BREEDING COWPEA (*Vigna unguiculata* (L.) Walp.) FOR RESISTANCE TO SPOTTED POD BORER (*Maruca vitrata* Fab.)

by

Ambavane Ajinkya Rajendra
(2015-21-031)

THESIS

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANT BREEDING AND GENETICS
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA

2018

DECLARATION

I, hereby declare that the thesis entitled **"Breeding cowpea (*Vigna unguiculata* (L.) Walp.) for resistance to spotted pod borer (*Maruca vitrata* Fab.)"** is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me of any degree, diploma, fellowship, or other similar title of any other University or Society.

Place : Vellanikkara
Date : 05/01/2019

Ambavane Ajinkya Rajendra
(2015-21-031)

CERTIFICATE

Certified that thesis entitled **"Breeding cowpea (*Vigna unguiculata* (L.) Walp.) for resistance to spotted pod borer (*Maruca vitrata* Fab.)"** is a record of research work done independently by Mr. Ambavane Ajinkya Rajendra under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Place : Vellanikkara
Date : 05 /01/2019

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

CERTIFICATE

We, the undersigned members of the advisory committee of Mr. Ambavane Ajinkya Rajendra, a candidate for the degree of **Doctor of Philosophy in Agriculture**, with major in **Plant Breeding and Genetics**, agree that the thesis entitled **"BREEDING COWPEA (*Vigna unguiculata* (L.) Walp.) FOR RESISTANCE TO SPOTTED POD BORER (*Maruca vitrata* Fab.)"** may be submitted by Mr. Ambavane Ajinkya Rajendra in partial fulfilment of the requirement for the degree.

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

Dr. Rose Mary Francies
(Member, Advisory Committee)
Professor and Head
Department of Seed Science and Technology
College of Horticulture, Vellanikkara

Dr. Madhu Subramanian
(Member, Advisory Committee)
Professor and Head
AICRP on BCCP & W
College of Horticulture, Vellanikkara

Dr. C. Beena
(Member, Advisory Committee)
Professor
AICRP on Medicinal and Aromatic Plants
College of Horticulture, Vellanikkara

Dr. Deepu Mathew
(Member, Advisory Committee)
Assistant Professor
CPBMB, Vellanikkara

Dr. K. N. Ganesan
(External Examiner)
Professor
Centre for Plant Breeding and Genetics
Tamil Nadu Agricultural University,
Coimbatore

ACKNOWLEDGEMENT

"Success is a journey, not a destination.

The doing is often more important than the outcome."

There are several occasions come in life when you want to express the deep sense of love and gratitude. I am at that golden moment of my life where I would like to express my heartfelt gratitude to ***Dr. Jiji Joseph****, Professor and Head, Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, my mentor and chairman of my Advisory Committee. I express my deep and sincere gratitude to her for valuable and inspirative guidance, keen interest, constant encouragement, enormous help throughout my academic career and above all, for having trust in me.*

I feel immense pleasure to express my deepest sense of gratitude to the members of my Advisory Committee ***Dr. Madhu Subramanian****,* ***Dr. Rose Mary Francies****,* ***Dr. C. Beena*** *and* ***Dr. Deepu Mathew*** *for their excellent guidance, consistent inspiration, keen interest and helpful discussion throughout the course of my degree programme.*

I extend my sincere thanks to ***Dr. C. George Thomas****, Associate Dean, College of Horticulture for rendering all support and supervision during my research programme.*

It is my prime responsibility to acknowledge all my teachers, ***Dr. Sindhumol****,* ***Dr. Dijee Bastian****,* ***Dr. Biju****,* ***Dr. Minimol****, for constantly encouraging me during the course of this study.*

With deep reverence, I express my heartfelt thanks to ***Dr. Mani Chellappan*** *for his crucial support and the facilities provided for taking the laboratory observations, technical guidance and the wholehearted co-operation throughout the period of investigation.*

I owe special thanks to Librarian, College of Horticulture, ***Dr. A. T. Francis*** *and all other staff members of the Central Library, who guided me in several ways, which immensely helped for the collection of literature for writing my thesis. I also thankfully remember the services rendered by all staff members of the Student's computer club, Library and Office of College of Horticulture, Vellanikkara, Kerala Agricultural University. I am thankful to the Kerala Agricultural University for technical and financial assistance for persuasion of my study and research programme.*

I would like to acknowledge the help extended by each of non-teaching staff of the Department of Plant Breeding and Genetics for all their remarkable support, especially, ***Mrs. Revathy V. S.*** *and* ***Mrs. Shymol P. M.***

It is my proud privilege to convey my heartfelt gratitude and humble respect to my beloved parents ***Shri. Rajendra Yashwant Ambavane*** *and to whom I owe my life* ***Sou. Snehal Rajendra Ambavane****. More words cannot express the sense of gratitude to my lovely sister* ***Pooja*** *and other family members who have been an inexhaustible source of inspiration and encouragement to me and whose long-cherished dreams are turning into reality in the form of this thesis.*

George Eliot has well said, "Friendship is feeling safe with a person, having neither to weigh thoughts nor measure words". With all my friends, I could be myself, I could share my feelings, experiences and my deepest thoughts. I would like to thank my classmate ***Manjunatha*** *and* ***Asna****, and my junior friends, without whom it was difficult to study here and thanking them for all the fun time spent together.*

No dictionary can provide me apposite words to express my heartfelt thanks to dearest friends ***Nagendra, Swapnil, Kishor*** *and* ***Debashish*** *whose strong hands were always with me during the research work. On a personal note, I am even more obliged and express my heartfelt thanks to* ***Vipul, Deepak*** *and* ***Neereja*** *for making my life full of joy and for giving me unforgettable memories.*

I avail this opportunity to thank my seniors and well-wisher ***Vijaykumar sir, Dr. Suma, Dr. Ashis*** *for their co-operation and lovely company during the Ph.D. degree Programme.*

Last but certain not least, thanks to my lifetime friends and well-wisher ***Riya, Reshma Raghu, Nasiya, Mujtaba sirji, Rajakumar, Garggi chechi, Manjusha chechi, Reshmika, Silpa, Milsha, Roshni chechi, Prakash sir, Om*** *and* ***Rakesh*** *for their presence and the good hours spent together.*

Finally, I thank and apologise to all those deserving people for their inspiration and encouragement whose names I have mistakably overlooked or forgot to mention in my acknowledgement.

Place : Vellanikkara

Date : 05 /01/2019

Ambavane Ajinkya Rajendra

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS USED

viz.	:	*Vi delicet* (namely)
etc.	:	*et cetera* (and the rest)
et al.	:	*et alii* (and co-workers)
i.e.	:	*Id est* (that is)
nm	:	Nanometer
mm	:	Millimeter
mm^2	:	Square millimeter
cm	:	Centimeter
ng	:	Nano gram
µg		Microgram
mg	:	Milligram
g	:	Gram
µl	:	Micro litre
ml	:	Millilitre/s
N	:	Normal solution
µmol		Micromole
pM	:	Pico molar
mM	:	Millimolar
M	:	Molar
%	:	Per cent
w/v	:	Weight/volume
CE	:	Catechol equivalent
EU	:	Enzyme units
pH	:	Hydrogen ion concentration
°C	:	Degree Celsius
min.	:	Minute/s
sec.	:	Second/s
rpm	:	Revolution per minute
OD	:	Optical Density
F_1	:	Filial 1 generation / hybrid
F_2	:	Filial 2 generation / hybrid

DNA	:	Deoxyribonucleic acid
RNA	:	Ribonucleic acid
dNTP	:	Deoxyribonucleotide triphosphat
$MgCl_2$	:	Magnesium Cloride
HCl	:	Hydrochloric acid
CTAB	:	Cetyl trimethylammonium bromide
NaCl	:	Sodium chloride
TAE	:	Tris-acetate-EDTA
EDTA	:	Ethylene Diamine Tetra Acetic Acid
PVP	:	Polyvinylpyrrolidone
PCR	:	Polymerase chain reaction
PIC	:	Polymorphic Information Content
bp	:	Base pair
KAU	:	Kerala Agricultural University
Fig.	:	Figure
p	:	Page
pp	:	Pages
/	:	Per
&	:	And
@	:	At the rate

Introduction

1. INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important pulse crop with a high amount of nutrients, especially proteins. This crop is cultivated in the tropics of Asia, Africa and other parts of the world. It is a food not only for human, but also serves as a feed for animals. With the nodules on root packs with *Rhizobium,* the cowpea plants are able to fix atmospheric nitrogen and enhances soil fertility, hence, plants require very less amount of resources which makes it a valued crop for resource-deprived farmers, and well-suited for intercropping.

Nevertheless, the economic production of cowpea is unable to achieve its summit. One of the prime reasons for this is the infestation of a notorious pest, spotted pod borer (*Maruca vitrata* Fab.; Lepidoptera: Crambidae). This pest was first reported by Dietz (1914) on beans in Indonesia. Singh and Allen (1980) reported 20 to 60 per cent loss in grain yield by spotted pod borer in cowpea, whereas, Kumar *et al.* (2013) reported pod damage varying from 22.81 to 32.56 per cent. It was also observed by a number of researchers that the pest infests plants at flower bud stage, flowering stage and pod maturity (Philip, 2004; Beegum, 2015). This pest has gained the tremendous attention of several researchers around the globe because the damage by this pest to cowpea almost always crosses the economic threshold level (Taylor and Ezedima, 1964).

Control of this pest with insecticides has not been widely adopted by farmers due to the prohibitive costs. Some farmers use excessive amount of pesticides on the crop against this pest which leads to health hazards and environmental pollution. Despite this desired protection could not be achieved. At the damaging stage, this pest feeds internally, which makes management of the pest through conventional chemical means very difficult. However, application of insecticides remains the prime means of management.

Breeding for resistance to the pest is of immense importance, both in terms of environmental well-being and reducing the cost of cultivation. Exploration and exploitation of host plant resistance against this insect is the most desired way. Sources of complete or partial resistance to many insect pests are available in different cultivars within the crop species itself (Singh, 1978; van Emden, 1989). It is also suggested by several researchers that the screening of commercial and local cultivars should be undertaken as the primary step in the search for resistance and to initiate a breeding programme.

Host plant resistance refers to those heritable characters possessed by the plant which influence the ultimate degree of damage done by the insect (Maxwell, 1972). Identification of such characters (morphological and biochemical) of the host plant conferring resistance to pests is very crucial in breeding for pest resistance (Snelling, 1941). Various biophysical characters *viz*., trichome length and density on plant parts, length of peduncle, pod wall thickness, and various biochemical parameters *viz*., total sugar, reducing sugar, non-reducing sugar, proteins, phenols, crude fibre as well as some enzymes activity in cowpea plays an important role in providing resistance to the plant against spotted pod borer (Phillip, 2004; Sunitha *et al*., 2008; Beegum, 2015; Barad *et al*., 2016; Jakhar *et al*., 2017; Tiwari *et al*., 2017).

A prime goal of cowpea breeding programmes around the world is to combine desirable agronomic traits with resistance to the major diseases and insect pests (Timko *et al*., 2007; Timko and Singh, 2008). The knowledge of the genetic diversity available within the indigenous and exotic germplasm collections can increase the overall efficacy of cowpea improvement programmes (Hegde and Mishra, 2009). Molecular markers play a major role in identifying specific traits at early stage thereby giving an option to select a right parents to achieve an early success in breeding programme.

Among the varieties of molecular markers used for assessment of genetic diversity in germplasm, SSR markers have found widespread application because of their high reproducibility, codominant nature and extensive genome coverage (Agarwal *et al*., 2008; Xu and Crouch, 2008) and easy detection by polymerase chain reaction (PCR) (Castillo *et al*., 2010).

Keeping all these points in view, the present study entitled 'Breeding cowpea (*Vigna unguiculata* (L.) Walp.) for resistance to spotted pod borer (*Maruca vitrata* Fab.)' was undertaken with the objective mentioned below.

Identification and incorporation of resistance against spotted pod borer in high yielding varieties of cowpea and assessment of parental polymorphism at the molecular level.

Review of literature

2. REVIEW OF LITERATURE

The damage caused by spotted pod borer (*Maruca vitrata* Fab.) infestation is very immense which makes this pest of grave concern with respect to cowpea and other pulse crops. Several studies have been conducted around the globe, wherever cowpea and other pulses are the economic crops, to search a potential source of resistance against this pest and to transfer that resistance into cultivated varieties. The work done on various aspects of spotted pod borer infestations and transferring resistance into cultivated varieties is comprehensively reviewed in this chapter.

2.1 SPOTTED POD BORER INFESTATION

The cowpea (*Vigna unguiculata* (L.) Walp.) is a major pulse crop and serves as an important source of protein and other nutrients for a vast number of people across the globe. However, the economic production of cowpea is seriously hampered by the infestation of a notorious pest, spotted pod borer (*M. vitrata* Fab.; Lepidoptera: Pyralidae). It is a polyphagous pyralid moth which causes damage to almost all kinds of pulses over wide range of environmental conditions in all areas where pulses cultivated as a major crop (Taylor, 1978; Singh and van Emden, 1979; Dabrowski *et al.*, 1983; Ezeuch and Taylor, 1984; Jackai and Daoust, 1986; Ngugi *et al.*, 1985; Suh, 1986).

It is one of the economically important pests of cowpea in the tropics, and damage by this pest almost always crosses the economic threshold level (Taylor and Ezedima, 1964). In India, it is a severe pest of cowpea, blackgram, greengram, pigeonpea, beans and soybean. The most frequent host plants are *V. unguiculata*, *Cajanus cajan*, *Phaseolus lunatus* and *Pueraria phaseoloids* (Mahalakshmi *et al.*, 2016). In India, the annual yield losses caused by spotted pod borer have been estimated to be around 30 million dollars (Saxena *et al.*, 2002).

Singh and Allen (1980) reported 20 to 60 per cent loss in cowpea grain yield by the incidence of spotted pod borer. Karel (1985) observed that the spotted pod borer larvae are more abundant and injurious to cowpea than any other pest. The pod damage due to the pest ranges from 13 to 31 per cent, the seed damage is about 16 per cent and the total yield loss averages between 33 to 53 per cent. In Kerala, 8 to 40 per cent pod damage have been estimated by Anithakumari (1992). She also observed that the high humidity and low temperature during the months of September and October favours the pest build up. Kumar *et al.* (2013) reported pod damage varying from 22.81 to 32.56 per cent by spotted pod borer in cowpea. Several reports around the world have confirmed

the economic importance of this pest (Attachi and Djihou, 1994; Dreyer *et al.*, 1994; Jackai and Adalla, 1997; Tamo *et al.*, 1997; IITA, 1998; Panicker *et al.*, 2002; Jayasinghe *et al.*, 2015; Rathwa *et al.*, 2018).

The moth is nocturnal in habit, and female moths lay flat scaly eggs on floral buds, flowers, leaves, leaf axils, terminal shoots and tender pods (Mahalakshmi *et al.*, 2016). The eggs hatch within three days and the first instar larvae start feeding at the place where the egg had been laid. The caterpillars are internal feeders and bore tiny holes to enter in flower buds and feed on internal developing organs or enters into a young pods to feed on immature seeds (Anithakumari, 1992). In pulses, seeds being the economic produce, infestation by spotted pod borer is considered as serious damage. The larval stage of this pest also known to attack terminal shoots of cowpea in addition to flower buds, flowers and pods (Veeranna *et al.*, 1999). There they cause damage by binding the plant parts together with silken thread and with the leftover faecal matter. The larval growth is of five instars and with the average life being around 15 days (Sravani and Mahalakshmi, 2015) cause heavy and irreversible damage.

According to Echendu and Akingbohungbe (1989), an infestation of two larvae per plant is sufficient to cause considerable yield damage in cowpea. Bindu (1997) observed the presence of spotted pod borer in the field throughout the cropping season, with the increased population during the post-flowering period, in spite of all chemical protection measures. As per the report of Attachi and Hountondji (2000), the spotted pod borer larvae infest the flower buds, flowers and pods of almost all types of cowpea. Considering yield loss caused by this pest, field screening for resistance and transferring that resistance into cultivated varieties is a need of the hour.

2.2 SOURCES AND NATURE OF RESISTANCE

As stated by Woolley (1976), resistance to spotted pod borer is dominant and possibly controlled by more than a few genes. Pathak (1985) studied the nature of inheritance and degree of dominance of resistance in relation to pod and seed damage, and reported partial dominance of susceptibility over dominance and also suggested polygenic inheritance for resistance against this pest.

Sources of complete or partial resistance to many insect pests are available in different cultivars within the crop species itself (Singh, 1978; van Emden, 1989). It is also suggested that the screening of commercial and local cultivars should be undertaken as the initial step in the search for resistance. Saxena and Khan (1991) reported that sources

of resistance should be looked for in traditional varieties or unimproved germplasm of the particular crop. Singh (1999) opined that the finding out and using a source of resistance from wild relatives for transferring the resistant genes to cultivated types may have a limited scope because of the retention of wild characters in the segregating generations.

2.3 SCREENING OF COWPEA GERMPLASM FOR RESISTANCE AGAINST SPOTTED POD BORER

The extent of cowpea resistance to spotted pod borer depends on plant growth stages, several morphological and biochemical parameters (Dabrowski *et al.*, 1983). Flower bud, flower, pod and seed damage (Jackai, 1982; Valdez, 1989), larval population on plant parts (Woolley and Evans, 1979) and pod evaluation index (ratio of number of pods per plant to pod damage) (Oghiakhe *et al.*, 1992a) have been suggested as selection criteria to screen plant population for resistance to spotted pod borer. Using these criteria, number of studies have been conducted to find out resistance against spotted pod borer in the area where cowpea has been grown for a long time. For instance, Usua (1975) reported that the cowpea lines 946 and 4557 were resistant to the attack of spotted pod borer, whereas, Singh (1978) screened 2800 accessions of cowpea at International Institute of Tropical Agriculture, Ibadan, Nigeria and reported the cultivars TVu-946 and TVu-4557 as resistant ones.

Woolley and Evans (1979) screened 140 genotypes of *V. unguiculata* and observed semi wild-type Wake Jaba was resistant to spotted pod borer. Jackai (1981) and Macfoy *et al.* (1983) screened a number of genotypes and reported TVu-946 as one of the most resistant. Echendu and Akingbohungbe (1989) identified four cowpea varieties (TVu-946, TVu-1896 AG, H 51-1 and 2 AK) as resistant and three varieties (Ife Brown, H 144-1 and 58-185) as susceptible in field screening trials. From Dharwad, Jagginavar *et al.* (1995) reported substantial variation in the level of resistance in a population of cowpea with respect to the spotted pod borer damage. In their study, genotypes P1201 was with the least pod damage, TVu-1631 was with the least seed damage, whereas, C11 recorded the lowest larval population.

Singh (1999) evaluated various improved lines of cowpea for resistance against spotted pod borer and observed that the lines IT90K-277-2, IT93K-452-1, IT94K-437-1, IT97K-569, IT95K-223-3, IT97K-838 and IT97K-499-38 suffered less damage in field conditions. He also observed unnoticeable reduction in yield of these lines even without

insecticidal sprays. Veeranna *et al.* (2000) screened 45 genotypes of cowpea and reported that the cultivar TVx-7 was completely resistant to spotted pod borer infestation.

A study was conducted by Vidya and Oommen (2001) with 50 accessions of yard-long bean at College of Agriculture, Vellayani, Kerala Agricultural University (KAU) to find out a source of resistance. Evaluation based on synchronised consideration of flower and pod damages revealed that Kottayam local (16 % pod damage), Palakkad local (18 % pod damage) and Chengannur local (18 % pod damage) were more resistant among the cultivars evaluated.

A study conducted by Philip (2004) at College of Agriculture, Vellayani, KAU to find out resistance against spotted pod borer revealed two local cultivars with least infestation, Palakkad local and Kottayam local (18 %), and two cultivars, Chengannur local and DCP 7, with 20 per cent infestation.

A screening trial conducted at Indian Institute Vegetable Research (IIVR), Varanasi publicised cowpea cultivar Pusa Komal as highly susceptible to flower damage (50 %) caused by spotted pod borer, whereas, CP-4 and CP-3 recorded 28 per cent and 34 per cent damage, respectively. Overall, CP-2 was the least damaged (19.44 %) (IIVR, 2008).

Fifty genotypes were screened for various damage parameters of spotted pod borers by Jithesh (2009) at College of Agriculture, Vellayani, KAU and noticed remarkable variability with respect to all the damage parameters. Based on all the damage parameters, he reported three genotypes *viz.*, Kurappunthara local, Kanichar local and KMV-1 with high plant resistance indices.

Kumar *et al.* (2013) screened one promising variety Pusa Komal and 14 genotypes of cowpea against spotted pod borer. The genotype KCP-6 recorded the least overall damage (22.81 %), while, KCP-1 was the most susceptible with 32.56 per cent damage. However, none of the cultivars recorded an accepted level of resistance to this pest.

Barad *et al.* (2016) conducted a field experiment involving 20 genotypes of cowpea. They found that the accession GC-706 was resistant with lower larval population (0.06 larvae per plant) and pod damage (8.89 %) and with maximum yield potential (981.48 kg/ha) under spotted pod borers infestation, while, maximum larval population (1.96 larvae per plant) and pod damage (24.30 %) were recorded in GC-12.

Beegum and Subramanian (2017) evaluated 48 accessions of cowpea against spotted pod borer and reported that the five accessions *viz.*, EC 100092, EC 98668,

IC 39945, IC 2918 and IC 52110 suffered no damage at all the three stages (flower bud, flower and pod). A research trial conducted by Asoontha (2017) at College of Agriculture, Vellayani, KAU, revealed Puthuppady local, Githika and IC 39947 as resistant accessions.

2.4 MORPHOLOGICAL BASIS OF RESISTANCE

Certain morphological characters of the plant act as a primary shield against the pest. These characters restrict the entry or movement of the pest on a plant body thereby impart mechanical resistance. The pod wall thickness, the presence of trichomes, podding habit, density and length of trichomes, pod angle, length of peduncle, plant architecture, *etc.* of different cultivars are associated with resistance to spotted pod borer (Oghiakhe *et al.*, 1991; Sharma, 1998; Sunitha, 2006; Halder and Srinivasan, 2011; Beegum, 2015; Asoontha, 2017). These characters alone or together act as a defence wall against spotted pod borer.

2.4.1 Pod wall thickness

The anatomical microenvironment of different plant parts plays an important role in resistance by limiting the larval movement and feeding. Thick and compact collenchyma cells in the pod wall and fibrous tissues on the pod surface contributed to resistance (Oghiakhe *et al.*, 1992b). Halder and Srinivasan (2005) observed a negative correlation between pod wall thickness and pod damage. Susceptible genotype of urd bean, LBG-17, possessed the lowest pod wall thickness (0.52 mm), compared to resistant genotypes LBG-611 (0.58 mm). Halder and Srinivasan (2011) supported the earlier results. They observed the lowest pod wall thickness (0.77 mm) in highly susceptible cv. GC-9708 and thick pod wall in the most tolerant cowpea cultivar HC-270 (0.89 mm) in their study. Beegum (2015) screened 48 accessions of cowpea and found a significant and negative correlation between pod wall thickness and level of spotted pod borer damage. However, Vidya (2000) observed no significant correlation between pod damage severity and pod wall thickness in cowpea.

2.4.2 Pubescence

Pubescence is one of the crucial physical character allied with insect resistance across the plant kingdom. A covering of hair or trichomes on plant organs is an indumentum, and the surface bearing them is said to be pubescent (Davis and Heywood, 1963). The defensive role of trichomes against insects has been very well documented by several researchers (Levin, 1973; Johnson, 1975; Webster, 1975; Stipanovic, 1983; Peter *et al.*, 1995).

It is a complex trait which involves several factors *viz*., distribution of the trichomes, the length of trichomes, the density of trichomes, disposition of trichomes and the type of trichomes (Verma and Afzal, 1940). The presence of trichomes has been reported by Oghiakhe (1995) as one of the mechanisms for ovipositional non-preference by spotted pod borer in cowpea. Presence of trichomes also causes hurdle in movement and restrict the larva from reaching flower bud and pod on the plant.

Pubescence can affect the activity of insects by both mechanical and chemical means. The mechanical effect depends on the physical characteristics of the trichomes which include density, erectness, length and shape (Dent, 1991). Some volatile components of trichome exudates serve as repellent or deterrent and in some cases also toxic to insects (Levin, 1973). Number of researchers have reported defensive role of trichomes against spotted pod borer (Chiang and Singh, 1988; Jackai and Oghiakhe, 1989; Oghiakhe, 1995; Veeranna and Hussain,1997; Philip, 2004; Halder and Srinivasan; 2005; Sunitha *et al*., 2008; Beegum, 2015; Asoontha, 2017).

Jackai and Oghiakhe (1989) examined the role of length and density of trichomes in the resistance by using two wild cowpea (*V. vexillata*) varieties, TVNu-72 and TVNu-73 with respect to spotted pod borer. They found that the feeding and development were daunted in insects on pods of TVNu-72 and TVNu-73, as compared to those on a susceptible variety, IT84E-124. However, when trichomes were removed, larvae fed and developed better on the above varieties. This clearly proved that the trichomes forms the first line of defense in the resistance against spotted pod borer. Oghiakhe (1995) also found the same streamline of results when studied three cultivars *viz*., TVnu-72 (wild, highly resistant and highly pubescent), TVu-946 (semi-wild, moderately resistant and pubescent) and IT82D-716 (cultivated, highly susceptible and less pubescent).

Veeranna and Hussain (1997) screened 45 cowpea genotypes for attack by spotted pod borer in Karnataka and observed that TVX-7, the most resistant genotype, had a high trichome density (24.41 /9 mm^2), while, DPCL-216, the most susceptible one, had a low trichome density (2.82 /9 mm^2), endorsing earlier findings that trichomes are important in reducing the attack by spotted pod borer. Sharma (1999) also reported significant negative correlation between trichome density and spotted pod borer damage.

Halder and Srinivasan (2005) revealed that the highly susceptible genotype of urd bean, LBG-17, had the least number of trichomes on stems (14.7 /mm^2), pods (3.4 /mm^2) and leaves (4.5 /mm^2) compared to the highly tolerant LBG-611, which had high trichome density (20.3 /mm^2, 10.1 /mm^2 and 8.2 /mm^2, respectively). Similarly,

trichome length was also the least (0.95 mm) in LBG-17 compared to LBG-611 (2.4 mm).

Sunitha *et al.* (2008) reported that the trichome density on upper and lower surfaces of the leaf and its length, and trichome density and its length on pods were positively correlated to resistance against spotted pod borer.

2.4.3 Plant architecture

Plant architecture also plays a noticeable role in defense against a number of pest including spotted pod borer. Distribution of spotted pod borer larvae is closely related to the distribution of reproductive structures which serve as the larval feeding sites, and hence, plant architecture is important in deciding the level of damage. Several researchers reported the importance of a position of the reproductive structure as a non-preference mechanism.

For instance, Oghiakhe *et al.* (1991) observed that the cowpea cultivars with pods held within the leaf canopy damaged significantly more than the cultivars with pods held above the canopy. Defoliated cultivars suffered significantly less infestation and damage than those with leaves. Cultivars with luxurious leafy growth can hold more relative humidity under the canopy than that of less luxurious cultivars. This also reduces soil and ambient temperatures, which in turn favour larvae of spotted pod borer. They also observed that the per cent pod damage and larval infestation by spotted pod borer were positively correlated with relative humidity and negatively correlated with high temperature. Canopy structure and pod position acting together or independently exerted a reflective effect on cowpea resistance to spotted pod borer. Beegum (2015) also recorded the same streamline of results while working with 48 accessions of cowpea.

2.5 BIOCHEMICAL BASIS OF RESISTANCE

A wide range of phytochemical substances including primary, secondary and intermediary metabolites play a crucial defensive role against spotted pod borer infestation. Biochemical parameters include total sugar content, reducing sugar content, total protein content, total phenol content, polyphenol oxidase activity, peroxidase activity, crude fibre content, *etc*. All these components play a deciding role in conferring resistance in one or other way. For instance, susceptible genotypes tend to have higher sugar content, reducing sugar, non-reducing sugar, protein content, and low phenolic content, crude fibre content, low enzymatic activity (polyphenol oxidase activity, peroxidase activity), *etc*., whereas, resistant genotypes often show overall reverse scenario.

Macfoy *et al.* (1983) recorded a higher content of sugars, amino acids and proteins in spotted pod borer susceptible cowpea variety Vita-1 and lower concentrations in resistant cowpea variety TVu-946. Moreover, the secondary metabolites, phenols, flavonoids, crude fibre and dry matter content were higher in resistant variety. This clearly indicates that the resistant varieties are less nutritionally suitable for larval development of spotted pod borer.

Anithakumari (1992) conducted an experiment to pinpoint the role of different biochemicals in resistance against spotted pod borer. The results of the study revealed that the total sugars, amino acids, total nitrogen and crude protein plays a deciding role in conferring resistance. She reported that the cowpea accessions *viz.*, V98, V30, V95, V61, V75 under the moderately resistant group possessed lower total sugar content of 2.90, 2.80, 3.37, 3.20 and 3.0 per cent, respectively. The susceptible accessions had higher total sugar (ranging from 4.5 to 5.7 %). Moreover, higher amino acid content was recorded in case of moderately and highly susceptible accessions (V13 - 0.85 µg /g, V41 - 0.843 µg /g, V90 - 0.833 µg /g, V89 - 0.877 µg /g, V2 - 0.967 µg /g and V1 - 1.83 µg /g).

Reports of Oghiakhe *et al.* (1993a) clearly indicate that the susceptible genotypes almost always tend to have high sugar and protein content in flower buds and pods, while, resistant genotypes tend to have high phenol concentration and low protein content. Veeranna (1998) observed higher phenol and tannin contents in tolerant genotypes than susceptible genotypes. In contrast to the report of Veeranna (1998), Oghiakhe *et al.* (1993b) reported that the phenol content does not play a significant role in spotted pod borer resistance in cowpea.

Halder *et al.* (2006) from Andhra Pradesh reported that the highly susceptible cultivar of mungbean, LGG- 450, had the highest amount of total sugar, reducing sugar, non-reducing sugar, amino acids and protein compared to the highly tolerant cultivar, whereas, phenols were highest in the resistant cultivar (LGG-497) than the susceptible cultivar. They also observed a significant positive correlation between total sugar, reducing sugar, non-reducing sugar, amino acids and proteins with pod damage, and a negative correlation between total phenol content in pods with pod damage. Sunitha *et al.* (2008) reported higher sugar content in flowers (22 %) and pods (10.6 %) as well as higher protein content in the susceptible genotype of pigeonpea, ICPL88034, as compared to the resistant genotype ICPL98003 with respect to spotted pod borer damage.

Singh and Singh (2014) screened 28 genotypes of cowpea against spotted pod borer at the Agricultural Research Farm, Banaras Hindu University, Varanasi (Uttar Pradesh). Their reports clearly established a high negative correlation between phenol in flowers and immature pods with the level of spotted pod borer infestation. They also reported that higher concentrations of carbohydrate and protein favour insect infestation.

Beegum (2015) observed a significant positive correlation between total damage caused by spotted pod borer and total protein content, moisture content, total sugars and reducing sugar content. However, the significant negative correlation was observed in peroxidase and polyphenol oxidase activities with total damage.

Barad *et al.* (2016) conducted a field experiment to find out biochemical basis of resistance in different genotypes of cowpea with respect to spotted pod borer. Amid the different genotypes evaluated, the resistant one, GC-706, had low amount of total amino acid (6.2 mg /g), total soluble sugar (4.5 %), nitrogen (2.9 %), proteins (25.4 %), moisture (85.9 %) and fibre content (1.8 %), whereas, anti-nutritional constituents *viz.*, total phenol (0.8 %), tannin (11.8 %) and flavonoid (1.0 %) were higher as compared to susceptible genotypes. These anti-nutritional constituents exhibited a significant negative correlation with pod damage.

Jakhar *et al.* (2017) carried out a research trial to associate resistance to spotted pod borer with biochemical traits of seven cowpea genotypes. They recorded high phenol content (428.63 mg /100 g, 326.33 mg /100 g) and high flavonoid concentration (484.08 mg /100 g, 458.81 mg /100 g) in the pods of resistant genotypes GC-5 and GC-0815, respectively.

Several researchers reported similar results while working with various resistant and susceptible varieties of cowpea. Above mentioned studies clearly indicate the existence of significant positive correlation between total sugar, reducing sugar, non-reducing sugar and proteins with overall damage, whereas, negative correlation between phenols content, crude fibre, peroxidase activity, polyphenol oxidase activity, *etc.* with spotted pod borer damage.

2.6 VARIABILITY STUDIES

Variability in a population is measured by the phenotypic and genotypic coefficients of variation (PCV and GCV). Assessment of PCV and GCV of genotypes under study is essential for a successful crop improvement programme.

Heritability estimates the degree of variation in quantitative traits that is due to genetic variation between genotypes. Burton and DeVane (1953) proposed the expected gain (genetic gain) from selection as a product of heritability, phenotypic standard deviation and selection differential. As per Johnson *et al.* (1955), high heritability and high genetic gain of the respective trait are more useful than high heritability alone in predicting the performance of the progenies of selected lines.

Hanson *et al.* (1956) defined heritability in a broad sense as the ratio of genotypic variance to the total variance in non-segregating populations. According to Panse and Khargonkar (1957), the genetic advance would be high if the heritability is due to additive gene action. Johnson *et al.* (1955) classified heritability into low (0-30 %), moderate (>30-60 %) and high (>60 %), and the genetic gain was categorised as low (0-10 %), moderate (>10-20 %) and high (>20 %). A number of researchers reported prominence of genetic parameters of different characters while working with different genotypes of cowpea around the globe. The important results of their studies are cited in the accompanying table (Table 1).

Table 1. Genetic parameters of different characters of cowpea

Characters	PCV	GCV	Heritability	Genetic gain	References
Days to 50 per cent flowering	-	-	-	Low	Khanpara *et al.*, 2015; Tudu *et al.*, 2015; Sharma, 2016
	Low	Low	-	-	Vidya, 2000; Borah and Khan, 2002
	-	-	High	High	Jana *et al.*, 1982; Roquib and Patnaik, 1990
	High	High	Moderate to high	-	Anbuselvam *et al.*, 2000
	Low	Low	High	Low	Mareena, 1989; Khanpara *et al.*, 2015
	Moderate to high	Moderate to high	High	High	Tyagi *et al.*, 2000; Srinivas *et al.*, 2017
	High	High	High	High	Sreekumar *et al.*, 1996; Sharma, 1999; Ajith, 2001

Table 1 continued

Characters	PCV	GCV	Heritability	Genetic gain	References
Plant height	-	-	-	Moderate	Nehru and Manjunath, 2001
	-	-	Moderate to high	High	Kumar and Sangwan, 2000
	High	High	-	-	Tyagi *et al.*, 2000; Anbuselvam *et al.*, 2001; Purushotham *et al.*, 2001; Singh and Verma, 2002; Prakash *et al.*, 2003; Ananda, 2012; Khanpara *et al.*, 2015; Dinesh *et al.*, 2017; Sarath and Reshma, 2017; Singh *et al.*, 2018
	-	-	High	High	Vidya, 2000; Borah and Khan, 2002; Dinesh *et al.*, 2017
	High	High	Moderate to high	High	Mareena, 1989; Anbuselvam *et al.*, 2000
	Moderate to high	Moderate to high	High	High	Tyagi *et al.*, 2000
	High	High	High	High	Ajith, 2001; Venkatesan *et al.*, 2003; Khanpara *et al.*, 2015; Srinivas *et al.*, 2017; Singh and Singh, 2018
Number of primary branches per plant	High	High	-	-	Radhakrishnan and Jebaraj, 1982; Anbuselvam *et al.*, 2000; Nehru and Manjunath, 2001
	High	High	High	High	Kalaiyarasi and Palanisamy, 2000; Borah and Khan, 2002; Khanpara *et al.*, 2015; Srinivas *et al.*, 2017
Number of pods per plant	Moderate	Moderate	Moderate	Moderate	Venkatesan *et al.*, 2003

Table 1 continued

Characters	PCV	GCV	Heritability	Genetic gain	References
Number of pods per plant	High	High	-	-	Gowda *et al.*, 1991; Backiyarani and Natarajan, 1996; Rangaiah, 2000; Chaudhari *et al.*, 2013
	-	-	Low	High	Ravindran and Das, 1997
	-	-	Moderate to high	High	Kumar and Sangwan, 2000
	-	-	High	High	Thiyagarajan, 1989; Ram *et al.*, 1994
	High	High	-	High	Renganayaki and Rengasamy, 1992
	High	High	Moderate to high	Moderate to high	Mareena, 1989; Rangaiah and Mahadevu, 1999
	Moderate to high	Moderate to high	High	Moderate	Tyagi *et al.*, 2000; Malarvizhi, 2002; Dinesh *et al.*, 2017
	High	High	High	High	Panicker, 2000; Vidya, 2000; Ajith, 2001; Nehru and Manjunath, 2001; Subbiah *et al.*, 2013; Khanpara *et al.*, 2015; Srinivas *et al.*, 2017; Singh and Singh, 2018
Pod length	-	-	Moderate to high	-	Anbuselvam *et al.*, 2000
	-	-	High	-	Siddique and Gupta, 1991; Savithramma, 1992; Ram and Singh, 1997; Ravindran and Das, 1997
	-	-	Moderate to high	High	Kumar and Sangwan, 2000
	-	-	High	High	Roquib and Patnaik, 1990; Sobha, 1994; Khanpara *et al.*, 2015
	Moderate	Moderate	Moderate	Moderate	Venkatesan *et al.*, 2003

Table 1 continued

Characters	PCV	GCV	Heritability	Genetic gain	References
Pod length	Moderate to high	Moderate to high	High	Moderate	Tyagi *et al.*, 2000; Srinivas *et al.*, 2017
	High	High	High	High	Sawant, 1994; Sreekumar *et al.*, 1996; Hazra *et al.*, 1999; Kalaiyarasi and Palanisamy, 2000; Ajith, 2001; Subbiah *et al.*, 2013; Singh and Singh, 2018
Number of seeds per pod	-	-	High	-	Siddique and Gupta, 1991; Ram and Singh, 1997; Arunachalam *et al.*, 2002
	-	-	High	High	Roquib and Patnaik, 1990; Thiyagarajan *et al.*, 1990; Mehta and Zaveri, 1998
	High	High	-	-	Jana *et al.*, 1982
	High	High	Moderate to high	-	Anbuselvam *et al.*, 2000
	High	High	High	-	Mathur, 1995
	Moderate	Moderate	High	Moderate	Khanpara *et al.*, 2015
	High	High	High	High	Kalaiyarasi and Palanisamy, 2000; Ajith, 2001; Srinivas *et al.*, 2017
Grain yield per plant	Low	Low	-	-	Indarsingh *et al.*, 2007; Mishra *et al.*, 2009
	High	High	-	-	Rangaiah, 2000; Borah and Khan, 2002
	-	-	Moderate to high	High	Kumar and Sangwan, 2000
	-	-	High	High	Ram *et al.*, 1994; Sobha, 1994; Backiyarani and Natarajan, 1996; Mehta and Zaveri, 1998
	High	High	-	Moderate	Nehru and Manjunath, 2001
	Moderate	Moderate	Moderate	Moderate	Venkatesan *et al.*, 2003
	High	High	Moderate	Moderate	Anbuselvam *et al.*, 2000

Table 1 continued

Characters	PCV	GCV	Heritability	Genetic gain	References
Grain yield per plant	Moderate to high	Moderate to high	High	High	Tyagi *et al.*, 2000
	High	High	High	High	Kalaiyarasi and Palanisamy, 2000; Panicker, 2000; Vidya, 2000; Ajith, 2001; Khanpara *et al.*, 2015; Singh and Singh, 2018
100 seed weight	-	-	High	-	Apte *et al.*, 1987; Damarany, 1994; Ram and Singh, 1997
	High	High	-	-	Gowda *et al.*, 1991
	-	-	High	High	Kandasamy *et al.*, 1989; Thiyagarajan, 1989; Rewale *et al.*, 1995; Sreekumar, 1995; Ram and Singh, 1997
	High	High	High	-	Patil and Baviskar, 1987; Siddique and Gupta, 1991
	High	High	Moderate to high	High	Mareena, 1989
	High	High	High	High	Kalaiyarasi and Palanisamy, 2000; Khanpara *et al.*, 2015

2.7 ASSESSMENT OF PARENTAL POLYMORPHISM THROUGH SSR

Successful breeding requires profound information of the diversity available within the species. This information helps breeder to decide appropriate and diverse parents as per the objective/s of the breeding programmes. The parents which are phenotypically dissimilar need not to be always dissimilar genotypically as the phenotypes is a product of genotype and micro/macro environment. Moreover, morphological and physiological traits of different members of same species have a high level of genetic variation associated with a population of different geographic origin (Libby *et al.*, 1969). These facts make an assessment of parental polymorphism in available genotypes of great importance. It is also presumed that the varieties developed through crossing will give a more frequency of transgressive segregants if the parents used for crossing are, to some extent, genetically diverse (Simioniuc *et al.*, 2002). Knowledge of genetic diversity among the genotypes also helps for determining core collection for plant biodiversity conservation.

2.7.1 Molecular DNA based markers

The development and use of molecular markers for the detection and exploitation of DNA level polymorphism is a single most momentous development in the field of molecular genetics. Molecular markers (DNA markers) are developed to overcome the limitations of morphological markers which tend to express differently as the environment around plants changes. However, it does not mean that any of the biochemical or molecular techniques or both have replaced morphological markers. Molecular markers own great potential for its use in the breeding programmes. These markers are distinguishable DNA sequences, found at specific loci and transmitted by the standard laws of inheritance from one generation to the next (Semagn *et al.*, 2006). Apart from this, DNA markers are stable in different environments and plant developmental stages. The polymorphism in these markers provide the ability to discriminate between individuals, thereby helps in the careful selection of parents for a breeding programme.

A large number of PCR-based DNA markers *viz.*, Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), microsatellites or Simple Sequence Repeats (SSR), Inter-Simple Sequence Repeats (ISSR), *etc.* provide an opportunity for fine-scale genetic characterisations. Nevertheless, they also generate a large amount of data in a short period of time (Powell *et al.*, 1996; Hokanson *et al.*, 1998). Therefore, these DNA markers are often used for assessment of genetic diversity and relationships, DNA fingerprinting, genome mapping, in the conservation of genetic resources, studies of phylogeny and evolutionary biology, gene tagging, selection of targeted traits, *etc.* (Tautz, 1989; Williams *et al.*, 1990; Reddy *et al.*, 2002). Among *Vigna* genotypes, genetic diversity and intraspecific or interspecific relationships have been evaluated based on several DNA markers *viz.*, RAPD, SSR, AFLP, ISSR, *etc.* by several researchers (Fatokun *et al.*, 1993; Kaga *et al.*, 1996; Ajibade *et al.*, 2000; Li *et al.*, 2001; Souframanien and Gopalakrishna, 2004). For the present study, we have used SSR markers in order to assess the diversity among 30 different cowpea genotypes.

2.7.2 Simple Sequence Repeats (SSR)

The term microsatellite was coined by Litt and Lutty (1989). They are also called Simple Sequence Repeats (SSR) which are species-specific and belong to the repetitive DNA family. The SSRs are short tandem repeats consist of 1-6 bp long monomer sequence that is repeated number of times (Joshi *et al.*, 2000). These sections of DNA

contain repeating mono, di, tri, tetra or pentanucleotide units (Powell *et al.*, 1996). Dinucleotides are generally abundant in genomes. The SSR markers are PCR based and genetically co-dominant in nature. They are robust, reproducible, hypervariable, abundant, with good genome coverage and uniformly dispersed in the plant genome, and have a significance in plant genetics and breeding (Powell *et al.*, 1996; Gupta and Prasad, 2009; Sharma *et al.*, 2015).

Microsatellite markers have applications in genetic mapping, functional diversity and comparative mapping (Jonah *et al.*, 2011). They have been successfully adopted to analyse the genetic diversity in a variety of different plant species (McCouch *et al.*, 1997; He *et al.*, 2003; Frary *et al.*, 2005; Sarikamis *et al.*, 2010). The SSRs have been widely used in major crops. The first attempt to map microsatellites in plants was in rice using (GGC)n by Zhao and Kochert (1993) followed by mapping of (GA)n and (GT)n by Tanksley *et al.* (1992) and (GA/AG)n, (ATC)10 and (ATT)14 by Panaud *et al.* (1995). Several researches have used SSR marker system to assess genetic diversity. It was used to assess genetic diversity in barley (Saghai Maroof *et al.*, 1994; Holton *et al.*, 2002), wheat (Gupta and Varshney, 2000), rice (Chakravarthi and Naravaneni, 2006), sugarcane (Sharma *et al.*, 2014), Brazilian barley (Ferreira *et al.*, 2016), Chinese jujube (Fu *et al.*, 2016), some accessions of African plum in Cameroon (Tchinda *et al.*, 2016), *etc.*

2.7.3 Assessment of diversity in cowpea through SSRs

The SSR markers are widely used in cowpea to have an insight look of diversity present in available germplasm. Several researchers around the globe have reported diversity in cowpea genotypes by using SSR markers. For instance, Ogunkanmi *et al.* (2008) used SSR markers to assess the genetic diversity in wild accessions of cowpea. They used 48 wild cowpea lines collected from different geographical locations in Africa. A total of 90 polymorphic bands produced by 12 selected SSR markers. The highest polymorphism information content (PIC) was recorded in the accessions which were collected from a Southern part of Africa. These high values suggested a high level of diversity present in wild cowpea. Another study by Asare *et al.* (2010) also suggested the usefulness of SSR markers in the study of assessing genotypic polymorphism. They screened 141 accessions collected from nine geographical regions of Ghana using SSR molecular markers. Out of the 25 markers used, 20 produced distinct polymorphism. In their study, they detected 74 alleles at 20 loci with an average of 3.8 alleles per locus.

Wamalwa *et al.* (2016) assessed the genetic diversity of 20 accessions of cowpea using SSR markers. They observed high divergence between accessions from Ethiopia and Australia and those from Western Kenya. Chen *et al.* (2017) screened 54 SSR markers to assess genotypic polymorphism in 105 cowpea genotypes. They identified a total of 155 alleles and 2.9 alleles per marker with the average PIC value 0.366. Mafakheri *et al.* (2017) used 22 SSR markers in order to assess polymorphism in 32 genotypes of cowpea. In the molecular analysis of generated data, they observed a total of 186 alleles with an average of 2 alleles for each locus, and the PIC ranged from 0.250 to 0.625 with an average of 0.445.

A vast number of researchers used SSR markers in their study to assess genetic polymorphism of various crops. The reviews enlisted above clearly explain the usefulness and importance of SSR markers in assessing the genetic polymorphism and diversity present in cowpea germplasm.

2.8 COMBINING ABILITY AND GENE ACTION

A higher magnitude of general combining ability (GCA) variance indicates the predominant role of additive gene action which is fixable and higher specific combining ability (SCA) variance indicates dominance deviation and epistatic effect.

The literature on gene action of resistance to spotted pod borer in cowpea is scanty. However, as quoted by Phillip (2004), Pathak (1985) suggested additive gene action for resistance to spotted pod borer in cowpea.

As per Anilkumar (1993), in cowpea, the non-additive components are more predominant in the expression of days to flowering and number of pods per plant. Number of seeds per pod and 100 seed weight are governed by additive gene effects. Jayarani (1993) also reported the dominance of non-additive gene effects for plant height, number of branches per plant, number of pods per plant, number of seeds per pod, 100 seed weight and seed yield per plant.

Madhusudan *et al.* (1995), while working with a cowpea segregating generation, reported the presence of both additive and non-additive genetic interaction. They also reported the importance of additive and non-additive genetic variances in the inheritance of major quantitative traits with a dominance of non-additive gene effects in most cases. Anbuselvam *et al.* (2000) and Bastian *et al.* (2001) reported the involvement of non-additive and additive gene effects in the expression of agronomic characters.

Philip (2004) reported substantial GCA effects in cowpea for inflorescence per plant, pods per plant, pod length, seeds per pod and grain yield per plant. Patil and Navale (2006) in their study synthesised 24 hybrids of cowpea by crossing four lines and six testers in a Line × Tester (L × T) fashion. The 24 hybrids along with their parents and standard check (Pusa Komal) were then evaluated to estimate GCA and SCA effects and variances for yield and various yield contributing characters. The local cultivar, Manjarkheda Local among the lines and IC-201097 among the testers recorded significant GCA effects for seed yield per plant and most of the yield attributes *viz*., plant height, pods per plants, pod length, seeds per pod and test weight. All the crosses with significant SCA effects involved parents with high × high, high × low and low × low combining ability suggesting the presence of allelic and non-allelic interaction in the expression of these characters.

Nair (2006) reported significant differences among different genotypes for all characters particularly pod yield per plant in yard-long bean. The magnitude of SCA variance alone was significant indicating the predominance of dominance gene action in controlling the quantitative and biochemical characters. The same streamline of result was also reported by Kwaye *et al*. (2008).

Valarmathi *et al*. (2007) synthesised 36 hybrids using nine genotypes from *V. unguiculata* subsp. *unguiculata* (grain type) as female parents and four genotypes from *V. unguiculata* subsp. *sesquipedalis* (vegetable type) as male parents following L × T mating design. They observed a preponderance of SCA variance over the GCA variance for all characters studied, and further suggested the predominant role of non-additive gene action in controlling these characters.

Patel *et al*. (2010) confirmed the preponderance of non-additive gene action in the inheritance of days to 50 per cent flowering, days to first picking and seeds per pod. They also observed of both additive and non-additive gene action for pod yield per plant, leaf area, branches per plant, plant height, pods per plant and protein content. However, in their study, the magnitude of dominant gene action was greater than their corresponding additive gene effects for all the traits. Finally, they concluded that the dominant genes played a significant role in the control of all the characters in cowpea.

Selvakumar *et al*. (2014) carried out a research trial to determine combining ability of 11 selected cowpea genotypes and derived crosses. Six lines and five testers were crossed in L × T fashion and 30 hybrids were developed. Genotypes GC3, Co6, ACM05-07, RC101, Co(CP)7 and ACM05-02 belonging to *V. unguiculata* were used as

lines, whereas, Vellayani Local, Ettumanoor Local, Vyjayanthi and Vellayani Jyothika belonging to *V. unguiculata* spp. *sesquipedalis* and VBN2 belonging to *V. unguiculata* were used as testers. The results indicated the presence of both additive and non-additive genetic components for most of the traits. Based on GCA, the parents GC3, RC101, Vyjayanthi and Vellayani Jyothika were selected as good combiners.

Dias *et al.* (2016) evaluated six cowpea genotypes and their F_1 hybrid combinations for GCA and SCA effects, and confirmed the presence of additive and non-additive gene effects for a number of characters. Moreover, they observed the predominance of additive gene effects in the trait expression.

Gupta *et al.* (2017) established the higher magnitude of dominance component than the additive component for a number of quantitative characters. By their study, both, additive and non-additive gene actions also confirmed to contribute significantly to the inheritance of various quantitative characters in cowpea.

Pethe *et al.* (2018) analysed eight lines, three testers and their 24 crosses of cowpea (developed through L × T mating design). The data clearly indicated the preponderance of non-additive gene action for all characters under study. They also observed that the characters *viz.*, pod length (46.61 %), number of grains per pod (40.36 %) and harvest index (33.21 %) had high heritability.

2.9 HETEROSIS IN YIELD CONTRIBUTING TRAITS

The term heterosis was first used by G. H. Shull in 1914 (Shull, 1914). The superiority of a hybrid in one or more quantitative traits over its parents is known as heterosis. Presence of significant amount of dominance variance is crucial for conducting heterosis breeding programmes. Even, the expression of small amounts of heterosis for yield contributing characters is also greatly desirable in breeding.

Danam and Chaudhari (2000) crossed nine parents of cowpea following diallel mating design and observed desired positive heterosis in seed yield over mid-parent, better parent and standard check. They also reported that the heterotic effect in yield was a cumulative effect of heterosis in yield contributing traits mainly pods per plant, seeds per pod, clusters per plant and branches per plant. Bhushana *et al.* (2000) also reported the similar results with respect to heterosis in yield contributing characters.

Philip (2004) reported desirable negative heterosis for days to flowering. In the study, she observed seven crosses with positive and significant estimates of all three types of heterosis (heterobeltiosis, average heterosis and standard heterosis) for pods per plant

whereas, three crosses had positive and significant estimates of heterosis for inflorescence per plant, pods per inflorescence and grain yield.

Several other researchers reported heterosis in segregating generations of different crosses of cowpea. Patel *et al.* (2009) reported desired heterosis in yield contributing characters like days to flowering, plant height, branches per plant, pods per plant, pod length, seeds per pod, 100 seed weight and seed yield per plant, Patel *et al.* (2013) reported heterosis in number of effective branches per plant, number of pods per plant, pod length, seeds per pod and 100 seed weight, Anitha *et al.* (2017) reported heterosis in days to 50 per cent flowering, plant height and number of branches per plant, Varan *et al.* (2017) reported heterosis in number of pods per plant, pod length, plant height, number of seeds per pods, 100 seed weight and seed yield per hectare.

Materials and methods

3. MATERIALS AND METHODS

The present study was taken up at the Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University (KAU), Thrissur from 2015 to 2018. Evaluation of cowpea genotypes for resistance to spotted pod borer (*Maruca vitrata* Fab.) was carried out as experiment 1. The selected parents from experiment 1 were hybridised with high yielding varieties to develop F_1s in experiment 2. Parental polymorphism was studied by molecular markers in experiment 3, and evaluation of F_1 and F_2 populations were done in experiments 4 and 5, respectively. Details of the materials used and methods followed in the study are described in this chapter.

3.1 EXPERIMENTAL DETAILS

3.1.1 Experimental material

Thirty genotypes of cowpea (Plate 1a and 1b) comprising of 20 genotypes from National Bureau of Plant Genetic Resources, Jodhpur Regional Station, Rajasthan (NBPGR RS), Jodhpur, six released varieties from KAU, one genotype each from University of Agricultural Sciences (UAS), Bengaluru, Vegetable and Fruits Promotion Council Keralam (VFPCK), Thiruvananthapuram, Indian Institute of Vegetable Research (IIVR), Varanasi and Indian Institute of Horticultural Research (IIHR), Bengaluru were evaluated for resistance to spotted pod borer. These genotypes constituted the treatments in the field experiment (Table 2).

3.1.2 Design and layout of field experiment

The experiment was laid out in Randomized Block Design (RBD) with 30 treatments and two replications, with 20 plants in each replication (Fig. 1). Agronomic practices were adopted as per the Package of Practices Recommendations of Kerala Agricultural University (KAU POP, 2011).

Table 2. Details of the cowpea genotypes evaluated for resistance to spotted pod borer

Treatment	Genotype	Source	Type and growth habit
T1	Geethika	KAU, Thrissur	Vegetable type- Trailing
T2	Vellayani Jyothika	KAU, Thrissur	Vegetable type- Trailing
T3	Lola	KAU, Thrissur	Vegetable type- Trailing
T4	Hridya	KAU, Thrissur	Grain type
T5	Palakkadan thandan payar	VFPCK, Thiruvananthapuram	Grain type

Table 2 continued

Treatment	Genotype	Source	Type and growth habit
T6	Kanakamony	KAU, Thrissur	Vegetable type- Semi trailing
T7	Mysore Local	IIHR, Bengaluru	Grain type
T8	Kashi Kanchan	IIVR, Varanasi	Dual purpose-Bushy
T9	EC 300039	NBPGR RS, Jodhpur	Grain type
T10	EC 98668	NBPGR RS, Jodhpur	Grain type
T11	EC 101216	NBPGR RS, Jodhpur	Grain type
T12	IC 52110	NBPGR RS, Jodhpur	Grain type
T13	IC 39945	NBPGR RS, Jodhpur	Grain type
T14	IC 2918	NBPGR RS, Jodhpur	Grain type
T15	IC 39922	NBPGR RS, Jodhpur	Grain type
T16	IC 52118	NBPGR RS, Jodhpur	Grain type
T17	IC 39916	NBPGR RS, Jodhpur	Grain type
T18	IC 2196	NBPGR RS, Jodhpur	Grain type
T19	IC 20645	NBPGR RS, Jodhpur	Grain type
T20	IC 26048	NBPGR RS, Jodhpur	Grain type
T21	IC 52107 A	NBPGR RS, Jodhpur	Grain type
T22	IC 39947	NBPGR RS, Jodhpur	Grain type
T23	IC 39921	NBPGR RS, Jodhpur	Grain type
T24	IC 26029	NBPGR RS, Jodhpur	Grain type
T25	IC 20720	NBPGR RS, Jodhpur	Grain type
T26	IC 39870	NBPGR RS, Jodhpur	Grain type
T27	IC 52105	NBPGR RS, Jodhpur	Grain type
T28	IC 9883	NBPGR RS, Jodhpur	Grain type
T29	TVX-944	UAS, Bengaluru	Grain type
T30	Bhagyalakshmy	KAU, Thrissur	Vegetable type- Bushy

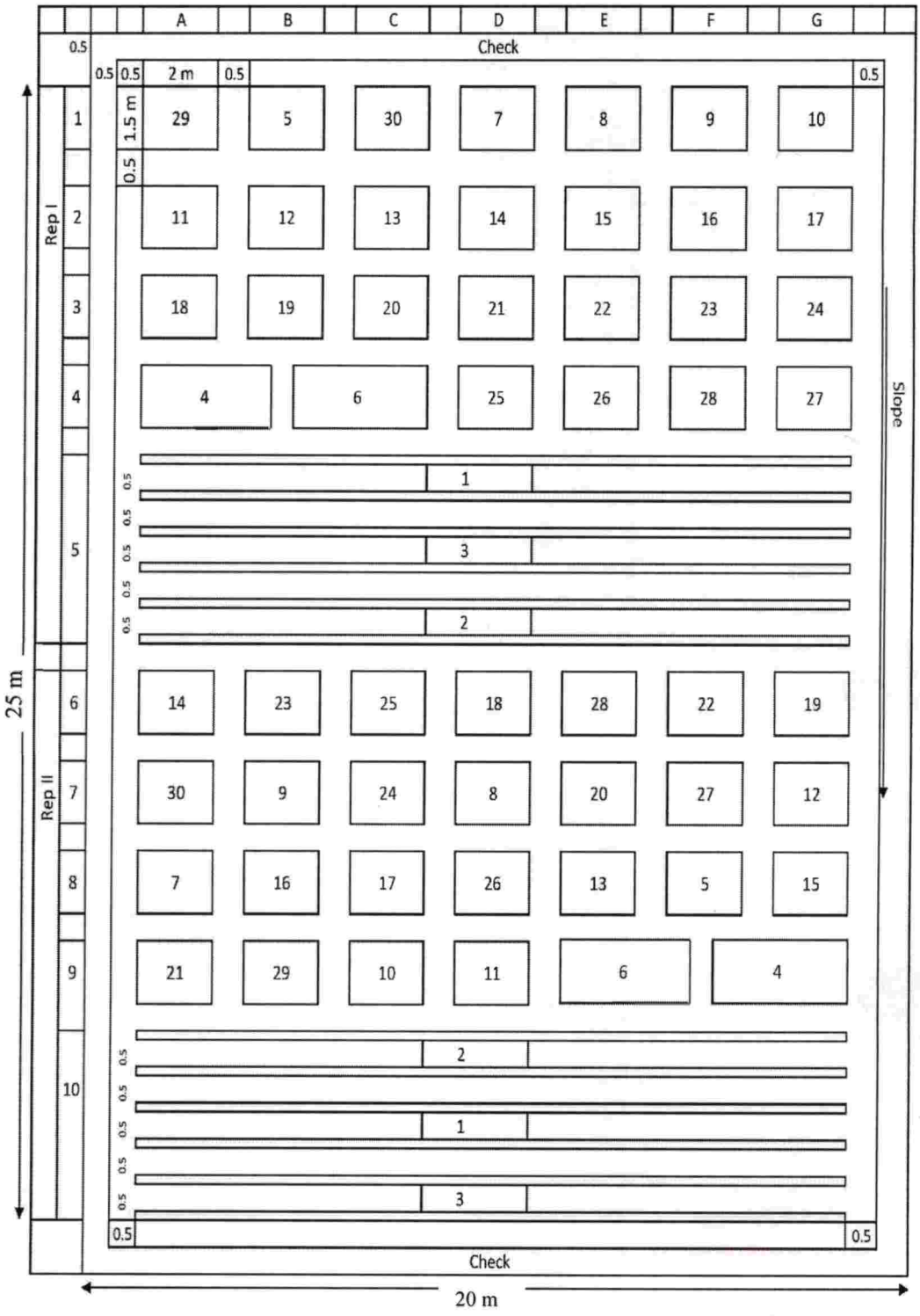

Fig. 1. Layout of experimental field

Plate 1a. Seeds of cowpea genotypes used in the study

Plate 1b. Seeds of cowpea genotypes used in the study

3.2 EXPERIMENT 1

3.2.1 Field screening of cowpea genotypes for resistance to spotted pod borer

Field evaluation of 30 cowpea genotypes was carried out by raising plants in an open field at a spacing of 30 × 15 cm, 45 × 15 cm and 2 × 2 m for bush, semi-trailing and trailing types, respectively (Plate 2) during *Kharif* 2016. Two weeks prior to planting, the variety Lola was sown along the border around the plot to serve as multiplication site for the test insect, spotted pod borer. Observations on the spotted pod borer incidence were recorded at three days interval starting from first flowering up to the end of flowering. Ten plants were selected at random from each genotype and tagged to record spotted pod borer incidence on flower buds, flowers and pods (Plate 3). Flower buds, flowers and pods once counted were tagged to avoid recounting. The per cent damage was calculated based on the ratio of infested flower buds, flowers and pods to the total number of flower buds, flowers and pods, respectively. Based on the extent of total damage caused by spotted pod borer, the cowpea genotypes were categorised into four groups *viz.*, resistant, moderately resistant, susceptible and highly susceptible (Beegum, 2015).

3.2.2 Evaluation of morphological basis of resistance in cowpea to spotted pod borer

In order to study the morphological basis of resistance in cowpea to spotted pod borer, a minimum of ten flower buds and pods were selected at random per replication for each genotype and the following observations were recorded. The mean value was worked out for each observation and expressed in corresponding units.

3.2.2.1 Pod wall thickness

The thickness of the pod wall of all genotypes were measured at vegetable maturity by using a digital Vernier calliper (Plate 4). The mean pod wall thickness was calculated and expressed in millimetres.

3.2.2.2 Trichome length and density

Leica-EZ4D stereomicroscope equipped with Leica Application Suite (LAS) image analysing software was used to observe trichomes on flower bud (calix) and pod. Trichomes were observed at 35X magnification.

Density of trichomes on the flower bud and pod surface was measured from an area of 1 mm^2 after marking out an area of 1 × 1 mm digitally by using Digimizer image analysing software (Plate 5). Length of ten trichomes were taken on flower buds and pods

Plate 2. Views of the experimental plot

3a. Adult moth of spotted pod borer

3b. Infestation to flower bud

3c. Infestation to flower

3d. Infestation to pod

Plate 3. Infestation by spotted pod borer during different stages of cowpea

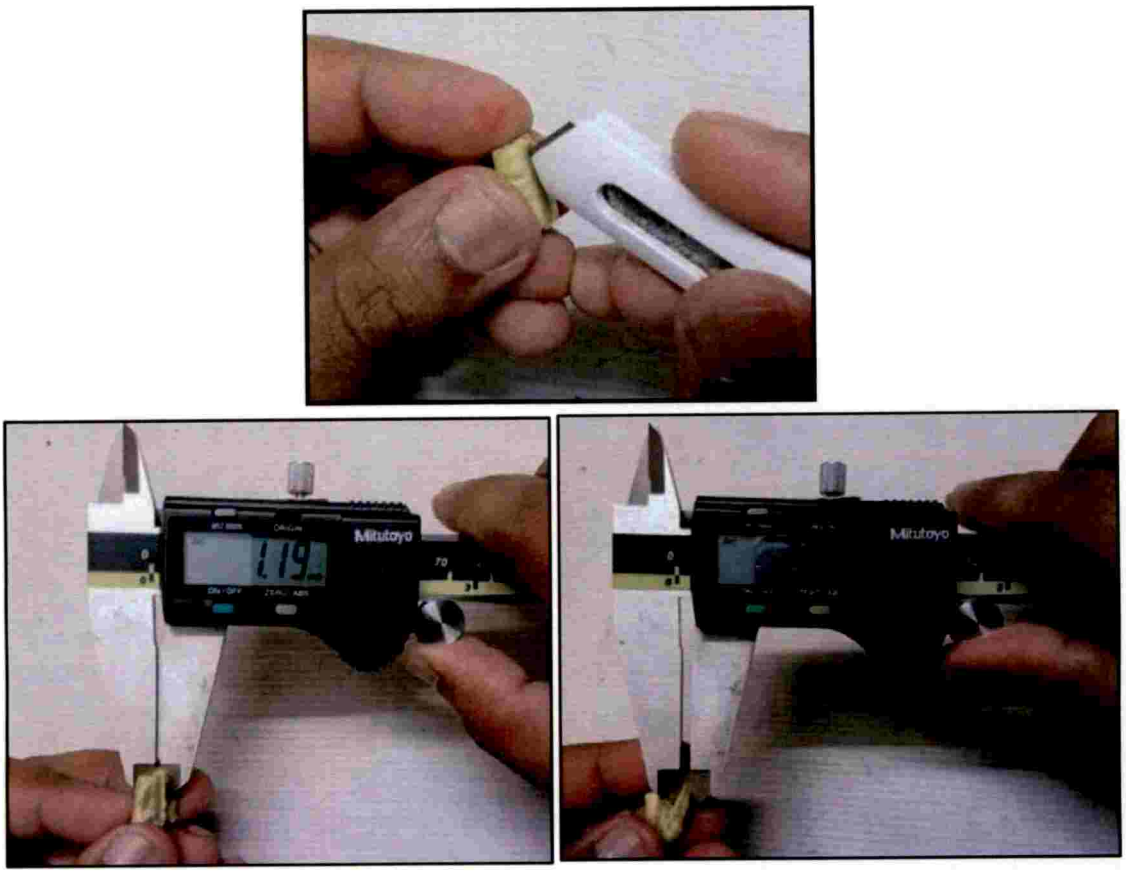

Plate 4. Measurement of pod wall thickness

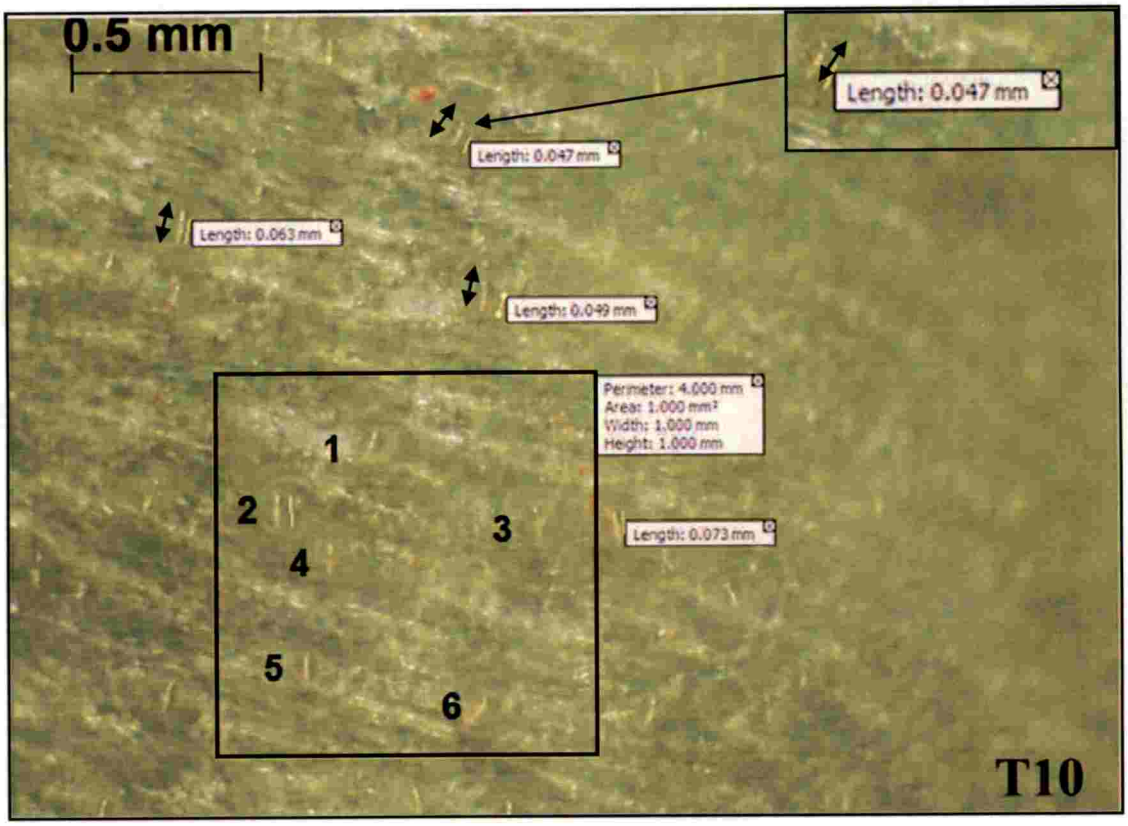

Plate 5. Measurement of trichome length and density

selected at random from each genotypes and the averages were worked out and expressed in millimeter. Counts were taken from three different points on each flower bud and pod and the averages were worked out.

3.2.3 Evaluation of biochemical basis of resistance in cowpea to spotted pod borer

Biochemical basis of resistance to spotted pod borer in cowpea was reconnoitered by estimating the total sugar, reducing sugar, non-reducing sugar, total protein, total phenol content of flower bud and pod, and crude fibre (only for pod wall) as well as through assaying peroxidase and polyphenol oxidase enzyme activities in flower bud and pod. The studies were carried out following the standard procedure as described below.

3.2.3.1 Total sugar content

Anthrone reagent method was followed to determine total sugar in test samples (Hedge and Hofreiter, 1962). Hundred milligrams of sample was hydrolysed with five millilitres of 2.5 *N* HCl in a boiling water bath for three hours. The hydrolysed content was then neutralised with solid sodium carbonate until effervescence ceased. The final volume of content was made up to 50 ml using DDH_2O (Doubled Distilled Water). From supernatant, one millilitre aliquot was used for analysis. To this one millilitre extract, four millilitres of Anthrone reagent was added followed by heating in a boiling water bath for eight minutes. Then, the hot content was cooled rapidly and the optical density was measured at 630 nm using a spectrophotometer. For standard readings, 0.1 mg /ml of glucose was used in the procedure. Regression factor was calculated using readings of standards and concentration of total sugars in the test sample (1 ml) was then calculated by multiplying the regression factor with the absorbance of samples and expressed in per cent.

3.2.3.2 Reducing sugar content

Estimation of reducing sugar was done by following dinitrosalicylic acid (DNS) method (Miller, 1959). Sugar extraction was done by grinding 500 mg of fresh cowpea pod and flower bud with hot 80 per cent ethanol twice (5 ml each time). After centrifugation, the supernatant was collected in a beaker and ethanol was evaporated by heating the beaker in a water bath at 80 °C. Then, 10 ml of distilled water was added to dissolve sugars. One millilitre of the extract was pipetted out and final volume was made to three millilitres by using DDH_2O. To this, three millilitres of DNS reagent was added followed by the heating content in a boiling water bath for five minutes. When the content was still warm, one millilitre of Rochelle salt solution (40 %) was added. The content

was allowed to cool and intensity of red colour (optical density) was recorded at 510 nm using a spectrophotometer. In the procedure, 0.1 mg /ml of glucose was used as standard. Regression factor was calculated using readings of standards. Concentration of reducing sugar in the test sample (1 ml) was then calculated by multiplying the regression factor with the absorbance of samples and expressed in per cent.

3.2.3.3 Non-reducing sugar content

Estimation of non-reducing sugar was done by subtracting the amount of reducing sugar from the amount of total sugar and expressed in per cent.

3.2.3.4 Total protein content

The protein content of the flower bud and pod was estimated as per the following procedure described by Lowry *et al.* (1951). Five hundred milligrams of the fresh cowpea flower buds and pods were weighed and macerated with a mortar and pestle in 10 ml phosphate buffer (0.1 *M*) of pH 7.0. This homogenate was centrifuged and the supernatant was used for protein estimation. In the test tubes, 0.1 ml of sample extract was added and the volume was made up to one millilitre. A tube with one millilitre of water served as the blank. Five millilitres of reagent C (50 ml of 2 % sodium carbonate in 0.1 *N* sodium hydroxide- reagent A and 1 ml of 0.5 % copper sulphate solution in 1 % sodium potassium tartarate- reagent B) was added to each tube including the blank. It was mixed well and allowed to stand for ten minutes. Then 0.5 ml of reagent D (Folin-Ciocalteu reagent) was added, mixed well, and incubated at room temperature in darkness for 30 min. The blue colour developed (optical density) was read at 660 nm using a spectrophotometer. Bovine Serum Albumin (BSA) (0.2 mg /ml) solution was used as a standard. Standards were also subjected to the same reaction as test samples, and the standard's graph was plotted. The concentration of total protein in the test samples were then calculated and expressed in per cent.

3.2.3.5 Total phenol content

Total phenol content in the flower bud and pod was estimated by Folin-Ciocalteu reagent method developed by Malik and Singh (1980). The fresh sample (100 mg) was macerated with 10 ml of ethanol (80 %) using mortar and pestle. The homogenate was then centrifuged at 10,000 rpm for 20 min. The supernatant was collected in a beaker. The remaining residue was then re-extracted with five millilitres of 80 per cent ethanol, centrifuged and the supernatant was collected in the same beaker. The ethanol was then allowed to evaporate. Five millilitres DDH_2O was added to the residue to dissolve the

phenols. From this solution, one millilitre of sample pipetted into a test tube and the volume was made up to three millilitres using DDH_2O followed by the addition of 0.5 ml of Folin-Ciocalteu reagent. This solution was then kept for three minutes and then two millilitres of 20 per cent sodium carbonate solution was added and mixed well. The test tubes were kept in a boiling water bath for one minute and then cooled to room temperature. The blue colour that developed following a complex redox reaction with phosphomolibdic acid present in Folin-Ciocalteu reagent in an alkaline medium. The intensity of the blue colour (optical density) was recorded at 650 nm using spectrophotometer.

For a standard, a stock solution prepared by dissolving 100 mg of catechol in 100 ml of distilled water. Working standards were prepared from this by diluting the stock solution ten times. Series of working standards were taken (0.2, 0.4, 0.6, 0.8 and 1 ml) and the final volume made to three millilitres and the same set of steps followed to develop blue colour, was followed to develop colour. The absorbance was recorded at 650 nm to plot a standard's graph.

Regression factor was calculated using readings of standards. The concentration of total phenol in the test sample (1 ml) was then calculated by multiplying the regression factor with the absorbance of the sample and expressed as milligram of catechol equivalent of phenol per gram sample (mg CE/g).

3.2.3.6 Peroxidase activity

Peroxidase activity was assayed by the method developed by Putter (1974). A known sample of fresh flower bud and pod were homogenised in 10 ml ice-cold 0.1 *M* phosphate buffer (pH 7.0). The homogenate was centrifuged for 15 min. at 11,000 rpm at 4 °C. The supernatant was immediately used for assaying the enzyme activity. Two millilitres of 0.1 *M* phosphate buffer (pH 7), one millilitre of 20 *mM* guaiacol and 40 µl of supernatant was taken in a clean dry cuvette which was transferred to a spectrophotometer. In order to start the reaction, 50 µL of 10 *mM* hydrogen peroxide was added to the cuvette. Initial absorbance and then change in absorbance were noted after an interval of 30 sec. for three minutes at 470 nm. Enzyme units were expressed in terms of the change in absorbance per minutes per gram of tissue weight (EU/g).

$$\frac{Peroxidase}{activity} = \frac{Average\ difference\ in\ OD\ per\ minute}{Volume\ of\ enzyme\ extracts} \times \frac{Total\ volume}{Weight\ of\ the\ tissue}$$

3.2.3.7 Polyphenol oxidase activity

Polyphenol oxidase activity was assayed by following the method of Esterbaner *et al.* (1977). A half gram of fresh tissue (flower bud and pod) was macerated with 10 ml of ice-cold 0.1 *M* phosphate buffer (pH 7.0) at 1:5 ratio. The final volume was adjusted to 10 ml. This mixture was centrifuged at 11,000 rpm at 4 °C for ten minutes and the supernatant was used for enzyme assay. Two millilitres of 0.1 *M* phosphate buffer (pH 7) and one millilitre of catechol solution (0.01 *M*) was mixed in a cuvette. The spectrophotometer was set at 495 nm. Then, one millilitre of enzyme extract was added to this mixture and initial absorbance and change in the absorbance recorded at every 30 sec. up to five minutes. One unit of catechol oxidase is defined as an enzyme which transforms 1 µmol of dihydrophenol to 1 µmol of product per minute under the assay condition. The activity of polyphenol oxidase was estimated by the formula given below, and enzyme units were expressed in terms of change in absorbance per minute per gram of tissue weight (EU/g).

$$\text{Enzymatic units in the test} = K \times (\Delta A / \text{minutes})$$

Where, K = 0.272 (for catechol oxidase)

ΔA = Initial value of absorbance –Final value of absorbance

3.2.3.8 Crude fibre content of immature pod

Estimation of crude fibre was done following method given by Maynard (1970). Two grams of dried ground pod wall was boiled with 200 ml of sulphuric acid solution (0.005 *N*) for 30 min. with bumping chip. The material was filtered through muslin cloth followed by multiple washing with warm DDH_2O until washings were no longer acidic. Then, the filtrate was boiled with 200 ml of sodium hydroxide (0.005 *N*). Filtration and washing were repeated. After making filtrate alkali-free, the filtrate was washed with 10 ml of acetone and DDH_2O. Residues were then removed carefully without losing any part and transferred to ashing dishes (pre-weighed dish W_1). These ashing dishes kept in the oven for two hours at 130±2 °C. Ashing dishes were then cooled in a desiccator and weighed (W_2). The samples in ashing dishes then ignited for 30 min. in a muffle furnace at 600±15 °C cooled and weighed (W_3). Amount of crude fibre was then estimated using the following formula and expressed in per cent.

$$Crude\ fibre\ (\%) = \frac{(W_2 - W_1) - (W_3 - W_1)}{Weight\ of\ sample} \times 100$$

3.3 EXPERIMENT 2: DEVELOPMENT OF F_1 HYBRIDS

The genotypes identified as resistant to spotted pod borer in experiment 1 were used for crossing with high yielding popular varieties *viz.*, Geethika, Vellayani Jyothika, Lola and Kashi Kanchan in Line × Tester mating design. experiments. From experiment 1, five testers were selected on the basis of damage parameters. The four lines and five testers were raised in a crossing block at the end of 2016 and hybridisation was done to obtain 20 F_1 hybrids.

The technique of artificial pollination suggested by Krishnaswamy (1970) was followed to produce hybrids. The flower buds which were about to bloom on the next day morning were selected on the lines on previous evening for emasculation. The flower bud was carefully opened by split opening standard and wing petal along the ridge by using a needle to expose the anthers. The ten immature stamens were then carefully taken out one at a time by grabbing the filament with forceps without damaging anthers or stigma. Care has been taken not to leave even a single anther in a flower bud. Butter paper bags were used to protect the emasculated flowers.

On the next early morning, pollination was done using pollens from freshly opened flowers of selected tester plants. The standard and wing petals of the male flowers were removed. The keel petal was gently pressed to expose the stamens covered with pollen grains. The exposed stamens were as such used to dust the pollen on the stigma of the emasculated flowers. The pollinated flower was then covered with butter paper bag and the cover was retained for another 2-3 days. Proper tagging was done with all the required information.

3.4 EXPERIMENT 3: ASSESSMENT OF PARENTAL POLYMORPHISM

3.4.1 Laboratory chemicals, glassware and equipment

The AR (analytical reagents) grade chemicals (extra pure) from Sisco Research Laboratories (SRL) were used in this study. The constituents for PCR reaction mixture *viz.*, Taq buffer, $MgCl_2$, dNTPs, Taq DNA polymerase, *etc.* used in this study were procured from Genei Pvt. Ltd., Bangalore. The plastic wares from Tarson India Ltd. were used. The SSR (Simple Sequence Repeats) primers synthesised by Sigma Aldrich Chemicals Pvt. Ltd., Bangalore were used.

For centrifugation, high-speed refrigerated centrifuge (Eppendorf 5804 R) was used. The DNA quality and quantity estimation were done using Nanodrop Spectrophotometer (Jenway- Genova Nano). Eppendorf Mastercycler® nexus gradient PCR machine was used for the DNA amplification. Horizontal gel electrophoresis unit by Bio-Rad, USA was used for Agarose gel electrophoresis

3.4.2 DNA isolation

3.4.2.1 Reagents used

I. Liquid nitrogen

II. CTAB extraction buffer (2 %)
   - 2 per cent CTAB (w/v)
   - 100 *mM* Tris (pH 8.0)
   - 20 *mM* EDTA (pH 8.0)
   - 1.4 *M* NaCl

III. 10 per cent CTAB solution
   - 10 per cent CTAB (w/v)
   - 0.7 *M* NaCl

IV. Chloroform: Isoamyl alcohol (24:1 v/v)

V. Isopropanol (100 %)

VI. Ethanol (70 % and 100 %)

VII. Sterile autoclaved distilled water

3.4.2.2 Procedure for extraction of genomic DNA

The DNA was isolated by following the CTAB protocol of Doyle and Doyle (1990) with the slight modifications of buffer concentration. The young newly flushing leaves were collected from seedlings grown in lab conditions. The extraction of genomic DNA was done using the following protocol.

1 Newly flushed tender leaf samples were collected and ground to a fine powder in liquid nitrogen using pre-chilled autoclaved mortar and pestle with 15 µl β-mercaptoethanol and a pinch of polyvinylpyrrolidone (PVP).

2 Homogenised samples were transferred to autoclaved 2 ml centrifuge tube with one millilitre of pre-warmed extraction buffer.

3 The tubes were inverted a few times to mix the contents and incubated at 65 °C in water-bath for 20 min. with gentle inversion once.

4 After incubation, the tubes were taken out and equal volume (1 ml) of chilled chloroform: isoamyl alcohol (24:1) was added, inverted to mix and emulsify. The contents centrifuged at 12,000 rpm for 15 min. at 4 °C.

5 After centrifugation, the contents got separated into three distinct layers.

- Aqueous topmost layer: containing DNA and RNA
- Interphase: containing fine particles and proteins
- Lower layer: containing chloroform and some pigments

6 The tubes were carefully taken out from the centrifuge without disturbing the three layers, and the top aqueous layer was carefully transferred to a fresh centrifuge tube. To this $1/10^{th}$ volume of 10 per cent CTAB solution and equal volume of chloroform: isoamyl alcohol (24:1) were added.

7 The content was mixed well with gentle inversions and centrifuged at 12,000 rpm for 15 min. at 4 °C.

8 After centrifugation, the tubes were taken out and the topmost layer was carefully transferred to a new centrifuge tube. To this 2 µl of RNase was added and incubated in the water bath at 37 °C for 15 min.

9 After incubation, an equal volume of chloroform: isoamyl alcohol (24:1) was added and centrifuged at 12,000 rpm for 15 min. at 4 °C.

10 After centrifugation, the aqueous phase was carefully transferred to a new 1.5 ml centrifuge tube. To this, 0.6 volume of chilled isopropanol was added and the tubes were incubated at -20 °C for two hours.

11 After incubation, the tubes were centrifuged at 10,000 rpm at 4 °C for ten minutes.

12 Then, the supernatant was discarded and to the pellet, 200 µl of 70 per cent ethanol was added. Then the tubes were centrifuged at 10,000 rpm for five minutes at 4 °C.

13 The 90 per cent ethanol wash was repeated. After centrifugation, the supernatant was discarded without disturbing the pellet.

14 The pellets were dried inside the laminar air flow until all the ethanol got evaporated and was dissolved in 70 µl autoclaved DDH_2O.

15 The tubes were gently tapped to dissolve pellet completely and then the DNA samples were stored at -20 °C.

3.4.3 Quality and quantity estimation of DNA with a spectrophotometer

The purity and quantity of the DNA were estimated using a Nanodrop Spectrophotometer (Jenway- Genova Nano). Since the absorption maxima for nucleic acids and proteins are at 260 and 280 nm, respectively, absorbance have been recorded at both the wavelengths and purity of the sample was estimated using the OD260/OD280 ratio. The DNA sample was considered to be pure if the OD260/OD280 value is between 1.8 and 2.0. Values below 1.8 and above 2.0 are due to contamination by protein and RNA, respectively. The concentration of DNA in the sample was estimated using the relation, 1 OD at 260 nm = 50 ng DNA/µl, hence, OD260×50 gave the quantity of DNA (ng/µl)

3.4.3.1 Procedure

1 The lid of spectrophotometer has been opened followed by the sampling arm, and the pedestal was wiped with tissue paper to remove any dust particles.

2 The reading was set to zero with a blank sample (DDH_2O which used to dissolve the DNA pellet).

3 Then, 1 µl of the test sample was loaded on to the pedestal and measure option was selected and necessary readings were recorded.

4 After the measurements, the pedestal was wiped clean with 70 per cent ethanol using a soft laboratory wipe.

3.4.4 Agarose gel electrophoresis

3.4.4.1 Reagents used

1. Agarose (0.8 %)
2. 50X TAE buffer (pH 8.0)
   - Tris buffer (1 *M*)
   - Glacial Acetic acid
   - 0.5 *M* EDTA
3. Tracking/loading dye (6X)
4. Ethidium bromide (stock 10 mg /ml, working concentration 0.5 µg/ml)

3.4.4.2 Procedure

1 The gel casting tray was placed appropriately in a gel caster and the movable wall was adjusted such that the gel casting tray was closed at both ends. A comb was

selected depending on the number of samples to be electrophoresed and positioned on the grooves provided on the gel casting tray.

2 The gel was prepared by adding 0.8 g of agarose in 100 ml of 1X TAE buffer in a glass conical flask. The mixture was heated in a microwave oven until all the agarose particles were completely dissolved and a clear solution was obtained.

3 Then the solution was allowed to cool down to 40 to 50 °C and an appropriate amount of ethidium bromide was added and mixed well. The warm gel was then poured into the gel casting tray and left to solidify for 20 min. at room temperature.

4 Special care was taken to avoid any air bubbles near the wells or on the gel

5 Once the gel was solidified, a small amount of 1X TAE was poured on top of the gel and the comb was removed carefully without breaking the gel. The TAE solution was discarded and the gel along with the tray was kept inside the electrophoresis tank with the wells on the negative electrode side.

6 The electrophoresis tank was filled with 1X TAE sufficient enough to submerge the wells.

7 The samples to be electrophoresed were prepared by mixing 5 µl of the DNA sample with 1 µl of 6X gel loading dye. After mixing, the total volume of 6 µl was loaded into individual wells.

8 The samples were electrophoresed at 75 volts until gel tracking dye reached two third of the gel length.

3.4.4.3 Gel documentation

Documentation of the electrophoresed gel was done under UV with E-Gel Imager gel documentation system using E-Gel Software.

3.4.5 Preparation of reaction mixture for thermal cycling

The reaction mixture was consists of template DNA, reaction buffer, $MgCl_2$, SSR primer (forward and reverse), dNTPs, DDH_2O and Taq DNA polymerase. The desired number of PCR cycles, time and temperatures for denaturation, annealing (AT) and extension were standardizsed based on the primers used (Table 3) and the conditions were programmed and saved in the thermal cycler (model- Mastercycler® nexus gradient PCR, made: Eppendorf).

3.4.5.1 Thermal cycling

1 PCR microcentrifuge tubes (0.2 ml) were numbered from 1 to 30.

2 1.5 µl of template DNA from individual genotypes was added to each tube.

3 18.5 µl of master mix was added to all the tubes and was given a short spin to mix the contents.

Thermal cycling was carried out with 20 µl reaction mixture. The composition of the reaction mixture used was

a.	Genomic DNA (25 ng/µl)	:	1.5 µl
b.	10X Taq assay buffer B	:	2.0 µl
c.	$MgCl_2$	:	0.7 µl
d.	dNTP mix (2.5 *mM* of each)	:	1.0 µl
e.	Taq DNA polymerase (3 Units)	:	0.3 µl
f.	Primer (10 *pM*)	:	1 µl each of forward and reverse primer
g.	Chilled autoclaved distilled water	:	12.5 µl
	Total reaction volume	:	20.0 µl

4 The tubes were placed in the thermal cycler for 35 cycles of PCR.
The PCR programme followed was

a.	94 °C for 4 min.	:	Initial denaturation	
b.	94 °C for 45 sec.	:	Denaturation	35 Cycles
c.	50 °C to 55 °C for 1 min.	:	Primer annealing	
d.	72 °C for 2 min.	:	Primer extension	
e.	72 °C for 8 min.	:	Final extension	
f.	4 °C hold for infinity	:	Storage	

5 Samples were held at 4 °C in the thermal cycler followed by storage at -20 °C until the contents were loaded on to the gel for electrophoresis.

6 The PCR amplified products were electrophoresed on 2 per cent agarose gel at 70 volts. A ProxiO 100 bp DNA Ladder Plus (SRL) was used. The gel profile was visualized under UV and was saved for further analysis.

Table 3. List of SSR primers (with their forward and reverse sequences) used in the study

Sl. No.	Primer name	Forward primer (5'-3')	Reverse primer (5'-3')	Average AT (°C)
1	CLM0002	ACAACAGCATCATCCCAAGT	ATCCACAGCCTTTATCACCA	53
2	CLM0009	AACTTCCCCGGAGTCTTCTA	GTGCGAGAAGAGAATCGAGA	55
3	CLM0010	CATTGCCTTGCATTTCTTTT	GAGTTTCTGGGACGATCAGA	52
4	CLM0014	CGTTCACCCATTTCTCATTC	CAAGATCACATCCAAGCACA	53
5	CLM0015	TGAAACGTGAAGCATCAAAA	CTGTTGGAACTGGAGGACAC	53
6	CLM0016	AGCAACACCAAAACACTCAAG	AGATTTGACCTAGCGCATTG	54
7	CLM0029	TGTGTGTGTTCGGTTTCTTG	GCTAGTTCCCCCTTCAGAAC	55
8	CLM0042	GAAAACAACATGGCTTCTGG	CATGGTGTTCCTGGTTGATT	53
9	CLM0061	AACATTTTCACCATTGATCG	CAAGCCACCAATCCTTTTAT	50
10	CLM0062	TGAAAGCTGCAAGATTGATG	AATTTTTGTTTGCGTGCTTC	50
11	CLM0137	CCATCAAACCATGGTCTCTC	GAACCATAGCAAGCAAGGAA	54
12	CLM0139	GTGCCGGGTATTTATTGTTG	TTTGTGGTGCTTATTGCACA	52
13	CLM0185	TCAAGGTCGTGTGAGGAAGT	GTGGAGGAGAGATGATGGTG	56
14	CLM0187	GTGCACAACCAATTCAATCA	CCCATGCAACATATCTACCC	53
15	CLM0190	TGAGTGGGATTGAAAGAAGTTT	TTATCAATGGACACTCAAGGG	55
16	CLM0191	TGGGATTCTTCTGCTGAGAT	TGCAAGCAAGTAATCCCTCT	53
17	CLM0192	CTGGTTCAAATATTTACAGAAA	ACGGGTTCAACATTCCAAC	51
18	CLM0193	ATCAACGGTGGTTGTTTCAG	TGAGGAAACTGAACTCAGGC	54
19	CLM0227	CCAAGAGTGGCCTGAGTAAA	TGCAATATTCTTAGGTCTAAAACG	57

Table 3 continued

Sl. No.	Primer name	Forward primer (5'-3')	Reverse primer (5'-3')	Average AT (°C)
20	CLM0230	TCATGAGTGCACG AGTGTTT	TTCCCAACAGAAGCA AGAAG	53
21	CLM0232	TGCTTCGACGAACT TTTACC	CAGCTAGCGGACCAA GATAA	54
22	CLM0243	ACCCTCTTTGGACT CTCACC	GATTCACGCTCTGAAG GAAA	55
23	CLM0245	TGCAGGATTCACTA GGAGGT	AGCAGGACTTATGCA AGCTG	55
24	CLM0247	CAGGAACACTTCC ACAACCT	GGGTGCGAGAATCAA TAACA	54
25	CLM0248	TGATTGGTGTTGTG ATGTCC	GGGTTCACCATTACAG ATGC	54
26	CLM0251	CTTTTCATGGGAAT TGTTGG	TGAACTTTCCAAGGA ACTCG	52
27	CLM0254	TGCATTCACAACCT GTTTTC	AGATCTATGATGGGC ACAGG	53
28	CLM0255	GGAGGCATAAAAA TGACACCT	CTCTTGGTTTGTGCAT TTCC	54
29	CLM0256	TCACCACACACAA ACACACA	AGATATCAGCGTGGC AGAAC	54
30	CLM0260	TCGATCAAATTTTC CTCTGC	TGCCACCATCTTTCAT TTCT	51
31	CLM0265	GATGTCTTCTCCCC CAAAGT	GTGGGTTCAAGAGGG AAAAT	54
32	CLM0273	AGCAACGAATCAA GAAAACG	ATCTCTCCGGCTATGG AATC	53
33	CLM0279	TGCAAAACGTGAA AGCAATA	ACAAGGAGACCAAGG AGCTT	52
34	CLM0291	ATGCCACTTCTCTG CTCATT	CCAGTGTTGGTTTCCT TGTC	54
35	CLM0292	GAGAGACGTGATG GAGAGGA	TCAATGATCGTATAAA GCCTCA	56
36	CLM0295	AGGGTTTTCACAGT GGGATT	AAGTGAAGCATCATG TTAGCC	54
37	CLM0298	GGTGAGAAACGCA GAAAGAT	CATTTGCTTCCTCCCA TTTT	52
38	CLM0300	TTTTGTTGGTTGAG CATCTG	GGTGTTCAATGTCAGG AATAACA	55
39	CLM0322	ACTGAACAGCAAG GACGTTT	TGTGTTTCCAGTGCAA GAAT	52
40	CLM0332	TGTCCTCAATTTCA ATAACAAG	CGAAACAGTTGGTCG GATAC	54

3.4.6 Scoring of primers for all genotypes

The gel profiles of individual SSR primer were carefully observed and scored and this data was used for further analysis.

3.4.7 Molecular weight analysis

The analysis of molecular weight of PCR images was done by using Navigating 1D MAX software, UVITECH Cambridge.

3.4.8 Statistical analysis of molecular data

The data generated from molecular weight analysis of all polymorphic SSR primers were compiled together to form a data sheet for cluster analysis. The SSR primers across the 30 genotypes were scored. For the presence of each band 1 code has been used, while, for its absence in another genotype, 0 code has been allotted for each primer.

Pair-wise similarity coefficient matrix was generated by Jaccard's coefficient of similarity by using MVSP-A Multivariate Statistical Package_5785 (Version 3.22). The cluster analysis was performed from the distance matrix using Jaccard's similarity coefficient. Distance matrix and dendrogram were constructed based on diversity coefficient generated from pooled data by using the unweighted pair group method of arithmetic means (UPGMA), a computer programme for distance estimation. Principal component analysis (PCA) of compiled data was performed by using Minitab V.18.

Other parameters *i.e.* PIC (Polymorphic Information Content), expected heterozygosity (H_e) and Shannon's diversity index (I) were calculated using the following formulas. A PIC and expected heterozygosity of each primer was determined using PIC calculator (Jan, 2002). Shannon's diversity index was measured by using the formula proposed by Shannon (1948).

$$PIC = \frac{Total\ no.of\ bands - Highest\ allelic\ Frequency}{Total\ no.of\ bands}$$

$$H_e = 1 - \sum p_i^2$$

$$Shannon's\ diversity\ index = -\sum p_i$$

Where, p_i represents the frequency of the i^{th} allele.

3.5 EXPERIMENT 4: EVALUATION OF F_1 HYBRIDS

The 20 hybrids, developed in experiment 2, were evaluated for yield and resistance to spotted pod borer along with the nine parents in a field experiment in a randomized block design with two replications during early *Kharif*, 2017. The seeds were sown at a spacing of 40 × 30 cm. The crop was raised following the Package of Practices Recommendations (KAU POP, 2011) of Kerala Agricultural University. However, insecticide application was avoided considering its possible adverse effect on buildup of targeted pest population. Insect damage observations were recorded from ten randomly selected plants from each cross progeny. The extent of damage was calculated based on the ratio of infested flower buds, flowers and pods to the total number of flower buds, flowers and pods, respectively. Flower buds, flowers and pods once counted were tagged to avoid recounting. Based on the level of infestation of flower buds, flowers and pods, the cowpea F_1s were categorised into four groups *viz*., resistant, moderately resistant, susceptible and highly susceptible. Apart from these observations, biophysical observations *viz*., trichome length and density on flower bud and trichome length and density on pod, and pod wall thickness were also recorded on the same plant.

3.5.1 Raising F_2 generation

The most promising hybrids in terms of spotted pod borer resistance and yield were selected based on the results of experiment 4. The F_1 hybrids were selfed to produce the corresponding F_2 population.

3.6 EXPERIMENT 5: EVALUATION OF F_2 PLANTS AND SCREENING FOR SPOTTED POD BORER RESISTANCE

The segregating generation (F_2) of selected hybrids (minimum 100 plants for each) were evaluated for yield and resistance to spotted pod borer during late *Kharif*, 2017. The seeds were sown at a spacing of 40 × 30 cm. The crop was raised following the Package of Practices Recommendations (KAU POP, 2011) of Kerala Agricultural University. However, insecticide application was avoided considering its possible adverse effect on buildup of targeted pest population. Insect damage observations were recorded on all plants of the F_2 populations. The extent of damage was calculated based on the ratio of infested flower buds, flowers and pods to the total number of flower buds, flowers and pods, respectively. Flower buds, flowers and pods once counted were tagged to avoid recounting. Based on the level of infestation of flower buds, flowers and pods, a

hybrid derived plants were categorised into four groups *viz.*, resistant, moderately resistant, susceptible and highly susceptible. Apart from these observations, biophysical observations, *viz.*, trichome length and density of flower bud, trichome length and density on pod and pod wall thickness were also recorded.

3.7 COLLECTION OF DATA

Observations were recorded from ten plants selected at random in each plot of screening trial, leaving the border rows. In experiments 1 and 4, ten plants were selected at random in each replication for recording observations of parents and F_1 generation, whereas, in F_2 generation, best-performing plants against spotted pod borer were selected to record biometric observations. The mean values as well as individual plant data (for F_2 generation) for each character were used for the statistical analysis.

3.7.1 Days to 50 per cent flowering

Number of days taken from sowing to 50 per cent of the plants to flower was recorded.

3.7.2 Plant height

Length of main stem was measured from the ground level to the tip of the plant at the time of final harvest and expressed in centimetre (cm).

3.7.3 Number of primary branches per plant

Number of primary branches were recorded on each observational plant at the time of final harvest.

3.7.4 Number of pods per plant

Pods obtained in each harvest from each of the observational plant were counted and added.

3.7.5 Pod length

Length of five randomly chosen mature pods from each observational plant was measured. The average value was worked out and expressed in centimetre (cm).

3.7.6 Number of seeds per pod

Number of seeds in five randomly selected mature pods on each observational plant were counted and mean value recorded.

3.7.7 Grain yield per plant

The yield of grains from each observational plant was recorded after each harvest. Total weight of grains separated from the harvested pods of each observational plant was calculated and expressed in grams (g).

3.7.8 100 seed weight

The weight of 100 randomly chosen seeds from plants of each genotype was recorded and expressed in grams (g).

3.8 STATISTICAL ANALYSIS

3.8.1 Analysis of variance(ANOVA)

Analysis of variance (Panse and Sukhatme, 1985) of the data collected from the various experiments was done to test the significance of differences among genotypes with respect to the characters and to estimate the variance components (Table 4).

Table 4. ANOVA for each character

Source of variation	Degrees of freedom	Mean square	F values
Replication	(r-1)	MSR	MSR / MSE
Treatment	(t-1)	MST	MST / MSE
Error	(r-1) (t-1)	MSE	
Total	(rt-1)		

r = Number of replications

t = Number of treatments

MSR = Replication mean square

MST = Treatment mean square

MSE = Error mean square

$$Standard\ Error\ of\ Mean\ (SEm) = \sqrt{\frac{MSE}{r}}$$

$$Critical\ Difference\ (CD)@5\ \% = SEm\sqrt{2} \times t_{(0.05,\ edf)}$$

Where, $t_{(0.05, edf)}$ is the student's t table value at error degrees of freedom and at five per cent level of significance.

3.9 ESTIMATION OF GENETIC PARAMETERS

3.9.1 Genetic components of variance

For each character, the phenotypic and genotypic components of variance were estimated by equating the expected values of mean squares (MS) to the respective variance components (Jain, 1982). Based on this, the following variance components were estimated.

i. Genotypic variance (Vo)

$$Vo = \frac{MST - MSE}{r}$$

ii. Environmental variance (VE)

$$VE = MSE$$

iii. Phenotypic variance (VP)

$$VP = Vo + VE$$

3.9.2 Coefficient of variation

Genotypic and phenotypic coefficients of variation (GCV and PCV, respectively) were worked out using the estimates, Vo and VP and was expressed as a per cent (Burton, 1952) for each trait.

$$PCV\ \% = \frac{\sqrt{Phenotypic\ variance\ (VP)}}{Mean} \times 100$$

$$GCV\ \% = \frac{\sqrt{Genotypic\ variance\ (Vo)}}{Mean} \times 100$$

PCV and GCV were classified as low (0-10 %), moderate (>10-20 %) and high (> 20 %) as per Sivasubramanian and Madhavamenon (1973).

3.9.3 Heritability in broad sense (H^2)

The broad sense heritability was calculated as the ratio of genotypic variance to the total or phenotypic variance as suggested by Lush (1949) and Hanson *et al.* (1956).

$$H^2 = \frac{Genotypic\ variance\ (Vo)}{Phenotypic\ variance\ (VP)} \times 100$$

The heritability estimates were categorized as low (0-30 %), Medium (>30-60 %) and high (> 60 %) as suggested by Johnson *et al.* (1955).

3.9.4 Genetic Advance (GA)

The genetic advance was estimated by following the formula given by Johnson *et al.* (1955).

$$GA = \frac{k\sqrt{Vo \times Vp}}{Vp}$$

Where,

K = Standard selection differential which is 2.06 at 5 per cent selection intensity

Vo = Genotypic variance

Vp = Phenotypic variance

The genetic advance was classified as low (0-10 %), moderate (>10-20 %) and high (>20 %) as suggested by Johnson *et al.* (1955).

3.9.5 Genetic gain

The genetic gain was estimated by following formula.

$$Genetic\ gain = \left(\frac{GA}{\bar{X}}\right) \times 100$$

Where,

GA = Genetic advance

$\bar{X}$ = General mean

The genetic gain was categorised as suggested by Johnson *et al.* (1955) as low (0-10 %), moderate (>10-20 %) and high (> 20%).

3.9.6 Correlation studies

The correlation coefficients were calculated to determine the degree of association of damage parameters with other characters related to resistance. Coefficients of correlation between character pairs were determined by using the variance and covariance components as suggested by Pearson (1905).

$$\rho(x,y) = \frac{Cov(x,y)}{\sigma x \times \sigma y}$$

Where,

ρ(x, y)	=	Pearson correlation coefficient between character x and y
Cov(x, y)	=	Covariance between character x and y
σx	=	Standard deviation of x
σy	=	Standard deviation of y

The calculated values of 'ρ' were compared with table 'ρ' values with n-2 degrees of freedom at 5 per cent, where, 'n' refers to the number of character combinations.

3.9.7 Line × Tester analysis

3.9.7.1 Combining ability

The general combining ability (GCA) of the parents and the specific combining ability (SCA) of the hybrids were estimated using the L × T analysis (Kempthorne, 1957). The mean squares due to various sources of variation and their genetic expectations were computed as per ANOVA given below (Table 5).

Table 5. ANOVA for Line × Tester analysis

Source of variation	Degrees of freedom	Mean sum of squares	Expected mean square
Replication	(r-1)		
Treatment	(e-1)		
Line	(l-1)	Me1	MSE + r (Cov F.S. - 2Cov H.S.) + rt (Cov H.S.)
Tester	(t-1)	Me2	MSE + r (Cov F.S. - 2Cov H.S.) + rl (Cov H.S.)
Parents	(l+t)-1		
Crosses	(lt-1)		
Parents *vs* crosses	1		
Line × Tester	(l -1) (t-1)	Me3	MSE + r (Cov F.S. - 2Cov H.S.)
Error	(r-1) (e-1)	Me4	MSE
Total	(re-1)		

Where,

r = Number of replications

l = Number of lines

t = Number of testers

e = Number of treatments (l+t+lt)

Where,

Cov H.S. = Covariance between half-sib families

Cov F.S. = Covariance between full-sib families

The GCA effects of parents and SCA effects of hybrids were estimated using the following model.

$$X_{ijk} = \mu + g_i + g_j + s_{ij} + e_{ijk}$$

Where,

μ = Population mean

g_i = GCA effect of the i^{th} line

g_j = GCA effect of the j^{th} tester

s_{ij} = SCA effect of the ij^{th} hybrid

e_{ijk} = error associated with ijk^{th} observation

Where,

i = 1,2, ...l

j = 1,2, ...t

k = 1,2, ...r

The individual effects were estimated as follows

1. Estimation of general mean

$$\bar{X} = \frac{x\ldots}{rtl}$$

Where, x…= Mean total of all hybrid combinations

2. Estimation of GCA effect of the i[th] line

$$g_i = \frac{x_i\ldots}{tr} \times \frac{x\ldots}{rtl}$$

Where, x_i...= Mean total of all i[th] male (tester) overall female (lines)

3. Estimation of GCA effect of j[th] tester

$$g_j = \frac{x_j\ldots}{tr} \times \frac{x\ldots}{rtl}$$

Where, x_j...= Mean total of all j[th] female overall males

4. Estimation of SCA effect of ij[th] hybrid

$$s_{ij} = \frac{x_{ij}}{r} - \frac{x_i\ldots}{rt} - \frac{x_j\ldots}{rl} - \frac{x\ldots}{rtl}$$

Where, x_{ij} = Mean total of ij[th] combination or performance of j[th] combination

5. Standard error for combining ability estimates

$$SE \pm (GCA\ of\ lines) = \left(\frac{Me}{rt}\right)^{1/2}$$

$$SE \pm (\text{GCA of testers}) = \left(\frac{Me}{rl}\right)^{1/2}$$

$$SE \pm (\text{sij of crosses}) = \left(\frac{Me}{r}\right)^{1/2}$$

Where,

Me	=	Error mean square
Significance of GCA effects of testers	=	SE (gi) × $t_{(edf)}$
Significance of GCA effects of lines	=	SE (gj) × $t_{(edf)}$
Significance of SCA effects	=	SE (sij) × $t_{(edf)}$

3.9.8 Estimation of heterosis

The heterotic effects were measured as the deviation of F_1 mean from mid-parent (relative heterosis) and better parent (heterobeltiosis) means as per the following formula given by Liang *et al.* (1971).

$$Relative\ heterosis = \frac{F_1 - Mean\ of\ mid\ parent}{Mean\ of\ mid\ parent} \times 100$$

$$Heterobeltiosis = \frac{F_1 - Mean\ of\ better\ parent}{Mean\ of\ better\ parent} \times 100$$

Results and Discussion

4. RESULTS AND DISCUSSION

The results obtained from the various experiments of the present study are explained below under the following headings.

1 **Experiment 1** : Identification of resistance against spotted pod borer in cowpea genotypes
   - Evaluation of morphological basis of resistance
   - Evaluation of biochemical basis of resistance

2 **Experiment 2** : The hybridisation of resistant genotypes (identified in experiment 1) with high yielding popular varieties

3 **Experiment 3** : Assessment of parental polymorphism at molecular level

4 **Experiment 4** : Evaluation of F_1 populations for resistance against spotted pod borer

5 **Experiment 5** : Evaluation of F_2 plants for resistance against spotted pod borer

4.1 EXPERIMENT 1: IDENTIFICATION OF RESISTANCE AGAINST SPOTTED POD BORER IN COWPEA GENOTYPES

4.1.1 Analysis of variance

Test of analysis of variance (ANOVA) for the different damage parameters and resistance/ susceptibility related characters of cowpea to spotted pod borer revealed the presence of significant variation among the 30 genotypes studied. Significant variation also existed for all the other characters considered. The mean values for the different spotted pod borer damage parameters, morphological and biochemical characters related to resistance/ susceptibility of the 30 cowpea genotypes are presented in Table 6, Table 8 and Table 10 and 11.

4.1.2 Damage parameters

Thirty genotypes of cowpea were evaluated for their reaction to the infestation of spotted pod borer. Significant variation was observed within the genotypes with respect to flower bud, flower, pod and overall damage (Table 6, Fig. 2).

Ten genotypes recorded the overall damage below five per cent (0.71 to 4.78 %). Among these ten genotypes, the low damage was recorded by IC 2918 (0.71 %), which was on par with IC 39922 (0.75 %), EC 98668 (1.14 %), IC 39945 (1.54 %), EC 101216 (2.61 %), IC 52110 (3.23 %), IC 39916 (3.83 %), Hridya (4.02 %), Palakkadan thandan payar (4.02 %) and EC 300039 (4.78 %). Three genotypes *viz*., IC 39947, IC 52107 A and IC 20645 recorded total damage of 6.88, 7.78 and 9.43 per cent, respectively.

Five genotypes recorded overall damage in the range of 10 to 15 per cent (Kanakamony, 11.08 %; IC 26048, 13.08 %; Geethika, 13.78 %; IC 52118, 14.81 % and IC 39921, 14.86 %). Remaining 12 genotypes recorded total damage more than 15 per cent. Among these, the higest damage was recorded by Bhagyalakshmy followed by variety Lola (Table 6). The damage caused by spotted pod borer in these varieties were 48.46 per cent and 30.04 per cent, respectively. Vellayani Jyothika and Kashi Kanchan also suffered a heavy damage (18.11 % and 28.74 %, respectively). Several researchers have reported variation in terms of total damage in different genotypes of cowpea (Jithesh, 2009; Kumar *et al.*, 2013; Barad *et al.*, 2016; Asoontha, 2017).

It was also observed that the vegetable type varieties *viz.*, Geethika, Vellayani Jyothika, Lola, Kashi Kanchan and Bhagyalakshmy suffered comparatively more damage by spotted pod borer than the grain type genotypes. The probable reason for this might be the succulent nature of the pods in vegetable cowpea, which makes these genotypes more attractive to spotted pod borer. These findings are in accordance with the report of Beegum and Subramanian (2017).

Variation was also observed in the infestation levels at different reproductive stages of the same genotype. Genotype IC 39922 recorded no flower bud damage. Among the remaining genotypes, 21 genotypes recorded flower bud damage below five per cent. Genotypes EC 300039, EC 98668, IC 52110, IC 39945, IC 2918, IC 39922 and IC 39916 recorded no flower damage. Amid remaining ones, 15 genotypes had flower damage below five per cent and the genotype EC 101216 observed to have least flower damage (0.79 %). Palakkadan thandan payar, IC 39945, IC 2918 and IC 39947 were free from pod damage, and with the remaining genotypes, 20 genotypes recorded pod damage below five per cent. The variety Bhagyalakshmy recorded the highest level of infestation at all three stages *viz.*, flower bud, flower and pod (13.85 %, 11.02 % and 9.49 %, respectively) followed by Lola (8.28 %, 10.85 % and 7.96 %, respectively).

In general, it was also observed that the flower buds of most genotypes suffered more damage than flowers and pods (Fig. 2). This can be explicated by the following reasons. The adult female of spotted pod borer prefers flower buds as oviposition site (Jackai, 1980). Hence, after emerging out from eggs, larva directly starts feeding on flower bud, resulting higher infestation of flower buds. According to Sharma *et al.* (1999), the first instar larvae of spotted pod borer shows a strong feeding liking for flower buds and flowers rather than pods. Smith (1979) stated that the pod infestation indicates the intensity of the overall larval migration or secondary infestation. As stated by Jackai

Table 6. The extent of damage caused by spotted pod borer in 30 genotypes of cowpea

Genotypes	Mean no. of flower buds	Flower bud damage (%)	Mean no. of flowers	Flower damage (%)	Mean no. of pods	Pod damage (%)	Total damage (%)
Geethika	35.00	6.01	28.90	3.46	22.60	2.45	13.78 ^{hij}
Vellayani Jyothika	42.10	6.39	35.40	3.64	19.20	7.50	18.11 ^{fgh}
Lola	37.40	8.28	31.30	10.85	21.60	7.96	30.04 ^{b}
Hridya	23.80	0.84	23.50	0.85	19.70	0.99	4.02 ^{mn}
Palakkadan thandan payar	22.20	0.91	17.30	1.16	12.40	0.00	4.02 ^{mn}
Kanakamony	24.90	3.21	17.70	1.14	12.00	1.68	11.08 ^{ijk}
Mysore Local	23.50	4.67	18.30	2.19	13.40	1.89	15.84 ^{ghi}
Kashi Kanchan	28.90	6.92	16.80	5.35	12.90	4.57	28.74 ^{bc}
EC 300039	20.70	0.97	16.00	0.00	11.40	1.34	4.78 ^{lmn}
EC 98668	38.90	0.25	31.60	0.00	24.20	0.83	1.14 ^{n}
EC 101216	35.10	0.56	24.80	0.79	19.80	0.75	2.61 ^{mn}
IC 52110	31.50	0.64	21.90	0.00	18.50	1.67	3.23 ^{mn}
IC 39945	29.20	1.03	21.20	0.00	16.30	0.00	1.54 ^{n}
IC 2918	23.80	0.42	19.70	0.00	15.50	0.00	0.71 ^{n}
IC 39922	22.10	0.00	17.50	0.00	13.40	0.38	0.75 ^{n}
IC 52118	35.40	6.82	27.20	4.12	22.40	1.52	14.81 ^{ghi}

Table 6 continued

Genotypes	Mean no. of flower buds	Flower bud damage (%)	Mean no. of flowers	Flower damage (%)	Mean no. of pods	Pod damage (%)	Total damage (%)
IC 39916	27.30	1.86	23.00	0.00	18.70	0.76	3.83 ^{mn}
IC 2196	33.50	6.00	28.20	5.32	20.60	5.06	19.94 ^{efg}
IC 20645	20.70	1.96	16.40	1.22	12.20	1.39	9.43 ^{jkl}
IC 26048	20.90	2.87	14.70	2.06	11.60	1.25	13.08 ^{hij}
IC 52107 A	30.60	2.63	23.90	1.63	15.80	1.21	7.78 ^{klm}
IC 39947	22.10	1.35	17.60	2.27	13.00	0.00	6.88 ^{klm}
IC 39921	23.70	2.53	16.80	3.57	12.10	1.71	14.86 ^{ghi}
IC 26029	29.60	3.03	20.50	5.38	15.20	6.88	23.57 ^{cde}
IC 20720	22.30	6.28	16.30	6.14	12.50	1.63	27.50 ^{bc}
IC 39870	27.90	4.65	19.90	5.03	15.40	3.79	21.33 ^{def}
IC 52105	22.30	2.69	16.90	5.31	13.10	5.52	25.84 ^{bcd}
IC 9883	20.00	4.49	15.70	4.45	11.80	2.97	25.09 ^{bcde}
TVX-944	14.20	2.09	9.50	1.04	7.70	2.58	18.07 ^{fgh}
Bhagyalakshmy	31.80	13.85	22.80	11.02	16.50	9.49	48.46 ^{a}
SEm±	-	**0.43**	-	**0.64**	-	**0.31**	
C.D.@ 5 %	-	**1.26**	-	**1.87**	-	**0.90**	

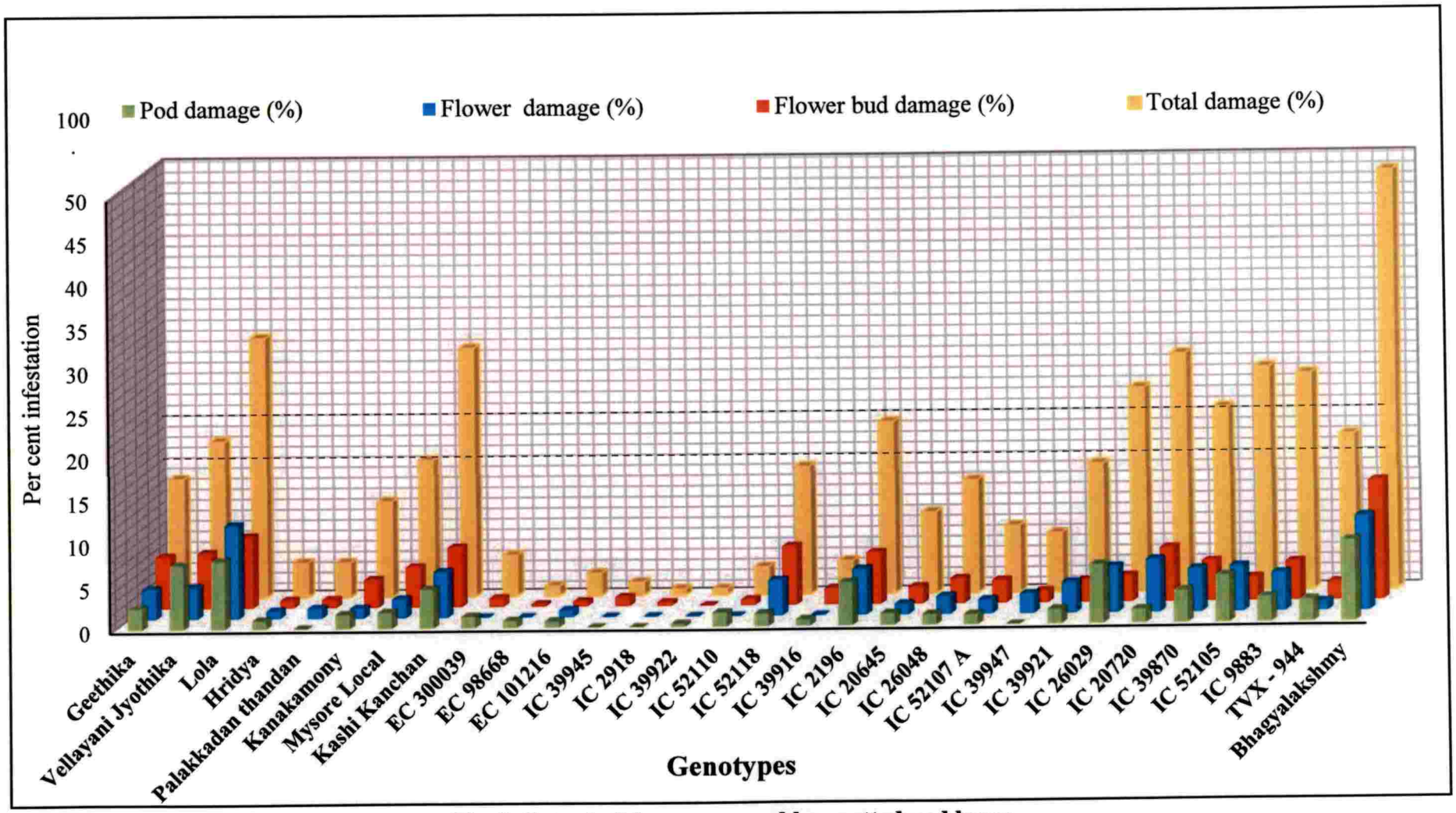

Fig. 2. Extent of damage caused by spotted pod borer

(1981), the intensity of spotted pod borer larval migration or secondary infestation is a product of age and the density of larvae on the plant parts at a given time. Hence, high densities of larvae on flower buds force the larvae to migrate to pods, and results in secondary infestation. Moreover, in field situations, usually, older instar larvae are found infesting pods and not first instar larvae (Oghiakhe *et al.*, 1995). The first few instar larvae feed on flower buds and cause more damage. The results of this study are in accordance with Phillip (2004), Jithesh (2009), Jayasinghe *et al.* (2015), Beegum and Subramanian (2017) and Asoontha (2017).

4.1.3 The categorisation of cowpea genotypes based on the extent of the damage

The 30 genotypes were subjected to Duncan's Multiple Range Test (DMRT) based on total damage caused by spotted pod borer, and were grouped as resistant, moderately resistant, susceptible and highly susceptible (Table 7). Ten genotypes were categorised as resistant genotypes (total damage ranging from 0 to 5 %). Three genotypes were categorised as moderately resistant (total damage ranging from 5 to 10 %). Four were categorised as susceptible (total damage between 10 to 15 %), and the remaining 13 genotypes, which recorded total damage more than 15 per cent, were categorised as highly susceptible.

Table 7. Classification of cowpea genotypes based on the extent of damage caused by spotted pod borer

Resistance rating	The extent of total damage (%)	Genotypes
Resistant	0-5	Hridya, Palakkadan thandan payar, EC 300039, EC 98668, EC 101216, IC 52110, IC 39945, IC 2918, IC 39922 and IC 39916
Moderately resistant	>5-10	IC 20645, IC 52107 A and IC 39947
Susceptible	>10-15	Geethika, Kanakamony, IC 52118, IC 26048 and IC 39921
Highly susceptible	>15	Vellayani Jyothika, Lola, Mysore Local, Kashi Kanchan, IC 2196, IC 26029, IC 20720, IC 39870, IC 52105, IC 9883, TVX-944 and Bhagyalakshmy

4.1.4 Morphological basis of resistance

Morphological basis of resistance was determined by recording the length and density of trichomes of a flower bud and pod, raceme position, length of the peduncle and pod wall thickness, and presented in Table 8.

4.1.4.1 Peduncle length and raceme position

Observations were recorded on the length of the peduncle and raceme position. The longer peduncle was observed in variety Lola (27.25 cm) followed by variety Geethika (23.15 cm). Short peduncle length observed in IC 20720 (5.55 cm), which was on par with variety Hridya (5.93 cm). There was no appeared relation between the length of peduncle and spotted pod borer infestation. A number of genotypes from resistant category had relatively long peduncle such as IC 2918 (22.54 cm), IC 39922 (17.12 cm) and EC 98668 (16.82 cm), whereas, few genotypes observed to have short peduncle such as Hridya (5.93 cm), Palakkadan thandan payar (7.95 cm) and IC 52110 (8.17 cm).

With respect to raceme position, again, there was no specific trend observed. However, few resistant genotypes were observed to bear pods above the canopy. Elongated peduncles enable the plant to hold the pods above the canopy level. Feeding on such pods could expose the larvae to other predators. This could be a reason for lower infestation in genotypes with long peduncle. Similar observations have also been made by IITA (1974), Singh (1978) and Oghiakhe *et al.* (1991).

4.1.4.2 Length and density of non-glandular trichome on flower bud and pod

The longest flower bud trichomes were observed in EC 101216 (0.213 mm) followed by IC 39947 (0.097 mm), whereas, short trichomes were observed in Vellayani Jyothika (0.020 mm) and Lola (0.020 mm), which was on par with IC 2196 (0.024 mm) and IC 20720 (0.027 mm). Least suffered genotype, IC 2918, was observed to have 0.077 mm long bud trichome, whereas, the most susceptible, Bhagyalakshmy, recorded 0.029 mm bud trichome length (Table 8).

Long pod trichomes were observed in genotype IC 20720 (0.081 mm) which was on par with Lola (0.078 mm), Hridya (0.074 mm), EC 101216 (0.073 mm) and IC 52110 (0.072 mm). Short trichomes were observed in Vellayani Jyothika (0.033 mm), which was on par with IC 52105 (0.035 mm), EC 98668 (0.040 mm), IC 39916 (0.042 mm) and IC 39921 (0.043 mm). Least suffered genotype, IC 2918, was observed to have 0.051 mm pod trichome length, whereas, the most susceptible, Bhagyalakshmy, recorded 0.050 mm pod trichome length, and both were on par with each other (Table 8).

With respect to trichome density on flower bud, high density was recorded by EC 300039 (6.33 /mm^2), which was on par with genotypes EC 101216 (5.33 /mm^2) and IC 26048 (5.33 /mm^2). Low trichome density was recorded by Lola (0.32 /mm^2), which was on par with IC 20720 (0.36 /mm^2), IC 52107 A (0.75 /mm^2), IC 9883 (1 mm^2), Bhagyalakshmy (1 /mm^2), IC 39916 (1.33 /mm^2) and IC 2196 (1.33 /mm^2). Least suffered genotype, IC 2918, recorded trichome density of 5 /mm^2. With respect to trichome density on the pod, IC 2918 recorded the highest trichome density (37.67 /mm^2) followed by Palakkadan thandan payar (17.67 /mm^2), Hridya (17 /mm^2) and Mysore Local (15 /mm^2). The minimum density of trichome on the pod was recorded by IC 39916 (2.67 /mm^2). The most susceptible variety, Bhagyalakshmy, recorded a comparatively low trichome density on the pod (4.67 /mm^2) (Table 8).

With respect to the length of non-glandular trichomes on the pod, no specific trend was observed. Few of the resistant genotypes recorded less trichome length on the pod and a few recorded more. The same scenario was also observed with respect to susceptible genotypes. This scenario indicates that the pod trichome length does not play a crucial role in imparting resistance against spotted pod borer. However, data revealed that the length of non-glandular trichomes on flower bud might play a deciding role in imparting resistance against spotted pod borer. Oghiakhe *et al.* (1992b) also made the same observations with respect to trichome length and density, and suggested the importance of density of trichome than its length in imparting resistance against spotted pod borer. They also mentioned that the sharp pointed tips of the non-glandular trichomes can sufficiently pierce larvae of spotted pod borer to cause mortality and also hinder movement. It is clear from the results of the present study that the higher density of non-glandular trichomes on flower buds and pods are responsible for the strong antixenotic effect imparting resistance against spotted pod borer. Higher trichome density could be one of the factor responsible for the comparatively low level of infestation and damage by spotted pod borer to cowpea pods. The greater number of trichomes per unit area on pods is evidently one of the important factor responsible for the increased resistance of IC 2918 compared to the susceptibility of Bhagyalakshmy. These results are in accordance with Panicker (2000), Phillip (2004), Sunitha *et al.* (2008), Jithesh (2009), Beegum (2015) and Asoontha (2017).

4.1.4.3 Pod wall thickness

With respect to pod wall thickness, a local genotype, Palakkadan thandan payar observed to have thick pod wall (0.93 mm), whereas, IC 39870 recorded the lowest pod

Table 8. Biophysical parameters of cowpea genotypes

Genotypes	Bud trichome length (mm)	Pod trichome length (mm)	No. of trichomes on bud (per mm^2)	No. of trichomes on pod (per mm^2)	Pod wall thickness (mm)	Length of peduncle (cm)	Raceme position
Geethika	0.054	0.068	4.00	5.33	0.64	23.15	3
Vellayani Jyothika	0.020	0.033	2.33	3.33	0.72	17.90	3
Lola	0.020	0.078	0.32	3.00	0.60	27.25	3
Hridya	0.043	0.074	2.67	17.00	0.51	5.93	1
Palakkadan thandan payar	0.066	0.062	4.33	17.67	0.93	7.95	2
Kanakamony	0.055	0.062	3.67	10.33	0.50	10.73	3
Mysore Local	0.033	0.060	2.33	15.00	0.55	14.65	1
Kashi Kanchan	0.038	0.054	2.33	3.00	0.46	13.83	2
EC 300039	0.056	0.070	6.33	8.67	0.62	16.70	3
EC 98668	0.051	0.040	5.00	6.33	0.72	16.82	3
EC 101216	0.213	0.073	5.33	8.33	0.71	13.80	3
IC 52110	0.042	0.072	2.67	9.33	0.58	8.17	2
IC 39945	0.058	0.047	3.67	10.00	0.64	14.89	2
IC 2918	0.077	0.051	5.00	37.67	0.39	22.54	3
IC 39922	0.074	0.054	3.67	4.33	0.71	17.12	1
IC 52118	0.034	0.054	2.00	4.67	0.35	13.50	2
IC 39916	0.067	0.042	1.33	2.67	0.91	15.84	2
IC 2196	0.024	0.066	1.33	4.00	0.31	7.82	2

Table 8 continued

Genotypes	Bud trichome length (mm)	Pod trichome length (mm)	No. of trichomes on bud (per mm^2)	No. of trichomes on pod (per mm^2)	Pod wall thickness (mm)	Length of peduncle (cm)	Raceme position
IC 20645	0.035	0.045	2.67	4.67	0.56	12.67	2
IC 26048	0.034	0.061	5.33	5.00	0.63	12.83	2
IC 52107 A	0.039	0.045	0.75	3.67	0.52	6.67	2
IC 39947	0.097	0.059	5.00	5.33	0.43	15.28	2
IC 39921	0.071	0.043	1.67	3.00	0.45	14.00	2
IC 26029	0.034	0.048	4.00	3.33	0.58	15.00	1
IC 20720	0.027	0.081	0.36	5.67	0.36	5.55	2
IC 39870	0.044	0.070	3.00	3.67	0.26	10.34	2
IC 52105	0.030	0.035	2.33	3.67	0.41	8.35	2
IC 9883	0.040	0.049	1.00	4.67	0.48	21.34	1
TVX-944	0.049	0.067	4.00	6.67	0.69	15.10	2
Bhagyalakshmy	0.029	0.050	1.00	4.67	0.39	13.34	2
SEm±	**0.027**	**0.004**	**0.37**	**0.77**	**0.01**	**0.79**	**-**
C.D.@ 5 %	**0.009**	**0.01**	**1.07**	**2.04**	**0.03**	**1.07**	**-**

Raceme positions

1 In upper canopy

2 Mostly above canopy

3 Throughout canopy

wall thickness (0.26 mm). Least suffered genotype, IC 2918, and the most susceptible variety, Bhagyalakshmy, recorded 0.39 mm pod wall thickness (Table 8). In general, thick pod wall was observed in the resistant group and thin pod wall thickness was observed in the susceptible group of genotypes.

The thicker pod wall could make it more difficult for the larvae to bore into the pods (Panda and Khush, 1995), thus forming an important morphological barrier and protects the economic part of the pods. However, in vegetable type varieties *viz.*, Geethika, Vellayani Jyothika and Lola, which were susceptible to spotted pod borer, thick pod wall was observed. The susceptible nature of these varieties could be attributed to soft and fleshy nature of pod wall. Halder and Srinivasan (2011) and Beegum (2015) also reported the similar results with respect to the pod wall thickness and level of resistance.

4.1.4.4 Correlation of morphological characters of cowpea with spotted pod borer infestation

Analysis was carried out to assess the correlation between morphological parameters of cowpea and the level of spotted pod borer infestation (Table 9).

Density of trichome on flower bud recorded negative and significant correlations with flower bud and flower damage (-0.596 and -0.597, respectively), whereas, length of trichomes recorded -0.460 and -0.412 correlation with flower bud and flower damage. Peduncle length showed a positive correlation with flower bud and flower damage (0.127 and 0.130, respectively), however, the correlation was not significant.

With regard to pod damage, trichome density on the pod registered strong and negative correlation (-0.400) since larval contact with trichomes increases with the high density of trichome on the pod. However, pod wall thickness and the length of trichome on pod showed negative but non-significant correlation (-0.257 and -0.120, respectively) with pod damage. Peduncle length showed positive but non-significant correlation with pod damage (0.171). With respect to total damage by spotted pod borer, trichome density on bud and pod, bud trichomes length and pod wall thickness registered strong negative correlation (-0.572, -0.414, -0.479 and -0.474, respectively). Pod trichome length and peduncle length showed positive but non-significant correlation with total damage. The same scenario was observed by a number of researchers (Oghiakhe *et al.*, 1992b; Sunitha *et al.*, 2008; Jithesh, 2009; Halder and Srinivasan, 2011; Beegum, 2015; Asoontha, 2017).

Table 9. Correlation between morphological parameters of cowpea and per cent damage

Variables	Flower bud damage	Flower damage	Pod damage	Total damage
No. of trichomes on bud	**-0.596**	**-0.597**	**-0.452**	**-0.572**
No. of trichomes on pod	-0.353	**-0.396**	**-0.400**	**-0.414**
Bud trichome length	**-0.460**	**-0.412**	**-0.458**	**-0.479**
Pod trichome length	0.052	0.096	-0.120	0.011
Pod wall thickness	**-0.414**	**-0.470**	-0.257	**-0.474**
Length of peduncle	0.127	0.130	0.171	0.038

Values in bold are significant at 5 % level of significance

4.1.5 Biochemical basis of resistance

Biochemical basis of resistance of the different cowpea genotypes to spotted pod borer were ascertained by estimating biochemical parameters such as total sugar content, reducing sugar content, non-reducing sugar content, total protein content, total phenol content, polyphenol oxidase activity, peroxidase activity of flower bud and immature pod as well as crude fibre content of pod. The results are presented in Table 10 and 11, and depicted in Fig. 3, 4 and 5. The results of correlation studies are presented in Table 12.

4.1.5.1 Total sugar content

The total sugar content in flower buds and pods varied significantly among all the cowpea genotypes evaluated. A variety Mysore Local (6.99 %) recorded the highest amount of total sugar in flower buds, followed by genotype IC 39921 (6.38 %). Variety Kanakamony observed to have low total sugar content in flower buds (0.65 %), which was on par with Bhagyalakshmy (0.73 %), Palakkadan thandan payar (0.74 %), Geethika (0.82 %), EC 300039 (0.83 %), IC 26048 (0.93 %), EC 98668 (0.94 %), IC 39922 (0.96 %), IC 52107 A (0.98 %) and IC 20645 (1.05 %). Least suffered genotype, IC 2918 was observed to have 1.37 per cent total sugar content in flower buds (Table 10).

With respect to total sugar content in a pod, genotype IC 20645 recorded the highest content, 12.65 per cent, followed by IC 39921 (10.43 %). Genotype EC 300039 recorded low total sugar content of 1.01 per cent, which was on par with EC 98668 (1.07 %), IC 39922 (1.24 %), Kanakamony (1.28 %) and IC 39916 (1.37 %). The least suffered genotype, IC 2918, recorded 2.03 per cent total sugar content and the most susceptible variety, Bhagyalakshmy, recorded 3.37 per cent total sugar content (Table 11).

Overall, the resistant genotypes were observed to have lesser total sugar content in flower buds and pods than the susceptible ones (Fig. 3 and 4). As per Ishikawa *et al.* (1969), sucrose acts as the strongest feeding stimulant for herbivorous insects. Naturally, spotted pod borer infests genotypes with more total sugar content in flower buds and pods. Correlation studies among the damage parameters (flower bud, pod and total damage) and total sugar content revealed positive correlations, but the correlations were not significant (Table 12). The present findings are in accordance with the findings of Halder and Srinivasan (2007), Sunitha *et al.* (2008), Beegum (2015), Barad *et al.* (2016), Jakhar *et al.* (2017) and Tiwari *et al.* (2017).

4.1.5.2 Reducing sugar content

With respect to reducing sugar content in flower buds, genotype IC 52118 recorded the highest value (2.45 %), followed by IC 20720 (2.11 %) and IC 26029 (2.11 %). Variety Kanakamony recorded the lowest reducing sugar content in flower buds (0.17 %), followed by Geethika (0.28 %) and IC 39870 (0.30 %). The least suffered genotype, IC 2918, recorded 1.10 per cent and the most susceptible variety, Bhagyalakshmy, recorded 0.59 per cent reducing sugar content (Table 10).

With regard to pods, IC 20720 (3.46 %) recorded more reducing sugar content, which was on par with IC 26029 (3.41 %) and IC 9883 (3.40 %), whereas, Kanakamony recorded low reducing sugar content (0.21 %), which was on par with genotypes IC 39922 (0.23 %) and IC 39870 (0.36 %). Least suffered genotype, IC 2918, recorded 1.27 per cent reducing sugar content and the most susceptible variety, Bhagyalakshmy, recorded 1.26 per cent reducing sugar content, and both were on par with each other (Table 11). Overall no specific trend was observed with respect to reducing sugar content in relation to damage by spotted pod borer. However, few of the genotypes from the resistant category recorded comparatively lower value for reducing sugar content. These results are in conformity with Halder *et al.* (2006), Halder and Srinivasan (2007) and Beegum (2015).

4.1.5.3 Non-reducing sugar content

In respect to flower buds, IC 39921 was observed to have more non-reducing sugar (5.00 %), which was on par with Mysore Local (4.95 %), whereas, genotype IC 26048 recorded low value (0.05 %), which was on par with Palakkadan thandan payar (0.08 %) and Bhagyalakshmy (0.15 %) (Table 10).

With regard to pods, the highest value for non-reducing sugar was recorded by IC 20645 (9.77 %), followed by IC 39921 (7.34 %). A low value of non-reducing sugar

was recorded by genotype EC 98668 (0.26 %), which was on par with IC 39916 (0.32 %), EC 300039 (0.41 %) and IC 2918 (0.76 %). The most susceptible variety, Bhagyalakshmy, recorded 2.11 per cent non-reducing sugar content (Table 11).

It was observed that, in general, the resistant genotypes recorded lesser non-reducing sugar content in flower buds and pod than the susceptible ones. These results are in conformity with Halder and Srinivasan (2007).

4.1.5.4 Total protein content

Pertaining to total protein content in flower buds, IC 39945 recorded highest value, 14.48 per cent, followed by EC 98668 (11.64 %). A local genotype Mysore Local recorded the low total protein content (7.31 %), which was on par with IC 39921 (7.64 %), TVX-944 (7.68 %), IC 52107 A (7.95 %), IC 39922 (8.01 %), IC 26029 (8.01 %) and IC 39947 (8.11 %). Least suffered genotype, IC 2918, recorded 10.00 per cent total protein content and the most susceptible variety, Bhagyalakshmy, recorded 8.63 per cent total protein content (Table 10, Fig. 3).

In pods, variety Vellayani Jyothika recorded high protein content of 30.78 per cent, which was on par with Bhagyalakshmy (29.82 %), followed by Hridya (24.71 %) and IC 39945 (23.88 %). A local genotype Palakkadan thandan payar recorded the lowest total protein content (5.51 %), followed by Kashi Kanchan (7.88 %) and IC 26048 (8.20 %). Least suffered genotype, IC 2918, recorded 13.36 per cent total protein content (Table 11, Fig. 4).

Plant proteins are the most significant sources of dietary nitrogen for herbivores. A number of researchers have reported the positive correlation between total protein content and pest infestation (Philip, 2004; Halder *et al.*, 2006; Sunitha *et al.*, 2008; Sharma *et al.*, 2009; Beegum, 2015; Barad *et al.*, 2016; Jakhar *et al.*, 2017; Tiwari *et al.*, 2017). The results of the present study affirm the above observations. However, there was no specific trend observed with respect to the total protein content of flower buds in relation with total damage by spotted pod borer. On the other hand, in pods, in general, the resistant genotypes recorded less total protein content than the susceptible ones. Correlation study between the damage parameters (flower bud, pod and total damage) and protein content in the pod also revealed the same scenario. Conversely, there was a strong and positive correlation between protein content and pod damage (0.436). The results of the study indicated the role of total protein content in determining the incidence of spotted pod borer.

4.1.5.5 Total phenol content

With regard to total phenol content in flower buds, IC 52110 recorded the highest value (419.35 mg CE /g) followed by IC 52105 (391.91 mg CE /g). Geethika recorded low total phenol content, 62.15 mg CE /g, which was on par with Lola (70.83 mg CE /g). Least suffered genotype, IC 2918, observed to have 104.37 mg CE /g total phenol content and the most susceptible variety, Bhagyalakshmy, recorded 74.11 mg CE /g total phenol content (Table 10).

In pods, a genotype EC 300039 recorded the highest total phenol content (549.52 mg CE /g), followed by IC 52110 (421.23 mg CE /g). A genotype IC 52118 recorded low total phenol content, 71.53 mg CE /g, which was on par with IC 52105 (72.94 mg CE /g), IC 52107 A (76.69 mg CE /g) and IC 2196 (79.74 mg CE /g). Least suffered genotype, IC 2918, recorded 217.65 mg CE /g total phenol content and the most susceptible variety, Bhagyalakshmy, recorded 113.75 CE mg /g total phenol content (Table 11).

With respect to total phenol content, the resistant genotypes recorded significantly higher phenolic content than the susceptible ones. These results are in conformity with Macfoy *et al.* (1983), Oghiakhe *et al.* (1993b), Halder *et al.* (2006), Sunitha *et al.* (2008), Barad *et al.* (2016) and Tiwari *et al.* (2017). It is an established fact that the phenolic compounds are anti-nutritional factors which reduce digestibility, palatability and nutritional value. From the results, it is evident that the total phenol content plays a crucial role in imparting resistance against spotted pod borer.

Correlation study revealed a negative correlation between damage parameters (flower buds, flower and pod damage) and total phenol content of flower buds and pods (Table 12, Fig. 5a). There was a significant negative correlation between flower bud damage and phenol content in flower bud, however, the correlation was not significant with respect to pod damage and phenol content in pods. This can be explained by the results given by Bressani and Elias (1980). They suggested that the high phenolic content in cowpea pod could react with the protein provided mainly by the cotyledons to decrease its digestibility. According to Goldstein and Swain (1965), the alkaline condition usually found in the gut of lepidopteran insect larvae is a mechanism of counter-adaptation to tannins and hence it decreases the ability of tannins to form complexes with proteins. The same phenomenon might have contributed partially to counteract with phenols giving the non-significant correlation between total phenol content and pod damage.

4.1.5.6 Polyphenol oxidase activity

With regard to flower bud, higher activity of polyphenol oxidase (PPO) was recorded by IC 39922 (0.0058 EU /g), which was on par with IC 52118 (0.0057 EU /g), IC 52107 A (0.0048 EU /g), Palakkadan thandan payar (0.0045 EU /g), EC 98668 (0.0044 EU /g), IC 26048 (0.0044 EU /g), EC 101216 (0.0042 EU /g), Geethika (0.0042 EU /g), IC 39921 (0.0040 EU /g), EC 300039 (0.0039 EU /g) and IC 2196 (0.0039 EU /g), whereas, the most susceptible, Bhagyalakshmy, recorded low PPO activity, (0.0003 EU /g). Least suffered genotype, IC 2918, recorded 0.0023 EU /g PPO activity (Table 10).

With respect to pod, high PPO activity was observed in IC 39922 (0.0056 EU /g), which was on par with a genotypes EC 98668 (0.0051 EU /g), IC 39947 (0.0049 EU /g) and EC 101216 (0.0043 EU /g), whereas, Vellayani Jyothika recorded very low PPO activity, (0.0002 EU /g). Least suffered genotype, IC 2918, recorded 0.0024 EU /g PPO activity, however, the most susceptible, Bhagyalakshmy, recorded low PPO activity (0.0003 EU /g) (Table 11).

In general, the resistant genotypes recorded higher PPO activity in flower buds and pods than the susceptible ones. The activity of the PPO enzyme increases in response to different types of stresses mostly due to physiological injury (Rivero *et al.*, 2001). As suggested by Felton *et al.* (1992), the activity of PPO under the high pH environments, such as the lepidopteran midgut, favour protein alkylation including several essential amino acids (lysine, histidine, cysteine, methionine, *etc.*) and significantly reduce protein quality which in turn influence the larval growth rate. However, PPO mediated resistance have been hardly reported in the case of cowpea against any pests.

The correlation study revealed an important role of PPO activity in imparting resistance (Fig. 5b). There was a strong negative correlation between damage parameters (flower buds flower and pod damage) and PPO activity in buds and pods (Table 12). These results are in conformity with Beegum (2015).

4.1.5.7 Peroxidase activity

With regard to flower bud, high peroxidase (POD) activity was recorded by genotype EC 101216 (24.65 EU /g), which was on par with IC 52118 (19.90 EU /g), whereas, low POD activity recorded by genotypes IC 9883 (0.37 EU /g), which was on par with IC 52105 (0.45 EU /g), IC 20720 (0.74 EU /g) and IC 39922 (1.18 EU /g). Least suffered genotype, IC 2918, recorded 4.76 EU /g, whereas, the most susceptible variety, Bhagyalakshmy, recorded 8.09 EU /g POD activity (Table 10).

With respect to the pod, the highest activity of POD was recorded by genotype IC 20645 (129.36 EU /g) followed by IC 39945 (81.99 EU /g). Low POD activity was recorded by genotype IC 52107 A (1.63 EU /g), which was on par with IC 20720 (3.82 EU /g), IC 26029 (4.52 EU /g), IC 52110 (4.87 EU /g) and Kashi Kanchan (4.92 EU /g). Least suffered genotype, IC 2918, recorded 42.83 EU /g POD activity, however, the most susceptible, Bhagyalakshmy, recorded 63.69 EU /g POD activity (Table 11).

With respect to damage parameters and POD activity, there was no specific trend observed as the most susceptible variety, Bhagyalakshmy, recorded higher POD activities than the resistant genotypes in both flower buds and pods. The scenario revealed by correlation study also support this result as there was a negative but non-significant correlation in between damage parameters and POD activities (Table 12). The defensive role of POD activity with respect to insects is somewhat controversial. Sometimes laboratory estimation of POD activity may not reflect field observations. Peroxidase activity believed to involve in disease/ insect resistance or susceptibility, even though POD alone has little effect against insects (Felton and Duffey, 1991; Rahbe and Febvay, 1993; Dowd and Vega, 1996).

4.1.5.8 The crude fibre content of the immature pod

A genotype IC 39922 had the highest crude fibre content (52.60 %) followed by EC 98668 (48.85 %) and IC 20645 (47.04 %). A low crude fibre content was recorded by IC 39945 (24.16 %), which was on par with IC 52105 (25.78 %). Least suffered genotype, IC 2918, recorded 42.83 per cent crude fibre content and the most susceptible variety, Bhagyalakshmy, recorded 32.89 per cent crude fibre content (Table 11).

Crude fibre is considered as indigestible cellulose, pentosans, lignin and other components of this type present in foods (Food Science, 2008). It indicates the antinutritional content of the tissue. It is very clear from the results of present study that the high content of crude fibre in a tissue results in the less preference by the insects as food. Correlation study revealed a strong and a negative correlation in between pod damage and crude fibre content (-0.479) (Table 12, Fig. 5c). The results of this study are in compliance with Macfoy *et al.* (1983) and Barad *et al.* (2016).

4.1.6 Principal component analysis for cowpea genotypes distribution

Principal component analysis (PCA) was carried out to visualise the dispersion of genotypes based on 25 different parameters which include all damage parameters, morphological and biochemical parameters in relation to spotted pod borer resistance. The two-dimensional PCA score plot, derived by considering all characters (Fig. 6),

Table 10. Biochemical parameters of cowpea genotypes (flower bud)

Genotypes	Total sugar content (%)	Reducing sugar content (%)	Non-reducing sugar content (%)	Total protein content (%)	Total phenol content (mg CE /g)	PPO activity (EU /g)	POD activity (EU /g)
Geethika	0.82	0.28	0.54	10.26	62.15	0.0042	12.20
Vellayani Jyothika	2.28	1.15	1.13	10.03	96.63	0.0007	11.25
Lola	2.03	1.82	0.21	9.41	70.83	0.0007	8.39
Hridya	1.51	1.23	0.28	10.56	94.05	0.0035	14.09
Palakkadan thandan payar	0.74	0.66	0.08	10.00	151.98	0.0045	7.92
Kanakamony	0.65	0.17	0.48	10.50	132.98	0.0007	5.14
Mysore Local	6.99	2.04	4.95	7.31	84.90	0.0007	7.77
Kashi Kanchan	1.10	0.66	0.44	8.65	137.44	0.0027	8.11
EC 300039	0.83	0.46	0.37	10.04	171.21	0.0039	9.51
EC 98668	0.94	0.68	0.26	11.64	165.11	0.0044	2.87
EC 101216	2.02	1.00	1.02	10.18	175.20	0.0042	24.65
IC 52110	1.10	0.72	0.38	10.11	419.35	0.0026	2.00
IC 39945	1.87	0.95	0.92	14.48	135.33	0.0015	17.77
IC 2918	1.37	1.10	0.27	10.00	104.37	0.0023	4.76
IC 39922	0.96	0.43	0.54	8.01	232.43	0.0058	1.18

Table 10 continued

Genotypes	Total sugar content (%)	Reducing sugar content (%)	Non-reducing sugar content (%)	Total protein content (%)	Total phenol content (mg CE /g)	PPO activity (EU /g)	POD activity (EU /g)
IC 52118	2.86	2.45	0.41	9.47	171.92	0.0057	19.90
IC 39916	3.05	1.78	1.27	9.04	104.37	0.0033	2.59
IC 2196	3.20	1.37	1.83	9.83	72.00	0.0039	1.57
IC 20645	1.05	0.76	0.29	10.34	94.75	0.0023	1.64
IC 26048	0.93	0.88	0.05	9.50	96.16	0.0044	5.55
IC 52107 A	0.98	0.73	0.25	7.95	264.09	0.0048	12.90
IC 39947	2.95	1.36	1.60	8.11	101.32	0.0024	15.25
IC 39921	6.38	1.38	5.00	7.64	138.85	0.0040	2.67
IC 26029	2.54	2.11	0.43	8.01	105.07	0.0003	1.45
IC 20720	2.55	2.11	0.43	9.01	73.88	0.0004	0.74
IC 39870	4.12	0.30	3.82	10.60	148.93	0.0030	11.81
IC 52105	1.64	0.99	0.64	9.41	391.91	0.0022	0.45
IC 9883	2.56	1.83	0.73	10.89	133.69	0.0006	0.37
TVX-944	1.27	0.77	0.50	7.68	168.16	0.0011	8.34
Bhagyalakshmy	0.73	0.59	0.15	8.63	74.11	0.0003	8.09
SEm±	**0.04**	**0.01**	**0.04**	**0.38**	**3.25**	**0.0001**	**0.28**
C.D.@ 1 %	**0.15**	**0.06**	**0.16**	**1.48**	**12.68**	**0.002**	**1.10**
C.D.@ 5 %	**0.11**	**0.04**	**0.12**	**1.09**	**9.41**	**0.002**	**0.82**

*PPO= Polyphenol oxidase and POD= Peroxidase

Table 11. Biochemical parameters of cowpea genotypes (pod)

Genotypes	Total sugar content (%)	Reducing sugar content (%)	Non-reducing sugar content (%)	Total protein content (%)	Total phenol content (mg CE /g)	PPO activity (EU /g)	POD activity (EU /g)	Crude fibre (%)
Geethika	5.00	2.29	2.71	12.05	185.99	0.0024	52.98	31.78
Vellayani Jyothika	3.56	1.77	1.79	30.78	119.38	0.0002	50.87	30.06
Lola	3.72	1.38	2.33	23.08	87.48	0.0004	42.03	35.76
Hridya	4.80	1.88	2.92	24.71	125.71	0.0027	63.94	41.67
Palakkadan thandan payar	5.15	2.21	2.95	5.51	127.59	0.0013	59.80	45.75
Kanakamony	1.28	0.21	1.07	10.16	107.18	0.0014	22.21	42.90
Mysore Local	8.74	3.07	5.67	10.22	117.97	0.0011	17.59	44.00
Kashi Kanchan	3.44	1.46	1.98	7.88	126.65	0.0015	4.92	33.68
EC 300039	1.01	0.60	0.41	14.68	549.52	0.0018	7.42	45.73
EC 98668	1.07	0.81	0.26	20.72	180.36	0.0051	12.19	48.85
EC 101216	4.69	1.52	3.17	13.75	272.53	0.0043	20.10	45.99
IC 52110	3.40	1.02	2.39	14.06	421.23	0.0016	4.87	33.26
IC 39945	2.89	1.23	1.66	23.88	202.87	0.0013	81.99	24.16
IC 2918	2.03	1.27	0.76	13.36	217.65	0.0024	42.83	42.83
IC 39922	1.24	0.23	1.01	18.29	152.68	0.0056	27.78	52.60

Table 11 continued

Genotypes	Total sugar content (%)	Reducing sugar content (%)	Non-reducing sugar content (%)	Total protein content (%)	Total phenol content (mg CE /g)	PPO activity (EU /g)	POD activity (EU /g)	Crude fibre (%)
IC 52118	4.35	2.65	1.71	10.16	71.53	0.0014	17.21	40.55
IC 39916	1.37	1.04	0.32	10.63	113.28	0.0004	20.55	41.30
IC 2196	4.48	1.52	2.97	10.39	79.74	0.0014	7.69	36.96
IC 20645	12.65	2.88	9.77	14.47	123.84	0.0009	129.36	47.04
IC 26048	3.43	1.47	1.96	8.20	267.84	0.0010	19.64	43.23
IC 52107 A	7.43	1.46	5.97	13.36	76.69	0.0022	1.63	29.01
IC 39947	2.59	1.64	0.96	14.62	157.14	0.0049	10.65	45.03
IC 39921	10.43	3.09	7.34	13.63	171.68	0.0027	32.54	31.10
IC 26029	9.47	3.41	6.05	14.79	177.08	0.0017	4.52	37.78
IC 20720	8.96	3.46	5.50	16.94	132.04	0.0007	3.82	29.03
IC 39870	5.86	0.36	5.50	13.49	152.45	0.0003	23.61	33.64
IC 52105	2.22	1.23	0.99	14.14	72.94	0.0014	11.02	25.78
IC 9883	8.33	3.40	4.93	11.19	180.36	0.0021	19.95	31.25
TVX-944	4.00	0.81	3.19	16.75	239.46	0.0004	7.96	45.83
Bhagyalakshmy	3.37	1.26	2.11	29.82	113.75	0.0003	63.69	32.89
SEm±	**0.12**	**0.12**	**0.22**	**0.56**	**5.51**	**0.0001**	**1.51**	**0.78**
C.D.@ 1 %	**0.48**	**0.47**	**0.85**	**2.17**	**21.47**	**0.003**	**5.87**	**3.06**
C.D.@ 5 %	**0.35**	**0.35**	**0.63**	**1.61**	**15.93**	**0.002**	**4.36**	**2.27**

*PPO= Polyphenol oxidase and POD= Peroxidase

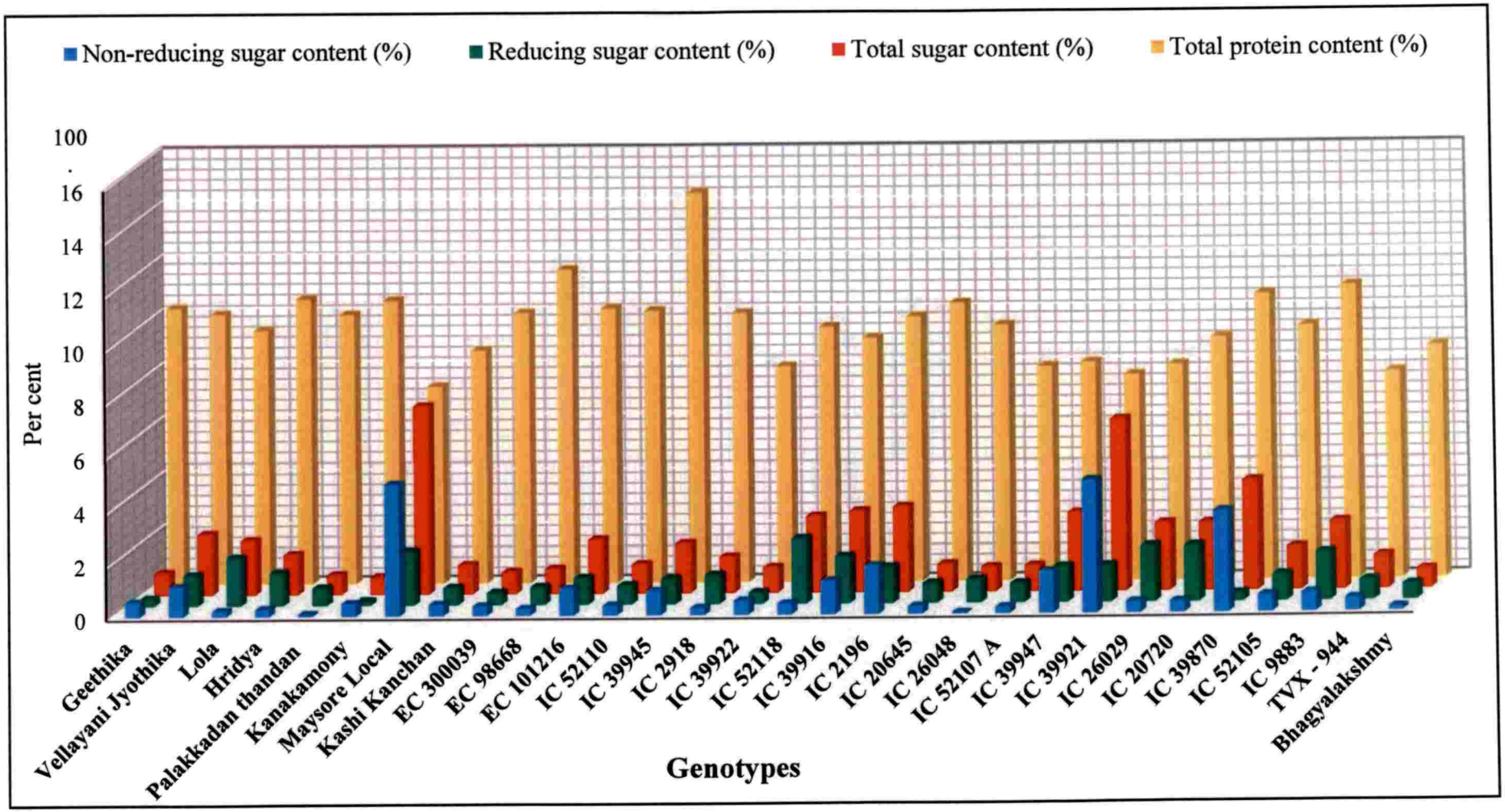

Fig. 3. Biochemical parameters of cowpea genotypes (flower bud)

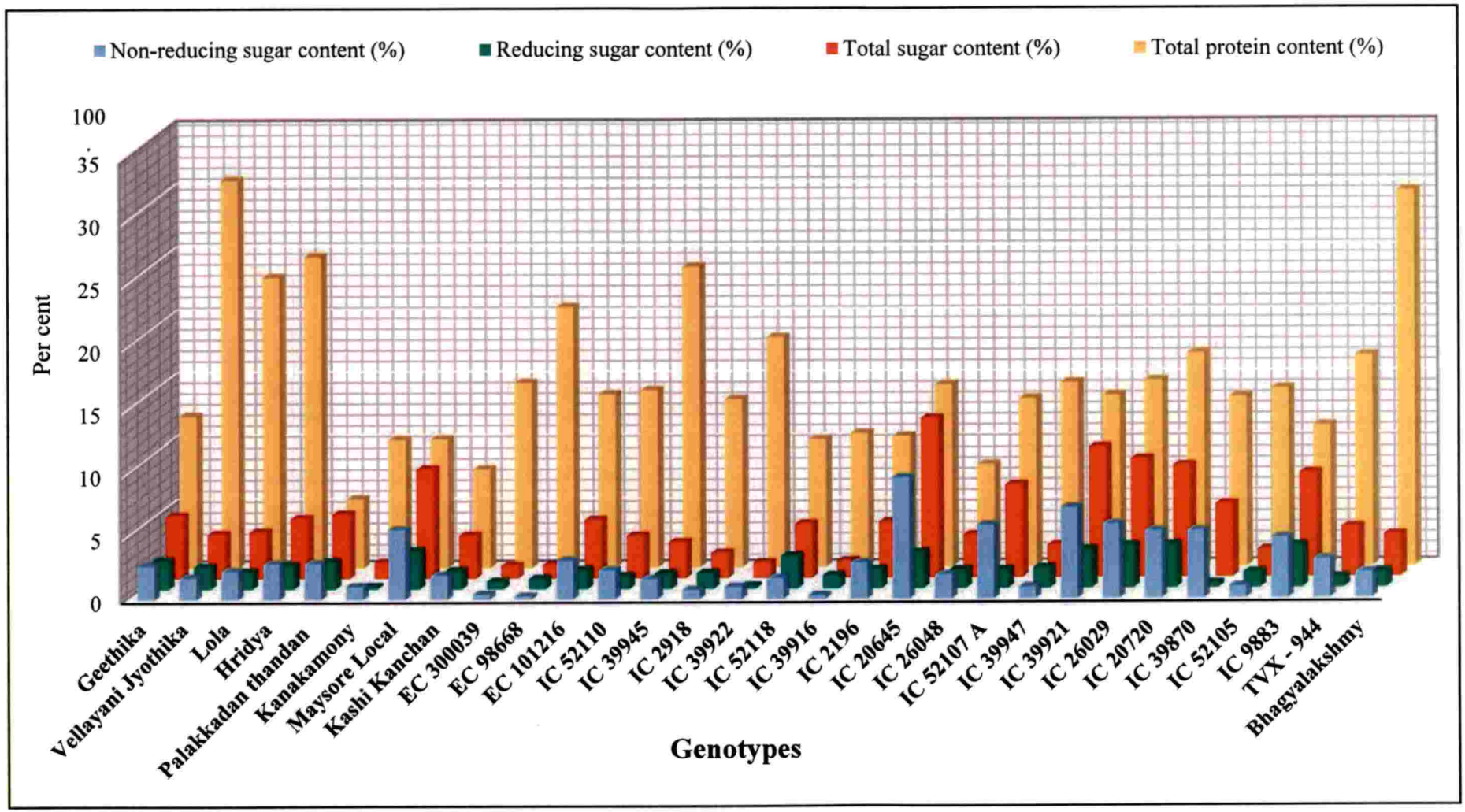

Fig. 4. Biochemical parameters of cowpea genotypes (pod)

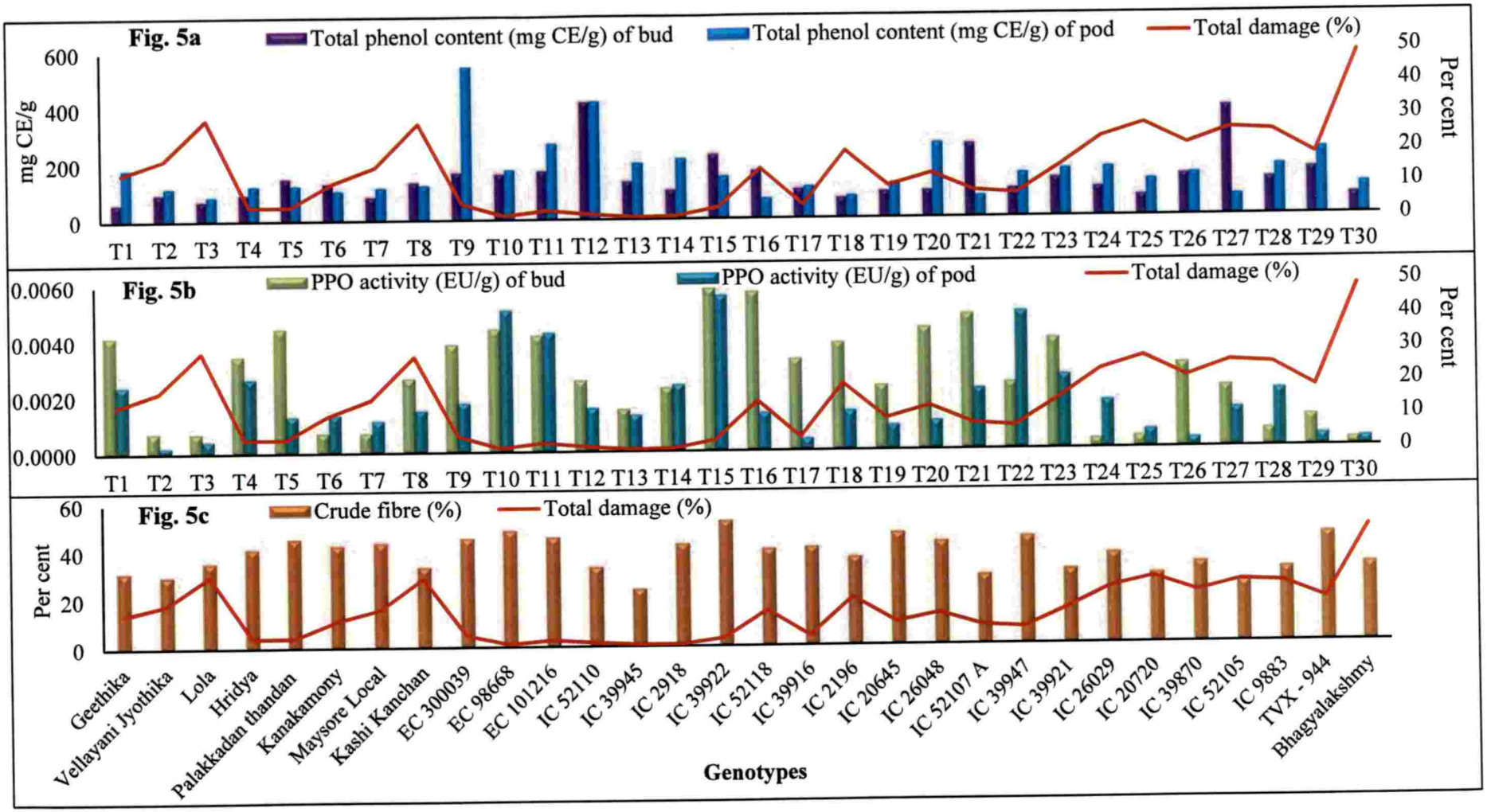

Fig. 5. Biochemical parameters of cowpea genotypes 5a. Total phenol content of flower bud and pod
5b. Polyphenol oxidase activity in flower bud and pod
5c. Crude fibre content of immature pod

Table 12. Correlation between biochemical parameters of cowpea and damage parameters

Variables	Flower bud damage	Flower damage	Pod damage	Total damage
Total sugar content of flower bud	0.100	0.146	0.022	0.129
Total sugar content of pod	0.129	0.21	0.063	0.245
Reducing sugar content of flower bud	0.198	0.292	0.12	0.216
Reducing sugar content of pod	0.207	0.259	0.063	0.261
Non-reducing sugar content of flower bud	0.027	0.037	-0.03	0.052
Non-reducing sugar content of pod	0.083	0.167	0.056	0.212
Total protein content of flower bud	-0.199	-0.244	-0.198	-0.306
Total protein content of pod	0.269	0.264	**0.436**	0.211
Total phenol content of flower bud	**-0.383**	-0.276	-0.138	-0.229
Total phenol content of pod	**-0.405**	**-0.423**	-0.278	-0.357
PPO activity of flower bud	**-0.392**	**-0.431**	**-0.518**	**-0.553**
PPO activity of pod	**-0.522**	**-0.417**	**-0.476**	**-0.525**
POD activity of flower bud	0.022	-0.099	-0.165	-0.204
POD activity of pod	0.046	-0.027	-0.011	-0.083
Crude fibre	**-0.436**	**-0.473**	**-0.428**	**-0.479**

*Values in bold are different from 0 at 5 % level of significance

**PPO= Polyphenol oxidase and POD= Peroxidase

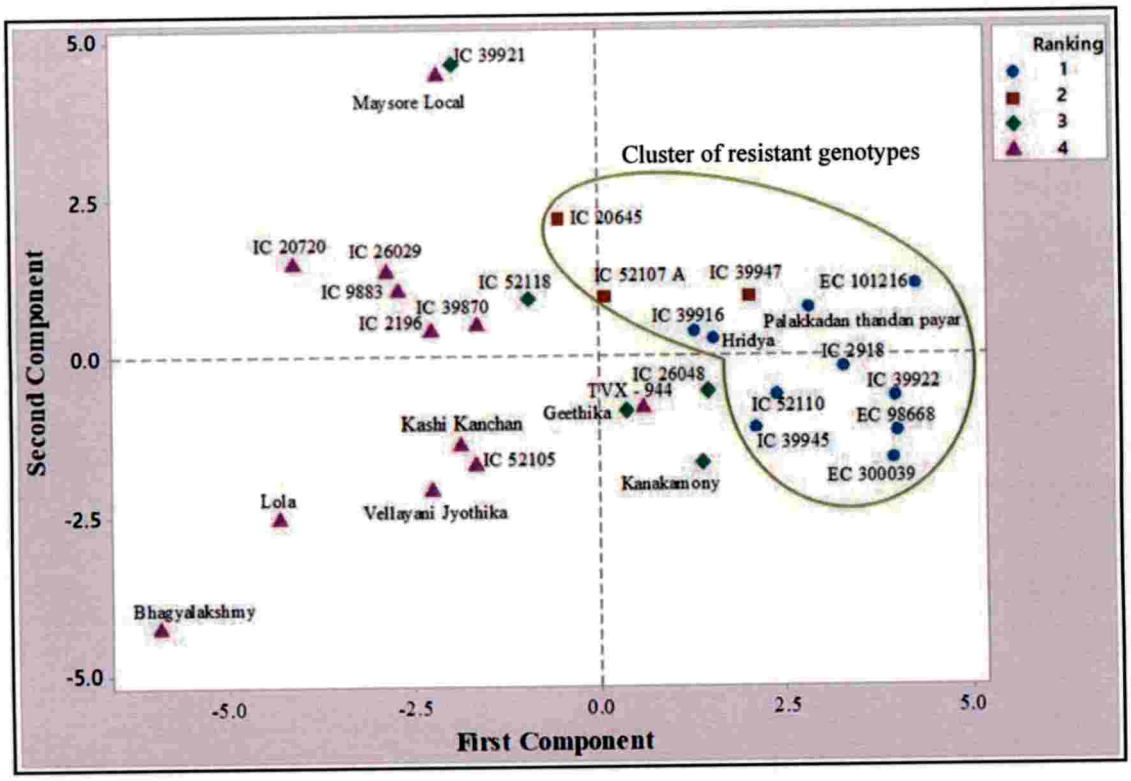

Fig. 6. Two-dimensional score plot of resistant and susceptible genotypes distribution

Ranking

1 : Total damage < 5 %
2 : Total damage > 5-10%
3 : Total damage > 10-15%
4 : Total damage > 15%

revealed a grouping of four main categories of genotypes with respect to resistance to spotted pod borer.

It is clearly evident from the score plot that almost all resistant genotypes discriminated to positive scores concerning the first principle component (PC1), and grouped in close proximity on the one side of the two-dimensional score plot. On the other hand, most of the susceptible genotypes discriminated for negative scores for PC1. Few genotypes *viz.*, Geethika, Kanakamony, IC 26048 and TVX-944 scored positively and clustered with resistant genotypes in PC1. The close immediacy of these genotypes with resistant genotypes can be attributed to one or few characters which have a positive correlation with the resistant nature of genotypes. However, resistant to the insects cannot be attributed to one or few characters, as the matter of fact, resistance to the insects is a product of multiple characters. Principal component analysis of a data also revealed the efficiency of characters examined to discriminate between the resistant and susceptible genotypes.

4.1.7 Biometric observations

The mean performances of different genotypes for different biometric characters are presented in Table 13 and described below.

4.1.7.1 Days to 50 per cent flowering

Among 30 genotypes, days to 50 per cent flowering was ranged from 41.50 to 71.50 days. Genotype Hridya and Bhagyalakshmy had taken less days for 50 per cent flowering, 41.50 days, which was on par with EC 98668 (44.50 days), IC 2196 (45.00 days), IC 52110 (45.50 days), Kashi Kanchan (46.50 days) and IC 20720 (47.00 days). Variety Lola (71.50) had taken more days for 50 per cent flowering, which was on par with IC 26029 (69.00).

4.1.7.2 Plant height

Plant height ranged from 26.40 cm to 492.10 cm. All three yard-long bean type genotypes *viz.*, Lola, Geethika and Vellayani Jyothika observed to have more plant height. Among them, Lola (492.10 cm) recorded the highest plant height, followed by Geethika and Vellayani Jyothika (465.60 cm and 389.00 cm, respectively). Genotype IC 52105 (26.40 cm) recorded short plant height, which was on par with IC 20645 (30.20 cm), IC 20720 (32.70 cm), TVX-944 (37.45 cm), Bhagyalakshmy (37.50 cm), IC 52107 A (38.50 cm) and Kashi Kanchan (43.70 cm).

4.1.7.3 Number of primary branches per plant

A number of primary branches per plant ranged between 2.2 to 4.2. Vellayani Jyothika (4.2) observed to have more number of primary branches per plant, which was on par with Geethika (4.1), Lola (3.8) and IC 52110 (3.8). Genotype IC 20720 (2.2) was noted for minimum number of branches per plant, which was on par with IC 52105 (2.3), EC 300039 (2.4), IC 39921 (2.4), IC 39870 (2.4), Palakkadan thandan payar (2.5), IC 2196 (2.5), IC 39922 (2.6), IC 52107 A (2.6) and IC 26029 (2.7). It was also observed that with respect to a number of primary branches per plant low variation was existed among all 30 genotypes.

4.1.7.4 Number of pods per plant

A number of pods per plant ranged between 11.40 to 26.40. Geethika observed to bear more number of pods per plant, 26.40, which was on par with IC 2196 (26.10), Bhagyalakshmy (25.50) and EC 98668 (25.20), whereas, EC 300039 (11.40) observed to bear less number of pods per plant, which was on par with TVX-944 (11.60) and Palakkadan thandan payar (12.80).

4.1.7.5 Pod length

The maximum pod length was recorded by Vellayani Jyothika (44.17 cm), followed by Lola (41.40 cm) and Geethika (40.80 cm), whereas, IC 39921 (7.76 cm) observed to have shorter pod length which was on par with IC 26029 (8.20 cm) (Table 13).

4.1.7.6 Number of seeds per pod

A wide range of variation was observed for seeds per pod among the 30 genotypes. Vellayani Jyothika (19.10) observed to have more number of seeds per pod, which was on par with Geethika (18.90). Genotype IC 20720 (7.10) observed to have less number of seeds per pod, which was on par with IC 39921 (7.20), IC 39945 (7.30), IC 39922 (7.40), IC 20645 (7.60), IC 39947 (7.90) and IC 26029 (7.90).

4.1.7.7 Grain yield per plant

Wide-ranging variation was observed for grain yield per plant. Vellayani Jyothika recorded the highest grain yield per plant, 81.85 g, followed by Lola (53.86 g), whereas, IC 20720 recorded less grain yield per plant, 9.38 g, which was on par with IC 20645 (9.54 g), IC 39922 (9.59 g), IC 39921 (9.74 g), IC 39947 (9.93 g), IC 39945 (11.45 g) and IC 9883 (11.72 g).

Table 13. The variability in quantitative characters in 30 genotypes of cowpea

Genotypes	Days to 50 per cent flowering	Plant height (cm)	No. of primary branches per plant	No. of pods per plant	Pod length (cm)	No. of seeds per pod	Grain yield per plant (g)	100 seed weight (g)
Geethika	58.50	465.60	4.1	26.40	40.80	18.90	45.70	9.15
Vellayani Jyothika	54.00	389.00	4.2	20.60	44.17	19.10	81.85	20.80
Lola	71.50	492.10	3.8	19.70	41.40	17.10	53.83	16.00
Hridya	41.50	54.40	3.4	21.90	9.38	9.10	18.22	4.50
Palakkadan thandan payar	68.00	101.80	2.5	12.80	14.52	10.90	12.80	14.00
Kanakamony	52.00	231.50	3.5	17.20	13.65	13.10	20.72	11.25
Mysore Local	57.00	115.50	3.2	16.60	14.52	15.20	23.13	8.95
Kashi Kanchan	46.50	43.70	3.3	17.90	17.54	11.30	18.49	9.30
EC 300039	54.50	134.00	2.4	11.40	19.15	16.80	17.47	10.80
EC 98668	44.50	82.50	3.1	25.20	13.20	12.40	28.22	7.15
EC 101216	50.00	124.00	3.4	20.60	11.15	10.60	19.94	6.05
IC 52110	45.50	68.90	3.8	20.30	9.40	9.40	17.51	6.15
IC 39945	50.50	148.20	3.1	17.10	9.41	7.30	11.45	10.10
IC 2918	65.00	135.60	3.2	15.90	12.46	14.60	21.22	9.50
IC 39922	49.00	70.00	2.6	14.10	12.52	7.40	9.59	9.05

Table 13 continued

Genotypes	Days to 50 per cent flowering	Plant height (cm)	No. of primary branches per plant	No. of pods per plant	Pod length (cm)	No. of seeds per pod	Grain yield per plant (g)	100 seed weight (g)
IC 52118	54.00	119.30	3.5	24.50	15.96	12.50	28.02	8.65
IC 39916	53.50	127.70	3.0	20.00	10.73	11.50	21.04	8.35
IC 2196	45.00	74.00	2.5	26.10	13.12	14.30	34.14	8.35
IC 20645	52.50	30.20	2.9	13.70	11.60	7.60	9.54	10.65
IC 26048	54.00	104.40	3.2	13.80	11.73	10.90	13.73	5.15
IC 52107 A	50.50	38.50	2.6	17.30	10.30	10.80	17.07	4.80
IC 39947	57.50	153.90	3.4	13.70	13.32	7.90	9.93	7.45
IC 39921	54.00	128.10	2.4	14.60	7.76	7.20	9.74	11.65
IC 26029	69.00	70.20	2.7	23.80	8.20	7.90	17.21	6.40
IC 20720	47.00	32.70	2.2	14.50	8.95	7.10	9.38	4.35
IC 39870	49.50	84.30	2.4	21.30	9.70	10.70	20.79	8.75
IC 52105	50.00	26.40	2.3	17.60	10.32	10.60	17.06	6.65
IC 9883	55.50	61.70	3.1	14.60	11.20	8.80	11.72	7.55
TVX-944	64.50	37.45	3.4	11.60	17.63	16.60	17.65	10.45
Bhagyalakshmy	41.50	37.50	3.5	25.50	13.98	13.40	31.26	9.30
SEm±	**1.06**	**7.49**	**0.21**	**0.62**	**0.37**	**0.29**	**0.86**	**0.28**
C.D.@ 5 %	**3.09**	**21.80**	**0.62**	**1.81**	**1.10**	**0.84**	**2.51**	**0.83**

4.1.7.8 100 seed weight

With respect to 100 seed weight, Vellayani Jyothika recorded the highest value, 20.80 g, followed by Lola (16.00 g). Genotype IC 20720 recorded low 100 seed weight, 4.35 g, which was on par with Hridya (4.50 g), IC 52107 A (4.80 g), IC 26048 (5.15 g), EC 101216 (6.05 g), IC 52110 (6.15 g), IC 26029 (6.4 g), IC 52105 (6.65 g) and EC 98668 (7.15 g) (Table 13).

4.1.8 Variability parameters

Assessment of genetic variability in the base population is a crucial step in the breeding programme. The genotypic and phenotypic coefficient of variation (GCV and PCV, respectively) are a simple measure of variability and commonly used for the assessment of variability. The relative values of these types of coefficients give an idea about the scale of variability present in a genetic population. However, it does not determine the proportion of heritable variation of the total variation present for a particular character. Johnson *et al.* (1955) suggested that heritability and genetic gain together would be more useful in predicting the effect of selection. Consequently, PCV and GCV, heritability (H^2) and genetic gain (GG) were estimated and results are presented in Table 14 and discussed as follows.

4.1.8.1 The genotypic and phenotypic coefficient of variation

The PCV was marginally higher than the corresponding GCV. This indicated the influence of environment on the expression of the characters under study. The high magnitude of PCV and GCV were recorded for plant height (39.11 % and 38.96 %, respectively) and pod length (21.62 % and 21.58 %, respectively). The high values of PCV and GCV for plant height and pod length indicates that these characters exhibited high variability among the genotypes under study. Moderate PCV and GCV were recorded in all remaining characters. Moderate to high PCV and GCV for the characters under study indicates the greater scope for selection to improve these characters in the breeding programme. The results of a study are in accordance with the results of Tyagi *et al.* (2000), Singh and Verma (2002), Prakash *et al.* (2003), Ananda (2012), Khanpara *et al.* (2015), Dinesh *et al.* (2017), Sarath and Reshma (2017) and Singh *et al.* (2018).

4.1.8.2 Heritability (H^2) and genetic advance as per cent of the mean (genetic gain)

The high heritability was observed for all characters. Character pod length (99.84 %) exhibited higher heritability, and low heritability was observed in number of primary branches per plant (85.14 %). With respect to genetic gain (GG), the characters number of primary branches per plant and 100 seed weight observed to have high values (56.84

% and 21.13 %, respectively). Characters *viz*., number of pods per plant, pod length and number of seeds per pod observed to have a moderate genetic gain. The moderate to the high value of heritability and genetic gain indicates the role of additive gene action. The selection may be effective for improvement of these characters. The same results are also reported by Malarvizhi and Rangasamy (2005), Girish *et al.* (2006), Nwosu *et al.* (2013), Khan *et al.* (2015), Tudu *et al.* (2015), Dinesh *et al.* (2017) and Singh *et al.* (2018).

However, with respect to characters *viz*., days to 50 per cent flowering, plant height and grain yield per plant, there was a low genetic gain (3.78 %, 1.63 % and 9.23 %, respectively). The low to moderate heritability coupled with low genetic gain indicates the role of non-additive gene action. Direct selection on the basis of these characters would not be effective. However, these characters can be improved by hybridisation. The same results are also reported by Khanpara *et al.* (2015), Tudu *et al.* (2015) and Sharma (2016).

Table 14. Variability parameters for various characters in cowpea

Characters	PCV (%)	GCV (%)	H^2 (%)	GG (%)
Days to 50 % flowering	11.01	10.81	98.16	3.78
Plant height	39.11	38.96	99.61	1.63
Number of primary branches /plant	13.84	11.79	85.14	56.84
Number of pods /plant	17.30	16.97	98.12	11.03
Pod length	21.62	21.58	99.84	13.50
Number of seeds /pod	18.77	18.65	99.34	17.52
Grain yield /plant	18.69	18.63	99.68	9.23
100 seed weight	16.77	15.24	90.88	21.13

4.2 EXPERIMENT 2: HYBRIDISATION OF RESISTANT GENOTYPES (IDENTIFIED IN EXPERIMENT 1) WITH HIGH YIELDING POPULAR VARIETIES

In the majority of agriculture experiments, the inferences are made considering a number of dependent characters. As it is very evident that the resistance is governed by not a single character but it is a product of a number of morphological and biochemical characters. Considering this fact, the parents for crossing programme were selected.

The analysis of variance (ANOVA) was carried out for a number of characters which are the characteristics feature of resistance or susceptibility as well as for the total damage caused by spotted pod borer. For these selected characters, ANOVA test revealed

a significant difference between the genotypes. Furthermore, stepwise multiple comparisons following the *post-hoc* test, Duncan's Multiple Range Test (DMRT) was carried out which grouped the genotypes by identifying the sample means which are significantly different. On the basis of total damage, all the genotypes divided into four categories *viz*., as resistant, moderately resistant, susceptible and highly susceptible (Table 7). From the resistant category, five genotypes were selected (Table 15) considering their biometric characters and crossed with four female parents in Line × Tester method to yield 20 hybrids (Table 16).

Table 15. Parents selected for hybridisation

Female parents (Lines)	**Selected male parents (testers)**	
Geethika	Palakkadan thandan payar	IC 2918
Vellayani Jyothika	EC 300039	
Lola	EC 98668	
Kashi Kanchan	IC 39945	

Table 16. Hybrid combinations and their coding

Sl. no.	**Cross**	**Code**
1	Geethika×Palakkadan thandan payar	Hybrid 1
2	Geethika×EC 300039	Hybrid 2
3	Geethika×EC 98668	Hybrid 3
4	Geethika×IC 39945	Hybrid 4
5	Geethika×IC 2918	Hybrid 5
6	Vellayani Jyothika×Palakkadan thandan payar	Hybrid 6
7	Vellayani Jyothika×EC 300039	Hybrid 7
8	Vellayani Jyothika×EC 98668	Hybrid 8
9	Vellayani Jyothika×IC 39945	Hybrid 9
10	Vellayani Jyothika×IC 2918	Hybrid 10
11	Lola×Palakkadan thandan payar	Hybrid 11
12	Lola×EC 300039	Hybrid 12
13	Lola×EC 98668	Hybrid 13
14	Lola×IC 39945	Hybrid 14
15	Lola×IC 2918	Hybrid 15
16	Kashi Kanchan×Palakkadan thandan payar	Hybrid 16
17	Kashi Kanchan×EC 300039	Hybrid 17
18	Kashi Kanchan×EC 98668	Hybrid 18
19	Kashi Kanchan×IC 39945	Hybrid 19
20	Kashi Kanchan×IC 2918	Hybrid 20

4.3 EXPERIMENT 3: ASSESSMENT OF PARENTAL POLYMORPHISM

A total of 40 SSR (Simple Sequence Repeats) primers were used to assess the genetic diversity and estimate genetic polymorphism in 30 cowpea genotypes. Out of these primers, 21 were polymorphic which produced polymorphic patterns in at least two genotypes (Plate 6a-6g). Data of these polymorphic primers were then used to study the molecular divergence among all genotypes and the analysis of this data revealed a high level of diversity among all genotypes. Alleles of primers CLM0251 and CLM0300 were in the heterozygous condition, so named as CLM0251A and CLM0251B as well CLM0300A and CLM0300B.

Different statistics parameters of SSR primers with respect to genetic diversity are presented in Table 17. Total of 86 polymorphic amplicons were amplified using these polymorphic primers. Among these, the number of alleles per locus ranged from 2 to 5. Primers CLM0061, CLM0279, CLM0291, CLM0295, CLM0300A and CLM0332 observed to produce five amplicons each, whereas, polymorphic primers CLM0245, CLM0247, CLM0248 and CLM0251B observed to have only two amplicons each. The results of this study are in conformity with Asare *et al.* (2010). They reported 4 to 13 alleles in cowpea genotypes collected from Ghana, whereas, Sawadogo *et al.* (2010) reported 5 to 12 amplicons in cowpea using SSR primers. Diouf and Hilu (2005) reported 1 to 9 amplicons in cowpea germplasm. With respect to amplicon size, primer CLM0190 recorded the maximum amplicons sizes ranging from 307.03 to 415.73 bp, whereas, primer CLM0251B recorded the minimum amplicons sizes, ranging from 105.67 to 133.49 bp.

Polymorphic Information Content (PIC) provides an estimate of the discriminatory power of a marker to differentiate genotypes based on both the number of alleles expressed and their relative frequencies (Nagl *et al.*, 2011). According to Taski-Ajdukovic *et al.* (2017), the high PIC of primers indicates the highly informative nature of the SSR primers and the diversity of the used populations. The PIC was calculated for 21 pairs of SSR primers representing the allelic diversity for a specific locus, varying from 0.33 to 0.76. The high PIC was recorded by CLM0300A (0.76), followed by CLM0295 (0.71) and CLM0061 (0.70), whereas, low PIC was recorded by CLM0247 (0.33), followed by CLM0248 (0.36) and CLM0245 (0.37). The results of this study supported with the results of Karuma *et al.* (2008). They observed PIC ranging from 0.09 to 0.87, whereas, Ogunkanmi *et al.* (2008) observed PIC ranging from 0.29 to 0.87.

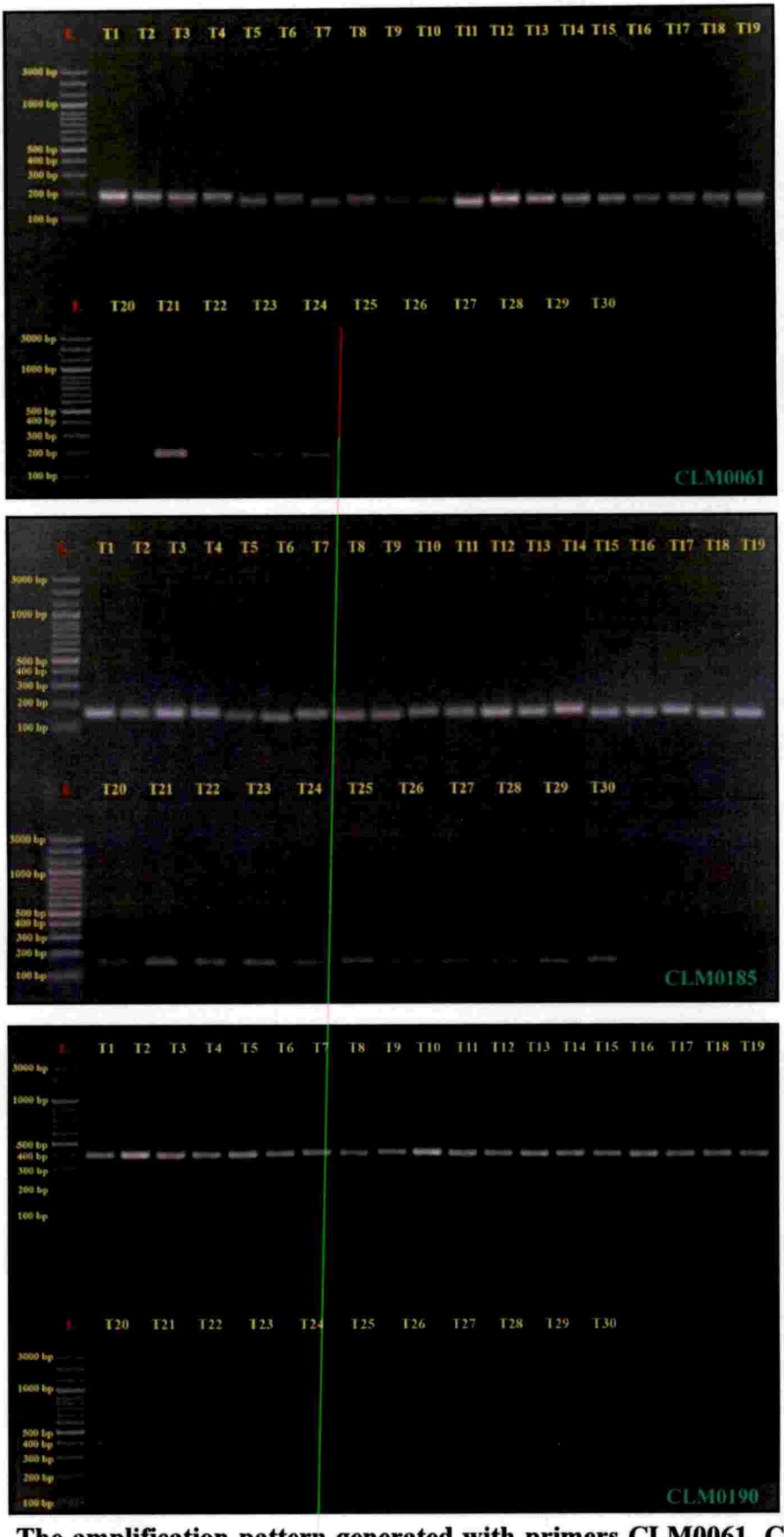

Plate 6a. The amplification pattern generated with primers CLM0061, CLM0185 and CLM0190

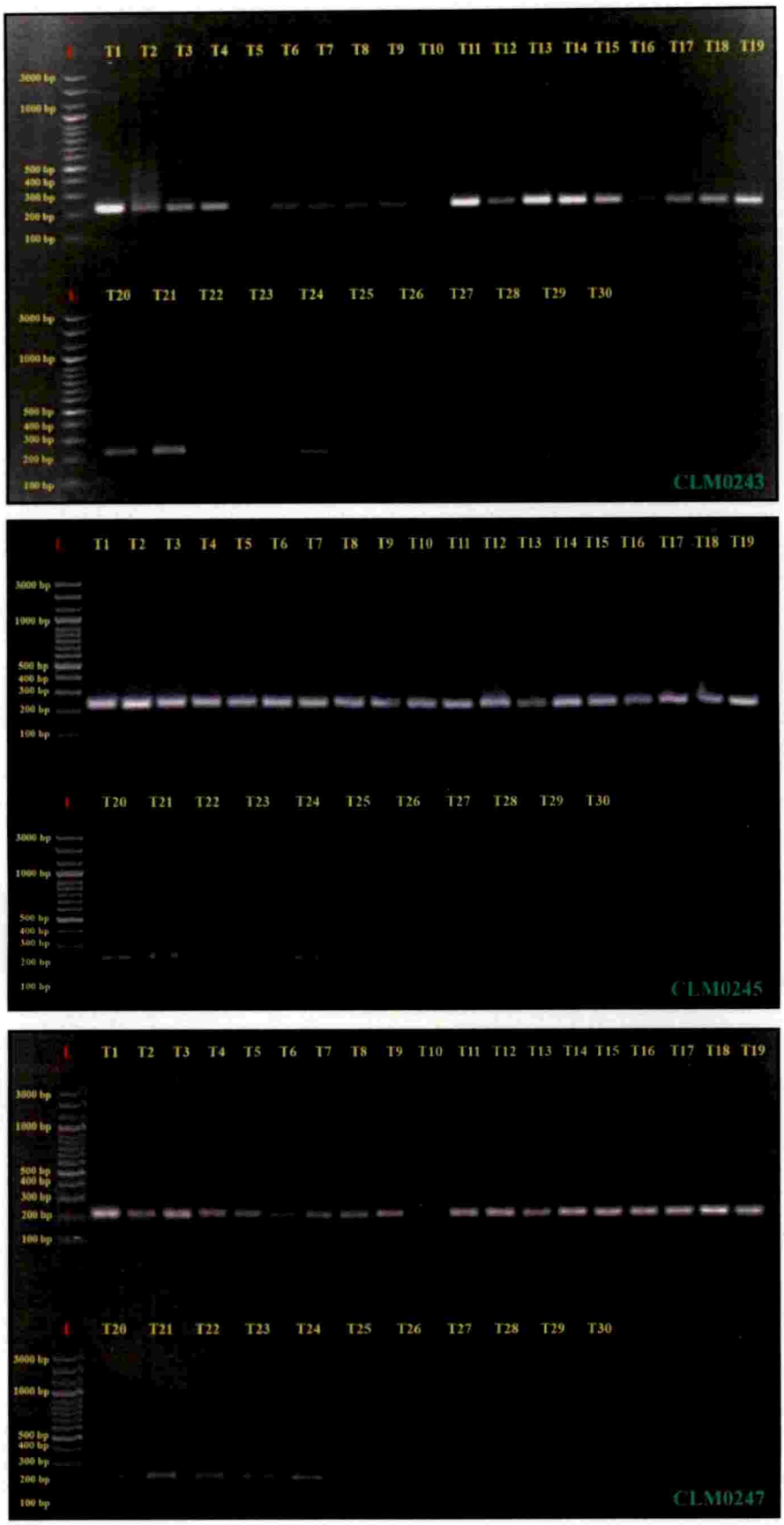

Plate 6b. The amplification pattern generated with primers CLM0243, CLM0245 and CLM0247

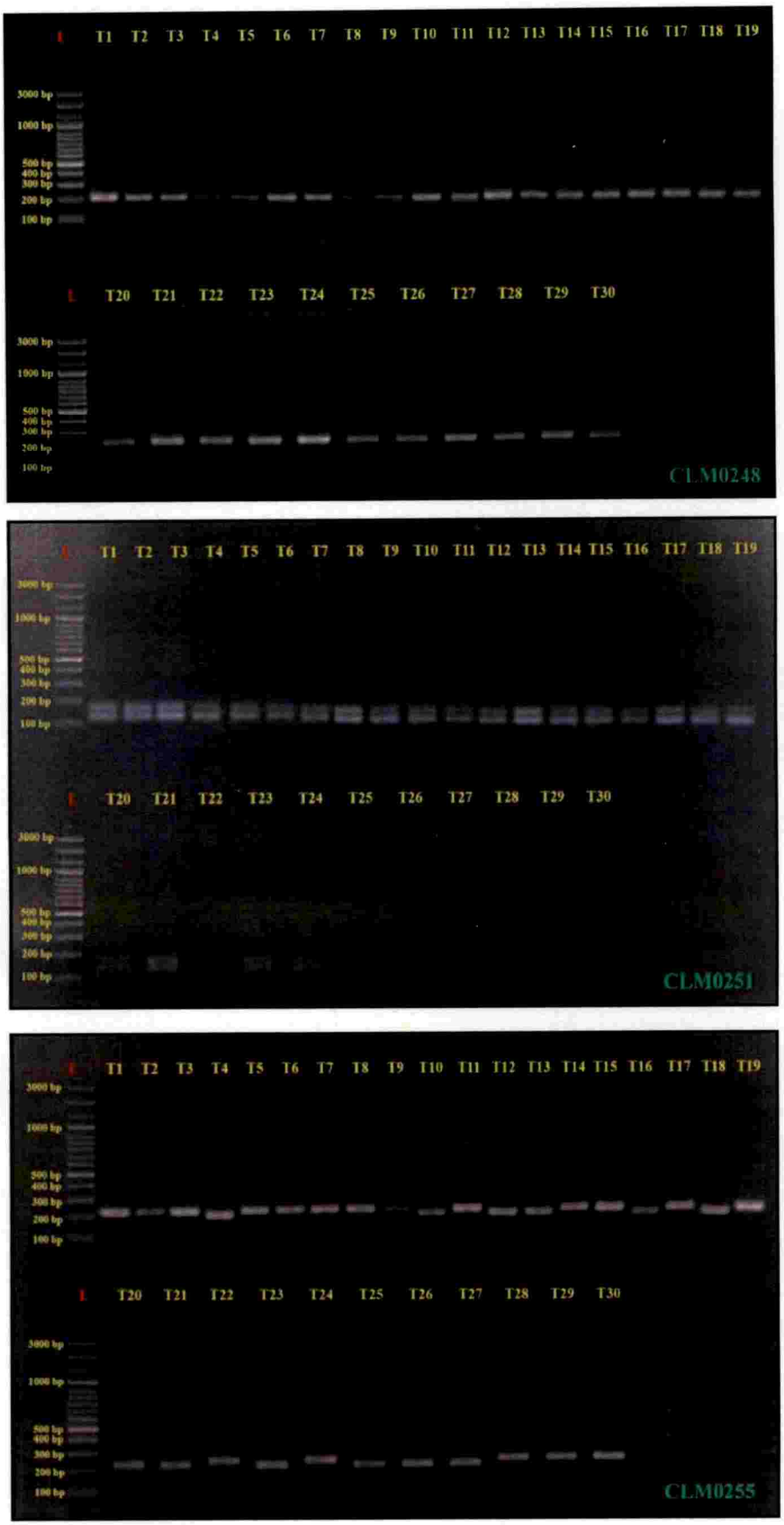

Plate 6c.The amplification pattern generated with primers CLM0248, CLM0251 and CLM0255

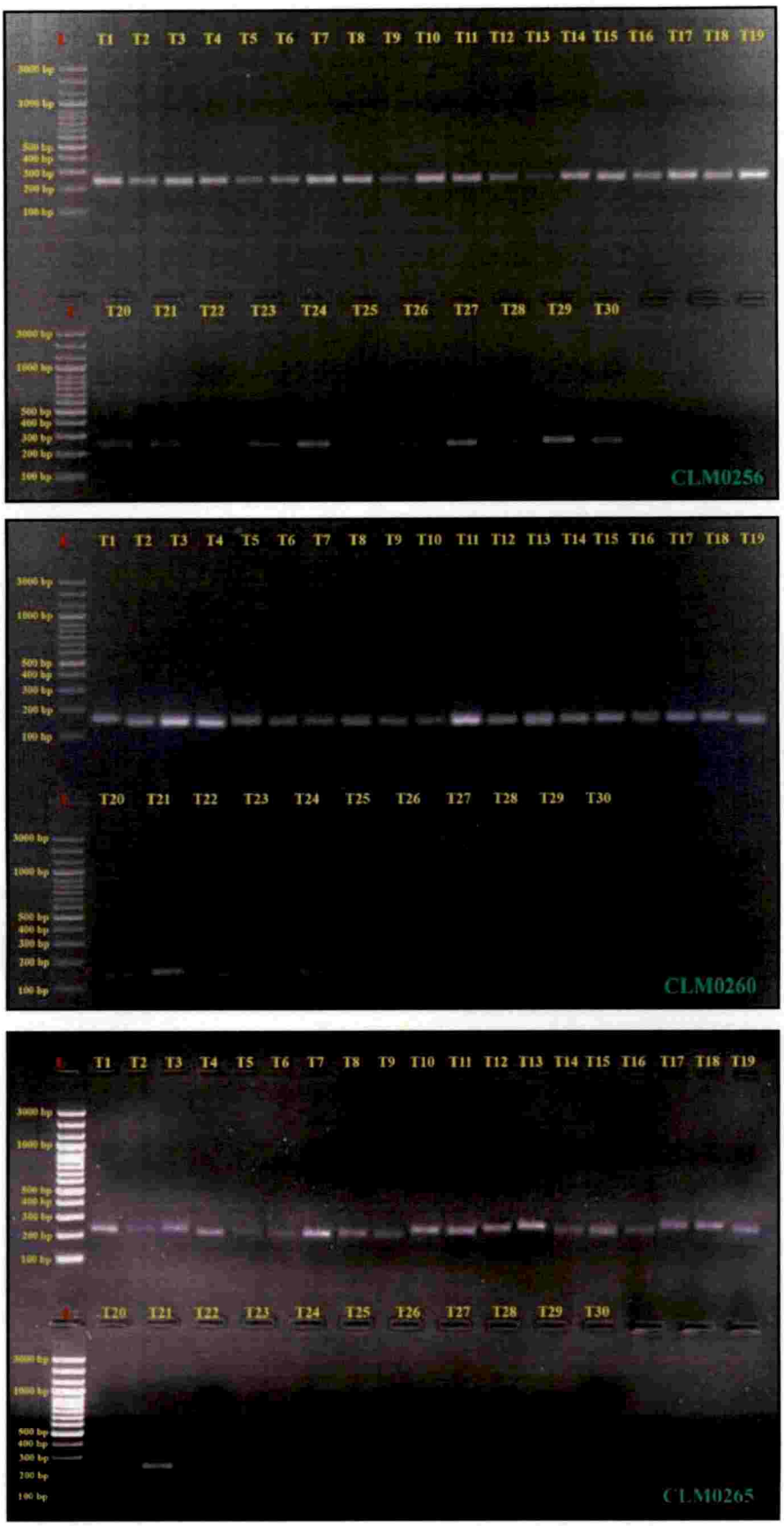

Plate 6d. The amplification pattern generated with primers CLM0256, CLM0260 and CLM0265

106 A

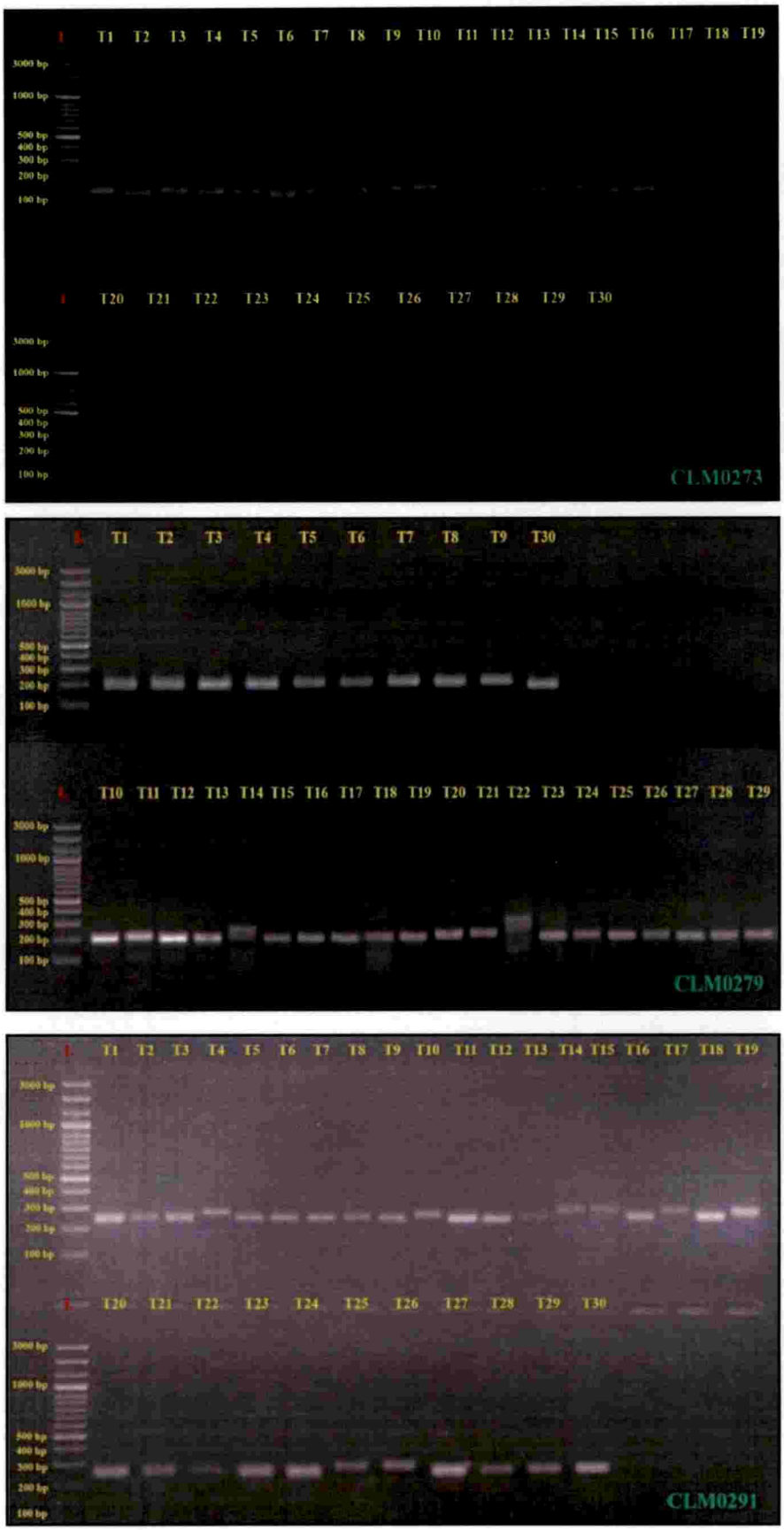

Plate 6e. The amplification pattern generated with primers CLM0273, CLM0279 and CLM0291

Plate 6f. The amplification pattern generated with primers CLM0292, CLM0295 and CLM0298

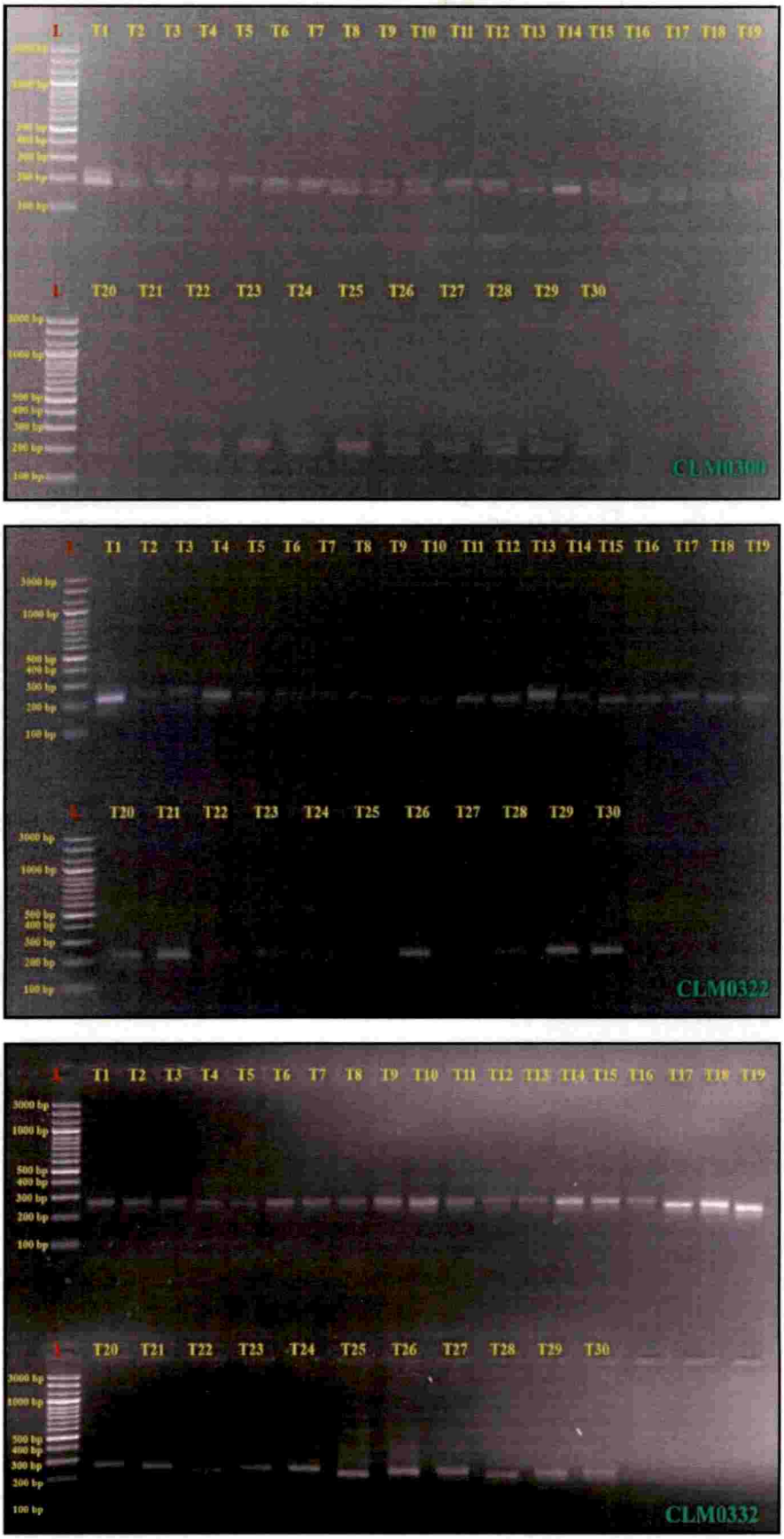

Plate 6g. The amplification pattern generated with primers CLM0300, CLM0322 and CLM0332

The high expected heterozygosity and Shannon's information index are two important and commonly used parameters which indicate the effectiveness of microsatellite loci to reveal the variation. In the experiment conducted, the high expected heterozygosity and Shannon's information index were recorded by CLM0300, whereas, the lowest values were recorded by CLM0247 (0.42 and 0.27, respectively).

Table 17. Genetic diversity statistics of SSR primers in cowpea

Primer	PIC	Amplicon size (bp)		No. of amplicons	Shannon's information index	Expected heterozygosity
		Min.	Max.			
CLM0061	0.70	151.91	196.99	5	0.63	0.74
CLM0185	0.51	142.48	169.50	3	0.42	0.58
CLM0190	0.67	307.03	415.73	4	0.61	0.69
CLM0243	0.54	238.50	274.16	3	0.44	0.61
CLM0245	0.37	227.53	253.90	2	0.30	0.50
CLM0247	0.33	209.28	233.24	2	0.27	0.42
CLM0248	0.36	196.32	215.20	2	0.29	0.48
CLM0251A	0.68	127.09	177.09	4	0.58	0.73
CLM0251B	0.36	105.67	133.49	2	0.29	0.48
CLM0255	0.67	206.09	274.42	4	0.57	0.72
CLM0256	0.67	246.52	273.72	3	0.41	0.57
CLM0260	0.52	142.42	182.92	4	0.45	0.59
CLM0265	0.67	208.55	251.65	4	0.58	0.72
CLM0273	0.66	100.00	141.85	4	0.55	0.69
CLM0279	0.46	192.91	261.97	5	0.44	0.48
CLM0291	0.68	243.27	303.09	5	0.61	0.73
CLM0292	0.63	155.62	209.88	4	0.54	0.68
CLM0295	0.71	206.82	270.73	5	0.63	0.75
CLM0298	0.68	124.73	171.93	4	0.59	0.73
CLM0300A	0.76	171.34	239.04	5	0.69	0.79
CLM0300B	0.70	155.80	208.52	4	0.60	0.74
CLM0322	0.56	224.79	261.14	3	0.46	0.64
CLM0332	0.69	212.93	284.73	5	0.63	0.74

4.3.1 Genetic diversity analysis

In plant breeding, genetic diversity is a precondition for genetic improvement of any agricultural crops. Prior knowledge of existing genetic diversity within the available germplasm is a key to the successful breeding programme. Therefore, assessment of genetical diversity based on molecular markers is of high interest for plant breeders. Results of this study confirmed the existence of a high genetical diversity among cowpea genotypes used.

The Jaccard's similarity coefficient values obtained are presented in Table 18. The maximum similarity coefficient was measured between IC 39916 and IC 52118 (0.643) followed by Kashi Kanchan and EC 300039 (0.586) and IC 39916 and IC 2196 (0.586). The minimum similarity was exhibited between Kashi Kanchan and TVX-944 (0.02). It was likewise noted that the three yard-long bean genotypes *viz.*, Geethika, Vellayani Jyothika and Lola showed comparatively higher values of similarity coefficient among themselves. This indicates that these three genotypes are more genetically similar to each other. However, Geethika recorded less value of similarity coefficients with Vellayani Jyothika and Lola (0.394) than the value of the similarity coefficient between Vellayani Jyothika and Lola (0.533). On the other hand, three yard-long bean genotypes showed less values of similarity coefficients with all other genotypes except Palakkadan thandan payar where they exhibited comparatively high values of similarity coefficient (0.314, 0.353 and 0.484, respectively).

Three exotic genotypes *viz.*, EC 300039, EC 98668 and EC 101216, in general, recorded less value of similarity coefficients with all other genotypes except Mysore Local and Kashi Kanchan. In between EC 300039 and Kashi Kanchan, the higher value was observed (0.586), whereas, EC 101216 observed to have a higher value of similarity coefficient with Mysore Local and Kashi Kanchan (0.484). Among the exotic genotypes, EC 101216 recorded higher values of similarity coefficient with EC 300039 and EC 98668 (0.438 and 0.533, respectively). However, less value of similarity coefficient was observed between EC 300039 and EC 98668 (0.278) (Table 18).

Cowpea is predominantly a self-pollinated crop and this ultimately resulted into a narrow genetic base (Asare *et al.*, 2010). Despite this, in our study, we observed significant divergence in the genotypes studied. The highest levels of divergence between the genotypes could be attributed to the fact that the genotypes used in the study were

Table 18. Genetic distances based on SSRs pooled over the 21 polymorphic primers in 30 cowpea genotypes

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20
T1	1.000																			
T2	0.394	1.000																		
T3	0.394	0.533	1.000																	
T4	0.243	0.278	0.353	1.000																
T5	0.314	0.353	0.484	0.353	1.000															
T6	0.211	0.278	0.438	0.394	0.533	1.000														
T7	0.211	0.278	0.394	0.394	0.353	0.438	1.000													
T8	0.278	0.179	0.243	0.314	0.278	0.278	0.438	1.000												
T9	0.179	0.179	0.179	0.211	0.314	0.243	0.353	0.586	1.000											
T10	0.122	0.150	0.070	0.070	0.095	0.095	0.211	0.243	0.278	1.000										
T11	0.179	0.179	0.179	0.150	0.179	0.179	0.484	0.484	0.438	0.533	1.000									
T12	0.122	0.150	0.179	0.070	0.211	0.150	0.243	0.211	0.278	0.484	0.314	1.000								
T13	0.150	0.278	0.211	0.179	0.211	0.095	0.150	0.179	0.179	0.243	0.211	0.394	1.000							
T14	0.070	0.150	0.179	0.211	0.243	0.122	0.243	0.179	0.179	0.278	0.278	0.394	0.353	1.000						
T15	0.095	0.095	0.095	0.179	0.095	0.070	0.150	0.179	0.211	0.243	0.278	0.243	0.278	0.394	1.000					
T16	0.243	0.095	0.211	0.122	0.211	0.070	0.150	0.278	0.278	0.211	0.243	0.314	0.278	0.438	0.353	1.000				
T17	0.211	0.150	0.211	0.211	0.150	0.070	0.122	0.243	0.243	0.150	0.179	0.278	0.314	0.438	0.394	0.643	1.000			
T18	0.243	0.179	0.314	0.150	0.211	0.122	0.211	0.243	0.243	0.122	0.278	0.278	0.353	0.314	0.314	0.586	0.586	1.000		
T19	0.095	0.045	0.095	0.122	0.179	0.179	0.179	0.278	0.314	0.243	0.353	0.278	0.179	0.278	0.278	0.278	0.211	0.243	1.000	
T20	0.243	0.278	0.394	0.243	0.179	0.211	0.314	0.243	0.150	0.278	0.278	0.243	0.243	0.211	0.150	0.243	0.179	0.179	0.211	1.000
T21	0.211	0.243	0.211	0.179	0.150	0.243	0.179	0.095	0.045	0.211	0.122	0.150	0.211	0.122	0.095	0.070	0.122	0.095	0.070	0.314
T22	0.150	0.211	0.211	0.211	0.122	0.211	0.179	0.150	0.070	0.243	0.150	0.179	0.122	0.150	0.122	0.095	0.070	0.045	0.150	0.353
T23	0.243	0.211	0.179	0.278	0.122	0.122	0.243	0.211	0.095	0.150	0.211	0.070	0.122	0.095	0.070	0.070	0.070	0.122	0.179	0.353
T24	0.211	0.353	0.211	0.278	0.150	0.179	0.211	0.179	0.095	0.095	0.150	0.045	0.243	0.150	0.211	0.122	0.179	0.095	0.150	0.314
T25	0.243	0.243	0.211	0.278	0.150	0.150	0.150	0.150	0.070	0.122	0.150	0.095	0.211	0.150	0.179	0.122	0.179	0.095	0.095	0.278
T26	0.243	0.211	0.150	0.278	0.122	0.211	0.179	0.150	0.070	0.070	0.150	0.045	0.150	0.070	0.150	0.095	0.095	0.122	0.070	0.243
T27	0.243	0.211	0.150	0.150	0.095	0.211	0.122	0.095	0.045	0.122	0.095	0.095	0.095	0.070	0.070	0.070	0.045	0.045	0.070	0.211
T28	0.070	0.278	0.179	0.211	0.095	0.122	0.179	0.070	0.122	0.095	0.150	0.070	0.150	0.095	0.150	0.070	0.122	0.122	0.095	0.150
T29	0.150	0.179	0.179	0.070	0.122	0.150	0.070	0.022	0.045	0.070	0.045	0.095	0.150	0.150	0.070	0.150	0.179	0.179	0.095	0.150
T30	0.122	0.211	0.243	0.150	0.150	0.179	0.095	0.045	0.045	0.070	0.045	0.122	0.122	0.211	0.122	0.179	0.243	0.150	0.070	0.243

Table 18 continued

	T21	T22	T23	T24	T25	T26	T27	T28	T29	T30
T21	1.000									
T22	0.484	1.000								
T23	0.394	0.438	1.000							
T24	0.314	0.314	0.394	1.000						
T25	0.278	0.278	0.353	0.484	1.000					
T26	0.314	0.314	0.438	0.438	0.394	1.000				
T27	0.353	0.353	0.353	0.278	0.278	0.533	1.000			
T28	0.243	0.211	0.314	0.314	0.314	0.278	0.314	1.000		
T29	0.211	0.150	0.211	0.150	0.179	0.179	0.353	0.394	1.000	
T30	0.211	0.179	0.122	0.179	0.150	0.150	0.179	0.243	0.533	1.000

collected from the different geographical zones of India and other parts of the world. Though, few genotypes observed to have less divergence and recorded higher values of similarity coefficients. The reason for the less divergence could be argued with the reason that these genotypes could have a common origin (Pasquet, 2000; Coulibaly *et al.*, 2002; Ba *et al.*, 2004; Karuma *et al.*, 2008; Magembe, 2008; Asare *et al.*, 2010).

4.3.2 Cluster analysis

Cluster analysis revealed the presence of high genetic variation among all genotypes studied. The dendrogram (Fig. 7) based on Jaccard's similarity coefficients was constructed using UPGMA after analysis of banding patterns generated by 21 polymorphic primers across the 30 cowpea genotypes. All three yard-long bean genotypes *viz.*, Geethika, Vellayani Jyothika and Lola occupied neighbouring places on the dendrogram and also on two-dimensional score plot (Fig. 8). The same scenario was observed with respect to three exotic genotypes (EC 300039, EC 98668 and EC 101216).

The two-dimensional graph of genotypes distribution was revealed by Principle component analysis (PCA) (Fig 8). This type of graphical presentation of the distribution of genotypes enables the assessment of the geometric distances among all of the genotypes in the study (De Sousa *et al.*, 2011). The distribution of the genotypes in the two-dimensional graph based on the first two principal components was similar to that clustering pattern which obtained through Jaccard's similarity coefficients.

Clusters analysis separated 30 cowpea genotypes into total 22 clusters at 50 per cent similarity (Table 19, Fig. 7). The cluster XXI was the larger cluster, which included three of genotypes (IC 52118, IC 39916 and IC 2196), whereas, nine clusters *viz.*, cluster I, III, V VI, VII, VIII, IX, XI, XII, XIII, XVII, X, VIII, XIX, XX and XXII observed to have only one genotype each. It was also observed that the most resistant variety, IC 2918, occupied a unique place in the constructed dendrogram (Fig. 7). The same genotype also showed less value of similarity coefficients with other genotypes under the study (Table 18). This clearly indicates that the genotype IC 2918 is highly diverse from other genotypes of this study and can be used as a donor parent against spotted pod borer. The same scenario was also observed with other few resistant genotypes.

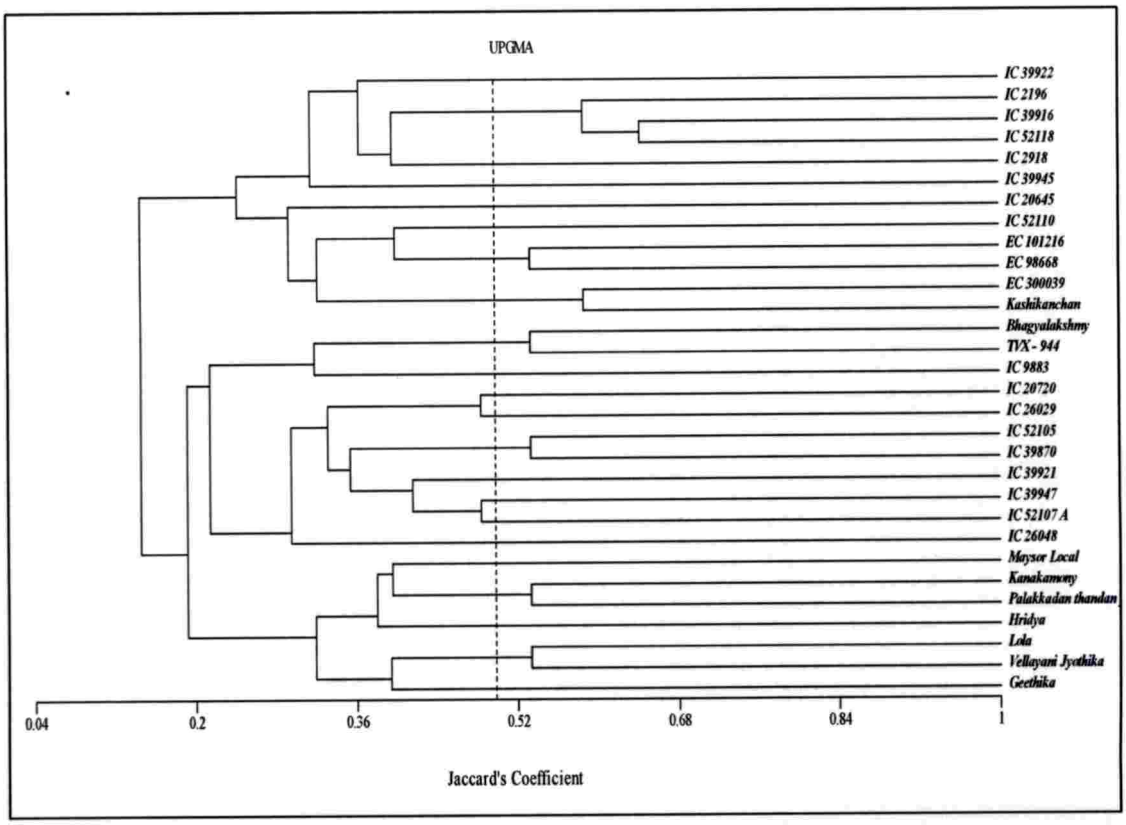

Fig.7. Dendrogram showing clustering of 30 cowpea genotypes

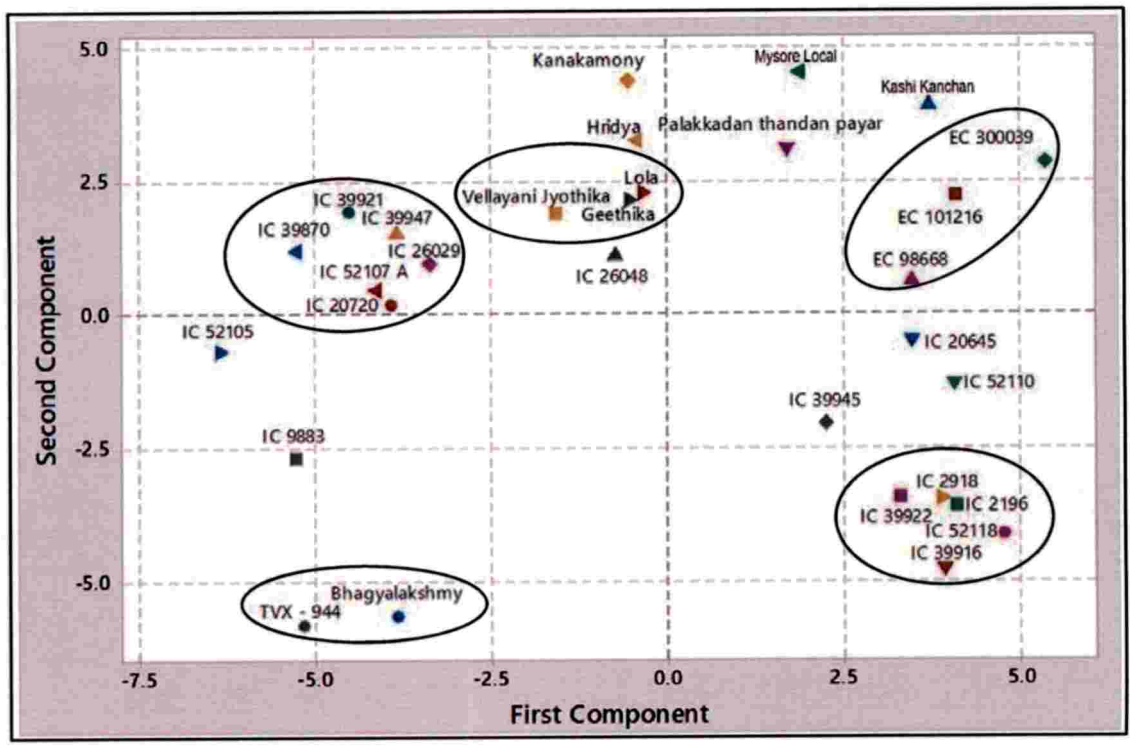

Fig. 8. Two-dimensional score plot of genotypes distribution

Table 19. Clustering pattern of 30 cowpea genotypes

Cluster	No. of genotypes	Genotype
I	1	Geethika
II	2	Vellayani Jyothika, Lola
III	1	Hridya
IV	2	Palakkadan thandan payar, Kanakamony
V	1	Mysore Local
VI	1	IC 26048
VII	1	IC 52107 A
VIII	1	IC 39947
IX	1	IC 39921
X	2	IC 39870, IC 52105
XI	1	IC 26029
XII	1	IC 20720
XIII	1	IC 9883
XIV	2	TVX-944, Bhagyalakshmy
XV	2	Kashi Kanchan, EC 300039
XVI	2	EC 98668, EC 101216
XVII	1	IC 52110
XVIII	1	IC 20645
XIX	1	IC 39945
XX	1	IC 2918
XXI	3	IC 52118, IC 39916, IC 2196
XXII	1	IC 39922

4.4 EXPERIMENT 4: EVALUATION OF F_1 HYBRIDS

4.4.1 Field screening of F_1 hybrids for spotted pod borer resistance

4.4.1.1 Damage parameters

Total 20 hybrids were evaluated for their reaction to the infestation of spotted pod borer. An abundant variation was observed in all hybrids with respect to flower bud, flower, pod and overall damage (Table 20, Fig. 9).

Total nine hybrids recorded the overall damage below 10 per cent (ranged between 3.79 to 18.83 %). Least total damage was noted in Hybrid 20 (3.79 %)

Table 20. The extent of damage caused by spotted pod borer to F_1 hybrids

Crosses	Mean no. of flower buds	Flower bud damage (%)	Mean no. of flowers	Flower damage (%)	Mean no. of pods	Pod damage (%)	Total damage (%)	Category
Hybrid 1	31.50	3.52	27.27	2.46	16.91	0.81	8.96 ^{f}	MR
Hybrid 2	24.70	4.65	19.04	0.94	16.25	2.75	13.91 ^{c}	S
Hybrid 3	33.83	4.98	26.29	1.65	16.39	0.89	9.83 ^{f}	MR
Hybrid 4	41.10	5.97	30.34	3.75	23.20	2.60	13.02 ^{cd}	S
Hybrid 5	32.19	1.96	28.01	1.43	19.55	0.65	5.08 ^{gh}	MR
Hybrid 6	29.29	4.35	22.49	2.24	13.30	0.00	10.12 ^{ef}	S
Hybrid 7	28.18	5.65	21.40	1.93	16.55	1.65	13.95 ^{c}	S
Hybrid 8	32.75	3.75	26.45	2.55	20.20	1.00	9.21 ^{f}	MR
Hybrid 9	33.73	5.30	23.08	3.03	18.50	1.90	13.58 ^{c}	S
Hybrid 10	30.78	2.55	25.62	1.38	15.30	0.00	5.47 ^{g}	MR
Hybrid 11	30.68	3.35	25.91	3.15	15.42	3.12	13.37 ^{cd}	S
Hybrid 12	28.08	4.55	22.51	3.35	15.06	2.96	16.54 ^{b}	HS
Hybrid 13	30.00	3.99	24.30	3.15	17.42	2.22	13.05 ^{cd}	S
Hybrid 14	33.28	5.40	27.28	5.25	18.13	4.13	18.83 ^{a}	HS
Hybrid 15	28.90	1.85	25.45	2.89	17.47	3.67	11.71 ^{de}	S
Hybrid 16	25.15	1.15	22.68	0.76	13.92	1.42	5.38 ^{gh}	MR
Hybrid 17	22.72	0.85	21.19	1.09	14.64	1.94	6.63 ^{g}	MR
Hybrid 18	30.78	1.40	28.14	1.80	23.12	1.92	6.24 ^{g}	MR

Table 20 continued

Crosses	Mean no. of flower buds	Flower bud damage (%)	Mean no. of flowers	Flower damage (%)	Mean no. of pods	Pod damage (%)	Total damage (%)	Category
Hybrid 19	32.25	4.60	26.00	2.85	17.49	2.49	13.12 [cd]	S
Hybrid 20	28.80	0.96	26.27	0.71	18.10	1.10	3.79 [h]	R
SEm±	**0.52**	**0.29**	**0.54**	**0.19**	**0.49**	**0.26**	**0.57**	**-**
C.D.@ 5 %	**1.55**	**0.87**	**1.62**	**0.58**	**1.46**	**0.78**	**1.69**	**-**
*R= Resistant, MR= Moderately Resistant, S= Susceptible, HS= Highly susceptible								

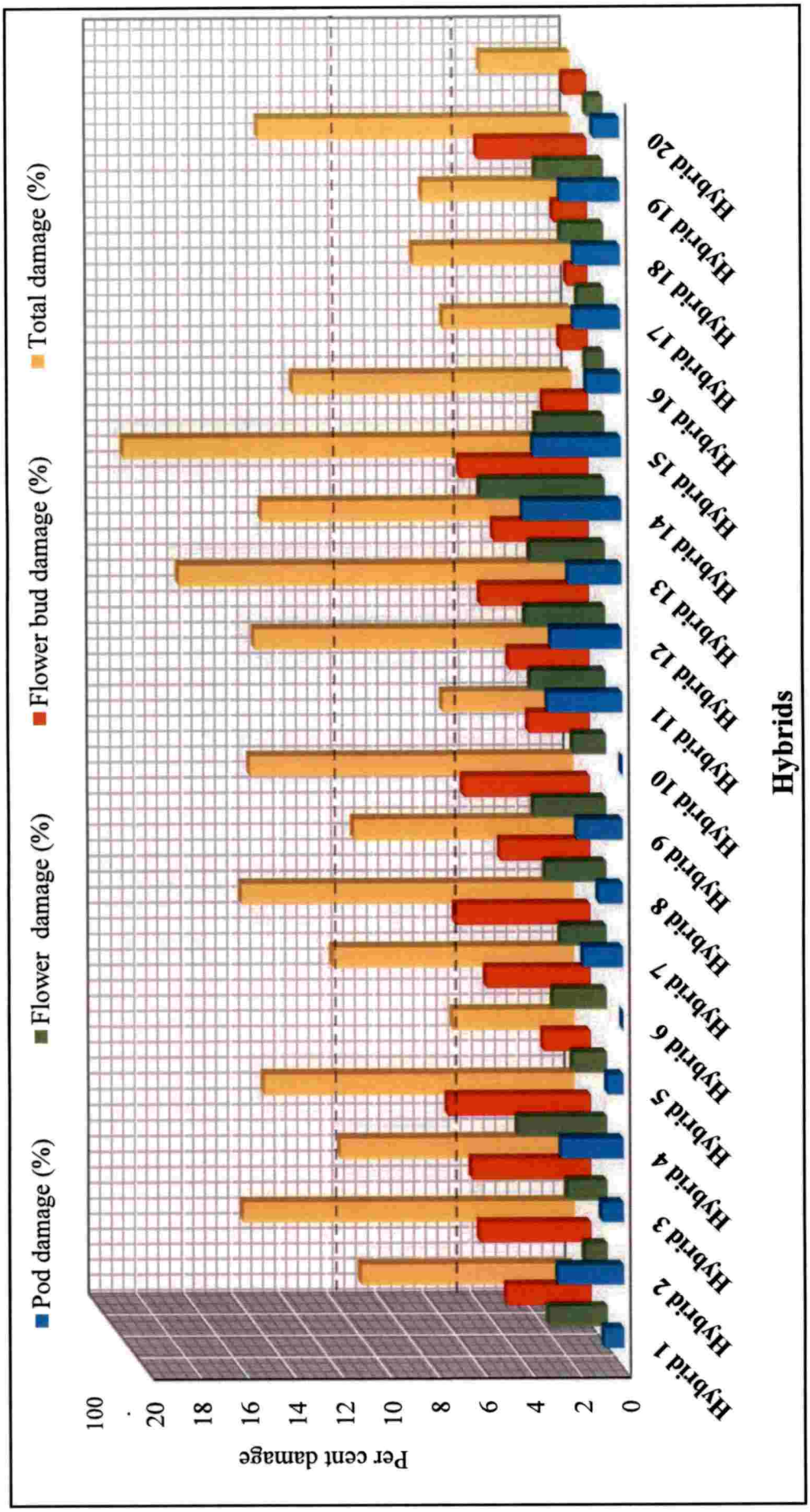

Fig. 9. The extent of damage in hybrids (F_1s) caused by spotted pod borer

categorised as a resistant hybrid. Out of remaining, eight hybrids recorded total damage between 5 to 10 per cent and categorised as moderately resistant. Those hybrids were Hybrid 1 (8.96 %), Hybrid 3 (9.83 %), Hybrid 5 (5.08 %), Hybrid 8 (9.21 %), Hybrid 10 (5.47 %), Hybrid 16 (5.38 %), Hybrid 17 (6.63 %) and Hybrid 18 (6.24 %).

Total eight hybrids recorded damage between 10 to 15 per cent and categorised as susceptible. Those hybrids were Hybrid 2 (13.91 %), Hybrid 4 (13.02 %), Hybrid 6 (10.12 %), Hybrid 7 (13.95 %), Hybrid 9 (13.58 %), Hybrid 11 (13.37 %), Hybrid 13 (13.05 %), Hybrid 15 (11.71 %) and Hybrid 19 (13.12 %). Two hybrids *viz.*, Hybrid 12 (16.54 %) and Hybrid 14 (18.83 %) registered heavy total damage (>15 %) and categorised as highly susceptible.

4.4.1.2 Morphological basis resistance to spotted pod borer (in hybrids)

Data pertaining to different morphological parameters *viz.*, number of trichomes on bud, number of trichomes on pod and pod wall thickness of hybrids with respect to resistance to spotted pod borer presented in Table 21.

4.4.1.2.1 Number of trichome on the bud

Hybrid 16 and Hybrid 17 recorded higher trichome density on bud, 4.33 /mm^2, which was on par with Hybrid 18 (4.00 /mm^2). Hybrid 11 recorded less trichome density on bud, 0.39 /mm^2, which was on par Hybrid 13 (0.40 /mm^2), Hybrid 14 (0.60 /mm^2), Hybrid 12 (0.77 /mm^2), Hybrid 15 (0.83 /mm^2) and Hybrid 2 (0.97 /mm^2). It was observed that all hybrids which include Lola as a parent recorded less trichome density on bud.

4.4.1.2.2 Number of trichome on the pod

With respect to trichome density on the pod, Hybrid 20 (15.33 /mm^2) recorded the highest value, which was followed by Hybrid 16 (11.33 /mm^2). Hybrid 13 observed to have less number of trichomes on the pod, 1.33 /mm^2, which was on par with Hybrid 9 (1.67 /mm^2), Hybrid 11 (2.00 /mm^2), Hybrid 12 (2.00 /mm^2), Hybrid 7 (2.33 /mm^2) and Hybrid 14 (2.33 /mm^2).

4.4.1.2.3 Pod wall thickness

Hybrid 6 observed to have the highest pod wall thickness, 0.83 mm, which was followed by Hybrid 1 (0.79 mm), whereas, Hybrid 20 recorded the lowest pod wall thickness, 0.42 mm, which was followed by Hybrid 15 (0.49 mm).

Table 21. Biophysical parameters of hybrids with respect to spotted pod borer resistance

Crosses	No. of trichomes on bud (per mm^2)	No. of trichomes on pod (per mm^2)	Pod wall thickness (mm)
Hybrid 1	2.67	3.67	0.79
Hybrid 2	0.97	2.67	0.63
Hybrid 3	1.67	3.67	0.68
Hybrid 4	1.67	3.33	0.64
Hybrid 5	2.33	5.67	0.52
Hybrid 6	1.33	2.67	0.83
Hybrid 7	1.33	2.33	0.67
Hybrid 8	2.33	3.33	0.72
Hybrid 9	1.33	1.67	0.68
Hybrid 10	2.67	5.33	0.56
Hybrid 11	0.39	2.00	0.67
Hybrid 12	0.77	2.00	0.61
Hybrid 13	0.40	1.33	0.66
Hybrid 14	0.60	2.33	0.61
Hybrid 15	0.83	3.00	0.49
Hybrid 16	4.33	11.33	0.69
Hybrid 17	4.33	5.00	0.54
Hybrid 18	4.00	5.33	0.58
Hybrid 19	2.67	5.00	0.54
Hybrid 20	3.67	15.33	0.42
SEm±	**0.30**	**0.37**	**0.01**
C.D.@ 5 %	**0.87**	**1.06**	**0.02**

4.4.2 Evaluation of hybrids for biometric characters

The mean performance of different hybrids for different biometric characters are presented in Table 22 and described below.

4.4.2.1 Days to 50 per cent flowering

Among 20 hybrids, days to 50 per cent flowering was ranged from 51.00 to 69.50 days. Hybrid 18 flowered early with 51.00 days, which was on par with Hybrid 19 (52.50 days). Hybrid 11 (69.50 days) had taken more days for 50 per cent flowering, which was on par with Hybrid 6 (68.00 days).

4.4.2.2 Plant height

Plant height ranged from 114.70 cm to 482.00 cm (Table 22). Hybrid 15 observed to have more plant of height (482.00 cm), which was on par with Hybrid 12 (477.90 cm), Hybrid 10 (458.00 cm), Hybrid 5 (451.10 cm) and Hybrid 2 (446.80 cm). Hybrid 16 recorded short plant height, 114.70 cm, which was on par with Hybrid 18 (134.80 cm) and Hybrid 17 (138.70 cm).

4.4.2.3 Number of primary branches per plant

A number of primary branches per plant ranged between 1.3 to 3.5 (Table 22). Hybrid 4 observed to have more number of primary branches per plant, 3.5, which was on par with Hybrid 7 (3.1), Hybrid 11 (3.1) and Hybrid 13 (3.1). Hybrid 17 (1.3) was noted for a minimum number of branches per plant, which was on par with Hybrid 18 (1.4), Hybrid 20 (1.4) and Hybrid 19 (1.7).

4.4.2.4 Number of pods per plant

A number of pods per plant ranged between 12.10 to 21.20 (Table 22). Hybrid 18 observed to bear more number of pods per plant, 21.20, which was on par with Hybrid 4 (20.60). Hybrid 12 produced a low number of pods per plant, 12.10, which was on par with Hybrid 11 (12.30), Hybrid 16 (12.50), Hybrid 17 (12.70), Hybrid 6 (13.30) and Hybrid 2 (13.50).

4.4.2.5 Pod length

The maximum pod length was recorded in Hybrid 8 (31.83 cm), followed by Hybrid 12 (26.75 cm), whereas, Hybrid 19 observed to have shorter pod length, 11.72 cm, which was on par with all remaining hybrids except Hybrid 8, Hybrid 10 and Hybrid 12.

4.4.2.6 Number of seeds per pod

Hybrid 12 observed to have more number of seeds per pod, 17.00, which was on par with Hybrid 3 (16.20). Hybrid 19 recorded the lowest value with nine seeds per pod.

4.4.2.7 Grain yield per plant

With respect to yield, Hybrid 4 recorded more grain yield per plant, 48.35 g, which was on par with Hybrid 5 (48.06 g) and Hybrid 8 (47.04 g). Hybrid 16 observed to produce less grain yield with 22.02 g per plant, which was on par with Hybrid 19 (22.04 g) and Hybrid 11 (23.26 g).

Table 22. Mean performance of F_1 hybrids for biometric characters

Crosses	Days to 50 per cent flowering	Plant height (cm)	No. of primary branches per plant	No. of pods per plant	Pod length (cm)	No. of seeds per pod	Grain yield per plant (g)	100 seed weight (g)
Hybrid 1	61.00	356.40	2.7	16.10	24.24	13.90	37.50	16.30
Hybrid 2	56.50	446.80	2.3	13.50	26.10	15.20	33.46	15.32
Hybrid 3	53.50	404.40	2.2	15.50	24.52	16.20	40.91	11.55
Hybrid 4	53.50	395.70	3.5	20.60	14.52	14.40	48.35	11.78
Hybrid 5	56.50	451.10	2.3	18.90	25.42	15.60	48.06	11.01
Hybrid 6	68.00	340.55	2.3	13.30	25.03	15.50	33.61	21.93
Hybrid 7	57.00	400.20	3.1	14.90	27.86	14.40	34.98	19.31
Hybrid 8	54.50	352.07	2.5	19.20	31.83	15.00	47.04	20.80
Hybrid 9	54.50	335.25	2.5	16.60	21.60	14.60	39.51	14.98
Hybrid 10	62.50	458.00	2.9	15.30	30.36	15.60	38.92	17.12
Hybrid 11	69.50	423.75	3.1	12.30	24.54	11.60	23.26	15.10
Hybrid 12	63.00	477.90	2.5	12.10	26.75	17.00	33.51	11.78
Hybrid 13	62.50	385.50	3.1	15.20	22.25	13.20	32.70	11.53
Hybrid 14	57.50	399.80	2.3	14.00	17.54	12.30	28.07	11.53
Hybrid 15	65.50	482.00	2.3	13.80	20.54	14.20	31.94	13.66

Table 22 continued

Crosses	Days to 50 per cent flowering	Plant height (cm)	No. of primary branches per plant	No. of pods per plant	Pod length (cm)	No. of seeds per pod	Grain yield per plant (g)	100 seed weight (g)
Hybrid 16	59.50	114.70	2.2	12.50	14.96	10.80	22.02	13.32
Hybrid 17	55.00	138.70	1.3	12.70	17.60	15.00	31.04	8.94
Hybrid 18	51.00	134.80	1.4	21.20	14.33	11.40	39.42	16.79
Hybrid 19	52.50	221.10	1.7	15.00	11.72	9.00	22.04	14.43
Hybrid 20	57.00	268.10	1.4	17.00	13.78	13.20	36.64	10.39
SEm±	**0.62**	**16.84**	**0.15**	**0.49**	**5.64**	**0.31**	**1.59**	**2.12**
C.D.@ 5 %	**1.83**	**49.85**	**0.45**	**1.44**	**16.68**	**0.91**	**4.70**	**0.79**

4.4.2.8 100 seed weight

In regards to 100 seed weight, Hybrid 6 recorded the highest weight, 21.93 g, which was followed by Hybrid 8 (20.80 g). The lowest 100 seed weight was recorded by Hybrid 17 (8.94 g).

4.4.3 Estimates of combining ability effects for total damage caused by spotted pod borer

For total damage by spotted pod borer, negative directional general combining ability (GCA) and specific combining ability (SCA) effects of the parents and hybrids, respectively, were considered to be desirable. These effects are presented in Table 23 and 24. The GCA effects of the parents varied from -4.08 to 4.11. Parent IC 2918 observed to have significant negative GCA effect, -4.08, indicating this parent as a good combiner to impart resistance to spotted pod borer. Parent Kashi Kanchan also recorded a significant negative GCA effect indicating that the susceptible nature of this parent can be cured by crossing with resistant donor parent. Genotypes Geethika, Vellayani Jyothika, Palakkadan thandan payar and EC 98668 observed as an average combiner for resistance. Parents Lola, EC 300039 and IC 39945 observed to have positive GCA effects indicating these genotypes as a poor combiner.

With respect to hybrids, three hybrids *viz.*, Hybrid 4, Hybrid 9 and Hybrid 17 observed to have significant negative SCA effects (-1.19, -0.94 and -2.57, respectively). Out of remaining hybrids, eight registered negative SCA effects and nine registered positive SCA effects. However, those effects were not desirable.

Table 23. General combining ability effects of parents for total damage caused by spotted pod borer

Sl. no.	Parents	GCA effects
1	Geethika	-0.43
2	Vellayani Jyothika	-0.12
3	Lola	4.11
4	Kashi Kanchan	-3.56**
5	Palakkadan thandan payar	-1.13
6	EC 300039	2.17*
7	EC 98668	-1.01**
8	IC 39945	4.05**
9	IC 2918	-4.08**

Table 24. Specific combining ability effects of hybrids for total damage caused by spotted pod borer

Sl. no.	Crosses	SCA effects
1	Hybrid 1	-0.07
2	Hybrid 2	1.58**
3	Hybrid 3	0.68**
4	Hybrid 4	-1.19**
5	Hybrid 5	-1.01
6	Hybrid 6	0.78**
7	Hybrid 7	1.32**
8	Hybrid 8	-0.25
9	Hybrid 9	-0.94**
10	Hybrid 10	-0.92
11	Hybrid 11	-0.20
12	Hybrid 12	-0.33
13	Hybrid 13	-0.64
14	Hybrid 14	0.08
15	Hybrid 15	1.09**
16	Hybrid 16	-0.52
17	Hybrid 17	-2.57**
18	Hybrid 18	0.21
19	Hybrid 19	2.04**
20	Hybrid 20	0.83**

*, ** Significant at 1 and 5 per cent level of significance, respectively

4.4.3.1 Magnitude of heterosis

Heterosis was measured as per cent increase or decrease over mid parent (relative heterosis) and better parent (heterobeltiosis).

The magnitude of these heterotic effects for resistance to spotted pod borer are presented in Table 25. The variation in relative heterosis for resistance to spotted pod borer ranged from -74.29 to 82.09. Total ten hybrids recorded desirable significant negative heterosis over mid parent. Those hybrids were Hybrid 5 (-29.93), Hybrid 10 (-41.86), Hybrid 11 (-21.51), Hybrid 13 (-16.30), Hybrid 15 (-23.85), Hybrid 16 (-67.16), Hybrid 17 (-60.44), Hybrid 18 (-58.26), Hybrid 19 (-10.34) and Hybrid 20 (-74.29). Hybrid 6 also recorded desirable negative relative heterosis (-8.59). However, all hybrids failed to register desirable better parent heterosis.

Table 25. The magnitude of heterosis for resistance to spotted pod borer in cowpea

Sl. no.	Crosses	Over mid parent	Over better parent
1	Hybrid 1	0.67	122.89**
2	Hybrid 2	49.89**	191.00**
3	Hybrid 3	31.81**	766.08**
4	Hybrid 4	82.09**	2427.18**
5	Hybrid 5	-29.93**	619.86**
6	Hybrid 6	-8.59*	151.62**
7	Hybrid 7	21.89**	191.84**
8	Hybrid 8	-4.29	711.45**
9	Hybrid 9	45.77**	2535.92**
10	Hybrid 10	-41.86**	675.89**
11	Hybrid 11	-21.51**	232.46**
12	Hybrid 12	-4.98	246.03**
13	Hybrid 13	-16.30**	1049.34**
14	Hybrid 14	23.24**	3555.34**
15	Hybrid 15	-23.85**	1560.28**
16	Hybrid 16	-67.16**	33.83**
17	Hybrid 17	-60.44**	38.70**
18	Hybrid 18	-58.26**	449.34**
19	Hybrid 19	-10.34**	2446.60**
20	Hybrid 20	-74.29**	436.88**

*, ** Significant at 1 and 5 per cent level of significance, respectively

4.4.4 Estimates of combining ability effects for biometric characters

According to Griffing (1956), the high GCA effects are related to additive gene effects and additive × additive interaction effect. This represents the fixable component of genetic variation.

The GCA effects of the five lines and four testers for different biometric observations are presented in Table 26. Scoring is given to each parent as a good combiner, average combiner or poor combiner and presented in Table 27. The SCA effects of the 20 hybrids with respect to each character presented in Table 28.

4.4.4.1 Days to 50 per cent flowering

For days to 50 per cent flowering, negative directional GCA and SCA effect of the parents and hybrids, respectively, were considered to be desirable as the early flowering varieties are preferred over the late flowering varieties. The GCA effects of the parents varied from -4.03 (IC 39945) to 5.98 (Palakkadan thandan payar). Parent IC 39945 showed highly significant negative GCA effect (-4.03), followed by Kashi Kanchan (-3.53) and EC 98668 (-3.15) indicating their good general combining ability for early flowering. Geethika (-2.33) reported significant negative GCA effect (Table 26). Lola and Palakkadan thandan payar observed as poor combiners. Genotypes *viz.*, Vellayani Jyothika, EC 300039 and IC 2918 were found to be average combiners for days to 50 per cent flowering (Table 27).

Among 20 hybrids tested, Hybrid 5, Hybrid 8, Hybrid 14 and Hybrid 16 were found to be significantly superior with respect to SCA by means of significant negative SCA effects for days to 50 per cent flowering. Hybrid 1, Hybrid 7, Hybrid 9 and Hybrid 11 also observed to have negative SCA effects, however, effects were not significant (Table 28).

4.4.4.2 Plant height

Significant positive GCA effects in Geethika (61.54), Lola (84.45), IC 2918 (65.46) and EC 300039 (16.56) indicated that these genotypes are good general combiners to achieve tallness, whereas, significant negative GCA effects were observed in Kashi Kanchan (-173.86), Palakkadan thandan payar (-40.49), EC 98668 (-30.15) and IC 39945 (-11.38) which indicates that this genotype can be used as a general combiner for dwarfness.

Hybrid 19 (57.00), Hybrid 11 (30.45), Hybrid 12 (27.55), Hybrid 20 (27.16), Hybrid 3 (23.67) and Hybrid 10 (15.33) recorded highly significant positive SCA effects, whereas, Hybrid 8 (5.00) observed to have significant positive SCA effect. Hybrid 17 (-53.34), Hybrid 5 (-25.24), Hybrid 16 (-20.29), Hybrid 13 (-18.14), Hybrid 15 (-17.25), Hybrid 18 (-10.53) and Hybrid 4 (-3.80) observed to have significant negative SCA effects.

4.4.4.3 Number of primary branches per plant

With respect to a number of primary branches per plant, only Geethika observed to have significant positive GCA effect, 0.22, whereas, Kashi Kanchan observed to have significant negative GCA effect (-0.78) (Table 26).

With regards to SCA effect, Hybrid 4 (0.78), Hybrid 7 (0.52) and Hybrid 13 (0.52) observed to have highly significant positive values. Hybrid 11 also recorded significant GCA effect (0.25). Hybrid 6 (-0.56) and Hybrid 14 (-0.48) showed highly significant negative GCA effects. Hybrid 9 (-0.28) and Hybrid 17 (-0.22) also recorded significant negative GCA effects.

4.4.4.4 Number of pods per plant

The GCA effects with respect to a number of pods per plant varied from -2.19 to 2.29. Significant GCA effects were noted in Geethika (1.44), IC 39945 (1.07) and IC 2918 (0.77). This indicated that these genotypes are good combiner in order to increase the number of pods per plant. Genotypes EC 300039 and Lola were observed as poor combiners with significant negative GCA effects (-2.19 and -2.01, respectively). Remaining parents were found to be average combiners (Table 26).

The range of SCA effects was observed from -3.71 to 3.23. Significant positive SCA effects were recorded by Hybrid 18 (3.23), Hybrid 4 (2.62), Hybrid 7 (1.23), Hybrid 5 (1.22) and Hybrid 11 (0.76). Hybrid 3 (-3.71), Hybrid 19 (-1.75), Hybrid 10 (-1.33), Hybrid 16 (-1.25), Hybrid 2 (-1.24), Hybrid 17 (-0.80), Hybrid 6 (-0.63) and Hybrid 14 (-0.55) observed to have significant negative SCA effects (Table 28).

4.4.4.5 Pod length

For pod length, three parents observed to exhibit significant positive GCA effect *viz.*, EC 300039 (7.80), Vellayani Jyothika (4.31) and Lola (4.30). Genotypes Kashi Kanchan and IC 39945 showed significant negative GCA effects (-8.55 and -6.68, respectively).

With respect to hybrids, SCA effects varied from -7.28 to 16.62. Seven hybrids registered significantly high SCA effects *viz.*, Hybrid 8 (4.29), Hybrid 19 (3.92), Hybrid 10 (3.52), Hybrid 5 (2.96), Hybrid 1 (2.11), Hybrid 16 (1.31) and Hybrid 9 (0.94). Out of remaining, eight hybrids recorded significant negative SCA effects *viz.*, Hybrid 7 (-7.28), Hybrid 15 (-6.28), Hybrid 13 (-5.28), Hybrid 17 (-4.68), Hybrid 2 (-4.66), Hybrid 14 (-3.10), Hybrid 4 (-1.76) and Hybrid 6 (-1.47).

4.4.4.6 Number of seeds per pod

The examination of the GCA effects of nine parents revealed the presence of significant positive GCA effect with the parents EC 300039 (1.50), Geethika (1.16), Vellayani Jyothika (1.12) and IC 2918 (0.75) indicating these varieties as a good combiner for number of seeds per pod (Table 27). However, Kashi Kanchan, IC 39945

Table 26. General combining ability effects of parents with respect to biometric traits

Characters / Parents	Days to 50 per cent flowering	Plant height	No. of primary branches per plant	No. of pods per plant	Pod length	No. of seeds per pod	Grain yield per plant	100 seed weight
Geethika	-2.33*	61.54**	0.22*	1.44**	-0.06	1.16**	6.51**	-1.19**
Vellayani Jyothika	0.78	27.87	0.28	0.38	4.31**	1.12**	3.66	4.45**
Lola	5.08**	84.45**	0.28	-2.01**	4.30**	-0.25	-5.25**	-1.66*
Kashi Kanchan	-3.53**	-173.86**	-0.78*	0.20	-8.55**	-2.03**	-4.92**	-1.61
Palakkadan thandan payar	5.98**	-40.49**	0.20	-1.94	-0.83	-0.96**	-6.05**	2.29
EC 300039	-0.65	16.56**	-0.08	-2.19**	7.80**	1.50**	-1.90**	-0.54*
EC 98668	-3.15**	-30.15*	-0.08	2.29	0.21	0.05	4.87	0.79**
IC 39945	-4.03**	-11.38**	0.12	1.07**	-6.68**	-1.33**	-0.66**	-1.20**
IC 2918	1.85	65.46**	-0.16	0.77**	-0.50	0.75**	3.74**	-1.34**

** Significant at 1 per cent level

* Significant at 5 per cent level

Table 27. GCA status of parents used in the study with respect to biometric traits

Characters / Parents	Days to 50 per cent flowering	Plant height	No. of primary branches per plant	No. of pods per plant	Pod length	No. of seeds per pod	Grain yield per plant	100 seed weight
Geethika	G	P	G	G	A	G	G	P
Vellayani Jyothika	G	A	A	A	G	G	A	A
Lola	P	P	A	P	G	A	P	P
Kashi Kanchan	A	G	P	A	P	P	P	A
Palakkadan thandan payar	P	G	A	A	A	P	P	A
EC 300039	A	P	A	P	G	G	P	P
EC 98668	G	G	A	A	A	A	A	G
IC 39945	G	G	A	P	P	P	P	P
IC 2918	A	P	A	P	A	G	G	P

G = Good combiner, A = Average combiner, P = Poor combiner

Table 28. Specific combining ability effects of hybrids with respect to biometric traits

Characters / Parents	Days to 50 per cent flowering	Plant height	No. of primary branches per plant	No. of pods per plant	Pod length	No. of seeds per pod	Grain yield per plant	100 seed weight
Hybrid 1	-1.18	-13.99	-0.10	1.12	2.11**	-0.21	1.90	0.82
Hybrid 2	0.95	19.36	-0.22	-1.24**	-4.66**	-1.36**	-6.30**	2.67**
Hybrid 3	0.45	23.67**	-0.32	-3.71**	1.35	1.10**	-5.62**	-2.43**
Hybrid 4	1.33*	-3.80**	0.78**	2.62**	-1.76**	0.67	7.35**	-0.21
Hybrid 5	-1.55**	-25.24**	-0.15	1.22**	2.96**	-0.21	2.66**	-0.85**
Hybrid 6	2.73*	3.83	-0.56**	-0.63**	-1.47**	1.44**	0.85	0.81**
Hybrid 7	-1.65	6.43	0.52**	1.23**	-7.28**	-2.12	-1.93**	1.02**
Hybrid 8	-1.65**	5.00*	-0.08	1.05	4.29**	-0.07	3.36	1.19**
Hybrid 9	-0.78	-30.59	-0.28*	-0.33	0.94**	0.91**	1.35*	-2.65**
Hybrid 10	1.35*	15.33**	0.40	-1.33**	3.52**	-0.17	-3.63**	-0.38
Hybrid 11	-0.08	30.45**	0.25*	0.76**	-1.95	-1.11	-0.58**	0.10
Hybrid 12	0.05	27.55**	-0.08	0.81	16.62	1.85**	5.51**	-0.40
Hybrid 13	2.05**	-18.14**	0.52**	-0.57	-5.28**	-0.51	-2.06	-1.98**
Hybrid 14	-2.08**	-22.61	-0.48**	-0.55*	-3.10**	-0.03	-1.17	0.01
Hybrid 15	0.05	-17.25**	-0.21	-0.45	-6.28**	-0.21	-1.70	2.28**
Hybrid 16	-1.48**	-20.29**	0.41	-1.25**	1.31**	-0.13	-2.16*	-1.74**

Table 28 continued

Characters / Parents	Days to 50 per cent flowering	Plant height	No. of primary branches per plant	No. of pods per plant	Pod length	No. of seeds per pod	Grain yield per plant	100 seed weight
Hybrid 17	0.65	-53.34**	-0.22*	-0.80**	-4.68**	1.63	2.71	-3.29**
Hybrid 18	-0.85	-10.53**	-0.12	3.23**	-0.36	-0.53*	4.32*	3.23**
Hybrid 19	1.53**	57.00**	-0.02	-1.75**	3.92**	-1.55**	-7.54**	2.86**
Hybrid 20	0.15	27.16**	-0.05	0.56	-0.20	0.58	2.67	-1.05*

** Significant at 1 per cent level of significance

* Significant at 5 per cent level of significance

and Palakkadan thandan payar recorded significant negative GCA effects (-2.03, -1.33 and -0.96, respectively).

With respect to SCA effects, four hybrids recorded significant positive values *viz.*, Hybrid 12 (1.85), Hybrid 6 (1.44), Hybrid 3 (1.10) and Hybrid 9 (0.91). However, Hybrid 19, Hybrid 2 and Hybrid 18 recorded significant negative SCA effects (-1.55, -1.36 and -0.53, respectively).

4.4.4.7 Grain yield per plant

With respect to grain yield per plant, only two genotypes, Geethika and IC 2918, observed to have high significant positive GCA effects (6.51 and 3.74, respectively) (Table 26). Genotypes Palakkadan thandan payar, Lola, Kashi Kanchan, EC 300039 and IC 39945 recorded significant negative GCA effects. Vellayani Jyothika and EC 98668 observed as average combiners (Table 27).

Among the hybrids, Hybrid 4, Hybrid 12 and Hybrid 5 had high significant positive SCA effects (7.35, 5.51 and 2.66, respectively). Hybrid 18 and Hybrid 9 also recorded significant positive SCA effects. Hybrid 2, Hybrid 3, Hybrid 7, Hybrid 10, Hybrid 16 and Hybrid 19 recorded significant negative SCA effects (Table 28).

4.4.4.8 100 seed weight

With respect to 100 seed weight, only two parents, Vellayani Jyothika and EC 98668, were observed as good combiners with significantly high GCA effects (4.45 and 0.79, respectively). Out of remaining parents, five parents *viz.*, IC 2918, IC 39945, Geethika, Lola and EC 300039 were found as poor combiners with significantly negative GCA effects (-1.34, -1.20, -1.19, -1.66 and -0.54, respectively) (Table 26).

Seven hybrids *viz.*, Hybrid 18, Hybrid 19, Hybrid 2, Hybrid 15, Hybrid 8, Hybrid 7 and Hybrid 6 recorded significant values of SCA effects (3.23, 2.86, 2.67, 2.28, 1.19, 1.02 and 0.81, respectively). Hybrid 17, Hybrid 9, Hybrid 3, Hybrid 13, Hybrid 16, Hybrid 20 and Hybrid 5were observed to have significantly negative values of SCA effects (-3.29, -2.65, -2.43, -1.98, -1.74, -1.05 and -0.85, respectively) (Table 28).

4.4.5 Magnitude of heterosis

Different estimates of heterosis for different characters are presented in Table 29 and 30. Character-wise heterosis over mid parent and better parent for seed yield and its related characters are described as follows.

4.4.5.1 Days to 50 per cent flowering

For this character, the parent of a respective cross which flowered earlier was considered as a better parent and accordingly heterotic effects were calculated. The estimate of relative heterosis ranged from -8.50 to 12.09. Six hybrids *viz.*, Hybrid 5, Hybrid 14, Hybrid 15, Hybrid 1, Hybrid 4 and Hybrid 11 registered desirable negative heterotic effect over mid parent (-8.50, -5.74, -4.03, -3.56, -1.84 and -0.36, respectively) (Table 29). Remaining hybrids registered undesirable positive heterosis. With respect to heterosis over the better parent, none of the hybrids registered significant heterotic effect. (Table 30)

4.4.5.2 Plant height

Negative heterosis is considered as desirable for plant height in cowpea. However, none of the hybrids registered desirable relative heterosis and heterobeltiosis as all hybrids recorded more plant height than their mid parent and better parent.

4.4.5.3 Number of primary branches per plant

Positive heterosis is preferred with respect to a number of primary branches per plant. However, none of the hybrids registered desirable relative heterosis and heterobeltiosis.

4.4.5.4 Number of pods per plant

With respect to number of pods per plant only one hybrid *i.e.* Hybrid 20 registered positive relative heterosis. All other hybrids registered negative relative heterosis and heterobeltiosis.

4.4.5.5 Pod length

In regard to pod length, only three hybrids *viz.*, Hybrid 12, Hybrid 8 and Hybrid 10 registered significant positive relative heterosis (70.93, 10.96 and 7.22, respectively). Remaining hybrids registered negative relative heterosis. With respect to heterobeltiosis, none of the hybrids registered desirable positive value.

4.4.5.6 Number of seeds per pod

Eight hybrids *viz.*, Hybrid 3, Hybrid 4, Hybrid 6, Hybrid 9, Hybrid 12, Hybrid 14, Hybrid 17 and Hybrid 20 registered significant values of relative heterosis (3.51, 9.92, 3.33, 10.61, 0.30, 0.82, 6.76 and 1.93, respectively. However, all hybrids failed to register positive heterobeltiosis.

Table 29. Relative heterosis for biometric characters in F_1 population

Crosses	Days to 50 per cent flowering	Plant height	No. of primary branches per plant	No. of pods per plant	Pod length	No. of seeds per pod	Grain yield per plant	100 seed weight
Hybrid 1	-3.56**	25.63**	-18.18**	-17.86**	-12.36**	-6.71**	28.22**	79.61**
Hybrid 2	0.00	49.03**	-29.23**	-28.57**	-12.93**	-14.85**	5.93**	53.58**
Hybrid 3	3.88	47.56**	-38.89**	-39.92**	-9.19**	3.51**	10.68**	41.72**
Hybrid 4	-1.84**	28.94**	-2.78**	-5.29**	-42.16**	9.92**	69.22**	22.34**
Hybrid 5	-8.50**	50.07**	-36.99**	-10.64**	-4.54**	-6.87**	43.65**	18.02**
Hybrid 6	11.48**	38.77**	-31.34**	-20.36**	-14.70**	3.33**	-28.99**	47.15**
Hybrid 7	5.07	53.04**	-6.06**	-6.88**	-12.00**	-19.78**	-29.57**	22.22**
Hybrid 8	10.66**	49.34**	-31.51**	-16.16**	10.96**	-4.76**	-14.54**	48.84**
Hybrid 9	4.31	24.81**	-31.51**	-11.94**	-19.37**	10.61**	-15.32**	-3.07**
Hybrid 10	5.04	74.61**	-21.62**	-16.16**	7.22**	-7.42**	-24.49**	12.97**
Hybrid 11	-0.36**	42.70**	-1.59**	-24.31**	-12.23**	-17.14**	-30.18**	20.80**
Hybrid 12	0.00	52.66**	-19.36**	-22.19**	70.93**	0.30**	-6.01**	-12.13**
Hybrid 13	7.76*	34.18**	-10.15**	-32.29**	-18.50**	-10.51**	-20.29**	-0.43**
Hybrid 14	-5.74**	24.88**	-33.33**	-23.91**	-30.96**	0.82**	-14.00**	-11.69**
Hybrid 15	-4.03**	53.58**	-34.29**	-22.47**	-23.73**	-10.41**	-14.88**	7.10**
Hybrid 16	3.93	57.66**	-24.14**	-18.57**	-6.68**	-2.70**	40.72**	45.57**
Hybrid 17	8.91*	56.11**	-54.39**	-13.31**	-4.06**	6.76**	72.64**	-11.05**
Hybrid 18	12.09**	113.63**	-56.25**	-1.62**	-6.77**	-3.80**	68.77**	104.07**
Hybrid 19	8.25*	130.43**	-46.88**	-14.29**	-13.02**	-3.23**	47.19**	48.71**
Hybrid 20	2.24	199.05**	-56.92**	0.59**	-8.13**	1.93**	84.51**	10.48**

*, ** significant at 5 per cent and 1 per cent levels of probability, respectively

Table 30. Heterobeltiosis for biometric characters in F_1 population

Crosses	Days to 50 per cent flowering	Plant height	No. of primary branches per plant	No. of pods per plant	Pod length	No. of seeds per pod	Grain yield per plant	100 seed weight
Hybrid 1	4.27	250.10*	-34.15**	-39.02**	-40.59*	-26.46**	-17.93**	78.14**
Hybrid 2	3.67	233.43	-43.90**	-48.86**	-36.03*	-19.58**	-26.79**	41.85**
Hybrid 3	20.22**	390.18**	-46.34**	-41.29**	-39.90*	-14.29**	-10.48*	26.23*
Hybrid 4	5.94	167.00	-14.63**	-21.97**	-64.41**	-23.81**	5.81	16.58
Hybrid 5	-3.42	232.67	-43.90**	-28.41**	-37.70*	-17.46**	5.18	15.84
Hybrid 6	25.93**	234.53	-45.24**	-35.44**	-43.33**	-18.85**	-58.94**	5.41**
Hybrid 7	5.56	198.66	-26.19**	-27.67**	-36.93*	-24.61**	-57.27**	-7.16**
Hybrid 8	22.47**	326.75**	-40.48**	-23.81**	-27.94	-21.47**	-42.54**	0.00
Hybrid 9	7.92	126.21	-40.48**	-19.42**	-51.10**	-23.56**	-51.74**	-28.01**
Hybrid 10	15.74*	237.76	-30.95**	-25.73**	-31.27*	-18.33**	-52.46**	-17.72**
Hybrid 11	2.21	316.26*	-18.42**	-37.56**	-40.73*	-32.16**	-56.79**	-5.63**
Hybrid 12	15.60*	256.64*	-34.21**	-38.58**	25.00	-0.59	-37.75**	-26.41**
Hybrid 13	40.45**	367.27**	-18.42**	-39.68**	-46.26**	-22.81**	-39.25**	-27.97**
Hybrid 14	13.86*	169.77	-39.47**	-28.93**	-57.63**	-28.07**	-47.85**	-27.97**
Hybrid 15	0.77	255.46*	-39.47**	-29.95**	-50.39**	-16.96**	-40.66**	-14.66**
Hybrid 16	27.96**	162.47	-33.33**	-30.17**	-14.71	-4.43	19.06	43.23
Hybrid 17	18.28**	217.39	-60.61**	-29.05**	-8.09	-10.71**	67.88**	-17.22**
Hybrid 18	14.61*	208.47	-57.58**	-15.87**	-18.30	-8.07*	39.67**	80.48**
Hybrid 19	12.90*	405.95**	-48.49**	-16.20**	-33.18	-20.35**	19.17	42.82
Hybrid 20	22.58**	513.50**	-57.58**	-5.03	-21.44	-9.59**	72.64**	9.32**

*, ** significant at 5 per cent and 1 per cent levels of probability, respectively

4.4.5.7 Grain yield per plant

The variation in relative heterosis for grain yield per plant ranged from -30.18 (Hybrid 11) to 84.51 (Hybrid 20). The data revealed that out of 20 hybrids, ten hybrids exhibited significant positive heterosis over mid parent. Those hybrids were Hybrid 1 (28.22), Hybrid 2 (5.93), Hybrid 3 (10.68), Hybrid 4 (69.22), Hybrid 5 (43.65), Hybrid 16 (40.72), Hybrid 17 (72.64), Hybrid 18 (68.77), Hybrid 19 (47.19) and Hybrid 20 (84.51) (Table 29). With respect to heterobeltiosis, three hybrids *viz.*, Hybrid 17, Hybrid 18 and Hybrid 20 observed to have significant positive values (67.88, 39.67 and 72.64) (Table 30).

4.4.5.8 100 seed weight

With respect to 100 seed weight, 15 hybrids observed to have significant positive relative heterosis. Those hybrids were Hybrid 1 (79.61), Hybrid 2 (53.58), Hybrid 3 (41.72), Hybrid 4 (22.34), Hybrid 5 (18.02), Hybrid 6 (47.15), Hybrid 7 (22.22), Hybrid 8 (48.84), Hybrid 10 (12.97), Hybrid 11 (20.80), Hybrid 15 (7.10), Hybrid 16 (45.57), Hybrid 18 (104.07), Hybrid 19 (48.71) and Hybrid 20 (10.48) (Table 29).

Five hybrids *viz.*, Hybrid 1, Hybrid 2, Hybrid 6, Hybrid 18 and Hybrid 20 observed to have highly significant positive values of better parent heterosis (78.14, 41.85, 5.41, 80.48 and 9.32, respectively). Hybrid 3 also recorded significant positive values of better parent heterosis (Table 30).

4.5 EXPERIMENT 5: EVALUATION OF F_2 PLANTS

Total nine hybrids, which registered total infestation less than ten per cent in experiment 4 (Table 20), were selected for F_2 plants evaluation. Those hybrids were Hybrid 1, Hybrid 3, Hybrid 5, Hybrid 8, Hybrid 10, Hybrid 16, Hybrid 17, Hybrid 18 and Hybrid 20.

4.5.1 Field screening of F_2 populations for spotted pod borer resistance

The data pertaining to different damage parameters in F_2 populations and selected individual plants are presented in Table 31 and 32, respectively.

4.5.1.1 Damage parameters

Total nine hybrids populations were evaluated for their reaction to the infestation of spotted pod borer. An abundant variation was observed in all hybrids populations with respect to flower bud, flower, pod and overall damage (Table 31).

Table 31. Extent of damage caused by spotted pod borer to the plants of F_2 populations

Crosses	Flower bud damage			Flower damage			Pod damage			Total damage (%)		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Hybrid 1	0.00	13.04	1.55	5.00	15.00	1.70	0.00	12.50	0.42	3.51	9.26	6.76
Hybrid 3	7.69	24.00	4.40	8.00	18.75	2.55	0.00	7.69	0.89	7.50	18.52	12.17
Hybrid 5	0.00	16.67	3.05	3.85	18.75	2.20	0.00	18.18	1.21	6.25	14.29	9.60
Hybrid 8	6.06	18.22	3.13	0.00	15.00	2.20	5.56	26.67	2.00	6.58	15.40	10.22
Hybrid 10	6.25	17.18	3.08	0.00	15.79	2.00	5.56	22.22	2.00	7.67	16.39	10.79
Hybrid 16	4.35	19.23	3.25	0.00	16.67	1.50	0.00	18.75	1.53	5.00	14.75	10.22
Hybrid 17	0.00	18.18	2.05	0.00	11.11	0.65	0.00	27.27	1.58	5.45	12.28	8.20
Hybrid 18	3.33	14.29	2.45	0.00	15.00	1.90	4.55	16.67	2.05	6.06	11.86	8.74
Hybrid 20	2.86	10.00	2.05	3.85	13.79	2.60	3.85	27.78	2.68	5.13	13.33	8.72

With Geethika as a female parent, out of three, two F_2 populations observed to have low total damage (below 10 %). Those populations were Hybrid 1 (total damage ranged from 3.51 % to 9.26 % with overall mean 6.76 %) and Hybrid 5 (total damage ranged from 6.25 % to 14.29 % with overall mean 9.60 %). Hybrid 3 F_2 population recorded a mean value of 12.17 per cent, however, few plants of this population recorded total damage between 5-10 per cent and categorised as moderately resistant (Table 32).

With respect to F_2 populations of Vellayani Jyothika as a female parent, both populations *i.e.* Hybrid 8 and Hybrid 10 observed to have a population mean above ten per cent. However, few plants from both populations observed to have total damage between 5-10 per cent and categorised as moderately resistant (Table 32).

With Kashi Kanchan as a female parent, out of four, three F_2 populations observed to have low total damage (below 10 %). Those populations were Hybrid 17 (total damage ranged from 5.45 % to 12.28 % with overall mean 8.20 %), Hybrid 18 (total damage ranged from 6.06 % to 11.86 % with overall mean 8.74 %) and Hybrid 20 (total damage ranged from 5.13 % to 13.33 % with overall mean 8.72 %). Hybrid 16 F_2 population recorded a mean value of 10.22 per cent, however, few plants of this population recorded total damage between 5-10 per cent and categorised as moderately resistant (Table 32).

Table 32. Selected F_2 plants on the basis of extent of damage caused by spotted pod borer

Hybrids	Plants	Flower bud damage (%)	Flower damage (%)	Pod damage (%)	Total damage (%)
Hybrid 1	1	2	2	1	8.93
	2	1	2	1	6.67
	3	2	2	0	7.41
	4	3	1	1	8.93
	5	3	2	0	8.33
	6	0	2	0	3.51
	7	1	1	1	5.17
Hybrid 3	1	3	2	1	9.38
	2	3	2	1	9.09
Hybrid 5	1	2	2	2	8.00
	2	1	2	2	7.69
	3	2	3	0	8.33
Hybrid 8	1	2	2	2	7.79
	2	2	3	2	8.97
	3	2	3	1	9.68
	4	3	2	1	9.09

Table 32 continued

Hybrids	Plants	Flower bud damage (%)	Flower damage (%)	Pod damage (%)	Total damage (%)
Hybrid 8	5	4	0	1	6.58
	6	2	3	2	8.64
	7	3	3	2	9.64
	8	2	1	2	7.12
	9	2	2	2	7.69
	10	2	2	2	8.33
Hybrid 10	1	2	2	1	8.33
	2	4	2	1	9.33
	3	2	1	2	7.94
	4	2	0	3	8.62
Hybrid 16	1	2	1	0	5.45
	2	2	1	2	7.25
Hybrid 17	1	2	1	1	8.70
	2	3	0	1	8.89
	3	1	0	3	7.02
Hybrid 18	1	4	1	1	7.59
	2	1	2	1	7.02
	3	3	0	2	8.62
Hybrid 20	1	1	4	2	8.43
	2	1	3	1	6.58
	3	2	3	3	9.52
	4	1	3	1	5.95

4.5.1.2 Morphological parameters of hybrids for resistance to spotted pod borer in F_2 populations

Data pertaining to different morphological parameters *viz*., number of trichomes on bud, number of trichomes on pod and pod wall thickness of F_2 populations for resistance to spotted pod borer presented in Table 33.

4.5.1.2.1 Number of trichome on bud

There was not much variation observed in all F_2 populations with respect to trichome density on bud. However, pods of Hybrid 17 F_2 population observed to have more number of trichome on the bud (ranged from 3 /mm^2 to 5 /mm^2 with a population mean of 4.00 /mm^2).

4.5.1.2.2 Number of trichomes on the pod

With respect to trichome density on the pod, all hybrid populations with Kashi Kanchan as female parent observed to have more number of trichomes on the pod.

Plants of Hybrid 20 recorded high trichome density (ranged from 8 /mm^2 to 12 /mm^2 population mean of 8.80 /mm^2).

Plants of F_2 populations of Hybrid 8 and Hybrid 1 observed to have less number of trichomes on the pod (ranged from 2 /mm^2 to 4 /mm^2 population mean of 2.80 /mm^2 and 3 /mm^2 to 5 /mm^2 with a population mean of 3.89 /mm^2, respectively).

4.5.1.2.3 Pod wall thickness

With Geethika as a female parent, pod wall thickness in Hybrid 1 ranged from 0.62 mm to 0.71 mm with population mean of 0.65 mm, in Hybrid 3 ranged from 0.67 mm to 0.72 mm with population mean of 0.68 mm and in Hybrid 5 ranged from 0.55 mm to 0.67 mm with population mean of 0.61 mm. With Vellayani Jyothika as a female parent, pod wall thickness in Hybrid 8 ranged from 0.69 mm to 0.75 mm with a population mean of 0.70 mm and in Hybrid 10 ranged from 0.60 mm to 0.66 mm with a population mean of 0.60 mm (Table 33).

In the crosses of Kashi Kanchan, pod wall thickness in Hybrid 16 ranged from 0.55 mm to 0.72 mm with population mean of 0.62 mm. In Hybrid 17 it ranged from 0.45 mm to 0.67 mm with population mean of 0.55 mm, in Hybrid 18 it ranged from 0.51 mm to 0.61 mm with population mean of 0.54 mm and in Hybrid 20 ranged from 0.40 mm to 0.52 mm with population mean of 0.45 mm.

Table 33. Biophysical parameters of F_2 populations with respect to spotted pod borer resistance

Crosses	No. of trichomes on bud (per mm^2)			No. of trichomes on pod (per mm^2)			Pod wall thickness (mm)		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Hybrid 1	2	3	2.5	3	5	3.89	0.62	0.71	0.65
Hybrid 3	1	2	1.5	4	6	4.55	0.67	0.72	0.68
Hybrid 5	2	3	2.5	3	6	4.50	0.55	0.67	0.61
Hybrid 8	1	2	1.5	2	4	2.80	0.69	0.75	0.70
Hybrid 10	2	4	3.0	3	5	3.55	0.6	0.66	0.60
Hybrid 16	2	3	2.5	8	10	8.40	0.55	0.72	0.62
Hybrid 17	3	5	4.0	5	9	6.80	0.45	0.67	0.55
Hybrid 18	2	4	3.0	5	7	5.40	0.51	0.61	0.54
Hybrid 20	2	4	3.0	8	12	8.80	0.4	0.52	0.45

4.5.2 Evaluation of F_2 populations for biometric characters

The mean performance of different hybrid populations for different biometric characters are presented in Table 34 and described below.

4.5.2.1 Days to 50 per cent flowering

Among nine hybrids, days to 50 per cent flowering ranged from 50 to 63 days. Hybrid 16 was earliest to flower with 50 days, whereas, Hybrid 10 took 63 days and was late in flowering.

4.5.2.2 Plant height

All four hybrids, with Kashi Kanchan as a female parent, recorded shorter plant height. Those were Hybrid 16, Hybrid 17, Hybrid 18 and Hybrid 20 with a population mean values of 82.22 cm, 91.94 cm, 55.73 cm and 92.95 cm, respectively. In hybrids *viz.*, Hybrid 1, Hybrid 3 and Hybrid 5, with Geethika as a female parent, Hybrid 5 observed to have a high population mean value for plant height *i.e.* 421.10 cm. In Hybrid 8 and Hybrid 10, with Vellayani Jyothika as a female parent, Hybrid 10 recorded higher population mean value *i.e.* 413.25 cm.

4.5.2.3 Number of primary branches per plant

In five F_2 populations of Hybrid 1, Hybrid 3, Hybrid 5, Hybrid 8, Hybrid 10 and Hybrid 20, a number of primary branches per plant ranged from 2 to 4 with a population mean of 3.15, 2.55, 2.90, 2.90, 2.55 and 2.95, respectively. Two F_2 populations *i.e.* Hybrid 16 and Hybrid 17 recorded less number of primary branches per plant ranging from 2.00 to 3.00 and 1.00 to 3.00 with population means of 2.40 and 2.20, respectively.

4.5.2.4 Number of pods per plant

With respect to number of pods per plant, Hybrid 18 and Hybrid 20 observed to have higher values. In these hybrids, pods per plant ranged from 13 to 23 and 13 to 25 with a population mean of 17.55 and 19.65, respectively. In the F_2 populations with Geethika as a female parent, Hybrid 3 and Hybrid 5 recorded higher population mean, 16.80 and 16.45, respectively. In the F_2 populations, with Vellayani Jyothika as a female parent, number of pods per plant ranged from 11.00 to 20.00 in Hybrid 8 with population mean of 15.65 pods per plant and 12.00 to 20.00 in Hybrid 10 with population mean of 14.40 pods per plant.

4.5.2.5 Pod length

With Geethika as a female parent, pod length in Hybrid 1 ranged from 17.00 cm to 25.50 cm with a population mean of 22.38 cm, in Hybrid 3 ranged from 21.50 cm to 25.20 cm with a population mean of 23.25 cm and in Hybrid 5 ranged from 20.00 cm to 28.00 cm with a population mean of 24.78 cm. With Vellayani Jyothika as a female parent, pod length in Hybrid 8 ranged from 23.50 cm to 27.50 cm with a population mean of 25.64 cm and in Hybrid 10 ranged from 21.00 cm to 28.50 cm with a population mean of 25.89 cm.

In the crosses of Kashi Kanchan, pod length in Hybrid 16 ranged from 14.50 cm to 20.00 cm with population mean of 17.40 cm, in Hybrid 17 ranged from 15.00 cm to 24.00 cm with population mean of 20.41 cm, in Hybrid 18 ranged from 12.50 cm to 18.50 cm with population mean of 15.35 cm and in Hybrid 20 ranged from 12.60 cm to 18.60 cm with population mean of 15.49 cm.

4.5.2.6 Number of seeds per pod

With Geethika as a female parent, a number of seeds per pod in Hybrid 1 ranged from 8 to 15 with a population mean of 12.85, in Hybrid 3 ranged from 6 to 18 with a population mean of 15.40 and in Hybrid 5 ranged from 8 to 18 with a population mean of 13.35. With Vellayani Jyothika as a female parent, a number of seeds per pod in Hybrid 8 ranged from 14 to 18 with a population mean of 15.65 and in Hybrid 10 ranged from 8 to 16 with a population mean of 13.70.

In the crosses of Kashi Kanchan as a female parent, number of seeds per pod in Hybrid 16 ranged from 9 to 15 with population mean of 13.30, in Hybrid 17 ranged from 12 to 17 with population mean of 14.35, in Hybrid 18 ranged from 12 to 15 with population mean of 13.65 and in Hybrid 20 ranged from 11 to 16 with population mean of 13.35.

4.5.2.7 Grain yield per plant

With respect to yield per plant, with Geethika as a female parent, Hybrid 3 recorded higher population mean *i.e.* 38.21 g. With Vellayani Jyothika as a female parent, both hybrids *viz.*, Hybrid 8 and Hybrid 10 observed to have comparatively the same population mean values (42.55 g and 42.19 g, respectively). In the F_2 populations of Kashi Kanchan as a female parent, Hybrid 17 recorded high value of the population mean with 26.43 g grain yield per plant, whereas, in Hybrid 18 the value was low (9.09 g).

4.5.2.8 100 seed weight

With respect to 100 seed weight, Hybrid 1 and Hybrid 10 observed to have a higher population mean with a range of 7.40 g to 15.40 g and 10.50 g to 16.60 g, respectively. In the F_2 populations, with Kashi Kanchan as a female parent, 100 seed weight ranged from 8.25 g to 12.50 g in Hybrid 17 with high population mean of 10.52 g, whereas, in Hybrid 18, 100 seed weight ranged from 5.00 g to 7.50 g with a low population mean of 6.41 g.

Table 34. The variability in biometric characters in nine F_2 populations

Crosses	Days to 50 % flowering	Plant height (cm)			No. of primary branches per plant			No. of pods per plant			Pod length (cm)		
		Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Hybrid 1	62.00	222.50	310.50	256.64	2.00	4.00	3.15	10.00	16.00	13.60	17.00	25.50	22.38
Hybrid 3	59.00	345.00	405.00	369.55	2.00	4.00	2.55	12.00	22.00	16.80	21.50	25.20	23.25
Hybrid 5	63.00	398.00	456.00	421.10	2.00	4.00	2.90	9.00	22.00	16.45	20.00	28.00	24.78
Hybrid 8	61.00	308.00	396.00	353.75	2.00	4.00	2.90	11.00	20.00	15.65	23.50	27.50	25.64
Hybrid 10	63.00	366.00	466.00	413.25	2.00	4.00	2.55	12.00	20.00	14.40	21.00	28.50	25.89
Hybrid 16	50.00	40.50	125.00	82.22	2.00	3.00	2.40	11.00	19.00	14.60	14.50	20.00	17.40
Hybrid 17	59.00	49.00	150.00	91.94	1.00	3.00	2.20	8.00	16.00	11.70	15.00	24.00	20.41
Hybrid 18	53.00	45.00	68.00	55.73	2.00	3.00	2.55	13.00	23.00	17.55	12.50	18.50	15.35
Hybrid 20	55.00	49.00	128.00	92.95	2.00	4.00	2.95	13.00	25.00	19.65	12.60	18.60	15.49

Crosses	No. of seeds per pod			Grain yield per plant (g)			100 seed weight (g)		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Hybrid 1	8.00	15.00	12.85	15.54	47.59	32.44	7.40	15.40	13.01
Hybrid 3	6.00	18.00	15.40	14.11	61.39	38.21	9.00	15.40	11.68
Hybrid 5	8.00	18.00	13.35	13.39	53.52	30.64	9.00	11.50	10.39
Hybrid 8	14.00	18.00	15.65	26.07	55.28	42.55	7.50	13.85	11.49
Hybrid 10	8.00	16.00	13.70	27.59	60.20	42.19	10.50	16.60	13.09
Hybrid 16	9.00	15.00	13.30	9.65	28.66	18.78	7.40	11.50	9.76
Hybrid 17	12.00	17.00	14.35	11.92	44.32	26.43	8.25	12.50	10.52
Hybrid 18	12.00	15.00	13.65	4.42	14.38	9.09	5.00	7.50	6.41
Hybrid 20	11.00	16.00	13.35	8.23	18.83	13.48	7.00	9.30	8.28

Summary

5. SUMMARY

Spotted pod borer (*Maruca vitrata* Fab.) is one of the most important post-flowering pests of cowpea in the tropics, and always a bottleneck in achieving high production in cowpea. It is a major lepidopteran pest and damage caused to cowpea by this pest almost always crosses an economic threshold level. Hence, identification of resistance against spotted pod borer in available germplasm and incorporation of resistance in high yielding varieties of cowpea becomes very essential, both in terms of environmental safety and reducing the cost of cultivation. Hence, the present investigation was conducted in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, Kerala Agricultural University, Thrissur during 2015 to 2018 with the objective of identification and incorporation of resistance against spotted pod borer in high yielding varieties of cowpea and assessment of parental polymorphism at the molecular level.

5.1 IDENTIFICATION OF RESISTANCE AGAINST SPOTTED POD BORER IN COWPEA GENOTYPES

5.1.1 Field screening of genotypes

Thirty cowpea genotypes were evaluated for resistance to spotted pod borer and yield. Flower bud, flower and pod damage measurements formed the basis of spotted pod borer resistance evaluation. Substantial variation was observed for all the damage parameters, and also in the related morphological and biochemical traits and yield contributing characters. Total damage was calculated for the 30 cowpea genotypes based on the simultaneous consideration of flower bud, flower and pod damage. Ten genotypes *viz*., Hridya, Palakkadan thandan payar, EC 300039, EC 98668, EC 101216, IC 52110, IC 39945, IC 2918, IC 39922 and IC 39916 recorded total damage below five per cent. Among them, IC 39922 observed to have no flower bud and flower damage. Genotypes EC 300039, EC 98668, IC 52110, IC 39945, IC 2918 and IC 39916 recorded no flower damage, whereas, Palakkadan thandan payar, IC 39945, IC 2918 and IC 39947 were free from pod damage. The highest damage was recorded in the variety Bhagyalakshmy (48.46 %) followed by variety Lola (30.04 %).

The 30 genotypes were subjected to Duncan's Multiple Range Test (DMRT), based on total damage caused by spotted pod borer, and were categorised as resistant, moderately resistant, susceptible and highly susceptible. Ten genotypes with damage below five per cent were categorised as resistant. Three genotypes with total damage

ranging from 5 to 10 per cent were classed as moderately resistant. Five genotypes with damage ranging from 10 to 15 per cent were grouped as susceptible and 12 genotypes with total damage more than 15 per cent were categorised as highly susceptible.

5.1.2 Evaluation of morphological basis of resistance

Analysis of the morphological basis of resistance to spotted pod borer revealed the negative correlation of trichome density and length on flower bud, trichome density on the pod and pod wall thickness with respective damage parameters. Length of peduncle was positively correlated with spotted pod borer damage, but the correlation was not significant.

5.1.3 Evaluation of biochemical basis of resistance

With respect to the biochemical basis of resistance to spotted pod borer, total sugar content, reducing sugar content and non-reducing sugar content of flower bud and pod showed a positive correlation with damage parameters, but the correlation was not significant. However, the total protein content of pod showed a strong and positive correlation with pod damage. The total phenol content of flower bud showed strong negative correlation with damage parameters. Polyphenol oxidase activity in flower bud and pod exhibited a strong negative correlation with damage parameters. The crude fibre content of pod also showed a strong negative correlation with pod damage.

5.1.4 Variability study for yield contributing characters

The phenotypic and genotypic coefficients of variation (PCV and GCV, respectively), heritability and genetic gain were worked out for each yield contributing characters. Moderate PCV and GCV were observed for days to 50 per cent flowering, number of primary branches per plant, number of pods per plant, number of seeds per pod, grain yield per plant and 100 seed weight, whereas, high PCV and GCV were observed for plant height and pod length. All characters exhibited high heritability, however, the high genetic gain was observed only with respect to a number of primary branches per plant and 100 seed weight. For days to 50 per cent flowering and grain yield per plant genetic gain were low. For remaining characters, the genetic gains were moderate.

5.2 THE HYBRIDISATION OF RESISTANT GENOTYPES WITH POPULAR HIGH YIELDING VARIETIES

Considering all damage parameters and agronomical performance of genotypes, five testers *viz.*, Palakkadan thandan payar, EC 300039, EC 98668, IC 39945 and IC 2918

were selected and crossed with Geethika, Vellayani Jyothika, Lola and Kashi Kanchan following Line × Tester mating and 20 hybrids were developed. These hybrids were used for further studies.

5.3 ASSESSMENT OF PARENTAL POLYMORPHISM AT THE MOLECULAR LEVEL

Among the 40 SSR primers used to assess the genetic diversity and estimate genetic polymorphism in 30 cowpea genotypes, 21 primers gave scorable DNA fragments and each of the 21 primers revealed polymorphism. Three primers *viz*., CLM0061, CLM0295 and CLM0300 observed to produce a high Polymorphic Information Content (above 0.70) with respect to 30 genotypes used in the study. Six primers produce five alleles with all genotypes. CLM0300 recorded higher Shannon's diversity index. The overall range of the similarity among 30 genotypes was found to be very wide-ranging (0.022 to 0.643) which revealed the presence of high variability among the cowpea genotypes under study.

The cluster analysis based on polymorphic SSR data, grouped 30 genotypes into 22 clusters at 50 per cent similarity. The 21st cluster was observed to have more members (3 genotypes). The least susceptible genotype, IC 2918, grouped in a separate cluster, which proved its diverse nature from other genotypes. Principal component analysis of SSR data placed three yard-long bean genotypes *viz*., Geethika, Vellayani Jyothika and Lola near to each other in a two-dimensional score plot. The same analysis also formed two clusters with more number of genotypes which placed resistant and susceptible genotypes separately.

5.4 EVALUATION OF F_1 HYBRIDS AGAINST SPOTTED POD BORER

In field screening of F_1 hybrids, Hybrid 20 observed to have total damage below 5 per cent. Eight hybrids recorded total damage in the range of 5 to 10 per cent. These hybrids were selected for the next experiment. Line × Tester analysis of F_1s revealed EC 98668 and IC 2918 as a good combiner for resistance against spotted pod borer. Hybrid 5, Hybrid 6, Hybrid 10, Hybrid 11, Hybrid 13, Hybrid 15, Hybrid 16, Hybrid 17, Hybrid 18, Hybrid 19 and Hybrid 20 observed to have desired negative heterosis (mid-parent) for total damage.

5.5 EVALUATION OF F_2 POPULATIONS AND SCREENING FOR SPOTTED POD BORER RESISTANCE

In F_2 plants screening, Hybrid 1 population recorded low mean for total damage (6.76 %), whereas, the population of Hybrid 3 recorded high population mean (12.17 %). Around 100 plants of F_2 generation recorded total damage below ten per cent. Out of them, around 38 plants also registered good yield.

As a future line of work, we suggest the evaluation of segregating progenies of isolated resistant plants, utilisation of identified resistant genotypes in breeding against spotted pod borer and screening of more SSR primers as well other marker systems to identify polymorphic pattern amid resistant and susceptible genotypes.

References

6. REFERENCES

Agarwal, M., Shrivastava, N., and Padh, H. 2008. Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Rep.* 27: 617-631.

Ajibade, S. R., Weeden, N. F., and Michite, S. 2000. Inter simple sequence repeat analysis of genetic relationships in the genus *Vigna*. *Euphytica* 111(1): 47-55.

Ajith, P. M. 2001. Variability and path analysis in bush type vegetable cowpea (*Vigna unguiculata* (L.) Walp.). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 64p.

Ananda, M. 2012. Comparative assessment of genetic variability in F_2, F_3 and biparental mating progenies in cowpea (*Vigna unguiculata* (L.) Walp.). M.Sc. (Ag.) thesis, University of Agricultural Sciences, Bengaluru, 88p.

Anbuselvam, Y., Manivannan, N., Murugan, S., Thangavelu, P., and Ganesan, J. 2000. Variability studies in cowpea (*Vigna unguiculata* (L.) Walp.). *Legume Res.* 23: 279-280.

Anbuselvam, Y., Manivannan, N., Saravanan, K., and Ganesan, J. 2001. Studies on genetic divergence in cowpea (*Vigna unguiculata* L. Walp.). *Madras Agric. J.* 87: 343-345.

Anilkumar, S. G. 1993. Combining ability for yield and drought tolerance in cowpea (*Vigna unguiculata* (L.) Walp.). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 99p.

Anitha, K. R., Thiyagarajan, K., Bharathi, S. P., and Rajendran, R. 2017. Combining ability analysis for yield and quality traits in fodder cowpea (*Vigna unguiculata* (L.) Walp.). *Electron. J. Plant Breed.* 8(1): 244-249.

Anithakumari, V. 1992. Host resistance in cowpea (*Vigna unguiculata* (L.) Walp.) to the pod borer *Maruca testulalis* (Geyer) (Pyralidae: Lepidoptera). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 98p.

Apte, U. B., Chavan, S. A., and Yadav, B. B. 1987. Genetic variability and heritability in cowpea. *Indian J. Agric. Sci.* 57: 596-598.

Arunachalam, P., Viswanatha, K. P., Chakravarthy, K. K., Manjunath, A., and Jayashree, M. K. 2002. Efficiency of breeding methods in early segregating generations of cowpea [*Vigna unguiculata* (L.) Walp.]. *Indian J. Genet.* 62: 228-231.

Asare, A. T., Gowda, B. S., Galyuon, I. K. A., Aboagye, L. L., Takrama, J. F., and Timko, M. P. 2010. Assessment of the genetic diversity in cowpea (*Vigna unguiculata* L. Walp.) germplasm from Ghana using simple sequence repeat markers. *Plant Genet. Resour.* 8(2): 142-150.

Asoontha, 2017. Genetic variability for yield and resistance to legume pod borer, *Maruca vitrata* (Fab.) in yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 86p.

Attachi, P. and Djihou, Z. C. 1994. Record of host plants of *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae) in Republic of Benin. *An. Soc. Ent. France* 30: 169-174.

Attachi, P. and Hountondji, F. C. C. 2000. Olfactory responses of *Maruca vitrata* (Fabricus) larvae to reproductive parts of different varieties of cowpea (*Vigna unguiculata* (L.) Walp.). *Insect Sci. Appl.* 20: 117-121.

Ba, F. S., Pasquet, R. S., and Gepts, P. 2004. Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] as revealed by RAPD markers. *Genet. Resour. Crop Evol.* 51(5): 539-550.

Backiyarani, S. and Natarajan, N. 1996. Variability studies in cowpea. *Legume Res.* 19: 59-61.

Barad, C. S., Patel, P. S., Rabari, G. N., and Panickar, B. 2016. Biochemical basis of resistance against *Maruca vitrata* in selected genotypes / varieties of cowpea. *Indian J. Plant Prot.* 44(1): 59-62.

Bastian, D., Vijendra Das, L. D., Kandasamy, G., and Sakila, M. 2001. Path analysis in cowpea (*Vigna unguiculata* L. Walp.) *Madras Agric. J.* 88(7-9): 526-527.

Beegum, N. A. N. 2015. Morphological and biochemical bases of resistance to spotted pod borer, *Maruca vitrata* (Fab.) (Lepidoptera: Crambidae) in cowpea. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 106p.

Beegum, N. A. N. and Subramanian, M. 2017. Screening for resistance in cowpea against spotted pod borer, *Maruca vitrata* (Fab.) (Lepidoptera: Crambidae). *Environ. Ecol.* 35(4B): 3185-3188.

Bhushana, H. O., Viswanatha, K. P., and Arunachalam, P. 2000. Heterosis in cowpea for seed yield and its attributes. *Crop Res.* 19(2): 277-280.

Bindu, S. S. 1997. Biocontrol of pests of vegetable cowpea (*Vigna unguiculata* sub sp. *sesquipedalis*) (L.) Verdcourt). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 84p.

Borah, H. K. and Khan, A. K. F. 2002. Genetic divergence in fodder cowpea. *Madras Agric. J.* 88: 625-628.

Bressani, R. and Elias, L. G. 1980. The nutritional role of polyphenols in beans. In: Hulse, J. H. (ed.), *Proceedings of Symposium on Polyphenols in Cereals and Legumes*, 10-13 June, 1979, St. Louis Missouri, USA. International Development Research Center, Ottawa, Canada, pp. 61-68.

Burton, G. M. and DeVane, E. H. 1953. Estimating heritability in tall fescue (*Fescuta arundinaceae*) from replicated clonal material. *Agron. J.* 45: 478-481.

Burton, G. W. 1952. Quantitative inheritance in grasses. In: *Proceedings of the VIth International Grassland Congress Vol. 1*. State College, Pennsylvania, USA, pp. 277-283.

Castillo, A., Budak, H., Martin, A., Dorado, G., Borner, A., Roder, M., and Hernandez, P. 2010. Interspecies and intergenus transferability of barley and wheat D-genome microsatellite markers. *An. Appl. Biol.* 156: 347-356.

Chakravarthi, B. K. and Naravaneni, R. 2006. SSR Marker Based DNA fingerprinting and diversity study in rice (*Oryza sativa*. L.). *Afr. J. Biotechnol.* 5: 684-688.

Chaudhari, S. B., Naik, M. R., and Patil, S. S. 2013. Combining ability for pod yield and seed protein content in cowpea over environment. *Trends Biosci.* 6: 395-398.

Chen, H., Chen, H., Hu, L., Wang, L., Wang, S., Wang, M. L., and Cheng, X. 2017. Genetic diversity and a population structure analysis of accessions in the Chinese cowpea [*Vigna unguiculata* (L.) Walp.] germplasm collection. *Crop J.* 5(5): 363-372.

Chiang, H. S. and Singh, S. R. 1988. Pod hairs as a factor in *Vigna vexillata* resistance to the pod-sucking bug, *Clavigralla tomentosicollis*. *Entomol. Exp. Appl.* 47(2): 195-199.

Coulibaly, S., Pasquet, R. S., Papa, R., and Gepts, P. 2002. AFLP analysis of the phenetic organization and genetic diversity of *Vigna unguiculata* L. Walp. reveals extensive gene flow between wild and domesticated types. *Theor. Appl. Genet.* 104(2-3): 358-366.

Dabrowski, Z. T., Bungu, O. O. M., and Ochieng, R. S. 1983. Studies on the legume pod borer, *Maruca testulalis* (Geyer): methods used in cowpea screening for resistance. *Insect Sci. Appl.* 4: 141-145.

Damarany, A. M. 1994. Estimation of genotypic and phenotypic correlation, heritability and potence of gene set in cowpea (*Vigna unguiculata* (L.) Walp.). *Indian J. Agric. Sci.* 25: 1-4.

Danam, S. and Chaudhari, F. P. 2000. Selection of superior combiners in cowpea. *Crop Res.* 20: 268-273.

Davis, P. H. and Heywood, V. H. 1963. *Principles of Angiosperm Taxonomy.* Van Nostrandpage, Princeton, New Jersey, 154p.

De Sousa, A., Jank, L., Campos, T., Sforca, D. A., and Zucchi, M. I. 2011. Molecular diversity and genetic structure of guineagrass (*Panicum maximum* Jacq.), a tropical pasture grass. *Trop. Plant Biol.* 4: 185-202.

Dent, D. R. 1991. Host plant resistance. In: *Insect Pest Management*. CAB International, Willingford, pp. 123-177.

Dias, F. T. C., Bertini, C. H. C. de M., and Freire Filho, F. R. 2016. Genetic effects and potential parents in cowpea. *Crop Breed. Appl. Biotechnol.* 16(4): 315-320.

Dietz, P. A. 1914. Het Katjand VI indertje (het verrmeende Toa-Toh-Motje). *Meded. Deli-Proefstu Medan.* 8: 273-278.

Dinesh, H. B., Viswanatha, K. P., Lohithaswa, H. C., Pavan, R., and Singh, P. 2017. Variability, correlation and path analysis studies in F_3 generation of cowpea [*Vigna unguiculata* (L.) Walp.]. *Int. J. Curr. Microbiol. App. Sci.* 6(9): 1420-1428.

Diouf, D. and Hilu, K. W. 2005. Microsatellites and RAPD markers to study genetic relationships among cowpea breeding lines and local varieties in Senegal. *Genet. Resour. Crop Evol.* 52: 1057-1067.

Dowd, F. P. and Vega, F. 1996. Enzymatic oxidation products of allelochemicals as a basis for resistance against insects: effects on the corn leafhopper *Dalbulus maidis*. *Nat. Toxins* 4: 85-91.

Doyle, J. J. and Doyle, J. L. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12(1): 13-15.

Dreyer, H., Baumgartner, J., and Tamo, H. 1994. Seed damaging field pests of cowpea (*Vigna unguiculata* L. Walp.) in Benin: occurrence and pest status. *Int. J. Pest Manag*. 40: 252-260.

Echendu, T. N. C. and Akingbohungbe, A. E. 1989. The larval population and crop growth phase for screening cowpea for resistance to *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae) in Nigeria based on flowers, pods and yield loss. *Trop. Pest Manag*. 35(2): 173-175.

Esterbaner, H., Schwarzl, E., and Hayn, M. 1977. A rapid assay for catechol oxidase and laccase using 2-nitro-5-thio benzoic acid. *Anal. Biochem.* 77: 486-494.

Ezeuch, M. I. and Taylor, T. A. 1984. Effects of time of intercropping with maize on cowpea susceptibility to three major pests. *Trop. Agric*. 61: 82-86.

Fatokun, C. A., Danesh, D., Young, N. D., and Stewart, E. L. 1993. Molecular taxonomic relationships in the genus *Vigna* based on RFLP analysis. *Theor. Appl. Genet*. 86: 97-104.

Felton, G. W. and Duffey, S. S. 1991. Protective action of midgut catalase in lepidopteran larvae against oxidative plant defenses. *J. Chem. Ecol*. 17: 1715-1732.

Felton, G. W., Donato, K. K., Broadway, R. M., and Duffey, S. S. 1992. Impact of oxidized plant phenolics on the nutritional quality of dietary protein to a noctuid herbivore, *Spodoptera exigua*. *J. Insect Physiol*. 38: 277-285.

Ferreira, J. R., Pereira, J. F., Turchetto, C., Minella, E., Consoli, L., and Delatorre, C. A. 2016. Assessment of genetic diversity in Brazilian barley using SSR markers. *Genet. Mol. Biol.* 39(1): 86-96.

Food Science, 2008. Crude fiber. Available: https://www.foodscience-avenue.com/2008/04/ crude-fiber.html [28 August 2018].

Frary, A., Xu, Y., Liu, J., Mitchell, S., Tedeschi, E., and Tanksley, S. 2005. Development of a set of PCR-based anchor markers encompassing the tomato genome and evaluation of their usefulness for genetics and breeding experiments. *Theor. Appl. Genet.* 111: 291-312.

Fu, P. C., Zhang, Y. Z., Ya, H., and Gao, Q. 2016. Characterization of SSR genomic abundance and identification of SSR markers for population genetics in Chinese jujube (*Ziziphus jujuba* Mill.). *PeerJ* 4: e1735.

Girish, G., Viswanatha, K. P., Manjunath, A., and Yogesh, L. N. 2006. Genetic variability, heritability and genetic advance analysis in cowpea [*Vigna unguiculata* (L.) Walp.]. *Environ. Ecol.* 24(4): 1172-1174.

Goldstein, J. L. and Swain, T. 1965. The inhibition of enzymes by tannins. *Phytochemistry* 4: 185-192.

Gowda, T. H., Shantha, R., Hiremath, S. R., and Salimath, P. M. 1991. Estimation of genetic parameters in intervarietal crosses of cowpea (*Vigna unguiculata* L.) and their implication in selection. *Legume Res.* 14: 15-19.

Griffing, B. 1956. Concept of general combining ability in relation to diallele system. *Aust. J. Bio. Sci.* 9: 463-493.

Gupta, P. K. and Varshney, R. K. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* 113(3): 163-185.

Gupta, R. P., Patel, S. R., Modha, K. G., and Wadekar, P. B. 2017. Generation mean analysis for yield and yield components in cowpea [*Vigna unguiculata* (L.) Walp.]. *Int. J. Curr. Microbiol. App. Sci.* 6(7): 2231-2240.

Gupta, S. and Prasad, M. 2009. Development and characterization of genic SSR markers in *Medicago truncatula* and their transferability in leguminous and non-leguminous species. *Genome* 52(9): 761-771.

Halder, J. and Srinivasan, S. 2005. Morphological basis of resistance to spotted pod borer, *Maruca vitrata* (Geyer) in urd bean. *SHASHPA J. Entomol. Res.* 12(2): 102-105.

Halder, J., Srinivasan, S., and Muralikrishna, T. 2006. Biochemical basis of resistance to spotted pod borer, *Maruca vitrata* (Geyer) in mungbean. *J. Entmol. Res.* 30(4): 313-316.

Halder, J. and Srinivasan, S. 2007. Biochemical basis of resistance to spotted pod borer, *Maruca vitrata* (Geyer) in urd bean. *An. Plant Prot. Sci.* 15(2): 287-290.

Halder, J. and Srinivasan, S. 2011. Varietal screening and role of morphological factors on distribution and abundance of spotted pod borer, *Maruca vitrata* (Geyer) on cowpea. *An. Plant Prot. Sci.* 19(1): 71-74.

Hanson, C. H., Robinson, H. F., and Comestock, R. E. 1956. Biometrical studies of yield in segregating populations of Korean lespedeza. *Agron. J.* 47: 314-318.

Hazra, P., Chattopadhyay, A., and Pandit, M. K. 1999. Genetic variability in three cultigroups of cowpea. *J. Interacademicia* 3: 263-268.

He, C., Poysa, V., and Yu, K. 2003. Development and characterization of simple sequence repeat (SSR) markers and their use in determining relationships among *Lycopersicon esculentum* cultivars. *Theor. Appl. Genet.* 106(2): 363-373.

Hedge, J. E. and Hofreiter, B. T. 1962. Determination of total carbohydrate by anthrone method. In: Whistler, R. L. and Be Miller, J. N (eds.), *Carbohydrate Chemistry.* Academic Press, New York, p. 17.

Hegde, V. S. and Mishra, S. K. 2009. Landraces of cowpea, *Vigna unguiculata* (L.) Walp., as potential sources of genes for unique characters in breeding. *Genet. Res. Crop Evol.* 56: 615-627.

Hokanson, S. C., Szewc-McFadden, A., Lamboy, W. F., and Mcferson, J. 1998. Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a *Malus domestica* Borkh. core subset collection. *Theor. Appl. Genet.* 97: 671-683.

Holton, T. A., Christopher, J. T., McClure, L., Harker, N., and Henty, R. J. 2002. Identification and mapping of polymorphism SSR markers from expressed sequences of barley and wheat. *Mol. Breed.* 9: 63-71.

IITA [International Institute of Tropical Agriculture]. 1974. *Annual Report.* International Institute of Tropical Agriculture, Ibadan, Nigeria, 160p.

IITA [International Institute of Tropical Agriculture]. 1998. IITA researches to reduce cowpea crop losses. In: *Towards Sustainable Agriculture for Developing Countries*: *Options from Life Sciences and Biotechnology*. Proceedings of the Conference, IITA, Ibadan, Nigeria, pp. 1-5.

IIVR [Indian Institute of Vegetable Research]. 2008. *Annual Report*. Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh, India, 108p.

Indarsingh, R., Singh, J., and Chaudhari, D. P. 2007. Genetic analysis of quality characters in cowpea (*V. unguiculata*). *Crop Improv*. 35: 35-41.

Ishikawa, S., Hirao, T., and Arai, N. 1969. Chemosensory basis of host plant selection in the silkworm. *Entomol. Exp. Appl*. 12: 544-554.

Jackai, L. E. N. 1980. Use of an oil-soluble dye to determine the oviposition sites of legume pod-borer, *Maruca testulalis* (Geyer) (Lepidoptera Pyralidae). *Trop. Grain Legumes Bull.* 19: 4-9.

Jackai, L. E. N. 1981. Relationship between cowpea crop phenology and field infestation by the legume pod borer, *Maruca testulalis*. *An. Entomol. Soc. Am*. 74(4): 402-408.

Jackai, L. E. N. 1982. A field screening technique for resistance of cowpea (*Vigna unguiculata*) to the pod-borer *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae). *Bull. Ent. Res*. 72: 145-156.

Jackai, L. E. N. and Adalla, C. B. 1997. Pest management practices in cowpea: a review. In: Singh, B. B., Mohan Raj, D. R., Dashiell, K. E., and Jackai, L. E. N. (eds.), *Advances in Cowpea Research*. IITA, Ibadan, Nigeria, pp. 240-258.

Jackai, L. E. N. and Daoust, R. A. 1986. Insect pests of cowpea. *An. Rev. Entomol*. 31: 95-119.

Jackai, L. E. N. and Oghiakhe, S. 1989. Pod wall trichomes and resistance of two wild cowpea, *Vigna vexillata*, accessions to *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae) and *Clavigralla tomentosicollis* Stal. (Hemiptera: Coreidae). *Bull. Entomol. Res*. 79: 595-605.

Jagginavar, S. B., Kulkarni, K. A., and Lingappa, S. 1995. Reaction of cowpea genotypes to the damage of pod borer complex. *Karnataka J. Agric. Sci*. 8(1): 90-93.

Jain, J. P. 1982. *Statistical Techniques in Quantitative Genetics*. Tata Mc Graw Hill Co., New Delhi, 281p.

Jakhar, B. L., Prajapati, D. M., and Ravindrababu, Y. 2017. Morphological and bio-chemical factors associated with resistance to *Maruca vitrata* (Testulalis) (Geyer) in cowpea. *Legume Res.* 40(5): 959-961.

Jan, S. J. K. 2002. PIC calculator. Available: http://www.liv.ac.uk/~kempsj/pic.html

Jana, S., Som, M. G., and Das, M. D. 1982. Genetic variability and correlation studies in cowpea. *Veg. Sci.* 9: 96-107.

Jayarani, L. S. 1993. Combining ability in grain cowpea (*Vigna unguiculata* (L.) Walp.). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 122p.

Jayasinghe, R. C., Premachandra, W. T. S. D., and Neilson, R. 2015. A study on *Maruca vitrata* infestation of yard-long beans (*Vigna unguiculata* subspecies *sesquipedalis*). *Heliyon* 1(1): e00014.

Jithesh, V. G. 2009. Genetic analysis of resistance to pod borers and yield in yard long bean (*Vigna unguiculata* subsp. *sequipedalis* (L.) Verdcourt). Ph.D. (Ag.) thesis, Kerala Agricultural University, Thrissur, 218p.

Johnson, H. B. 1975. Plant pubescence: An ecological perspective. *Bot. Rev.* 41: 233-258.

Johnson, H. W., Robinson, H. F., and Comstock, R. E. 1955. Estimation of genetic variability and environmental stability in soyabean. *Agron. J.* 55: 516-532.

Jonah, P. M., Bello, L. L., Lucky, O., Midau, A., and Moruppa, S. M. 2011. Review: The importance of molecular markers in plant breeding programmes. *Glob. J. Sci. Frontier Res.* 11(5): 5-12.

Joshi, S. P., Gupta, V. S., Aggarwal, R. K., Ranjekar, P. K., and Brar, D. S. 2000. Genetic diversity and phylogenetic relationship as revealed by inter-simple sequence repeat (ISSR) polymorphism in the genus *Oryza*. *Theor. Appl. Genet.* 100: 1311-1320.

Kaga, A., Tomooka, N., Egawa, Y., Hosaka, K., and Kamijima, O. 1996. Species relationships in the subgenus *Ceratotropis* (genus *Vigna*) as revealed by RAPD analysis. *Euphytica* 88: 17-24.

Kalaiyarasi, R. and Palanisamy, G. A. 2000. Parent-progeny regression analysis and variability studies in cowpea (*Vigna unguiculata* (L.) Walp.). *Legume Res.* 23: 237-240.

Kandasamy, G., Kadambavanasundaram, M., and Rajasekaran, S. 1989. Variability in cowpea (*Vigna unguiculata* (L.) Walp.) under different environmental conditions. *Madras Agric. J.* 76: 197-199.

Karel, A. K. 1985. Yield losses from and control of bean pod borer, *Maruca testulalis* (Lep: Pyr) and *Heliothis armigera* (Lep: Noct). *J. Econ. Entomol.* 78: 1323-1326.

Karuma, R. W., Kiplagat, O., Ateka, E., and Owuoche, G. 2008. Genetic diversity of Kenyan cowpea accessions based on morphological and microsatellite markers. *E. Afr. Agric. For. J.* 76: 3-4.

KAU POP [Kerala Agricultural University]. 2011. *Package of Practices Recommendations: Crops (14th Ed.)*. Kerala Agricultural University, Thrissur, 360p.

Kempthorne, O. 1957. *An Introduction to Genetic Statistics*. John Wiley and Sons Inc., New York, 126p.

Khan, H., Viswanatha, K. P., and Sowmya, H. C. 2015. Study of genetic variability parameters in cowpea [*Vigna unguiculata* (L.) Walp.] germplasm lines. *Int. Q. J. Life Sci.* 10(2): 747-750.

Khanpara, S. V., Jivani, L. L., Vachhani, J. H., and Kachhadia, V. H. 2015. Genetic variability, heritability and genetic advance studies in vegetable cowpea [*Vigna unguiculata* (L.) Walp.]. *Electron. J. Plant Breed.* 7(2): 408-413.

Krishnaswamy, N. 1970. Cowpea. In: Kachroo, P. (ed.), *Pulse Crops of India*. Indian Council of Agricultural Research, Krishi Bhavan, New Delhi, India, pp. 201-232.

Kumar, A., Kumar, A., Satpathy, S., Singh S. M., and Lal, H. 2013. Legume pod borer (*Maruca testulalis* Geyer) and their relative yield losses in cowpea cultivars. *Prog. Hortic.* 45(1): 229-232.

Kumar, R. and Sangwan, R. S. 2000. Genetic variability and heritability in cowpea (*Vigna unguiculata* (L.) Walp.). *An. Biol.* 16: 181-183.

Kwaye, R., Shimelis, H., and Mashela, P. 2008. Combining ability analysis and association of yield and yield components among selected cowpea lines. *Euphytica* 162: 205-210.

Levin, D. A. 1973. The role of trichomes in plant defense. *Q. Rev. Biol.* 48(1): 3-15.

Li, C., Fatokun, C. A, Ubi, B., Singh, B., and Scoles, G. J. 2001. Determining genetic similarities and relationships among cowpea breeding lines and cultivars by microsatellite markers. *Crop Sci.* 41(1): 189-197.

Liang, G. H., Reddy, C. R., and Dayton, A. D. 1971. Heterosis, inbreeding depression and heritability estimates in a systematic series of grain sorghum genotypes. *Crop Sci.* 12: 400-411.

Libby, W. J., Steller, R. F., and Seitz, F. W. 1969. Forest genetics and forest breeding. *Annu. Rev. Genet.* 3: 469-494.

Litt, M. and Luty, J. A. 1989. A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am. J. Hum. Genet.* 44: 397-401.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193: 265-275.

Lush, J. L. 1949. *Animal Breeding Plan (3rd Ed.).* IOWA State College Press, Amer, 216p.

Macfoy, G. A., Dabrowski, Z. T., and Okech, S. 1983. Studies on the legume pod-borer, *Maruca testulalis* (Geyer)-VI. Cowpea resistance to oviposition and larval feeding. *Int. J. Trop. Insect Sci.* 4: 147-152.

Madhusudan, K., Ramesh, S. M., Rao, A. M., Kulkami, R. S., and Savithralnma, D. L. 1995. Combining ability in cowpea. *Crop Improv.* 22(2): 241-243.

Mafakheri, K., Bihamta, M. R., and Abbasi, A. R. 2017. Assessment of genetic diversity in cowpea (*Vigna unguiculata* L.) germplasm using morphological and molecular characterisation. *Cogent Food Agric.* 3(1): 1327092.

Magembe, E. M. 2008. Genetic diversity analysis among cowpea (*Vigna unguiculata* (L.) Walp.) accessions from Sub-Saharan Africa using simple sequence repeats (SSR's). University of Nairobi Publication, Available: https://profiles.uonbi.ac.ke/ericmagembe/publications [19 August 2018].

Mahalakshmi, M. S., Sreekanth, M., Adinarayana, M., Reni, Y. P., Rao, Y. K., and Narayana, E. 2016. Incidence, bionomics and management of spotted pod borer [*Maruca vitrata* (Geyer)] in major pulse crops in India-A review. *Agric. Rev.* 37(1): 19-26.

Malarvizhi, D. 2002. Genetic variability, heritability and genetic advance for leaf, pod and seed protein content in cowpea *Vigna unguiculata* (L.) Walp. *Legume Res.* 25: 196-198.

Malarvizhi, D. and Rangasamy, P. 2005. Character association and component analysis in F_2 generation of cowpea [*Vigna unguiculata* (L.) Walp.]. *Legume Res.* 26: 264-267.

Malik, E. P. and Singh, M. B. 1980. *Plant Enzymology and Hittoenzymology (1st Ed.).* Kalyani Publishers, New Delhi, 286p.

Mareena, M. 1989. Potential for drought tolerance in cowpea (*Vigna unguiculata* (L.) Walp.). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 102p.

Mathur, R. 1995. Genetic variability and correlation studies in segregating generations of cowpea. *Madras Agric. J.* 82: 150-152.

Maxwell, F. G. 1972. Morphological and chemical changes that evolve in the development of host plant resistance to insects. *J. Environ. Qual.* 1: 265-270.

Maynard, A. J. 1970. *Methods in Food Analysis.* Academic Press, New York, 176p.

McCouch, S. R., Chen, X., Panaud, O., Temnykh, S., Xu, Y., Cho, Y. G., and Blair, M. 1997. Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Mol. Biol.* 35(1): 89-99.

Mehta, D. R. and Zaveri, P. P. 1998. Genetic variability and association analysis in F_2 generation resulted from three selection schemes in cowpea. *J. Maharashtra Agric. Univ.* 23: 238-240.

Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31(3): 426-428.

Mishra, S. K., Singh, B. B., Chand, D., and Neena, K. N. 2009. Diversity for economic traits in cowpea (*V. unguiculata*). *Afr. J. Agric. Res.* 2: 54-58.

Nagl, N., Taski-Ajdukovic, K., Popovic, A., Curcic, A., Danojevic, D., and Kovacev, L. 2011. Estimation of genetic variation among related sugar beet genotypes by using RAPD. *Genetika* 43: 575-582.

Nair, R. G. 2006. Genetic analysis for yield and fusarium wilt resistance in line × tester progeny of yard long bean. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 120p.

Nehru, S. D. and Manjunath, A. 2001. Genetic variability for yield and accessory characters in cowpea (*Vigna unguiculata* Walp.). *Indian Agricst.* 45: 99-101.

Ngugi, E. C. K., Shakoor, A., and Omanga, P. G. A. 1985. Breeding for resistance against some major insect pests of cowpea and pigeon pea. *Insect Sci. Appl.* 6: 365-367.

Nwosu, D. J., Olatunbosun, B. D., and Adetiloye, I. S. 2013. Genetic variability, heritability and genetic advance in cowpea genotypes in two agro-ecological environments. *Greener J. Biol. Sci.* 3(5): 202-207.

Oghiakhe, S. 1995. Effect of pubescence in cowpea resistance to the legume pod borer *Maruca testulalis* (Lepidoptera: Pyralidae). *Crop Prot.* 14: 379-387.

Oghiakhe, S., Jackai, L. E. N., and Makanjuola, W. A. 1991. Anatomical parameters of cowpea, *Vigna unguiculata* (L.) Walp. stem and pod wall resistance to the legume pod borer *Maruca testulalis* Geyer (Lepidoptera: Pyralidae). *Int. J. Trop. Insect Sci.* 12: 171-176.

Oghiakhe, S., Jackai, L. E. N., and Makanjuola, W. A. 1992a. A rapid visual field screening technique for resistance of cowpea (*Vigna unguiculata*) to the legume pod borer *Maruca testulalis* (Lepidoptera: Pyralidae). *Bull. Entomol. Res.* 82(4): 507-512.

Oghiakhe, S., Jackai, L. E. N., Makanjuola, W. A., and Hodgson, C. J. 1992b. Morphology, distribution and the role of trichomes in cowpea (*Vigna unguiculata*) resistance to the legume pod borer, *Maruca testulalis* (Lepidoptera, Pyralidae). *Bull. Entomol. Res.* 82(4): 499-505.

Oghiakhe, S., Makanjuola, W. A., and Jackai, L. E. N. 1993a. Anatomical and biochemical parameters of resistance of the wild cowpea, *Vigna vexillata* Benth. (ACC. TVNU 72) to *Maruca testulalis* Geyer (Lepidoptera: Pyralidae). *Int. J. Trop. Insect Sci.* 14(03): 315-323.

Oghiakhe, S., Makanjuola, W. A., and Jackai, L. E. N. 1993b. The relationship between the concentration of phenol in cowpea and field resistance to the legume pod borer, *Maruca testulalis* Geyer (Lepidoptera: Pyralidae). *Int. J. Pest Manag.* 39(3): 261-264.

Oghiakhe, S., Jackai, L. E. N., and Makanjuola, W. A. 1995. Evaluation of cowpea genotypes for field resistance to the legume pod borer, *Maruca testulalis*, in Nigeria. *Crop Prot.* 14(05): 389-394.

Ogunkanmi, L. A., Ogundipe, O. T., Ng, N. Q., and Fatokun, C. A. 2008. Genetic diversity in wild relatives of cowpea (*Vigna unguiculata*) as revealed by simple sequence repeats (SSR) markers. *J. Food Agric. Environ.* 6(3&4): 132-137.

Panaud, O., Chen, X., and McCouch, S. R. 1995. Frequency of microsatellite sequences in rice (*Oryza sativa* L.). *Genome* 38(6): 1170-1176.

Panda, N. and Khush, G. S. 1995. *Host Plant Resistance in Insects*. CABI International, Wallingford, 431p.

Panicker, P. R. 2000. Evaluation of vegetable cowpea (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) for legume pod borer, *Maruca vitrata* (Fab.) resistance and yield. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 92p.

Panicker, P. R., Oommen, S. K., Mathew, T. B., and Saraswathy, P. 2002. Inter-relationships of flower, pod and seed damages by legume pod borer (*Maruca vitrata*) (Lepidoptera: Pyralidae) in yard long bean (*Vigna unguiculata* subsp. sesquipedalis). *Indian J. Agric. Sci.* 72: 51-53.

Panse, V. G. and Khargonkar, S. S. 1957. Genetics of quantitative characters in relation to plant breeding. *Indian J. Genet.* 17: 318-328.

Panse, V. G. and Sukhatme, P. V. 1985. Randomized blocks and Latin square. In: *Statistical Methods for Agricultural Workers*. ICAR, New Delhi, pp. 152-165.

Pasquet, R. S. 2000. Allozyme diversity of cultivated cowpea *Vigna unguiculata* (L.) Walp. *Theor. Appl. Genet.* 101(1-2): 211-219.

Patel, M. D., Ravindrababu, Y., Patel, A. M., and Sharma, S. C. 2013. Heterosis studies for seed yield and its contributing traits in cowpea [*Vigna unguiculata* (L.) Walp.]. *Environ. Ecol.* 31(2C): 1051-1053.

Patel, N. K., Desai, D. T., and Bhakta, R. S. 2010. Combining ability studies in cowpea [*Vigna unguiculata* (L.) Walp.]. *Green Farming* 1(3): 236-238.

Patel, S. J., Desai, R. T., Bhakta, R. S., Patel, D. U., Kodappully, V. C., and Mali, S. C. 2009. Heterosis studies in cowpea [*Vigna unguiculata* (L.) Walp]. *Legume Res.* 32(3): 199-202.

Pathak, R. S. 1985. Genetics of cowpea resistance to pod borer, *Maruca testulalis*. In: Napompeth, B. and Subhadrabandhu, S. (eds.), *New Frontiers in Breeding Researches.* Proceedings of the Vth SABRAO Congress, Bangkok, Thailand 25-29, November, 1985. Society for the Advancement of Breeding Researches in Asia and Oceania (SABRAO), Bangkok, Thailand, pp. 56.

Patil, H. E. and Navale, P. A. 2006. Combining ability in cowpea [*Vigna unguiculata* (L.) Walp.]. *Legume Res.* 29(4): 270-273.

Patil, R. B. and Baviskar, A. P. 1987. Correlation studies in cowpea. *J. Maharashtra Agric. Univ.* 12: 43-47.

Pearson, K. 1905. *On the General Theory of Skew Correlation and Non-linear Regression.* Dulau & Co., London, 74p.

Peter, A. J., Shanower, T. G., and Romeis, J. 1995. The role of plant trichomes in insect resistance a selective review. *Phytophaga* 7(1995): 41-63.

Pethe, U. B., Dodiya, N. S., Bhave, S. G., and Dalvi, V. V. 2018. Line × tester analysis for yield and rust resistance in cowpea (*Vigna unguiculata* L. Walp.). *Int. J. Curr. Microbiol. App. Sci.* 7(1): 511-515.

Philip, A. M. C. 2004. Genetic analysis of legume pod borer [*Maruca Vitrata* (Feb.)] resistance and yield in cowpea [*Vigna Unguiculata* (L.) Walp.]. Ph.D. (Ag.) thesis, Kerala Agricultural University, Thrissur, 232p.

Powell, W., Morgante, M., Doyle, J. J., McNicol, J. W., Tingey, S. V., and Rafalski, A. J. 1996. Genepool variation in genus Glycine subgenus Soja revealed by polymorphic nuclear and chloroplast microsatellites. *Genetics* 144: 793-803.

Prakash, M., Ganesan, J., and Venkatesan, M. 2003. Genetic variability, herirtability and genetic advance analysis in cowpea [*Vigna unguiculata* (L.) Walp.]. *Legume Res.* 26: 155-156.

Purushotham, S., Narayanaswamy, G. V., Siddaraju, R., and Girejesh, G. K. 2001. Production potential of fodder cowpea genotypes under rainfed conditions. *Karnataka J. Agric. Sci.* 14: 446-448.

Putter, J. 1974. Peroxidases. In: Bergmeyer, H. U. (ed.), *Methods of Enzymatic Analysis.* Verlag Chemie, Weinheim, pp. 685-690.

Radhakrishnan, T. and Jebaraj, S. 1982. Genetic variability in cowpea (*Vigna unguiculata* (L.) Walp.). *Madras Agric. J.* 69: 216-219.

Rahbe, Y. and Febvay, G. 1993. Protein toxicity to aphids: an *in vitro* test on *Acyrthosiphon pisum*. *Entomol. Exp. Appl.* 67: 149-160.

Ram, D. and Singh, K. P. 1997. Variation and character association studies in cowpea (*Vigna unguiculata* (L.) Walp.). *Hort. J.* 10: 93-99.

Ram, T., Ansari, M. M., and Shanna, T. R. V. S. 1994. Relative performance of cowpea genotypes in rainfed conditions in Andaman and their genetic parameter analysis for seed yield. *Indian J. Pulses Res.* 7: 72-75.

Rangaiah, S. 2000. Studies on genetic variability and component analysis in cowpea. *Curr. Res.* 29: 16-18.

Rangaiah, S. and Mahadevu, P. 1999. Genetic variability, correlation and path coefficient analysis in cowpea. *Madras Agric. J.* 86: 381-384.

Rathwa, M. G., Patel, V. N., and Makwana, A. B. 2018. Estimation of yield losses due to spotted pod borer, *Maruca testulalis* in cowpea. *Int. J. Chem. Stud.* 6(2): 322-324.

Ravindran, R. R. and Das, L. D. V. 1997. Variability, heritability and genetic advance in cowpea. *Madras Agric. J.* 84: 702-703.

Reddy, P. M., Sarla, N., and Siddiq, E. A. 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica* 128: 9-17.

Renganayaki, K. and Rengasamy, S. R. 1992. Path coefficient analysis in cowpea (*Vigna unguiculata* (L.) Walp.). *Madras Agric. J.* 79: 476-481.

Rewale, A. P., Birari, S. P., and Jamadagni, B. M. 1995. Genetic variability and heritability in cowpea. *Agric. Sci. Digest* 15: 73-76.

Rivero, R. M., Ruiz, M. J., Garcia, P. C., Lopez-Lefebre, R. L., Sanchez, E., and Romero, L. 2001. Resistance to cold and heat stress: Accumulation of phenolic compounds in tomato and watermelon plants. *Plant Sci.* 160: 315-321.

Roquib, M. A. and Patnaik, R. K. 1990. Genetic variability in grain yield and its components in cowpea, *Vigna unguiculata*. *Environ. Ecol.* 8: 197-200.

Saghai Maroof, M. A., Biyashev, R. M., Yang, G. P., Zhang, Q., and Allard, R. W. 1994. Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. *Proc. Natl. Acad. Sci. USA* 91: 5466-5470.

Sarath, P. S. and Reshma, T. 2017. Heterosis in cowpea (*Vigna unguiculata* L. Walp.) for selected traits. *Int. J. Curr. Microbiol. App. Sci.* 6(7): 522-526.

Sarikamis, G., Yanmaz, R., Ermis, S., Bakir, M., and Yuksel, C. 2010. Genetic characterisation of pea (*Pisum sativum*) germplasm from Turkey using morphological and SSR markers. *Genet. Mol. Res.* 9(1): 591-600.

Savithramma, D. L. 1992. Genetic variability in cowpea. *Agric. Res. J. Kerala* 31: 50-52.

Sawadogo, M., Ouedraogo, J. T., Gowda, B. S., and Timko, M. P. 2010. Genetic diversity of cowpea [*Vigna unguiculata* (L.) Walp.] cultivars in Burkina Faso resistance to *Striga gesnerioides*. *Afr. J. Biotechnol.* 9: 8146-8153.

Sawant, D. S. 1994. Association and path analysis in cowpea. *An. Agric. Res.* 15: 134-139.

Saxena, K. B., Chandrasena, G. D. S. N., Hettiarachchi, K., Iqbal, Y. B., Foneska, H. H. D., and Jayasekhara, S. J. B. 2002. Evaluation of pigeonpea accessions and selected lines for reaction to *Maruca*. *Crop Sci.* 42: 615-618.

Saxena, R. C. and Khan, Z. R. 1991. Genetics of insect- host plant interactions: Concepts, old and new. Mandal, A. K., Gauguli, P. K., and Banerjee, S. P. (eds.), *Advances in Plant Breeding, Volume I.* CBS Publishers and Distributors, New Delhi, pp. 111-120.

Selvakumar, G., Anandakumar, C. R., Chinniah, C., and Ushakumari, C. 2014. Combining ability analysis in the inter sub specific crosses of *Vigna unguiculata* and yard long bean (*Vigna sesquipedalis*). *Electr. J. Plant Breed.* 5: 187-191.

Semagn, K., Bjornstad, A., and Ndjiondjop, M. N. 2006. An overview of molecular marker methods for plants. *Afr. J. Biotechnol.* 5: 2540-2568.

Shannon, C. E. 1948. A mathematical theory of communication. *Bell Syst. Tech. J.* 27(3): 379-423.

Sharma, H. C. 1998. Bionomics, host plant resistance and management of the legume pod borer, *Maruca vitrata* - a review. *Crop Prot.* 17(5): 373-386.

Sharma, H. C., Saxena, K. B., and Bhagwat, V. R. 1999. The legume pod borer, *Maruca vitrata*: bionomics and management. In: *Information Bulletin no. 55*. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India, p. 44.

Sharma, H. C., Sujana, G., and Manohar Rao, D. 2009. Morphological and chemical components of resistance to pod borer, *Helicoverpa armigera* in wild relatives of pigeonpea. *Arthropod Plant Interactions* 3: 151-161.

Sharma, M. 2016. Character associations in cowpea [*Vigna unguiculata* (L.) Walp.]. M.Sc. (Ag.) thesis, Maharana Pratap University of Agriculture and Technology, Udaipur, 82p.

Sharma, M. D., Dhobal, U., Singh, P., Kumar, S., Gaur, K., Singh, S. P., Jeena, A. S., Koshy, E. P., and Kumar, S. 2014. Assessment of genetic diversity among sugarcane cultivars using novel microsatellite markers. *Afr. J. Biotechnol.* 13(13): 1444-1451.

Sharma, T. R. 1999. Genetic variability studies in cowpea. *Legume Res.* 22: 65-66.

Sharma, V., Rana, M., Katoch, M., Sharma, P. K., Ghani, M., Rana, J. C., and Chahota, R. K. 2015. Development of SSR and ILP markers in horsegram (*Macrotyloma uniflorum*), their characterization, cross-transferability and relevance for mapping. *Mol. Breed.* 35(4): 102-110.

Shull, G. H. 1914. Duplicated genes for capsule form in *Bursa bursabrids*. *Z. Indukt. Abstammungs u. Vererbungsl.* 12: 97-149.

Siddique, A. K. M. A. R. and Gupta, S. N. 1991. Genotypic and phenotypic variability for seed yield and other traits in cowpea (*Vigna unguiculata* (L.) Walp.). *Int. J. Trop. Agric.* 9: 144-148.

Simioniuc, D., Uptmoor, R., Friedt, W., and Ordon, F. 2002. Genetic diversity and relationships among pea cultivars revealed by RAPDs and AFLPs. *Plant Breed.* 121: 429-435.

Singh, A. and Singh, V. 2018. Estimates of genetic variability, heritability and genetic advance for yield and yield component traits in Indian cowpea [*Vigna unguiculata* (L.) Walp.]. *Int. J. Pure App. Biosci.* 6(1): 1142-1147.

Singh, B. B. 1999. Evaluation of new improved breeding lines without insecticide sprays. In: *Annual Report, 1999 Project.* International Institute of Tropical Agriculture, pp. 11-26.

Singh, C. and Singh, N. N. 2014. Evaluation of responses of cowpea (*Vigna unguiculata* Walpers) genotypes to infestation of legume pod borer (*Maruca vitrata* Fabricius). *J. Food Legumes* 27(4): 334-339.

Singh, M. K. and Verma, J. S. 2002. Variation and character association for certain quantitative traits in cowpea germplasm. *Forage Res.* 27: 251-253.

Singh, O. V., Shekhawat, N., Singh, K., and Gowthami, R. 2018. Genetic divergence studies in cowpea [*Vigna unguiculata* (L.) Walp.] germplasm using Mahalanobis D^2 analysis. *Int. J. Curr. Microbiol. App. Sci.* 7(03): 2616-2624.

Singh, S. R. 1978. Resistance to pest of cowpea in Nigeria. In: Singh, S. R., van Emden, H. F. and Taylor, T. A. (eds.), *Pests of Grain Legumes: Ecology and Control.* Academic Press, London, pp. 267-279.

Singh, S. R. and Allen, D. J. 1980. Pests, diseases, resistance and protection in cowpea. In: Summerfield, R. A. and Bunting, H. H. (eds.), *Advances in Legume Science.* Royal Botanical Garden, Kew, Ministry of Agriculture, Fisheries and Food, pp. 419-433.

Singh, S. R. and van Emden, H. F. 1979. Insect pests of grain legumes. *An. Rev. Entomol.* 24: 255-278.

Sivasubramanian, V. and Madhavamenon, P. 1973. Path analysis for yield and yield components of rice. *Madras Agric. J.* 60: 1217-1221.

Smith, J. G. 1979. Pests of soybean in Brazil. In: Singh, S. R., van Emden, H. F., and Taylor, T. A. (eds.), *Pests of Grain Legumes: Ecology and Control.* Academic Press, New York, pp. 167-177.

Snelling, R. O. 1941. Resistance of plants to insect attack. *Bot. Rev.* 7: 543-586.

Sobha, P. P. 1994. Variability and heterosis in bush type vegetable cowpea (*Vigna unguiculata* (L.) Walp.). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 120p.

Souframanien, J. and Gopalakrishna, T. 2004. A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. *Theor. Appl. Genet.* 109(8): 1687-1693.

Sravani, D. and Mahalakshmi, M. S. 2015. Life cycle of spotted pod borer, *Maruca vitrata* (Fabricius) (Crambidae, Lepidoptera) on greengram under laboratory conditions. *Int. J. Plant Anim. Environ. Sci.* 6(1): 31-34.

Sreekumar, K. 1995. Genetic analysis of biological nitrogen fixation traits and yield components in cowpea (*Vigna unguiculata* (L.) Walp.). Ph.D. thesis, Kerala Agricultural University, Thrissur, 175p.

Sreekumar, K., Inasi, K. A., Antony, A., and Nair, R. R. 1996. Genetic variability, heritability and correlation studies in vegetable cowpea (*Vigna unguiculata* var. *sesquipedalis*). *S. Indian Hort.* 44: 15-18.

Srinivas, J., Kale, V. S., and Nagre, P. K. 2017. Study of genetic variability, heritability and genetic advance in cowpea [*Vigna unguiculata* (L.) Walp.]. *Int. J. Curr. Microbiol. App. Sci.* 6(6): 3314-3318.

Stipanovic, R. D. 1983. Function and chemistry of plant trichomes and glands in insect resistance. In: Hedin, P. A. (ed.), *Plant Resistance to Insects*. American Chemical Society Symposium Series 208, Washington, DC, USA, pp. 70-100.

Subbiah, A., Prabhu, M., Rajangam, J., Jagadeeson, R., and Anbu, S. 2013. Genetic analysis of vegetable cowpea [*Vigna unguiculata* (L.) Walp]. *Legume Res.* 36(1): 1-9.

Suh, J. B. 1986. Report of the working group on cowpea entomology. In: *Proceedings of the International Seminar on CILSS Project on Integrated Pest Management, Niger*, 6-13 December, 1986, pp. 192-193.

Sunitha, V. 2006. Varietal screening and insecticidal evaluation against *Maruca vitrata* (Geyer) in pigeonpea. M.Sc. (Ag.) thesis, Acharya N. G. Ranga Agricultural University, 189p.

Sunitha, V., Ranga Rao, G. V, Vijaya Lakshmi, K., Saxena, K., Rameshwar Rao, V., and Reddy, Y. V. R. 2008. Morphological and biochemical factors associated with resistance to *Maruca vitrata* (Lepidoptera: Pyralidae) in short-duration pigeonpea. *Int. J. Trop. Insect Sci.* 28: 1-22.

Tamo, M., Bottenberg, H., Arodokoun, D., and Adeoti, R. 1997. The feasibility of classical biological control of two major cowpea pests. In: Singh, B. B., Mohan Raj, D. R., Dashiell, K. E., and Jackai, L. E. N. (eds.), *Advances in Cowpea Research*. International Institute of Tropical Agriculture, Ibadan, Nigeria, pp. 259-270.

Tanksley, S. D., Ganal, M. W., Prince, J. P., de Vicente, M. C., Bonierbale, M. W., Broun, P., and Martin, G. B. 1992. High density molecular linkage maps of the tomato and potato genomes. *Genetics* 132(4): 1141-1160.

Taski-Ajdukovic, K., Nagl, N., Curcic, Z., and Zoric, M. 2017. Estimation of genetic diversity and relationship in sugar beet pollinators based on SSR markers. *Electron. J. Biotechnol.* [e-journal]. Available: https://doi.org/10.1016/j.ejbt. 2017.02.001 [5 September 2018].

Tautz, D. 1989. Hyper variability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res.* 17: 6463-6471.

Taylor, T. A. 1978. *Maruca testulalis*: an important pest of tropical grain legumes. In: Singh, S. R., Taylor, T. A., and van Emden, H. F. (eds.), *Insect Pests of Grain Legumes: Ecology and Control*. London Academic Press, pp. 193-200.

Taylor, T. A. and Ezedima, F. O. G. 1964. Preliminary investigation of field pests of cowpea and methods of control. *Nigerian Agric. J.* 1: 8-11.

Tchinda, N. D., Wanjala, B. W., Muchugi, A., Fotso, Nzweundji, G., Ndoumou, D. O., and Skilton, R. 2016. Genetic diversity and gene flow revealed by microsatellite DNA markers in some accessions of African Plum (*Dacryodes edulis*) in Cameroon. *Afr. J. Biotechnol.* 15(13): 511-517.

Thiyagarajan, K. 1989. Genetic variability for yield and component characters in cowpea (*Vigna unguiculata* (L.) Walp.). *Madras Agric. J.* 76: 564-567.

Thiyagarajan, K., Natarajan, C., and Rathinaswamy, R. 1990. Combining ability and inheritance studies in cowpea (*Vigna unguiculata* (L.) Walp.). *Madras Agric. J.* 77: 305-309.

Timko, M. P. and Singh, B. B. 2008. Cowpea, a multifunctional legume. In: Moore, P. H. and Ming, R. (eds.), *Genomics of Tropical Crop Plants.* Springer Science+Business Media, New York, pp. 227-258.

Timko, M. P., Ehlers, J. D., and Roberts, P. A. 2007. Cowpea. In: Kole, C. (ed.), *Pulses, Sugar and Tuber Crops, Genome Mapping and Molecular Breeding in Plants Vol. 3*. Springer-Verlag, Berlin, Heidelberg, pp. 49-67.

Tiwari, S., Yadav, S., and Nadaf, A. 2017. Influence of morphological factors on the incidence of *Maruca vitrata* (Geyer) on short duration pigeonpea *Cajanus cajan* (L.) Millsp. *Int. J. Chem. Stud.* 5(5): 190-193.

Tudu, D., Mishra, H. N., Dishri, M., Rao, K. M., and Toppo, R. 2015. Variability and correlation studies in cowpea [*Vigna unguiculata* L. Walp.]. *Trends Biosci.* 8(1): 193-196.

Tyagi, P. C., Kumar, N., and Agarwal, M. C. 2000. Genetic variability and association of component characters for seed yield in cowpea (*Vigna unguiculata* (L.) Walp.). *Legume Res.* 23: 92-96.

Usua, E. J. 1975. Studies in relation to *Maruca testulalis*. In: *Proceedings of IITA Collaborators' Meeting on Grain Legume Improvement*, 9-13 June 1975, pp. 52-54.

Valarmathi, G., Surendran, C., and Muthiah, A. R. 2007. Studies on combining ability for yield and yield traits in inter subspecies of cowpea (*Vigna unguiculata* ssp. *unguiculata* and *Vigna unguiculata* ssp. *sesquipedalis*). *Legume Res.* 30: 173-179.

Valdez, P. C. 1989. Host plant resistance in cowpea, *Vigna unguiculata* (L.) Walp. var. *unguiculata*, to the pod borer, *Maruca testulalis* (Geyer) (Pyralidae: Lepidoptera). M.Sc. thesis, Philippines University College, Los Banos, Laguna, 112p.

van Emden, H. F. 1989. *Pest Control*. Edward Arnold, London, 117p.

Varan, R., Singh, Y. V., Jain, V. K., and Bisen, P. 2017. Heterosis studies in vegetable cow pea [*Vigna unguiculata* (L.) Walp.] for yield and quality traits heterosis. *Indian J. Ecol.* 44(4): 190-194.

Veeranna, R. 1998. Phenol and tannin reduce the damage of cowpea pod borer *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae). *Insect Environ.* 4(1): 5-6.

Veeranna, R. and Hussain, M. H. 1997. Trichomes as physical barriers for cowpea pod borer, *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae). *Insect Environ.* 3: 15.

Veeranna, R., Jayaramaiah, M., and Sreeramulu, K. R. 1999. Biology of cowpea pod borer, *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae). *Legume Res.* 22: 51-54.

Veeranna, R., Jayaramaiah, M., and Sreeramulu, K. R. 2000. Selection of resistant genotypes of cowpea and efficient insecticide against cowpea pod borer, *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae). *J. Exp. Zool.* 3: 97-100.

Venkatesan, M., Prakash, M., and Ganesan, J. 2003. Genetic variability, heritability and genetic advance analysis in cowpea (*Vigna unguiculata* (L.) Walp.). *Legume Res.* 26: 155-156.

Verma, P. M. and Afzal, M. 1940. Studies on the cotton jassid (*Empoasca devastans* Distant) in Punjab, I-varietal susceptibility and development of pest on different varieties of cotton. *Indian J. Agric. Sci.* 10: 914-956.

Vidya, C. 2000. Legume pod borer resistance and genetic divergence in domestic germplasm of yard-long bean (*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdcourt). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 121p.

Vidya, C. and Oommen, S. K. 2001. Evaluation of legume pod-borer (*Maruca vitrata*) resistance in yard-long bean (*Vigna unguiculata* subsp. *sesquipedalis*). *Indian J. Agric. Sci.* 71(12): 800-801.

Wamalwa, E. N., Muoma, J., and Wekesa, C. 2016. Genetic diversity of cowpea (*Vigna unguiculata* (L.) Walp.) accession in Kenya gene bank based on simple sequence repeat markers. *Int. J. Genomics* 2016: 1-5.

Webster, J. A. 1975. *Association of Plant Hairs and Insect Resistance: An Annotated Bibliography (Vol. no.1297).* Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C. 17p.

Williams, J. G. K., Kubelik, A. R., Lavak, K. J., Rafalski, J. A., and Tingey, S. V. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6531-6535.

Woolley, J. and Evans, A. 1979. Screening for resistance to *Maruca testulalis* Geyer in cowpea (*Vigna unguiculata* L. Walp.). *J. Agric. Sci.* 92(2): 417-425.

Woolley, J. N. 1976. Breeding cowpea for resistance to *Maruca testulalis*: methods and preliminary results. *Trop. Grain Legume Bull.* 4: 13-14.

Xu, Y. and Crouch, J. H. 2008. Marker-assisted selection in plant breeding: from publications to practice. *Crop Sci.* 48(2): 391-407.

Zhao, X. and Kochert, G. 1993. Phylogenetic distribution and genetic mapping of a (GGC)n microsatellite from rice (*Oryza sativa* L.). *Plant Mol. Biol.* 21(4): 607-614.

BREEDING COWPEA (*Vigna unguiculata* (L.) Walp.) FOR RESISTANCE TO SPOTTED POD BORER (*Maruca vitrata* Fab.)

by

Ambavane Ajinkya Rajendra
(2015-21-031)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANT BREEDING AND GENETICS
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA

2018

ABSTRACT

Cowpea [*Vigna unguiculata* (L.) Walp.] is important pulse crop rich in nutrients, especially proteins. This crop is cultivated in the tropics of Asia, Africa and other parts of the world. Nevertheless, the production of cowpea is unable to achieve its summit. One of the prime reasons for this is the infestation of a notorious pest, the spotted pod borer, (*Maruca vitrata* Fab.; Lepidoptera: Crambidae). Spotted pod borer is one of the most important post-flowering pests of cowpea in the tropics. It is a major lepidopteran pest and damage cause to cowpea by the pest almost always crosses economic threshold level. Hence, the present investigation was conducted in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, Kerala Agricultural University, Thrissur during 2015 to 2018 with the objective of identification and incorporation of resistance against spotted pod borer in high yielding varieties of cowpea and assessment of parental polymorphism at the molecular level

Thirty genotypes of cowpea formed the material for the study. These genotypes were subjected to field screening against spotted pod borer. These genotypes were also evaluated for morphological and biochemical basis of resistance. Five selected genotypes from experiment 1 then hybridised with four high yielding genotypes *viz*., Geethika, Vellayani Jyothika, Lola and Kashi Kanchan following Line × Tester mating design. Twenty F_1 hybrids evaluated for field resistance and the morphological basis of resistance. Progenies of selected F_1 hybrids grown as F_2 populations and evaluated for same parameters as like F_1s. Thirty genotypes were also subjected to molecular screening by 40 SSR primers.

Wide variation was observed in terms of different damage parameters. Ten genotypes *viz*., Hridya, Palakkadan thandan payar, EC 300039, EC 98668, EC 101216, IC 52110, IC 39945, IC 2918, IC 39922 and IC 39916 recorded total damage below five per cent. Among them, IC 39922 observed to have no flower bud and flower damage, EC 300039, EC 98668, IC 52110, IC 39945, IC 2918 and IC 39916 recorded no flower damage, whereas, Palakkadan thandan payar, IC 39945, IC 2918 and IC 39947 were free from pod damage. The highest damage was recorded in the variety Bhagyalakshmy (48.46 %) followed by variety Lola (30.04 %).

Analysis of the morphological basis of resistance to spotted pod borer revealed the negative correlation of trichome density and length on flower bud, trichome density on the pod and pod wall thickness with respective damage parameters. With respect to

the biochemical basis of resistance to spotted pod borer, total sugar content, reducing sugar content and non-reducing sugar content of flower bud and pod showed a positive correlation with damage parameters, but the correlation was not significant. However, the total protein content of pod showed a strong and positive correlation with pod damage. Total phenol content of flower bud showed strong negative correlation with damage parameters. Polyphenol oxidase activity in flower bud and pod exhibited a strong negative correlation with damage parameters. The crude fibre content of pod also showed a strong negative correlation with pod damage.

In experiment 3, three SSR primers *viz*., CLM0061, CLM0295 and CLM0300 recorded high polymorphic information content (0.70, 0.71 and 0.76, respectively). Primer CLM0190 observed to have high amplicon size (307.03-415.73 bp). Jaccard's similarity coefficient was highest between IC 52118 and IC 39916 (0.643) and was lowest between Kashi Kanchan and TVX-944 (0.022). Cluster analysis of SSR data grouped 30 genotypes in 22 clusters, and the 21st cluster was observed to have more members (3 genotypes). Most resistant genotype, IC 2918, grouped in a separate cluster which proved its diverse nature from other genotypes. Principal component analysis of SSR data placed three yard-long bean genotypes *viz*., Geethika, Vellayani Jyothika and Lola near to each other in a two-dimensional score plot. The same analysis also formed two clusters with more number of genotypes which placed resistant and susceptible genotypes separately.

In field screening of F_1 hybrids, Hybrid 20 observed to have total damage below 5 per cent. Eight hybrids recorded total damage in the range of 5 to 10 per cent. These hybrids were selected for next experiment. Line × Tester analysis of F_1s revealed Kashi Kanchan, EC 98668 and IC 2918 as a good combiner for resistance against spotted pod borer. Hybrid 5, Hybrid 6, Hybrid 10, Hybrid 11, Hybrid 13, Hybrid 15, Hybrid 16, Hybrid 17, Hybrid 18, Hybrid 19 and Hybrid 20 observed to have desired negative heterosis (mid-parent) for total damage. In F_2 plant screening, Hybrid 1 population recorded low mean for total damage (6.76 %), whereas, the population of Hybrid 3 recorded high mean (12.17 %). Around 100 plants of F_2 generation recorded total damage below ten per cent. Out of them, around 38 plants also registered good yield. These plants should be further evaluated to isolate high yielding resistant segregants.