GENERAL COMBINING ABILITY OF SELECTED BLACK POD DISEASE RESISTANT COCOA(*Theobroma cacao* L.) HYBRIDS

By

VARSHA BABU.

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THESIS

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DEPARTMENT OF PLANTATION CROPS AND SPICES

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VELLANIKKARA, THRISSUR - 680656

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DECLARATION

I hereby declare that this thesis entitled **"General combining ability of selected black pod disease resistant cocoa(*Theobroma cacao* L.) hybrids"** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara

Date: 29|11|16

Varsha Babu.

2014-12-101

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Vellanikkara

Date: 29|11|16

Dr. B. Suma

Chairman, Advisory Committee

Professor

Dept. of Plantation Crops and Spices

College of Horticulture,

Vellanikkara

CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Varsha Babu (2014-12-101)**, a candidate for the degree of **Master of Science in Horticulture** with major field in **Plantation Crops and Spices,** agree that this thesis entitled **"General combining ability of selected black pod disease resistant cocoa(*Theobroma cacao* L.) hybrids"** may be submitted by **Ms. Varsha Babu,** in partial fulfilment of the requirement for the degree.
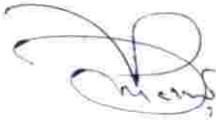
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(Chairman, Advisory Committee)

Professor

Dept. of Plantation Crops and Spices
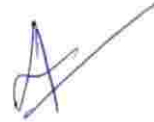
College of Horticulture, Vellanikkara

Dr. V. S. Sujatha

(Member, Advisory Committee)

Professor and Head

Dept. of Plantation Crops and Spices

College of Horticulture, Vellanikkara

Dr. Minimol J. S.

(Member, Advisory Committee)

Assistant Professor

Plant Breeding and Genetics

Cocoa Research Centre, Vellanikkara

Dr. Sainamol P. Kurian

(Member, Advisory Committee)

Associate professor

Dept. of plant pathology

AICVIP, College of Horticulture, Vellanikkara

Dr. S. Prasannakumari Amma

(External Examiner)

Professor (Retd.)

College of Horticulture, Vellanikkara

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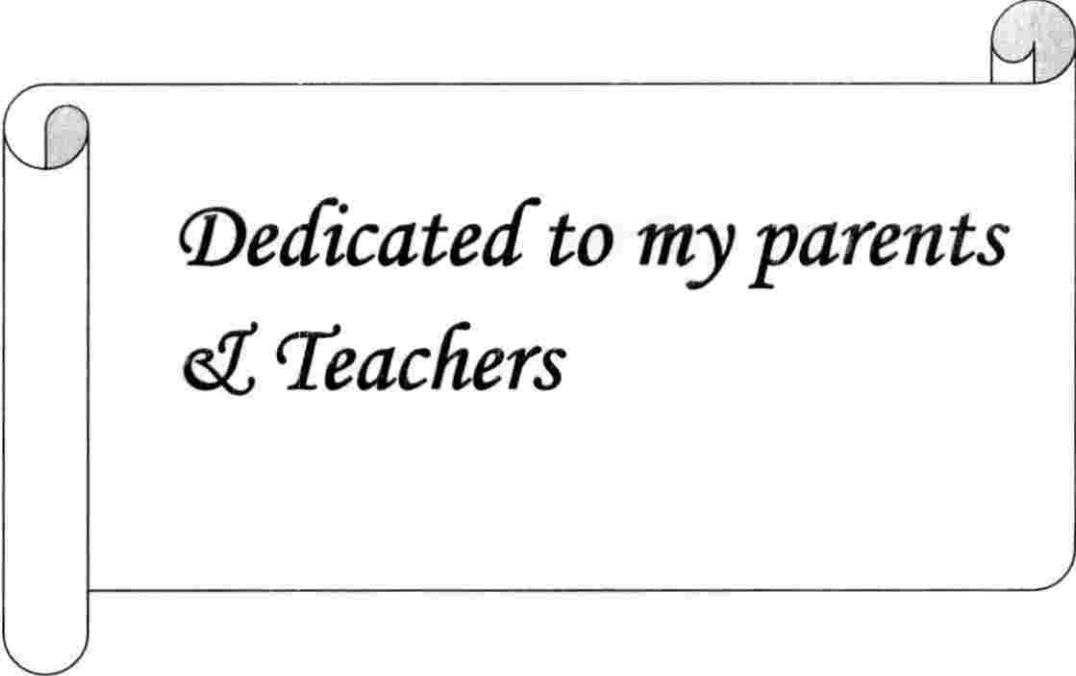
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Finally, I take this opportunity to express the profound gratitude from my deep heart to my beloved parents and my lovable brother for their prayers, moral support, constant encouragement, love and warm blessings.

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Varsha Babu.

Dedicated to my parents & Teachers

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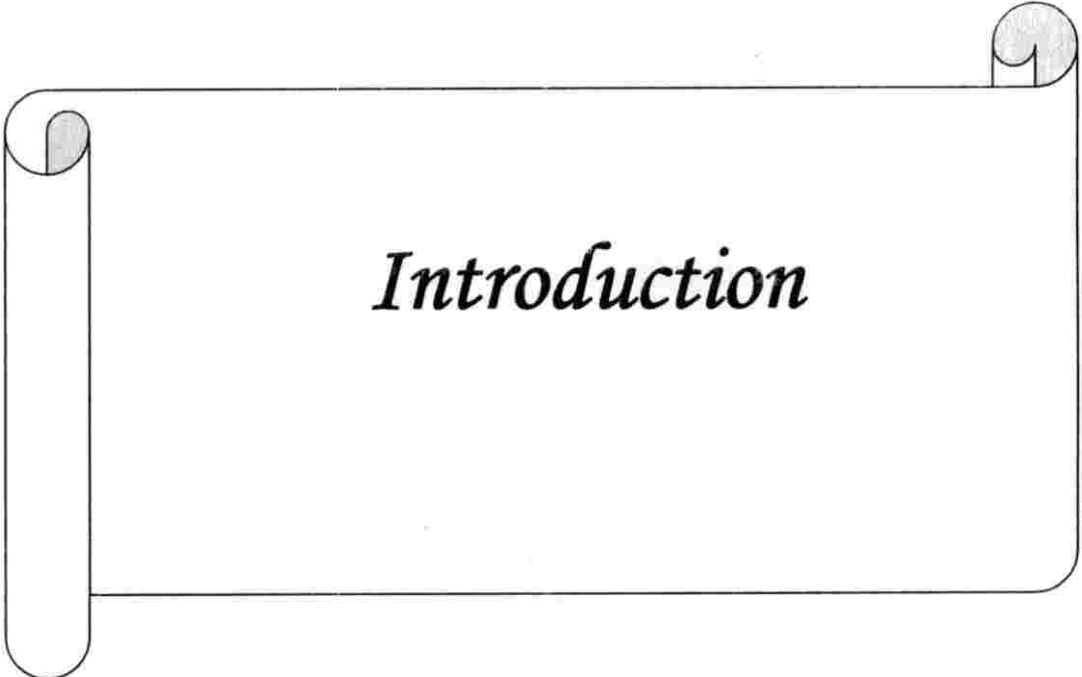
Introduction

1. INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a major beverage crop now gaining importance due to its high remunerative price. The genus is indigenous to the new world and the centre of origin is considered to be the Amazon river basin of South America. It was early classified under family Sterculiaceae and now being re classified in the expanded family of Malvaceae (Alverson *et al.*, 1999). Because of its high economic importance and perennial nature, it is grown by more than two million farmers in more than 50 countries. It is commercially cultivated for its nibs which are used in the production of an array of products like chocolate and cocoa butter (Amma *et al.*, 2011).

The term 'Cocoa' is believed to have been derived from 'Cacahoatl'. It is used by Aztec Indians of high Mexican plateau. According to them it was brought to earth by the God QuetZacoatl whom they called as Xocohatl. Hence it is popularly known as *'food of god'* and is believed to have a divine origin. So with this legend in mind, using the Greek words *'theos'* meaning God and *'broma'* meaning food, Linnaeus gave the name *Theobroma cacao* to the cultivated cocoa plant.

The crop was introduced to India during 1798 (Ratnam, 1961) but its commercial cultivation started only during 1970's (Nair *et al.*, 2002). In India, cocoa is widely cultivated as an inter crop in coconut and arecanut gardens of Kerala, Karnataka and Tamil nadu. Recently in India there has been a massive increase in area under this crop. And now the cultivation had spread even to the non-traditional areas like Andhra Pradesh. But the productivity of cocoa is very low due to various constraints. It includes both biotic and abiotic factors. Among the biotic factors, *Phytophthora* pod rot (PPR) or Black pod is an important fungal disease of cocoa caused by *Phytophthora palmivora*(Butl.). It causes a heavy crop loss of 40 to 60 per cent at global level (ICCO, 2013).The disease is very serious during rainy period. The predominant symptom on pod is the brownish or black lesion on the husk, which finally leads to blackening and rotting of the pods.

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Initially there occurs a minute translucent water soaked spots on the surface of pod which gradually turns to chocolate brown, darkens and increases in size. Finally the whole pod is invaded by the fungus and the pods become completely black. In case of ripe pod, beans escape partially or wholly from the infection as it gets separated from the pod husk on ripening stage. The yield loss was largely due to the black pod which accounts for 64.1% of total yield loss (Adomako, 2007).

The pathogen also causes cankers on stem which appears in different part of the tree including jorquette and fan branches. The symptoms appear as greyish brown water soaked lesions with broad dark brown to black margins on the bark. From the infected region, reddish brown liquid oozes out which dries and forms a rusty deposit on it. Beneath the tissue occurs characteristic reddish brown discolouration. These lesions coalesce which finally leads to extensive rotting. It spreads and leads to wood infection which appears as grayish brown to black discolouration with black streaks. Die- back occurs when canker girdles the stem. The leaves get wilted, turn to yellow and fall off. The pods also show wilting and finally the whole tree dies. On the internal bark, the spread of infection is faster than in the surface of bark (Chandramohanan, 1978).

In the nursery stage, the pathogen causes seedling blight (Chandramohanan et al., 1979; Guerrero et al., 2011). The symptoms appear on the leaves and stem of seedlings. Initially on the young leaves, the symptom appears as small water soaked lesions on the under surface of the lamina. These lesions gets scattered all over the leaves or seen at the distal end and margins of the leaves and ultimately defoliation occurs. On the mature leaves, there occurs water soaked lesions along and near the veins. In case of immature stem, initially the symptom appears as water soaked linear brown lesion which later turns to black colour. On the seedling, the infection starts from the tip, spreads downwards which results in defoliation and die back. At the cotyledonary region, the infection spreads both upward and downward which result in wilting.

The control of *Phytophthora* is a major challenge in cocoa cultivation. The farmers adopt several measures to control the disease, of which the use of copper based fungicides is the most predominant one (Tan and Tan, 1990). Although it is reasonably effective, their indiscriminate use possesses serious environmental issues.Moreover, due to the occurrence of the disease during peak rainy season,farmers face inconvenience in timely application of the fungicide.

To overcome this situation, breeding for resistance to *Phytophthora* is the most economical, environmental friendly and effective control method (Iwaro and Singh,2004). The resistance to *Phytophthora* is polygenically inherited and could be improved by recurrent selection (Iwaro *et al.*, 1999). Hence an organized breeding programme was initiated at Cocoa Research Center (CRC), KAU, Vellanikkara during 2005 for the development of varieties resistance to *Phytophthora* pod rot disease and high yield.

It was with this background the present study entitled 'General combining ability of selected black pod disease resistant cocoa(*Theobroma cacao* L.) hybrids' which forms a part of the ongoing project at Cocoa Research Centre (CRC), was taken up with the following objectives:

To evaluate the incompatibility and general combining ability of hybrids resistant to *Phytophthora* pod rot for identifying superior genotypes for further crop improvement programmes.

Review of literature

2. REVIEW OF LITERATURE

Cocoa (*Theobroma cacao* L.) has originated from the Amazon river basin of South America. The genus *Theobroma* includes 22 species classified into six sections (Cuatrecasas, 1964). The presence of cocoa had been clearly mentioned in the archaeological records dated to 2000-4000 years back in Central America (Bergman, 1969). The cultivation of cocoa had extended from Mexico to Costa Rica and over the time it spread to Caribbean and other parts of South America (Wood and Lass, 1985).The scientific name *Theobroma* is derived from two greek words '*Theo*' means god and '*broma*' means food, hence known as "*food of god*". The beans are the source for chocolate and other edible products. There is an immense scope for its cultivation due to the diversified climate in India.

The worldwide cultivation of cocoa on tropical lowlands is over five million hectares (Kraus and Soberanis, 2001). Around 72% of world's cocoa production is met by Africa and in that 40% is from Ivory coast (Anonymous, 2015). Cocoa was introduced to India during 1798 (Ratnam, 1961). The area during 2014-15 was13,183 ha in Kerala and Idukki stands 1st with an area of 8,831 ha (Agricultural statistics, 2014-15). It has now spread to all south Indian states. Cultivation of cocoa during early years were comparatively problem free. However as the area extended, new biotic and abiotic challenges aroused. Among the biotic stresses faced by cocoa, *Phytophthora* pod rot is the most serious one.

2.1 *Phytophthora* pod rot

Phytophthora pod rot is an important fungal disease of cocoa in the world and accounts crop loss to the tune of 64 per cent of world production (Nyadanu *et al.*, 2012). Jenman and Harrison (1897), first reported black pod from Guyana and West Indies.The etiology of the disease was reported in 1898 (Carruthers, 1898) however the causal organism was identified as the fungus which belongs to the genus *Phytophthora* in 1899 (Massee, 1899).

In India, Ramakrishnan and Thankappan, 1965 identified the fungus as a species of *phytophthora*. The pathogen causes various other diseases in cocoa excluding black pod *viz.*, canker (Chandramohanan, 1978), seedling dieback (Chandramohanan, 1979), twig dieback and chupon blight (Chandramohanan *et al.*, 1979). Many other species of *Phytophthora* have been reported to cause black pod *viz.*,*Phytophthora megakarya* in West Africa (Griffin, 1977), *Phytophthora citrophthora* in Brazil (Campello and Luz, 1981), *Phytophthora megasperma* in Venezuela (Zentmyer, 1988), *Phytophthora nicotianae* in Malaysia (Tey and Bong, 1990) and *Phytophthora capsici* in Central and South America (Zentmyer, 1988).

Chowdappa *et al.* (1993) reported that *Phytophthora palmivora* is the predominant species causing black pod in India.

2.2 Symptomatology

The cocoa pods are infected at any of its age by the pathogen (Ramakrishnan and Thankappan, 1965). There are about 80 species of *Phytophthora* and all causes damage to plants. It has four types of spores that causes infection. They are sporangia, zoospores, chlamydospores and oospores. The sporangia are produced on infected leaves, fruits, stem or roots. It can also germinate to produce zoospores which can swim in soil water or on wet plant surface. Both of these structures are spread by rain splash, wind-blown rain soil and soil water. The chlamydospores is produced by the mycelium of some isolates. It germinates under certain conditions and form sporangia. Oospores are produced when two mating system called as A_1 and A_2 are present(Vanegtern *et al.*, 2015).

The symptom appears as a brown discolouration, which begins from apical or pedicel end of pod and spreads rapidly and cover the entire pod surface. A white web of mycelial growth was observed on the infected pods. The tissue has a shrunk and corky appearance with dark brown colour. In advanced stage of infection, the pathogen invades the internal pod tissues, causing discolouration and shriveling of cocoa beans. Finally, the affected pod gets shrivelled, hard,

mummified and turn into black. This infected pods act as a massive source of inoculum to infect other pods of cocoa. The sporangia which forms on the surface of infected parts under humid condition, act as the secondary source of inoculum. It is dispersed by rainfall, splashing water or water moving over the soil surface. The motile zoospores (primary infective propagules) formed inside sporangia are released in water and moves to the infection site. Hence, windborne rain has a primary role in the spread of the disease.

Other symptoms like seedling blight, trunk canker, twig dieback, blight and necrosis of leaves were reported by Gregory (1974). Firman (1974) found that *Phytophthorapalmivora* attacks all parts of cocoa plant. Abraham *et al.* (1992), observed abnormal symptoms on immature pods infected by *Phytophthora palmivora* during rainy season. Initially there is a concentric ring in the sub-epidermal region of infected pods and there will be cementing of beans with placenta and husk. Finally a watery consistency in kernel of infected beans is observed.

2.3 Morphology of the pathogen

Phytophthora produces zoospores in a structure called sporangia. According to Basier and Griffin (1979), the shape of sporangia can be spherical, ovoid, obovoid, subspherical, ellipsoid, pyriform, turbinate, obturbinate, obpyriform, limoniform and is often species specific. Appiah *et al.* (2003) observed that the mycelium of the pathogen is hyaline, coenocytic which measures 3.22-6.45 µm in width. Bhavani(2004) observed that the sporangia of *P. palmivora* causing *Phytophthora* pod rot were ellipsoid to ovoid, papillate and caduceus with L/B ratio 1.2-2.2. Waterhouse *et al.*, (1983) reported that the size, shape and length to breadth ratio of sporangia are marked characters in identification of species of *Phytophthora*. The recent studies on taxonomy found that the genus *Phytophthora* belongs to the class Oomycetes. Based on molecular phylogeny, Oomycetes are not included in the kingdom fungi (Hawksworth *et*

al.,1995) and are considered to be a group of fungus-like mycelial organisms that belong to the kingdom straminopila (Dube, 2005).

2.4 Resistance breeding

Tan and Tan (1990) reported several methods adopted by farmers to control black pod disease. Even though fungicidal control is very effective, but is not suitable in areas receiving heavy and continuous monsoon rains (Amma *et al.*, 2009). Due to the large variation in the accession of cocoa, genetic resistance to the disease is getting more attention. Hence, breeding for resistance to *Phytophthora* pod rot disease agreed to be more effective, environmentally friendly and effective control method (Iwaro *et al.*, 2004).

The varying levels of resistance to *Phytophthora* species has been identified earlier in clones of cocoa (Iwaro *et al.*, 1997a; Iwaro *et al.*, 1997b; Sreenivasan, 1980).According to Cilas *et al.* (1998) genetic factors of resistance are important to make the selection of resistance more effective. As a result of many investigations done in genetic population of cocoa, the predominant factor controlling the resistance to black pod is the additive genetic effects (Iwaro *et al.*, 1999; Adomako, 2006).

The screening of fifty one cultivars of cocoa seedlings using glass house method against black pod revealed that the cultivars EET59, EET 376, Pound 7, UF 713, UF 715, SCA 12, Catongo and Diamantes 800 expressed promising level of resistance (Asare-Nyako and Amponsah, 1973). Sri-Sukamato and Marwadi (1986) reported DR 16, SCA 6, SCA 12 and ICS 6 clones of cocoa to be resistant to *Phytophthora palmivora* from East Java. *Phytophthora* pod rot resistance screening on different cocoa types using detached pod method showed G IV 14 with lowest percentage of infection on pod area against *Phytophthora palmivora*(CCRP, 2000).

Iwaro *et al.* (2004) selected 816 cacao accessions from ICG (International Cocoa Genebank, Trinidad) and evaluated for *Phytophthora* pod rot disease

resistance. After evaluation, the level of resistance to pod rot was compared among wild and cultivated types of cocoa, then with Forastero, Trinitario, Refractario and also with accession (JA, LP, MOQ, NA, B, AM, ICS, IMC, PA, CL and TRD). The score distribution indicates that 68.9% of the sample was susceptible (disease scoring 6-8), 12.9% was resistant (disease scoring 1-3) and 18.2% moderately resistant (disease scoring 4-5). Significant variance was observed between the wild and cultivated types for which higher per cent of resistant (17.7%) and moderately resistant (22.6%) genotypes in wild accession as compared to the cultivated varieties (9.4% resistant and 14.4% moderately resistant) and also with Forastero, Trinitario and Refractario. Forastero was found to be more resistant comprising 18% resistant genotypes and 23.1% moderately resistant genotypes than the Refractario (11.3% resistant and 15.4% moderately resistant) or Trinitario (4.8% resistant and 13.6% moderately resistant). The accession groups showed a marked difference in disease rating and identified in PA (Forastero) of 24.2% resistant and 28.8% moderately resistant genotypes. Out of 816 accessions evaluated, 105 promising genotypes with resistance to black pod were identified in the study.

Djocgoue *et al.* (2006) conducted a study in Cameroon to assess the susceptibility of seedling progenies from (SNK 10×SNK413; ICS84×ICS95) to *Phyophthora megakarya* by measuring lesion size along the midrib. Significant variation in lesion size was observed among parental clones. Heterosis value confirmed the vigor of hybrid progenies. The result showed significant effect of day on necrotic size of lesion after inoculation to all progenies (1% probability level). They also observed that narrow sense of heritability was average for SNK clones and high for ICS clones. Hence, ICS clones appeared to be the best promising parents than SNKconfirmed it as the best promising genotype resistant to black pod disease. Adomako (2006) observed significant variation between cocoa genotypes in the level of black pod attack in field trials.

Adomako (2007) and Nyadanu *et al.* (2009) identified lines of partial resistance to black pod disease but not with complete resistance in cocoa. Rubiyo

and Rivaie (2013) conducted a study to determine the genetic parameters of resistance to black pod disease in cocoa using half diallel crossing analysis. They had taken ICCRI 3, TSH 858, DR 1, ICS 13, and Sca 6 as parental clones from in the order range from susceptible to resistant. The experiment was designed in RBD with 3 replications, 10 F_1 hybrids and 5 parental clones were taken as treatments.The result of diallel crossing analysis gave a highly significant difference between the genotypes when the parameter was taken as spot width of disease, occurred after inoculation with *Phytophthora palmivora*. They observed that there was no interaction between genes in predisposing the resistance to black pod disease in cocoa and was more affected by additive gene effect. They also found that dominant genes were more in parents and it is the recessive genes which controlled the characters of resistance to the disease. It revealed that there is an opportunity to produce a hybrid of cocoa which is owned by ICCRI 3 and Sca6. The estimated value of heritability (broad sense heritability and narrow sense heritability) was high for the spot area observed and moderate to high for the intensity of disease.

Barreto *et al.* (2015) selected 262 genotypes from segregating progeny of cacao (TSH 1188× CCN 51) and evaluated the genetic resistance to black pod disease caused by three species of *Phytophthora*. Results was significant and a high level of heritability (h^2 = 0.79, 0.839, 0.799) was observed for *P.citrophthora, P. palmivora, P. capsici*. They identified ten genotypes which are resistant to the species of *Phytophthora* and have opined that resistance was likely to be oligogenic and was important for breeding programmes.

2.5 Assessment of self incompatibility

The first experimental results based on the existence of incompatibility in cocoa were published by Pound in the year 1931. Further reports also confirmed the earlier findings (Marshall, 1933;Voelcker, 1936; 1937). Cope (1939) studied the cytological basis of incompatibility. Through statistical comparison, he observed that growth of pollen tube in compatible and incompatible stigmas were insignificant. The method followed in cocoa for cytological study is different

since it is late acting self-incompatibility as the growth of pollen tube is similar in compatible and incompatible types. They grow down to the ovule and one male gamete is fused with the endosperm nuclei in both compatible and incompatible reaction. But in self-incompatible type the second male gamete will not fuse with egg and division of zygote is affected resulting in incompatibility. After 24 hours of pollination the pollen tube reaches the synergid cells, two spermatic nuclei can be seen in the synergid, one sperm nuclei fuse with the polar nucleus, development of endosperm nucleus and fusion between egg and sperm has not been affected it will result in formation of irregular ovule (Cope, 1939).

Gerstel (1950) credited Hughes (1943) as first recognizer in case of incompatibility governed by multiple alleles. It exhibited dominance combined with sporophytic control of pollen reaction.

Knight and Rogers (1953) studied the allelic interaction in cocoa. They have selected three clones Pa-7, Pa-35 and Na-32. Five alleles were reported in cocoa as S_1, S_2, S_3, S_4, and S_5 and the interaction of alleles was $S_1>S_2=S_3>S_4>S_5$. They assumed the genetic constitution of Pa-7 as S_1S_5, Pa-35 as S_3S_5 and Na-32 as S_2S_4. Cross combinations were made between them and interaction was studied. In the cross between Pa-7 and Na-32, Pa-7 produced two gametes S_1 and S_5 and Na-32 produced two gametes S_2 and S_4. Four types of classes are expected in nature i.e., S_1S_2, S_1S_4, S_2S_5 and S_4S_5. But when progenies were crossed with each other, only three groups were able to identify i.e., individual in group A can be crossed with B and C, B with A and C and C with B. But individuals within group A were cross incompatible i.e., between $S_1S_2 \times S_1S_4$. This is because all the individuals in this group behave as S_1, Since $S_1>S_2$ and S_4 and reaction is sporophytic self-incompatibility. Similarly in the cross between Pa-35 and Pa-7, the expected classes were four, but in nature only three classes were identified since all individuals in class A S_1S_3 and S_1S_5 behave in the same manner and they cannot cross each other. This is because $S_1>S_3$ and S_5, and the self-incompatibility is sporophytic.In another cross compatible relationship between Pa-35 and Na-32, four crosses were expected but only two classes were found in nature. All

individuals under class A, S_2S_3, S_3S_4 and S_2S_5 have same behavior and are not cross compatible. This is because S_2 is co-dominant to S_3 and S_2 and S_3 are dominant over S_4 and S_5 and reaction is sporophytic.

Minimol and Prasannakumari (2013) conducted a study in cocoa on assessment of self-incompatibility in fifty field established hybrids resistant to vascular streak dieback. After assessment of 50 hybrids, 21 turned to be self-incompatible, 9 self-compatible and remaining 20 did not produce any flowers. They also observed that there was no similarity in their compatibility position in hybrid progenies belonging to the same cross.

2.4 Combining ability

Sprague and Taturn (1942) gave the concept of combining ability on the basis of genetic variation in maize crop by using single cross. This gives information about the parental selection with respect to the performance of their hybrids. If the parental line possessed a high GCA value, then their hybrid will express a high SCA value which is necessary for the improvement of desirable character.

Ilchovska (2013) carried out the preliminary evaluation of the combining ability for grain yield of 12 mutant maize lines using top cross method for early testing and analysis of the general combining ability and specific combining ability. Mutant lines are tested by three testers that have proven high general combining ability. Inorder to know the productive abilities of the received top crosses, three preliminary varietal experiments were carried at the experimental field of Maize Institute, Knezha. The results revealed that the lines XM 42-7-1*-1, XM 34-1-1*-1 and XM 33-4-4-1 as high general combiners and can be used as components for obtaining synthetics with high yields or as testers in analyzing crosses for determining the general combining ability at earlier stages of the selection process. The lines XM 4-1-1-1, XM 31-1-3*-1, XM 44-6-2*-1, XM 34-1-1*-1 and XM 3-5-1-1found to have high specific combining ability can be included in the combination for obtaining hybrids with high yields. Two mutant

lines of the spectrum – XM 33-4-4-1 and XM 42-7-1*-1, found to have high general combining ability and specific combining ability were identified in the study.

Topp and Sherman (1993) studied the combining ability for resistance to stem canker (*Xanthomonas campestris*pv. *Pruni*) in five Japanese plum cultivar using diallel mating design under greenhouse condition. They measured the length of inoculated cankers, its appearance rate and expansion rate. On the basis of canker appearance rate, 'Wade' was found to be the best parent in transmitting canker resistance, even though 'Burbank', 'Wilson' had similar GCA value as that of Wade in length of inoculated cankers. The cultivar 'Friar' and 'Gulfruby' were found as most susceptible. Canker appearance rating identified as best for measuring resistance to stem canker under greenhouse, provided lower coefficient of variation and greater separation of estimated GCA value.

Lashermes *et al.* (1994) estimated combining ability of fifty-five double haploids (DH) of *Coffea canephora* P which was crossed with either heterozygous genotypes or with double haploids. Strong hybrid vigour was observed for all the characters evaluated. Results revealed that from the evaluation of twenty-four top crosses involving DH which are derived from IF 200 and DH-160-02, significant variation was observed for height, girth, leaf characteristics, susceptibility to leaf rust, cherry maturity and yield. Variance for GCA was prominent, although additive effects due to interactions were identified. They have correlated the yield observed from hybrid combination with that of predicted yield from parental GCA and found that GCA accounts for larger proportion of difference between hybrid combination and in some cases, the hybrid yield was comparable with that of the standard clone. Implications of these results for the breeding programme and thereby development of F_1hybrid varieties is still envisaged.

Gayathri (1997) reported that parents with mean performance higher for a specific trait are generally good combiners for that particular trait on the basis of a trial work on cucumber at KAU. After evaluation of seven parental lines, CS-12 and CS-9 were found to be good combiners based on its yield. The SCA effects

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were high for the hybrids of CS-12× Punerikhira, BSS-169× Arc-1 and CS9× Arc-1.

A study was carried out by Cilas *et al.* (1998) to determine the genetic analyses of yield and its morphological characters in *coffea arabica* using half-diallel method. Significant SCA variance was observed for all the traits. The morphological traits were genetically correlated to yield and the hybrid showed better performance than parental line. There was no clear relation between performance of parental line and GCA. They observed different levels in interaction which seems to be related to residual heterozygosity among parents and identified Java with high interactivity in the diallel analysis with favorable SCA. They also analyzed that indirect yield prediction by using morphological traits was in concurrence with expected genetic gain based on cumulative data of yield over four years. These results can be incorporated in further breeding programme involving selection of hybrid.

Verma *et al.* (2000) generated 21 hybrids of cucumber by using line× tester method (7 line and 3 tester) and studied the combining ability of seven traits. The results of GCA and SCA was significant for parents and hybrids. They observed that parental lines K 27080, LC-3, CS-12 and GY-2 were good general combiners for yield and its related components.

In line × tester (2×4) mating design to study the combining ability and genetic analysis of drought responsive physiological characters in coconut, Rajagopal *et al.* (2007) observed significant difference among parents and hybrids. The photosynthetic rate under stress was additive with good GCA, non-stress and recovery were influenced by non-additive gene action which can be exploited for heterosis.

A study was carried out by Marchesan *et al.* (2009) in triple hybrid of sweet pepper to assess the combining ability of agronomic performance and resistance to powdery mildew in a partial diallel method. Estimates of GCA effects demonstrated the female parent 'P36-R' and 'Platero' as good combiners

for most agronomic traits evaluated. They also observed additive effects to be greater than non-additive effects. The triple hybrids 'Quantum-R×HV-12' and 'P36-R×HV-12' were identified as best specific combiners. The mean square of SCA was significant for the severity of disease which indicated the dominant and epistatic effect of genes. However, the triple hybrid obtained by crossing with 'Quantum-R' and 'Rubia-R' showed a negative GCA and best reaction to powdery mildew disease.

Combining ability for yield and its attributes was studied by Reddy *et al.* (2011) following line×tester approach in ridge gourd. Six lines and three testers were crossed and estimated for combining ability effects. RGP-26 and LA-31 among lines and PusaNasdar and Jaipur long among tester were identified as good general combiners. Among crosses, LA-31× PusaNasdar was found to be the superior, based on SCA effects for fruit weight, number of female flowers/ vine, node number of first female flower appearance, sex ratio, average weight of fruit and fruit girth. GCA and SCA variance revealed the presence of non-additive gene effects for all the characters studied.

Tamilsevi *et al.* (2015) investigated the combining ability for yield and its contributing characters in pumpkin using line× tester mating design. For all the characters observed, SCA variance was higher than GCA which indicated the preponderance of non-additive gene effect. After evaluation of 15 parental lines, Vadhalgundu local which is followed by KashiHarit were identified as best combiners for both quantitative and qualitative characters. They also reported that the tester CO 2 expressed higher GCA value than ArkaSuryamukhi. Among the crosses, KashiHarit × Avinash local were good specific combiners for days to first emergence of female flower, sex ratio, number of fruits per vine, flesh thickness, total carotenoids content followed by Vadhalgundu local × C0 2 for carbohydrate content, total carotenoids content, sex ratio, number of fruits per vine, node number of first female flower and also fruit yield per vine.

2.4.1 Combining Ability Studies in Cocoa

Tan (1990) conducted a study to evaluate the combining ability of yield and its components of 54 F_1 hybrids generated from 6 Trinitario females and 9 Amazonian males of cocoa. GCA differed significantly for all the characters from female + male, female and male source and SCA (female× male) was also significantly different. GCA to SCA ratio for number of beans per pod and pod weight was 7.1 and 25.7, suggests that genetic variability was additive in nature for all characters evaluated. KA-106 identified as best female parent among Trinitario for high yield, pod and bean values. Among Amazonians, KEE 42 and KEE 43 combined high yield and transmitting pod and bean characteristics into progenies. On the basis of its GCA value and mean performance of hybrids, a multi-line cultivar with good pod and bean values is being produced for commercial planting in seed gardens.

Cuauhtemoc *et al.* (2006) evaluated the combining ability for disease resistance, yield and its components in 25 hybrids of cocoa obtained as a result of partial full-sib mating from one to eight trees of each parental clone. The clones UF 273, UF 712, ICS 95 constituted parental set 1 and CC137, CC252, Tree 81, CCN 51, SCA 6, ICS 6, ICS 44,CATIE 1000, EET 75 and pound 7 formed parental set 2. Results showed that six parental clones differed in the identity and was presumed to be similar from other germplasm collection. Significant GCA effect was observed for all characters in type 1 and type 2 parental set except for per cent pods with black pod attack and monthwise production of first jorquette. Additive gene action was seen predominantly for the traits assessed of per cent healthy pods and per cent pods with frost pods, while for the traits total number of pods and diameter of trunk expressed both additive and non-additive gene action. They identified the crosses of two clones UF 712 and UF 273 of type 1with favorable GCA estimate for the traits of frost pod resistance and total production of pod. It was hereby suggested that the crosses of these two clones will act as a potential candidates in breeding population for QTL analysis.

Nyadanu *et al.* (2012) studied inheritance and general combining ability of resistance to *Phytophthora palmivora* and *Phytophthora megakarya* using leaf disc, detached pod and natural field observation. It was observed that inheritance of pod lesion number and leaf disc scores were influenced by GCA and SCA effect which implies that inheritance to black pod resistance was influenced by dominance or epistatic effect. SCA effect was not significant for size of pod lesion under natural field condition which suggested the predominance of additive effect of gene on mode of inheritance of these traits. The reciprocal effects were non-significant and it was due to the absence of cytoplasmic inheritance in pod rot resistance. Based on the results it was suggested that reciprocal selection method was effective for the improvement of resistance against black pod.

A study was carried out by Adewale *et al.* (2014) to determine the breeding value of cocoa (*Theobroma cacao* L.) for pod and bean traits and a consequential advance in Nigerian cocoa breeding program. Fourteen genotypes were evaluated for pod length, weight, number of beans/pod, bean length, width and thickness following line × tester approach. They observed that the hybrids from same female parent differed significantly for all the traits studied. The GCA effect was highest inT65/7 for the trait pod weight (0.42) and the least was for pod length in T86/2(-0.081).It was found that GCA and SCA differed significantly for all the traits. The ratio of GCA/SCA revealed that the inheritance of all characters were additive in nature except number of beans/pod and bean length which are non- additive in nature. Heterosis was in the range of -17.82% for bean thickness to 52.40% for pod weight.

2.5 Screening of seedling for disease resistance

The zoospores of *Phytophthora* species was inoculated on leaves or leaf-disc from 1972 onwards, as an early screening method for resistance. Many researchers have reported that after inoculation, scores of disease severity increased with time and older leaves were showing more resistance than young leaves. The leaf stages, interflush (2&3) can be used for screening were identified

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by Greathouse *et al.* (1971). Tahi *et al.* (2000) selected nine clones of cocoa and inoculated with *Phytophthora palmivora* at hardening stage and found that the result showed good correlation with infection of pods under natural field observation.

Cilas and Despreaux (2004) suggested suitable efforts to improve the screening methods for resistance to black pod using the leaf inoculation, leaf disc and attached or detached pods. It was observed that the results of detached pod test and leaf disc obtained significant correlation with the level of field infection when performed under standard conditions (Iwaro *et al.*, 2005; Nyadanu *et al.*, 2009).

Iwaro *et al.* (2006) carried out genetic analysis of resistance of cocoa to *Phytophthora* pod rot using 5×5 diallel and 4×2NC II in factorial design. The study was conducted in crossed progenies and parental clones which were in nursery and in field. The results of scoring for resistance were compared with per cent of infected pods estimated over the past seven year period and found to be significant. They observed that heritability values (Narrow sense heritability-0.34&0.67, Broad sense heritability-0.60&0.67) were higher when compared with per cent of infected pods (0.42&0.47) in both designs. They also observed significant genotypic and phenotypic coefficient of correlation for resistance on leaves and per cent of infected pods in field. It confirmed the feasibility of leaf disc method in early selection of *Phytophthora* pod rot resistance in cocoa. According to Tahi *et al.* (2006), 75-90% of genetic variation of cocoa genotype for field resistance to *Phytophthora palmivora* can be explained by leaf-disc method when carried under standard condition.

Nyadanu *et al.* (2009) selected 25 international genotypes of cocoa for laboratory and field observation at Cocoa Research Institute, Ghana to evaluate the efficacy of screening methods for resistance used in breeding of black pod disease resistance in cocoa. Significant clonal difference was observed for leaf and pod resistance at penetration and post-penetration infection stage. Correlation

studies were positive and significant between resistance of leaves and pod at both stages of infection. This peculiarity of cocoa leaf suggested the value of use of seedling leaves for the prediction of pod resistance to black pod. The reliability of the test was conducted by correlating the results of inoculation tests with the level of field infection and was found positive for both detached pod and leaf tests. Finally, they concluded that for the convenience of operations and reliability of results, leaf disc test was found to be the best screening method.

Eight cocoa clones selected from cocoa research institute of Nigeria (CRIN) headquarters, Ibadan were evaluated for susceptibility to black pod disease using leaf disc inoculation test (Otuonye *et al.*, 2007). Screening of cocoa clones was done with four isolates of *Phytophthora megakarya* (NGRI$_4$) and observed a significant variation in the level of resistance to *P. megakarya* isolate. It had been reported that clone T12/5 developed a fairly low lesion number in response to the isolate (NGRI$_4$) and subsequent to these findings, the susceptibility-resistant ranking of this germplasm is resistant. Other clones identified as T17/11, T86/2(moderately resistant) and T85/5, T85/45 and T20/11(moderately susceptible). It was suggested that clone T12/5 can be incorporated into breeding programmes for the development of high yielding *Phytophthora* pod rot resistant cultivars in cocoa.

Iwaro *et al.* (1998) investigated the effect of two species of *Phytophthora* (P. *palmivora* and P. *capsici*), inoculation at two depths (3mm and 9mm) and stages of pod maturity on the assessment of cacao resistance to *Phytophthora*. They observed a significant difference in the two pathogens tested and *Phytophthora palmivora* showed more aggressive than the other. There was no interaction between pathogen and clone and observed similarity in the ranking of clonal response to two *Phytophthora* species. The study revealed that *Phytophthora palmivora* being more aggressive than the other preferred as a choice of screening for resistance. They also reported that there was significant variation in inoculation depths and also between maturity stages of the pod with larger lesion recorded at 3mm and on unripe pod as compared to other. The

magnitude of lesion size varied with genotype which indicated that depth of inoculation and stage of pod maturity is to be standardized before screening for resistance to *Phytophthora* in cacao germplasm.

Iwaro *et al.* (2005) selected 40 genotypes from various groups of cocoa and screened for resistance to *Phytophthora* pod rot using the detached pod test by spray method (DPT-SM). Significance variation was observed among the 40 genotypes based on disease rating scale for DPT-SM and percentage of pod rot under field observation. Higher level of susceptibility was observed in the third year (63%) of field observation than in first (15%) or second year (25%). This suggested that susceptible factor for *phytophthora* pod rot (PPR) were unstable between years of field observation. They have correlated the results with amount of rainfall obtained during main pod harvest season (Nov-Feb) and high rainfall in November accounts for increasing PPR incidence. Similar results were obtained from DPT-SM was in concurrence with field observation suggesting that there is a strong association existing between the result of DPT-SM and cumulative data for field observation for a period longer than three years. Hence DPT-SM being cost effective and non-destructive inoculation method, provide a suitable option for cocoa collections in gene banks to be assessed.

According to Efombagn *et al.* (2011), under standard condition the leaf disc and detached pod method can be applied in selection activities for *Phytophthora* pod rot resistance in Cameroon. The clones screened with these two methods were assessed for *Phytophthora* pod rot resistance in field (Efombagn *et al.*, 2004;Ndoumbo, 2002).

2.6 Pod and bean characters

According to Vanderknaap (1954), the seedlings produced by beans originated from different position in pods showed no difference in its growth pattern. They did not find any association between the colour of cotyledon and bean weight. The variety with high bean index fetches better economic value for

the production of chocolate (Ruinard, 1961). So the breeders mainly focus on the bean yield and its resistance to pest and disease incidence.

Findings by Mora and Bullard (1961) indicated significant correlation (5% level) with bean size and fat content and are non-significant with hull per cent and bean size. They also observed no significant variation among hybrids or clones in fermentation rates or flavour.

Enriquez and Soria (1966) reported that bean yield in cocoa expressed as dry or wet weight of bean. The dry weight varied from 0.5 to 2.5g per bean or seed and opined high variability in weight of bean within a single pod. They also suggested that even though the ridge thickness and furrow depth are descriptive traits, are partially influenced by environment.

Adenikinju (1977) reported that the dry matter accumulation in seedlings was influenced by bean size and was found to increase in a linear manner during nursery stage. Engles (1982) compared the phenotypic relationships of 32 clones of cocoa with its genetic relationships, observed that for higher production of dry cocoa per fruit, selection of seed size per fruit was important than that for seed number.

Cilas et al. (1989) reported that variability of bean size was found highest in Trinitario than upper Amazon and Amelonado and seeds produced from fruit apex were smaller and absence of flat beans. In another study, EET 400 and SPA 9 werevarieties of cocoa with flat beans present only in apical areas (Mora, 1989).

Bekele et al. (2008) selected 164 cocoa accessions and evaluated for promising type using morphological descriptors. They identified Trinitario accessions as promising on the basis of pod index(less than 20), thickness of pod wall, bean size and pale colour of cotyledon.

Apshara et al. (2009) studied the growth and yield performance of 44 Nigerian clones in the field gene bank, regional station of CPCRI Vittal. They identified the clones NC-25, 27,51,20,50,26,23,37 as heavy bearers with an

average pod yield per year ranging from 43-61.9. In addition, these clones were identified to be well suitable for industries because of its characteristics like shelling per cent(10-15%), fat content(> 50%) and single bean weight(>1g).

According to Monterio *et al.* (2009), cocoa bean weight exceeding 1g are superior genotypes. Oyedokun *et al.* (2011) investigated bean value of 14 genotypes of cocoa with dried beans. Significant variation was observed among the genotypes for the traits of length of bean, weight of individual bean, width of bean, thickness, weight of 100 beans, ratio of bean width to thickness, bean length to thickness and bean length to width. In a study conducted by Minimol *et al.* (2011) involving twenty three accession of cocoa reported the influence of fruit apex on fruit shape. Asna *et al.* (2013) analysed the performance of fifty clonal accessions of cocoa comprising both exotic and indigenous at Cocoa Research Centre, Vellanikkara. They observed wide variability among the accessions for all qualitative traits. After evaluating the traits for pod weight, number of beans per pod, pod index, per cent of flat beans, dry weight of peeled bean, identified the accession R (10) (MEX) to be desirable with more number of beans per pod (49.20), dry weight of peeled bean (1.68g), low pod index value (less than 12) and flat bean content per pod (0.81%). They also reported the accession COCA 3370-3 can be used in breeding for resistance as donor parent having high husk thickness and tolerance to pests and diseases.

Rubeena (2015) evaluated the inheritance of various bean characters and assessed the threshold bean size as well as its heterotic effect of the selected forty hybrid progenies maintained at Cocoa Research Centre, Vellanikkara. Significant variation was observed among the hybrid progenies in terms of pod and bean quantitative traits, indicating the heterogeneity among them and also for all qualitative characters evaluated. The hybrid 13.7 was found to be the desirable one with low pod index value (PI value), high pod weight, high pod breadth and wet weight of unpeeled bean.

2.7 Estimation of heterosis

A line × tester analysis was carried out in maize by crossing 20 inbred lines with 2 testers in maize. Forty F_1 hybrids along with 22 parents (20 S_2 lines and 2 testers) and two standard checks were evaluated in partially balanced lattice square design with two replications. Highly significant differences were observed among the testcrosses for all the characters. Among the crosses (40 x Kiramat and 3 x Jalal) exhibited maximum value of mid parent (47.36) and better parent heterosis (13.63), with respect to 100 grain weight. They identified fifteen testcrosses with highest significant positive mid parent heterosis for grain yield. Fifteen hybrid showed highest significant positive standard heterosis over both the checks. The result revealed that these lines were having promising performance which could be used in further breeding programme (Ali *et al.*, 2001).

Kurian*et al.* (2001) investigated heterosis for yield related components and fruit using line x tester analysis in between bacterial wilt resistant / tolerant accessions and processing varieties in tomato. Heterotic hybrids were identified for average fruit weight. The cross Sakthi x Fresh Market 9, Sakthi x HW 208F (average fruit weight), Sakthi x TH 318, Sakthi x Fresh Market 9 (yield/ plant), LE 206 x Ohio 8129, LE 214 x St 64 (locule number) and Sakthi x St64, LE 206 x 64, LE 214 x St 64 (for pericarp thickness) were identified as heterotic. All the hybrids produced round shaped fruits and were late to harvest.

In a study conducted by Patel *et al.* (2010), observed higher magnitude of heterobeltiosis and standard heterosis for green fruit yield per plant and number of fruits per plant in hybrids of chilli. Generally the hybrids which exhibited higher magnitude of heterotic effects also exerted greater amount of heterotic effects for various growth attributes and yield related traits. So the heterotic effects for green fruit yield could be because of direct and indirect effects of various yield contributing traits. The desired level of heterosis of each component trait should be determined to identify superior hybrids with respect to quality parameters, yield and its contributing characters to obtain maximum benefit of heterotic effects for green fruit yield. They also identified a cross (ACMS 8×IPS -2005 -15)

as consistent across the environments and therefore can be used for commercial exploitation.

Vasudevan *et al.* (2011) evaluated heterosis in hybrids of cocoa and found that all the hybrids expressed high relative heterosis resulted in the existence of wide variability among the parents. They identified eleven superior hybrids based on the evaluation of heterosis over open pollinated variety. They also observed that the selected hybrids were not superior over the standard check variety (CCRP 8).Three hybrids were found to be superior with positive heterotic effect with the hybrid H4 showed maximum heterosis over open pollinated variety.

For the estimation of mid-parent and better-parent heterosis in *Linum usitatissimum* L. genotypes was conducted by Reddy *et al.* (2013) at College of Agriculture, Nagpur using line x tester analysis. Out of 60 hybrids evolved, significant positive heterosis were estimated in 51 and 41 crosses for number of branches plant-1. Among parents, Karthika, ACC NO 4/47, PKVNL-260, EC-9825 and GS-234 proved to be superior in most of the hybrid combinations. Maximum positive significant heterosis over better-parent were observed in crosses Padmini x ACCNO4/47, Karthika x JRF-5, Padmini x LCK-8605, PKVNL-260 x ACCNO4/47, PKVNL-260 x Eita and Karthika x EC-9825 for early flowering, plant height, branches plant-1, days to physiological maturity, yield, number of capsules/ plant and 1000 seed weight respectively.

Sadaiah *et al.* (2013) investigated the combining ability and to estimate heterosis of yield and its contributing characters in sweet corn were selected and crossed in diallel fashion excluding reciprocals during kharif, 2010. Twenty eight crosses were evolved from eight divergent parents and a standard check Sugar 75 and Madhuri in diallel manner at Agricultural Research Institute, Rajendranagar, Hyderabad. The estimates of heterosis, heterobeltiosis and standard heterosis were variable among crosses. The hybrids 6072-3 x 6069, 6072-3 x 6100-2, 6069 x 6122-1, 6122-1 x 6127, 6072-3 x 6127 and 6104 x 6082 performed well over standard Madhuri for sugar content in kernel. Based on heterosis and combining ability, they identified four superior cross combinations (6072-3 x 6100-2, 6072-3

x 6069 and 6104 x 6082 which showed good performance for sugar content in the kernel. It can be used as single cross hybrids after evaluation in multi-location trials.

The inheritance patterns of some traits in generated progenies of eleven parental clones of cocoa were studied by Sobowale *et al.* (2016). Hybridization was followed by randomized complete block design of six replications in a hybrid evaluation trial plot at the Cocoa Research Institute of Nigeria (CRIN), Ibadan in 1999.They estimated heritability, component of genetic variation and heterosis for the traits observed and found significant variation ($P<0.05$) among the nineteen genotypes for all the characters studied except the placental weight and bean weight per pod. Pound 7 recorded the highest pod weight among the parents. The cross P7xPA150 had the highest number of bean per pod and some hybrids exhibited significant heterosis for most of the yield components.

2.8 Analysis of genetic parameters

Soria *et al.* (1974) studied the inheritance of fruit size in cocoa and evaluated the heritability for fruit diameter (63%), fruit length (55%) total weight of fruit (55%), revealed these traits are highly transmissible. In another study, parameters like fruit length, fruit diameter, husk weight, total weight and weight of seed observed great variation in each pod (Soria, 1975).

Francis (1998) observed high PCV and GCV for the characters like pod weight, dry bean weight^{-1}pod, wet bean weight^{-1}pod, bean thickness, pod index pod value and which indicated a greater contribution of these traits towards total genetic divergence in cocoa.

Birhan *et al.* (2013) reported high phenotypic (PCV) and genotypic coefficient of variation (GCV) for harvest index and biomass yield per plant in pigeon pea genotypes. High heritability coupled with high expected genetic advance was recorded for 100-seed weight, seed yield per plot, biomass yield per plant, harvest index and plant height.

Osekita and Ademiluyi (2013) conducted a study in tomato (*Lycopersicon esculentum*(Mill.)) during the growing seasons of 2011 and 2012 to find the interrelationship among quantitative traits. The analysis of variance were found significant at (p>0.05) and the highest coefficient of variation (66.56%) was observed for average fruit weight. Phenotypic and genotypic coefficients of variation were determined to show the degree of heritable variation on the component traits. Wide variability was observed for the traits and hence can be exploited by direct selection for improving yield.

Vashistha *et al.* (2013) observed high to moderate heritability with moderate estimates of genetic advance for biological yield, grain yield per plant, plant height and ear height in maize genotypes. Hence, provides better opportunities for selecting plant material for these traits in maize.

Wolie *et al.* (2013) studied heritability, variance components, variability and genetic advance for yield and yield related agronomic characters in eighty-eight finger millet (*Eleusine coracana* L.Gaertn.)germplasm collections at Adet Agricultural Research Station in the year 2008. Significant difference (p<0.01) was observed among the genotypes tested for characters indicating the presence of variability and the usefulness of selection for these traits in the genetic constitution used for future crop improvement. The PCV and GCV values were high for number of tillers per plant, number of ears per plant, number of fingers per ear, length of finger, yield of biomass, grain yield, lodging and blast severity. High heritability coupled with high expected genetic advance as percent of mean was observed for the traits number of ears per plant, number of finger per ear, finger length, days to heading, biomass yield, 1000 kernel weight, lodging susceptibility and blast severity indicating the presence of more additive gene effects for crop improvement.

In a study conducted by Minimol *et al.* (2014) to select superior hybrids as a part of breeding programme, identified the hybrid VSDI 23.21 which showed highest pod number with reduction in pod size and weight. The hybrid PIV 59.8 ranked first in pod weight and dry bean weight but was not released due to its

inferiority with check variety. High PCV and GCV ratio was due to the genetic constitution of hybrids. Eventhough heritability for single dry bean weight was high, its genetic advance was very low. So it cannot be considered as selection criteria for further breeding programme.

Paikhomba *et al.* (2014) evaluated thirty F1 hybrids of rice along with complete set of 13 parents and checks for variability, heritability and genetic advance in different yield and its contributing traits. Significant differences was observed for 14 characters studied, indicating the presence of high genetic variability among the genotypes. GCV were lower than the respective PCV, indicating the influence of environmental factors on the expression of the traits studied. High heritability coupled with moderate genetic advance was observed for characters like percent pollen fertility (99.9, 33.33), grain yield hill^{-1} (99.2, 31.13), harvest index (98.5, 30.42) and number of filled grains panicle^{-1} (97.8, 30.04) reveals that selection for the improvement of these characters may be rewarding and non-additive gene action plays a major role in their inheritance. Hence heterosis breeding could be used to improve these characters.

Gite *et al.* (2015) identified high mean value, heterobeltiosis and standard heterosis for three hybrid combination (185 A × RSV 458, 185 A × RSV 1093, 185 A × RSV 1145) in sorghum for grain yield and its components.

Ten diverse genotypes were crossed in a diallel manner to study variability, heritability and genetic advance for 12 quantitative characters rice (*Oryza sativa* L.). Variability (gcv) ranged from 5.95 for no. of leaves per tiller to 17.40 for grain yield per plant and pcv varied from 7.08 for days to 50% flowering to 17.49 for grain yield per plant. Heritability estimates ranged from 0.721 for total biological yield per plant to 1.000 for plant height. The genetic advance varied from 0.71 for no. of leaves per tiller to 46.23 for no. of spikelets per panicle. High estimates of genetic advance were reported for plant height, days to maturity, days to 50% flowering and total biological yield per plant. High heritability coupled with high predicted genetic advance was observed for plant height, days to maturity, days to 50% flowering and no. of spikelets per panicle.

Hence selection based on these characters being of additive in nature, is likely to be more effective for their improvement. The estimates of phenotypic coefficient of variation were found higher than those of genotypic coefficient of variation for all the traits except plant height. High heritability and genetic advance were obtained for plant height, days to 50 per cent flowering, number of spikelets per panicle and days to maturity. These traits were mostly governed by additive gene action and are important for the breeder to construct selection indices (Tiwari, 2015).

2.8 Diversity analysis

Bekele and Bekele (1996) studied the diversity of hundred accessions maintained in the germplasm at International Cocoa Gene Bank, Trinidad with morphological descriptors and associations among them were investigated by average linkage cluster analysis. They identified rich phenetic diversity by cluster analysis and observed nine accessions remained ungrouped at 75 per cent similarity level with remaining grouped into eleven clusters.

Dias and Kageyama (1997) investigated the genetic distance among cultivars of cocoa selected based on multivariate analysis using the D^2 statistics and a 5×5 complete diallel was evaluated. Based upon five yield components, they selected five cultivars of S_1 generation belonged to the Lower Amazon Forastero and Trinitario, 20 crosses between S_0 parents. The diversity analysis suggested a close relationship between the Trinitario and Amazon Forastero groups. They found that D^2 statistics was found to be linearly related to the average performance of hybrids for wet seed weight/plant and wet seed weight/fruit. They also observed that the most divergent cultivar exhibited high general combining ability and thereby generating the best performing parents.

Multivariate phenetic divergence among SIC and SIAL series by cluster and PCA (Principal Component Analysis) were investigated by Santos et al. (1997). They identified the clones SIC 17 and SIAL 244 with highest genetic

divergence of 3.05. Based on Euclidean distance matrix, highest similar pair (0.33) was observed in SIC 18 an SIC 765.

To study the relationships among 20 Trinidad Selected Hybrids (TSH) of cocoa and five parental types, Maharaj *et al.* (2011) employed the cluster analysis using 15 quantitative traits. They observed that SCA 6, ICS 95 and ICS 1 were very distinct from the TSH progeny. SCA 6 was found to be very unique even at the lowest level of similarity. They also observed two TSH types with descriptive fruit values which were similar to the parents and possessed strong IMC ancestry.

Oyedokun *et al.* (2011) selected 14 genotypes of cocoa and carried out Principal Component Analysis (PCA) to identify the distinguishing characters and to group them based on similarities. Four distinct clusters were formed with five and seven genotypes in clusters I and II with a mean bean weight of 1.07g and 1.02g. Cluster III and IV had unique member with an outstanding bean weight of 1.12g and 1.30g.

Asna (2013) carried out the performance analysis of selected fifty accessions of cocoa maintained at Cocoa Research Centre, Vellanikkara to evaluate and characterize the accessions and to assess the genetic divergence. Based on qualitative and quantitative characters, cluster analysis revealed nine and seven clusters for exotic accessions with maximum genetic divergence in quantitative cluster I and V as indicated by its highest inter cluster distance(33763.40) and five and three clusters for indigenous ones with maximum genetic divergence in cluster I and II(148447.4).
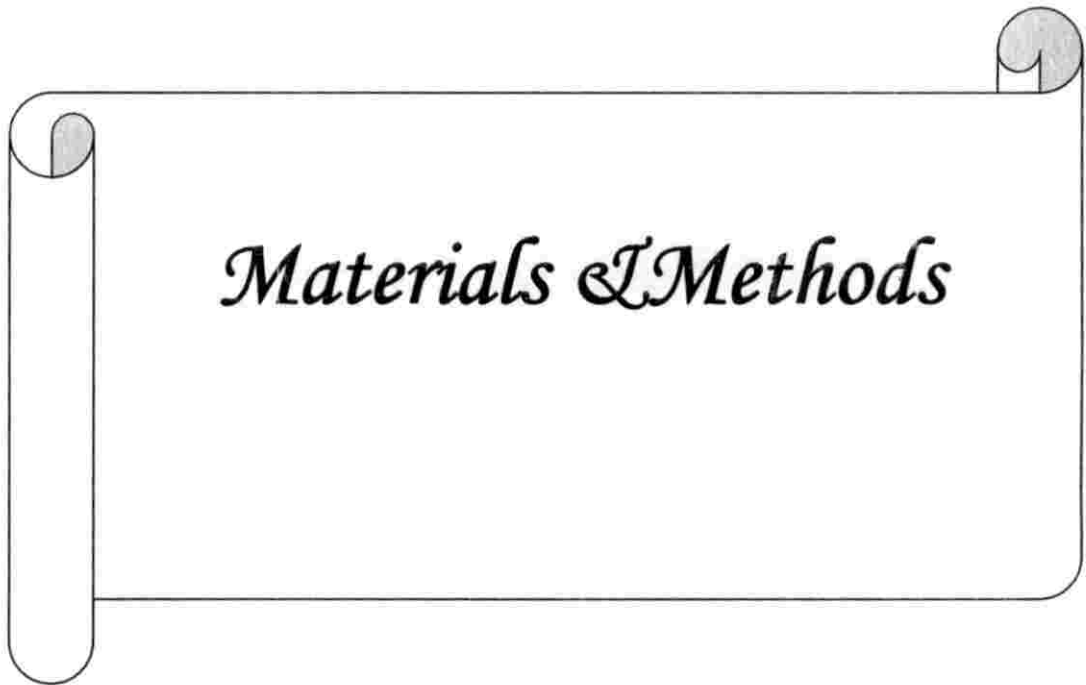
Materials &Methods

3. MATERIALS AND METHODS

The present study 'General combining ability of selected black pod disease resistant cocoa (*Theobroma cacao* L.) hybrids' was carried out at Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during the period from August 2014 to May 2016.

3. 1. Experimental materials and methods

3. 1.1 Experimental materials

An organized breeding programme was initiated at Cocoa Research Centre, Vellanikkara for the development of superior varieties with resistance to *Phytophthora* pod rot disease in 2005. After the initial screening in the nursery, 615 hybrids seedlings were field planted and screened for vigour and disease incidence. Out of 615 hybrids, 25 hybrids did not show any symptom of *Phytophthora* pod rot incidence in the field after six years of field screening. These hybrids formed the base material for the study. The lists of hybrids are presented in Table 1.

Table 1.List of hybrids selected for the study

Sl. No.	Stand No.	Genotype
1	2.4	SIV 1.26 × TISSA
2	3.8	SIV 1.26 × TISSA
3	6.5	SIV 1.26 × P II 12.11
4	12.3	SIV 1.26 × P II 12.11
5	18.5	G VI 188 × GVI 287
6	18.6	G VI 188 × GVI 287
7	22.4	G VI 188 ×GVI 287
8	22.7	G VI 188 × GVI 287

9	25.4	G VI 188 × GVI 287
10	26.4	GVI 188 × GVI 304
11	27.4	GVI 188 × GVI 304
12	29.6	SIV 5.20 × TISSA
13	29.7	SIV 5.20 × TISSA
14	32.5	GVI 216 × GVI 294
15	33.4	GVI 216 × GVI 294
16	34.4	GVI 216 × GVI 294
17	34.6	GVI 216 × GVI 294
18	35.4	GVI 216 × GVI 304
19	35.5	GVI 216 × GVI 304
20	35.9	GVI 216 × GVI 304
21	36.5	GVI 216 × GVI 304
22	36.6	GVI 216 × GVI 304
23	37.4	GVI 216 × GVI 304
24	38.4	GVI 216 × GVI 304
25	38.9	GVI 216 × GVI 304

Plate 1. Field view

3. 1.2 Efficiency of parents in producing resistant hybrids

Efficiency of male and female parents in producing resistant hybrids were estimated. This is to know the extent of contribution of parental genotypes in producing resistant hybrids.

3. 1.3 Morphological evaluation

Based on the descriptor developed by Bekele and Butler (2000), qualitative and quantitative characters of pod and bean were recorded. The descriptor and the descriptor states for recording observations are mentioned in Table 2.

Table 2. Descriptor and descriptor states used for morphological characters

Sl. No	Character	Descriptor state	Description
1	Colour of ripe pod	3	Light
		5	Intermediate
		9	Dark green
2	Pod shape	1	Cundeamor
		2	Angoleta
		3	Amelonado
		4	Calabacillo
		5	Criollo
3	Colour of ripe pod (Ridge and furrow colour)	0	Absent(green)
		3	Slight(greenish yellow
		5	Intermediate (yellowish green)
		7	Intense (yellow)
4	Pod apex form	1	Attenuate
		2	Acute
		3	Obtuse
		4	Rounded
		5	Mammelate

5	Pod basal constriction	0	Absent
		1	Slight
		2	Intermediate
		3	Strong
6	Husk hardiness(cm) (difference between ridge and furrow)	3	Soft(<1)
		5	Intermediate (1-1.5)
		7	Hard(>1.5)
7	Pod rugosity	0	Absent
		3	Slight
		5	Intermediate
		7	Intense
8	Cotyledon Colour (Bean Colour)	1	White
		2	Grey
		3	Light purple
		4	Medium purple
		5	Dark purple
		6	Mottled
		7	Mixed

3.1.3.1 Qualitative evaluation

Observation recorded on eight qualitative characters were used for further evaluation. The genetic association among genotypes were evaluated by Jaccard's similarity coefficients (Jaccard, 1908) using NTSYS pc version 2.1(Rohlf, 1992). Based on similarity matrix, clustering was performed and the dendrograms were constructed by Unweighted Pair- Group Method (UPGMA) (Sneath and Sokal, 1973).

3. 1. 3. 2 Quantitative evaluation

Observation recorded on ten quantitative characters *viz.* pod weight (g), pod length (cm), pod breadth (cm), husk thickness (cm), number of beans/pod, number of flat beans/pod, total wet bean weight (g) and single dry bean weight(g) were utilized for further evaluation. A measuring device designed by Cocoa Research Centre was used for measuring the length and breadth of pods (Plate 2). Husk

HC

thickness measured as an average of ridge thickness and furrow thickness with the help of vernier calipers (Plate 3).The quantitative characters recorded are detailed below. The data for all quantitative traits recorded were subjected to analysis of variance.

3.1.3.2.1 Number of pods

The data of previous two years on the total number of pods (matured) including the damaged pods due to biotic factors were considered for the analysis.

3.1.3.2.2 Pod weight (g)

The weight of five pods from individual tree was recorded and the average value was calculated to obtain single pod weight.

3.1.3.2.3 Pod length (cm)

The length of pod is measured as its distance from the base of the pod to its apex. The length of five pods from individual tree was recorded and the average value was calculated.

3.1.3.2.4 Pod breadth (cm)

The breadth of five pods from individual tree was recorded and the average value was calculated.

3.1.3.2.5 Wet bean weight/pod (g)

The average weight of wet bean (with mucilage) per pod was calculated from five fresh healthy pod harvested from each tree.

3.1.3.2.6 Number of beans/pod

The number of beans per pod was calculated as the average taken from five fresh pods.
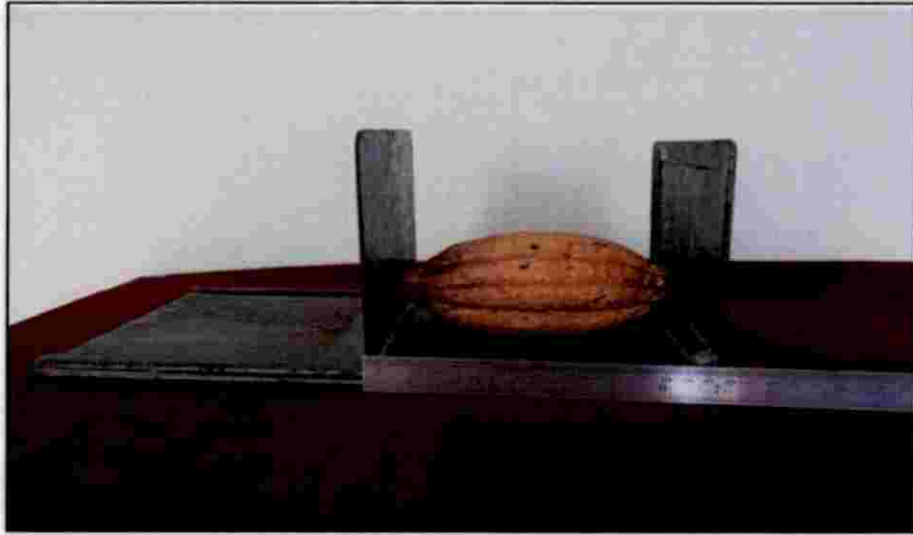
a. Length of pod

b. Breadth of pod

Plate 2. Measurement of length and breadth of pod

Plate 3. Measurement of husk thickness

3.1.3.2.7 Dry weight/bean (g)

The dry weight of a single bean was recorded from the measure of average value calculated over twenty beans which was sun dried.

Clustering of hybrids based on quantitative characters was performed with the help of NTSYS pc version 2.1 software (Rohlf, 1992) and a dendrogram was constructed.

3.2 Comparison of qualitative and quantitative clustering pattern

The percentage of hybrids distributed to various quantitative cluster based on each qualitative clusters were worked out. This will help to find out homology between qualitative and quantitative clustering pattern.

3.3 Clustering based on D^2 statistics

Genetic divergence among the hybrids were computed following the D^2 statistics developed by Mahalanobis (1936).

3.4 Estimation of heterosis in hybrids

To know the superiority of hybrids relative heterosis, heterobeltiosis and standard heterosis were computed using the following formula as proposed by Hayes *et al.* (1965) and Briggle (1963).

3. 4. 1 Relative heterosis (%)

Relative heterosis is the superiority of the hybrid over mid parental value

$$\text{Relative heterosis (\%)} = \frac{F_1 - \text{average performance of both the parents}}{\text{average performance of both the parents}} \times 100$$

3.4.2 Heterobeltiosis (%)

Heterobeltiosis is the superiority of hybrids over better parent

$$\text{Heterobeltiosis (\%)} = \frac{F_1 - \text{performance of better parent}}{\text{performance of better parent}} \times 100$$

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3. 4. 3 Standard heterosis (%)

CCRP 15 was taken as standard check variety. This is a variety released for resistance to another major disease vascular streak die back (VSD) disease. The superiority of hybrid over check was computed.

$$\text{Standard heterosis (\%)} = \frac{F1 - \text{performance of check variety}}{\text{performance of check variety}} \times 100$$

To test the significance of difference of F_1 mean over mid and better parents and check variety, critical difference (CD) was computed. It was calculated from the standard error of difference as given below (Briggle, 1963).

$$CD(0.05) = \text{te' } (0.05) \times \sqrt{2MSE}/r$$
$$= \text{te' } (0.05) \times SE$$

Where, te' - critical value at 5 % level of significance at (p -2df); p- number of parents

MSE - Error mean square

r   - Number of replications

SE   - Standard error of difference between two means

3.5 Estimation of genetic parameters

The descriptive statistics viz., range, mean, standard deviation (SD), standard error (SE), phenotypic coefficient of variation(PCV), genotypic coefficient of variation(GCV), heritability(h^2), genetic advance(GA) for these ten characters were estimated using standard formula.

3.5.1 Range: The range is the size of the smallest interval which contains all the data and provides an indication of statistical dispersion.

3.5.2 Mean: It is the average of all the observations.

3.5.3 Standard Error (SE): Dispersion of family means(X) around the experimental or estimated population mean (X) is SE

$SE = \sqrt{2EMSS}/r$

3.5.4 Standard deviation : It is a measure of how much variability is there between members. It is expressed as σ

$\sigma = \sqrt{\text{Variance}}$

3.5.5 Analysis of variance: It means the splitting of total variation into different components.

Phenotypic variance = Genetic variance + environmental variance

$\sigma^2 p = \sigma^2 g + \sigma^2 e$

Genotypic variance can be calculated using the formula

$V_G = \dfrac{\text{mean sum of squares of treatments} - \text{Error mean sum of squares}}{\text{Number of replications (r)}}$

$V_G = \dfrac{TrMSS - EMSS}{r}$

Phenotypic variance $(V_p) = V_G + V_E$

Environmental variance $(V_E) = ErMSS$

3.5.6 Coefficient of variation (CV): It shows the extent of variability in relation to the mean population and is calculated as the ratio of standard deviation to mean.

Phenotypic Coefficient of variation (PCV) (%) = $\dfrac{\sigma p}{Mean} x\ 100$

Genotypic Coefficient of variation (GCV) (%)= $\dfrac{\sigma g}{Mean} x\ 100$

The PCV and GCV value were classified by Sivasubramanian and Madhavamenon (1973).

0 – 10% - low

10.1 – 20% - moderate; > 20% - high

3.5.7 Heritability (H^2) (%): Heritability is defined as the measure of the correspondence between breeding values and phenotypic values. It gives an estimate of broad sense heritability.

$$H^2 = \frac{vg}{vp} x100$$

The rank of heritability was classified by Robinson et al.,(1949). 0-30% - low; 31-60% - moderate; 61% and above – high

3.5.8 Genetic Advance (GA): It is a measure of genetic gain under selection. The expected genetic advance is estimated with the formula

$$= \frac{\sigma^2 g}{\sigma p} xK; \text{ k=2.06(Selection differential)}$$

GA value was classified by Johnson et al., (1955) .0 – 10% - low; 10.1 – 20% - moderate; > 20% - high

3.5.9 Genetic Advance (GA %): It is usually expressed as the percentage of mean

$$(GA \%) = \frac{GA}{Mean} x\ 100$$

3.6 Assessment of self incompatibility

To assess self incompatibility position in cocoa hybrids hand pollination was carried out. A minimum of 100 flowers were selfed to find out the status of incompatibility (Gigord et al., 1998). Procedure described by Mallika et al. (2000) was followed for the pollination. The day before pollination, the flower buds which would open on the next day were covered with pollination hood, which is a piece of rubber hose closed with muslin cloth and tied with rubber band at one end and sealed to the trunk with glaze putty at other end. This technique restricts the

stigma from receiving any foreign pollen grains. Self-pollination was done by placing anthers of same flower in the stigmatic lobe during early morning hours before stigma loosing viability. Pollination hood is retained for one more day in order to avoid any outcrossing. The pollinated flowers are labeled by using aluminum foil pieces inserted into cushions using ball pins. On the next day after pollination the hoods were removed and the success was ascertained. If no fruit set or if cherelles formed were not retained for more than 15 days the genotypes is classified as self incompatible (SIC). Observations were recorded on number of flowers pollinated, number of fruit set and number of cherelles retained after 15 days.

3. 7 Assessment of General Combining Ability(GCA)

The hybrid progenies which are found to be self-incompatible in nature were considered for further analysis. Among the self incompatible hybrids superior ones were selected based on number of pods/tree/year.

Top cross design was employed and the tester used was GI 5.9. The pollination technique is same as that followed in self pollination. Only difference is that the pollen grains are collected from a tester (G. I. 5.9) which has been used as a tester at CRC from 1989 to estimate the general combining ability (GCA). The seeds obtained from the successful cross was raised in the nursery and screened for initial vigour. Observations like germination percentage, height (cm), girth (cm), diameter (cm), height x diameter^2 (HD^2), number of leaves, presence or absence of early jorquetting and chlorophyll content(Plate 4) were recorded at three month stage since it is reported that early vigour is correlated with final yield (Toxopeus, 1985).The mean data computed were subjected to combining ability analysis by following top cross method developed by Sprague and Tautum (1942). The analysis of variance for parents and top crosses is carried out based on the skeleton of ANOVA (Table 3).

a. Recording height of seedling

b. Recording girth of seedling   c. Recording chlorophyll using SPAD meter

Plate 4. Nursery evaluation of top cross progenies

Table 3. Skeleton of ANOVA for topcross

Sl.no.	Sources of variation	d.f.	SS	MSS
i.	Replication (r)	(r-1)	rSS	rMS
ii.	Entries (g)	(g-1)	gSS	gMS
iii.	Parents (p)	(p-1)	pSS	pMS
iv.	Topcrosses (c)	(c-1)	cSS	cMS
v.	p *vs* c	(p-1) - (c-1)	pcSS	pcMS
vi.	Error (e)	(g-1) (r-1)	eSS	eMS
vii.	Total	(gr-1)	TSS	

CF (correction factor) = GT^2/N; where GT= Grand total and N= number of observations.

(i) Replication sum of squares(rSS) $= \sum\limits_{j}^{r} Tr_j^2 / g - CF$

Replication mean sum of square (rMS) = rSS/ (r-1)

(ii)Entries sum of squares(gSS)= $\sum\limits_{i}^{g} T^2_{gi} / r - CF$

Entries mean sum of squares (gMS) = gSS / (g-1)

(iii)Parent sum of squares(pSS) = $\sum\limits_{i}^{p} T^2_{gi} / r - T^2_p / pr$

Parent mean sum of squares (pMS) = pSS (p-1)

(iv) Top crosses sum of squares (cSS) = $\sum\limits_{i}^{c} T^2_{gi} / r - T^2_c / cr$

Top crosses mean sum of squares(cMS) = cSS/ (c-1)

(v)Parent *vs* top crosses sum of squares(pcSS) = T_p^2 /pr + T_c^2 / cr – CF

(vi)Total sum of squares(TSS) = $\sum\limits_{i}^{g} \sum\limits_{j}^{r} x_{ij}^2$ - CF

(vii) Error sum of square (eSS) = TSS - gSS – rSS

Error mean sum of squares (eMS) = eSS/ (g-1) (r-1)

Breeding value (A) denotes the GCA effect of the individual in top cross analysis. Higher the value of A, greater is the GCA effect which is a fixable component. It is calculated as per the standard normal deviate procedure (Sharma, 1988).

Breeding value (A) = A'_i/ SD (A')

$= (\bar{c_i} - \bar{c}) / SD (A')$

Where, SD – Standard deviation

Variance $(A'_i) = \sum A_i^2 / (p-1)$

Var (A) = $\sum A_i^2$

For hybrids which did not yield any pod by top crossing, open pollinated pods were selected for further analysis considering the fact that since they are self incompatible all the pods produced will be hybrid pods. The observations on germination percentage, height (cm), girth (cm), diameter (cm), height x diameter^2 (HD^2), number of leaves, presence or absence of early jorquetting and chlorophyll content were recorded at three month stage and data were subjected to analysis of variance

3.8 Screening of seedling for disease resistance

3.8.1. Nursery screening of seedling for disease resistance:

The seeds developed were raised in the nursery. The visual screening of seedlings was done in nursery for every two weeks interval and observations were recorded.

3.8.2. *In vitro* screening of seedling

The *in vitro* screening of seedlings to *Phytophthora palmivora* was assessed by using a modified method of detached leaf disc assays (Tahi *et al.*, 2000, Tahi *et al.*, 2006, Kurian, 2011).

3.8.2.1 Isolation of the pathogen

The naturally infected pods from the CRC cocoa farm were collected and washed thoroughly in running water. The pods were wiped with ethyl alcohol (70%). These bits were then surface sterilized in one per cent sodium hypochlorite solution for one minute. They were washed in sterile water for three times followed by placing the bits in sterile filter paper in the petriplate for the absorption of excess water. These bits were transferred to the petridishes containing Potato Dextrose Agar media (PDA) (Table 4) and incubated at 28+/- 2^0C.The growth of fungal hyphae starts four days after inoculation. Sub culturing was done at an interval of fifteen days. The subcultured seven day old culture were used for the leaf inoculation for *in vitro* screening of seedling for disease resistance (Plate 5).

Plate 5. Seven day old culture of *Phytophthora palmivora*

Table 4.Composition of PDA media

Potato	200g
Dextrose	20g
Agar	20g

3.8.2.2 Leaf inoculation technique

Leaf inoculation was done on immature green leaves of 10-15 cm long, flexuous and semi translucent (Bailey *et al.*, 2005). The leaves were first washed three times with sterile water and disinfested with ethyl alcohol (70%). It was placed with adaxial side up on moist sterile filter paper discs placed in sterile petri dishes (20cm). Pinpricks were given at the centre of midrib of leaves using sterilized needles. Over the pinpricks, placed the culture disc (10mm) of *Phytophthora palmivora* taken from seven day old culture grown on PDA media. The petridishes were covered with lid which is laden with a piece of moist cotton. It is done in three replications and leaves taken from susceptible clone inoculated with pathogen served as control. It was observed for 10 days for intensity of infestation and compared with the control. The per cent of leaf area infected was calculated as per the formula (Tahi *et al.*, 2000;Tahi *et al.*, 2006; Kurian, 2011).

$$\text{Per cent leaf area infection (\%)} = \frac{\text{Length} \times \text{breadth of lesion}}{\text{Length} \times \text{breadth of leaf}} \times 100$$

Results & Discussion

4. RESULTS AND DISCUSSION

The present study was conducted in the Department of Plantation Crops and Spices, College of Horticulture and the Cocoa Research Centre (CRC), Vellanikkara during 2014-2016 with the objective to evaluate the incompatibility and general combining ability of selected cocoa hybrids resistant to *Phytophthora palmivora* so as to identify superior parents for further use in breeding programme.

The results of the study are presented below and the details of hybrids are presented in Table 1.

4.1 Efficiency of parents in producing resistant hybrids

Five female and five male parents were used in crossing programme resulted in 615 hybrids and screened for vigour and disease incidence in the nursery. After the initial screening in the nursery, 615 hybrids seedlings were field planted and 160 hybrids established in field. Out of 615 hybrids, 25 hybrids did not show any symptom of *Phytophthora* pod rot disease incidence for six years served as the material for the study.

4.1.1 Efficiency of female parent in producing resistant hybrids

Table 5 comprises the relative efficiency of female parent to transfer *Phytophthora* pod rot resistance to their progenies. Here five female parents were used to evolve 160 field established hybrids. When the potential of these parents with respect to producing resistant hybrids were estimated, it was seen that G VI 216 produced maximum number of resistant hybrids(12). But the recovery percentage was more for S IV 5.20 (40%).

Table 5. Efficiency of female parent in producing the resistant hybrids

Female parent	Total no. of hybrids field planted	No. of hybrids with field resistance after six years	Recovery % of resistant hybrids
SIV 1.26	50	4	8
GVI 188	54	7	13
SIV 5.20	5	2	40
SIV 6.18	5	0	0
GIV 216	46	12	26
Total		160	25

4.1.2 Efficiency of male parent in producing the resistant hybrids

Five male parents were used in the breeding programme. Both maximum number of hybrids and recovery percent were recorded in G VI 304. Hence it can be considered as a potential parent.

Table 6. Efficiency of male parent producing the resistant hybrids

Male parent	Total no. of hybrids field planted	No. of hybrids shown field resistance after six yrs.	Recovery % of resistant hybrids
TISSA	25	4	16
PII 12.11	35	2	5.7
GVI 287	42	4	10
GVI 304	41	11	27
GVI 294	17	4	23.5
	160	25	15.6

Combined efficiency of both the parents (G VI 216 × G VI 304) resulted in production of maximum number of resistant hybrids (10). The genotype G VI 216 is ICS 47 and G VI 304 is a local collection from Wayanad. ICS 47 was reported to show ample resistance to *Phytophthora* pod rot (Iwaro *et al.*, 2006)

and G VI 304 was collected from farmer's field based on their vigour and resistance to disease (CCRP report, 1993).

4. 2 Qualitative evaluation

The observations recorded on various qualitative characters of hybrids are presented in Table 7 and Table 8.

Wood and Lass (1955) had described the characters of different types of pods and according to their findings the morphology of pod was determined by different characters of independent inheritance. They had described the angoleta types assquare shaped at the stalk end and deeply rigid, warty and devoid of bottleneck. Cundeamor types are similar to the angoleta but characterized by bottle neck. Amelando types are characterized by the presence of pods having smooth, shallow furrow, slight bottle neck and are in melon shape with blunt end. The pods with nearly spherical shape, small size and with a point at its apex are the features of calabacillo type. Criollo types are with high rugosity, red colour and with pointed tip. From the twenty five hybrids evaluated, hybrid H2 alone was observed to be having calabacillo type pods. Angoleta, amelonado, cundemor and criollos were noticed in other hybrids.

The pod apex was found to be mammelate only in the hybrid H2 and the remaining hybrids were having acute, obtuse and types of attenuate pod apex. The hybrid H22 evolved from the cross of GV1 216 × GV1 304 alone was having intermediate type of basal constriction. However in most of the hybrids, the rugosity of the pods was intense or the surface was rough. This may be one of the factor contributing to *Phytophthora* resistance expressed by these hybrids as discussed by some early workers (Nydanu *et al.*, 2012).

Table 7. Pod shape and rugosity of hybrids

Hybrids	Genotype	Pod shape	Pod apex	Pod basal constriction	Pod rugosity
H1	SIV 1.26 x TISSA	Angoleta	Acute	Slight	Intermediate
H2	SIV 1.26 x TISSA	Calabacillo	Mammelate	Absent	Slight
H3	SIV 1.26 x P II 12.11	Angoleta	Acute	Slight	Intermediate
H4	SIV 1.26 x P II 12.11	Amelonado	Obtuse	Absent	Slight
H5	G VI 188 X GVI 287	Angoleta	Acute	Slight	Intermediate
H6	G VI 188 X GVI 287	Angoleta	Acute	Slight	Intermediate
H7	G VI 188 X GVI 287	Angoleta	Acute	Absent	Intermediate
H8	G VI 188 X GVI 287	Amelonado	Acute	Absent	Slight
H9	GVI 188 X GVI 304	Amelonado	Acute	Absent	Slight
H10	GVI 188 X GVI 304	Angoleta	Acute	Absent	Intermediate
H11	GVI 188 X GVI 304	Angoleta	Slight	Absent	Intense
H12	SIV 5.20 X TISSA	Angoleta	Attenuate	Absent	Intermediate
H13	SIV 5.20 X TISSA	Angoleta	Attenuate	Absent	Intermediate
H14	GVI 216 X GVI 294	Angoleta	Acute	Absent	Slight
H15	GVI 216 X GVI 294	Angoleta	Acute	Absent	Intermediate
H16	GVI 216 X GVI 294	Cundeamor	Attenuate	Slight	Intense
H17	GVI 216 X GVI 294	Cundeamor	Attenuate	Slight	Intense
H18	GVI 216 X GVI 304	Criollo	Acute	Slight	Intense
H19	GVI 216 X GVI 304	Criollo	Acute	Slight	Intense
H20	GVI 216 X GVI 304	Angoleta	Acute	Slight	Intense
H21	GVI 216 X GVI 304	Angoleta	Acute	Slight	Intermediate
H22	GVI 216 X GVI 304	Cundeamor	Attenuate	Intermediate	Intense
H23	GVI 216 X GVI 304	Criollo	Acute	Slight	Intense
H24	GVI 216 X GVI 304	Criollo	Acute	Absent	Intense
H25	GVI 216 X GVI 304	Criollo	Attenuate	Slight	Intense

Table 8. Pod and bean colour of cocoa hybrids

Hybrids	Colour of unripe pod	Colour of ripe pod		Bean colour
		Ridges	Furrows	
H1	Light green	Yellow	Yellow	Dark purple
H2	Light green	Greenish yellow	Greenish yellow	Dark purple
H3	Intermediate green	Greenish yellow	Greenish yellow	Light Purple
H4	Intermediate green	Greenish yellow	Greenish yellow	Light Purple
H5	Light green	Yellow	Yellow	Medium Purple
H6	Light green	Greenish yellow	Greenish yellow	Light Purple
H7	Light green	Greenish yellow	Greenish yellow	Medium Purple
H8	Light green	Greenish yellow	Greenish yellow	Light Purple
H9	Light green	Yellow	Yellow	Light Purple
H10	Light green	Yellow	Yellow	Medium Purple
H11	Light green	Greenish yellow	Greenish yellow	Light Purple
H12	Light green	Greenish yellow	Greenish yellow	Light Purple
H13	Light green	Greenish yellow	Greenish yellow	Light Purple
H14	Light green	Greenish yellow	Greenish yellow	Dark purple
H15	Light green	Greenish yellow	Greenish yellow	Medium Purple
H16	Light green	Yellow	Yellow	Dark purple
H17	Light green	Greenish yellow	Greenish yellow	Light Purple
H18	Light green	Greenish yellow	Greenish yellow	Dark Purple
H19	Light green	Greenish yellow	Greenish yellow	Dark Purple
H20	Light green	Greenish yellow	Greenish yellow	Medium Purple
H21	Light green	Greenish yellow	Greenish yellow	Dark purple
H22	Light green	Greenish yellow	Greenish yellow	Dark purple
H23	Light green	Greenish yellow	Greenish yellow	Medium Purple
H24	Light green	Greenish yellow	Greenish yellow	Medium Purple
H25	Light green	Greenish yellow	Greenish yellow	Light Purple

The phenotypic appearance of cocoa pods plays an important role in defining the types and populations (Efombagn *et al.*, 2009). The colour of unripe pod was observed to be light green in majority of the hybrids except in the hybrid H3and H4 (Table 9). The colour of the ripe pod was marked by the colour of its ridges and furrows. It was observed that the progenies from the same cross exhibited variation in the colour of ridges and furrows. This is evident from the hybrids H1 and H2 of the crosses(S IV 1.26 × TISSA), H5 and H6 (G VI 188 × G VI 287), H10 and H11 (G VI 188 × G VI 304), and H15 and 16(G VI 216 × G VI 294). However all the hybrids exhibited forester character.

Among the hybrids, variability was observed for bean colour. From Table 9 it is clear that the progenies from the same parents showed colour variations. The hybrids H14 and H16 from the cross G VI 216 × G VI 294 showed dark purple, H17 light purple and H15 with medium purple colour. Velayutham *et al.* (2013) in their study on cocoa reported that there was wide variability among progenies obtained from same cross.

4. 3 Quantitative evaluation

4.3.1 Yield contributing characters

Analysis of variance was done for yield contributing characters and presented in Table 9. Significant variation was observed between the hybrids for all the characters studied.

Among the hybrids, hybrid H22 recorded the highest pod weight (724g) followed by H18 (644g). It was also having the highest value of total wet bean weight/ pod (176.98g) and fourth highest value for number of beans (44.20). The character number of beans alone does not contribute to final yield. High bean count with small sized beans is an undesirable character (Engles, 1982). Here even though the hybrid had less number of beans, single peeled dry bean weight was above international standard of 0.8 g (GOI, 1997).

The hybrid H9 recorded lowest pod weight and wet bean weight^{-1} pod ie.,176g and 42.10g respectively. The hybrid H6 with the lowest value of pod length(8.04cm), pod breadth (5.14 cm) recorded the highest number of beans (46.40).

The pod length was highest for hybrid H16 (17.36 cm) followed by hybrid H22 (17.26 cm) and pod breadth was observed to be higher in the hybrid H18 (8.58 cm) followed by hybrid H13 (8.40 cm).

The single peeled dry bean weight/ pod recorded highest in the hybrid H17(1.16g) followed by hybrid H15 and H5 (1.09g) and was lowest in the hybrid H2 (0.64g). Enriquez and Soria (1966) explained that bean yield was expressed as the dry or wet weight of bean. They opined high variability in weight of single bean within a single pod. The desirable dry weight of peeled bean was 0.8g or more as per the international standard(GOI, 1997).Out of the twenty five hybrids evaluated, five failed to satisfy the international standard. Monteiro *et al.* (2009) also reported that cocoa accessions with dry weight/ bean (peeled or not) more than 0.8g were superior.

Table 9. Mean values of yield contributing characters of hybrids

Sl.no	Hybrid no.	Pod weight (g)	Pod length (cm)	Pod breadth (cm)	Number of beans	Total wet bean weight(g)	Single dry bean weight(g)
1	H1	199.00^{hi}	14.80^{cde}	7.40^{de}	33.60^{fg}	48.02^{jk}	0.85^{fg}
2	H2	238.00^{fghi}	12.22^{hi}	6.22^{g}	35.40^{efg}	63.34^{ij}	0.64^{j}
3	H3	272.00^{efg}	11.56^{hi}	6.12^{g}	28.20^{h}	87.98^{fg}	1.01^{bcd}
4	H4	584.00^{bc}	14.60^{cde}	7.70^{bcd}	39.60^{cde}	134.26^{b}	0.85^{g}
5	H5	324.00^{e}	12.24^{hi}	6.70^{efg}	33.60^{fg}	67.38^{hi}	1.09^{ab,}
6	H6	296.00^{ef}	8.04^{j}	5.14^{h}	46.40^{a}	94.72^{dfg}	0.96^{cde}
7	H7	538.00^{c}	15.92^{abc}	8.32^{abc}	45.40^{a}	135.54^{b}	1.04^{bc}
8	H8	432.00^{d}	11.90^{hi}	7.78^{abcd}	42.40^{abc}	104.56^{def}	0.83^{gh}
9	H9	176.00^{i}	13.88^{efg}	5.98^{gh}	36.80^{def}	42.10^{k}	0.82^{gh}
10	H10	312.00^{e}	12.28^{hi}	7.56^{cd}	40.60^{bcd}	107.44^{de}	0.84^{gh}

11	H11	240.00^{fgh}	12.60^{fghi}	6.66^{efg}	36.20^{efg}	82.84^{gh}	0.81^{gh}
12	H12	228.00^{ghi}	11.34^{i}	6.42^{g}	34.60^{fg}	68.96^{hi}	0.75^{hi}
13	H13	462.00^{d}	15.76^{bc}	8.40^{abc}	44.00^{ab}	130.10^{bc}	0.86^{g}
14	H14	286.00^{efg}	12.60^{fghi}	7.32^{def}	32.00^{gh}	61.84^{ij}	0.72^{ij}
15	H15	474.00^{d}	14.84^{cde}	7.98^{abcd}	37.00^{def}	126.96^{bc}	1.09^{ab}
16	H16	444.00^{d}	17.36^{a}	8.42^{ab}	35.60^{efg}	126.16^{bc}	1.00^{bcd}
17	H17	544.00^{c}	15.92^{abc}	7.92^{abcd}	44.80^{ab}	131.50^{bc}	1.16^{a}
18	H18	644.00^{b}	15.22^{cde}	8.58^{a}	40.60^{bcd}	118.62^{bcd}	0.83^{gh}
19	H19	328.00^{e}	12.94^{fgh}	6.44^{g}	32.00^{gh}	88.04^{fg}	0.83^{gh}
20	H20	468.00^{d}	15.50^{cd}	7.86^{abcd}	37.20^{def}	116.20^{cd}	0.90^{efg}
21	H21	432.00^{d}	14.64^{cde}	7.36^{de}	44.00^{ab}	89.61^{fg}	0.66^{ij}
22	H22	724.00^{a}	17.26^{ab}	7.44^{de}	44.20^{ab}	176.98^{a}	0.94^{def}
23	H23	310.00^{e}	12.36^{ghi}	6.04^{g}	42.40^{abc}	83.84^{gh}	0.86^{fg}
24	H24	246.00^{fgh}	14.00^{def}	8.02^{abcd}	33.40^{fg}	57.83^{ijk}	0.88^{efg}
25	H25	230.00^{ghi}	12.94^{fgh}	6.50^{fg}	34.40^{fg}	57.56^{ijk}	0.68^{ij}
	CD(0.05)	62.43	1.56	0.85	4.34	16.93	0.09

Table 10. Husk thickness and number of flat beans in hybrids

Hybrids	Husk thickness (cm)	Number of flat bean/pod
H1	0.96^{ef}	4.20^{ab}
H2	0.97^{ef}	3.20^{bc}
H3	1.84^{a}	2.80^{cde}
H4	1.31^{bcde}	4.20^{ab}
H5	1.15^{ef}	2.20^{cdefg}
H6	1.12^{ef}	2.60^{cdef}
H7	1.33^{bcde}	5.00^{a}
H8	1.13^{ef}	2.00^{defg}
H9	1.27^{cdef}	3.00^{cd}
H10	0.94^{ef}	1.60^{fg}
H11	1.69^{abc}	1.40^{g}
H12	0.95^{ef}	2.20^{cdefg}

H13	1.24^{def}	2.00^{defg}
H14	0.92^{ef}	2.80^{cde}
H15	1.64^{abcd}	1.80^{efg}
H16	0.86^{f}	1.80^{efg}
H17	1.27^{cdef}	2.40^{cdefg}
H18	1.00^{ef}	2.60^{cdef}
H19	1.37^{bcde}	1.80^{efg}
H20	1.03^{ef}	2.20^{cdefg}
H21	1.17^{ef}	2.00^{defg}
H22	1.09^{ef}	2.20^{cdefg}
H23	1.72^{ab}	2.80^{cde}
H24	0.96^{ef}	2.60^{cdef}
H25	1.14^{ef}	1.60^{fg}
CD(0.05)	0.44	1.20
CV	29.47	37.81

The husk thickness was highest for the hybrid H3 (1.84 cm), followed by hybrid H23 (1.72cm) and was lowest in hybrid H16 (0.86 cm).Variability of husk thickness was observed between pods (Soria, 1975). As described by Enriquez and Soria (1966), husk thicknesses are descriptive traits and are partially influenced by environment. Lower husk thickness is the desirable character. Flat bean in cocoa is considered to be unfertilized ovule and it is an undesirable character (Asna *et al.*, 2014). The hybrid H7reported highest average number of flat beans (5), followed by hybrid H1 and H4 (4.20) and was lowest in hybrid H11 (1.40). The breeding programme has to be designed in such a manner to reduce the number of flat beans (Rubeena, 2015).

4.4 Diversity analysis

4.4.1 Cluster analysis based on qualitative characters

Cluster analysis was done based on Jaccards similarity coefficient using UPGMA methods considering the qualitative traits and the dendrogram was constructed. In the present study, 25 hybrids selected were grouped into 10 clusters at 60% similarity level (Fig.1) and the details are presented in Table 11.

It was found that majority of the accessions were grouped in cluster V and the hybrids H3, H6, H7, H12, H13, H14, H15, H20, H21, and H22 belonged to the same cluster showed high amount of similarity to one another with respect to qualitative characters. From the dendrogram (Fig. 1) it can be seen that hybrids H14 and H15 evolved from the same parental cross combination, G VI 216 × G VI 294 and H20, H21, H22 from G VI 216 × G VI 304 shared some amount of similarity at qualitative level. The similar results with the hybrids H23 and H24 from the parental combination, G VI 216 × G VI 304. The results indicated that parental characters were inherited to the progeny *ie* the qualitative characters considered were heritable in nature (Kosev and Naydenova, 2015). The clusters II, III, IV, VI, VII, and X were found to have single accessions in each of them.
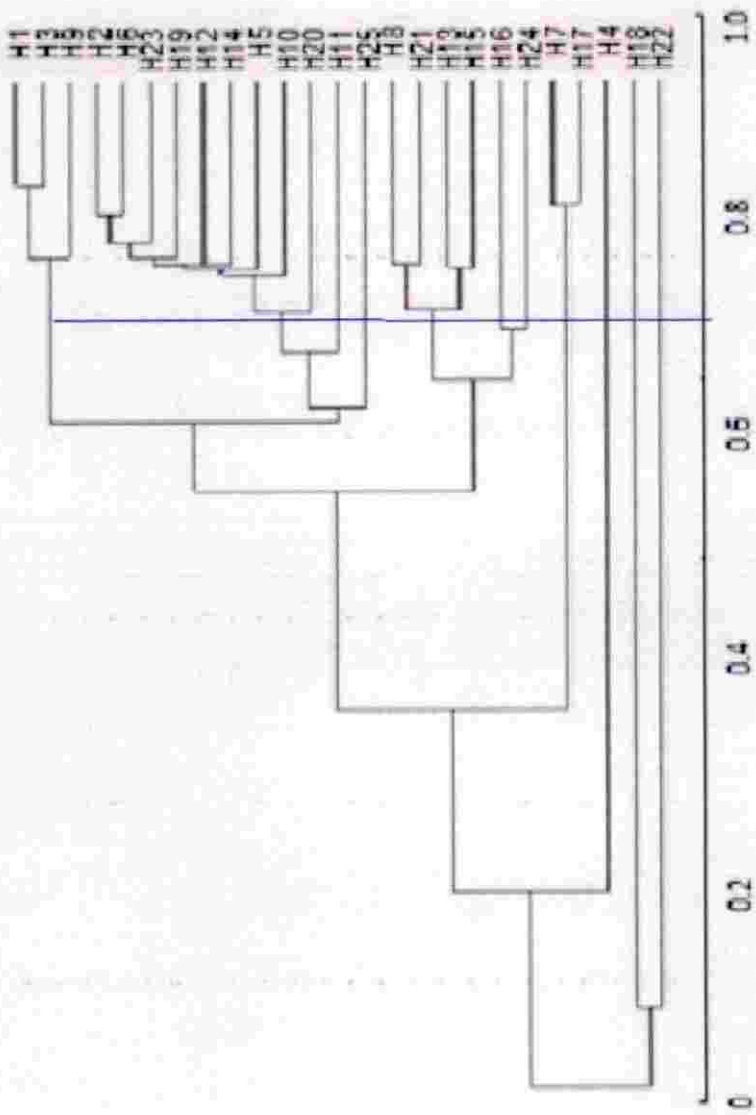
Fig 1 . Dendrogram based on similarity coefficient for qualitative character

Table 11. Clustering of cocoa hybrids based on qualitative characters

Cluster No.	No. of accession	Name of accession
I	3	H1, H5, H10
II	1	H16
III	1	H9
IV	1	H2
V	10	H3, H6, H12, H13, H7, H15, H21, H14, H20, H22
VI	1	H8
VII	1	H4
VIII	3	H11, H17, H18
IX	3	H19, H23, H24
X	1	H25

4.4.2 Cluster analysis based on quantitative characters

Cluster analysis was done based on Jaccards similarity coefficient using UPGMA methods considering ten quantitative traits and the dendrogram was constructed. In the present study, 25 hybrids selected were grouped into 11 clusters at 70% similarity level (Fig. 2) and the details are presented in Table 12.

Cluster II was the biggest one with 9 hybrids (H2, H6, H23, H19, H12, H14, H5, H10 and H20).The cluster III, IV, VI, VII, VIII, IX, X and XI were found to have only one hybrid each.

From the dendrogram (Fig 2), it can be seen that the hybrids H1 and H2evolved from the same cross S IV 1.26 × TISSA were placed in clusters I & II respectively. Similarly the hybrids H3 and H4 (S IV 1.26 × P II 12.11) in cluster I and IX, hybrids H5, H6, H7, H8 (G VI 188 × G VI 287) in cluster II, V, VIII, hybrids H9, H10, H11 (G VI 188× G VI 304) in cluster I, II, III, hybrids H12 & H13(S IV 5.20 × TISSA) in cluster II & V, hybrids H14, H15, H16, H17(G VI

216 × G VI 294) in cluster II, V, VI, VII and hybrids H18, H19, H20, H21, H22, H23, H24 & H25(G VI 216 × G VI 304) in cluster II, IV, V, VII, X, XI. This indicated that progenies belonging to the same crosses showed variation in their quantitative character. The result was in accordance with the findings of some early workers (Pang and Lockwood, 2008; Minimol *et al.*, 2016) that the progenies of same cross exhibited wide variability among themselves.
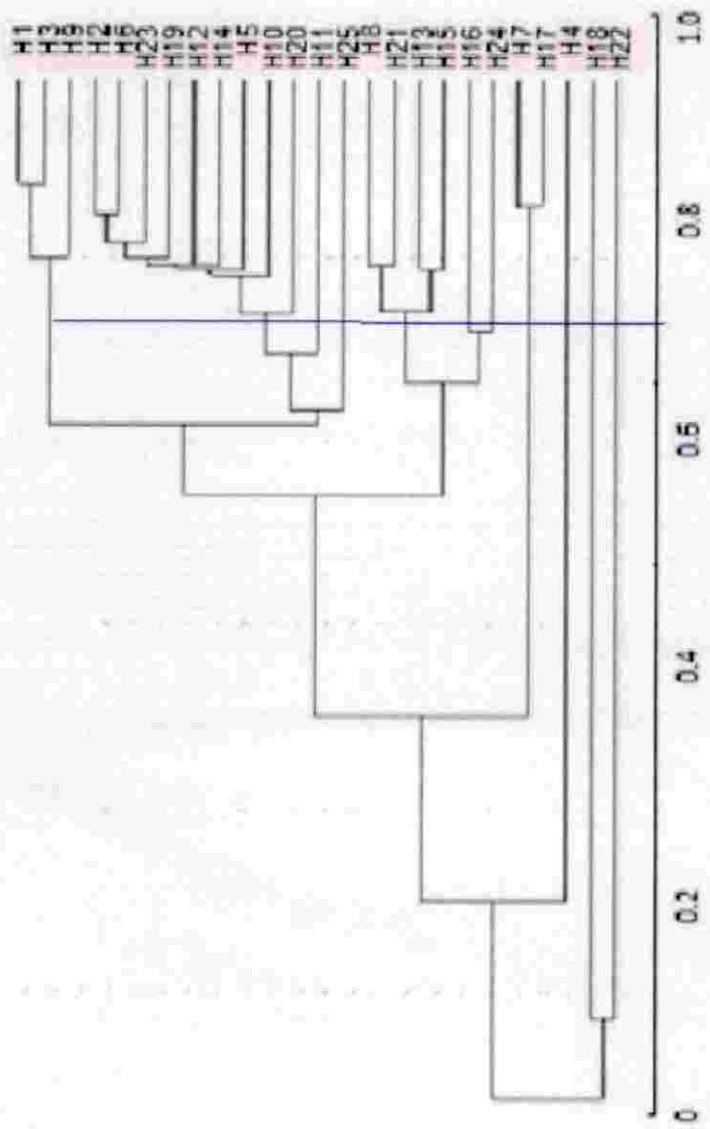
Fig 2. Dendrogram based on similarity coefficient for quantitative character

Table 12.Clustering of cocoa based on quantitative characters

Cluster No.	No. of accession	Name of accession
I	3	H1, H3, H9
II	9	H2, H6, H23, H19, H12, H14, H5, H10, H20
III	1	H11
IV	1	H25
V	4	H8, H21, H13, H15
VI	1	H16
VII	1	H24
VIII	2	H7, H17
IX	1	H4
X	1	H18
XI	1	H22

4.4.3 Comparison of qualitative and quantitative clustering patterns

In order to find out the homology between the qualitative and quantitative clustering patterns, the percentage of distribution of cocoa hybrids belonging to each cluster of qualitative traits into different quantitative clusters were estimated and presented in Table 13.

All the hybrids present together in each qualitative cluster were distributed among different cluster when they were grouped based on quantitative characters. The ten hybrids were present together in qualitative cluster V were distributed among five quantitative clusters ie.cluster I (10%), cluster II (40%), cluster V (30%), cluster VIII (10%) and cluster XI (10%). Similarly three hybrids clustered together in qualitative cluster VIII were distributed among three quantitative clusters and three hybrids in qualitative cluster IX were distributed among two clusters (II and IV) of quantitative clusters with a percentage of 66.66 and 33.33 respectively. This indicated that similarity expressed based on visual appearance

like colour, shape etc was not actually correlated with quantitative traits that decide the yield and yield contributing character of that genotype. Similar results were reported by Asna (2014) and Rubeena (2015).

Table 13.Homology between the qualitative and quantitative clustering patterns of cocoa hybrids.

Qualitative cluster	No. of hybrids	Quantitative cluster										
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	3	33.33	66.66									
II	1						100					
III	1	100										
IV	1		100									
V	10	10	40			30						10
VI	1					100						
VII	1									100		
VIII	3			33.33					33.33		33.33	
IX	3		66.66					33.33				
X	1				100							

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4.4.4 D^2 statistics

D^2 statistics based on the concept for measuring the group distance developed by Mahalanobis (1928) was carried out for 25 hybrids. This had been used as a potential technique for assessment of genetic diversity in plant breeding by number of scientists (Murthy *et al.*, 1967; Ram and Panwar, 1970; Engles, 1986). According to Dias and Kageyama(1997), there existed a relationship between genetic divergence and combining ability and the most divergent cultivar exhibited a high general combining ability, thereby generating the best performing hybrids.

In the present study, all the 25 hybrids could be meaningfully grouped into eight clusters (Table 14). Only one cluster(cluster VIII) was having a solitary hybrid(H22). The cluster I was with maximum number of hybrids(6) followed by cluster II (5).

Table 14.Clustering based on D^2 Statistics

Cluster no.	No. of hybrids	Name of hybrids
1	6	H1,H2,H11,H12,H14,H25
2	5	H7,H13,H15,H16,H17
3	3	H8, H10, H20
4	2	H6, H23
5	4	H3, H5, H19,H24
6	2	H9,H21
7	2	H4,H18
8	1	H22

The intra and inter cluster distance between the clusters are presented in the Table 15. The maximum intra cluster distance (50.86) was observed in cluster VI eventhough only two hybrids were grouped together. This indicated an amount of much high divergence among these hybrids eventhough they were clustered together. Cluster I with maximum number of hybrids showed an intra cluster distance of only 22.25 indicated high level of similarity between these hybrids.

Maximum inter cluster divergence was observed between cluster I and cluster VIII which indicated the genetic diversification among these clusters (Table 16) and (Fig.3). Similarly large divergence was also expressed between members of cluster IV and clusters VIII (117.91), cluster V and cluster VIII(121.17), cluster VI and cluster VIII (126.10). This indicated that the unique member placed in cluster VIII (H22) is highly divergent from other hybrids.

Table 15. Intra and inter cluster distances between clusters of cocoa hybrids

Cluster	I	II	III	IV	V	VI	VII
I	22.25						
II	99.05	23.26					
III	46.51	42.53	26.37				
IV	63.78	63.95	42.96	37.00			
V	44.15	49.92	38.52	47.10	23.44		
VI	57.82	72.40	61.66	56.63	49.63	50.86	
VII	97.82	35.39	40.91	73.39	64.88	76.42	14.28
VIII	174.81	45.41	86.87	117.91	121.17	126.10	36.31
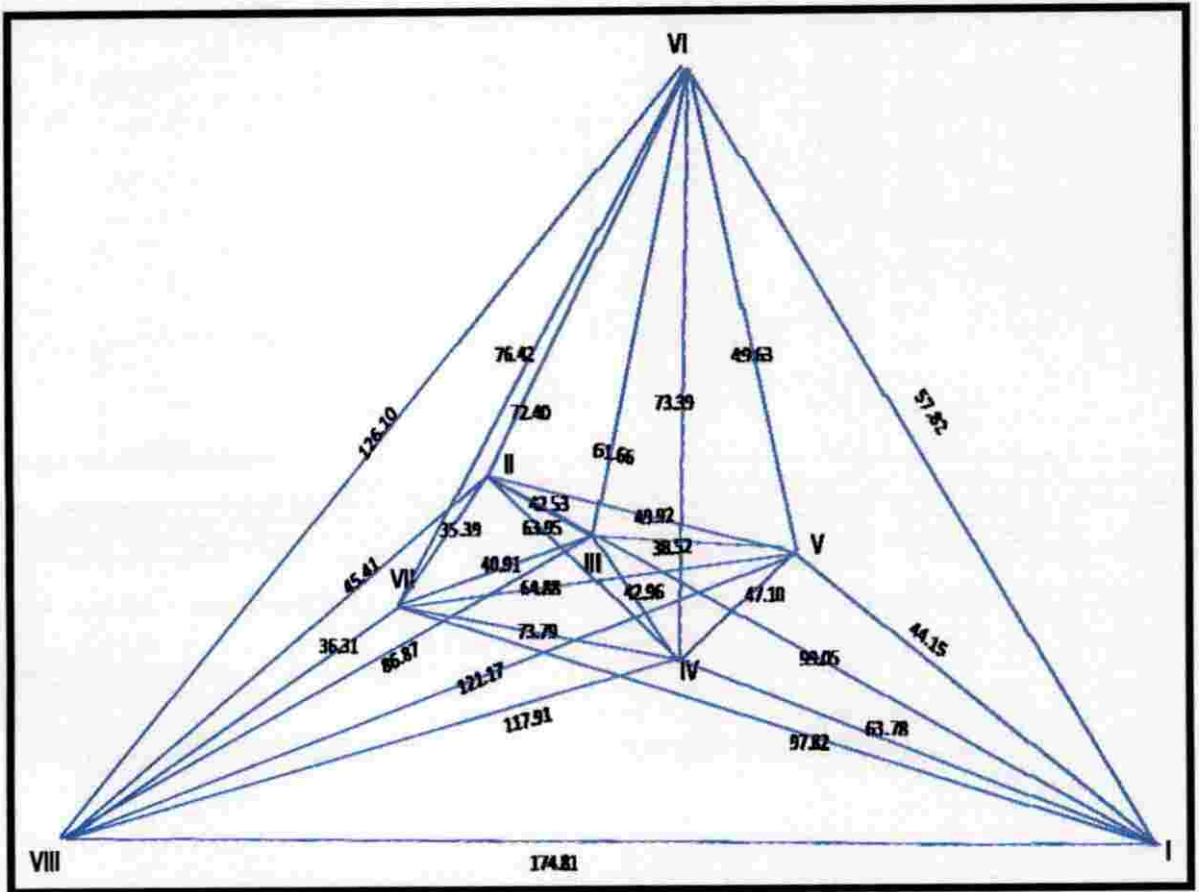
Fig. 3.Cluster diagram for cocoa hybrids

4.4.5 Estimation of heterosis

Heterosis is an unexpected significant deviation from the average of two parental lines (Kendall *et al.*, 2013). High level of heterosis was exhibited in cocoa for yield and yield contributing characters (Atlanda and Toxopeus, 1971; Dias and Kagemah, 1997). Posnette (1943) was the first to explain inter population heterosis in cocoa and later it was widely exploited by many breeders (Toxopeus, 1985; Minimol *et al.*, 2011).

4.4.5.1Relative heterosis

Relative heterosis was estimated as the superiority of hybrids over mid parental value. The parental characters were represented in Table 16. Most of the hybrids expressed significant negative heterosis (Table 17) for pod length, pod breadth, total wet bean weight and single dry bean weight.

4.4.5.2 Heterobeltiosis

It refers to superiority of hybrid over the better parent and the value is presented in Table 18. Here also the result is in tune with relative heterosis showing a significant negative value.

The field was maintained under overshaded condition to facilitate maximum infestation to *Phytophthora palmivora*. The inferiority of performance of the hybrids may be due to this over shaded condition maintained in the field. This conclusion was supported by the findings of many scientist (Mooleedhar and Lauckner, 1990; Lockwood and Pang, 1996) stating that yield and general vigour of cocoa was heavily influenced by planting density and shade. Lockwood and Pang(1996) reported that Upper Amazon clones with high vigour showed a reduction of three times when grown under high shaded condition.

Table 16. Parental characters of the hybrids

Hybrid No.	Pod length (cm)		Pod breadth (cm)		Number of beans		Pod weight (g)		Total wet bean wt.(g)		Single dry bean wt.(g)	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
H1	12.70	13.90	6.90	7.50	36.93	44.20	639.50	240.00	160.30	120.40	1.30	0.87
H2	12.70	13.90	6.90	7.50	36.93	44.20	639.50	240.00	160.30	120.40	1.30	0.87
H3	12.70	14.00	6.90	16.30	36.93	45.50	639.50	280.00	160.30	170.00	1.30	1.09
H4	12.70	14.00	6.90	16.30	36.93	45.50	639.50	280.00	160.30	170.00	1.30	1.09
H5	12.80	14.70	6.90	8.10	36.93	39.80	249.00	464.60	80.40	113.00	1.30	1.00
H6	12.80	14.70	6.90	8.10	36.93	39.80	249.00	464.60	80.40	113.00	1.30	1.00
H7	12.80	14.70	6.90	8.10	36.93	39.80	249.00	464.60	80.40	113.00	1.30	1.00
H8	12.80	14.70	6.90	8.10	36.93	39.80	249.00	464.60	80.40	113.00	1.30	1.00
H9	12.80	16.00	6.90	8.50	36.93	31.00	249.00	360.00	80.40	85.00	1.30	1.10
H10	12.80	16.00	6.90	8.50	36.93	31.00	249.00	360.00	80.40	85.00	1.30	1.10
H11	12.80	16.00	6.90	8.50	36.93	31.00	249.00	360.00	80.40	85.00	1.30	1.10
H12	14.50	13.90	8.20	8.20	55.10	44.20	300.00	240.00	115.50	120.40	0.82	0.87
H13	14.50	13.90	8.20	8.20	55.10	44.20	300.00	240.00	115.50	120.40	0.82	0.87
H14	18.28	18.00	9.00	8.00	31.85	23.00	637.14	530.00	121.40	100.00	1.16	1.20
H15	18.28	18.00	9.00	8.00	31.85	23.00	637.14	530.00	121.40	100.00	1.16	1.20

H16	18.28	18.00	9.00	8.00	31.85	23.00	637.14	530.00	121.40	100.00	1.16	1.20
H17	18.28	18.00	9.00	8.00	31.85	23.00	637.14	530.00	121.40	100.00	1.16	1.20
H18	18.28	16.00	9.00	8.50	31.85	31.00	637.14	360.00	121.40	85.00	1.16	1.10
H19	18.28	16.00	9.00	8.50	31.85	31.00	637.14	360.00	121.40	85.00	1.16	1.10
H20	18.28	16.00	9.00	8.50	31.85	31.00	637.14	360.00	121.40	85.00	1.16	1.10
H21	18.28	16.00	9.00	8.50	31.85	31.00	637.14	360.00	121.40	85.00	1.16	1.10
H22	18.28	16.00	9.00	8.50	31.85	31.00	637.14	360.00	121.40	85.00	1.16	1.10
H23	18.28	16.00	9.00	8.50	31.85	31.00	637.14	360.00	121.40	85.00	1.16	1.10
H24	18.28	16.00	9.00	8.50	31.85	31.00	637.14	360.00	121.40	85.00	1.16	1.10
H25	18.28	16.00	9.00	8.50	31.85	31.00	637.14	360.00	121.40	85.00	1.16	1.10

Table 17. Relative heterosis of the hybrids

Hybrid	Pod weight (g)	Pod length (cm)	Pod breadth (cm)	Number of beans	Total wet bean weight (g)	Single dry bean weight (g)
H1	11.28	2.78*	-17.17*	-54.75*	-65.79*	-21.47*
H2	-8.12	-13.61*	-12.73*	-45.88*	-54.87*	-41.38*
H3	-13.41	-47.24*	-31.58*	-40.84*	-46.73*	-15.31*
H4	9.36	-33.62*	-3.92*	27.03*	-18.70	-29.04*
H5	-10.98	-10.67*	-12.42*	-9.19*	-30.32	-5.39*
H6	-41.53	-31.47*	20.94*	-17.04*	-2.05	-16.52*
H7	15.78	10.93*	18.34*	50.78*	40.17*	-9.57*
H8	-13.45	3.73*	10.52*	21.08*	8.13	-28.00*
H9	-3.61	-22.34*	8.35*	-42.20*	-49.09*	-32.00*
H10	-14.72	-1.82	19.53*	2.46	29.92*	-30.00*
H11	-12.50	-13.51*	6.58*	-21.18*	0.17	-32.33*
H12	-20.14	-21.71*	-30.31*	-15.56*	-41.53*	-11.01*
H13	10.99	2.44*	-11.38*	71.11*	10.30	0.36
H14	-30.54	-13.88*	16.68*	-50.99*	-44.14*	-39.32*
H15	-18.19	-6.12*	34.91*	-18.78*	14.69	-7.80*
H16	-4.30	-0.94	29.81*	-23.92*	13.97	-15.25*
H17	-12.24	-6.82*	63.35*	-6.78*	18.79	-2.03*
H18	-11.20	-1.94*	29.20*	29.17*	14.94	-26.73*
H19	-24.50	-26.40*	1.83*	-34.21*	-14.69	-27.79*
H20	-9.57	-10.17*	18.38*	-6.13	12.60	-20.35*
H21	-14.59	-15.89*	40.02*	-13.35*	-13.16	-41.24*
H22	0.70	-14.97*	40.65*	45.22*	71.49*	-16.46*
H23	-27.89	-30.97*	34.92*	-37.82*	-18.76	-23.89*
H24	-18.32	-8.34*	6.28*	-50.66*	-43.96*	-21.77*
H25	-24.50	-25.71*	9.47*	-53.87*	-44.22*	-40.18*
CD(0.05)	111.89	1.91	1.06	6.5	32.17	1.08

* Significant at 5 % level

Table 18. Heterobeltiosis of the hybrids

Hybrids	Pod weight (g)	Pod length (cm)	Pod breadth (cm)	Number of beans	Total wet bean weight(g)	Single dry bean weight(g)
H1	6.47	-1.33	-23.98	-68.88*	-70.04*	-34.46*
H2	-62.78	-12.09*	-17.07	-19.91*	-60.49*	-51.08*
H3	-17.43	-62.45*	-38.02	-57.47*	-48.25*	-22.15*
H4	4.29	-52.76*	-12.97	-8.68*	-21.02	-34.77*
H5	-16.73	-17.28*	-15.58	-56.47*	-40.37*	-16.31*
H6	-45.31	-36.54*	16.58*	-67.71*	-16.18	-26.15*
H7	8.30	2.72*	14.07*	29.48*	19.95	-20.00*
H8	-19.05	-3.95*	6.53*	-13.09*	-7.47	-36.31*
H9	-13.25	-29.65*	-0.42	-73.90*	-50.47*	-37.23*
H10	-23.25	-11.06*	11.84*	-19.28*	26.40	-35.38*
H11	-21.25	-21.65*	-2.35	-48.19*	-2.54	-37.54*
H12	-22.73	-21.71*	-46.38	-24.00*	-42.72*	-14.39*
H13	9.06	2.44*	-25.11	54.00*	8.06	-2.68*
H14	-31.56	-18.67*	0.65	-55.11*	-49.06*	-41.72*
H15	-19.11	-11.33*	22.39*	-25.61*	4.58	-9.66*
H16	-5.11	-6.44*	16.30*	-30.31*	3.92	-17.24*
H17	-13.11	-12.00*	56.30*	-14.62*	8.32	-3.79*
H18	-19.13	-4.67*	28.23*	1.08	-2.29	-28.62*
H19	-33.38	-28.44*	0.48	-48.52*	-27.48	-29.66*
H20	-17.38	-12.67*	17.26*	-26.55*	-4.28	-22.41*
H21	-22.75	-18.22*	39.19*	-32.20*	-26.18	-42.76*
H22	-6.38	-17.33*	39.84*	13.63*	45.78*	-18.62*
H23	-37.00	-32.89*	34.03*	-51.35*	-30.94	-25.86*
H24	-26.75	-10.89*	5.00*	-61.39*	-52.36*	-23.79*
H25	-33.38	-27.78*	8.23*	-63.90*	-52.59*	-41.72*
CD(0.05)	111.89	1.91	1.06	6.5	32.17	1.08

* Significant at 5 % level

4.4.5.3 Standard heterosis

It was estimated as the superiority of hybrid over the check variety. The check variety used here was CCRP- 15 with pod weight (870g), pod length(11.7 cm), pod breadth (6.4 cm), number of beans (58), total wet bean weight per pod(200g) and single dry bean weight(1.4g). Standard heterosis computed for different traits are presented in Table 19. Compared to relative heterosis and heterobeltiosis more significant positive values were expressed for standard heterosis. This may be due to the reason that the variety taken for comparison was not the top performer in yield but a variety released for resistance to another important disease, Vascular Streak Dieback disease (Minimol *et al.*, 2015). Generally in resistance breeding programme yield is sacrificed to certain extent for resistance (Smith and Campbell, 1996).Hence from the present study it can be concluded that these hybrids expressed a moderate vigour coupled with ample resistance to *Phytophthora*.

To study the overall positive heterotic effect of each hybrid they were reclassified and presented in the Table 20. Maximum hybrids showed positive heterotic effect over standard variety. Two hybrids (H7 and H13) were superior with respect to their better parent when pod length was considered. H7 showed maximum heterotic effect for the character pod length (RH, HB) and H18 (SH). In case of pod breadth, two hybrids (H7, H13) were superior to their parent. None of the hybrids expressed superiority with reference to pod weight (g) and total wet when compared to the parent. Five hybrids expressed superiority in number of beans but none was superior with respect to single dry bean weight (g) when compared with parent. Most of the hybrids expressed positive heterotic vigour over the check variety except for pod weight (g) and pod breadth (cm). Eventhough there is no significant heterotic effect for pod weight in any of the hybrids studied, three hybrids showed superiority in total wet bean weight^{-1}pod when average of two parents is considered and one hybrid showed superiority with respect to better parent. This may be due to the fact that increase in total pod weight expressed in parents was due to husk thickness ((Enriquez and Soria, 1966).

Table 19. Standard heterosis of the hybrids

Hybrids	Pod weight (g)	Pod length (cm)	Pod breadth (cm)	Number of beans	Total wet bean weight (g)	Single dry bean weight (g)
H1	-77.13	15.63*	-42.07*	-26.30*	-75.99*	6.50*
H2	-72.64	-2.81*	-38.97*	-11.85*	-68.33*	-20.50*
H3	-68.74	-4.38*	-51.38*	0.74	-56.01*	26.50*
H4	-32.87	20.31*	-31.72*	116.30*	-32.87*	6.00*
H5	-62.76	4.69*	-42.07*	20.00*	-66.31*	36.00*
H6	-65.98	-19.69*	-20.00*	9.63*	-52.64*	20.00*
H7	-38.16	30.00*	-21.72*	99.26*	-32.23*	30.00*
H8	-50.34	21.56*	-26.90*	60.00*	-47.72*	3.50*
H9	-79.77	-6.56*	-36.55*	-34.81*	-78.95*	2.00*
H10	-64.14	18.13*	-30.00*	15.56*	-46.28*	5.00*
H11	-72.41	4.06*	-37.59*	-11.11*	-58.58*	1.50*
H12	-73.79	0.31	-40.34*	-15.56*	-65.52*	-6.00*
H13	-46.90	31.25*	-24.14*	71.11*	-34.95*	6.00*
H14	-67.13	14.38*	-44.83*	5.93	-69.08*	-10.50*
H15	-45.52	24.69*	-36.21*	75.56*	-36.52*	36.00*
H16	-48.97	31.56*	-38.62*	64.44*	-36.92*	25.00*
H17	-37.47	23.75*	-22.76*	101.48*	-34.25*	44.50*
H18	-25.98	34.06*	-30.00*	138.52*	-40.69*	3.50*
H19	-62.30	0.63	-44.83*	21.48*	-55.98*	2.00*
H20	-46.21	22.81*	-35.86*	73.33*	-41.90*	12.50*
H21	-50.34	15.00*	-24.14*	60.00*	-55.195*	-17.00*
H22	-16.78	16.25*	-23.79*	168.15*	-11.51	18.00*
H23	-64.37	-5.63*	-26.90*	14.81*	-58.08*	7.50*
H24	-71.72	25.31*	-42.41*	-8.89*	-71.08*	10.50*
H25	-73.56	1.56	-40.69*	-14.81*	-71.22*	-15.50*
CD(0.05)	111.89	1.91	1.06	6.5	32.17	1.08

*Significant at 5% level

Table 20. Hybrids showing significant positive heterotic vigour

Character	Relative heterosis	Heterobeltiosis	Standard heterosis
Pod length(cm)	H1, H7, H8, H13	H7, H13	H1, H4, H5, H7, H8, H10, H11, H13, H14, H15, H16, H17, H18, H20, H21, H22, H24
Pod breadth(cm)	H6, H7, H8, H9, H10, H11, H14, H15, H16, H17, H18, H19, H20, H21, H22, H23, H24, H25	H6, H7, H8, H10, H13, H15, H16, H17, H18, H20, H21, H22, H23, H24, H25	-
Number of beans	H4, H7, H8, H18	H7, H13	H4,H5, H6, H7, H8, H10, H13, H15, H16, H17, H18, H19, H20, H21, H22, H23
Pod weight (g)		-	-
Total wet bean weight (g)	H7, H10, H22	H22	-
Single dry bean weight(g)	-	-	H1, H3, H4, H5, H6, H7, H8, H9, H10, H11, H13 ,H15, H16, H17, H18, H19, H20, H22, H23, H24

The result of low heterotic value can be discussed as reduction in yield and vigour due to mobilization of energy in the plant for imparting high resistance. This concept was reported in the work done by Blum (2013). The reduction in

yield can also be attributed to overshaded condition of the experimental plot. Since the plot was designed for natural screening of *Phytophthora* pod rot incidence, the field was maintained under over crowded trees to provide natural optimum condition for the disease incidence. Samuels *et al.* (2012) had also reported that there will be yield reduction under overshaded condition.

4.4.6 Descriptive statistics

Analysis of variance was done for each of the ten quantitative characters in the hybrids and genetic parameters like phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) (Sivasubramaniam & Madhavamenon, 1973), heritability and genetic advance (Johnson *et al.* 1955) were estimated.

PCV and GCV were highest for all characters studied as per the classification of Johnson *et al.* (1955). This indicated high amount of variability among the genotype and the variation was due to the genetic constitution of hybrids. Similar results were reported by Lachenaud and Oliver (2005) in their study. Velayuthan *et al.* (2013) also suggested high amount of variability for yield and yield contributing traits in cocoa hybrids or clones. From the Table 21, it was observed that highest PCV was recorded for the character single dry bean weight (g) (109.05) and it recorded a GCV value of 78.19 which was classified as high (Johnson *et al.* 1955). Since GCV value was high for all the characters under study it can be concluded that variation is due to genetic factors.

According to Falconer and Mackay (1996), heritability was defined as the measure of the correspondence between breeding values and phenotypic values. High broad sense heritability is a good indicator of reliability of genetic improvement of phenotypic trait (Adewale *et al.*, 2010).In the present study as per the classification given by Johnson *et al.* (1955) heritability was high for all the characters except single dry bean weight (16.05 g). Even though the character single dry bean weight expressed high variability, it is not heritable and cannot be used as selection criteria. All the characters expressed high GA(%) value. High

heritability combined with high genetic advance indicated that the characters were controlled by additive genes and can be used as selection criteria. Similar findings were reported by Asna *et al.* (2014). However Rubeena(2015) reported high heritability for single dry bean weight. Heritability and genetic advance are important selection parameters which help to predict the gain under selection. Sobowale *et al.* (2016) reported that high variability coupled with high heritability for the phenotypic characters makes most of the parental clones convenient for selection for further crop improvement through hybridization.

Table 21. Descriptive statistics of selected 25 cocoa hybrids

Character	Range		Mean	SE	PCV (%)	GCV (%)	H2 (%)	GA (%)
	Minimum	Maximum						
Pod length(cm)	19.50	6.50	13.72	0.21	34.63	33.32	92.44	65.97
Pod breadth (cm)	10.00	4.50	7.18	0.10	28.83	27.02	87.58	51.99
Husk thickness(cm)	4.00	0.70	1.20	0.04	57.45	48.29	73.18	85.46
Number of beans/pod	51.00	20.00	38.25	0.57	30.82	28.55	85.67	54.47
Number of flat beans	7.00	1.00	2.51	0.11	84.00	74.84	79.34	137.35
Pod weight (g)	800.00	120.00	370.84	13.91	87.47	84.99	94.41	170.11
Total wet bean wt.(g)	194.70	26.80	91.30	3.42	84.19	80.64	91.76	159.14
Wet bean wt. /pod wt. (%)	46.67	4.54	25.75	0.68	55.65	51.76	86.59	99.26
Single dry bean wt.(g)	9.20	0.58	0.97	0.07	109.05	78.19	16.05	2993.07
TSS (%)	24.00	8.00	14.22	0.34	54.43	52.67	93.61	104.91

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

*H^2 (Johnson et al., 1955) - Low: Less than 30%, Moderate: 30-60%, High: More than 60%

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

4.5 Assessment of self incompatibility

Self incompatibility is defined as the inability of a pollen of flower to fertilize the egg of same flower (Gowers, 1998) and was first reported by Koelreuter in the middle of eighteenth century. It is used as an efficient tool to exploit hybrid vigour in many crops(Meer and Nieuwhof, 1968). It helps to avoid tedious and laborious step of emasculation in hybridization programme.

Knight and Rogers (1953) reported that self incompatibility in cocoa is controlled by a gene having five alleles (S_1, S_2, S_3, S_4, S_5). The allelic interaction is reported to be $S_1 > S_2 = S_3 > S_4 = S_5$. Cope (1962) observed that the site of incompatibility is within the embryo sac which is unique in cocoa. He also suggested that the mechanism causing flower abscission depends on the nuclear activity within the ovules.

After incompatible pollination, pollen tube grows faster and releases the gametes into the embryo sac. There occurs failure of male nuclei to unite with the egg. This is referred to as "prefertilization inhibition in the ovule" and is genetically controlled. Royaert et al. (2011) reported that if there is no fruit set or if fruit sets fails to retain for more than 15 days, then the genotype can be classified as self incompatible.

To assess self incompatible position of twenty-five hybrids, hundred flowers were selfed per plant (Plate 6) by assisted pollination as suggested by Mallika et al.(2000). The result is depicted in Table 22. Out of 25 hybrids, two didn't yield sufficient flowers (H5, H14) for confirming the incompatibility position. In hybrid H9 (G VI 188 × G VI 304) when fifty five flowers were selfed, pod set was observed and it was rated as self compatible.

Twenty-two hybrids turned to be self incompatible without producing any pod even after selfing hundred flowers. The hybrids H9, H10, H11 were having the same parentage G VI 188 × G VI 304. Out of these three, H9 was self compatible and H10 and H11 are self incompatible in nature ie progenies derived from the

same cross showed different level of incompatibility reaction. This result was in tune with the early reports (Nair and Rekha, 1996; Minimol and Prasannakumari, 2013) indicating that hybrids from same cross do not show identical compatibility reaction.

a. Flower bud

b. Covering with plastic hood

c. Hand pollination

d. Pod set

Plate 6. Hand pollination technique in cocoa

Table 22. Incompatibility position of selected hybrids

Sl.no	Hybrid no	Genotype	No. of flowers selfed	Pod set	Incompatibility position
1	H1	SIV 1.26×TISSA	100		SIC
2	H2	SIV 1.26×TISSA	100		SIC
3	H3	SIV1.26×PII 12.11	100		SIC
4	H4	SIV1.26×PII 12.11	100		SIC
5	H5	GIV188×GVI 287	42		NC
6	H6	GIV188×GVI 287	100		SIC
7	H7	GIV188×GVI 287	100		SIC
8	H8	GIV188×GVI 287	100		SIC
9	H9	GVI 188×GVI 304	55	1	SC
10	H10	GVI 188×GVI 304	100		SIC
11	H11	GVI 188×GVI 304	100		SIC
12	H12	SIV 5.20×TISSA	100		SIC
13	H13	SIV 5.20×TISSA	100		SIC
14	H14	GVI 216×GVI 294	30		NC
15	H15	GVI 216×GVI 294	100		SIC
16	H16	GVI 216×GVI 294	100		SIC
17	H17	GVI 216×GVI 294	100		SIC
18	H18	GVI 216×GVI 304	100		SIC
19	H19	GVI 216×GVI 304	100		SIC
20	H20	GVI 216×GVI 304	100		SIC
21	H21	GVI 216×GVI 304	100		SIC
22	H22	GVI 216×GVI 304	100		SIC
23	H23	GVI 216×GVI 304	100		SIC
24	H24	GVI 216×GVI 304	100		SIC
25	H25	GVI 216×GVI 304	100		SIC

4.6 Assessment of general combining ability

4.6.1 Selection of hybrids

Yield data recorded in the station for twenty two self incompatible hybrids for the previous two years of the study is explained in the Table 23.

Table 23. Mean value for number of pods/tree/year obtained from previous records.

Hybrid no.	Stand No.	Genotype	Yield (2012-13)	Yield (2013-14)	Pooled mean yield
H1	2.4	SIV 1.26 x TISSA	54.00	56.00	55.00
H2	3.8	SIV 1.26 x TISSA	51.00	54.00	52.50
H3	6.5	SIV 1.26 x P II 12.11	54.00	56.00	55.00
H4	12.3	SIV 1.26 x P II 12.11	93.00	95.00	94.00
H6	18.6	G VI 188 X GVI 287	50.00	49.00	49.50
H7	22.4	G VI 188 X GVI 287	71.00	74.00	72.50
H8	22.7	G VI 188 X GVI 287	85.00	87.00	86.00
H10	26.4	G VI 188 X GVI 304	70.00	72.00	71.00
H11	27.4	GVI 188 X GVI 304	44.00	45.50	44.75
H12	29.6	SIV 5.20 X TISSA	49.00	51.00	50.00
H13	29.7	SIV 5.20 X TISSA	83.00	88.00	85.50
H15	33.4	GVI 216 X GVI 294	95.00	92.00	93.50
H16	34.4	GVI 216 X GVI 294	101.00	106.00	103.5
H17	34.6	GVI 216 X GVI 294	131.00	125.00	128.00
H18	35.4	GVI 216 X GVI 304	71.00	73.00	72.00
H19	35.5	GVI 216 X GVI 304	47.50	49.00	48.25
H20	35.9	GVI 216 X GVI 304	69.50	72.00	70.75
H21	36.5	GVI 216 X GVI 304	48.50	50.00	48.25
H22	36.6	GVI 216 X GVI 304	104.00	102.00	103.00
H23	37.4	GVI 216 X GVI 304	44.00	45.00	44.50
H24	38.4	GVI 216 X GVI 304	50.00	48.00	49.00
H25	38.9	GVI 216 X GVI 304	51.00	50.00	50.50

From this eleven hybrids were selected based on maximum value for number of pods^{-1}tree^{-1}year and the list is given in Table 24.

Table 24. List of selected hybrids for further evaluation

Sl. No.	Hybrid no	Genotype
1	H4	SIV1.26×PII 12.11
2	H7	GVI 188× G 287
3	H8	GIV188×GVI 287
4	H10	GVI 188×GVI 304
5	H13	S IV 5.20 × TISSA
6	H15	GVI 216×GVI 294
7	H16	GVI 216×GVI 294
8	H17	GVI 216×GVI 294
9	H18	GVI 216×GVI 304
10	H20	GVI 216×GVI 304
11	H22	GVI 216×GVI 304

The difference between mean pod weight (g), wet bean weight (g) and single dry bean weight (g) of each pair of hybrids are presented in Table 25.

Table 25. Difference between mean pod weight (g), wet bean weight (g) and single dry bean weight (g) for each pair of treatment of data

H4	Characters	H4	H7	H8	H10	H13	H15	H16	H17	H18	H20	H22
H4	Pod weight(g)	0.00	38.00	132.00*	252.00*	122.00*	110.00*	184.00*	40.00	80.00	228.00*	160.00*
	Wet bean wt.(g)	0.00	92.68*	69.70*	72.58*	95.24*	80.10*	81.70*	80.64*	77.76*	81.34*	142.12*
	Single dry bean wt(g)	0.00	0.21	0.02	0.03	0.00	0.28	0.15	0.35	0.02	0.03	1.79
H7	Pod weight(g)		0.00	94.00	214.00*	84.00	72.00	146.00*	2.00	118.00*	190.00*	198.00*
	Wet bean wt.(g)		0.00	22.98	20.10	2.56	12.58	10.98	12.04	14.92	11.34	49.44*
	Single dry bean wt(g)		0.00	0.19	0.18	0.21	0.07	0.06	0.14	0.19	0.17	1.58
H8	Pod weight(g)			0.00	120.00*	10.00	22.00	52.00	92.00	212.00*	96.00	292.00*
	Wet bean wt.(g)			0.00	2.88	25.54*	10.40	11.98	10.94	8.06	11.64	72.42*
	Single dry bean wt(g)			0	0.01	0.02	0.26	0.13	0.33	0.00	0.01	1.77
H10	Pod weight(g)				0.00	130.00*	142.00*	68.00	212.00*	332.00*	24.00	412.00*
	Wet bean wt.(g)				0.00	22.66	7.52	9.12	8.06	5.18	8.76	69.54*
	Single dry bean wt(g)				0	0.03	0.25	0.12	0.32	0.01	0.00	1.76
H13	Pod weight(g)					0.00	12.00	62.00	82.00	202.00*	106.00*	282.00*
	Wet bean wt.(g)					0.00	15.14	13.54	14.60	17.48	13.90	46.88*
	Single dry bean wt(g)					0.00	0.28	0.15	0.35	0.02	0.03	1.79
H15	Pod weight(g)						0.00	74.00	70.00	190.00*	118.00*	270.00*
	Wet bean wt.(g)						0.00	1.60	0.54	2.34	1.24	62.02*
	Single dry bean wt(g)						0.00	0.13	0.07	0.26	0.25	1.51

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H16	Pod weight(g)	0.00	144.00*	264.00*	44.00	344.00*
	Wet bean wt.(g)	0.00	1.06	3.94	0.36	60.42*
	Single dry bean wt(g)	0.00	0.19	0.13	0.12	1.64
H17	Pod weight(g)		0.00	120.00*	188.00*	200.00*
	Wet bean wt.(g)		0.00	2.88	0.70	61.48*
	Single dry bean wt(g)		0.00	0.33	0.32	1.44
H18	Pod weight(g)			0.00	308.00*	80.00
	Wet bean wt.(g)			0.00	3.58	64.36*
	Single dry bean wt(g)			0.00	0.01	1.77
H20	Pod weight(g)				0.00	388.00*
	Wet bean wt.(g)				0.00	60.78*
	Single dry bean wt(g)				0.00	1.76
H22	Pod weight(g)					0.00
	Wet bean wt.(g)					0.00
	Single dry bean wt(g)					0.00

*Significant at 5% level

It showed that majority of selected hybrids had no significant difference over mean wet bean weight (g) and single dry bean weight (g).It indicated that they all were superior and not much variation exhibited between them for yield contributing characters. Pod weight showed significant variation, however it alone cannot be considered as yield contributing character(Minimol *et al.*, 2014), since it is a total of husk thickness and wet bean weight.

4. 6. 2Assisted pollination with tester

The selected eleven hybrids were crossed with a tester G. I. 5.9 and the result is presented in Table 26.

Table 26. Crossing with the tester G I 5.9

Sl. No.	Hybrid no	Genotype	No. of flowers crossed	Male parent	Pod sets
1	H4	SIV1.26×PII 12.11	55		
2	H7	GVI 188× G 287	60	G.I.5.9	1
3	H8	IV188×GVI 287	62		
4	H10	GVI 188×GVI 304	25		
5	H13	S IV 5.20 × TISSA	103		
6	H15	GVI 216×GVI 294	30		
7	H16	GVI 216×GVI 294	88	G.I.5.9	2
8	H17	GVI 216×GVI 294	46	G.I.5.9	2
9	H18	GVI 216×GVI 304	45		
10	H20	GVI 216×GVI 304	80		
11	H22	GVI 216×GVI 304	25	G.I.5.9	1

After crossing eleven hybrids with a common tester G. I. 5.9, six pods were obtained from four parents and the remaining did not set fruits. It may be due to cross incompatibility mechanism. It indirectly measures the degree of closeness between the genotypes. Mallika *et al.* (2000) had reported that the compatibility reaction between the genotypes varied widely. When crossing between more genetically similar genotypes were attempted incompatibility mechanism operated and resulted in no fruit set.

4. 6.3 Combining ability analysis

Top cross analysis was employed for analysis of variance. Even though top cross is the simplest method employed, it is reported to be an efficient tool as that of polycross (Gopal *et al.*, 2008).

As discussed by Bhatt, 1971 the estimate of combining ability analysis will classify the parents in terms of their hybrid performance. The analysis of variance was carried out and results are presented in the Table 27. High p *vs* c value indicated high value for average heterosis (Beck *et al.*, 1989)and can be concluded that this character can be considered as selection criteria for parental selection. Mean sum of squares of parent vs cross was significant for the character girth and Height × Diameter^2 (HD^2). HD^2 value can be considered as an indicator of vigour and it is used as selection criteria in seedlings. Progenies with high HD^2 value was reported to be best performer during its yielding stage(Enriquez, 1981). Significance (P < 0.01) of single degree of comparison variance (p *vs* c) indicated substantial difference between the parent as a group and their top cross progenies as another group (Sharma, 1988). There by indicating the superiority of parents in producing heterotic progenies. Since early jorquetting was absent in the seedlings, it is not mentioned in the Table 28.

Table 27.Topcross ANOVA for general combining ability

Source of variation	Df	Height(cm)	Girth(cm)	Diameter(cm)	HD²	Chlorophyll	Number of leaves
Replications	4	70.74	0.027	0.005	25.885	6151.06	3.41
Entries	7	382.72**	2.31**	0.23**	866.816**	109.16	62.62**
Parents	3	635.78**	4.05**	0.4**	1957.04**	233.59	127.35**
Topcrosses	3	249.5**	0.87**	0.09	31.92	20.62	18.22**
p vs c	1	23.19	1.433**	0.14	7709.814**	1.47	1.65
Error	28	17.9	0.063**	0.055	34.44	872.66	1.56

*Significant at 5% level

**Significant at 1% level

4.6.3.1 Breeding value

Breeding value (A) represents the general combining ability effect (GCA) of individual test entries. It is the main parameter in initial screening through topcross analysis. Combining ability had been investigated in cacao on yield characteristics and pest and disease incidences (Tan and Tan, 1988). Tan, 1990 suggested that major part of genetic variability was additive in action. The GCA effects of four parents were estimated and presented in Table 28.

Table 28. Breeding value for hybrids

Character	Hybrids			
	H16	H22	H17	H7
Height (cm)	0.69*	-0.55	-1.12	0.99*
Girth (cm)	-0.35	0.61*	1.00*	-1.27
Diameter (cm)	-0.10	0.20	0.32	-0.39
HD^2	-0.07	-0.10	1.31*	-1.15
Number of leaves	-0.20	0.44*	-1.29	1.04*
Chlorophyll	-0.08	-1.33	0.33	1.08*
CD(0.05) = 0.38				

4.6.3.1.1 Height

Two hybrids (H16, H7) manifested a high GCA effect for height with a breeding value of 0.69 and 0.99 respectively. But for hybrid H17 and H22, breeding value was negative (-1.12, -0.55).

GCA effect of genotypes for various traits in *Brassica juncea* revealed plant height in the range of -14.09 to 9.47 and the variation among genotypes for plant height were controlled by non- additive gene action (Ali *et al.* 2015).

4.6.3.1.2 Girth

Highest GCA effect for girth was observed for the hybrid H 17 (1.0) followed by H22 (0.61). But for the hybrids H16 and H7were having negative value (-0.35, -1.27).

4.6.3.1.3 Diameter

Two hybrids (H22, H17) manifested a high GCA effect for diameter with a breeding value of 0.20 and 0.32 respectively. The other two hybrids (H16, H7) were observed to have negative breeding value of -0.10 and 0.39.

4.6.3.1.4 HD^2

H17 had the highest significant GCA effect (1.31) for HD^2. Since HD2 is considered as the selection criteria, it can be considered as the most desirable and potential parent for producing superior hybrids. The other three hybrids showed a negative breeding value of -0.07, -0.10 and -1.15.

4.6.3.1.5 Number of leaves

Breeding value for number of leaves was highest for the hybrid H7(1.04) followed by H22 (0.44). The other two hybrids expressed a negative GCA effect of-0.20 and -1.29 respectively.

4.6.3.1.6 Chlorophyll

Two hybrids (H17, H7) manifested a high GCA effect for height with a breeding value of 0.33 and 1.08 respectively. But for hybrid H16 and H22, breeding value was negative (-0.08, -1.33).

HD^2 is considered as the selection criterion which is directly correlated to final yield (Toxopeus, 1985). Only one hybrid (H 17) (Plate 7) expressed a positive significant value (1.31) for HD^2. Hence only this parent can be selected for further improvement programme. Adewale *et al.* (2014) in their study reported that negative GCA is undesirable for selection and only parents with positive significant GCA can be considered for further hybrid development with improved pod and bean qualities.
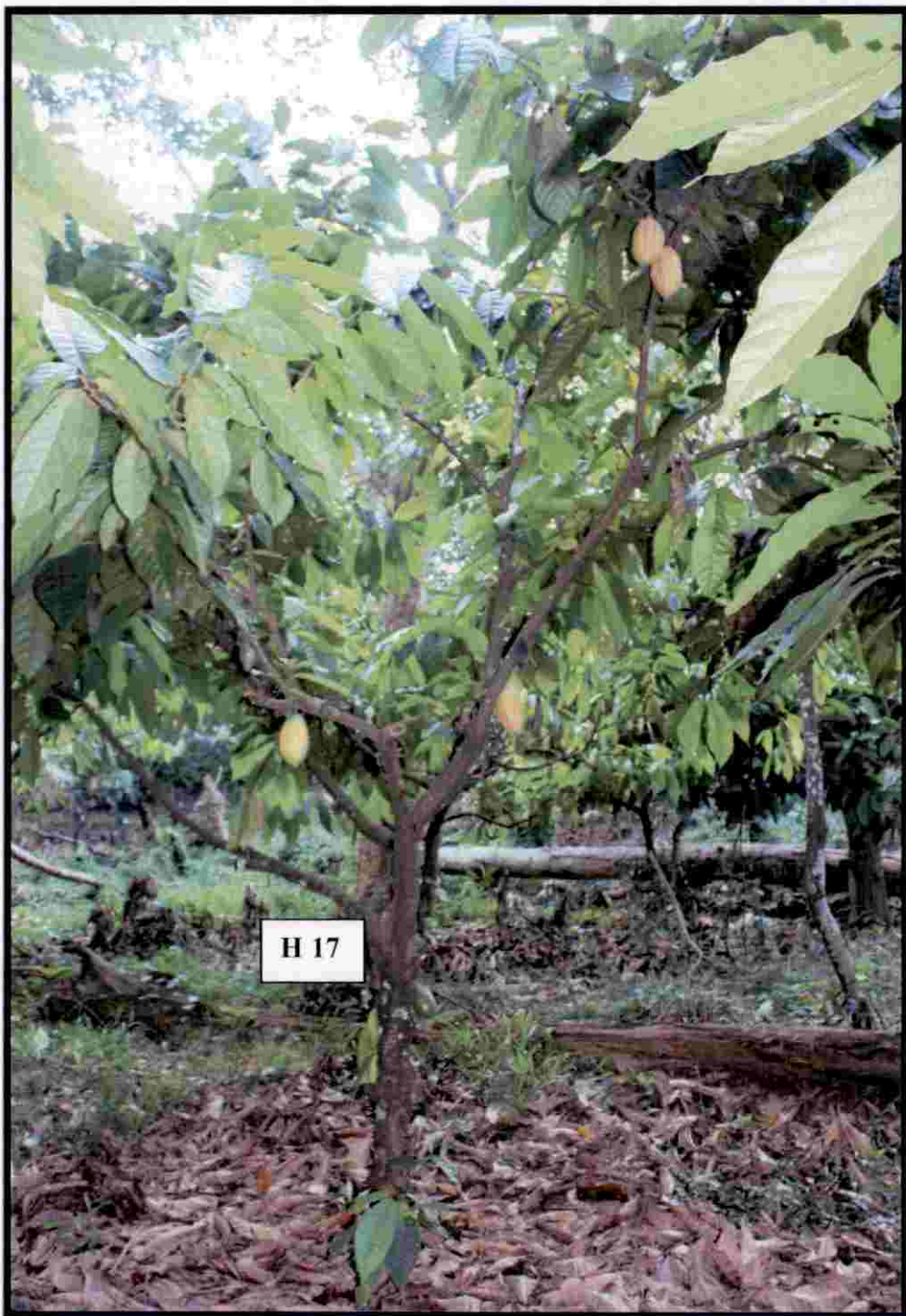
Plate 7. Selected hybrid (H 17)

4.6.3.2 General performance of open pollinated progenies

Seven hybrids out of selected eleven did not yield any pod on cross pollination. However progenies developed from open pollinated pods of these hybrids were evaluated to predict the performance of the parent based on the concept that since they are self pollinated, any pod developed in them will be a hybrid. Results are presented in Table 29.

Three hybrids were selected H4 (38.61), H15 (35.57), H8 (29.49), based on the HD^2 value of their progeny. These parents can be considered as better combiners, but has to be confirmed by crossing it with a specific tester.

Table 29. General performance of open pollinated hybrids

Hybrid no.	Height	Girth	Diameter	HD^2	Number of leaves	Chlorophyll
H4	39.99^a	3.06^a	0.97^a	38.61^a	17.20^a	34.06^b
H8	36.53^b	2.78^{bc}	0.88^{bc}	29.49^b	16.08^{ab}	37.28^{ab}
H10	24.73^c	1.39^c	0.44^c	4.88^d	9.93^d	21.74^{de}
H13	36.21^b	2.48^d	0.79^d	22.96^c	13.95^c	19.74^c
H15	41.42^a	2.99^{ab}	0.95^{ab}	37.57^a	16.80^a	39.43^a
H18	21.51^d	1.34^c	0.43^c	3.97^d	11.18^d	23.90^d
H20	34.31^b	2.68^{cd}	0.86^{cd}	26.08^{bc}	14.97^{bc}	29.95^c
C. V (%)	6.53	7.32	7.45	18.40	9.64	9.28
C. D(0.05)	2.84	0.23	0.07	5.57	1.79	3.54

4.7 Combining ability for disease resistance

4.7.1 Screening of seedlings for disease incidence

Seedlings were screened in the nursery at two weeks interval for *Phytophthora* incidence for three months and no seedlings were found to be infected.

4.7.2 Lab screening for disease resistance

Iwaro *et al.* (2006) observed significant genotypic and phenotypic coefficient of correlation for resistance on leaves and per cent of infected pods in field. It confirmed the feasibility of leaf disc method in early selection of black pod resistance in cocoa. The study conducted by Tahi *et al.* (2006), revealed that 75-90% of genetic variation of cocoa genotype for field resistance to *Phytophthora palmivora* can be explained by leaf-disc method when carried under standard condition.

Semi translucent leaves from all the progenies raised through top cross and open pollinated method were screened in the lab along with control for eight days by providing artificial inoculums and percentage of infection was calculated. Result presented in (Table30 and Table 31)and (plate 8 and plate 9). It was observed that either top cross progeny or open pollinated progeny showed any infection. Result indicated that the disease is controlled by poly genes (Barreto *et al.,* 2015), highly inherited and additive in nature (Dias, 2001).All the eleven hybrids can be used for further breeding programme.

Table 30. Leaf inoculation for topcross hybrid for assessment of combining ability

Sl. no	Per cent leaf area infection after 8 days							
	Control	H16	Control	H22	Control	H17	Control	H7
1	100	0	100	0	100	0	100	0

Table 31. Leaf inoculation for open pollinated seedlings for assessment of combining ability

Sl. no	Per cent leaf area infection after 8 days													
	Control	H4	Control	H8	Control	H10	Control	H13	Control	H15	Control	H18	Control	H20
1	100	0	100	0	100	0	100	0	100	0	100	0	100	0

a. H16

b. H22

c. H17

d. H7

Plate 8. Lab screening of top cross hybrid for *Phytophthora* pod rot resistance

a. H4


b.H8

c.H10

d.H13

e.H15

f.H18

g.H20

Plate 9. Lab screening for open pollinated hybrid for *Phytophthora* pod rot resistance

4.8 Future line of work

I. H 17 found to be good combiner will be used to establish clonal garden

II. Genetic potential of H 17 will be further evaluated in CYT(Comparitive Yiled Trial)

III. General combining ability of remaining seven hybrids has to be confirmed by using an another tester
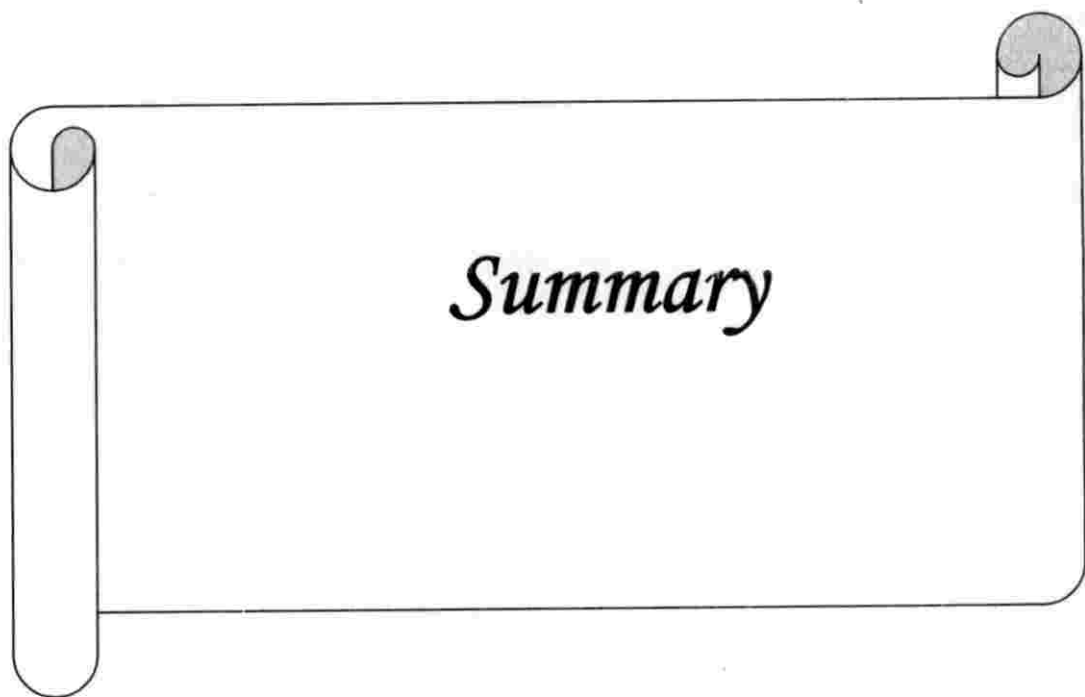
Summary

5. SUMMARY

The study entitled, "General combining ability of selected black pod disease resistant cocoa (*Theobroma cacao* L.) hybrids" was carried out at Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during the period from August 2014 to May 2016. The objective of the study were to evaluate the incompatibility and general combining ability of hybrids resistant to *Phytophthora* pod rot for identifying superior genotypes for further crop improvement programmes.

For this an organized breeding programme was initiated at Cocoa Research Centre, Vellanikkara for the development of superior varieties with resistance to *Phytophthora* pod rot disease in 2005. Five female and five male parents were used in crossing programme resulted in 615 hybrids and screened for vigour and disease incidence in the nursery. After the initial screening in the nursery, 615 hybrids seedlings were field planted and 160 hybrids established in field. Out of 615 hybrids, 25 hybrids did not show any symptom of *Phytophthora* pod rot disease incidence for six years. This served as the material for the study. When the potential of these parents with respect to production of resistant hybrids were estimated, it was seen that the female parent G VI 216 produced maximum number of resistant hybrids (12) and the recovery percentage was more for the S IV 5.20 (40%). In case of male parent, both maximum number of hybrids and recovery percent were recorded in G VI 304. The morphological evaluation of these hybrids was carried out based on eight qualitative and eight quantitative characters. Variability was observed among the hybrids for all the qualitative characters and quantitative characters. Among the hybrids, hybrid H 22 recorded the highest pod weight (724g) and total wet bean weight per pod (176.98g). The husk thickness and number of flat beans were lowest for hybrid H16 (0.86 cm) and H11 (1.40). From the twenty five hybrids evaluated, hybrid H2 alone was observed to be having calabacillo type pods. Angoleta, amelonado, cundemor and criollos were noticed in other hybrids. The pod apex was found to be mammelate only in the hybrid H2 and the remaining hybrids were having acute, obtuse and

types of attenuate pod apex. The hybrid H2 evolved from the cross of GV1 216 × GV1 304 alone was having intermediate type of basal construction. However in most of the hybrids, the rugosity of the pods was intense or the surface was rough. The colour of unripe pod observed to be light green in majority of the hybrids except in the hybrid H3and H4. The variability was observed for bean colour also.The hybrids H14 and H16 from the cross G VI 216 × G VI 294 showed dark purple, H17 light purple and H15 with medium purple colour.

Among the hybrids, hybrid H 22 recorded the highest pod weight (724g) and total wet bean weight per pod (176.98g). The husk thickness and number of flat beans were lowest for hybrid H16 (0.86 cm) and H11 (1.40).

Cluster analysis based on qualitative and quantitative characters resulted in 10 clusters at 60 % similarity level and 11 clusters at 70 % similarity level following the unweighted pair group method as suggested by Sneath and Sokal, 1973.

In order to know the homology between the qualitative and quantitative clustering patterns, the percentage of distribution of cocoa hybrids belonging to each cluster of qualitative traits into different quantitative clusters were estimated and found that all the hybrids present together in each qualitative cluster were distributed among different cluster when they were grouped based on quantitative characters.

Clustering of the 25 hybrids following D^2 statistics developed by Mahalanobis (1936) resulted in eight clusters with maximum inter cluster divergence was observed between cluster I and cluster VIII.

Analysis of variance was done for each of the ten quantitative characters in the hybrids. PCV and GCV were highest for all characters studied as per the classification of Johnson et al. (1955). Heritability was also high for all the characters except single dry bean weight (16.05 g) as per the classification.

Self incompatibility was assessed by self pollinating 100 flowers per hybrids. Twenty two hybrids turned to be self incompatible, one self compatible and rest with insufficient number of flowers. Out of 22 self incompatible hybrids eleven were selected for further study based on their yield (No. of pods/ tree/ year). selection differential was calculated inorder to know the the efficiency of selection pressure applied on the basal self incompatible population.

They were crossed with tester (G.I 5.9) in top cross model. Only four hybrids (H16, H22, H17, H8) yielded fruits. The fruits were raised in the nursery and analysis of variance was carried out for the characters like height, girth, diameter, HD^2, number of leaves, chlorophyll and presence or absence of jorquetting. Single degree of comparison of variance (p vs c) between the parent as a group and their top cross progenies as another group recorded a high value for HD^2. High p vs c value indicated high value for average heterosis (Beck $et\ al.$, 1989). To know the potentiality of individual parent, breeding value was computed. Since HD^2 considered as the indicator of vigour, it is used as selection criteria in seedlings. Only one hybrid (H17) expressed a positive significant value (1.31) for HD^2. Hence only this parent can be selected for further improvement programme.

Seven hybrids out of selected eleven one did not yield any pod on cross pollination. So progenies developed from open pollinated pods of these hybrids were evaluated to predict the performance of the parent. Three hybrids were selected H4 (38.61), H15 (35.57), H8 (29.49), based on the criteria HD^2 value of their progeny. These parents can be considered as better combiners, but has to be confirmed by crossing it with a specific tester. Seedlings were raised in the nursery and observations were recorded in the third month.

Nursery screening for $Phytophthora$ resistance of top cross progeny and open pollinated progeny of all eleven selected hybrids were done at two weeks interval for three months. Lab screening for disease resistance was carried out in semi translucent leaves from all the progenies raised through top cross and open

pollinated method along with control for eight days by providing artificial inoculation and percentage of infection was calculated.

No disease incidence was noticed in any progenies. H 17, found to be superior combiner can be evaluated in comparative yield trial. H4, H15 and H8 were selected as superior ones based on the performance of open pollinated progeny which has to be further evaluated with another tester.

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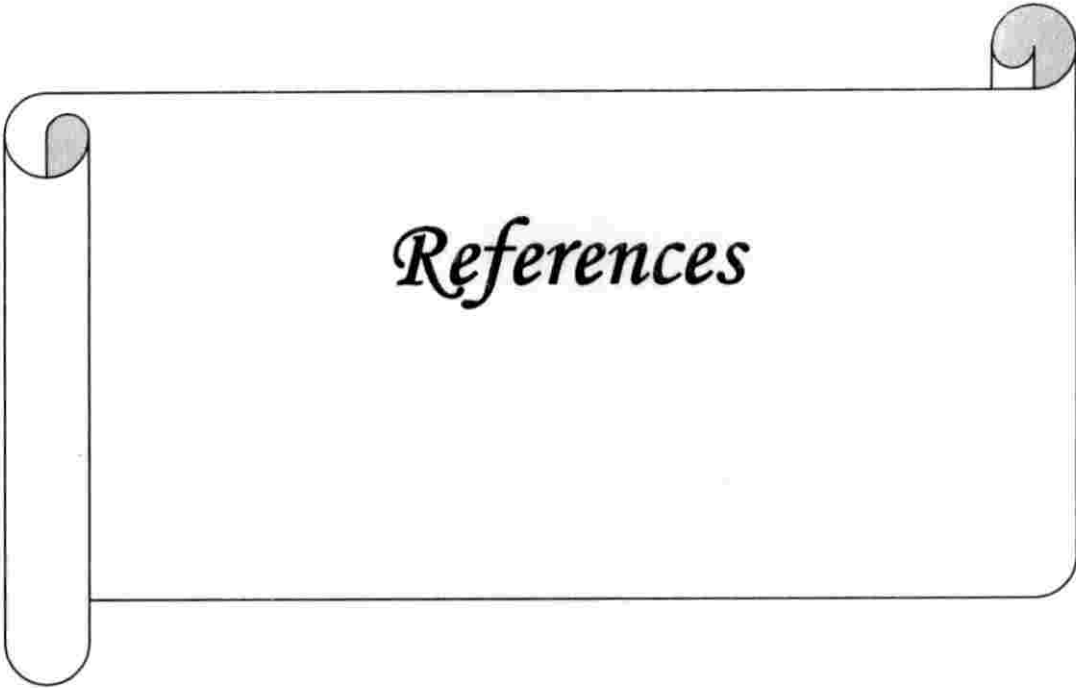
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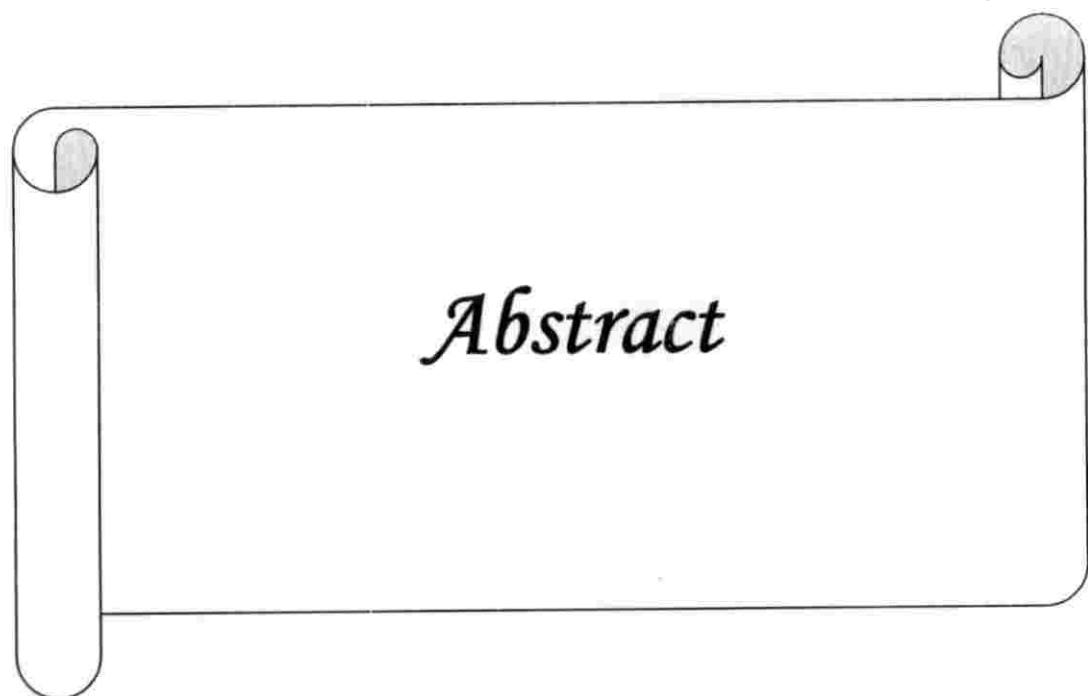
Abstract

GENERAL COMBINING ABILITY OF SELECTED BLACK POD DISEASE RESISTANT COCOA(*Theobroma cacao* L.) HYBRIDS

By

VARSHA BABU.

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ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

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Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF PLANTATION CROPS AND SPICES

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680656

KERALA, INDIA

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ABSTRACT

Cocoa originated in the Amazon riverbasin of South America. The cultivation of cocoa had extended from Mexico to Costa Rica and over the time it had spread to Caribbean and other parts of South America (Wood and Lass, 1985). It was introduced to India during 1979. The generic name *Theobroma* was derived from two greek words '*Theo*' means god and '*broma*' means food, hence known as "*food of god*". The beans are the only source for chocolate.

Cocoa is affected by many biotic and abiotic stresses. Among the biotic stresses *Phytophthora* pod rot is the most serious one affecting cocoa, leading to a total of 64% yield loss (Adomako, 2007). Therefore the control of black pod is a major challenge for cultivation of cocoa. The farmers adopt several measures to control the disease of which the use of copper based fungicides is the most predominant one (Tan and Tan, 1990). Although it is reasonably effective, their indiscriminate use poses serious environmental issues. To overcome this situation breedingfor resistance to black pod is the most economical, environmental friendly and effective control method (Iwaro *et al.*, 2004). Hence an organized breeding programmewas initiated at Cocoa Research Center (CRC), KAU, Vellanikkara during 2005 for the development of varieties resistance to black pod disease and high yield.

Twenty five hybrids showing considerable level of resistance after 6 years of screening were selected for the present study. The morphological evaluation of these hybrids were carried out based on eight qualitative and eight quantitative characters.Variability was observed among the hybrids for all the qualitative characters and quantitative characters. Among the hybrids, hybrid H22 recorded the highest pod weight (724g) and total wet bean weight per pod (176.98g). The husk thickness and number of flat beans were lowest for hybrid H16 (0.86 cm) and H11 (1.40).

Self incompatibility was assessed by self pollinating 100 flowers per hybrids. Twenty two hybrids turned to be self incompatible, one self compatible and rest with insufficient number of flowers. Out of 22 self incompatible hybrids eleven were selected for further study based on their yield (No. of pods/ tree/ year). They were crossed with tester (G.I 5.9) in top cross model and only four yielded fruits. Seedlings were raised in the nursery and observations were recorded in the third month.

The analysis of variance of top cross showed significant variation for two characters. The characters like height, diameter, chlorophyll and number of leaves did not express any significant difference. HD^2 is considered as an indicator of initial vigour and it is found to be directly correlated to the final vigour. The significant value for variance (p Vs c) for HD^2 indicated substantial difference between the parents as agroup and their hybrid progenies as another group. This shows that the average heterosis is significantly high. Seven hybrids out of selected eleven did not yield any pod on cross pollination, progenies developed from open pollinated pods of these hybrids were evaluated to predict the performance of the parent. Progenies expressed high HD^2 value. Based on this, these parents can be considered as superior ones. However this has to be confirmed further by crossing them withanother tester.

Nursery screening for *Phytophthora* resistance of top cross progeny and open pollinated progeny of all eleven selected hybrids were done at two weeks interval for three months. Lab screening for disease resistance was carried out in semi translucent leaves from all the progenies raised through top cross and open pollinated method along with control for eight days by providing artificial inoculation and percentage of infection was calculated. No disease incidence was noticed in any progenies indicating high GCA for this character.

H 17 found to be superior combiner can be evaluated in comparative yield trial. H4, H15 and H8 were selected as superior ones based on the performance of open pollinated progeny which has to be further evaluated with another tester.