17.37	17.63	18.75
Hybrids	27.03	26.83	34.81	30.90

Table 86. Comparison of RWC during April 2017 content among inbreds, inbred crosses and hybrids

	Inbred self (45.58)	Inbred cross (15.49)
Inbred cross	45.58 / 15.49 = 2.94	
Hybrid(27.03)	45.58 /27.03 = 1.68	15.49 /27.03 =0.57

Table 87. Comparison of RWC during June 2017 content among inbreds, inbred crosses and hybrids

	Inbred self (53.80)	Inbred cross (17.37)
Inbred cross	53.80 /17.37 = 3.09	
Hybrid(26.83)	53.80 /26.83 = 2.00	17.37 /26.83 =0.65

Table 88. Comparison of RWC during September 2017 content among inbreds, inbred crosses and hybrids

	Inbred self (63.75)	Inbred cross (17.63)
Inbred cross	63.75 /17.63 = 3.61	
Hybrid(34.81)	63.75 /34.81 = 1.83	17.63 /34.81 = 0.51

Table 89. Comparison of RWC during December 2017 among inbreds, inbred crosses and hybrids

	Inbred self (47.79)	Inbred cross (18.75)
Inbred cross	47.79/18.75 = 2.54	
Hybrid(30.90)	47.79/30.90 = 1.54	18.75 /30.90 = 0.61

Table 90. The mean leaf nutrient content in inbreds, inbred crosses and hybrids of cocoa

	N	P	K
Inbred self	1.69	0.18	1.29
Inbred cross	1.52	0.23	1.31
Hybrids	1.14	0.18	1.21

RWC during April 2017 was in the order of inbred self > hybrids > inbred cross. RWC in inbred cross was the lowest.

The relative content during June 2017 is presented in table 85. Inbred self had high RWC than inbred cross with a comparison ratio of 3.09. Among the hybrids and inbred self, inbred self had more relative water content than hybrid with a comparison ratio of 2.00. Hybrids had higher relative water than inbred cross with a comparison ratio of 0.65. The RWC during June 2016 was in the order of inbred self > hybrids> inbred cross. RWC in inbred cross was the lowest.

The relative content during September 2017 is presented in table 86. RWC was higher in inbred self than inbred cross with a comparison ratio of 3.61. Among the hybrids and inbred self, inbred self had more relative water content than hybrid with a comparison ratio of 1.83. Hybrids had higher relative water than inbred cross with a comparison ratio of 0.51. The RWC during September 2017 was in the order of inbred self > hybrids > inbred cross. RWC in inbred self was the highest.

The relative content during December 2017 is presented in table 87. RWC was high in inbred self than inbred cross with a comparison ratio of 2.54. Among the hybrids and inbred self, inbred self had more relative water content than hybrid with a comparison ratio of 1.54. Hybrids had high relative water than inbred cross with a comparison ratio of 0.61. The RWC during December 2017 was in the order of inbred self > hybrids > inbred cross. RWC in inbred self was the highest.

4.2.6.7 Leaf nutrient content (%)

The details of leaf nutrient content in inbred self, inbred cross and hybrids is presented in table 90, 91, 92 and 93.

The nitrogen content in leaf of inbred self, inbred crosses and hybrids are presented in table 91. The leaf nitrogen content inbred self was higher than inbred cross with a comparison ratio of 1.11. Among the hybrids and inbred self, inbred self had more nitrogen content than hybrid with a comparison ratio of 1.48. Inbred cross had high

Table 91. Comparison of nitrogen content in leaf among inbreds, inbred crosses and hybrids

	Inbred self (1.69)	**Inbred cross (1.52)**
Inbred cross	1.69/1.52 = 1.11	
Hybrid(1.14)	1.69/1.14 = 1.48	1.52 /1.14=1.33

Table 92. Comparison of phosphorus content in leaf among inbreds, inbred crosses and hybrids

	Inbred self (0.18)	**Inbred cross (0.23)**
Inbred cross	0.18 /0.23 = 0.78	
Hybrid(0.18)	0.18 /0.18 = 1.00	0.23 /0.18 = 1.27

Table 93. Comparison of potassium content in leaf among inbreds, inbred crosses and hybrids

	Inbred self (1.29)	**Inbred cross (1.31)**
Inbred cross	1.29 /1.31 = 0.98	
Hybrid(1.21)	1.29 /1.21 = 1.07	1.31 /1.21 = 1.08

nitrogen than hybrids with a comparison ratio of 1.33. The nitrogen content was in the order of inbred self > inbred cross > hybrids. The nitrogen content in hybrids was the lowest.

The phosphorus content in leaf of inbred self, inbred crosses and hybrids are presented in table 92. The leaf phosphorus content in inbred self was lower than inbred self with a comparison ratio of 0.78. The hybrids and inbred self were on par in their phosphorus leaf content. Inbred cross had higher phosphorus than hybrids with a comparison ratio of 1.27. The phosphorus content was in the order of inbred inbred cross > inbred self and hybrids. The phosphorus content in inbred cross was the highest.

The potassium content in leaf of inbred self, inbred crosses and hybrids are presented in table 93. The leaf potassium content in inbred self was lower than inbred self with a comparison ratio of 0.98. The inbred self had high potassium content than hybrids with a comparison ratio of 1.07. Inbred cross had higher potassium content than hybrids with a comparison ratio of 1.08. The potassium content was in the order of inbred cross > inbred self > hybrids. The phosphorus content in inbred cross was the highest.

4.3.3 Techniques and methods to overcome self incompatibility in cocoa

Self incompatibility is a mechanism in which the pollen or pollen tubes are inhibited on the surface of stigma or style (Gowers, 1989). This is considered as a useful tool for production of hybrids. However, the existence of self incompatibility in many crops leads a problem in obtaining parental inbred lines and is a hindrance in producing inbreds which is an essential step for producing stable hybrids. This can be overcome to certain extent by temporary suppression of self incompatibility by various methods. The reported methods for temporary breaking of self incompatibility in various plants were tried in selected self incompatible cocoa inbreds. A total of nine methods have been tried to overcome the self incompatibility in cocoa at CRC, Vellanikkara and the results thus obtained are presented here under.

The number of flower pollinated under different methods are presented in table 94

A total of nine techniques and methods to overcome self incompatibility barriers such as bud pollination, surgical technique, intra-ovarian technique, salt spray (3% and 1%), high humidity, high temperature, Naphthalene Acetic Acid (100 and 200 ppm), gamma irradiation and flower organ extract were attempted from September 2016 to March 2017 and September 2017 to March 2018. In all the methods, a minimum of 100 flowers were pollinated and tested for success of pollination and fertilization.

The following techniques and methods were tried in self incompatible inbreds belonging to S_1 to S_5 generation. A total of 46 self incompatible genotypes were tested by 9 different pollination techniques to overcome the self incompatibility.

A total of 5086 flowers were pollinated before opening of the flowers (bud pollination) (Plate 5a), however, no pod set was observed in bud pollination method. In *Petunia axillaries*, self pollination of buds, two days before anthesis resulted in seed set (Shivanna and Rangaswamy, 1969) that was mainly due to the protein secretion covers the stigmatic surface after anthesis, which acts as a barrier to penetration of stigma by germinating pollen grains.

Table 94. Number of flowers pollinated in different methods of pollination

S. No.	Stand No.	Bud pollination	Surgical Technique	Intra ovarian technique	High temperature	High Humidity	Salt (1%)	Salt Spray (3%)	Gamma irradiation	Flower organ extract	NAA 100 ppm	NAA 200 ppm
1.	3.2	104	146	130	110	114	114	137	102	101	120	101
2.	3.3	108	128	132	102	113	112	117	105	104	105	102
3.	3.6	148	139	138	100	148	114	148	108	105	104	104
4.	3.7	109	113	142	102	143	112	143	109	104	108	106
5.	3.9	118	133	136	99	114	114	149	107	102	101	105
6.	10.12	114	143	145	114	123	111	114	109	101	102	106
7.	20.2	48	114	130	143	124	102	113	106	101	100	105
8.	20.3	108	126	113	119	123	103	112	108	112	142	114
9.	20.10	143	114	118	119	143	105	143	106	102	135	112
10.	20.12	124	119	112	117	142	108	128	106	114	128	121
11.	20.13	114	118	114	125	112	102	124	118	121	156	123
12.	20.14	134	119	133	179	131	112	113	102	112	120	121
13.	20.15	131	48	137	147	141	99	114	103	101	110	124
14.	20.16	106	118	118	105	113	125	117	108	106	120	125
15.	20.18	99	119	119	148	114	121	119	109	104	112	112
16.	25.9	134	134	118	119	113	112	117	104	104	115	114
17.	25.12	138	139	139	112	114	110	147	105	105	98	101
18.	25.15	106	108	114	121	114	109	112	102	114	142	102
19.	26.9	44	134	147	133	112	102	114	106	121	153	103
20.	27.3	69	104	103	113	108	101	104	107	112	142	105
21.	27.4	151	133	119	112	124	102	114	108	114	132	101
22.	28.3	109	134	149	106	124	103	114	106	113	105	102
23.	29.3	131	153	119	98	124	104	114	109	124	110	112
24.	29.4	134	133	138	114	141	105	134	104	114	120	121
25.	29.7	114	118	119	119	111	106	143	106	114	118	104

26.	29.9	44	122	117	112	112	108	141	105	105	117	101
27.	29.12	49	129	114	142	124	109	113	102	104	112	102
28.	29.13	98	118	139	142	98	114	117	102	124	112	102
29.	29.16	99	131	136	141	104	115	114	104	112	114	112
30.	30.4	114	114	144	134	104	126	114	102	115	124	112
31.	30.5	112	113	142	156	124	124	113	105	108	115	106
32.	30.6	110	112	147	149	122	112	114	108	98	114	105
33.	30.8	130	125	127	124	122	102	117	109	105	101	108
34.	30.9	89	119	143	141	126	102	141	107	107	120	107
35.	30.10	99	122	137	142	114	104	119	109	107	124	106
36.	30.11	112	159	143	118	121	106	118	105	108	145	102
37.	30.13	114	143	149	149	124	105	114	112	105	114	102
38.	30.14	140	113	141	135	129	102	117	114	105	142	106
39.	30.16	112	11	105	113	114	102	114	104	104	124	108
40.	6.1	112	102	113	124	113	110	115	115	121	115	107
41.	6.2	110	125	115	121	102	102	112	106	114	105	105
42.	6.4	112	114	115	126	112	104	114	103	114	105	113
43.	6.5	112	112	121	132	121	102	102	102	115	102	114
44.	6.6	120	124	121	148	121	106	106	105	112	102	112
45.	6.7	120	163	120	129	122	102	105	102	113	106	106
46.	6.10	140	142	102	104	102	105	102	105	105	105	105

In intra ovarian technique method (Plate 5b), a total of 5873 flowers were pollinated and no fruit set was observed. However, viable seeds have been obtained by this method in *Argemone mexicana* and *A. ochroleuca* by Kanta and Maheshwari (1963). This method was also successful in other members of *Papaveraceae,* like *Papaver rhoeas* and *P. somniferum* (Allard, 1960).

In high temperature treatment techniques of pollination, total of 5758 number of flowers were pollinated in 46 self incompatible plants belonging to S_3 to S_5 generation by closing the pollination hood with a small polythene bag. Okazaki and Hinata (1987) succeed in breaking self incompatibility in *Lilium longiflorum* by increasing the temperature to 30°C for 6 minutes before pollination. High temperatures reduced the incompatibility in cole crops also (Kalloo, 1988).

No fruit set was observed under high humidity after pollinating 5514 flowers in 46 self incompatible plants. On contrary Kalloo (1988) obtained fruit set under high humidity in cole crops.

One and three per cent salt spray was sprayed on the 4970 and 5526 flowers respectively before covering the flower with pollination hood and just before pollination but no fruit set was observed. Contrary to this result, Monteiro (1988) overcome the self-incompatibility in *Brassica campestris* by spraying sodium chloride solution to the stigmas of self-incompatible plants. Wang *et al.,* 2012 reported the effectiveness of NaCl in overcoming self incompatibility in Chinese cabbage. The spray of NaCl solution on the stigma surface before self pollination at anthesis, more pollen grains attached to the stigma surface and more pollen grains germinated, pollen tube entered and further penetrated in to the stigma.

Under gamma irradiation, a total of 4889 flowers were pollinated with gamma irradiated (10Gy) pollen grains and no fruit set was observed. According to Kalloo (1988), irradiation of pollen is effective in overcoming the self-incompatibility reaction of pollen in *Brassica campestris.* Falque *et al*., (1992) reported the pollen fertilization capabilities in cocoa at different doses of gamma irradiation. In all the doses the pollen grain viability and in vitro germination was not affected, the pollen tubes penetrated into the styles and reached the ovules after pollination. In the present study, the few

flowers set in to fruits after gamma irradiation @ 10 Gy. The very low level of pod survival was observed after 30 days of pollination. The similar results were observed by Adu-Ampomah *et al*.,(1991) in cocoa. This plant seems to present much higher radio sensitive for fruit setting than other plants such as Malus (Zhang and Lespinasse, 1991), Pyrus (Sniezko and Visser, 1987) and Citrus (De Lange and Vincent, 1988) wherein the gamma irradiation up to 50Gy resulted in fruit setting. However, in the present study no fruit set was observed.

A total of 5026 flowers were pollinated by applying flower organ extract along with pollen grains. However, no fruit set was observed. On contrary, Matsubara (1981) was successful in obtaining the fruits by treating with flower organ extract in *Lilium longiflorum.*

In another method, 100 and 200 ppm NAA hormone was sprayed on the 5416 and 4987 flowers respectively before covering the flower with pollination hood and just before pollination but no fruit set was observed.

In all of the above methods, the covering of flower bud was done a day before opening of flower as in case of selfing. The details of all the pollination methods are presented in table 61.

In surgical method, the majority of the self incompatibility plants belonging to S_3 and S_5 generation set into fruits (Table 96).

The fruit set was observed in surgical pollination method, the reasons for the fruit set in this method may be due to the compound responsible for rejection of union of gamete might have synthesized in the stigmatic surface. Once, the one third portion of style is removed the fruit set was observed. The sporophytic self incompatibility exists in cocoa (Knight and Rogers, 1953). The site of action for sporophytic self incompatibility is in upper ⅓ portion of the style.

4.3.1 Studies on pollen tube growth and fluorescent microscopy results

The pollen tube growth studies were conducted by following standard procedure using aniline blue dye (Martin, 1959) at 4 to 8 hours after pollination. In case of compatible flowers, the pollen tube growth was found normal and it reached till to the ovary (Plate 9a, 9c & 9e). A large number of pollen grains adhered to the stigma after self-pollination and some pollen grains germinated. At 4-8 h after self-pollination, most pollen grains were able to germinate and produce pollen tubes, resulted in the penetration through the stigmatic cell wall. At 8 hours after pollination, the pollen tubes had grown through the style, reached the ovary (Plate 9a, 9c & 9e). Whereas, in the self incompatible flowers, the pollen grains germinated, pollen tube growth was observed in the ⅓ portion of the style. After ⅓ portion penetration in the style the pollen tube degraded resulting in no fertilization and finally no fruit set (Plate 9b, 9d and 9f).

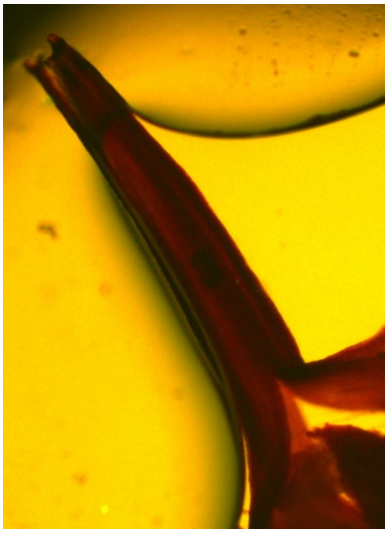
4.3.2 Characters of pod and bean obtained after surgical technique.

The details of pod characters are presented in table 95

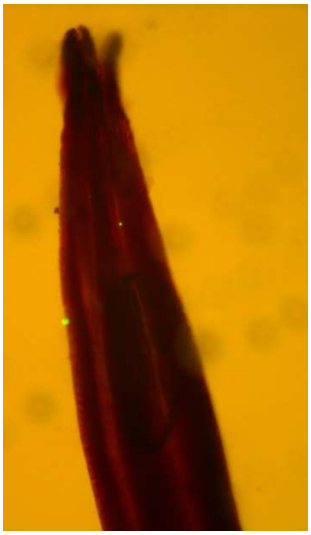
Total of 17 pods were produced in self incompatible plants. Of the 17 pods, nine pods are produced from S_5 generation inbreds and achieved to produce S_6 inbreds. Remaining 8 pods were produced in S_3 generation inbreds and succeed to produce S_4 generation inbreds.

The details of time taken from pollination to harvesting are presented in table 63.

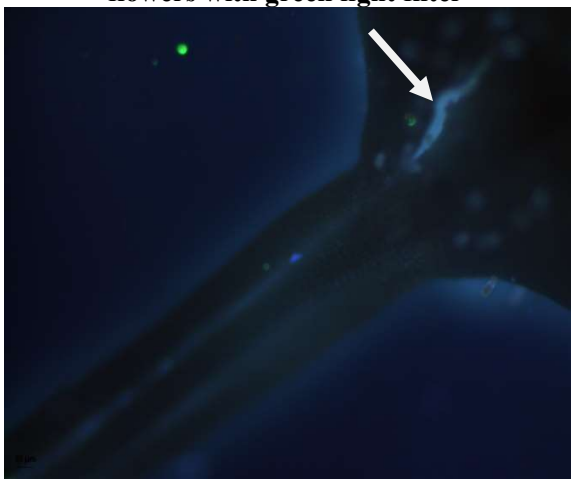
The pod weight in advanced generation of inbreds ranged from 170 g to 450 g with an average pod weight of 284.71 g. The pod length ranged from 8 cm to 17 cm with a mean of 12.96 cm. The pod breadth ranged from 5.5cm to 10 cm with a mean of 7.26 cm. The number of beans per pod ranged from 20 to 47 with an average of 36.09 beans per pod. The total wet bean weight ranged from 35.90 g to 110 g with an average of 77.17 g. The maximum number of flat beans per pod was 3 with an average of 0.91. The ridge thickness of advanced generation pod varied from 0.90cm to 1.21 cm with an



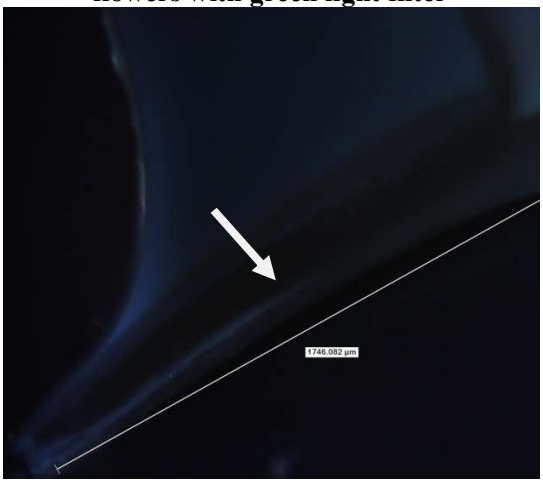
a.Pollen tube growth in self compatible flowers with green light filter



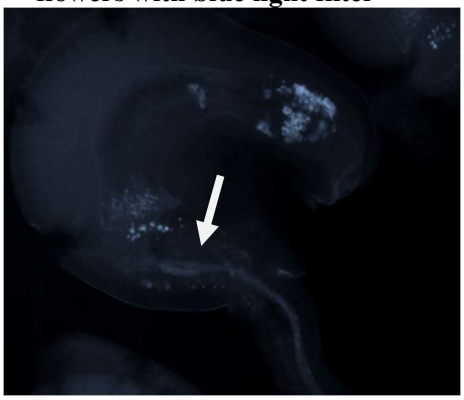
b.Pollen tube growth in self incompatible flowers with green light filter



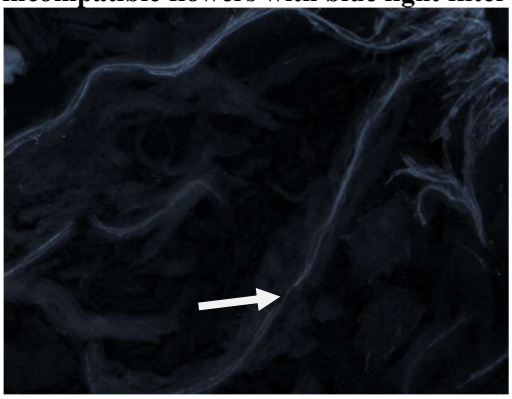
c. Pollen tube growth in self compatible flowers with blue light filter



d. Degradation of pollen tube growth in self incompatible flowers with blue light filter



e. Pollen tube reaching the ovary in self compatible flowers



f. Degradation of pollen tube in the style region in self incompatible flower

Plate 9. Pollen tube growth of self compatible and self incompatible flowers

Table 95. Details of pods characters produced through surgical method of pollination in self incompatible plants

S. No.	Genotype	Generation	Stand No.	Pollination date	Harvesting date	Pod weight (g)	Pod length (cm)	Pod breadth (cm)	No. of beans	Total bean wt. (g)	Flat bean	Ridge thickness (mm)	Furrow thickness (mm)	20 Bean weight (g)
1	G II 7.4	S_5 to S_6	6.4	24.08.17	20.3.18	450.0	15.50	8.00	43	110	1	1.3	0.9	60.00
2	G II 7.4	S_5 to S_6	6.4	14.09.17	24.3.18	420.0	17.00	10.00	42	100	0	1.3	1.0	40.00
3	G II 7.4	S_5 to S_6	6.4	11.11.17	14.5.18	340.0	15.50	8.50	39	100	2	1.5	1.0	40.00
4	G II 7.4	S_5 to S_6	6.4	13.12.17	18.6.18	180.0	8.50	5.50	33	55	0	1.2	1.0	28.00
5	G II 7.4	S_5 to S_6	6.6	21.12.17	28.6.18	240.0	13.20	6.50	42	63.5	3	0.9	0.7	30.60
6	G II 7.4	S_5 to S_6	6.10	25.08.17	26.2.18	381.2	14.82	7.82	32	94.5	3	1.0	0.9	41.50
7	G II 7.4	S_5 toS_6	6.10	20.10.17	14.5.18	300.0	15.00	9.00	30	50	1	1.4	1.0	42.00
8	G II 7.4	$S_5$to S_6	6.10	28.11.17	18.6.18	170.0	8.00	5.50	36	70	0	1.1	0.9	24.50
9	G II 7.4	S_5 to S_6	6.10	13.12.17	18.6.18	180.0	9.00	6.50	33	85	0	1.0	0.8	42.00
10	G II 7.4	S_3 to S_4	20.10	21.10.16	20.3.17	240.0	12.50	6.00	47	85	0	1.3	1.0	36.00
11	G II 7.4	S_3 to S_4	20.18	29.09.16	14.3.17	430.0	15.00	7.50	43	100	0	1.0	0.8	46.50
12	G IV 35.7	S_3 to S_4	27.4	04.10.16	05.7.17	230.6	13.50	6.50	20	35.9	0	1.3	1.1	35.90
13	G IV 35.7	S_3 to S_4	27.4	20.10.16	30.3.17	318.0	13.00	6.50	40	86	2	1.3	1.0	43.00
14	M 18.7	S_3 to S_4	29.3	20.10.16	20.3.17	350.0	14.50	6.50	37	90	0	1.0	0.8	48.00
15	M 18.7	S_3 to S_4	29.4	20.10.16	20.3.17	310.0	13.50	6.50	47	100	0	1.3	1.1	42.50
16	M 18.7	S_3 to S_4	29.7	20.10.16	30.3.17	364.0	15.00	7.50	38	81	0	1.4	1.1	42.00
17	M 18.7	S_3 to S_4	29.13	30.09.16	14.3.17	420.0	14.50	7.00	44	90	0	1.2	0.9	41.00

Table 96. Number of days taken pollination to harvesting

S. No.	Genotype	Generation	Stand No.	Pollination date	Harvesting date	No. of days for harvest after pollination
1	G II 7.4	S_5 to S_6	6.4	24.08.2017	20.03.2018	6 months 26 days
2	G II 7.4	S_5 to S_6	6.4	14.09.2017	24.03.2018	6 months 10 days
3	G II 7.4	S_5 to S_6	6.4	11.11.2017	14.05.2018	6 months 3 days
4	G II 7.4	S_5 to S_6	6.4	13.12.2017	18.06.2018	6 months 5 days
5	G II 7.4	S_5 to S_6	6.6	21.12.2017	28.06.2018	6 months 7 days
6	G II 7.4	S_5 to S_6	6.10	25.08.2017	26.02.2018	6 months 1 day
7	G II 7.4	S_5 to S_6	6.10	20.10.2017	14.05.2018	6 months 24 days
8	G II 7.4	S_5 to S_6	6.10	28.11.2017	18.06.2018	6 months 20 days
9	G II 7.4	S_5 to S_6	6.10	13.12.2017	18.06.2018	6 months 5 days
10	G II 7.4	S_3 to S_4	20.10	21.10.2016	20.03.2017	4 months 29 days
11	G II 7.4	S_3 to S_4	20.18	29.09.2016	14.03.2017	5 months 15 days
12	G IV 35.7	S_3 to S_4	27.4	04.10.2016	05.07.2017	9 months 1 days
13	G IV 35.7	S_3 to S_4	27.4	20.10.2016	30.03.2017	6 months 10 days
14	M 18.7	S_3 to S_4	29.3	20.10.2016	20.03.2017	5 months
15	M 18.7	S_3 to S_4	29.4	20.10.2016	20.03.2017	5 months
16	M 18.7	S_3 to S_4	29.7	20.10.2016	30.03.2017	5 months 10 days
17	M 18.7	S_3 to S_4	29.13	30.09.2016	14.03.2017	5 months 16 days

a. S_6 pod of developed through surgical method of pollination

b. Field view of S_6 generation of cocoa

Plate 10. Pods and field view of S_6 generation of cocoa

average thickness of 1.21 cm. The furrow thickness ranged from 0.70 to 1.10cm with an average of 0.94 cm. All maximum values are observed in S_3 to S_4 generation except the number of beans per pod. The number of beans per pod was the highest in S_5 to S_6 generation.

4.4. Study of self incompatibility mechanism in S_5/S_4

The results obtained in the investigations on characterization of proteins in S_5 generations of cocoa in relation to self-incompatibility are presented in this chapter.

4.4.1. Isolation of protein from flowers

Flowers from fifth generation cocoa inbreds were collected from the inbred cocoa block in icebox and subsequently directly placed in liquid nitrogen until it is used for protein extraction.

Ovaries from the self-incompatible flowers were separated and placed in a solution containing 7 % PVP followed by 0.3 % ascorbic acid to avoid browning of flowers and to avoid oxidation.

The ovaries after extraction from flowers were used for protein isolation. The ovaries were ground in pre-chilled pestle and mortar in liquid nitrogen by adding pinch of PVP.

The finely ground flower powder was used for extraction of protein. Available procedures for extraction of protein were tried with suitable modification to get sufficient quantity of protein for loading into the SDS gel. The following methods of protein extraction demonstrated in plants such as black pepper, cotton and avocado, which are having large quantity of secondary metabolites and polyphenols have been attempted in the present study.

Standard protocols have been proposed for various types of samples, but the particularities of many samples require the use of specific protocols that are optimized according to the objective of the study, the specific type of tissue, and the age of the organ (Gorg *et al.,* 2004; Islam *et al.,* 2004). *T. cacao*, in particular, has a very high level of interfering compounds, such as polysaccharides and phenolic compounds (Gesteira *et al.,* 2003), that possibly explain the absence of data in the literature for obtaining quality proteins and studies of successful 2-DE proteome analysis of *T. cacao* flower, making it

necessary to develop an efficient protocol for protein extraction from flowers of this species.

Protein extraction from plant samples is often challenging, especially in woody plants, which have higher lignin and secondary metabolite contents than other plant species, making the disruption of the cell wall problematic. Flowers, especially of recalcitrant species such as *T. cacao* (Figueira *et al.,* 1994; Gesteira *et al.,* 2003), besides being an organ containing compounds responsible for colour and odour, have low protein content (Isaacson et al., 2006). Flowers have many non-protein contaminants that affect 1-DE, including polysaccharides, polyphenols, nucleic acids, terpenes, and organic acids; these contaminants accumulate mainly in the vacuole in various soluble forms (Pan, 2000). The contaminants can be co-extracted with protein and affect protein migration in 1-DE, resulting in streaking and disintegration of protein also (Gorg *et al*., 2000).

The following available methods have been tried and each method is repeated at thrice to confirm the quantity and quality of protein.

4.4.1.1 Method I (Modified TCA/Acetone method)

The modified TCA/Acetone method was reported by Damerval *et al.,* 1986. The flower sample crushed in liquid nitrogen in pre-chilled pestle and mortar with pinch of PVP. The finely ground powder is then used immediately for protein isolation or stored at -80 ˚C until protein extraction. The precipitation of protein was done in 10 percent TCA and 0.07 percent β-mercaptoethanol in acetone for 12 hours. The precipitate was washed with β-mercapto ethanol in cold acetone. The protein pellet obtained after washing was air dried. The protein pellet was dissolved in 0.1 N NaOH (Laemmli, 1970). The quantification was initially done in NanoDrop spectrophotometer and finally the concentration was confirmed by Bradford method (Bradford, 1976)

The quantity of protein obtained by this was only 3-4 µg/ mL (Plate 11a. The minimum concentration of protein to be loaded in SDS PAGE gel electrophoresis is 15 µg/ mL (Umadevi and Anandraj, 2015). With this minimum concentration of protein

the SDS PAGE was carried out. Then staining and destaining with Coomasei brilliant blue was done by following standard procedures. After destaining, protein bands did not appear in the gel. The major reason for low protein yield in TCA/Acetone method of extraction may be due to the insolubility of the pellet in 0.1N.NaOH (Chen and Harmon 2006), compared to other methods. Though PVP is known to be effective in absorbing poly phenols it has been found ineffective in extraction of proteins in cocoa. This could be due to high pH of extraction buffer, at which phenols get oxidized and it cannot be absorbed by the PVP. Similar finding was reported by Carpentier *et al*., (2005) on protein extraction from banana meristems. The cocoa has phenol content up to 3 % which interfere in the expression of protein in 1DE. According to Vâlcu and Schlink (2006), the grinding of the flowers in liquid nitrogen, followed by precipitation by TCA/acetone and sonication, is one of the most effective approaches for plant samples that are rich in secondary metabolites. In this study, preparing the ADP of the *T. cacao* flower required precipitation with TCA/acetone overnight with several stages of vortexing and centrifugation to obtain a high yield of protein.

4.4.1.2 Method II (Dense SDS/Phenol method)

In this, method reported by Wang *et al.,* 2003 was followed. Cocoa flowers were collected from the self-incompatible plants, placed directly in liquid nitrogen and ground in autoclaved pre-chilled pestle and mortar. Finely ground powder was digested with 0.3 M Tris hydrochloric acid, pH 8.65, 30 % sucrose, 2 % sodium dodysyl sulphate, 1 mM PMSF, 2 % β-mercapto ethanol and equal volume of Tris buffered phenol (pH 8.0). After 24 hours, the precipitation was obtained by centrifugation at 8000 rpm for 10 min. at 4 ˚C. To this precipitate, 1:5 volumes of 0.1M cold ammonium acetate was added, stored at -20 ˚C for overnight. On the next day, the protein pellet was obtained by centrifugation at 20000 rpm for 10 minutes at 4 ℃. The protein pellet was washed with cold acetone with β–mercapto ethanol twice and final washing with 80 % ethanol. The protein pellet was air dried and stored at -80^0 C. The protein pellet was dissolve in rehydration buffer for running in SDS PAGE electrophoresis.

After ammonium acetate precipitation, the extract was dissolved in 10 % TCA and 0.07 % β-mercapto ethanol in cold acetone and stored at -20 ºC.

On contrary to Wang *et al* (2003), in the present study the addition of SDS to the extraction buffer did not improve extraction. Similar result was reported by Carpentier *et al.,* (2005) in banana meristem, reporting inefficiency of SDS for improved solvent action. In general, the phenol has the tendency to dissolve some polysaccharides and nucleic acids. This could be the reason for prolonged extraction time and streaks in the high pH range in SDS PAGE gels (Carpentier *et al.,* 2005).

The extraction of proteins from ADP was performed using a mixture of phenol and dense SDS buffer (Wang *et al.,* 2003; Pirovani *et al.,* 2008). SDS is a good solubilizing agent, and phenol minimizes the protein degradation that often occurs during sample preparation because of the action of proteolytic enzymes that are found in the sample itself (Schuster and Davies, 1983); phenol has been reported to remove interfering compounds, such as polyphenols, efficiently prior to electrophoresis (Wang *et al.,* 2003). The protein concentration in the sample was only 5µg/mL. The bands did not appear in the gel. (Plate 11b and 11c))

4.4.1.3 Method III (PVP/TCA acetone method with some modification).

This method was reported by Shen *et al.,* 2002. In PVP/TCA acetone method with some modification, the collected flowers were crushed in liquid nitrogen along with PVP. For homogenization and extraction, a solution containing 40 mM Tris Cl, pH 7.5, 250 mM Sucrose, 10 mM EDTA 1 % Tritonx-100 1 mM DTT 2% (v/v) 2ME was used for complete homogenization and extraction of protein. After complete homogenization and extraction, protein precipitation was done with 1:5 volume 0.1 M ammonium acetate in cold methanol stored at -20 °C for 2 hours and stored at -20 °C overnight. After complete precipitation, the protein pellet was obtained by centrifugation at 20,000 rpm for 10 min. at 4 °C. The protein pellet was washed twice with acetone and 0.07 % β-mercapto ethanol followed by washing with 80 % ethanol.

After homogenization and extraction, the precipitation of protein was done using 1.5 volume of 10 % TCA and 0.07 % β-mercapto ethanol in cold acetone. The protein pellet was washed in cold acetone containing 0.07 % β-mercapto ethanol till the pellet turn to white colour. The final washing was done 80 % ethanol

By PVP/Acetone method, the protein was extracted, a white crystal pellet was observed. The protein quantification was done by Bradford method and it yielded 4µg/mL. The SDS PAGE was run and it did not yield any bands (Plate 11e).

4.4.1.4 Method IV (Phenol method with slight modification)

The phenol method with slight modification was reported by Hurkman and Tanaka, (1986). The flower was placed directly in the liquid nitrogen. The flowers and leaf are crushed in liquid nitrogen with pinch of PVP. Homogenization and extraction of sample was done using 0.1 M Tris HCl (pH 8.8), 10 mM EDTA, 0.4 % ME, 0.9 M sucrose and equal volume of Tris buffered phenol (pH 8.0), 2 µL protease inhibitor cocktail. Re-extraction and pooling of phenol phase and precipitation of protein was done by dissolving in 1.5 volume of 0.1 M ammonium acetate in cold methanol and stored at -20 ºC for 2 hours, then stored at -20^0 C for overnight for complete precipitation. The protein pellet was obtained centrifugation and washing twice cold acetone containing 0.07 % β-mercapto ethanol till the pellet turn to white colour. The final washing was done 80 % ethanol

After precipitation, the protein pellet was generated by dissolving the extract with 10 % TCA and 0.07 % ME in cold acetone followed by storage at -20 ºC for 2 hours, then stored at -20^0 C for overnight for complete precipitation. The protein pellet was washed twice with acetone and 0.07 % ME and 80 per cent ethanol.

In general, the phenol has the tendency to dissolve some polysaccharides and nucleic acids. This could be the reason for prolonged focusing time and streaks in the high pH range in 2D gels (Carpentier *et al*., 2005). The protein concentration in this

method was quantified by Bradford. The protein concentration was 4µg/ mL. The SDS PAGE was run. The bands did not appear in this method (Fig.4.4).

4.4.1.5 Method V (Lysis buffer extraction with some modification.)

This method of protein extraction was reported by Farrel (1975). The leaf and flower samples were placed directly in the liquid nitrogen. The flowers were crushed in liquid nitrogen with pinch of PVP. Homogenization and extraction of sample was done using 7 M urea, 2 M thiourea, 2 % CHAPS, 40 mM DTT and 2 µL protease inhibitor cocktail. Precipitation of protein was done by dissolving in 1.5 volume of 0.1 M ammonium acetate in cold methanol and stored at -20 0C for 2 hours, then stored at -20 °C for overnight for complete precipitation. The protein pellet was obtained by washing twice with cold acetone and final washing with 80 per cent ethanol (Farrell, 1975).

After precipitation of the protein the pellet was generated as mentioned earlier.

The protein concentration was quantified by Bradford analysis in all the methods, the protein concentration ranged from 3-5 µg / mL. The maximum of 6-7 µg / µL was obtained in Method V. The protein was run in SDS PAGE, the faint bands appeared (Plate 11g). Compared to other four methods in the study, the bands are observed only in this method. The possible reasons for this may be the phenols got observed by the components of lysis buffer is having a higher pH of more than 8.0. At this pH, the oxidation of phenol don't take place (Farrel, 1975)

In all the methods the protein was quantified by Lawry's method and Bradford assay. The protein yield in TCA-acetone method was extremely low (3-4 µg per mL). The dense SDS, PVP/ TCA-acetone method yielded next least protein concentration (5µg per mL). The protein concentration was less than 6 µg per mL of sample in all the methods except the modified lysis buffer method.. To increase the concentration of protein in the extract, variation in the components of protocol was attempted as suggested by experts in proteome. The protein was loaded in SDS PAGE as per the standard procedure along with BSA as check. In all the methods, the protein bands did

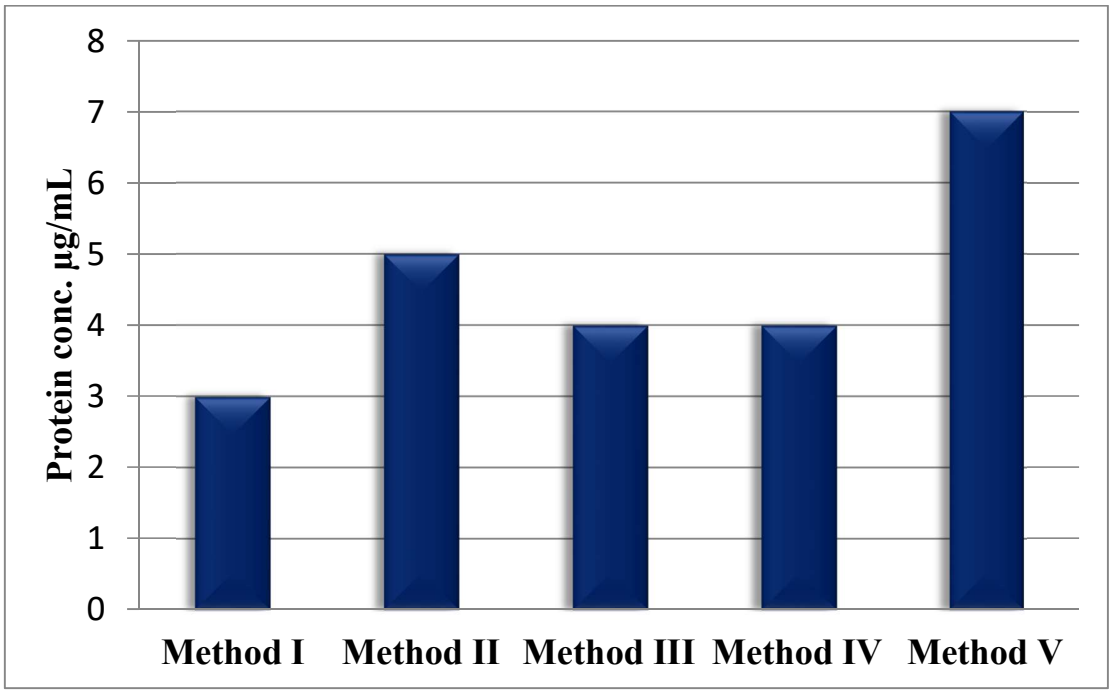


Fig. 59 Protein concentration in different methods of extraction

not appear with the cocoa flower and leaf protein whereas, the protein bands appeared in BSA (Plate 11g). Very faint bands were appeared with leaf protein (Plate 11g)). The protein bands were not clearly visible even with leaf protein. The minimum concentration of protein required for loading in 2D is 15 µg/ mL of sample (Umadevi and Anandaraj, 2015). Because of low concentration of protein in flower and leaf of cocoa, it was decided to go for LC-MS of the same to find the possible reasons for low concentration of protein in the final pellet. The flower and leaf proteins from self-incompatible lines were sent for LC-Q-ToF. The protein concentration of cocoa flower was not sufficient to load into the LC-Q-ToF. Hence, the LC-Q-ToF was done for leaf protein only. The results of LC-Q-ToF are presented in Table 97.

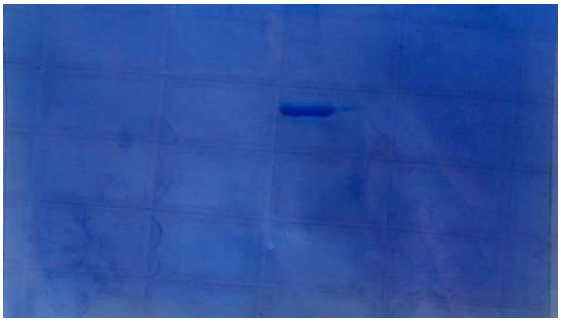
Table 97. Protein summery of cocoa leaf proteome through LC-Q-ToF

S. No	Name	Species	Peptides (95%)
1	ribulose bisphosphate carboxylase large chain (chloroplast) [*Theobroma cacao*]	*T. cacao*	11
2	ribulose bisphosphate carboxylase large chain (chloroplast) [*Theobroma cacao*]	*T. cacao*	11
3	ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [*Theobroma cacao*]	*T. cacao*	11
4	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast) [*Theobroma cacao]*	*T. cacao*	11
5	DNA-directed RNA polymerase II subunit 1, partial [*Theobroma cacao]*	*T. cacao*	1
6	NADH-plastoquinone oxidoreductase subunit 1 (chloroplast) [*Theobroma cacao]*	*T. cacao*	1
7	NADH-plastoquinone oxidoreductase subunit 1 (chloroplast) [*Theobroma cacao]*	*T. cacao*	1
8	NADH-plastoquinone oxidoreductase subunit 1 (chloroplast) [*Theobroma cacao]*	*T. cacao*	1
9	putative DNA-directed RNA polymerase IV subunit 1, partial [*Theobroma cacao]*	*T. cacao*	1
10	initiation factor 1 (chloroplast) [*Theobroma cacao]*	*T. cacao*	1
11	initiation factor 1 (chloroplast) [*Theobroma cacao]*	*T. cacao*	1
12	translational initiation factor 1 (chloroplast) [*Theobroma cacao]*	*T. cacao*	1

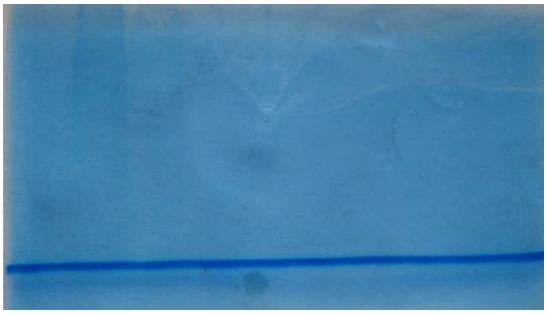
13	putative endoribonuclease dicer 3b-like protein, partial [*Theobroma cacao]*	*T. cacao*	0
14	REVERSED putative argonaute 2, partial [*Theobroma cacao]*	*T. cacao*	0
15	maturase K, partial (chloroplast) [*Theobroma cacao]*	*T. cacao*	0
16	maturase K (chloroplast) [*Theobroma cacao]*	*T. cacao*	0
17	maturase K (chloroplast) [*Theobroma cacao]*	*T. cacao*	0
18	maturase K (chloroplast) [*Theobroma cacao]*	*T. cacao*	0
19	maturase K, partial (chloroplast) [*Theobroma cacao]*	*T. cacao*	0
20	maturase K, partial (chloroplast) *[Theobroma cacao]*	*T. cacao*	0
21	maturase K, partial (chloroplast) *[Theobroma cacao]*	*T. cacao*	0
22	maturase K, partial (chloroplast) *[Theobroma cacao]*	*T. cacao*	0
23	maturase K, partial (chloroplast) *[Theobroma cacao]*	*T. cacao*	0
24	maturase K, partial (chloroplast) [*Theobroma cacao]*	*T. cacao*	0
25	hypothetical chloroplast RF19 (chloroplast) [*Theobroma cacao]*	*T. cacao*	0
26	hypothetical chloroplast RF19 (chloroplast) [*Theobroma cacao]*	*T. cacao*	0
27	hypothetical chloroplast RF19 (chloroplast) [*Theobroma cacao]*	*T. cacao*	0
28	stearoyl-acyl-carrier protein desaturase [*Theobroma cacao]*	*T. cacao*	0
29	RNA polymerase C1, partial (plastid) [*Theobroma cacao]*	*T. cacao*	0
30	maturase (mitochondrion) [*Theobroma cacao*]	*T. cacao*	0
31	maturase (mitochondrion) [*Theobroma cacao*]	*T. cacao*	0
32	REVERSED phospholipase D alpha 1-like protein [*Theobroma cacao*]	*T. cacao*	0
33	ribosomal protein S15 (chloroplast) [*Theobroma cacao*]	*T. cacao*	0
34	ribosomal protein S15 (chloroplast) [*Theobroma cacao*]	*T. cacao*	0

35	ribosomal protein S15 (chloroplast) [*Theobroma cacao*]	*T. cacao*	0
36	RecName: Full=ATP synthase subunit beta, chloroplastic; AltName: Full=ATP synthase F1 sector subunit beta; AltName: Full=F-ATPase subunit beta	*T. cacao*	0
37	ATP synthase CF1 beta chain (chloroplast) [*Theobroma cacao]*	*T. cacao*	0
38	ATP synthase CF1 beta chain (chloroplast) [*Theobroma cacao]*	*T. cacao*	0
39	ATP synthase beta subunit (chloroplast) [*Theobroma cacao]*	*T. cacao*	0
40	ATP synthase CF1 beta subunit (chloroplast) [*Theobroma cacao]*	*T. cacao*	0
41	ribosomal protein L2 (mitochondrion) [*Theobroma cacao*]	*T. cacao*	0
42	ribosomal protein L2 (mitochondrion) [*Theobroma cacao*]	*T. cacao*	0
43	non-expressor of pathogenesis-related protein 1 [*Theobroma cacao*]	*T. cacao*	0
44	putative argonaute 1 [*Theobroma cacao*]	*T. cacao*	0
45	Gibberellin 2-beta-dioxygenase [*Theobroma cacao*]	*T. cacao*	0
46	REVERSED hypothetical chloroplast RF21 (chloroplast) [*Theobroma cacao*]	*T. cacao*	0
47	REVERSED hypothetical chloroplast RF21 (chloroplast) [*Theobroma cacao*]	*T. cacao*	0
48	REVERSED hypothetical chloroplast RF21 (chloroplast) [*Theobroma cacao*]	*T. cacao*	0
49	REVERSED hypothetical chloroplast RF21 (chloroplast) [*Theobroma cacao*]	*T. cacao*	0
50	REVERSED hypothetical chloroplast RF21 (chloroplast) [*Theobroma cacao*]	*T. cacao*	0
51	REVERSED hypothetical chloroplast RF21 (chloroplast) [Theobroma cacao]	*T. cacao*	0
52	leucine-rich repeat receptor-like protein kinase [*Theobroma cacao]*	*T. cacao*	0
53	polyA binding protein, partial [*Theobroma cacao*]	*T. cacao*	0
54	GAI-like protein 1, partial [*Theobroma cacao*]	*T. cacao*	0
55	REVERSED putative endoribonuclease dicer-like protein 1, partial [*Theobroma cacao*]	*T. cacao*	0

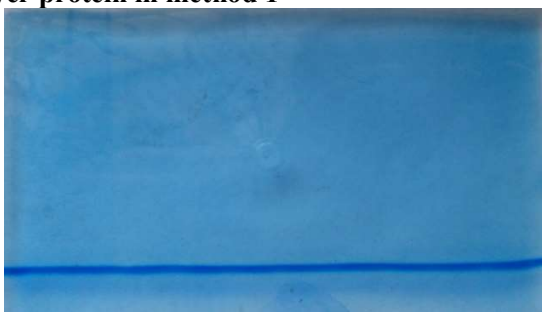
56	REVERSED ribosomal protein L2 (chloroplast) [*Theobroma cacao*]	*T. cacao*	0
57	REVERSED ribosomal protein L2 (chloroplast) [*Theobroma cacao*]	*T. cacao*	0
58	REVERSED ribosomal protein L2 (chloroplast*)* [*Theobroma cacao*]	*T. cacao*	0
59	REVERSED ribosomal protein L2 (chloroplast) [*Theobroma cacao*]	*T. cacao*	0
60	REVERSED ribosomal protein L2 (chloroplast) [*Theobroma cacao*]	*T. cacao*	0
61	REVERSED ribosomal protein L2 (chloroplast) [*Theobroma cacao*]	*T. cacao*	0
62	REVERSED phosphoribulokinase-like protein 2, partial [*Voanioala gerardii*]	*Voanioala gerardii*	0
63	REVERSED phosphoribulokinase-like protein 2, partial [*Voanioala gerardii*]	*Voanioala gerardii*	0
64	putative DNA-methyltransferase, partial [Theobroma cacao]	*T. cacao*	0
65	REVERSED hypothetical protein (mitochondrion) [*Theobroma cacao*]	*T. cacao*	0
66	REVERSED hypothetical protein (mitochondrion) [*Theobroma cacao*]	*T. cacao*	0
67	REVERSED ribosomal protein S4 (mitochondrion) [*Theobroma cacao*]	*T. cacao*	0
68	REVERSED ribosomal protein S4 (mitochondrion) [*Theobroma cacao*]	*T. cacao*	0
69	REVERSED alpha-D-galactosidase, partial [*Theobroma cacao*]	*T. cacao*	0
70	REVERSED hypothetical chloroplast RF19 (chloroplast) [*Theobroma cacao]*	*T. cacao*	0
71	REVERSED hypothetical chloroplast RF19 (chloroplast) [*Theobroma cacao*]	*T. cacao*	0
72	REVERSED hypothetical chloroplast RF19 (chloroplast) [*Theobroma cacao*]	*T. cacao*	0
73	11S globulin isoform 2 [*Theobroma cacao*]	*T. cacao*	0
74	pyruvate kinase, partial [*Theobroma cacao*]	*T. cacao*	0
75	E3 UFM1-protein ligase [*Theobroma cacao*]	*T. cacao*	0
76	REVERSED DNA-directed RNA polymerase V subunit 1, partial [*Theobroma cacao*]	*T. cacao*	0
77	NADH dehydrogenase subunit 9 (mitochondrion) [*Theobroma cacao*]	*T. cacao*	0



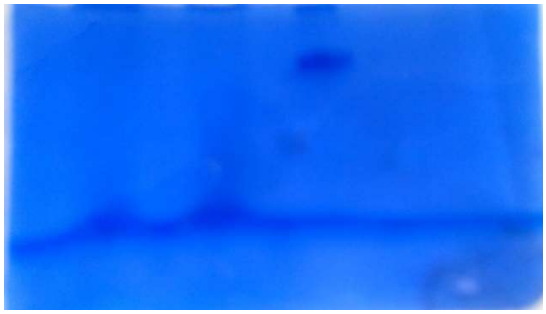
a. SDS PAGE of cocoa flower protein in method 1



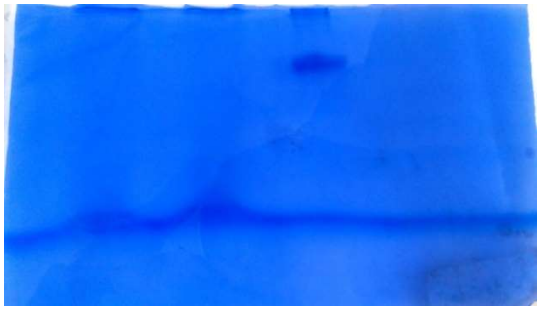
b. SDS PAGE of cocoa flower protein in method II



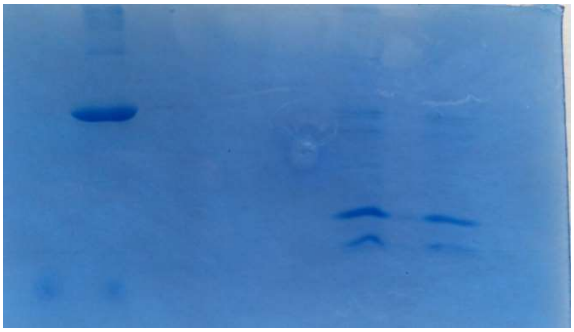
c. SDS PAGE of cocoa leaf protein in method II



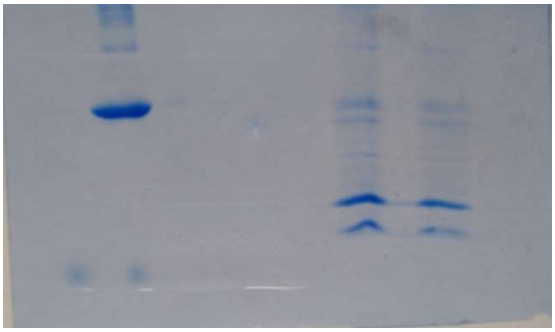
d. SDS PAGE of cocoa flower protein in method III



e. SDS PAGE of cocoa leaf protein in method III



f. SDS PAGE of cocoa leaf protein in method IV



g. SDS PAGE of cocoa leaf protein in method V

Plate 11. SDS PAGE of cocoa leaf and flower protein in different methods of protein extraction

78	NADH dehydrogenase subunit 9 (mitochondrion) [*Theobroma cacao*]	*T. cacao*	0
79	REVERSED NBS-LRR resistance protein RGC45, partial [*Theobroma cacao*]	*T. cacao*	0
80	REVERSED DNA repair and recombination protein [*Theobroma cacao*]	*T. cacao*	0
81	REVERSED exosome complex exonuclease RRP44 [*Theobroma cacao*]	*T. cacao*	0
82	alpha-D-galactosidase, partial [*Theobroma cacao*]	*T. cacao*	0
83	alpha-D-galactosidase, partial [*Theobroma cacao*]	*T. cacao*	0
84	alpha-D-galactosidase, partial [*Theobroma cacao*]	*T. cacao*	0
85	alpha-D-galactosidase, partial [*Theobroma cacao*]	*T. cacao*	0
86	alpha-D-galactosidase, partial [*Theobroma cacao*]	*T. cacao*	0
87	alpha-D-galactosidase, partial [*Theobroma cacao*]	*T. cacao*	0

A total of eighty seven proteins have been identified in leaf sample of cocoa which were identical to cocoa proteins in library. Two proteins were identical to *Voanioala gerardii*.

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Summary

5. SUMMARY

The present study entitled "Genetic analysis of inbreds, inbred crosses and hybrids of cocoa (*Theobroma cacao* L.) was carried out in the Department of Plantation Crops and Spices, College of Horticulture, Cocoa Research Centre, Vellanikkara during the period 2015-2018. The objective of the study was to evaluate the inbreds to quantify the magnitude of inbreeding depression in yield and yield attributes in various self-generations, to establish a physiological relationship between the vigour of inbreds, inbred crosses and hybrids, to standardise pollination techniques to break self-incompatibility in fifth generation self-incompatible inbred and to study the self incompatibility mechanism using protein profiling at critical stages of pollination using two dimensional gel electrophoresis protein profile analysis.

A total of 113 inbreds were evaluated on the basis of availability of preceding and succeeding generations to establish the magnitude of inbreeding depression over generations in the field maintained at CRC, Vellanikkara. The inbreds of various generations differed for qualitative characters like pod shape, colour, apex, basal constriction, rugosity and bean colour. Among the inbreds, 22 inbreds (19.16 per cent) had cundeamore, 32 inbreds (28.31 per cent) were angoleta, 41 inbreds (36 per cent) were amelonado and 18 inbreds (15.92 per cent) were calabacillo pods shape. The colour was absent in 85 genotypes and yellowish ridge color was observed in 28 genotypes. Acute, obtuse, rounded, intermediate, attenuate and mammaleate pod apex was observed in 41, 37, 13, 2, 11 and 7 inbreds respectively. The basal constriction was absent in 41 inbreds and it was strong in 7 inbreds. Slight and intermediate basal constriction was observed in 55 and 10 inbreds respectively. Slight and medium pod rugosity was observed in 69 and 9 inbreds respectively. Pod rugosity was absent in 35 inbreds. Light and medium colour beans were observed in 26 and 44 inbreds respectively. Mixed colour beans were observed in 26 inbreds.

The plant height during 2018 ranged between 110 cm and 780cm. The maximum plant height of 780 cm was recorded in inbred P II 4.8 (Plant number 19.1) and P II

13.12 (Plant number 16.2). During 2018, the maximum collar girth of 78 cm was observed in S_1 inbred H 7.3 (Plant number 1.12) followed by 74 cm girth in G VI 135 (Plant number 12.6) and the least collar girth of 30 cm was observed in P II 13.8 (Plant number 21.3).

The mean pod weight varied between 104 g and 532 g among the S_1 generation inbreds. The mean pod weight recorded among the S_1 inbreds was 303.02 g. The mean pod length varied between 8.1 cm and 17.34 cm among the S_1 generation inbreds. The mean pod length recorded among the S_1 inbreds was 13.24 cm. The pod breadth significantly varied among the S_1 generation inbreds. The pod breadth ranged from 5.76 cm in plant number 4.18 to 8.90 cm in plant number 21.3. The ridge thickness ranged from 0.72 cm in plant number 6.3 to 2.54 cm in plant number 18.6. Furrow thickness among the S_1 generation inbred varied from 0.34 in plant number 6.2 to 1.94 cm in plant number 23.2. The number of beans per pod ranged from 35.80 to 42.20, 31.80 to 44.20 and 26.60 to 35.6 in inbred H1 1.2, G VI 135 and G VI 141 respectively. The number of flat bean per pod ranged from 0.2 in plant number 4.14 to 6.2 in plant number 4.11

Important bean characters such as wet bean weight per pod, dry bean weight per pod, single dry bean weight (SDBW), bean length, bean breadth, and bean thickness ranged from 31.26 to 224 g, 9.58 to 51.62 g, 0.57 to 1.19 g, 11.22 to 22.28 mm, 9.25 to 13.31 mm, 5.48 to 8.55 mm respectively.

The pod value ranged from 9.58 to 51.61. Minimum (23.94) pod index was observed in H7.3 (Plant number 4.12). The minimum efficiency index (6.24) was observed in genotype H 7.3 (Plant number 4.9) followed by H7.3 (Plant number 4.16), H1 1.2 (Plant number 6.1). Maximum conversion index of 0.5 was recorded in P II 12.9 (Plant number 23.5) followed by H 7.3 (Plant number 4.3). The maximum dry matter recovery (50.71 %) was observed in P II 12.9 (Plant number 23.2) whereas, minimum dry matter recovery (13.41 %) in P II 13.8 (Plant number 21.2).

The fat content ranged from 38.63 in genotype P II 13.12 (plant number12.3) to maximum of 64.93 per cent in genotype PII 12.9 (Plant number 16.1). The poly phenols

ranged between 1.47 to 3.6 percent among the S_1 inbreds. The maximum poly phenols (3.6 per cent) are observed in G VI 141(Plant number 14.2) followed by P II 13.12 (3.57 per cent). The least poly phenol content estimated in P II 13.8 inbred (Plant number 21.1).

The maximum plant height of 375 cm was recorded in S_2 inbred G VI 141 (Plant number 15.9), whereas, the least plant height of 135 cm was recorded in S_2 inbred P II 4.8 (plant number 20.4) during 2018. The collar girth in S_2 during 2018, ranged from 19 cm to 58 cm in P II 13.2 (Plant number 17.6) and H 7.3 (Plant number 5.1) respectively.

The maximum pod weight of 464 g was recorded in PII 13.12 (Plant number 17.4) inbred and the least pod weight of 106g was observed in P II 4.8 (Plant number 20.1) inbred plant. The maximum pod length of 19.16 cm was observed in PII 4.8(Plant number 20.6) inbred and the minimum pod length (9.20cm) was recorded in P II 12.9 (Plant number 24.2) inbred. The pod breadth ranged between 4.68 and 10.40 cm. The maximum pod breadth of 10.40 cm was observed in PII 13.12 (Plant number 17.4) inbred and the minimum pod breadth (4.80cm) was recorded in P II 4.8 (Plant number 20.1) inbred. The ridge thickness ranged between 0.80 and 1.84 cm. The maximum (1.84cm) and minimum (0.80cm) ridge thickness were observed in H 7.3 (86) (Plant number 5.1) and H 7.3 (Plant number 5.3) inbreds respectively. The inbred H 7.3 (86) (plant number 5.3sn) and G VI 295.4 has shown the maximum (1.50cm) and minimum (0.44cm) furrow thickness among the S_2 inbreds. The maximum number of beans per pod in S_2 inbreds was 44.6 in G VI 141(Plant number 15.8). P II 4.8(Plant number 20.1) recorded the minimum number of beans per pod. . Maximum of 8 flat beans per pod was observed in PII 13.12 (plant number 17.2).

The wet bean weight per pod ranged between 35.28g in inbred P II 13.8 (Plant number 17.8) and 128g in H 7.3 (Plant number 5.1). The average wet bean weight of S_2 generation inbred was 67.63 g. The dry bean weight per pod in S_2 inbreds ranged from 10.44 to 38.77 g. The single dry bean weight ranged between 0.47 and 0.91g in S_2 inbreds

The pod value ranged from 10.44 to 38.77 among the S_2 generation inbreds. The pod index ranged from 26.16 to 96.85. Minimum efficiency index of 5.94 was observed in G VI 141. Maximum conversion index (0.61) was in P II 13.12. Minimum conversion index was observed in H 7.3. The dry matter recovery ranged from 14.63 to 61.40 per cent among the S_2 inbreds.

The maximum fat content of 63.10 per cent was observed in G VI 295.4 (Plant number 1.3) followed by G VI 295.4 (62.93 per cent)(Plant number 1.2). The minimum fat content of 32.97 per cent was estimated in PII 12.9 inbred (Plant number 24.1). The maximum poly phenols (3.74 per cent) are observed in G VI 141(Plant number 15.6) followed by P II 13.12 (3.68 per cent) (Plant number 17.8). The least poly phenol content (2.15 per cent) was observed in H 7.3 inbred (Plant number 5.2).

In S_3 genotype, the maximum plant height of 620 cm was observed in M 18.7 (Plant number 2.4) and the minimum plant height of 225 cm was observed in M 18.7 (Plant number 2.5) during 2018. The maximum collar girth of 60 cm was observed in M 18.7 (Plant number 2.5) and the minimum collar girth of 34 cm was observed in M 18.7 (Plant number 2.4).

The pod length in S_3 inbred ranged between 10.70 and 14.10 cm. The maximum (1.82cm) and minimum (1.22 cm) ridge thickness were observed in plant number 2.1 and 2.5 respectively. The wet bean weight per pod ranged between 66.3 g in plant number 2.5 and 103.20 g in plant number 2.1.

The pod value ranged from 20.86 to 35.77 among the S_3 generation M 18.7 inbreds. The minimum pod index was observed in M 18.7 (Plant number 2.2) inbred. The maximum pod index was observed in M 18.7(Plant number 2.3). The dry bean weight per pod ranged from 20.86 to 35.77 g. The dry bean weight per pod ranged from 20.86 to 35.77 g.

The maximum fat content of 63.77 per cent was observed in G II 7.4 (Plant number 8.1). The minimum fat content of 42.33 was estimated in M 18.7 inbred (Plant number 2.4). The poly phenols ranged between 2.01 to 3.42 percent among the inbreds.

The inbreeding depression was studied generation wise. In S_1 generation of M 18.7, the husk furrow thickness and number of flat beans expressed negative inbreeding depression of -12 and -7.5 per cent respectively. The average yield per tree per year has shown a very meager inbreeding depression of 4.21 per cent over the preceding generation. In S_2 generation of M 18.7, the inbreeding expression was to a tune of less than five per cent for 16 characters. Pod value, conversion index, dry matter recovery have shown an inbreeding depression between 5 to 10 percent. Negative inbreeding depression was observed for husk ridge thickness, number of flat bean per pod, pod index, efficiency index and fat content. In S_3 generation of M 18.7, maximum inbreeding depression of 10 per cent was observed for wet bean weight per pod followed by pod length (6.86 per cent). The negative inbreeding depression for number of flat bean per pod, pod value and efficiency index persist in S_3 generation. In S_4 generation of M 18.7, highest inbreeding depression of 43.58 per cent was observed for number of pods/tree/year. The negative inbreeding depression for number of flat bean pod, pod index, conversion index, dry matter recovery and fat estimation persisted and was more than in the preceding generation.

In S_1 generation of G II 7.4, positive inbreeding depression was observed for 10 characters out of 21. The husk ridge and furrow thickness and number of flat beans expressed negative inbreeding depression of -64.52, -44.29 and -9.09 respectively. In S_2 generation of G II 7.4, positive inbreeding depression was observed for 15 characters. The maximum inbreeding depression was observed for bean thickness (31.80%) followed by fat content (26.46%), and efficiency index (23.17%). Only seven characters expressed positive inbreeding depression in S_3 generation of G II 7.4 genotype. In S_5 generation inbred, the maximum inbreeding depression (63.95%) was observed for husk furrow thickness followed by husk ridge thickness (49.02), pod weight (39.11 per cent),

efficiency index (33.39 per cent). Negative inbreeding depression was observed for number of beans per pod (-11.50), number of flat per pod (-11.11).

In S_1 and S_2 generation of H1 1.2, positive inbreeding was observed in 16 characters out of 21 characters indicating the reduction in their values over the preceding generation

Positive inbreeding depression was observed for 13 characters in G IV 35.7. Av. Yield (No. of pods per tree per year, pod weight, husk ridge thickness, husk furrow thickness, wet bean weight per pod, single bean weight, efficiency index have shown the inbreeding depression over S_0 generation. In S_2 generation of G IV 35.7, positive inbreeding depression was observed for fifteen characters out of 21. In S_3 generation of G IV 35.7, the maximum negative inbreeding depression was observed for wet bean weight per pod (-79.70) followed by average pod weight (-44.09) and efficiency index (-44.21).

Positive inbreeding depression over the preceding generation was observed for majority of the characters except wet bean weight per pod (-5.44) and pod index (-2.33) in S_1 generation in G VI 135and number of flat beans per pod (-71.43), conversion index (-44.37) and dry matter recovery (-44.56) and pod index (-27.62) in S_2 generations in G VI 135.

In P II 13.12, the positive inbreeding depression was expressed for majority of the characters in S_1 generation over S_0 generation. In P II 13.8, negative and less inbreeding depression was observed for conversion index (-13.82) and fat content (-15.21),

The comparative evaluation of inbreds, inbred crosses and hybrids planted in 2015 was carried out CRC farm, Vellanikkara. The plant height in inbreds ranged between 70 and 310 cm in S_4 generation of M 18.7 (Stand number 2.8) and S_4 generation of G 4 35.7(Stand number 10.3) respectively. The plant spread in east-west and north-south direction ranged between 20 to 385 and 18 to 400 cm. The girth at collar

region ranged between 7 cm and 28cm in S_4 generation of M 18.7(Stand number 5.2) and M 18.7(Stand number 4.3) respectively. The leaf area in inbred self ranged from 44.45cm^2 to 531cm^2.

In inbred crosses, the plant height among the inbred crosses range between 90 cm to 200cm in S_3H_7 3(86) X S_3G_4 35.7(Stand number 2.6) and S_3H_7 3(86) X S_3G_4 35.7 (Stand number 4.4) respectively. The plant spread in EW and NS direction ranged between 20 to 150 cm and 25 to 170 cm respectively. The plant girth ranged from 6 to 14 cm in S_3H_7 3(86) X S_3G_4 35.7(Stand number 4.7 and 7.5) respectively. The leaf area in inbred cross ranged between 50.50 to 414.66 cm^2.

In hybrids, the maximum plant height of 200 cm was observed in CCRP 13 (Stand number 28.6). The maximum plant spread in EW and NS directions were recorded in CCRP 13 (Stand No.28.6) and CCRP 15(Stand No.31.6). In hybrids, the leaf area measured from 44.49 to 414.49 cm^2 with an average leaf area of 172.09cm^2

Significant variation in chlorophyll content was observed among the inbreds of cocoa. The chlorophyll A content in inbred ranged from 1.746 to 6.352, Chlorophyll B ranged from 0.299 to 3.557 and total chlorophyll content ranged from 5.490 to 9.627.

The average chlorophyll A, Chlorophyll B and total Chlorophyll in inbred crosses was 3.785, .671 and 4.457 mg/g. Chlorophyll A ranged from 1.823mg/g in S_5G_1 7.4 X S_3G_4 35.7 (Stand number 3.7) to 5.816 mg/g in S_3H_7 3 (86) X S_3G_4 35.7 (Stand number 4.4). The average chlorophyll A, Chlorophyll B and total Chlorophyll observed was 2.885, .594 and 3.461 mg/g. was observed in hybrids.

The maximum leaf nitrogen content of 2.65 per cent was observed in M 18.7 (Stand number 5.7 and 5.12) followed by M 18.7 (Stand number 5.9). The phosphorus content in leaf ranged from 0.04 per cent in S1 generation of G IV 35.7 (Stand number 8.16) to .0.5 per cent in M 18.7 (Stand number 4.7). The average potassium content in the leaf of inbred self was 1.29 per cent. The nitrogen content in leaf varied from 0.85 per cent in S_3H_7 3 (86) X S_3G_4 35.7 (Stand number 4.5) to 2.12 per cent in S_3H_7 3 (86) X

S_3G_4 35.7 (Stand number 7.5). The phosphorus content in inbred cross ranged from 0.11 per cent in S_3H_7 3 (86) X S_3G_4 35.7 (Stand number 4.6) to 0.64 per cent in S_3H_7 3 (86) X S_3G_4 35.7 (Stand number 4.4).

The nitrogen content in hybrids varied significantly, the maximum nitrogen content of 1.75 per cent was registered in CCRP 11 (Stand number 26.2). The phosphorus content in hybrids ranged between 0.05 per cent in CCRP 13 (Stand number 28.1 and 28.3) and 0.81 per cent in CCRP 9 (Stand number 9.5 and 9.6). The maximum potassium content of 1.43 per cent was observed in CCRP 8(Stand number 8.1) and the minimum potassium content (1.06 per cent) in hybrids was registered in CCRP 12 (Stand number 27.2).

. In inbreds, the bud break was between 18 and 21 days. In inbred cross, the flowering and bud break was not observed. In hybrids, the bud break occurred between 18 to 20 days.

The flushing in inbred self took place in the month of July and December months. In inbred cross, flushing took place in the month of August and September. In hybrids, the flushing took place in the month of July and November.

The maximum number of cushions (18 No's) were formed in M 18.7 (stand number 3.8). In inbred cross, cushions did not form. The flowering took place in the month of September to December in inbred self. In inbred cross, flowering did not occur

The inbred cross and inbred self were on par with a comparison ratio of 1.00. Among the hybrids and inbred self, the inbred self plants were superior with a comparison ratio of 1.15. When inbred crosses and hybrids are compared, the inbred crosses are superior over with respect to growth parameters. The girth of inbred self was superior over inbred crosses and hybrids with a comparison ratio of 1.59 and 1.70 respectively. Among the inbred crosses and hybrids, inbred crosses were superior with a comparison ratio of 1.07

With respect to chlorophyll A, The inbred self had slight high chlorophyll content than inbred cross with a comparison ratio of 0.98. Among the hybrids and inbred self, the inbred self plants had high chlorophyll content with a comparison ratio of 1.29.

The leaf nitrogen content inbred self was higher than inbred cross with a comparison ratio of 1.11. Among the hybrids and inbred self, inbred self had more nitrogen content than hybrid with a comparison ratio of 1.48. Inbred cross had high nitrogen than hybrids with a comparison ratio of 1.33. The leaf phosphorus content in inbred self was lower than inbred self with a comparison ratio of 0.78. The leaf potassium content in inbred self was lower than inbred self with a comparison ratio of 0.98. The inbred self had high potassium content than hybrids with a comparison ratio of 1.07. Inbred cross had high potassium content than hybrids with a comparison ratio of 1.08.

A total of nine techniques and methods to overcome self incompatibility barriers such as bud pollination, surgical technique, intra-ovarian technique, salt spray (3% and 1%), high humidity, high temperature, Naphthalene Acetic Acid (100 and 200 ppm), gamma irradiation and flower organ extract were attempted from September 2016 to March 2017 and September 2017 to March 2018. In all the methods, a minimum of 100 flowers were pollinated and tested for success of pollination and fertilization. In all the methods, the fruit set did not occur except in surgical technique. The majority of S_3 and S_5 inbreds set in to pods through surgical techniques. The main reason for success may be the self incompatibility proteins are synthesized in the upper one third portion of the stigma. In the fluorescent microscope, the clear disintegration of pollen tube was observed in self incompatible flower.

Flowers from fifth generation cocoa inbreds were used for extraction of protein. All the available protein extraction methods were tried and the protein was quantified. In all the methods, the protein content was bellow 5µg per mL in the cocoa flower. The protein from cocoa leaf was extracted and quantified. In Lysis buffer extraction with some modification, a protein content of 7 µg per mL was obtained. Faint bands were

observed in SDS PAGE. The flower and leaf protein were subjected for LC Q ToF. The protein content in flower was insufficient to run LC Q ToF. A total of 87 proteins were found in cocoa leaf sample. Of which, 85 proteins are similar to cocoa and two proteins are similar to forest coconut.

References

REFERENCES

Abdul-Karimu, A., Butler, D. R., Iwaro, A. D., Sukha, D.A., Bekele, F., Mooledhar, V., and Shripat, C. 2003. Farmers' perceptions of cocoa planting material and factors affecting the cocoa industry in Trinidad and Tobago. *Trop. Agric. Trin*. 80 (4): 261-266.

Abraham, C.C. and Remamony, K. S. 1979. Pests that damage cocoa plants in Kerala. *India Arecanut Spices Cocoa J.* 2: 77-81.

Abraham, E.V. and Padmanabhan, M. D. 1967. Pests that damage cocoa in Madras. *Indian Hortic.* 11 (3): 11-12.

Adewale, B.D., Okonji, A. A., Akintobi, D. A. C., and Aremu, C. O. 2010. Genotypic variability and stability of some grain yield components of cowpea. *Afr. J. of Agric. Res.* 5: 874-880.

Adjaloo, M. K., Oduro, W., and Banful, B. K. 2012. Floral phenology of upper amazon cocoa trees: Implications for reproduction and productivity of cocoa. Agronomy. 2: 8-16.

Adomako, B and Y. Adu-Ampomah. 2003. Bean characteristics of progenies of upper Amazon cacao in Ghana. *Trop. Agric.,* **80:** 41-47.

Adu-Ampomah, Y., Novak, F. J., Klu, G. Y. P. and Lamptey, T. V. O. 1991. Use of irradiated pollen as mentor pollen to induce self-fertilization of two self-incompatible Upper Amazon cacao clones. Euphytica. 51:219-225

Advani, R. 1982. Vertebrate pest problems in the Indian desert and their bio toxicological management. *Sci. Rev.* 1: 87-118.

Advani, R. 1984. Ecology, status and postnatal development of the black rat infesting coconut and cocoa. In: Bhavappa, K.V.A., *Proceedings of the PLACROSYM V.* Central Plantation Crops Research Institute, Kasargod, pp. 626-636.

Afoakwa, E.O., Peterson, A., Fowler, M., and Ryan, A. 2008, Flavour formation and character in cocoa and chocolate: A critical review. *Crit. Rev. Food Sci. Nutr*. 48: 840-857.

Afoakwa, P. O. 2010. Variation in agro-morphological characteristics of cacao, *Theobroma cacao* L. in farmers fields in Nigeria. *N. Z. J. Crop Hortic. Sci.* 38: 157-170.

Ahnert, D. 2006. Ideotype breeding in cocoa. In**:** *Proceedings of the International Workshop on Cocoa Breeding for Farmers' Needs, San Jose, Costa Rica,* 15th – 17th October. 2006. p.157-166.

Aikipondian, P. O. 2010. Variation in agro-morphological characteristics of cacao, *Theobroma cacao L.,* in farmers' field in Nigeria. N. Z. *J. Crop Hortic. Sci.* 38: 157-170.

Aikpokpodion, P. O., K. Badaru, L. O. Raji, and A. B. Eskes. 2011. Selection of new cocoa (*Theobroma cacao* L.) varieties in on-station and on-farm trials in Nigeria. In: *Final Report of the CFC/ICCO/Bioversity International Project on "Cocoa Productivity and Quality Improvement: a Participatory Approach"* 2004-2010. p. 85-93.

Ajmal, P. M. 2016. Evaluation of selected cocoa (*Theobroma cacao* L.) hybrid breds for quality. M.Sc. ((Ag) thesis, Kerala Agricultural Sciences, Thrissur, 67p.

Alagar, M. and Subaharan, K. 2011. Management of tea mosquito bug for enhancing the productivity of cocoa. In: Apshara, E. S., Jaganathan, D., and Balasimha, E. (eds.),

Proceedings of seminar on strategies for enhancing productivity of Cocoa. Central Plantation Crops Research Institute, Vittal, pp. 128-134.

Allard RW. 1990. Principles of plant breeding. *John Wiley and Sons. Inc.,* New York and London, pp. 224-232.

Allard, A. W. 1971. *Principles of Plant Breeding*. Wiley International, New York, 264p.

Allard, R.W. 1960. *Principles of Plant Breeding.* John Willey and Sons Inc., New York.216

Alverson, W.S., Whitlock, B. A., Nyfeller, R., Bayer, C., and Baum, D. A. 1999. Phylogeny of the core Malvales: Evidence from ndhF sequence data. *Am. J. Bot.* 86: 1474-1486.

Alvim de, P. 1966. Factors affecting the flowering of cocoa tree. *Cocoa Growers Bulletin*. 7:15-19.

Alvim de, P. 1984. Flowering of cocoa. *Cocoa Growers Bulletin*. 35: 23-31.

Alvim, P. T. 1954. Studies on the cause of cherelle wilt of cacao. *Turrialba*. 4: 72-78.

Amma S. P., Minimol, J. S., and Lalithatbai, E. S. 2011. Utilization of introduced genetic resources in cocoa. *Cashew Cocoa J.* 3(1): 16-18.

Amores, F. M., Vasco, S. A., Eskes, A. B., Suarez, C., Quiroz, T. G., Loor, R. G., Jimenez, J. C., Zambrano, J., Bolanos, M. J., Reynel, V. H., Teran, M. M., and Quijano. G. C. 2011. On-farm and on-station selection of new cocoa varieties in Ecuador. In: *Final Report of the CFC/ICCO/Bioversity International Project on Cocoa Productivity and Quality Improvement: a Participatory Approach* : 2004-2010. P 59-72.

Amoye, S. 2006. Cocoa sourcing, world economics and supply. *The Manuf. Conf.* 86 (1): 81-85.

Anderson, N.L. and Anderson, N.G. 1998. Proteome and proteomics: new technologies, new concepts and new words. *Electrophoresis* 19(11): 1853-1861.

Aneja, M.,Gianfagna, T., and Ng. E. 1999. The roles of abscisicacid and ethylene in the abscission and senescence of cocoa flowers. *Plant Growth Regulation.* 27:149-15.

Asna, A. C., Prasannakumari, K. T., Minimol, J. S., and Krishnan, S. 2014. Variability analysis in bean characters of selected accessions of cocoa (*Theobroma cacao* L.). *J. Plantation Crops,* 42 (2): 246 -251.

Assemat, S., Lachenaud, P. H., Ribeyre, F., Davrieux, F., Pradon J. L., and Cros, E. 2005. Bean quality traits and sensory evaluation of wild Guianan cocoa populations. (*Theobroma cacao* L.). *Genet. Resour.Crop Evol.* 52 (7): 911-917.

Atta Ofori, Francis, K.P., Frank, O. A., Abraham, A., Gilbert, A. K., 2016. Genetic variation for vigour and yield of cocoa (*Theobroma cacao* L.) clones in Ghana. *Scientia Horticulture*, 231: 124-142.

Awua, P. K. 2002. *Cocoa Processing and Chocolate Manufacture in Ghana.* David Jamieson and Associates Press Inc., Essex, UK. 118p.

Barrs, H.D. &Weatherley, P.E. 1962. A re-examination of the relative turgidity for estimating water deficits in *Aus. J. Biol. Sci.* 15:413-428,

Barrs, H.D., 1968. Determination of water deficits in plant tissues. In: T.T. Kozolvski, *Water Deficits and Plant Growth*, Vol 1, pp 235–368. Academic Press, New Delhi.

Bartley, B. G. D. 2005. *The Genetic Diversity of Cacao and Its Utilization.* Oxford University Press (CABI Publishing), Oxford, UK.

Bayer, C., Fay, M. F., De Bruijn, P. Y., Savolainen, V., Morton, C. M., and Kubitzki, K. 1999. Concept of Malvaceae within a recircumscribed order Malvales: a combined

analysis of plastid atpB and rbcL DNA sequences. *Bot. J. Linnean Soc.,* 129(4): 267-303.

Bekele, F. & D.R. Butler, 2000. Proposed list of cocoa descriptors for characterisation. Working procedures for cocoa germplasm evaluation and selection. In: A.B. Eskes, J.M.M. Engels & R.A. Lass (Eds.), Proc CFC/ICCO/IPGRI Project Workshop, pp. 41– 48. Montpellier, France, February 1–6, 1998.

Bekele, F. and Butler, D.R. 2000. Proposed list of cocoa descriptors for characterization. Working procedures for cocoa germplasm evaluation and selection, Proceedings of the CFC/ICCO/IPGRI Project Workshop (eds. Eskes, A.B., Engels, J.M.M. and Lass, R.A.), February 1998, Montpellier, France, IPGRI, pp. 41- 48.

Bekele, F. L. and Bidaisee, G. G. 2006. Characterization of germplasm in the International Cocoa Genebank, Trinidad. In**:** Global Approaches to Cocoa Germplasm Utilization and Conservation. Final report of the CFC/ICCO/IPGRI project on "Cocoa Germplasm Utilization and Conservation: a Global Approach" 1998-2004. CFC, Amsterdam, The Netherlands/ICCO, London, UK/IPGRI, Rome, Italy.

Bekele, F. L. and Butler, D. R. 2000. Proposed short list of cocoa descriptors for characterization. In: *Working Procedures for cocoa Germplasm Evaluation and Selection*. Eskes, A. B., Engels, J. M. M., and Lass, R. A. (Eds.). Proceedings of the CFC/ICCO/IPGRI Project workshop, Montepellier, France. pp. 41- 48.

Bekele, F., Bekele, L. I., Butler, D. R., and Bidaisee, G. G. 2006. Patterns of morphological variation in a sample of cacao (*Theobroma cacao* L.) germplasm from the International Cocoa Genebank, Trinidad. *Genet. Resour.Crop Evol.* 53: 933-948.

Bertolde F. Z, Almeida A-AF, Pirovani, C. P. 2014. Analysis of Gene Expression and Proteomic Profiles of Clonal Genotypes from *Theobroma cacao* Subjected to Soil Flooding.*PLoSONE* 9(10): e108705. https://doi.org/10.1371/journal.pone.0108705.

Bhat, S. K., 1978. Rodent damage to cocoa in South India. *Rodent News Lett.* 2(3): 1.

Boardman, N.K. 1977. Comparative photosynthesis of sun and shade plants. *Annu. Rev. Plant Physiol.* 28:355–377.

Borhi, B., Bonalli, F., and Boggini, G. 1973. Induction of male sterility in wheat with Ethephon for hybrid seed production, Proceedings of 4th Interanat. Wheat genetics symposium, pp 337-343

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-254.

Brew, A. H .1988. Cocoa pod husk as a breeding substrate for forcipomyia midges and related species which pollinate cocoa in Ghana. Cocoa Growers Bulletin. p40.

Brown, A.H.D., D.R. Marshall, O.H. Frankel, and J.T. Williams (Eds). 1989. The Use of Plant Genetic Resources. International Board for Plant Genetic Resources: Cambridge University Press, Cambridge, UK. 382 pp.

Bucheli, P., Rousseau, G., Alvarez, M., Laloi, M., and McCarthy, J. 2001. Developmental variation of sugars, carboxylic acids, purine alkaloids, fatty acids, and endoproteinase activity during maturation of *Theobroma cacao* L. seeds. *J. Agric. Food Chem.* 49: 5046-5051.

Bushnell, J.W. 1922. Isolation of uniform types of hubbard squash by inbreeding. Proceedings of American Soc. Hortic. Sci.. 19:139-144.

Candiano, G., Bruschi, M., Musante, L., Santucci, L., Ghiggeri, G.M., Carnemolla, B., Orecchia, P., Zardi, L., and Righetti, P.G. 2004. Blue silver: a very sensitive colloidal Coomassie G-50 staining for proteome analysis. *Electrophoresis* 25(9): 1327-1333.

Carpentier, S.C., Witters, E., Laukens, K., Deckers, P., Swennen, R., and Panis, B. 2005. Preparation of protein extracts from recalcitrant plant tissues: an evaluation of different methods for two-dimensional gel electrophoresis analysis. *Proteomics* 5(10): 2497-2507.

CCRP [Cadbury- KAU Co-operative Cocoa Research Project] 2013. Annual Report 2012-13. Cocoa Research Centre, Vellanikkara, pp. 159.

Chambach, A. and Rodbard, D. 1971. Polyacrylamide gel electrophoresis. *Science* 172: 440-451.

Chandramohanan, R. 1979. Cocoa seedling dieback. *Indian Cocoa Arecanut Spices J.* 3:5-6.

Chandramohanan, R. 1982. Studies on the reaction of pods of different cocoa accessions to *Phytophthora palmivora.Plater*. 58: 99-103.

Cheesman, E. E. 1944. Notes on the nomenclature classification and possible relationships of cacao populations. *Trop. Agric.* 21 (8): 144-159.

Chekalina, I.N. 1976. Effect of inbreeding on variability of cucurbits (*Cucurbita maxima* Dusch and *Cucurbita pepo* L.) *Genetika*. 12:45- 49.

Chen, S. and Harmon, A. C. 2006. Advances in plant proteomics. *Proteomics.* 6 (20): 5502-16.

Chesney, P. E. 2007. Preliminary characterization and evaluation of cocoa on-farm genetic diversity in the north-west region of guyana. *Ingenic. News letter*11: 2-5.

Chetana, S. 2016. Analysis of inbreeding depression in west coast tall coconut (*Cocos nucifera* L.). M. Sc. Agric, thesis submitted to Kerala Agric. University, pp. 186.

Ciferri, R. and Ciferri, F. 1957. The evolution of cultivated cacao. *Evol*. 11: 381-397.

Cilas, C., C. Duchemin and R. Lotode. 1989. Lamelioration genetique de la qualite du cacao: etude de la granulometric. Cafe, Cacao, 33 (1): 3-8.

Clapperton, J., Yow, S., Chan, J., Lim, D., Lockwood, R., Romanczyk, L., and Hammerstone, J. 1994. The contribution of genotype to cocoa (*Theobroma cacao* L.) flavor. *Trop. Agric.* 71(4): 303-308.

Coe, S. D. and Coe, M. D. 1996. *The True History of Chocolate.* Thames and Hudson, London, United Kingdom, 416p.

Cope, F. W. 1962. The mechanism of pollen incompatibility in *Theobroma cacao*L. *Heredity*. 17: 157-182.

Cornic, G., 2000. Drought stress inhibits photosynthesis by decreasing stomatal aperture–not by affecting ATP synthesis. *Trends in Plant Science 5 (5),* 187–188.

Counet, C., Ouwerx, C., Rosoux, D., and Collin, S. 2004. Relationship between procyanidin and flavor contents of cocoa liquors from different origins. *J. Agric. Food Chem.* 52: 6243-6249.

Cuatrecasas, J. 1964. Cocoa and its alleles - A taxonomic revision of the genus *Theobroma* contribution from the U. S. *Herbarium.* 35(6): 379-414.

Cummings, M.B. 1928. Pure lines studies with ten generations of hubbard squash. Vermont: Agricultural Experimental Station, 29p. (Bulletin, 280).

Damerval, C., De Vienne, D., Zivy, M., and Thiellement, H. 1986. Technical improvements in two-dimensional electrophoresis increase the level of genetic variation detected in wheat-seedling proteins. *Electrophoresis* 7(1): 52-54.

Darwin, C. R. 1876. *The Effects of Cross and Self-Fertilization in the Vegetable Crops*: Tropical Species. Springer Science, Business Media.

Davis, B.J., 1964. Disc electrophoresis-II method and application to human serum proteins. *Ann. New York Acad. Sci.* 121(1): 404-427.

DCCD [Directorate of Cashewnut and Cocoa Development]., 2017. Area, Production and Productivity of cocoa in India. [Online]. Available : http://dccd.gov.in /Content. Aspx?mid=1072&tid=1(01.08.2019).

De Lange L. A. and Vincent, A. A. 1988. Self and Cross incompatibility systems. In: *Plant Breeding*, Springrer, Dordrecht, pp. 203-212.

Deberdt, P., Mfegue, C. V., Tondje, P. R., Bon, M. C., Ducamp, M., Hurard, C., Begoude, B.A. D., Ndoumbe-Nkeng, M., Hebbar, P. K., and Cilas, C. 2008. Impact of environmental factors, chemical fungicides and biological control on cacao pod production dynamics and black pod disease (*Phytophthora megakarya*) in Cameroon. *Biol*. Control. 44:149-159.

Delpinalrivero, C. S and Acunagale, J. 1967. Floral physiology of cocoa. *Rev. Agric*. Cuba. 1(3): 82-111.

Dias, L. A. S. and Kageyama, P.Y. 1997. Temporal stability of multivariate genetic divergence in cacao (*Theobroma cacao* L.) in Southern Bahia conditions. *Euphytica*. 93:181-187.

Donovan, J. 2006. Diversification in international cacao markets: opportunities and challenges for smallholder cacao enterprises in Central America [online]. Available: http://ibcperu.org/doc/isis

Efombagan, M. I. B., Sounigo, O., Nyasse. S., Manzanares-Dauleux, M., and Eskes, A. B. 2009. Phenotypic variation of cacao (*Theobroma cacao* L.) on farms and in the gene bank in Cameroon. *J. Plant Breed. Crop Sci.,* 1(6): 258-264.

Efron, Y., Epaina, P., and Marfu. J. 2006. Guidelines for accelerated clone development (ACD). In: Eskes AB & Efron Y (Eds.) Global approaches to cocoa germplasm utilization and conservation. Final Report of the CFC/ICCO/IPGRI Project on Cocoa Germplasm Utilization and Conservation: A Global Approach 1998-2004. pp.87-89.

Elain Apshara, S., Bhat V. R., and Nair. R. V. 2008. Comparative studies on elite cocoa progenies in their initial years of growth. *J. Plant. Crops.* 36 (1): 38-44.

Elain Apshara, S., Bhat, V. R., Ananda, K. S., Nair R. V., and Suma. D. 2009. Evaluation and identification of high yielding trees in Nigerian cocoa germplasm. *J. Plant. Crops.* 37 (2): 111-116.

Engels, J. M. M. 1986. The systematic description of cacao clones and its significance for taxonomy and plant breeding. Ph.D. (Ag) thesis, Agricultural University of Wageningen, Ntherlands, 90p.

Enriquez, G.A. and Soria, J. 1966. Study of the availability of several characteristics of the cocoa beans (*Theobroma cacao* L.*). Fitotcnia Latino americana* 12(3): 99-118.

Falque, M., A. Vincent and Beaucamp. J. 1993. Relation between amount of pollen deposited on the styles and pod set in *Theobroma cacao* L. **In:** Proceedings of the 11th International Cocoa Research Conference, Book of Summaries. Yamoussoukro, Cote d Ivoire. Montpellier, France. pp.18-24.

Falque, M., A. Vincent and J. Beaucamp. 1992. Relation between amount of pollen deposited on the styles and pod set in *Theobroma cacao* L. p. 18-24. In: Proceedings of the 11th International Cocoa Research Conference, Book of Summaries. Yamoussoukro, Cote dIvoire.

FAOSTAT [Food and Agriculture Organization Corporate Statistical Database]. 2017. *Online Database* [on-line]. Available: http://faostat.fao.org/[15.5.2018].

Farquhar G.D. and Richards R. A. 1984. Isotopic Composition of Plant Carbon Correlates With Water-Use Efficiency of Wheat Genotypes. *Australian Journal of Plant Physiology* 11(6):539-552

Farrell O. R. H. 1975. High resolution two dimensional electrophoresis of proteins. *J. Biol. Chem.* 250 (10): 4007-21

Figueira, A. Lambert, S. Carpenter, D. Pires, J. L. Cascardo, J.C.M. Romanczyk, L. 1997.The similarity of cocoa flavour of fermented seeds from fingerprinted genotypes of *Theobroma cacao* L. from Brazil and Malaysia. *Trop Agric.* 74: 132 - 139.

Fowler, M. F. 1999. Cocoa beans: From tree to factory. In: Beckett, S. T., (ed.), Industrial Chocolate Manufacture and Use (3rd Ed.). Blackwell Science, Oxford, pp. 8-35.

Frost, D. J., Adhikari, K., and Lewis, D. S. 2011. Effect of barley flour on the physical and sensory characteristics of chocolate chip cookies. *J. Food Sci. Technol*. 22: 114-117.

Giorgianni, F., Desiderio, D.M., and Beranova-Giorgianni, S. 2003. Proteome analysis using isoelectric focusing in immobilized pH gradient gels followed by mass spectrometry. *Electrophoresis* 24(1-2): 253-259.

Goenaga, R., Irizarry, H., and Irish, B. 2009. TARS Series of Cacao Germplasm Selections. *Hortic. Sci.*44(3):826-827.

Gonzalez, R. V., Giron, C., Sanchez, P., Castillo, A., Movil, O., Parra D., and Vidal. R., 2011. Selection of new varieties on-farm and on-station in Venezuela. **In:** Final Report of the CFC/ICCO/Bioversity International Project on "Cocoa Productivity and Quality Improvement: a Participatory Approach" 2004-2010. P. 113-122.

Gorg, A., Weiss, W., and Dunn, M.J. 2004. Current two-dimensional electrophoresis technology for proteomics. *Proteomics* 4(12): 3665-3685.

Govindaraj. 2012. Studies on canopy management in cocoa (*Theobroma cacao* L.). M.Sc. (Hort.) thesis, Tamil Nadu Agricultural University, Coimbatore. 69p.

Gowers, S. 1989. Self-incompatibility interactions of *Brassica napus*. *Euphytica* 42: 99-103.

Gratz, N. G. and Arata, A. A. 1975. Problems associated with the control of rodents in Tropical Africa. *Bull. Wld. Hlth. Org*. 52: 697-706.

Gregory, Z. 1983. Genetic variability and correlation studies in cocoa (*Theobroma cacao* L.) M. Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur. 120p.

Guiltinan, M. J., Verica, J., Zhang, D., and Figuera, A. 2008. Genomics of Theobroma cacao, "The Food of Gods". In: Genomics of Tropical Crop Plants. Springer, New York, pp. 145-170.

Hasenstein, K. H. and Zavada. M. S. 2001. Auxin modification of the incompatibility response in *Theobroma cacao. Physiologia Plantarum.* 112(1):113-118.

Hawkes, J.G. 1983. Diversity of Crop Plants. Harvard University Press, Cambridge, Massachusetts. 189 pp.

Hewison, H. K. and Ababio. N. K. 1929. Flower and fruit production of *Theobroma cacao*. Gold coast. Dept. of Agri Year Book., 1929: 87-94.

Hiscox, J. D. and Israelstam, G. F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian J. Bot.* 57 (12): 1332-1334. *https://doi.org/10.1139/b79-163.*

Homey, C .1993. Genetic analysis of yield attributes in cocoa (*Theobroma cacao* L.). M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur. 59p.

Humphries, E. C. 1943. Studies in the physiology of *Theobroma cacao* with special reference to cherelle wilting. In**:** *AM. Rep. Cacao Res. Imp. Coil. Trap. Agric.* St. Agustine, Trinidad. P. 23-27.

Hurkman, W.J. and Tanaka, C.K. 1986. Solubilization of plant membrane proteins for analysis by two-dimensional gel electrophoresis. *Plant Physiol.* 81(3): 802-806.

ICCO [International Cocoa Organization]. 2016. Production of cocoa beans[online]. Available: http://www.icco.org/statistics/[05 July 2018].

INGENIC [International Group for Genetic Improvement of Cocoa] Workshop. 1994. Cocoa Breeding Strategies, Kaula Lumpore, Malaysia, 18th to 19th October, 1994, 204p.

Irish Brian and Goenaga Ricardo. 2012. Five-year agronomic and phenotypic data summary for a replicated cacao (*Theobroma cacao* L.) germplasm collection. Available[online] http://www.ars.usda.gov/research/publications/publications.html.

Islam, F.S., Gault, A.G., Boothman, C., Polya, D.A., Charnock, J.M., Chatterjee, D., and Lloyd, J.R. 2004. Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. *Nature* 430(6995): 68-71.

Iwaro, A. D. Bekele, F. L., and Butler, D. R. 2003. Evaluation and utilization of cacao (*Theobroma cacao* L.) germplasm at the International Cocoa Genebank, Trinidad. *Euphytica*. 130(2): 207-221.

Iwaro, A. D., Bekele, F. L. and Butler., D. R. 2003. Evaluation and utilisation of cacao (*Theobroma cacao* L.) germplasm at the International Cocoa Genebank, Trinidad. *Euphytica*, 130: 207-221.

Jackson, M. L. 1958. *Soil Chemical Analysis*. Prentice Hall of India, New Delhi, 478p.

Jacoba, V. J. and Atanda, O. A., 1971. Pod value studies of Amelonado and Amazon cacao. Turrialba. 23 (3): 347-351.

Jenman, G. S. and Harrison, J. B. 1897. Pests affecting cocoa plantations. In: *British Guyana Report on the Agricultural Work in the Botanical Gardens for the Years,* George Town, Denmarara, pp. 100-116.

Judd, W. S and Manchester, S. R. 1997. Circumscription of Malvaceae (Malvales) as determined by a preliminary cladistic analysis of morphological, anatomical, palynological, and chemical characters. *Brittonia*. 49: 384-405.

Kalloo, G. 1988. Vegetable breeding Vol. 1. CRC Press. New Delhi 239

Kanta, K. and Maheshwari, P. 1963. Test tube fertilization in some angiosperms. *Phytomorphology.* 13: 230-237.

Khader, P. K. A. 2005. A study on the prospects and problems of cocoa cultivators in Kerala with special reference to marketing. Ph.D thesis, University of Calicut, 82p.

Kim A. and Keeny, P. G. 1984.A. Epicatechin content in fermented and unfermented cocoa beans. *J. Food Sci*. 49 (1984), pp. 1090-1092

Knight, R. and Rogers, H. H. 1953. Sterility in *Theobroma cacao* L. *Nature* 172: 164-169.

Kochhar, S. L. 1986. *Tropical Crops:* A Textbook of Economic Botany (2nd Ed.). MacMillan Publishers Ltd., London, pp. 310-328.

Lachenaud, P. H and Oliver, G. 2005. Variability and selection for morphological bean traits in wild cocoa trees (*Theobroma cacao* L.) from French Guiana. *Genet. Resour. Crop Evol.* 52 (3): 225-231.

Lachenaud, P. H., Clement, D. Sallee, D., and Bastide, P. H. 1994. Le comportement en Guyane de cacaoyers selectionne senCote dIvoire.*Cafe Cacao The.*38**:** 91-102.

Laemmli, U.K. 1970. Denaturing (SDS) discontinuous gel electrophoresis. *Nature* 277: 680-685.

Lambert, S., Ismail, D., Hidayat, M., and Burhanuddin, M. 2009. Field-testing of local farmer's selections in sulawesi and results on their quality parameters. In**:** Proceedings of the International Workshop on Cocoa Breeding for Farmers' Needs, 15th- 17th October 2006, San Jose, Costa Rica. pp.123-130.

Lamin, K., Francis, A. Mohd. Jaafar, H., Shari Fuddin, S. Haya, R., Navies, M., and Nuraziawati, M. Y. 2011. Farmer participatory and collaborative approaches to cocoa breeding in Malaysia. In:Final Report of the CFC/ICCO/Biodiversity International Project on "Cocoa Productivity and Quality Improvement: a Participatory Approach 2004-2010. pp. 80-93.

Lewandowska M., Linder, V. B., and P. G. Jarvis. 1977. Changes in Chlorophyll and Carotenoid Content, Specific Leaf Area and Dry Weight Fraction in Sitka Spruce, in Response to Shading and Season. *The New Phytologist.* 79 (2): 247-256

Li, Y., Nie, Y., Zhang, Z., Ye, Z., Zou, X., Zhang, L., and Wang, Z. 2014. Comparative proteomic analysis of methyl jasmonate-induced defense responses in different rice cultivars. *Proteomics* 14(9): 1088-1101.

Loveless, M.D. and Hamrick, J.L. .1984. Ecological Determinants of Genetic Structure in Plant Populations. *Annual Review of Ecology and Systematics*, 15, 65-95.

Luiz, Antonio dos, Santos, Dias and Paulo, Yoshio Kageyama. 1997. Multivariate genetic divergence and hybrid performance of cocoa (*Theobroma cacao* L.) . Braz. J. Genet. 20. doi.org.10.1590/SO 100-84551997000100012.

Luna, F., Crouzillat, D., Cirou, L., and Bucheli, P. 2002. Chemical composition and flavor of Ecuadorian cocoa liquor. *J. Agric. Food Chem*. 50: 3527-3532.

Magnuson, K. and Otto, S. P. 2012. Linking the Investigations of Character Evolution and Species Diversification. the american naturalist. 180, (2): 226-244.

Maharaj, P., Maharaj, K., Persad, C., Ramnath, D., Jennings, K., and Sankar, R. 2011. Selection of new varieties on-farm and on-station in Trinidad and Tobago. In:Final Report of the CFC/ICCO/Bioversity International Project on "Cocoa Productivity and Quality Improvement: a Participatory Approach" 2004-2010. pp. 108-112.

Malik, C. P. and Singh, M. B. 1980. *Plants Enzymology and Histo-Enzymology*. Kalyani Publishers, New Delhi, 286p.

Mallika, V. K., Amma, P. S., Vikraman Nair, R., and Namboothiri, R. 2002. An overview of inbreeding in cocoa (*Theobroma cacao* L.) In: Ravi Bhat, Balasimha, D. and Jayasekhar, S. (eds.), *Extended summeries of the papers presented in the National Seminar on Technologies for enhancing productivity in cocoa,* 29-30th November 2002, Central Plantation Crops Research Institute, Regional Station, Vittal, Karnataka, pp. 50-51.

Marfu, J., Epaina, P. Butubu, J. and Efron, Y. 2011. On-farm and on-station trials implemented in Papua New Guinea. In:Final Report of the CFC/ICCO/Bioversity International Project on "Cocoa Productivity and Quality Improvement: a Participatory Approach" 2004-2010. pp. 94-101.

Marquez, A. M., Jaime Alfredo, M.C., and Roque, B. M. 2017. A comparison of tissue preparation methods for protein extraction of cocoa (*Theobroma cacao* L.) pod. *Genomics, Molecular Genetics and Biotechnology*. 66 (2): 248-253.

Martin, F. W. 1959. Staining and observing pollen tubes in the style by means of fluorescence. *Stain Technol*. 34: 125-128.

Martinson, V.A. 1966. Hybridization of cacao and *Theobroma grandiflora*. *J. Heredity*. 57: 134-136.

Matos, G., Blaha, G., Rodriguez, F., Cabrera, M., Marquez, J., Martinez, F., Lotode, R., and Cilas, C. 1998. Losses due to *Phytophthora palmivora* and other agents on cocoa plantations in Baracoa. *Café Cacao*. 1**:**7-11.

Matsubara, S. 1981. Overcoming the self-incompatibility of Raphanus sativus by application of amino acids, vitamins and phytohormones. Japan. Soc. Hort. Sci. 54:46-57

Minimol, J. S. Amma, S. P., Krishnan, S., and Vasudevan, N. R. 2011. Influence of fruit apex on fruit shape in selected accessions of cocoa. In: *Proceedings of Seminar on Strategies for Enhancing Productivity of Cocoa,* 28-29 January 2011, Central Plantation Crops Research Institute, Regional Station, Vittal, Karnataka, pp. 23-25.

Minimol, J. S., Prasannakumari Amma, Suma, B. and Shahanas, E. 2015. Breeding cycle of fifth generation inbred of cocoa and performance analysis of progenies over generations. *Indian J. of Hortic.*72 (4): 566-570.

Minimol, J. S., Shija, T. K., Vasanthan, N., Sunil, K. M., Suma, B., and Krishnan, S. 2015. Seasonality in cocoa: weather influence on pod characters of cocoa clones. *Int. J. Plant Sci.* 10: 102-107.

Minimol, J. S., Suma, B., and Mathew, M. 2015b. Importance of polyclonal garden in production of superior planting material in cocoa. *Cashew Cocoa J.* 16: 19-21.

Minimol, J. S., Suma, B., Ummer, M., and Chithira, P. G. 2015a. Genetic improvement of cocoa by developing superior hybrids. *J. Trop. Agric*. 53: 157-165.

Mirinda, F. 1962. Wild cacao in the Lacandona forest, Chiapas, Mexico. Cacao. 7: 7-13.

Monteiro, W. R., U. V. Lopez and D. Clement. 1988. Genetic Improvement in Cocoa.

Monteiro, W. R., Vanderlei Lopes, U., Pires, J. L., Newman Luz, E. D. M., Midlej Silva, S. D. V., Silva Reis, D., and Silva de Araujo., D. C. 2011. On-farm and on-station

selection of new cocoa varieties in the State of Bahia, Brazil. In:Final Report of the CFC/ICCO/Bioversity International Project on “Cocoa Productivity and Quality Improvement: a Participatory Approach” 2004-2010. pp. 8-30.

Mooledhar, V. 1995. “Fine” flavor cocoa – a Trinidadian and Tobagonian tradition. Cocoa Research Unit, Newsletter No. 2, Trinidad and Tobago, 6p.

Morera, D. C., Yadav, V. B. and Patil, H. S. 1991. Heterosis and inbreeding depression for yield and yield components in okra. Indian J. Agric. Res.31 (3): 141-148.

Mossu, G. 1992. *Cocoa*. The Macmillan Press Ltd. London, 103p.

Motamayor, J. C., Risterucci, A. M., Heath M., and Lanaud. C. 2002. Cacao domestication II: progenitor germplasm of the Trinitario cacao cultivar. *Heredity*. 91: 322-330.

Motamayor, J. C., Risterucci, A. M., Lopez, P. A., Ortiz, C. F., Moreno A., and Lanaud, C. 2008. Cacao domestication I: the origin of the cacao cultivated by the Mayas. *Heredity***.** 89**:** 380-386.

Nageswara Rao, R.C., H.S. Talwar and G.C. Wright, 2001. Rapid assessment of specific leaf area and leaf nitrogen in peanut (*Arachis hypogaea* L.) using a chlorophyll meter. J. Agron. Crop Sci., 189: 175-182.

Nair, P. K. P. 2010. The agronomy and economy of important tree crops of the developing world. Sci. Direct: 131-180.

Neville Jr, D.M. 1974. Molecular weight determination of protein-dodecyl-sulfate complexes by gel electrophoresis in a discontinuous buffer system. *J. Biol. Chem*. 246: 6328-6334.

NurgulErcan and Ranakurum. 2003. Plant, flower, fruit and seed characteristics of five generation inbred summer squash lines (*Cucurbitapepo* L.) Pak. J. Bot., 35(2): 237-241,

O'Farrell, P.H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 250 (10) : 4007-4021.

Okazaki, K. and Hinata, K., 1987. Repressing the expression of self incompatibility in crucifers by short term high temperature. *Theor. Appl. Genet*. 73: 496-500

Omolaja, S. S. P., Aikpokpodion, S., Adedeji and Vwioko, D. E., 2009. Rainfall and temperature effects on flowering and pollen productions in cocoa. *African Crop Sci. J.* 17 (1) : 41-48.

Opoku, S. Y., M. A. Dadzie, F. K. Padi, I. Y. Opoku, Y. Adu-Ampomah and B. Adomako. 2011. Selection of new varieties on-farm and on-station in Ghana**.** In: *Final Report of the CFC/ICCO/Bioversity. International Project on "Cocoa Productivity and Quality Improvement: a Participatory Approach" 2004-2010*. pp.73-79.

Ornstein, L. 1964. Disc electrophoresis-i background and theory. *An. New York Acad. Sci.* 121(1): 321-349.

Oviedo, N.J., Nicolas, C. L., Adams, D. S. and Levin, M. 2008. Live imaging of planarian membrane potential using DiBAC4. *Int. J. of Dev. Biol.* 56: 193-196

p: 589-626. In: Jain, S.M. and D. Priyadarshan (Eds.), Breeding Plantation Tree

Pessarkli, M.1999. Hand book of plant and crop stress. Marcel Dekker Inc. 697 p.

Peter J. M, Agung W. S., Ayu K. P. , Susanna, R. B., Nurlailae, Sahardi, M., Ashar, S., Agus, P., Hussin bin P., Smilja, L., David, I. G., Philip J. K. 2018. Testing local cacao selections in Sulawesi for resistance to vascular streak dieback. *Crop Protection*. 109: 24 - 32.

Piper, C. S. 1966. *Soil and Plant Analysis.* Hans Publishers, Bombay, 368p.

Pirovani, C.P., Carvalho, H.A., Machado, R.C., Gomes, D.S., Alvim, F. C., Pomella, A. W. 2008. Protein extraction for proteome analysis from cacao leaves and

meristems, organs infected by *Moniliophthora perniciosa*, the causal agent of the witches' broom disease. *Electrophoresis*.29: 391–401.

Pound, E. J .1932. The genetic constitution of the cacao crop. In::*First Annual Report of Cacao Research, Imperial College of Tropical Agriculture*, Trinidad and Tobago. pp. 25-28.

Pound, E. J. 1933. Criteria and method of selection in cacao. In: Annual Report of Cacao Research, Imperial College of Tropical Agriculture, Trinidad. pp.27-29.

Prabha, K. P. and Chandramohanan, R. 2011. Occurrence and distribution of cocoa (*Theobroma cacao* L.) diseases in India. *J. Res. Agron.* 39(4): 44-50.

Purseglove, J. W. 1974. *Tropical Crops, Dicotyledons*. John Wiley and Sons, New York, 634p.

Rajamony, L. and Mohankumaran. N. 1995. Studies on the floral biology of cocoa. *Planter*. 71: 119-127.

Ramakrishnan, K. and Thankappan, M. 1965. First report of black pod disease of India. *South Indian Hortic.* 13: 33-34.

Reynolds, J.A. and Tanford, C. 1970. Binding of dodecyl sulfate to proteins at high binding ratios. Possible implications for the state of proteins in biological membranes. *Proc. Natl. Acad. Sci.* 66 (3) : 1002-1007.

Ritchie,S.W.,Nguyan ,H.T., Holaday. A.S.1990. Leaf Water content and gas exchange parameters of two wheat genotypes differing in drought resistance. *Crop Sci.*30:105-111.

Robinson, R.W. 1999. Rationale and methods for producing hybrid cucurbit seed. Journal of New Seeds. 1:.1-47.

Rosemary Francies, 1998. Genetic analysis of certain clones, hybrids and inbreds of cocoa. Ph.D. (Ag) thesis, Kerala Agricultural University, Thrissur, 182p.

Rubino, D.B. wehner, T. C. 1986. Effect of inbreeding on horticultural performance of lines developed from an open-pollinated cucumber population. *Euphytica*, 35 :459-464.

Sadasivam, S. and Manickam. A. 2008. *Biochemical Methods* (3rd Ed.). New Age International Publishers, New Delhi, India. 120p.

Sailaja, D., Srilakshmi, P., Puneeth, K., and Krishna, C. R. 2015. Estimation of protein content and phytochemical studies in cocoa fruit outer covering. *Int. J. Plant Ani. Environ. Sci.* 5 (1): 111-115.

Saravanan R. S. and Rose J. K.2004. A critical evaluation of sample extraction techniques for enhanced proteomic analysis of recalcitrant plant tissues. *Proteomics*. 4 (9) : 2522-2532

Satheesan, K. V. and Ramadasan, A. 1992. Growth and productivity of turmeric grown as pure and as an intercrop in coconut garden. National seminar on Ginger and Turmeric. Central Plantation Crops Research Institute. Calicut. India. Pp. 69-75

Scopes, R. K. 1994. Separation by precipitation. In: *Protein purification.* Springer, New York, NY, pp. 71-101.

Selles, M. S., Luque, I., Casado, V.J., Martinez, E. M. J., and Bru-Martinez, R. 2008. Proteomics of multigenic families from species underrepresented in databases: the case of loquat (*Eriobotrya japonica* Lindl) Polyphenol oxidases. *J. Proteome Res.* 7: 4095 – 4106.

Shagger, M. G. 1987. Tricine sodium dodecyl sulfate poliacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem*. 166: 378-379.

Shapiro, A.L. and Maizel, J.V. 1969. Molecular weight estimation of polypeptides by SDS-polyacrylamide gel electrophoresis: further data concerning resolving power and general considerations. *Anal. Biochem.* 29(3): 505-514.

Shaw, M.M. and Riederer, B.M. 2003. Sample preparation for two-dimensional gel electrophoresis. *Proteomics* 3(8): 1408-1417.

Shen-Orr, S.S., Milo, R., Mangan, S., and Alon, U. 2002. Network motifs in the transcriptional regulation network of *Escherichia coli*. *Nat. Genet.* 31(1): 64-68.

Singh, N., Jain, N., Ramkumar, Jain, A., Singh, N. K., and Rai V. 2015. A comparative method for protein extraction and 2-D gel electrophoresis from different tissues of *Cajanus cajan. Frontiers in Plant Sci.* 6: 1-7.

Smith, N. 1999. *The Amazon River Forest: ANatural History of Plants, Animals, and People*. Oxford University Press, New York, 208p.

Sniezko, R. & T., Visser, 1987. Embryo development and fruit-set in pear induced by untreated and irradiated pollen. Euphytica 36: 287–294.

Taylor, A. J. and Roberts, D. D. 2004. *Flavour Perception.* Blackwell Publishing Limited, Oxford, U. K. 283p.

Taylor, K. D. 1972. Rodentproblems in tropical agriculture. Pest Articles News Summaries. 18: 81-88.

Thondaiman, V., Rajamani, K., Senthil, N., Shoba, N., and Joel, A. J. 2013. Variability studies and identification of high yielding plus trees of cocoa (*Theobroma cacao* L.) in Tamil Nadu. *African J. of Agric. Res.* 8 (26): 3444-3453.

Toxopeus, H. and Jacob, V. J. 1968. Studies on pod and bean values of *Theobroma cacao* L. in Nigeria – Number of beans per pod with special reference to the natural pollination process. *J. Agric. Sci.* 18: 188-194.

Umadevi, P. and Anandaraj, M. 2015. An efficient protein extraction method for proteomic analysis of black pepper (*Piper nigrum* L.) and generation of protein map using nano LC-LTQ Orbitrap mass spectrometry. *Plant Omics* 8(6): 500-512.

Valle, R.R., De Almeida,A. F. and Deo. Leite, R. M. 1990. Energy costs of flowering, fruiting and cherelle wilt in cacao. *Tree Physiol.*6:329-336.

Veeresh, S. A. 2018. Genetic stock development for Phytophthora pod rot disease resistance in cocoa (*Theobroma cacao* L.). M.Sc. (Agri.) thesis. Kerala Agricultural University,85p.

Voelcker, O. J. 1938. A note on the behavior of cacao flowers after pollination and on the incidence of cherelle wilt. p.**7:** 5-8. In. Ann. Rep. Cacao Res. Imp. Coil. Trop Agric. St. Agustine, Trinidad. cacao: etude de la granulometric. *Cafe, Cacao, The,*33 (1)**:** 3-8.

Voight, J. 2013. *Chocolate and Cocoa Aroma*. In : Watson, R., Preedy, V. R., and Zibadi, S. (Eds.), Chocolate in health and nutrition. Humana Press, New York, pp. 89-101.

Weatherley, P. E. l951. Studies in the water relations of the cotton plant. II. Diurnal and seasonal variations in relative turgidity and environmental factors. New Phytol. 50: 36-51.

Wang, C.Y., Ru, C.Q., and Mioduchowski, A. 2003. Axially compressed buckling of pressured multiwall carbon nanotubes. *Int. J. Solids Structures* 40(15): 3893-3911.

Wasinger, V.C., Cordwell, S.J., Cerpa-Poljak, A., Yan, J.X., Gooley, A.A., Wilkins, M.R., Duncan, M.W., Harris, R., Williams, K.L., and Humphery-Smith, I. 1995. Progress with gene-product mapping of the Mollicutes: *Mycoplasma genitalium*. *Electrophoresis* 16 (1): 1090-1094.

Weatherley, P. E. 1950. Studies in the water relations of the cotton plant. I. The field measurement of water deficits in leaves. *New Phytol*. 49: 81-97.

Weatherley, P. E. 1962. In "The Water Relations of Plants". (Symp. Brit. Ecol. Soc.) (in press) *Kingdom.* John Murray, London, 482p

Weatherley, P.E. and Barrs, H.D. 1962. A Re-examination of the relative turgidity techniques for estimating water deficits in leaves. *Aus. J. Biol. Sci.* 15: 413-428.

Weber, G., Shinitzky, M., Dianoux, A.C., and Gitler, C. 1971. Micro viscosity and order in the hydrocarbon region of micelles and membranes determined with fluorescent probes. I. Synthetic micelles. *Biochemistry* 10(11): 2106-2113.

Whitaker, T.W. Robinson, R.W. 1986. Squash breeding. In. Basset, M.J. (Ed.) Breeding vegetable crops. Westport: Avi Publishing Company,. cap.6, p.209-242.

Williams, J. M. 1973. Rat damage assessment and control in cocoa. *Fiji Agric. J.* 35: 15-25.

Wilson, A. W. 1991. Effects of gasteroid fruiting body morphology on diversification rates in three inde-pendent clades of fungi estimated using binary state speciation and extinction analysis. Evolution 65:1305–1322

Wood, G. A. R. and Lass, R. A.1985. *Cocoa*, 4thEdn. Longman Scientific and Technical, New York, USA.

Wright, G. C., Hubick, K.T., Farquhar, G. D. and Nageswara Rao, R. C. 1994. Genetic and environmental variation in transpiration efficiency and its correlation with carbon isotope discrimination and specific leaf area in peanut. In: stable isotope and carbon-water relations, Ehleringer, J.R., A.E. Hall and G.D. Farquhar (Eds.). Academic Press Inc., New York, ISBN: 0-12-233380-2, pp: 247-267.

Xie, Y. Kerscher, O. Kroetz, M. B. McConchie, H. F., Sung, P. Hochstrasser, M. 2007. The yeast Hex3.Slx8 heterodimer is aubiquitin ligase stimulated by substrate sumoylation, *J. Biol Chem.* 282(47): 3417-84

Yan, J.X., Wait, R., Berkelman, T., Harry, R.A., Westbrook, J.A., Wheeler, C.H., and Dunn, M.J. 2000. A modified silver staining protocol for visualization of proteins compatible with matrix-assisted laser desorption/ionization and electrospray ionization-mass spectrometry. *Electrophoresis* 21(17): 3666-3672.

Young, A. and Smith A. B. 1997. Carotenoids in higher plant photosynthesis. In: Pessarakli, M. (Ed.), Handbook of Photosynthesis. Marcel Dekker, New York, pp. 575–596.

Zhang, D., and Lespinasse 1991. Genetic diversity and structure of managed and seminatural populations of cacao (*Theobroma cacao* L.) in the Huallaga and Ucayalivalleys of Peru. Ann. Bot., 98: 647-655.

Zhang, Z.G., Zhang, J.Y., Zheng, X.B., Yang, Y.W., and Ko, W.H. 2004. Molecular distinctions between *Phytophthora capsici* and *P. tropicalis* based on ITS sequences of ribosomal DNA. *J. Phytopathol.* 152: 358-364.

Zhao, P., Liu, F., Zhang, B., Liu., X., Wanga, B., and Gong, J. 2013. MAIGO2 is involved in abscisic acid-mediated response to abiotic stresses and Golgi-to-ER retrograde transport. *Physiol Plant* 148 (2) : 246-60.

Annexures

ANNEXURE I

Cocoa inbreds evaluated

S. No.	Plant No.	Genotype	Generation	Stand No.
1	1.1	G VI 295.4	S_2	1.7
2	1.2	G VI 295.4	S_2	1.9
3	1.3	G VI 295.4	S_2	2.6
4	1.4	G VI 295.4	S_2	2.13
5	1.5	G VI 295.4	S_2	2.16
6	1.6	G VI 295.4	S_2	2.18
7	1.7	G VI 295.4	S_2	3.18
8	1.8	G VI 295.4	S_2	3.19
9	1.9	G VI 295.4	S_2	4.11
10	1.10	G VI 295.4	S_2	4.12
11	1.11	G VI 295.4	S_2	4.20
12	2.1	M 18.7	S_3	29.3
13	2.2	M 18.7	S_3	29.6
14	2.3	M 18.7	S_3	29.8

15	2.4	M 18.7	S_3	29.12
16	2.5	M 18.7	S_3	29.14
17	3.1	M 18.7	S_4	5.10
18	3.2	M 18.7	S_4	5.12
19	3.3	M 18.7	S_4	5.13
20	3.4	M 18.7	S_4	5.14
21	4.1	H 7.3 (86)	S_1	12.3
22	4.2	H 7.3 (86)	S_1	12.4
23	4.3	H 7.3 (86)	S_1	12.5
24	4.4	H 7.3 (86)	S_1	12.6
25	4.5	H 7.3 (86)	S_1	12.7
26	4.6	H 7.3 (86)	S_1	12.8
27	4.7	H 7.3 (86)	S_1	12.9
28	4.8	H 7.3 (86)	S_1	12.10
29	4.9	H 7.3 (86)	S_1	12.11
30	4.10	H 7.3 (86)	S_1	12.12
31	4.11	H 7.3 (86)	S_1	12.13

32	4.12	H 7.3 (86)	S_1	12.14
33	4.13	H 7.3 (86)	S_1	12.15
34	4.14	H 7.3 (86)	S_1	12.16
35	4.15	H 7.3 (86)	S_1	12.17
36	4.16	H 7.3 (86)	S_1	12.18
37	4.17	H 7.3 (86)	S_1	12.19
38	4.18	H 7.3 (86)	S_1	12.20
39	5.1	H 7.3 (86)	S_2	25.7
40	5.2	H 7.3 (86)	S_2	25.8
41	5.3	H 7.3 (86)	S_2	25.10
42	6.1	H1 1.2 (86)	S_1	16.19
43	6.2	H1 1.2 (86)	S_1	16.20
44	6.3	H1 1.2 (86)	S_1	17.9
45	6.4	H1 1.2 (86)	S_1	17.11
46	7.1	H1 1.2 (86)	S_2	3.15
47	8.1	GII 7.4	S_3	20.2
48	9.1	GII 7.4	S_4	25.15

49	10.1	GII 7.4	S_5	1.15
50	11.1	G IV 35.7	S_4	6.11
51	12.1	G VI 135	S_1	23.5
52	12.2	G VI 135	S_1	23.6
53	12.3	G VI 135	S_1	23.9
54	12.4	G VI 135	S_1	23.14
55	12.5	G VI 135	S_1	23.15
56	12.6	G VI 135	S_1	23.18
57	12.7	G VI 135	S_1	23.19
58	12.8	G VI 135	S_1	23.20
59	13.1	G VI 135	S_2	2.9
60	14.1	G VI 141	S_1	18.11
61	14.2	G VI 141	S_1	18.18
62	15.1	G VI 141	S_2	1.1
63	15.2	G VI 141	S_2	1.2
64	15.3	G VI 141	S_2	1.5
65	15.4	G VI 141	S_2	1.10

66	15.5	G VI 141	S_2	1.11
67	15.6	G VI 141	S_2	1.12
68	15.7	G VI 141	S_2	1.13
69	15.8	G VI 141	S_2	1.18
70	15.9	G VI 141	S_2	1.19
71	16.1	P II 13.12	S_1	39.3
72	16.2	P II 13.12	S_1	39.4
73	16.3	P II 13.12	S_1	39.7
74	17.1	PII 13.12	S_2	8.11
75	17.2	PII 13.12	S_2	8.12
76	17.3	PII 13.12	S_2	8.13
77	17.4	PII 13.12	S_2	8.14
78	17.5	PII 13.12	S_2	8.16
79	17.6	PII 13.12	S_2	8.18
80	17.7	PII 13.12	S_2	8.19
81	17.8	PII 13.12	S_2	8.20
82	18.1	G VI 256.5	S_1	31.5

83	18.2	G VI 256.5	S_1	31.7
84	18.3	G VI 256.5	S_1	31.12
85	18.4	G VI 256.5	S_1	31.16
86	18.5	G VI 256.5	S_1	31.17
87	18.6	G VI 256.5	S_1	31.18
88	19.1	P II 4.8	S_1	39.8
89	19.2	P II 4.8	S_1	39.9
90	19.3	P II 4.8	S_1	39.10
91	19.4	P II 4.8	S_1	39.11
92	20.1	P II 4.8	S_2	6.19
93	20.2	P II 4.8	S_2	6.20
94	20.3	P II 4.8	S_2	7.1
95	20.4	P II 4.8	S_2	7.2
96	20.5	P II 4.8	S_2	7.3
97	20.6	P II 4.8	S_2	7.4
98	20.7	P II 4.8	S_2	7.5
99	21.1	P II 13.8	S_1	34.5

100	21.2	P II 13.8	S_1	34.7
101	21.3	P II 13.8	S_1	34.9
102	22.1	P II 13.8	S_2	4.13
103	22.2	P II 13.8	S_2	4.16
104	22.3	P II 13.8	S_2	4.18
105	22.4	P II 13.8	S_2	5.17
106	22.5	P II 13.8	S_2	8.10
107	23.1	P II 12.9	S_1	41.4
108	23.2	P II 12.9	S_1	41.5
109	23.3	P II 12.9	S_1	41.6
110	23.4	P II 12.9	S_1	41.8
111	23.5	P II 12.9	S_1	48.6
112	24.1	P II 12.9	S_2	7.15
113	24.2	P II 12.9	S_2	7.20

Abstract

GENETIC ANALYSIS OF INBREDS, INBRED CROSSES AND HYBRIDS OF COCOA (*Theobroma cacao* L.)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

The genetic analysis of inbreds, inbred crosses and hybrid was carried out at College of Horticulture and Cocoa Research Centre (CRC), Vellanikkara, Thrissur. A total of 113 inbreds was evaluated for qualitative and quantitative characters. Cocoa is predominantly out breeding with highly complex genetic structure. The cross pollinating nature coupled with existence of self/cross incompatibility, poses much difficulty to the cocoa breeders. The hybrid progeny from the same cross exhibit high level of variability due to heterogeneous nature of the parents. This can be overcome to certain extent by using fully homozygous inbreds of diverse genotypes. The CRC succeeded in producing first ever fifth generation inbred and it was proved self incompatible. In this context, the present study was formulated to quantify the magnitude of inbreeding depression in yield and yield attributes in various self-generations and to establish a physiological relationship between the vigour of inbreds, inbred crosses and hybrids in the early stages of plant growth. The different pollination techniques were tested to overcome the self incompatibility and the extraction of proteins from self incompatible plants was attempted.

Morphological characterization of 113 inbreds were carried out by recording qualitative characters such as pod shape, ridge colour, pod apex, pod basal constriction, pod rugosity and bean colour. High variability was observed for all qualitative characters except ridge colour. The pod and bean characterization for 21 characters expressed wide variation among the inbreds and within the inbreds of same genotype. Characterization of inbreds based on the biochemical parameters such as fat and phenol expressed wide variability.

The inbreeding depression was estimated for 21 characters in 12 genotypes over generations. In S_1 generation of M 18.7, the husk furrow thickness and number of flat beans expressed negative inbreeding depression, whereas the average yield per tree per year has shown a very meager inbreeding depression over the preceding generation. In S_2 generation of M 18.7, pod value, conversion index and dry matter recovery have shown an inbreeding depression between 5 to 10 percent. Negative inbreeding depression was observed for husk ridge thickness, number of flat bean per pod, pod index, efficiency index and fat content. In S_3 generation of M 18.7, maximum inbreeding depression was observed for wet bean weight per pod followed by pod length. In S_4 generation of M 18.7, the highest inbreeding depression of 43.58 per cent was observed for number of pods/tree/year. In S_1 generation of G II 7.4, positive inbreeding depression was observed for 10 characters out of 21. In S_2 generation of G II 7.4, positive inbreeding depression was observed for 15 characters. Only seven characters expressed positive inbreeding depression in S_3 generation of G II 7.4 genotype. In S_5 generation inbred, the maximum inbreeding depression (63.95%) was observed for husk furrow thickness, followed by husk ridge thickness (49.02%), In S_1 and S_2 generation of H1 1.2, positive inbreeding was observed in 16 characters.

In general, inbreeding depression was less for economic characters confirming that most of the characters are controlled by additive gene action and lethal gene canceled in heterozygous condition is less.

In the comparative evaluation of inbreds, inbred crosses and hybrids, the inbreds were found superior over inbred crosses and hybrids for morphological characters such as plant height, collar girth, plant spread and leaf area. The inbreds also had maximum chlorophyll content, leaf nutrient status and relative water content. The superiority of inbreds was mainly attributed to the growing environmental condition with more of openness in the inbred plantation, which was confirmed with spherical densiometer, an instrument for measuring plantation overstory density.

A total of nine techniques to overcome self incompatibility barriers such as bud pollination, surgical technique, intra-ovarian technique, salt spray (1% and 3%), high humidity, high temperature, Naphthalene Acetic Acid (100 and 200 ppm), gamma irradiation and flower organ extract were attempted from September 2016 to March 2017 and September 2017 to March 2018. In all the methods, the fruit set was not obtained except in surgical technique. The majority of self incompatible S_3 and S_5 inbreds set in to pods through surgical techniques. In the fluorescent microscopic study, the clear disintegration of pollen tube was observed in self incompatible flower in the style.

Flowers from fifth generation cocoa inbreds were used for extraction of protein. All the available protein extraction methods were tried and the protein was quantified. In all the methods, the protein content was below 5µg per ml in the cocoa flower. The protein from cocoa leaf was extracted and quantified. In lysis buffer extraction with some modification, a protein content of 7 µg per ml was obtained. Faint bands were observed in SDS PAGE. The flower and leaf protein were subjected for LC Q ToF. The protein content obtained from the flower was insufficient to run LC Q ToF. A total of 87 proteins were found in cocoa leaf sample, of which, 85 proteins were similar to cocoa and two proteins were similar to forest coconut.

From the above, it can be concluded that the genotypes in S_5/S_6 can be crossed to get highly heterotic hybrids. For extraction of protein from cocoa flowers, fine tuning of the available methods under ideal laboratory conditions must be employed. Molecular basis of self-incompatibility has to be studied in detail.