CHARACTERIZATION OF VEGETABLE COWPEA
(*Vigna unguiculata* (L.) Walp.)

MANJU P. R.

Thesis submitted in partial fulfilment of the requirement for the degree of

Doctor of Philosophy in Horticulture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2006

Department of Olericulture
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM - 695 522

I

DECLARATION

I hereby declare that this thesis entitled **"Characterization of vegetable cowpea (*Vigna unguiculata* (L.) Walp.)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any other university or society.

Vellayani,
10-3-2006

MANJU P. R.
(2002-22-05)

II

CERTIFICATE

Certified that this thesis entitled **"Characterization of vegetable cowpea (*Vigna unguiculata* (L.) Walp.)"** is a record of research work done independently by Ms. Manju P. R. (2002-22-05) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Vellayani,
10-3-2006

Dr. L. Rajamony
(Chairman, Advisory Committee),
Associate Director of Research (Planning)
Kerala Agricultural University,
Thrissur – 680 656

APPROVED BY:

CHAIRMAN

Dr. L. RAJAMONY,
Associate Director of Research (Planning),
Kerala Agricultural University, Vellanikkara,
Thrissur – 680 656.

3.06.2006

MEMBERS

Dr. M. ABDUL VAHAB
Associate Professor and Head,
Department of Olericulture,
College of Agriculture, Vellayani,
Thiruvananthapuram – 695 522.

3/06/06

Dr.V. A. CELINE,
Associate Professor,
Department of Olericulture,
College of Agriculture, Vellayani,
Thiruvananthapuram – 695 522.

8/6/06

Dr. SUNNY K. OOMMEN,
Associate Professor,
Department of Plant Breeding and Genetics,
College of Agriculture, Vellayani,
Thiruvananthapuram – 695 522.

3.6.06

Dr. K. B. SONI,
Assistant Professor,
Department of Plant Biotechnology,
College of Agriculture, Vellayani,
Thiruvananthapuram – 695 522.

3.6.06

Dr. C. R. SUDHARMAIDEVI,
Associate Professor,
Department of Soil Science and Agricultural Chemistry,
College of Agriculture, Vellayani,
Thiruvananthapuram – 695 522.

3/6/06

EXTERNAL EXAMINER

3.6.06

(S. NATARAJAN)
Dean, HC&RI, TNAU
Periyakulam

ACKNOWLEDGEMENT

I wish to express my deep sense of gratitude and indebtedness to :

The 'God Almighty' for his unspeakable help rendered through various hands, which helped me pursue this endeavor to completion.

Dr. L. Rajamony, Associate Director of Research (Planning) and Chairman of the advisory committee for his valuable guidance and suggestions which contributed much to my work. I express my heartfelt gratitude and indebtness to him for his critical scrutiny of the manuscript and sustained interest which greatly facilitated in the preparation of the thesis.

Dr. M. Abdul Vahab, Associate Professor and Head, Department of Olericulture, for his genuine interest, periodic suggestions and whole-hearted help.

Dr. V. A. Celine, Associate Professor, Department of Olericulture for her valuable comments and encouragement throughout the study.

Dr. K. B. Soni, Assistant Professor, Department of Plant Biotechnology, with her expertise in molecular biology has always been there to provide guidance and direction to my work.

Dr. Sunny K. Oommen, Associate Professor, Department of Plant Breeding and Genetics for critical scrutiny of the manuscript and valuable suggestions.

Dr. C. R. Sudharmaidevi, Associate Professor, Department of Soil Science and Agricultural Chemistry for helping me to conduct the biochemical analysis.

Dr. M. M. Viji, Dr. Manju, R. V., Dr. Roy Stephen of the Department of Plant Physiology and Dr. Philippose Joshua, Department of Processing Technology for providing the lab facilities for doing the experiments.

All the teaching and non-teaching staff of the Department of Olericulture as well as Instructional farm, Vellayani for their sincere help during the course of investigation.

Dr. P. Janardhanan Pillai, Director of Research, Dr. P. Santhakumari, Associate Professor, Department of Plant Pathology and Reshmi, C. R., Agricultural Officer for helping me in seed collection.

Krishnan Sir, Department of Agricultural Statistics, College of Horticulture, Vellanikkara, Jacob and Eldo, students of the same college for statistical analysis of the experimental data.

Kerala Agricultural University for the award of Senior Research Fellowship.

Prajeesh, Satheesh, Anita chechi as well as all Research Assistants of the Department of Plant Biotechnology for being with me throughout my research.

My friends Simi and Geethalakshmy for their noble help and moral support throughout the course of study and research.

Jayakumar and Prasad chettan for taking photographs for my thesis work, Biju, P. and staff of Photopark, Thiruvananthapuram for editing them.

Vishnu S. Nair (Agrl. Officer), Suresh Sir (Deputy Director) and Muneera Madam (Joint Director of Agriculture), Kannur District for helping me to get leave for completing my thesis work. My friend Leaya (Agrl. Officer) for her love and moral support.

Somasundaran and Aboobacker (Agrl. Assistants) and Durgapriya Madam (Assistant Director of Agriculture), Malappuram District for their support and co-operation.

My husband for his love and understanding, without which I could not have accomplished this endeavor.

My father and mother for their blessings and for being instrumental throughout my work and making me what I am today.

My brother Hari, Ambili, Anu, Roshni, Shaju, Sooraj, Shiva, Harish chettan, Sandhya, Harsha and above all my in-laws and ammumma for their prayers and moral support.

And finally I convey my deep feeling of indebtness to my daughter Parukutty for her love, for never being demanding and bearing with an ever absentee mother. To her I owe everything.

MANJU, P. R.

Dedicated to
my husband, parukutty,
amma and achan

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

Sl. No.	Title	Appendix No.
1.	Score card for the organoleptic evaluation of cooked cowpea fruits	I

Introduction

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.), one of the most important cosmopolitan crops, is grown in many parts of India and else where in the world. It is important as a pulse and vegetable crop and enriches soil fertility by fixing atmospheric nitrogen. Cowpeas are of ancient cultivation in Asia and Africa where immense diversity in *V. unguiculata* occur. The subspecies *sesquipedalis* is widespread in the humid tropics of India (Verdcourt, 1970 ; Purseglove, 1974).

In Kerala, vegetable cowpea is one of the most favourite crops as it ensures a stable market throughout the year. The traditional vernaculars *viz.*, 'Achingapayar', 'Kurutholapayar', 'Vanpayar', 'Pathinettumaniyan' etc., used to refer vegetable cowpea / yard long bean indicates that Kerala is the land of vegetable cowpea. Perhaps cowpea is the only vegetable evenly distributed and preferred in all the 14 districts of Kerala.

Over several decades of cultivation, genetically diverse types of the crop got evolved and maintained in the state. Inspite of large genetic diversity in the crop, the variability utilized for crop improvement in general is quite restricted. This may be due to poor characterization of germplasm and lack of understanding of the relations existing among cultivars.

In recent years, molecular markers have been developed that could aid in better management of genetic resources and could enhance the benefits from them. The information generated through DNA markers like RAPD (Random amplified polymorphic DNA) as well as protein markers gives a clear picture on domestication, the structure of variability within and between species, the relationship between populations and the gene flow between wild and cultivated species.

Now-a-days, plant DNA fingerprinting using molecular markers has come into the limelight because of two multilateral agreements namely, Trade Related Intellectual Property Rights (TRIPS) and the Convention on Biological Diversity (CBD). Enforcement of their provisions is possible only if the identity and ownership of the genotypes can be established unequivocally. DNA fingerprints are accurate as they emanate from nucleotide sequence differences between individuals.

In this context, it is high time to characterize the available landraces and cultivars of vegetable cowpea in Kerala. Such a data-base could be used as a powerful document for genetic exchange and future crop improvement programmes.

Taking into consideration of all these aspects, the present study was undertaken with the following objectives:

1. To genetically catalogue the available landraces of vegetable cowpea.
2. To identify superior genotypes based on yield, quality and pest and disease resistance.
3. To estimate the extent of available variability for important characters.
4. To estimate the role of genetic contribution in the expression of each character.
5. To measure the degree and pattern of association between the characters.
6. To study the extent of genetic divergence among the landraces and to group them into clusters based on genetic distance.
7. To characterize as well as to study the extent of variability in the available germplasm using RAPD (Random amplified polymorphic DNA) based DNA markers and protein markers.

Review of Literature

2. REVIEW OF LITERATURE

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most important leguminous vegetable crops of Kerala. The morphotypes grown in the state mainly belongs to three groups *viz.*, grain type (*V. unguiculata* ssp. *catjang* Wall), vegetable type (yard long bean) (*V. unguiculata* ssp. *sesquipedalis* (L.) Verdcourt) and dual purpose type (*V. unguiculata* ssp. *cylindrica*) (Gopalakrishnan, 2004). Eventhough a lot of work has been done on grain cowpea, very little attention has been paid to the improvement of vegetable types.

The available literature on variability studies in cowpea in general as well as other vegetables are reviewed under the following subheads.

2.1 Genetic cataloguing

2.2 Variability

2.3 Heritability and genetic advance

2.4 Correlation studies

2.5 Path coefficient analysis

2.6 Selection index

2.7 Genetic divergence

2.8 Quality characters

2.9 Reaction towards pest and disease incidence

2.10 Anatomical characters

2.11 Biochemical characters

2.12 Seed protein electrophoresis

2.13 Molecular characterization based on RAPD

2.1 Genetic cataloguing

The cowpea gene pool is characterized by its unusually large size with wide morphological variations (Pasquet, 2000).

Thirty yard long bean genotypes were scored for morphological characters using IPGRI descriptor by Resmi (1998). Association was found for flower colour with stem pigmentation, pod pigmentation and seed colour. Wide variability was noticed upon cataloguing 330 vegetable cowpea accessions under NATP on "Sustainable management of biodiversity of vegetable cowpea and amaranthus", implemented at the Department of Olericulture, College of Horticulture, Vellanikkara (Gopalakrishnan, 2004).

Classification of cowpea cultivars into three subspecies based on various growth and reproductive characters were attempted by Ebong (1970) and Hazra *et al.* (1993).

Variability in 1200 genetic stocks of cowpea was studied by Magoon *et al.* (1973), and identified some Indian stocks as sources of genes for vigorous growth and leafiness. Marker genes affecting pigmentation of stem, petiole, flower, pod and seed were also identified.

DeMooy (1985) described 180 accessions of Botswana cowpea germplasm (*V. unguiculata*) based on morphological characters. Similar works were also done on Nigerian vegetable cowpea (*V. unguiculata* subsp. *unguiculata*) by Uguru (1996).

A key to cowpea varieties (*V. unguiculata* subsp. *unguiculata*) based on seed characteristics such as seed coat pigmentation and texture, seed size and 1000-seed weight was presented by (Asante *et al.*, 2004). . Seed that had high tannin level was reported to have either brown mottled, dark mottled, brown or flesh-coloured testa.

Padi (2003) studied the genetic control of pigmentation in different parts of cowpea (*V. unguiculata* (L.) Walp.). A monogenic control for colour expression was found in leaf node pigmentation, flower colour, immature pod colour, seed coat colour, seed eye colour and seed eye colour pattern.

2.2 Variability

Genetic variability in the base population is a pre-requisite for effective crop improvement. Considerable variation for several characters in cowpea was reported by Radhakrishnan and Jebaraj (1982), Sobha and Vahab (1998), Kumar and Sangwan (2000) and Venkatesan *et al.* (2003).

2.2.1 Morphological characters

2.2.1.1 Growth characters

Wide range of variation for plant height was reported by Pandita *et al.* (1982), Anbuselvam *et al.* (2000), Rangaiah and Mahadevu (2000) and Singh and Verma (2002). Pekoen and Artuk (2004) compared some cowpea genotypes from Turkey and reported significant differences for days to seedling emergence.

High phenotypic and genotypic variances were observed for the length of main stem by Vidya (2000) in yard long bean and by Ajith (2001) in bush type vegetable cowpea.

Moderate values of phenotypic and genotypic coefficients of variation (PCV and GCV respectively) were recorded for plant height by Kalaiyarasi and Palanisamy (2000), while Philip (2004) found low values.

Number of branches per plant was reported to have high variability by Bapna and Joshi (1973) and Borah and Khan (2000). Relatively high PCV and GCV were also recorded for primary branches (Nehru and Manjunath, 2001 ; Pal *et al.*, 2003).

In yard long bean, significant differences among genotypes were observed for petiole length and length and breadth of terminal as well as lateral leaflets (Resmi, 1998). Borah and Khan (2000) reported low PCV and GCV for leaflet length. Ogbonnaya *et al.* (2003) evaluated cowpea genotypes varying in drought tolerance for collar diameter and root : shoot ratio in hydroponics. Based on

the results, selection of cowpea for vigorous growth under well watered conditions could be conducted by means of hydroponics.

2.2.1.2 Flowering characters

Wide range of variability was reported for days to first flowering by Pandita *et al.* (1982), while moderate and low PCV and GCV were found by Singh and Verma (2002) and Philip (2004) respectively.

High genetic variability was observed for peduncle length by several workers (Trehan *et al.*, 1970). The genomic relationship between subspecies *unguiculata* and *sesquipedalis* was studied by Neema (1986) and reported higher pollen fertility in subspecies *sesquipedalis*. Singh and Dapaah (1998) characterized a partial male sterile line of cowpea (IT85D-3626) based on morphology, pollen viability and pod set. Partial male sterile plants were found to have 77.0 per cent pollen viability compared to 98.3 per cent in normal plants.

2.2.1.3 Pod and yield characters

High PCV and GCV were recorded for pods per plant and yield by Lakshmi and Goud (1977), Kumari *et al.* (2003) and Kutty *et al.* (2003).

Pod length was reported to have high genetic variability by Bapna and Joshi (1973), while moderate values of PCV and GCV were also observed (Kalaiyarasi and Palanisamy, 2000 ; Singh and Verma, 2002).

Veeraswamy *et al.* (1973) and Resmi (1998) found significant differences among vegetable cowpea genotypes for pod girth. High phenotypic and genotypic variances were observed for pod weight by Rangaiah (2000) and Ajith (2001).

Sobha (1994) observed high coefficient of variation for seeds per pod, while less variability was reported by Singh and Verma (2002). 100-seed weight was also reported to have high PCV and GCV values (Kumari *et al.*, 2003 ; Philip, 2004).

Jalajakumari (1981) observed significant variation among 17 varieties of cowpea for seed length and seed width, whereas seed thickness recorded the least variation.

2.3 Heritability and genetic advance

Effectiveness of selection depends upon the heritability and genetic advance of the character studied. High heritability coupled with high genetic advance for several characters was reported by several workers in cowpea (Ajith, 2001 ; Philip, 2004).

2.3.1 Morphological characters

2.3.1.1 Growth characters

Vine length was reported to have high heritability and moderate genetic advance by Resmi (1998), while Vidya (2000) observed high genetic advance.

In yard long bean, Resmi (1998) found high heritability and low genetic advance for primary branches per plant while high genetic advance was reported by Ajith (2001) in bush type vegetable cowpea.

High heritability and low genetic advance was recorded for petiole length and length and breadth of terminal and lateral leaflets (Resmi, 1998). Similar results were also reported for stem thickness, leaf length and width by Borah and Khan (2001).

2.3.1.2 Flowering characters

Sreekumar *et al.* (1996) observed high heritability and low genetic advance for days to flowering, whereas Tyagi *et al.* (2000) reported high genetic advance. Peduncle length was found to have high heritability along with high genetic advance by Panicker (2000) and Pal *et al.* (2003).

2.3.1.3 Pod and yield characters

In the case of pod characters, Roquib and Patnaik (1990) reported high heritability and genetic advance for pod length, while Panicker (2000) recorded low genetic advance.

Sobha (1994) observed high genetic advance for pod girth, while low values were reported by Ajith (2001).

In vegetable cowpea, high heritability and genetic advance was recorded for pods per plant and yield by Tikka *et al.* (1977) and Angadi *et al.* (1978). Umaharan *et al.* (1997) reported high heritability for pod weight and can be effectively selected for in the early generations of improvement of the crop.

Jalajakumari (1981) observed high heritability and genetic advance for seed characters *viz.*, seed length, seed width and seed thickness. Based on high heritability and genetic advance, Apte *et al.* (1987) suggested 100-seed weight and seeds per pod as selection criteria for cowpea improvement. On the other hand, low heritability and genetic advance was reported by Patil and Patil (1987). High heritability and low genetic advance was also recorded in cowpea (Pal *et al.*, 2003).

2.4 Correlation studies

A thorough knowledge of the relationship between yield and its component characters make crop improvement more effective. Genotypic correlation coefficients were found higher than phenotypic and environment ones for most of the traits in cowpea (Singh *et al.*, 1982 ; Tyagi *et al.*, 2000).

Based on correlation coefficients, selection for pods per plant, seeds per pod and test weight for yield improvement in cowpea was suggested by Chikkadyavaiah (1985), Patil and Bhapkar (1987) and Subbiah *et al.* (2003).

Days to first flowering was observed to have high negative correlation with yield in yard long bean (Panicker, 2000). Positive and significant association was found between primary branches per plant and yield (Vidya, 2000). Plant height

exhibited high positive correlation with yield (Kalaiyarasi and Palanisamy, 2001 ; Neema and Palanisamy, 2003).

Trehan *et al.* (1970) and Kumar *et al.* (1983) observed high positive correlation between yield and peduncle length. Pod length, pod girth and pod weight showed significant correlation with yield (Vidya, 2000). Kutty *et al.* (2003) found number of pickings positively correlated with yield at both phenotypic and genotypic levels.

2.5 Path coefficient analysis

Path coefficient analysis provides an effective means of partitioning the genotypic correlation coefficients into direct and indirect effects of the component characters on yield. In yard long bean, pods per plant was found to exert the highest direct effect on yield followed by pod weight (Resmi, 1998 ; Vidya, 2000 ; Kutty *et al.*, 2003).

Panicker (2000) reported that the highest direct effect on yield was contributed by days to first flowering, whereas Tyagi *et al.* (2000) observed a negative direct effect of days to first flowering on yield.

Hanchinal *et al.* (1979) suggested that emphasis should be given on branches per plant while selecting a good genotype for enhancing the yield of cowpea. In bush type vegetable cowpea, Sobha (1994) recorded pod weight and pod girth as the most important yield components, whereas Ajith (2001) found pods per plant and pod weight contributing more towards yield.

Pod length exhibited high direct effects on yield (Choulwar and Borikar, 1987). High positive indirect effects of pod length through pod weight and days to flowering is also reported (Resmi, 1998). Stem weight showed the greatest direct contribution to green fodder yield. Primary branches, stem diameter and petiole length also contributed to green fodder yield through stem weight (Kutty *et al.*, 2003).

Jalajakumari (1981) observed that seed width exerted a positive direct effect on seed yield. Obisesan (1985) reported high indirect effects of peduncle

length. Seeds per pod and 100-seed weight also recorded high positive effects on yield (Venkatesan *et al.*, 2003).

2.6 Selection index

Average selection index is more effective than visual pedigree or bulk population methods for developing high yielding lines in cowpea (Yap, 1983).

Tikka *et al.* (1977) proposed an efficient selection index involving the characters *viz.*, plant height, pods per plant and test weight. Jalajakumari (1981) applied discriminant function analysis on 17 varieties of cowpea. Superior genotypes of yard long bean were identified by constructing selection indices using the characters namely, vine length, primary branches, petiole length, length and breadth of lateral leaflets, days to flowering, pod length, pod girth, pod weight, pods per inflorescence, pods per kilogram, pods per plant and yield (Resmi, 1998).

Philip (2004) worked out selection indices for 50 genotypes of cowpea on the basis of pods per plant, number of inflorescence per plant, pods per inflorescence, pod length, seeds per pod and 100-seed weight. Five superior genotypes were selected for hybridization programme as female parents to develop F_1 hybrids.

2.7 Genetic divergence

A knowledge of genetic diversity, its nature and degree is useful in the improvement of any heritable character. Sobha (1994) assessed genetic diversity among bush type vegetable cowpea genotypes, while Resmi (1998) studied the clustering pattern in yard long bean. Anbuselvam *et al.* (2000) grouped 50 genotypes of cowpea into four different clusters. Based on the *per se* performance and genetic divergence, desirable genotypes were suggested for use in crossing programme. Kapoor *et al.* (2000) also identified divergent genotypes based on Mahalanobi's D^2 statistic.

Kumar *et al.* (1982) observed that days to 50 per cent maturity, pod length, pod width and 100-seed weight contributed to genetic divergence.

Ushakumari *et al.* (2000) found that the highest contribution towards divergence were for plant height, seeds per pod, number of branches, pods per cluster and pod length.

Backiyarani *et al.* (2000) reported that geographic diversity was not an index of genetic diversity. Similar results were also reported by Borah and Khan (2001). Venkatesan *et al.* (2004) suggested a possible genetic drift and selection under different environment could have caused greater divergence than geographical distance and hence desirable diverse plants may be selected from the locally adapted varieties.

2.8 Quality characters

2.8.1 Keeping quality, pod protein and pod fibre contents

In yard long bean, Resmi (1998) observed a shelf life of 2.00 to 4.22 days. The PCV and GCV recorded was 20.89 and 20.18 per cent respectively along with high heritability and genetic advance. A negative correlation of keeping quality of pods with breadth of terminal leaflets and with length and breadth of lateral leaflets was noticed.

Cowpea provides an uninterrupted protein supply throughout the year as fresh immature pods or as dry grains (Uguru, 1996).

Significant differences among landraces of cowpea for crude protein content (12.64 to 16.19 per cent) were observed by Gupta and Pradhan (1974). Kochhar *et al.* (1988) analyzed ground seeds of 24 cowpea cultivars from Nigeria and reported a crude protein content of 23 to 31 per cent and true protein content of 20.7 to 27.3 per cent on dry weight basis. In vegetable cowpea, Aghora *et al.* (1994) recorded 2.5 to 5.9 per cent protein, whereas in yard long bean, a narrow range of 4.75 to 5.90 per cent was observed with high heritability and low genetic advance (Resmi, 1998). Similar results were also reported by Borah and Khan (2000).

A mean pod fibre content of 1.97 per cent was reported by Resmi (1998) in yard long bean. The PCV and GCV were 14.39 and 13.32 per cent respectively with moderately high heritability and high genetic advance. Significant

differences for crude fibre content among yard long bean genotypes (1.92 to 2.20 per cent) were also reported by Vidya (2000), while Philip (2004) observed 1.95 to 2.65 per cent crude fibre content in grain cowpea. Wide range of variation for crude fibre content in yard long bean was also reported by Lovely (2005).

Correlation and path analysis revealed that fibre content of pods is one of the main determinants of pod yield (Kar *et al.*, 1995). Similar reports were also given by Peksen *et al.* (2002).

Omueti *et al.* (1986) analysed pods of vegetable cowpea at various developmental stages and found that crude fibre decreased with age of pods. Negri *et al.* (2001) observed no significant correlation between crude protein and crude fibre or between them and organoleptic characters.

2.8.2 Organoleptic analysis

Omueti *et al.* (1986) observed that cowpea pods harvested between seven and ten days after flowering were more crispy, tasty and high in nutrients and therefore it is nutritionally acceptable for consumption. Umaharan *et al.* (1997) conducted a preliminary study of consumer preferences for pod characteristics in vegetable cowpea, which showed a general preference for greener, longer, fleshier pods that are less seedy. It was found that these characters could be improved by carefully selecting the parents in hybridization programmes.

Negri *et al.* (2001) evaluated Italian cowpea landraces based on visual appearance, first impression in the mouth, taste, tenderness of the skin, overall judgement and chemical composition. There were significant differences among the cultivars for most of the organoleptic characters. Overall judgement was significantly correlated with visual appearance, first impression in the mouth and taste. Taste was also correlated with visual appearance and first impression.

2.9 Reaction towards pest and disease incidence

Legume pod borer [*Maruca vitrata* (Fab.)]

Legume pod borer, *Maruca vitrata* (Fab.) (Syn. *Maruca testulalis* Geyer) is a major limitation to successful cultivation of cowpea in many countries (Singh and Jackai, 1988). The crop loss caused by the pest is tremendous since the larvae feed on flowers and developing pods (Jackai and Adalla, 1997). The moth lays eggs on the flower buds, flowers, and young pods and the first instar larvae start feeding at the oviposition sites. It then bores into the pods and devours the ripening seeds one after another. The larval burrow is marked by a mass of brownish excrement at the entrance of the gallery (Panicker, 2000).

Source of resistance

Screening of cowpea, *Vigna unguiculata* (L.) Walp. germplasm for pod borer resistance resulted in the identification of tolerant lines / varieties (Singh, 1978). A field screening technique for locating resistance in cowpea to pod borer, *M. vitrata* was developed by Jackai (1982). Based on this technique, TVu 946 was the most resistant cowpea cultivar. Studies conducted in the screen house showed that females had non-preference for TVu 946, while biochemical studies provided evidence of antibiosis due to nutritional and antibiotic factors (Macfoy *et al.*, 1983).

A large number of selected wild *Vigna* accessions were evaluated by Jackai *et al.* (1996) and found that *V. vexillata* had the most resistant accession. Both antibiosis and antixenosis modalities of resistance were expected to be involved.

In yard long bean, screening for legume pod borer resistance was done by Panicker (2000), who observed a plant susceptibility index ranging from 33.13 to 109.37. Larval count in flowers was not correlated with any of the damage parameters. Significant and positive correlation was found among percentage pod infestation, pod damage severity and seed damage index. No significant correlation was noted between pod fibre content and percentage pod infestation.

Employing Mahalanobi's D^2 statistic, 50 yard long bean varieties were grouped into seven clusters based on the different legume pod borer damage parameters (Vidya, 2000). In grain cowpea, Philip (2004) observed a seed damage index of 40 to 192 and plant susceptibility index of 16.09 to 66.50. Flower damage was positively correlated with pod damage parameters and negatively with peduncle length.

Role of plant characters in host plant resistance

Oghiakhe *et al.* (1992) found a negative and significant correlation between pod wall trichome density and pod damage by legume pod borer in cowpea and highlighted the role of trichome density in reducing pod damage. Pubescence (trichomes) in wild and cultivated cowpea adversely affected oviposition, mobility, food consumption and utilization by the pod borer (Oghiakhe, 1995). Veeranna and Hussain (1997) observed a trichome density of 24.41 / $9mm^2$ in the resistant genotype(TVX-7), while the susceptible genotype DPCL-216 had a low trichome density of 12.82 / $9mm^2$.

Thick and compact collenchyma cells in the stems and fibrous tissues on the petal surface contributed to pod borer resistance in the resistant variety TVNu 72, with trichomes as the principal factors in the resistance (Oghiakhe *et al.*,1993).

Cowpea varieties with upright and long peduncles that hold pods away from the canopy as well as from each other suffer less damage by legume pod borer (Singh, 1978). Oghiakhe *et al.* (1991) found that *V. unguiculata* cultivars with pods held within the canopy suffered significantly more damage than cultivars with pods held above the canopy. They opined that larvae penetrate the pods more successfully when pods are in contact with each other or with the foliage. Pods with wide angles were damaged only on one and rarely on both pods. Selection and breeding for wide pod angle was suggested for reducing pod borer damage in cowpea pods (Oghiakhe *et al.*,1992a).

Pod size and rate of pod growth are important factors in the susceptibility of cowpea to attack by pod borer (Tayo, 1988). Oghiakhe *et al.* (1992b) reported that eventhough the pressure required to penetrate pod wall increases with pod age, the correlation between pod damage severity and pod wall toughness was not significant.

2.10 Anatomical characters

2.10.1 Trichomes

Trichomes (pubescence), hair like outgrowths from the aerial plant parts, have been gradually eliminated from cultivars by selection, although they show great promise towards the development of multiple pest-resistant cultivars. The role of trichomes, as evidenced from highly pubescent wild *Vigna* species in resistance to *Maruca vitrata*, *Clavigralla tomentosicollis* and *Callosobruchus maculatus* was described by Oghiakhe (1997).

Jackai and Oghiakhe (1989) reported glandular and non-glandular trichomes to be present in both cultivated and wild cowpea. Trichomes in the two types of cowpea differ significantly only in their number and non-glandular trichome length. Rather than density, trichome length and angle to pod surface seemed to be more important for resistance. Significantly lower densities of glandular trichomes was observed in cultivated genotypes of cowpea (*Vigna unguiculata* ssp. *unguiculata*) when compared to wild *Vigna* species (*V. vexillata*) which suffered less damage due to *Clavigralla.*

Studies have shown that glandular trichomes contain high concentration of phenol and alkaloids which enhance their biochemical defence against insects (Oghiakhe *et al.*, 1992). In yard long bean, Panicker (2000) reported a non-glandular trichome density range of 1.50 to 7.00 / mm^2 area of pod wall surface, while Philip (2004) observed a pod trichome count of 1.67 to 6.83 / mm^2 in grain cowpea.

2.10.2 Stomata, vascular bundle and cuticle

Ghimiray and Das (1996) observed higher mean stomatal frequency / mm^2 in cultigroup *unguiculata* than *sesquipedalis*, while *sesquipedalis* had somewhat larger stomatal size and pore dimension than *unguiculata*. High variability along with high heritability and moderately high genetic advance was noticed for stomatal frequency, stomatal length and breadth by Hazra *et al.* (1996). The adaxial stomatal frequency was highest in cultivar group *biflora*, lowest in *sesquipedalis* and intermediate in *unguiculata*.

Hazra *et al.* (1988) crossed genotypes representing subspecies *unguiculata*, *sesquipedalis* and *cylindrica*. Some hybrids (*unguiculata* x *unguiculata*) showed marked heterosis over the mid-parental and better parent values for stomatal frequency, length and breadth, while others showed hybrid depression. The functional composition of herbivorous insect assemblages was correlated with new and mature leaf anatomy in 18 plant species. Densities of sessile phloem feeders, rostrum chewers and all herbivores were significantly negatively correlated with cuticle thickness, vascular tissue depth and stomatal length and positively correlated with stomatal density.

2.11 Biochemical characters

2.11.1 Phenol

Phenolic content of seeds of six genotypes of cowpea resistant to various insect pests (*Helicoverpa armigera*, *Maruca vitrata*, *Psidia tikora* and bruchids) ranged from 0.01 to 0.05 per cent of defatted meal (Prasad *et al.*, 1996).

Oghiakhe *et al.* (1993) observed that phenol concentration varied between different plant parts of cowpea cultivars at the same growth stage, and generally decreased with increase in plant age. Despite the difference in phenol concentration between cultivars, correlation showed that phenol does not play any significant role in cowpea resistance to *Maruca vitrata*. On the other hand, Veeranna (1998)

recorded a higher phenol in tolerant genotypes of cowpea to *Maruca* than susceptible genotypes.

Infection of blackeye cowpea mosaic virus caused an initial increase in total phenolics which later on decreased in the susceptible cowpea variety Sharika, while the phenol content of resistant variety CO 6 remained constant (Sindhu, 2001). Kumar *et al.* (2003) analysed the total phenols in 34 genotypes of cowpea. PCV was higher than GCV indicating that environment had an effect on the expression of the character. Moreover, high estimates of GCV, high heritability coupled with high genetic advance was observed which revealed that selection could be effective.

2.11.2 Proline

Less than five percentage of the total pool of free amino acids in plants under stress-free conditions is provided by proline. In many plants, under various forms of stress, proline concentration increases up to 80 per cent of the amino acid pool (Matysik *et al.*, 2002).

In chilli, Sreelathakumary (2000) observed a proline content of 1.953 to 2.086 mg/g of leaf tissue in vegetative growth stage. An increase in proline content was noticed with growth stages in shade tolerant and shade susceptible genotypes of chilli.

Geetha (2004) studied the variation in proline content in response to shade on various vegetables like chilli (217.52 to 281.53 $\mu g\ g^{-1}$), tomato (44.53 to 81.77 $\mu g\ g^{-1}$) and sword bean (35.71 to 77.92 $\mu g\ g^{-1}$) .

2.11.3 Pigments (chlorophyll)

Oghiakhe *et al.* (1991) reported a negative correlation between total leaf chlorophyll content and plant susceptibility index. Based on the different levels of chlorophyll, cultivars were divided into three groups – resistant, moderately resistant and susceptible. This profile based on the level of total leaf chlorophyll was simple, fast and could be readily used in classifying cultivars for resistance to *Maruca vitrata.*

Panicker (2000) evaluated yard long bean genotypes and observed total chlorophyll content of 0.710 to 1.692 mg/g of leaf tissue. Philip (2004) analyzed 50 genotypes of grain cowpea for variability in chlorophyll a (0.70 to 0.97 mg/g), chlorophyll b (0.31 to 0.56 mg/g) and total chlorophyll (1.01 to 1.53 mg/g). A positive correlation was observed by chlorophyll a, chlorophyll b and total chlorophyll with flower damage, percentage pod infestation and plant susceptibility index for legume pod borer incidence.

2.12 Seed protein electrophoresis

Seed protein electrophoresis has been utilized as a tool for resolving specific taxonomic and evolutionary problems, species and cultivar identification in many crops and for a range of application in breeding studies (Ladizinsky and Hymovitz, 1979 ; Hussain *et al.*, 1986 ; Moustakas *et al.*, 1986 ; Lanham *et al.*, 1994).

SDS-polyacrylamide gel electrophoresis (PAGE) and native-PAGE of seed proteins of different varieties have been increasingly utilized during the past years (Iwasaki *et al.*, 1982). Most of the works in cowpea storage protein were confined to regulation of storage protein degradation, accumulation, quality as well as characterization of storage protein (Awolumate, 1983 ; Misra and Kar, 1990).

There are a number of electrophoretic techniques and procedures to distinguish among closely related varieties *viz.*, in french bean (Hussain *et al.*, 1986 ; Hussain *et al.*, 1988) and in chick pea (Singh *et al.*,1991), but the available information for varietal identification of cowpea is scanty.

Seed protein are mainly storage protein and not likely to change in dry mature seed and the comparison of seed proteins is highly constant and is not likely to be affected by environment or seasonal fluctuations. Data from protein electrophoresis seem to give more accurate information on phylogenetic relationships than isozymes. Proteins separated by electrophoretic methods are thought to undergo the process of evolution with relative slowness due to their 'non-essential' nature,

while enzymes are thought to be extremely sensitive to selection pressures in evolution and thus to the survival of the organism (McDaniel, 1970).

Rao *et al.* (1992) analysed the seed storage proteins of ten *Vigna* species by means of SDS-PAGE and reported great variation both in number and molecular weight (MW) of the polypeptides. They also reported that proteins extracted from different accessions of the same species revealed the presence of an electrophoretic pattern typical for each species and these specific bands allowed the identification of ten *Vigna* species analysed.

Vaillancourt *et al.* (1993) compared cultivated and wild cowpea for their isozyme diversity and reported that cultivated cowpea were characterized by very low genetic diversity with only six polymorphic loci. Wild cowpea was highly diverse with 19 polymorphic out of 26 loci, and six wild accessions displayed identity with the cultivated cowpea.

Oghiakhe *et al.* (1993) reported that no inter varietal differences existed for total protein content, but water soluble seed proteins proved useful in distinguishing cultivars. A key for the classification of the fifteen cultivars into five groups was developed based on the presence or absence of three proteins following PAGE of the water soluble proteins.

SDS-PAGE is a useful tool for discriminating and estimating genetic similarities among selections. Protein bands with molecular weight ranging from 12.0 to 94.5 kDa was observed by Osanyinpeju and Odeigah (1998). A number of lines with particular traits were found to be characterized by the presence of specific polypeptide bands. Kalloo *et al.* (2001) suggested that both SDS-PAGE and native-PAGE can be used by breeders to characterize differences among closely related varieties of cowpea.

Odeigah and Osanyinpeju (1996) reported two main total seed protein electrophoretic patterns with respect to 39 and 20 kDa subunits. While there was no correlation between seed colour and total seed protein banding pattern, while six

insect resistant cultivars were characterized by the presence of the 39 and 20 kDa subunits. Iqbal *et al.* (2003) found that variation in quantitative traits was significantly correlated with various protein subunits.

The trend of changing pod length was found related to number of bands of seed proteins, where the least number of bands (two) were observed for shorter pods in bush type vegetable cowpea (Sobha, 1994).

2.13 Molecular characterization

Molecular markers are genotypic markers. Unlike morphological characters, molecular markers characterize diversity at the molecular level, and therefore are environmentally independent. The use of these markers provide a potential effective selection technique for crop improvement and has advantage over selection based on phenotype alone. Molecular markers have been widely used in genetic analysis and diversity assessment in a number of plant species (Bretting and Widerlechner, 1995 ; Staub *et al.*, 2004).

Molecular markers that reveal polymorphism at the DNA level are known as DNA markers. They provide an opportunity to characterize genotypes and to measure genetic relationships more precisely than other markers (Soller and Beckmann, 1983). Various types of molecular markers are utilized to evaluate DNA polymorphism and among them the most important is polymerase chain reaction (PCR) based DNA markers.

Polymerase chain reaction (PCR) based DNA markers

PCR based DNA marker techniques are fingerprinting techniques that use an *in vitro* enzymatic reaction to specifically amplify a multiplicity of target sites in one or more nucleic acid molecules. Among the PCR based marker techniques, the important ones are Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (RFLP) and microsatellite.

Random Amplified Polymorphic DNA (RAPD)

RAPD is a multiplex marker system that conventionally uses single-primer PCR to amplify random DNA fragments. RAPD technique is particularly well suited to high through-put systems required for germplasm assessment because of their simplicity, speed and relatively low cost. RAPD markers are commonly used for molecular characterization studies despite disadvantages in reliability.

RAPD is now being applied to a wide range of research activities including genome fingerprinting (Welsh and McClelland, 1990), identification of genome specific markers (Williams *et al.*, 1990 ; Erlich *et al.*, 1991), population biology studies (Astley, 1992), discrimination among specific genotypes, estimation of genetic variation and systematics (Lee *et al.*, 1996 ; Youn and Chung, 1998).

RAPD for detection of genetic variability

In cowpea, a high degree of genetic diversity was observed by Mignouna *et al.* (1998), when 95 accessions involving three cultivar groups of *Vigna unguiculata* from diverse geographical origin was subjected to RAPD analysis. Shim *et al.* (2001) screened for parental polymorphism in cowpea using random RAPD primers for construction of molecular genetic linkage map. Analysis of genetic diversity in cowpea varieties by RAPD technique was also done by Fall *et al.* (2003). It was suggested that the method can be used to reorganize the national germplasm in order to eliminate the putative duplicates and to identify elite varieties.

Nkongolo (2003) determined the pattern and extend of RAPD variation within and among cowpea populations from different agroecological zones and found a general lack of agreement between clustering and morphological features. The high within-accession variability observed in the study was suggested to be due to uncontrolled gene flow among polulation. Similarly, Ba *et al.* (2004) reported high variability in domesticated cowpea based on RAPD analysis. But the cultivar groups were poorly resolved and several results obtained with isozyme data were not confirmed with RAPD data.

Thirteen radiation – induced mutants as well as the parent lines of cowpea were screened for random amplified polymorphic DNA (RAPD) variation. Mutant – specific polymorphic markers were detected which will facilitate in their identification, registration and determination of seed purity (Pandey *et al.*, 2004). Roychoudhury *et al.* (2005) grouped nineteen diverse germplasm belonging to the genus *Vigna* into four clusters following RAPD technique. Crossing between different clusters was suggested to generate new genotypes with wide variability. RAPD technique can be used to find out the relationship within and among the various cultivated *Vigna* species and their polymorphic forms.

In azuki bean (*Vigna angularis*), RAPD variation was assessed among wild, weedy and cultivated races by Mimura *et al.* (2000). High variation was observed among wild races compared to weedy and cultivated ones. Afzal *et al.* (2004) subjected 21 mung bean (*Vigna radiata*) cultivars to RAPD analysis and observed a narrow genetic base among them. Genetic similarity obtained in the study may be used for selecting parents for breeding purposes.

Materials and Methods

3. MATERIALS AND METHODS

The present investigation was carried out in the Department of Olericulture and Department of Plant Biotechnology, College of Agriculture, Vellayani during 2002 to 2005. The area is located at 8.5° N latitude and at an altitude of 29.0 m above mean sea level. Experimental site has a laterite red loam soil with a pH of 5.2. The area enjoys a warm humid tropical climate.

The study consisted of the following experiments :

3.1 Genetic cataloguing of vegetable cowpea

3.2 Variability of vegetable cowpea

3.2.1 Morphological, anatomical and biochemical characterization

3.2.2 Molecular characterization

3.1 Genetic cataloguing of vegetable cowpea

The basic material for the study consisted of 66 diverse accessions of vegetable cowpea (*Vigna unguiculata*) collected through survey and correspondence. The details of the accessions and their sources are presented in Table 1.

The accessions were described morphologically using descriptors developed by IPGRI (IBPGR, 1983) for cowpea (Table 2).

3.2 Variability in vegetable cowpea

Sixty six accessions of *V. unguiculata* were grown during May 2004 to September 2004 to characterize them based on morphological, anatomical, biochemical and molecular parameters as well as their quality and reaction towards pests and diseases.

Statistical details were as furnished below :

Design	:	RBD
Replications	:	2
Treatments	:	66 accessions
Number of plants per plot	:	10 (microplot)

Table 1. Particulars of vegetable cowpea accessions used for the study and their sources

Sl. No.	Accession Number	Source
1.	VS 1	Hosdurg, Kasargode
2.	VS 2	Kumarapuram, Thiruvananthapuram
3.	VS 3 (KMV-1)	RARS, Kumarakom
4.	VS 4 (Kanjikuzhi payar)	College of Agriculture, Vellayani
5.	VS 5 (Ajeet-11)	College of Horticulture, Vellanikkara
6.	VS 6	College of Agriculture, Vellayani
7.	VS 7 (IVRCP-2)	College of Horticulture, Vellanikkara
8.	VS 8 (CHCP-1)	College of Horticulture, Vellanikkara
9.	VS 9 (Vyjayanthi)	Kerala Agricultural University
10.	VS 10 (BCP-3)	College of Horticulture, Vellanikkara
11.	VS 11 (VS 386/Kuttimulla)	State Seed Farm, Palakkad
12.	VS 12 (IVRCP-1)	College of Horticulture, Vellanikkara
13.	VS 13	Thalasserry, Kannur
14.	VS 14 (Sarika)	State Seed Farm, Ananganadi, Palakkad
15.	VS 15	Pattambi, Palakkad
16.	VS 16	Payyannur, Kannur
17.	VS 17	College of Agriculture, Vellayani
18.	VS 18	Thaliparambu, Kannur
19.	VS 19	Aryanad, Thiruvananthapuram
20.	VS 20	Vengad, Kannur
21.	VS 21	Kuttippuram, Malappuram
22.	VS 22 (Lola)	College of Horticulture, Vellanikkara
23.	VS 23	Thrippunithura, Ernakulam
24.	VS 24	College of Horticulture, Vellanikkara
25.	VS 25	Pilicode, Kasargode
26.	VS 26 (Vellayani local)	College of Agriculture, Vellayani
27.	VS 27	Pattom, Thiruvananthapuram
28.	VS 28	Paudikkonam, Thiruvananthapuram
29.	VS 29	Mitraniketan, Vellanad
30.	VS 30	Sreekaryam, Thiruvananthapuram
31.	VS 31	Neyyattinkara, Thiruvananthapuram
32.	VS 32 (Malika)	Kerala Agricultural University
33.	VS 33 (Bhagyalakshmi)	State Seed Farm, Ananganadi, Palakkad
34.	VS 34 (Anaswara)	RARS, Pattambi, Palakkad
35.	VS 35	State Seed Farm, Ananganadi, Palakkad
36.	VS 36	Pattom, Thiruvananthapuram
37.	VS 37	Koothattukulam,Ernakulam

Contd...

Table 1. Continued..

Sl. No.	Accession Number	Source
38.	VS 38 (V-118)	College of Agriculture, Vellayani
39.	VS 39 (V - 629)	RARS, Pattambi, Palakkad
40.	VS 40 (V-16)	College of Agriculture, Vellayani
41.	VS 41	Brahmamangalam, Kottayam
42.	VS 42	Pilicode, Kasargode
43.	VS 43 (Pusa Komal)	College of Agriculture, Vellayani
44.	VS 44	College of Agriculture, Vellayani
45.	VS 45	College of Agriculture, Vellayani
46.	VS 46	College of Agriculture, Vellayani
47.	VS 48	College of Agriculture, Vellayani
48.	VS 49	College of Agriculture, Vellayani
49.	VS 50	Koliyoor, Thiruvananthapuram
50.	VS 52 (V-15)	College of Agriculture, Vellayani
51.	VS 53	Brahmamangalam, Kottayam
52.	VS 54	Vaikom, Kottayam
53.	VS 55 (GC-9732)	RARS, Pattambi, Palakkad
54.	VS 56 (CO-2)	TNAU, Coimbatore
55.	VS 57 (Pusa Phalguni)	College of Agriculture, Vellayani
56.	VS 58	Kothamangalam, Ernakulam
57.	VS 59 (Kanakamony)	RARS, Pattambi, Palakkad
58.	VS 60	Koliyakode, Thiruvananthapuram
59.	VS 61 (GC-3)	College of Agriculture, Vellayani
60.	VS 62	Thodupuzha, Idukki
61.	VS 63 (Pusa Karnal)	College of Agriculture, Vellayani
62.	VS 64 (Krishnamony)	RARS, Pattambi, Palakkad
63.	VS 65	Kalliyoor, Thiruvananthapuram
64.	VS 66 (CO-26)	College of Agriculture, Vellayani
65.	VS 68 (COVU-6233)	College of Agriculture, Vellayani
66.	VS 69 (C-152)	College of Agriculture, Vellayani

Table 2. Genetic cataloguing of vegetable cowpea

1. Vegetative characters	
1.1 Growth habit	- 1- Acute erect / 2- erect / 3- semi-erect / 4- intermediate / 5- semi-prostrate / 6- prostrate / 7- climbing
1.2 Growth pattern	- 1- Determinate / 2- indeterminate
1.3 Twining tendency	- 1- None / 3- slight / 5- intermediate / 7- pronounced
1.4 Leaf colour	- 3- Pale green / 5- intermediate green / 7- dark green
1.5 Plant pigmentation (stem, branches, petioles and peduncles)	- 0-None /1-very slight /3-moderate /5-intermediate / 7-extensive / 9-solid
2.Inflorescence and fruit characters	
2.1 Flowering pigment pattern	- 0- Not pigmented (white) /1- wing pigmented, standard with light v-shaped pattern at top centre / 2- pigmented margins on wing and standard /3- wing pigmented, standard lightly pigmented /4- wing with pigmented upper margin, standard is pigmented /5-completely pigmented / 6- others
2.2 Calyx colour	- 0- Green / 3- lightly pigmented / 5- deeply pigmented
2.3 Duration (nature) of flowering	- 1- Asynchronous / 2- intermediate /3- synchronous
2.4 Raceme position	- 1- Mostly above canopy / 2-in upper canopy / 3-throughout canopy
2.5 Pod attachment to peduncle	- 3-Pendant /5- 30-90° down from erect / 7-erect
2.6 Immature pod pigmentation	- 0-None /1-pigmented tip/ 3-pigmented sutures / 4-pigmented valves, green sutures / 5-splashes of pigment / 6-uniformly pigmented / 7-other
2.7 Pod curvature	- 0-Straight /3- slightly curved / 5-curved / 7-coiled
3. Seed characters	
3.1 Seed shape	- 1-Kidney / 2-ovoid / 3-crowder / 4-globose / 5-rhomboid
3.2 Seed colour	- 1-Light brown / 2-light brown and white / 3-brown / 4-brown with stripes / 5-brown with white tip / 6-brown and white / 7-dark brown / 8-dark brown and white / 9-black
3.3 Testa texture	- 1-Smooth /2- smooth to rough /3- rough / 4-rough to wrinkled / 5-wrinkled
4. Pest and disease scoring	- 0- No incidence/3-low /5-medium/7-high/9-very high

The crop was raised as per package of practices recommendation of Kerala Agricultural University (Kerala Agricultural University, 2002). No insecticide or fungicide was applied on the plants during the course of experimentation to observe the reaction of the accessions towards pests and diseases.

3.2.1 Morphological, anatomical and biochemical characterization

All the observations were recorded from plants selected at random in each replication and the mean was taken for further analysis. For recording observations on pod characters, ten fully grown tender pods were selected at random from each accession in each replication. Observations on the following characters were recorded :

3.2.1.1 Morphological characterization

3.2.1.1.1 Growth characters

(a) Days to seedling emergence

Number of days taken from sowing to germination.

(b) Vine length at final harvest (m)

Length of the vines from ground level to the tip was measured at final harvest.

(c) Collar girth (cm)

Girth of the stem at collar region was measured using twine and scale.

(d) Primary branches

The number of primary branches arising from the main stem in each plant was recorded.

(e) Petiole length (cm)

Length of petiole of five leaves selected at random was measured in each observational plant.

(f) Terminal leaflet length (cm)

Length of five terminal leaflets selected at random from each observational plant.

(g) Terminal leaflet width (cm)

The widest dimension of the terminal leaflets whose length was measured was recorded.

(h) Lateral leaflet length (cm)

Length of five lateral leaflets selected at random from each observational plant.

(i) Lateral leaflet width (cm)

The widest dimension of the lateral leaflets whose length was measured was recorded.

(j) Root : shoot ratio

Root : shoot ratio was calculated as the ratio between the averages of root dry weight and shoot dry weight of each observational plant at final harvest.

3.2.1.1.2 Flowering characters

(a) Days to first flowering

Number of days taken from sowing until 50 per cent of the plants in each accession have at least one open flower.

(b) Pollen viability (%)

Pollen viability was determined by the acetocarmine staining technique. Anthers about to dehisce were collected separately from each accession and the pollen grains were mounted on a drop of acetocarmine : glycerine mixture (1 : 1). The slides were kept for 30 minutes to allow pollen grains to take stain properly before examining under a microscope. Pollen viability was studied by counting the well filled and stained pollen grains. An average of 100 pollen were counted in different microscopic fields (10X) in each accession. Unfilled and unstained pollen grains were considered as sterile. Pollen viability was calculated as follows :

$$\% \text{ of pollen viability} = \frac{\text{Number of well filled and stained pollen grains}}{\text{Total number of pollen grains counted}} \times 100$$

(c) Peduncle length (cm)

Length of peduncle was measured from five randomly selected inflorescence in each observational plant.

3.2.1.1.3 Pod and yield characters

(a) Pod length (cm)

Length of pods were measured using an ordinary scale and average was worked out.

(b) Pod girth (cm)

The same pods used for measuring length were taken to measure the girth using twine and scale.

(c) Pod weight (g)

Individual pods which were used for measurement of length and girth were weighed and average was worked out.

(d) Pods per plant

The total number of pods from each observational plant was recorded.

(e) Seeds per pod

Seeds from each pod was extracted, counted and average was worked out.

(f) 100-seed weight (g)

The dry weight of randomly selected hundred seeds were weighed using an electronic balance.

(g) Seed length (mm)

Mean of ten mature seeds excluding those from the extremities of pods.

(h) Seed width (mm)

Mean width from hilum to heel of the ten seeds was measured for length.

(i) Seed thickness (mm)

Mean thickness was measured perpendicular to length and width of the same ten seeds taken for measuring length and width.

(j) Number of harvests

Total number of harvests from each plant was recorded.

(k) Yield per plant (g)

Weight of pods harvested from each plant was recorded.

3.2.1.1.4 Quality characters

(a) Keeping quality (days)

The harvested pods were kept under ordinary room conditions to study their shelf life and the number of days up to which the pods remained fresh for consumption without loss of colour and firmness were recorded.

(b) Pod protein (%)

Fresh green pod samples were subjected to protein estimation using Lowry's method (Sadasivam and Manickam, 1992).

Reagents

(i) 2 % Sodium carbonate in 0.1 N Sodium hydroxide (Reagent A).

(ii) 0.5 % Copper sulphate in 1.0 % Potassium sodium tartrate (Reagent B).

(iii) Alkaline copper solution

Mixed 50 ml of A and 1 ml of B prior to use (Reagent C).

(iv) Folin – Ciocalteau reagent (Reagent D).

Refluxed gently for 10 h a mixture consisting of 100 g sodium tungstate, 25 g sodium molybdate, 700 ml water, 50 ml of 85 % phosphoric acid and 100 ml of concentrated hydrochloric acid in a 1.5 L flask. Added 150 g lithium sulphate, 50 ml water and a few drops of bromine water. Boiled the mixture for 15 minutes without condenser to remove excess bromine, cooled, diluted to 1 L and filtered.

(v) Protein solution (stock standard)

Weighed accurately 50 mg of bovine serum albumin and dissolved in distilled water and made up to 50 ml in a standard flask.

(vi) Working standard

Diluted 10 ml of the stock solution to 50 ml with distilled water in a standard flask. One ml of this solution contains 200 µg of protein.

Procedure

Extraction of protein from sample

Extraction was carried out with Tris-HCl buffer (62.5 mM, pH 6.8). Weighed 200 mg of fresh pod at vegetable maturity and ground well with a pestle and mortar in 3.8 ml of the buffer. Centrifuged and the supernatant was taken for protein estimation.

Estimation of protein

Pipetted out 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard into a series of test tubes. 0.1 ml of the sample extract was taken in another test tube. Made up the volume to 1 ml in all the test tubes. A tube with 1 ml of water served as the blank. Added 5 ml of reagent C to each tube including the blank. Mixed well and allowed to stand for 10 minutes. Then added 0.5 ml of reagent D, mixed well and incubated at room temperature in the dark for 30 minutes. A blue colour was developed and the optical density was measured at 660 nm using a UV spectrophotometer. Standard graph was prepared and calculated the amount of protein in the samples

(c) Crude fibre content of pods (%)

Crude fibre content of whole dried pods along with seeds was estimated by acid and alkali digestion method (Sadasivam and Manickam, 1992).

3.3 Statistical analysis

3.3.1 Analysis of variance (ANOVA) and covariance (ANCOVA) for Randomized Block Design (RBD) in respect of the various characters was done (Panse and Sukhatme, 1967).

3.3.2 Mean : The mean of the character X_i ($\overline{X}_i$) was worked out.

3.3.3 Variability components (phenotypic and genotypic) for different characters was estimated as suggested by Kempthorne (1977).

(a) The variance and covariance components were calculated as per the following formulae :

For the character X_i,

Environmental variance, $\sigma_{ei}^2 = MSE$

Genotypic variance, $\sigma_{gi}^2 = \frac{MST - MSE}{r}$

Phenotypic variance, $\sigma_{pi}^2 = \sigma_{gi}^2 + \sigma_{ei}^2$

where, MST and MSE are respectively, the mean sum of squares for treatment and error from ANOVA and 'r', the number of replications.

For two characters X_i and X_j,

Environmental covariance, $\sigma_{eij} = MSPE$

Genotypic covariance, $\sigma_{gij} = \frac{MSPT - MSPE}{r}$

Phenotypic variance, $\sigma_{pij} = \sigma_{gij} + \sigma_{eij}$

where, MSPT and MSPE are respectively, the mean sum of products between the i^{th} and j^{th} characters for genotype and environment respectively from Analysis of Covariance (ANCOVA).

(b) Coefficient of variation

Variability that existed in the population for various characters were apportioned using the estimates of coefficient of variation (Singh and Chaudhary, 1985).

For the character X_i,

$$\text{Phenotypic coefficient of variation, PCV} = \frac{\sigma_{pi}}{\overline{X_i}} \times 100$$

$$\text{Genotypic coefficient of variation, GCV} = \frac{\sigma_{gi}}{\overline{X_i}} \times 100$$

$$\text{Environmental coefficient of variation, ECV} = \frac{\sigma_{ei}}{\overline{X_i}} \times 100$$

where, σ_{pi}, σ_{gi} and σ_{ei} are respectively the phenotypic, genotypic and environmental standard deviations with respect to each character.

3.3.4 Heritability

Hanson *et al.* (1956) proposed the mathematical relationship of variance estimates on computation of heritability, which is usually expressed as a percentage :

$$\text{Heritability (broad sense), } H^2 = \frac{\sigma_{gi}^2}{\sigma_{pi}^2} \times 100$$

The range of heritability was categorized as suggested by Robinson *et al.* (1949) as follows :

Definition	Category
0 – 30 per cent	Low
31 – 60 per cent	Medium
61 per cent and above	High

3.3.5 Genetic advance

Genetic advance as percentage over mean was calculated as per the formula given by Lush (1949) and Johnson *et al.* (1955) :

$$\text{Genetic advance, GA} = \frac{kH^2 \sigma_{pi}}{\overline{X}_i} \times 100$$

where, H^2 - heritability in broad sense.

σ_{pi} - phenotypic standard deviation

k - selection differential which is 2.06 in case of 5 % selection in large samples (Miller *et al.*, 1958 and Allard, 1960).

Genetic advance was categorized according to Robinson *et al.* (1949) as follows :

Definition		Category
Less than 20 per cent	:	Low
Greater than 20 per cent	:	High

3.3.6 Correlation analysis

Phenotypic, genotypic and environmental correlation coefficients were worked out according to the procedure suggested by Singh and Choudhary (1985).

3.3.7 Path analysis

The direct and indirect effects of yield contributing factors were estimated through path analysis technique (Wright, 1954).

3.3.8 Mahalanobi's D^2 analysis

Genetic divergence was studied based on 16 characters taken together using Mahalanobi's D^2 statistic as described by Rao (1952). The genotypes were clustered by Tochers method.

3.3.9 Selection index

The various genotypes were discriminated based on 16 characters using the selection index developed by Smith (1936) using the discriminant function of Fisher (1936). The selection index is described by the function

$$I = b_1 X_1 + b_2 X_2 + \ldots b_k X_k$$

The function $H = a_1G_1 + a_2G_2 + \ldots a_k G_k$ describes the merit of a plant, where X_1, X_2, ..., X_k are the phenotypic values and G_1, G_2, ..., G_k are the genotypic values of the plant with respect to the characters X_1, X_2, ..., X_k. H denotes the genetic worth of the plant. The economic worth assigned to each character is assumed to be equal to unity. i.e., a_1, a_2, ..., $a_k = 1$. The regression coefficients b_1, b_2, ..., b_k are estimated in such a way that the correlation between H and I is maximum.

i.e., $b = P^{-1}Ga$, where P and G are the phenotypic and genotypic variance–covariance matrices respectively. Based on the 'b' estimates and the mean values for the 16 characters with respect to each accession, scores were calculated and the accessions were ranked.

(d) Organoleptic analysis

The organoleptic quality and acceptability traits were done using a scoring method proposed by Swaminathan (1974). The major quality attributes included in the score were colour, doneness, flavour, taste, texture and overall acceptability (Appendix I). Each of the above mentioned quality was assessed by a four point rating scale.

The pods were washed thoroughly in water and cut into pieces. 25 g of pods were boiled in 50 ml water containing 0.5 g salt for five minutes. The prepared sample was used for organoleptic quality scoring.

The panel members were selected from a group of healthy adults in the age group of 25 to 35. They were requested to taste one sample and score it. Each quality was assessed by the panel member after tasting the same sample several times if needed.

3.2.1.1.5 Reaction towards pest and disease incidence

Legume pod borer (*Maruca vitrata*)

Different damage parameters were measured employing the field screening technique developed by Jackai (1982) as detailed below :

(i) Number of larvae per 25 flowers

(Severity of larval infestation of flowers)

This was determined by randomly collecting 25 flowers 10 weeks after planting from each plot. The samples were collected in vials containing 30 per cent alcohol and subsequently examined for larval counts.

(ii) Percentage infestation of pods

Twenty five pods at vegetable maturity stage were harvested at peak podding phase from each plot. Each sample was examined in the laboratory to determine the number of pods with entry/exit holes made by *M. vitrata*. Pod infestation was expressed as a percentage of total number of pods collected from each plot.

(iii) Pod damage severity

Pod samples used for the assessment of percentage pod infestation were examined for the number of larval entry/exit holes. The results were expressed as the number of holes per pod.

(iv) Seed damage assessment

The sample used for assessing pod infestation was also used for assessing seed damage measurements. A seed damage index (Isd) was worked out using the following formula.

$$Isd = \frac{ds \times 100}{pt}$$

where, ds = number of damaged seeds

pt = total number of pods sampled

(v) Plant susceptibility index (Ips)

This was computed for each variety using a combination of the following parameters :

Number of larvae per 25 flowers

Percentage pod infestation

Seed damage index (Isd)

$$Ips = \frac{SW_1 + TW_2 + MW_3}{W_1 + W_2 + W_3}$$

where, S, T and M are measurements of damage of seed (S), pods (T) and flowers (M) respectively, with weights W_1, W_2 and W_3 are1, 2 and 3 respectively. These weighted measurements reflect the relative importance attached to each.

3.2.1.2 Anatomical characterization

Representative plants from resistant and susceptible categories of pest incidence was analyzed for anatomical features like pod trichome density, stomatal density, vascular bundle thickness and cuticle thickness.

(a) Pod trichome density

Five pods at vegetable maturity stage (eight days after flowering) were taken from each selected accession at random. The skin was peeled from the middle portion of the pods and observed under a compound microscope with a magnification of 10X objective. The number of glandular and non-glandular trichomes observed in a microscopic field was counted. The non-glandular type of trichome consists of single, long cell with enlarged base which tapers towards the distal portion to form a narrow, needle-like and filiform tip. Glandular trichomes are four lobed, bulbous and distended towards the upper part. A fissure divides the first, and sometimes the second upper lobe into two halves. The area of the microscopic field was calculated using stage micrometer. The mean value of glandular and non-glandular trichome counts per mm^2 area of pod wall surface was calculated and expressed as glandular and non-glandular trichome density on pods.

(b) Stomatal density

A thin film of quick fix was applied over the adaxial surface of three randomly selected leaves in each selected accession. The film was peeled off after a few minutes and the number of stomatal impressions were counted using a compound microscope (10X objective). The area of the microscopic field was calculated using a stage micrometer and the number of stomata per mm^2 was calculated and recorded.

(c) Vascular bundle thickness (µm)

Third leaf from the tip was selected in each accession. A portion of the leaf lamina including the midrib was cut off. Fine sections were taken and the thickness of vascular bundle was measured using an ocular micrometer in a compound microscope (10X objective).

(d) Cuticle thickness (µm)

The same leaf sections taken for measuring vascular bundle thickness was used for measuring cuticle thickness also (40X objective).

3.2.1.3 Biochemical characterization

Biochemical characters governing pest resistance like phenol, proline and pigments (chlorophyll) were estimated from representative plants of tolerant and susceptible categories.

(a) Phenol

Fresh tender leaves were subjected to total phenol estimation with the Folin-Ciocalteau reagent (Sadasivam and Manickam, 1992).

Reagents

(i) 80 % Ethanol

(ii) Folin-Ciocalteau reagent

(iii) 20 % Sodium carbonate

(iv) Standard (100 mg Catechol in 100 ml water). Diluted 10 times for a working standard.

Procedure

Weighed exactly 0.5 g of the leaf sample and ground with a pestle and mortar in 10-time volume of 80 % ethanol. Centrifuged the homogenate at 10 000 rpm for 20 minutes. Supernatant was collected and re-extracted the residue with five times the volume of 80 % ethanol, centrifuged and pooled the supernatants. Evaporated the supernatant to dryness. Dissolved the residue in 5 ml of distilled water. Pipetted out 0.2 ml into test tubes and made up the volume to 3 ml with water. Added 0.5 ml of Folin-Ciocalteau reagent. After 3 minutes, added 2 ml of 20 % sodium carbonate and mixed thoroughly. Placed the tubes in boiling water for exactly one minute, cooled and measured the absorbance at 650 nm against reagent blank. Prepared a standard curve using different concentrations of catechol. From the standard curve, phenol concentration in the test sample was found out and expressed as mg/g leaf material.

(b) Proline

Proline content of fresh tender leaves was estimated as per the procedure suggested by Sadasivam and Manickam (1992).

Reagents

(i) Acid Ninhydrin

Warmed 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid until dissolved.

(ii) 3 % Aqueous sulphosalicylic acid

(iii) Glacial acetic acid

(v) Toluene

(vi) Proline

Procedure

Extracted 0.5 g of leaf material by homogenizing in 10 ml of 3 % aqueous sulphosalicylic acid. Filtered out 2 ml of filtrate in a test tube and added 2 ml of glacial acetic acid and 2 ml of acid ninhydrin. Heated it in boiling water bath for 1 hour and terminated the reaction by placing the tubes in ice bath. Added 4 ml toluene to the reaction mixture and stirred well for 20-30 sec. Separated the toluene layer and warmed to room temperature. Measured the red colour intensity at 520 nm. A series of standards with pure proline was run in a similar way and prepared a standard curve. The amount of proline in the test sample was found out from the standard curve and expressed on fresh weight basis as follows :

$$\text{Proline (}\mu\text{moles per g tissue)} = \frac{\mu\text{g proline/ml x ml of toluene x 5}}{115.5 \text{ x g sample}}$$

(c) Pigments (Chlorophyll, mg/g tissue)

Estimation of chlorophyll content of leaves was done by the Dimethyl Sulfoxide (DMSO) method (Hiscox and Israelstam, 1979).

Reagents

Dimethyl sulfoxide : 80 % acetone (1 : 1) mixture

Procedure

A known weight (0.1 g) of the leaf material was taken in a test tube and cut into small bits. Added 10 ml of DMSO-acetone mixture and incubated the test tubes

overnight at room temperature. All the pigments got extracted into the solution. Decanted the coloured solution into a measuring cylinder and made up the volume to 25 ml with DMSO-acetone mixture. Recorded the absorbance at 645 and 663 nm using a spectrophotometer. Calculated the chlorophyll content by substituting the absorbance values in the given formula.

$$\text{Chlorophyll a} = (12.7\ A_{663} - 2.69\ A_{645}) \times \frac{V}{1000} \times \frac{1}{\text{fresh weight}}$$

$$\text{Chlorophyll b} = (22.9\ A_{645} - 4.68\ A_{663}) \times \frac{V}{1000} \times \frac{1}{\text{fresh weight}}$$

$$\text{Total chlorophyll (a+b)} = (8.02\ A_{663} + 20.20\ A_{645}) \times \frac{V}{1000} \times \frac{1}{\text{fresh weight}}$$

where, A is the absorbance at specific wavelengths and V, the volume of the extract.

Statistical Analysis

Analysis of variance

The data on plant susceptibility index, damage parameters as well as anatomical and biochemical characters were subjected to analysis of variance for varietal differentiation.

Correlation analysis

A correlation analysis was done to determine the degree of association of plant susceptibility index with the various damage parameters as well as anatomical and biochemical characters.

(d) Seed protein electrophoresis

Seeds of all the sixty six accessions taken for morphological characterization were used in the present investigation.

Sample preparation

Reagents

(i) Extraction buffer (pH 6.8)

Tris-HCl buffer (62.5 mM) - 0.985 g

Water - 100 ml

Procedure

The samples of dry mature seeds were prepared by removing the seed coat manually and grinding the cotyledons by mortar and pestle. 0.2 g powder of different accessions was dissolved in 3.8 ml of Tris-HCl buffer. The samples were centrifuged at 15,000 rpm for 15 minutes at 4°C. The pellets were discarded and supernatant were stored at -20°C for protein estimation by Folin-Ciocalteau method (Sadasivam and Manickam, 1992) using Bovine Serum Albumin as standard.

Polyacrylamide Gel Electrophoresis (PAGE)

SDS-PAGE using 12 per cent separating and 5 per cent stacking gel was performed following the procedure of Laemmli (1970) with slight modifications.

Reagents

(i) Stock acrylamide solution

Acrylamide (30 %) - 30 g

Bisacrylamide (0.8 %) - 0.8 g

Water - 100 ml

(ii) Separating/resolving gel buffer (pH 8.8)

Tris-HCl buffer (1.5 M) - 23.64 g

Water - 100 ml

(iii) Stacking gel buffer (pH 6.8)

Tris-HCl buffer (0.5 M) - 7.88 g

Water - 100 ml

(iv) Polymerising agents

Ammonium persulphate (10 %) - 0.1 g/ml, prepared freshly before use

TEMED - fresh from the refrigerator

(v) Tank / Electrophoresis buffer

Tris base (25 mM) - 6.057 g

Glycine (250 mM) - 37.535 g

SDS (0.1 %) - 2.0 g

Water - 2 L

(vi) Sample buffer

Tris-HCl (50 mM) - 0.788 g

SDS (2 %) - 2.0 g

β-mercaptoethanol (5 %) - 5.0 ml

Bromophenol Blue (0.1 %) - 0.1 g

Glycerol (10 %) - 10 ml

Water - 100 ml

(vii) Sodium dodecyl sulphate (SDS) solution (10 %)

SDS - 10 g

Water - 100 ml

(viii) Standard marker proteins

The molecular weight marker contains a mixture of the proteins listed below. The total protein concentration is 3 mg/ml.

Protein	Approximate MW Daltons
Phosphorylase b	97 400
Bovine Serum Albumin	68 000
Ovalbumin	43 000
Carbonic Anhydrase	29 000
Soybean Trypsin Inhibitor	20 000
Lysozyme	14 300

(ix) Protein staining solution

Coomassie brilliant blue R 250 (0.1 %) - 0.1 g
Methanol (40 %) - 40 ml
Glacial acetic acid (8 %) - 8 ml
Water - 52 ml

(x) Destaining solution

Methanol (40 %) - 40 ml
Glacial acetic acid (8 %) - 8 ml
Water - 52 ml

Procedure

Thoroughly cleaned and dried the glass plates and spacers and assembled them properly in an upright position. Two per cent agar solution was then applied around the edges of the spacers to seal the chamber between the glass plates. Prepared sufficient volume of separating gel mixture (30 ml) by mixing the following:

For 12 % gel

Stock acrylamide solution - 12.0 ml
Separating buffer - 7.5 ml
Water - 9.9 ml
SDS - 0.3 ml
Ammonium persulphate solution - 0.3 ml
TEMED - 0.012 ml (12 µl)

The gel solution was mixed gently and poured into the chamber between the glass plates. distilled water was then layered on top of the gel and allowed to set for 30 minutes. Prepared stacking gel (10 ml) by mixing the following solutions :

For 5 % gel

Stock acrylamide solution - 1.7 ml

Stacking buffer - 1.25 ml

Water - 6.8 ml

SDS - 0.1 ml

Ammonium persulphate solution - 0.1 ml

TEMED - 0.01ml (10 µl)

Water was removed from the top of the gel and poured the stacking gel mixture. Placed the comb in the stacking gel and allowed to set (30 min.). After the stacking gel has polymerized, the comb was removed without distorting the shapes of the well. After removing the clips, agar etc., the gel was carefully installed in the electrophoresis apparatus and filled it with tank buffer. Seed protein extracted was then prepared for electrophoresis. The protein concentration was adjusted in each sample to a strength of 100 µg following Lowry's method. About 30 µl of protein sample was mixed with 30 µl of sample buffer in eppendorf tubes and heated in boiling water for 5 minutes. Similarly, 15 µl of protein molecular weight marker mixed with same quantity of sample buffer was also treated as above. The position of wells was marked on the glass plate with a marker pen to facilitate easy loading of the samples. After cooling, the sample solution was loaded into sample wells using a micropipette. Each gel had one well of protein marker. The DC-power was turned on to get a current of around 10-15 mA. The gel was run until the bromophenol blue reaches the bottom of the gel (12 hours). After electrophoresis, the gel was carefully removed from between the glass plates and immersed in staining solution overnight. For destaining, the gel was transferred to a suitable container with about 200-300 ml of destaining solution and shaken gently using a shaker for about 1 hour. Protein appeared as bands and the gel was photographed after plating it on to a transilluminator (Appligene Model white/UV TMV-20).

3.2.2 Molecular characterization

Materials

Fifty accessions of vegetable cowpea selected based on distinct morphological characters were studied for molecular characterization.

Procedure

1. Isolation of genomic DNA

For the isolation of genomic DNA, leaf samples were collected from young leaves of cowpea plants. The method of isolation followed was modified from that of Murray and Thompson (1980).

Approximately 0.5 g of leaf material was first washed in running tap water and later in distilled water two or three times after chopping the leaves coarsely. After wiping off the water using tissue paper, the chopped leaves were pulverized in liquid nitrogen in a pre-cooled mortar by rapid grinding to a fine powder. It was then transferred to a 2 ml centrifuge tube and enough extraction buffer (0.7 N NaCl, 1 % CTAB, 50 mM Tris HCl (pH 8.0) and 10 mM EDTA) was added to it so that clumps can easily be dispersed but the solution remains somewhat viscous. For this, 1 ml of buffer per 30-100 mg dry weight of powdered leaf material was required. 200-300 µg of PVP and 5 µl of β-mercaptoethanol was also added to the centrifuge tube and incubated in water bath at 60°C for 1 hour with occasional vortexing. This mixture was then subjected to centrifugation at 15 000 rpm for 10 minutes. The clear supernatant was taken and the remaining extraneous matter was discarded. About one-third volume of Phenol : Chloroform : Isoamyl alcohol (25:24:1) solution was added to the supernatant. The two phases were mixed gently and centrifuged at 12 000 rpm for 10 minutes at 4°C. The supernatant was collected and the same step was repeated twice. After collecting the upper phase, one-third volume of Chloroform : Isoamyl alcohol (24:1) solution was added and centrifuged as in the previous step after thorough mixing. Then one-tenth volume of 3 M sodium acetate followed by

double the volume of chilled absolute ethanol were added to the supernatant. Upon gentle vortexing, the DNA was precipitated which was then pelleted by centrifuging at 10 000 rpm for 10 minutes at 4°C. The supernatant was discarded and the pellet was washed in 70 per cent ethanol by centrifuging at 10 000 rpm for 5 minutes. The supernatant was again discarded and the pellet was air dried for 20 minutes, which was then dissolved in 0.5 ml of 1X Tris EDTA buffer (10 mM Tris HCl, 1 mM EDTA at pH 8.0) and stored at -20°C.

All the materials used in the preparation and storage of reagents including reagent bottles, conical flasks, centrifuge tubes, spatula, glass rod and tips of micropipettes were washed with labolin solution, rinsed with distilled water and autoclaved.

2. Quantification of DNA

The quantification of DNA is necessary before it is subjected to amplification by PCR. DNA quantification was carried out with the help of UV-Vis Spectrophotometer (Spectronic Genesys 5).

The buffer in which the DNA was already dissolved was taken in a cuvette to calibrate the spectrophotometer at 260 and 280 nm wavelengths. The optical density (O.D.) of the samples dissolved in the buffer was recorded at both 260 and 280 nm.

The quantity of DNA in the sample was estimated by employing the following formula :

$$\text{Amount of DNA } (\mu g\ ml^{-1}) = \frac{A_{260} \times 50 \times \text{dilution factor}}{1000}$$

where, A_{260} is the absorbance at 260 nm.

The quality of DNA could be judged from the ratio of the O.D. values recorded at 260 and 280 nm. The A_{260}/A_{280} ratio around 1.8 indicates good quality of DNA.

3. Agarose gel electrophoresis

Agarose gel electrophoresis was carried out in a horizontal gel electrophoresis unit (GENEI, Bangalore). The required amount of agarose was weighed out (0.8 per cent) and dissolved in 1X TAE buffer (0.04 mM Tris acetate, 0.001 mM EDTA, pH 8) by boiling. After cooling to about 50° C, ethidium bromide was added to a final concentration of 0.5 μg ml^{-1}. The mixture was then poured to a preset template with appropriate comb. After solidification, the comb and the sealing tapes were removed and the gel was mounted in an electrophoresis tank filled with 1X TAE buffer, so that the gel was fully immersed in the buffer. The DNA samples (10 μl) were mixed with the required volume of gel loading buffer (6X loading dye *viz.*, 40 per cent sucrose, 0.25 per cent bromophenol blue) and loaded in separate wells. One of the wells was loaded with 5.0 μl of molecular weight marker along with the required volume of gel loading buffer. Electrophoresis was performed at 50 volts until the loading dye reached $3/4^{th}$ of the length of the gel. The gel was visualized using a gel documentation system (BIO RAD, USA).

Random Amplified Polymorphic DNA (RAPD) analysis

DNA amplification was done on selected 50 accessions using arbitrarily designed decamer primers (Operon Technologies, CA, USA) adopting the procedure of Pandey *et al.* (2004) with required modifications.

Polymerase chain reactions (PCR)of genomic DNA were performed in PCR tubes using 25 μl mixture containing 2.5 μl of 10X PCR buffer, 1.0 μl $MgCl_2$, 0.5 μl each of dNTPs, 20 pM primer, 0.6 unit of Taq DNA polymerase (GENEI, Bangalore) and 100 ng genomic DNA. Amplification was performed in a Programmable Thermal Controller (PTC – 100, MJ Research Inc.) using the following programme : 1 cycle at 94° C for 4 min., 45 cycles at 94° C for 1 min., 37° C for 1 min., 72° C for 2 min., followed by an extension at 72° C for 5 min. Finally the products of amplification were cooled to 4° C. A negative control containing sterile water instead of template was included in each reaction set.

The PCR product was size fractioned on a 1.2 per cent agarose gel prepared in 1X TAE buffer and stained with ethidium bromide. DNA fragments were visualized and photographed using a gel documentation system (BIO RAD, USA). The amplified products of five primers which could produce amplification for most of the accessions were used for further analysis. The PCR was repeated twice in order to confirm the reproducibility.

Statistical analysis

RAPD bands were represented as '+' for presence and '-' for absence and recorded. A genetic similarity coefficient (Sj) matrix was constructed using Jaccard's coefficient method as given below :

$$Sj = a / (a+b+c)$$

where,

a : number of bands present in both the accessions in a pair

b : number of bands present in the first accession but not in the second

c : number of bands present in the second accession but not in the first

Based on the similarity coefficient, the distance between the accessions was computed with the help of the software package NTSYS – PC (Version 2.02i). Using these values of distances between accessions, a dendrogram was constructed by UPGMA (Unweighted pair group method with arithmetic average). Association between the various accessions was found out from the dendrogram.

Results

4. RESULTS

Experimental data recorded during the course of investigation were subjected to statistical analysis and are presented under the following headings.

4.1 Genetic cataloguing in vegetable cowpea

4.2 Variability in vegetable cowpea

4.3 Screening for pest and disease resistance

4.4 Organoleptic analysis

4.5 Seed protein electrophoresis

4.6 Molecular characterization based on RAPD

4.1 Genetic cataloguing in vegetable cowpea

The 66 accessions were catalogued based on the IPGRI descriptor list for cowpea. The morphological characters that were catalogued included vegetative, inflorescence, fruit and seed characters (Tables 3, 4 and 5).

Majority of the accessions had a climbing growth habit, followed by semi-prostrate, acute erect and prostrate habits. VS 12 and VS 69 were semi-erect, while VS 54 was an erect accession. Growth pattern was either indeterminate or determinate with varying twining tendency from none, slight, intermediate to pronounced levels.

The accessions had either intermediate to dark green leaf colour. Only six accessions had pale green leaves. Wide range of variation was noticed in pigmentation of stem, branches and petioles. Slight to intermediate pigmentation was observed in most of them. VS 9 and VS 53 had extensive pigmentation along the main stem. On the other hand, pigmentation on branches were mostly slight to moderate, with few of them showing none, intermediate or extensive pigmentation. Similarly, petiole pigmentation was also slight in most of the accessions.

Flowering pigmentation pattern varied from none having no pigmentation to completely pigmented flowers. Majority of the accessions had pigmented wings and

Table 3.Vegetative characters in vegetable cowpea

Accession	Growth habit	Growth pattern	Twining tendency	Leaf colour	Plant pigmentation		
					Stem	Branches	Petioles
VS 1	7	2	7	5	0	0	1
VS 2	7	2	7	7	1	3	5
VS 3	7	2	7	7	0	0	1
VS 4	7	2	7	5	0	0	0
VS 5	1	1	1	5	0	1	1
VS 6	5	2	3	7	0	0	3
VS 7	1	1	1	5	0	3	1
VS 8	7	2	7	7	0	3	1
VS 9	7	2	7	7	7	5	3
VS 10	7	2	7	7	0	3	1
VS 11	7	1	1	5	0	1	1
VS 12	7	1	1	7	0	1	1
VS 13	7	2	7	7	3	3	1
VS 14	7	2	7	7	0	3	1
VS 15	7	2	7	7	1	3	1
VS 16	7	2	7	7	0	1	1
VS 17	7	2	7	5	0	1	1
VS 18	7	2	7	7	0	1	1
VS 19	7	2	7	7	0	0	0
VS 20	7	2	7	7	5	3	1
VS 21	7	2	7	7	0	1	1
VS 22	7	2	7	7	5	3	1
VS 23	6	2	3	7	0	3	1
VS 24	7	2	7	7	1	3	1
VS 25	7	2	7	7	1	3	1
VS 26	7	2	7	7	0	3	1
VS 27	7	2	7	7	0	3	1
VS 28	7	2	7	7	0	1	1
VS 29	7	2	7	7	0	1	1
VS 30	7	2	7	5	0	1	3
VS 31	7	2	7	7	5	3	3
VS 32	7	2	7	5	0	1	1
VS 33	5	1	3	5	0	0	1

Contd...

Table 3. Continued...

Accession	Growth habit	Growth pattern	Twining tendency	Leaf colour	Plant pigmentation		
					Stem	Branches	Petioles
VS 34	7	2	7	7	0	3	1
VS 35	7	2	7	3	0	3	1
VS 36	7	2	7	3	5	3	1
VS 37	7	2	7	5	1	3	1
VS 38	7	2	7	7	1	5	1
VS 39	7	2	7	7	1	5	1
VS 40	7	2	7	5	0	1	1
VS 41	7	2	7	5	1	3	1
VS 42	7	2	7	5	3	5	3
VS 43	1	1	1	7	1	3	1
VS 44	7	2	7	3	3	7	3
VS 45	7	2	7	3	3	3	1
VS 46	7	2	7	7	0	1	0
VS 48	6	2	7	5	3	3	1
VS 49	6	2	7	5	1	5	3
VS 50	5	1	3	5	0	3	1
VS 52	1	1	3	5	0	3	1
VS 53	5	1	3	7	7	3	3
VS 54	2	1	1	7	0	1	1
VS 55	7	2	7	7	1	3	1
VS 56	5	2	7	7	1	3	1
VS 57	5	1	3	7	1	3	1
VS 58	1	1	3	5	0	1	0
VS 59	5	1	3	5	0	1	0
VS 60	6	2	5	7	1	3	0
VS 61	5	1	3	7	0	1	1
VS 62	5	1	3	7	1	3	1
VS 63	1	1	1	5	0	1	1
VS 64	5	2	3	5	0	1	1
VS 65	5	2	3	3	0	1	0
VS 66	5	2	3	5	0	1	0
VS 68	5	2	5	5	1	3	3
VS 69	3	2	3	5	1	3	1

Table 4. Inflorescence and fruit characters in vegetable cowpea

Accession	Flowering pigment pattern	Calyx colour	Nature of flowering	Raceme position	Pod attachment to peduncle	Immature pod pigmentation	Pod curvature
VS 1	3	3	3	3	3	1	0
VS 2	5	5	3	3	3	3	0
VS 3	3	0	3	3	3	0	0
VS 4	2	0	3	3	3	0	0
VS 5	3	0	1	1	3	0	3
VS 6	3	0	3	3	3	0	0
VS 7	3	0	1	1	3	0	3
VS 8	3	0	3	3	3	0	0
VS 9	5	5	3	3	3	3	0
VS 10	3	0	3	3	3	0	0
VS 11	0	0	1	2	3	0	3
VS 12	5	3	1	2	3	1	3
VS 13	3	0	3	3	3	0	0
VS 14	3	3	3	3	3	1	0
VS 15	3	3	3	3	3	1	0
VS 16	0	0	3	3	3	0	0
VS 17	0	0	3	3	3	0	0
VS 18	5	3	3	3	3	1	3
VS 19	2	0	3	3	3	0	3
VS 20	3	0	3	3	3	1	3
VS 21	5	3	3	3	3	1	0
VS 22	5	3	3	3	3	1	0
VS 23	3	0	1	3	3	0	5
VS 24	5	3	3	3	3	1	5
VS 25	5	3	3	3	3	1	0
VS 26	0	0	3	3	3	0	0
VS 27	3	0	3	3	3	0	0
VS 28	3	0	3	3	3	0	0
VS 29	5	3	3	3	3	1	0
VS 30	3	0	3	3	3	0	0
VS 31	5	5	3	3	3	0	0
VS 32	3	0	3	3	3	0	0
VS 33	0	0	1	3	5	0	3

Contd...

Table 4. Continued...

Accession	Flowering pigment pattern	Calyx colour	Nature of flowering	Raceme position	Pod attachment to peduncle	Immature pod pigmentation	Pod curvature
VS 34	3	0	3	3	3	0	3
VS 35	2	0	3	3	3	0	3
VS 36	3	0	3	3	3	0	0
VS 37	2	0	3	3	3	0	3
VS 38	3	3	3	3	3	0	0
VS 39	3	5	3	3	3	0	0
VS 40	3	0	3	3	3	0	3
VS 41	2	0	3	3	3	0	3
VS 42	5	5	3	3	3	3	3
VS 43	0	0	1	2	3	0	3
VS 44	5	5	3	2	3	3	3
VS 45	2	0	3	2	3	0	0
VS 46	2	0	3	2	5	0	0
VS 48	3	0	3	2	3	0	0
VS 49	3	0	3	2	3	0	3
VS 50	3	0	2	2	3	0	0
VS 52	3	0	2	2	3	0	0
VS 53	3	5	2	2	3	3	3
VS 54	0	0	1	1	3	0	3
VS 55	3	5	3	3	3	0	0
VS 56	3	0	3	3	3	0	0
VS 57	0	0	2	3	3	0	3
VS 58	2	0	1	2	3	0	3
VS 59	2	0	2	2	3	0	0
VS 60	3	0	3	2	3	0	0
VS 61	3	0	2	2	3	0	0
VS 62	3	0	2	2	3	0	0
VS 63	3	0	1	2	5	0	0
VS 64	3	3	3	3	3	0	0
VS 65	3	0	3	3	3	0	0
VS 66	3	3	3	3	3	0	0
VS 68	2	3	3	2	7	0	0
VS 69	2	0	3	3	3	0	0

Table 5. Seed characters and incidence of Fusarium wilt in vegetable cowpea

Accession	Seed shape	Seed colour	Testa texture	Incidence of Fusarium wilt
VS 1	1	9	2	0
VS 2	1	7	1	3
VS 3	1	7	3	5
VS 4	1	5	3	3
VS 5	4	2	3	0
VS 6	5	1	1	3
VS 7	1	3	3	0
VS 8	1	7	2	3
VS 9	1	7	1	3
VS 10	1	7	1	3
VS 11	1	2	3	0
VS 12	1	9	3	0
VS 13	1	7	1	3
VS 14	1	9	2	0
VS 15	1	9	3	5
VS 16	1	8	2	3
VS 17	1	5	1	3
VS 18	1	9	3	0
VS 19	1	7	2	0
VS 20	1	7	3	0
VS 21	1	9	2	0
VS 22	1	9	1	3
VS 23	2	1	1	0
VS 24	1	9	2	5
VS 25	1	9	2	5
VS 26	1	5	1	3
VS 27	1	3	4	3
VS 28	1	7	2	3
VS 29	1	9	2	3
VS 30	1	3	1	3
VS 31	1	7	2	3
VS 32	1	5	2	5
VS 33	1	6	2	0

Contd...

Table 5. Continued...

Accession	Seed shape	Seed colour	Testa texture	Incidence of Fusarium wilt
VS 34	1	1	1	0
VS 35	1	4	1	0
VS 36	1	7	1	0
VS 37	2	1	1	0
VS 38	5	2	1	0
VS 39	5	7	1	0
VS 40	1	7	1	0
VS 41	5	1	1	0
VS 42	2	7	1	0
VS 43	1	2	2	0
VS 44	2	3	1	0
VS 45	1	4	1	0
VS 46	5	7	1	0
VS 48	5	7	1	0
VS 49	5	3	1	0
VS 50	2	3	1	0
VS 52	2	3	1	0
VS 53	1	1	1	0
VS 54	1	2	2	0
VS 55	5	7	1	0
VS 56	2	4	1	0
VS 57	5	2	1	0
VS 58	5	7	1	0
VS 59	2	7	1	0
VS 60	2	3	1	0
VS 61	1	2	2	0
VS 62	5	3	1	0
VS 63	5	1	1	0
VS 64	5	8	1	0
VS 65	2	7	1	0
VS 66	2	7	1	0
VS 68	5	1	1	0
VS 69	5	7	1	0

lightly pigmented standards. Calyx colour was mostly green, while a few accessions had light to deep pigmented calyx. Variation was also noticed in the nature of flowering as well as in the position of racemes. Majority of the accessions had synchronous flowering throughout the canopy.

Pod attachment to peduncle was mostly pendant with no pigmentation on pods. VS 2, VS 9, VS 31, VS 42, VS 44 and VS 53 had pigmented valves and green sutures, while ten others had pigmentation only on the tips. Pod curvature was either straight, slightly curved or curved.

Most of the accessions had kidney shaped seeds, while a few of them possessed ovoid, globose or rhomboid shape as well. Wide variation was noticed in seed colour ranging from light brown to black, majority having dark brown coloured seeds. VS 35, VS 45 and VS 56 had brown seeds with stripes, while VS 4, VS 17 and VS 26 and VS 32 had seeds which are brown with white tip. VS 16 was noticed with brown and white seeds, whereas dark brown and white seeds were characteristic to VS 33. Testa texture ranged from smooth, smooth to rough and rough types.

Fusarium wilt incidence was noticed among yard long beans only. There was no symptom in few of the accessions, while majority showed mild incidence of the disease. VS 3, VS 15, VS 24, VS 25 and VS 32 recorded medium incidence of *Fusarium* wilt.

4.2 Variability in vegetable cowpea

4.2.1 Mean performance

Analysis of variance showed significant differences among the accessions for all the characters studied (Table 6). The mean values of 66 accessions for different characters are presented in Table 7.

Days to seedling emergence

There was significant difference among the accessions for seedling emergence. It ranged from 3.50 to 6.00 days. VS 5 was the earliest to emerge (3.50 days), while VS 38 was the latest (6.00 days).

Table 6. Analysis of variance for 27 characters in 66 accessions of vegetable cowpea (Mean squares)

Source	df	Days to seedling emergence	Vine length at final harvest	Collar girth	Primary branches	Petiole length	Terminal leaflet length	Terminal leaflet width	Lateral leaflet length	Lateral leaflet width
Replication	1	0.37	0.002	0.05	0.03	0.72	0.38	0.26	1.14	0.12
Genotype	65	0.48**	5.60**	2.41**	1.53**	7.71**	7.34**	2.46**	7.47**	2.60**
Error	65	0.18	0.07	0.19	0.27	1.19	0.31	0.19	0.40	0.22

Source	df	Root:shoot ratio	Days to first flowering	Pollen viability	Peduncle length	Pod length	Pod girth	Pod weight	Pods per plant	Seeds per pod
Replication	1	0.07*	10.02	0.63	0.13	2.75	0.03	1.63	8.25	0.23
Genotype	65	0.06**	22.42**	99.41**	118.48**	448.02**	0.44**	131.61**	910.11**	11.51**
Error	65	0.01	3.69	13.08	3.17	4.05	0.03	1.05	158.97	1.50

Source	df	100-seed weight	Seed length	Seed width	Seed thickness	Number of harvests	Yield per plant	Keeping quality	Pod protein	Pod fibre
Replication	1	2.38	6.04**	0.43*	0.24*	2.45*	545.00	3.85**	0.03	0.05
Genotype	65	25.12**	9.24**	0.86**	0.32**	3.93**	83650.43**	1.37**	2.70**	0.25**
Error	65	0.68	0.30	0.07	0.06	0.56	6342.35	0.24	0.05	0.03

*Significant at 5 per cent level ** Significant at 1 per cent level

Table 7. Mean value of biometric characters

Accession No.	Days to seedling emergence	Vine length at final harvest (m)	Stem girth at collar region (cm)	Primary branches	Petiole length (cm)	Terminal leaflet length (cm)	Terminal leaflet width (cm)	Lateral leaflet length (cm)	Lateral leaflet width (cm)	Root : shoot ratio	Days to first flowering	Pollen viability (%)	Peduncle length (cm)
VS 1	4.59	4.53	6.48	3.50	8.03	18.75	14.57	10.12	5.88	0.03	44.75	94.62	15.13
VS 2	4.93	5.52	4.35	3.25	10.60	17.50	15.20	10.47	5.67	0.08	48.50	94.41	10.38
VS 3	4.00	4.51	6.38	3.75	7.05	17.12	6.80	7.06	4.93	0.09	45.00	72.90	12.50
VS 4	4.35	6.08	5.88	2.75	12.48	17.00	10.35	6.51	5.30	0.07	45.00	90.37	22.80
VS 5	3.50	0.50	5.13	4.50	6.65	14.84	8.94	6.39	4.43	0.21	38.40	95.27	40.50
VS 6	4.00	1.77	3.13	6.75	11.54	11.80	7.96	6.59	5.00	0.38	45.00	91.05	38.68
VS 7	4.30	0.62	5.28	4.53	10.35	15.46	9.09	6.91	5.11	0.23	34.67	91.43	39.58
VS 8	4.25	3.91	4.13	4.00	9.55	16.39	9.47	6.81	5.14	0.13	39.83	91.90	14.93
VS 9	4.25	4.53	3.85	3.50	8.55	13.92	10.57	8.51	5.87	0.04	51.17	94.79	12.98
VS 10	4.00	3.62	4.05	4.25	7.20	13.84	7.77	6.89	4.62	0.06	40.00	97.46	14.58
VS 11	4.27	0.65	4.83	4.25	10.25	14.40	11.97	8.75	6.45	0.32	37.17	91.89	36.95
VS 12	4.80	0.83	4.68	4.00	12.55	12.50	9.63	8.04	4.60	0.20	37.25	90.89	37.18
VS 13	4.84	4.80	5.10	4.25	11.78	7.80	5.11	5.77	3.80	0.10	44.75	91.30	18.95
VS 14	4.59	4.88	4.78	5.00	8.18	15.42	9.86	7.41	5.10	0.11	44.50	91.47	30.34
VS 15	4.17	4.16	3.83	5.50	8.25	14.17	9.03	6.01	4.67	0.03	40.83	99.02	13.13
VS 16	5.25	4.65	4.33	3.25	7.95	16.25	7.15	6.01	5.06	0.08	44.92	91.56	15.25
VS 17	4.58	5.48	5.35	3.75	11.34	17.24	7.90	6.50	5.48	0.05	44.00	88.00	17.91
VS 18	4.42	4.28	4.88	3.50	10.36	15.84	9.87	7.51	5.59	0.12	38.34	96.07	14.60
VS 19	4.09	6.17	5.73	4.00	10.43	15.50	12.29	9.29	5.54	0.06	39.17	96.29	17.80
VS 20	4.59	4.28	5.18	4.75	10.95	19.00	10.09	6.59	4.97	0.10	42.25	91.70	15.81
VS 21	4.00	4.32	5.45	4.50	11.05	17.84	10.91	6.41	5.85	0.08	41.25	94.24	14.38
VS 22	4.42	5.98	6.05	3.75	9.33	16.00	7.21	6.70	4.61	0.07	41.42	92.71	22.34
VS 23	5.30	4.59	5.40	4.25	9.85	18.34	10.22	6.53	5.59	0.03	50.34	91.56	25.98
VS 24	4.75	4.11	5.90	5.25	9.05	12.09	11.18	6.71	5.82	0.09	44.17	92.81	12.38
VS 25	4.80	4.20	5.45	4.75	9.68	14.17	9.68	6.90	5.68	0.12	44.50	95.65	12.68
VS 26	4.13	5.07	5.53	3.50	12.38	15.63	12.86	7.80	5.34	0.04	45.50	94.27	14.78
VS 27	4.60	5.85	5.70	3.25	10.08	17.83	9.53	6.52	5.39	0.04	44.50	93.44	12.83
VS 28	5.00	3.85	4.90	4.00	8.50	14.84	7.55	6.72	4.84	0.15	42.84	61.71	15.80
VS 29	4.75	4.66	5.65	3.75	7.35	15.33	7.00	6.18	4.66	0.18	42.17	86.32	16.38
VS 30	4.40	4.63	5.68	2.50	11.60	17.84	9.62	6.52	5.37	0.06	44.17	94.31	12.83
VS 31	5.09	5.33	4.25	3.00	9.65	14.42	10.44	5.98	5.83	0.06	42.17	94.17	19.78
VS 32	4.67	4.73	5.80	3.75	12.54	11.67	4.51	5.08	3.57	0.05	44.67	93.48	26.29
VS 33	4.62	1.71	3.65	3.50	9.10	15.84	9.88	6.42	5.16	0.15	41.17	89.68	26.00
CD (5%)	0.8436	0.5455	0.8753	1.0443	2.1776	1.1126	0.8775	1.2670	0.9398	0.2303	3.8408	7.2319	3.5593

Contd...

Table 7.Continued...

Accession No.	Pod length (cm)	Pod girth (cm)	Pod weight (g)	Pods per plant	Seeds per pod	100 – seed weight (g)	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	Number of harvests	Yield per plant (g)	Keeping quality (days)	Pod protein (%)	Pod fibre (%)
VS 1	43.35	2.65	14.58	6.87	18.17	16.85	11.13	6.55	5.10	5.00	91.95	5.25	3.90	2.51
VS 2	58.08	3.08	18.15	28.09	20.25	12.38	11.37	6.19	3.93	4.00	299.59	3.25	4.98	2.28
VS 3	43.66	2.88	14.85	41.67	18.50	12.99	9.30	4.79	3.22	5.00	441.58	3.50	5.59	2.35
VS 4	67.63	3.90	43.60	25.00	21.34	19.38	11.02	5.63	3.33	5.50	662.73	3.50	6.90	3.64
VS 5	21.63	2.52	6.20	48.59	16.67	10.20	7.31	5.24	4.87	3.50	245.82	5.50	5.78	2.70
VS 6	13.62	2.04	2.82	25.70	16.17	6.34	5.39	4.49	3.39	3.00	72.50	2.50	5.41	2.04
VS 7	26.09	2.82	7.50	27.10	13.84	10.82	10.51	5.48	3.51	2.50	185.08	3.00	6.36	2.02
VS 8	32.64	3.08	12.70	102.59	17.34	9.55	9.55	5.27	3.18	6.00	1136.89	3.77	6.10	2.33
VS 9	53.62	3.09	19.27	40.88	19.17	11.52	10.66	5.79	3.49	4.00	432.86	3.34	6.41	3.30
VS 10	22.25	3.13	10.46	39.27	16.50	11.97	9.11	4.63	3.84	5.50	339.69	4.70	4.71	2.50
VS 11	25.26	2.89	10.94	23.98	14.40	11.63	10.02	5.78	4.29	4.00	177.67	4.75	4.90	2.48
VS 12	29.84	2.64	7.48	32.60	15.00	14.72	10.48	5.08	3.54	3.50	213.57	5.00	5.59	3.08
VS 13	45.80	2.94	14.32	40.19	18.34	16.48	10.83	5.96	4.00	4.50	400.72	4.17	6.29	2.53
VS 14	46.37	3.13	17.12	34.79	19.13	16.08	10.75	5.94	4.21	5.00	457.22	4.50	5.50	2.99
VS 15	40.58	2.74	14.00	18.50	19.17	10.73	10.38	5.86	4.31	4.50	217.07	3.50	5.22	2.20
VS 16	43.95	3.13	18.88	31.50	19.75	17.14	11.19	5.67	3.94	3.50	311.25	3.34	5.32	1.99
VS 17	52.91	3.64	20.26	47.25	17.50	13.80	12.23	6.00	3.76	3.50	627.94	3.00	5.87	2.39
VS 18	39.93	2.85	10.60	88.37	17.67	10.95	9.67	5.23	3.95	4.50	761.91	3.67	4.08	2.92
VS 19	76.08	4.43	42.78	21.39	18.75	20.77	13.03	6.03	4.35	5.50	564.44	3.46	6.46	3.72
VS 20	38.88	3.40	15.90	41.38	16.25	12.72	10.48	5.98	3.63	5.00	371.25	3.50	5.69	2.21
VS 21	35.60	3.30	13.39	43.93	17.88	17.31	11.54	6.64	4.15	3.00	214.17	5.00	6.38	2.95
VS 22	48.52	3.02	16.88	49.50	19.13	16.56	11.18	6.08	3.90	5.00	550.83	4.00	7.52	2.29
VS 23	26.14	2.94	10.78	13.90	15.50	16.38	9.66	6.75	4.44	2.50	139.00	4.13	8.09	2.34
VS 24	33.33	2.59	9.58	67.34	20.84	12.72	10.50	5.65	3.85	4.00	622.09	3.00	7.57	2.10
VS 25	39.61	3.04	15.22	57.11	18.45	18.08	11.75	6.60	3.95	4.50	620.04	4.00	8.26	2.27
VS 26	51.14	2.68	22.98	39.60	19.10	14.37	10.87	5.16	3.83	4.00	497.44	4.50	7.62	2.73
VS 27	57.87	3.50	23.91	60.55	18.00	16.49	11.68	6.14	4.00	5.50	815.92	4.00	8.27	2.46
VS 28	27.83	2.90	14.37	47.70	16.50	15.76	10.64	6.28	4.01	6.00	489.30	4.13	6.75	2.14
VS 29	43.60	2.75	11.87	66.25	20.00	16.65	11.65	6.51	3.75	6.00	696.23	4.50	7.40	2.20
VS 30	42.55	2.58	12.23	71.88	17.88	12.38	10.09	5.66	3.87	5.00	695.45	4.67	7.66	2.44
VS 31	51.88	2.88	15.58	36.50	18.50	12.97	10.74	5.81	3.31	3.00	411.67	3.50	7.81	2.32
VS 32	36.28	3.30	14.75	27.25	19.00	12.33	10.82	5.44	3.49	3.50	261.46	2.00	7.31	1.95
VS 33	20.43	2.35	3.79	21.25	13.97	11.10	10.04	5.95	4.09	3.00	134.38	2.84	6.48	2.31
CD (5%)	4.0266	0.3642	2.0475	25.2165	2.4456	1.6526	1.0911	0.5162	0.4735	1.4996	159.2778	0.9781	0.4591	0.3197

Contd...

Table 7.Continued...

Accession No.	Days to seedling emergence	Vine length at final harvest (m)	Stem girth at collar region (cm)	Primary branches	Petiole length (cm)	Terminal leaflet length (cm)	Terminal leaflet width (cm)	Lateral leaflet length (cm)	Lateral leaflet width (cm)	Root : shoot ratio	Days to first flowering	Pollen viability (%)	Peduncle length (cm)
VS 34	4.50	2.58	2.90	3.25	8.65	17.98	10.85	15.25	9.90	0.07	50.00	75.95	20.60
VS 35	4.42	3.50	2.95	2.00	11.20	14.70	9.50	16.83	10.68	0.07	45.00	93.52	21.70
VS 36	3.67	3.30	4.50	3.50	12.23	12.55	8.73	11.33	7.73	0.30	47.67	92.90	21.63
VS 37	4.29	2.63	3.00	3.00	12.40	14.10	9.40	12.58	8.55	0.08	44.67	89.00	24.53
VS 38	6.00	3.03	3.40	3.25	8.88	8.55	6.15	8.23	5.40	0.16	47.00	60.41	30.98
VS 39	4.35	3.68	3.05	3.25	10.20	12.15	9.38	12.20	8.20	0.59	42.67	89.37	21.98
VS 40	4.50	3.03	4.08	2.75	12.85	12.00	9.05	11.68	8.10	0.22	44.50	98.41	29.85
VS 41	3.92	3.01	3.25	3.75	12.28	13.48	8.43	11.68	7.85	0.13	41.50	97.22	26.88
VS 42	4.92	2.45	2.95	2.00	7.75	16.90	10.95	15.13	9.40	0.45	42.34	96.29	28.00
VS 43	4.15	0.58	3.93	4.00	12.88	12.10	8.05	11.95	9.13	0.14	38.17	91.95	30.30
VS 44	5.00	2.90	2.68	3.00	15.15	15.30	9.15	13.68	8.55	0.25	42.34	90.29	27.18
VS 45	4.50	2.13	3.55	4.25	8.70	12.78	8.05	11.98	7.78	0.18	42.17	91.26	20.15
VS 46	5.60	1.73	2.35	4.75	11.25	10.03	7.78	8.60	7.08	0.37	44.84	94.08	22.68
VS 48	4.90	2.95	4.10	4.00	12.93	12.20	8.65	10.33	7.23	0.29	43.92	97.09	27.80
VS 49	4.10	2.43	5.10	5.75	8.35	11.13	9.55	10.33	8.60	0.76	41.75	97.93	28.13
VS 50	5.75	1.38	3.35	4.00	9.55	12.00	8.43	12.00	8.33	0.14	36.17	90.73	27.40
VS 52	4.08	0.82	4.25	5.25	12.53	10.50	7.39	10.35	7.20	0.25	37.00	84.98	26.43
VS 53	4.00	2.17	3.60	3.75	9.25	13.45	8.58	11.40	7.95	0.46	42.50	95.59	22.25
VS 54	4.17	0.56	5.25	4.25	9.48	13.23	9.00	12.55	8.93	0.16	40.34	88.83	32.78
VS 55	3.92	1.52	2.90	3.75	14.45	12.10	7.83	10.70	7.40	0.25	40.50	97.08	27.70
VS 56	4.84	1.85	4.65	4.00	10.55	11.10	7.53	10.35	7.20	0.55	42.67	95.69	20.93
VS 57	5.23	1.12	2.55	4.50	9.00	10.95	7.23	8.95	6.05	0.23	40.50	93.70	27.13
VS 58	4.09	0.42	2.20	2.75	11.88	10.35	7.13	9.88	7.05	1.00	42.00	96.21	13.25
VS 59	4.20	2.41	4.75	3.50	10.55	10.43	8.18	9.95	8.00	0.08	40.09	92.92	17.88
VS 60	5.34	0.86	3.75	2.50	11.18	11.73	8.00	11.53	7.15	0.24	39.34	93.12	17.25
VS 61	5.13	1.26	3.95	2.00	12.75	12.63	8.40	12.48	8.30	0.16	40.67	93.32	25.33
VS 62	4.83	0.68	3.50	3.25	14.50	11.65	7.78	11.13	7.98	0.26	39.59	94.91	20.93
VS 63	5.14	1.47	3.38	3.50	9.90	9.42	6.45	8.75	6.18	0.20	40.42	97.75	16.23
VS 64	4.47	1.53	3.28	4.00	12.10	13.50	7.73	13.13	7.80	0.21	37.00	92.45	23.88
VS 65	4.17	2.18	3.70	3.25	13.58	13.58	9.03	12.70	8.60	0.15	42.75	96.22	26.58
VS 66	4.67	2.38	4.05	3.75	11.88	8.83	6.98	8.93	6.33	0.20	41.75	98.67	25.43
VS 68	4.84	2.05	4.40	3.00	8.10	10.88	6.63	10.28	6.13	0.19	39.17	96.35	25.23
VS 69	4.75	1.53	2.33	4.25	8.80	10.63	6.65	9.73	6.43	0.39	43.67	87.93	39.43
CD (5%)	0.8436	0.5455	0.8753	1.0443	2.1776	1.1126	0.8775	1.2670	0.9398	0.2303	3.8408	7.2319	3.5593

Contd...

Table 7.Continued...

Accession No.	Pod length (cm)	Pod girth (cm)	Pod weight (g)	Pods per plant	Seeds per plant	100 – seed weight (g)	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	Number of harvests	Yield per plant (g)	Keeping quality (days)	Pod protein (%)	Pod fibre (%)
VS 34	40.62	3.10	11.39	16.33	18.75	14.57	10.12	5.88	4.58	2.50	177.90	3.25	7.42	2.61
VS 35	23.80	2.75	9.38	35.65	17.50	15.20	10.47	5.67	4.11	3.50	335.00	3.13	6.54	2.30
VS 36	16.95	2.17	3.27	26.84	17.12	6.80	7.06	4.93	3.36	3.00	137.09	2.59	7.95	2.27
VS 37	22.90	2.65	6.07	33.00	17.00	10.35	6.51	5.30	4.18	3.00	149.72	2.63	5.96	2.55
VS 38	16.10	2.06	5.72	78.17	14.84	8.94	6.39	4.43	3.73	5.50	298.67	3.38	3.73	2.67
VS 39	18.98	2.73	4.75	83.25	16.40	7.96	6.59	5.00	3.66	4.50	397.13	3.50	6.19	2.65
VS 40	25.90	2.38	3.92	46.09	15.46	9.09	6.91	5.11	3.69	3.50	182.59	3.00	5.44	2.94
VS 41	19.85	2.50	5.00	68.25	16.39	9.47	6.81	5.14	3.88	5.00	300.42	2.75	3.50	2.39
VS 42	22.20	2.35	5.38	28.00	13.92	10.57	8.51	5.87	4.38	3.00	157.50	2.84	4.18	2.59
VS 43	18.19	2.65	5.35	30.92	13.84	7.77	6.89	4.62	3.67	4.50	142.92	3.00	6.59	2.36
VS 44	22.55	2.68	5.82	48.42	14.40	11.97	8.75	6.45	4.21	6.00	296.34	2.88	8.75	3.05
VS 45	21.75	3.28	6.88	47.84	12.50	9.63	8.04	4.60	3.73	5.50	309.17	2.00	5.82	2.40
VS 46	12.35	2.38	2.49	39.63	7.80	5.11	5.77	3.80	2.98	3.00	98.47	2.13	5.30	2.26
VS 48	15.60	2.30	4.71	35.00	15.42	9.86	7.41	5.10	4.05	3.00	157.50	3.13	5.25	2.73
VS 49	20.45	2.85	5.98	86.63	14.17	9.03	6.01	4.67	3.61	5.50	495.00	3.75	5.97	2.73
VS 50	18.98	2.35	5.91	55.42	16.25	7.15	6.01	5.06	3.50	7.50	317.92	3.00	6.36	2.77
VS 52	17.60	2.41	5.36	77.63	17.24	7.90	6.50	5.48	3.96	8.00	445.50	3.38	5.72	2.76
VS 53	23.80	2.42	5.12	75.84	15.84	9.87	7.51	5.59	3.97	5.50	367.50	2.88	6.43	2.51
VS 54	22.28	2.56	5.99	56.39	15.50	12.29	9.29	5.54	4.05	5.50	305.28	3.38	6.41	2.77
VS 55	18.62	2.63	6.00	28.59	19.00	10.09	6.59	4.97	4.05	5.00	113.17	2.25	5.87	2.30
VS 56	17.98	2.57	5.34	58.34	17.84	10.91	6.41	5.85	4.22	6.50	277.09	2.25	6.94	2.39
VS 57	17.08	2.14	3.44	82.84	16.00	7.21	6.70	4.61	3.83	6.00	280.00	3.00	6.36	2.55
VS 58	16.84	2.85	5.04	44.34	18.34	10.22	6.53	5.59	4.13	6.50	219.34	2.34	6.42	2.28
VS 59	18.95	1.99	5.25	53.67	12.09	11.18	6.71	5.82	4.13	8.00	282.92	3.13	7.21	2.25
VS 60	13.95	2.09	4.24	67.09	14.17	9.68	6.90	5.68	4.25	5.50	288.75	2.75	7.95	2.73
VS 61	17.50	2.17	3.15	66.50	15.63	12.86	7.80	5.34	4.33	7.50	217.59	2.55	5.57	2.34
VS 62	18.51	2.83	5.29	51.92	17.83	9.53	6.52	5.39	3.80	7.50	275.92	2.84	6.34	3.03
VS 63	15.27	2.22	3.01	79.34	14.84	7.55	6.72	4.84	3.95	5.00	265.42	2.00	6.72	2.44
VS 64	13.30	1.99	2.42	80.16	15.33	7.00	6.18	4.66	3.71	5.50	192.75	3.00	6.31	2.38
VS 65	17.75	2.53	4.98	62.58	17.84	9.62	6.52	5.37	3.64	6.50	302.05	3.25	6.31	2.83
VS 66	17.42	2.34	4.02	59.60	14.42	10.44	5.98	5.83	4.10	5.00	237.83	2.84	8.26	2.67
VS 68	12.75	2.10	2.47	69.57	11.67	4.51	5.08	3.57	2.93	4.50	93.94	3.34	5.21	2.83
VS 69	16.95	2.18	3.27	28.79	15.84	9.88	6.42	5.16	3.81	3.00	171.50	2.13	6.42	2.38
CD (5%)	4.0266	0.3642	2.0475	25.2165	2.4456	1.6526	1.0911	0.5162	0.4735	1.4996	159.2778	0.9781	0.4591	0.3197

Vine length

Vine length was found to vary from 0.42 to 6.17 m with an overall mean of 3.04 m. VS 19 was the longest (6.17 m) and VS 58 was the shortest (0.42 m).

Collar girth

Collar girth ranged from 2.20 to 6.48 cm with a general mean of 4.31 cm. It was highest in VS 1 (6.48 cm) and lowest in VS 58 (2.20 cm).

Primary branches per plant

It was found to vary from 2.00 (VS 42) to 6.75 (VS 6). The accessions on an average had 3.79 primary branches per plant.

Petiole length

Wide variation among the accessions was observed for petiole length. It ranged from 6.65 cm in VS 5 to15.15 cm in VS 44.

Terminal leaflet length

Significant differences among the accessions were observed for terminal leaflet length, having an overall mean of 12.96 cm. VS 34 had the longest (17.98 cm) and VS 38 had the shortest terminal leaflet length (8.55 cm).

Terminal leaflet width

Width of terminal leaflets varied from 6.03 in VS 3 to 10.95 cm in VS 42, with a general mean of 8.26 cm.

Lateral leaflet length

The accessions varied considerably for lateral leaflet length from 8.23 cm in VS 38 to 16.83 cm in VS 35 with an overall mean of 12.14 cm.

Lateral leaflet width

Width of lateral leaflets varied significantly among the accessions from 5.40 (VS 38) to 10.68 cm (VS 35).

Root : shoot ratio

It was observed to range from 0.03 in VS 15 to 1.00 in VS 58, with an average of 0.19.

Days to first flowering

Days to first flowering exhibited a range of 34.67 days in VS 7 to 51.17 in VS 9

Pollen viability

Wide variation was observed among the accessions for pollen viability. It ranged from 60.14 per cent in VS 38 to 99.02 per cent in VS 15, with an overall mean of 91.80 per cent.

Peduncle length

Peduncle length was found to vary from 10.38 to 40.50 cm. The accessions on an average had 22.59 cm long peduncles, the longest being recorded in VS 5 (40.50 cm) and the shortest in VS 2 (10.38 cm).

Pod length

Pod length varied considerably from 12.35 cm in VS 46 to 76.08 cm in VS 19 with an overall mean of 29.86 cm (Plates 1, 2 and 3).

Pod girth

Girth of pods was found to range from 1.99 to 4.43 cm. Fruit girth was highest in VS 19 (4.43 cm) and smallest in VS 59 (1.99 cm).

Pod weight

Range in pod weight among the accessions was from 2.42 to 43.60 g, the highest in VS 4 (43.60 g) and the lowest in VS 64 (2.42 g).

Pods per plant

A wide range of variation was noticed for pods per plant, which was from 6.87 in VS 1 to 102.59 in VS 58.

Seeds per pod

Seeds per pod observed a range from 7.80 in VS 46 to 21.34 in VS 4 with an overall mean of 16.66.

Plate 1.Variability in pod characters – Accessions VS 1 to VS 15

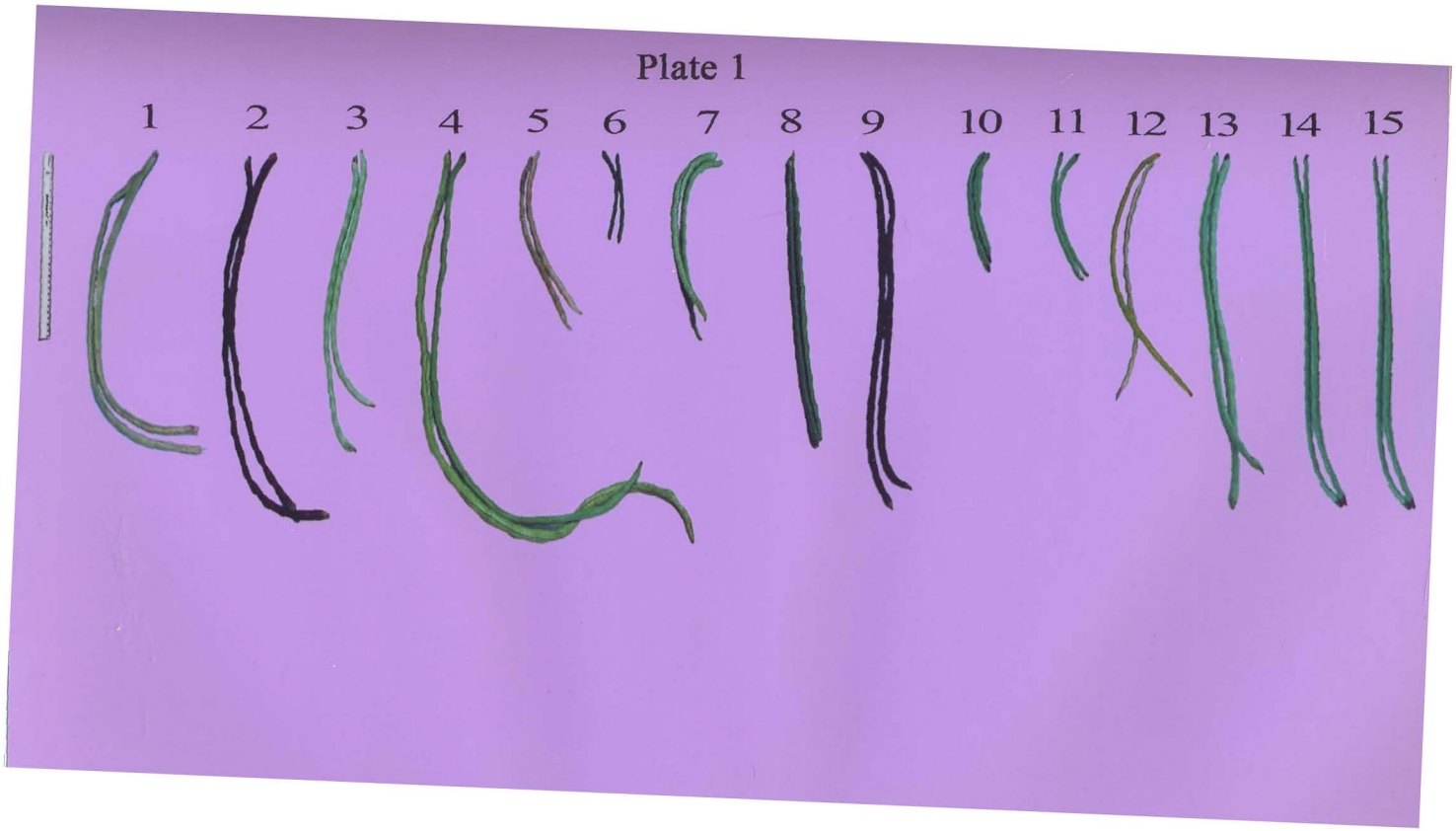


Plate 1
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

Plate 2.Variability in pod characters – Accessions VS 16 to VS 32

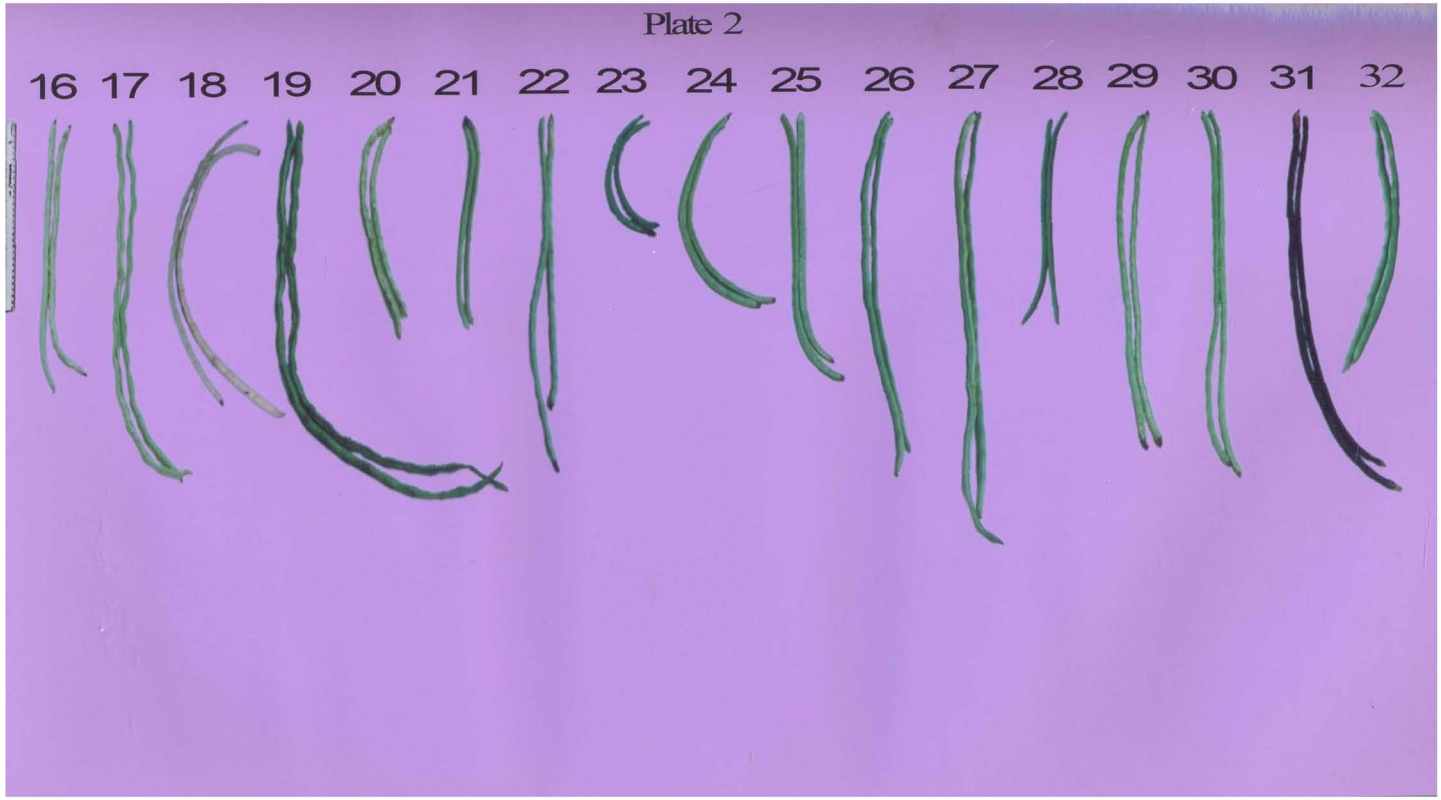


Plate 2
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32

Plate 3. Variability in pod characters – Accessions VS 33 to VS 69

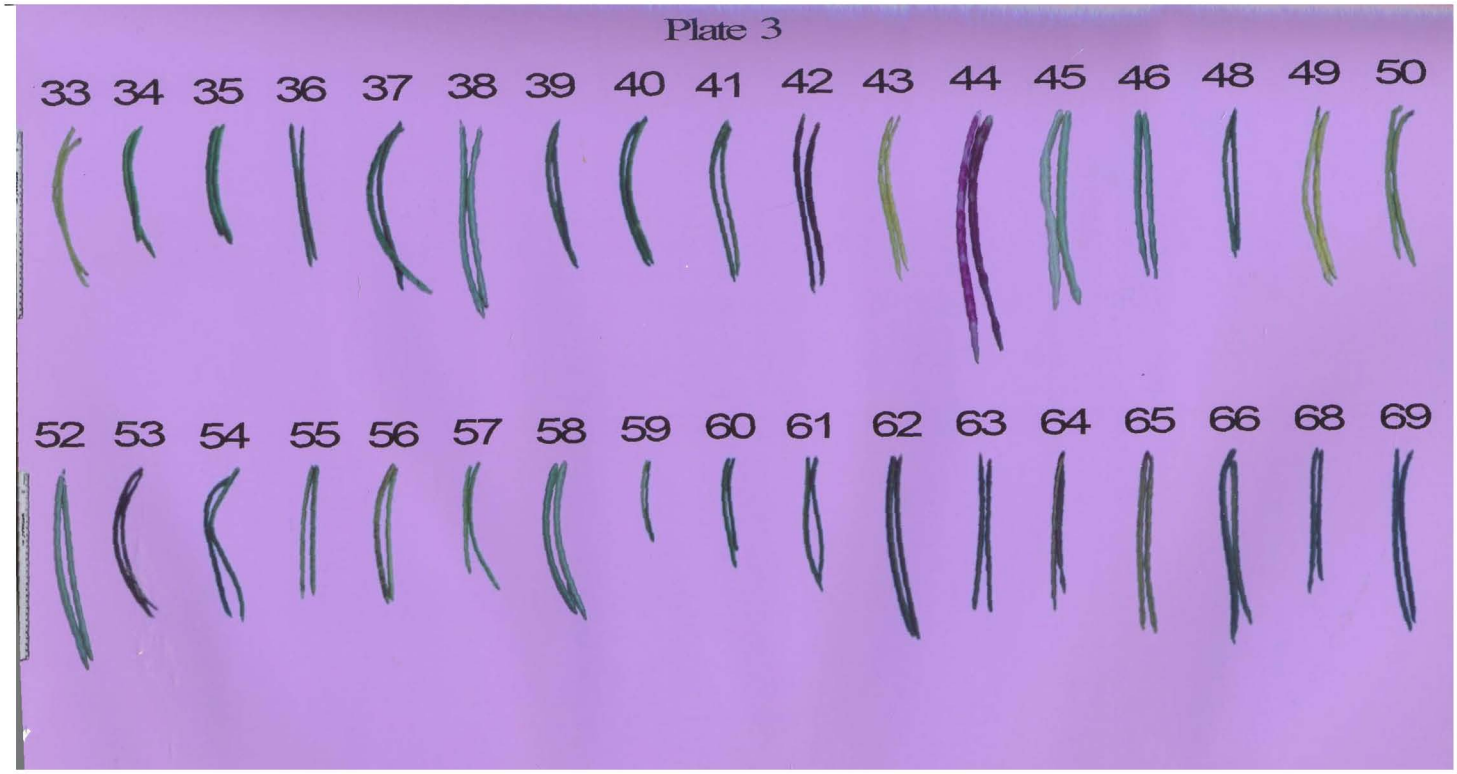


Plate 3
33 34 35 36 37 38 39 40 41 42 43 44 45 46 48 49 50
52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 68 69

100-seed weight

Wide variation in 100-seed weight was observed among the accessions with VS 19 showing the highest value of 20.77 g and VS 68 with the lowest (4.51 g).

Seed length

Seed length observed a range of 5.08 mm in VS 68 to 13.03 mm in VS 19, with an average of 8.76 mm (Plate 4).

Seed width

Among the accessions, seed width was found to vary from 3.57 mm in VS 68 to 6.75 mm in VS 23.

Seed thickness

Seed thickness was found to range from 2.93 (VS 68) to 5.10 mm (VS 1).

Number of harvests

Among the accessions, number of harvests was found to vary from 2.50 in VS 23 to 8.00 in VS 52.

Yield per plant

A wide range of variation was noticed for yield per plant. VS 8 had the highest yield (1136.89 g) which was significantly different from all other accessions. The lowest yield was recorded in VS 6 (72.5 g).

Keeping quality

Keeping quality observed a range of 2.00 to 5.50 days. Among the 66 accessions, VS 5 had the longest keeping quality (5.50 days) while VS 45 had the shortest (2.00 days).

Pod protein

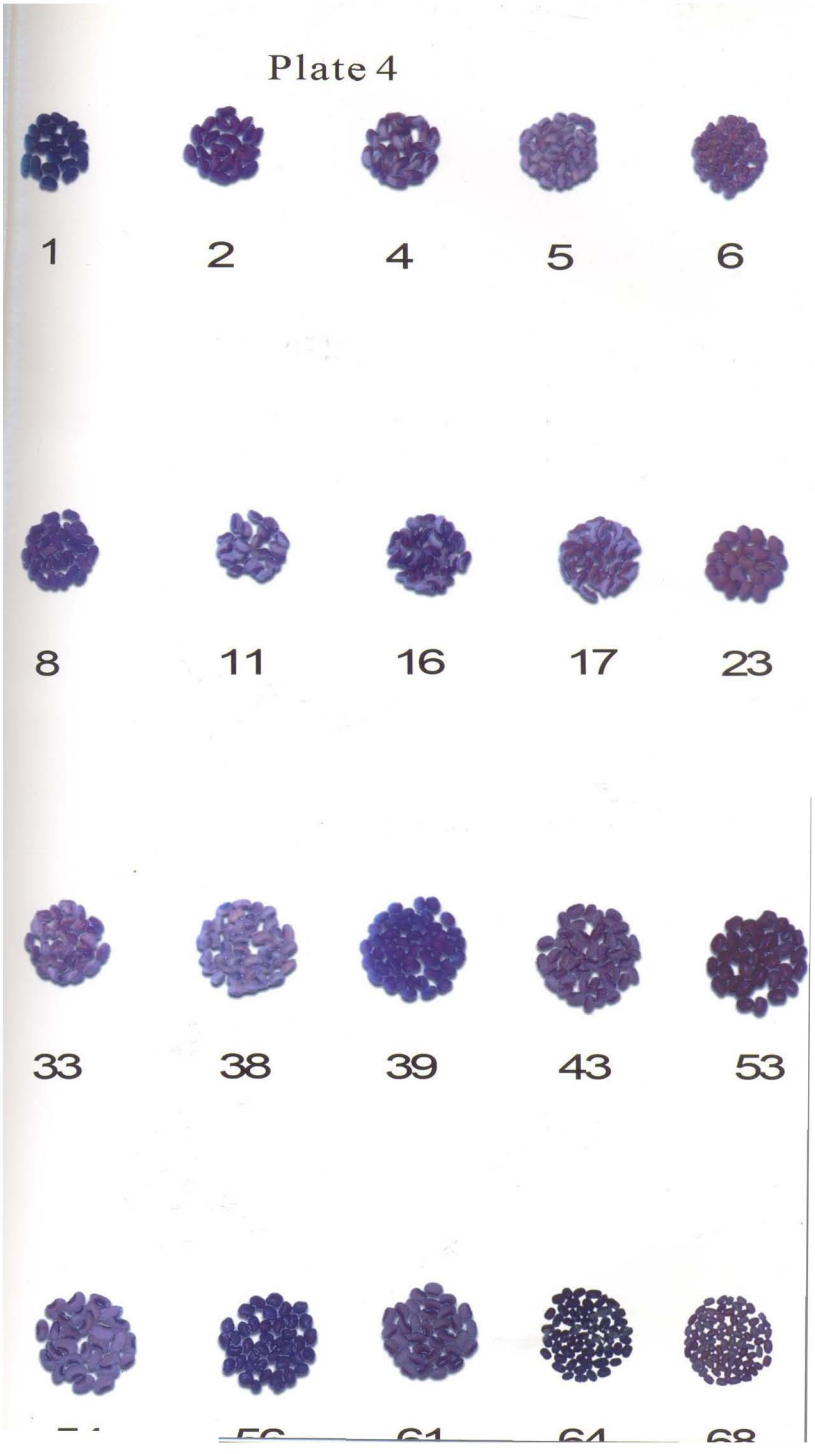
Pod protein content ranged from 3.50 per cent in VS 41 to 8.75 per cent in VS 44 with an overall mean of 6.27 per cent.

Pod fibre

Pod fibre observed a range of 1.95 to 3.72 per cent. VS 19 had the highest content of pod fibre (3.72 per cent), where as VS 32 had the lowest (1.95 per cent).

Plate 4. Variability in seed characters in vegetable cowpea

Plate 4



4.2.2 Genetic parameters

The mean, range, phenotypic, genotypic and environmental variances, phenotypic and genotypic coefficients of variation are given in Table 8.

High phenotypic and genotypic variances were observed for several characters including yield per plant, pods per plant, pod length and pod weight. Wide variation was observed in phenotypic and genotypic variances for all the characters. A close association between phenotypic and genotypic variances were noticed for yield per plant, pods per plant, pod length and pod weight. For most of the characters, genotypic variance makes up the major portion of phenotypic variance, with very little effect of environment.

Phenotypic and genotypic coefficients of variation (PCV and GCV respectively) observed were high for most of the characters (Fig.1). Root : shoot ratio had the highest PCV (101.83) and GCV (82.30), followed by pod weight (79.74 and 79.12 respectively) and yield per plant (62.33 and 57.84 respectively). The lowest PCV and GCV were exhibited by pollen viability (8.16 and 7.17 respectively), followed by days to first flowering (8.51 and 7.23 respectively).

4.2.3 Heritability and genetic advance

Heritability and genetic advance for different characters are presented in Table 9 (Fig.2).

High heritability coupled with high genetic advance was observed for several characters including yield per plant, pods per plant, pod length and pod weight.

Heritability estimate was highest for pod weight (98.44), followed by pod length (98.23), vine length (97.42) and pod protein (96.22). Days to seedling emergence recorded the lowest but a medium heritability (46.67).

Genetic advance was highest for pod weight (161.75), followed by root : shoot ratio (138.14), vine length (111.19), yield per plant (110.56) and pod length (101.88). Low genetic advance was recorded for days to seedling emergence (12.08), days to first flowering (12.64), pollen viability (12.96) and seed thickness (16.03).

Table 8. Range, mean, phenotypic, genotypic and environmental variances, phenotypic and genotypic coefficients of variation for different characters in vegetable cowpea

Sl. No.	Character	Range	Mean ± SE_m	σp^2	σg^2	σe^2	PCV (%)	GCV (%)
1.	Days to seedling emergence	3.50-6.00	4.56±0.30	0.33	0.15	0.18	12.58	8.60
2.	Vine length at final harvest (m)	0.42-6.17	3.04±0.19	2.84	2.76	0.07	55.39	54.66
3.	Collar girth (cm)	2.20-6.48	4.31±0.31	1.30	1.11	0.19	26.46	24.46
4.	Primary branches	2.00-6.75	3.79±0.37	0.90	0.63	0.27	24.97	20.90
5.	Petiole length (cm)	6.65-15.15	10.45±0.77	4.44	3.27	1.19	20.15	17.30
6.	Terminal leaflet length (cm)	8.55-17.98	12.96±0.39	3.82	3.52	0.31	15.09	14.47
7.	Terminal leaflet width (cm)	6.03-10.95	8.26±0.31	1.33	1.14	0.19	13.93	12.90
8.	Lateral leaflet length (cm)	8.60-16.83	12.14±0.45	3.93	3.54	0.40	16.34	15.49
9.	Lateral leaflet width (cm)	5.40-10.68	7.97±0.33	1.41	1.19	0.22	14.82	13.69
10.	Root:shoot ratio	0.03-1.00	0.19±0.08	0.04	0.02	0.01	101.83	82.30
11.	Days to first flowering	34.67-51.17	42.41±1.36	13.03	9.39	3.69	8.51	7.23
12.	Pollen viability (%)	60.41-99.02	91.80±2.56	56.14	43.27	13.08	8.16	7.17
13.	Peduncle length (cm)	10.38-40.50	22.59±1.26	60.80	57.74	3.11	34.52	33.64

Contd...

Table 8. Continued...

Sl. No.	Character	Range	Mean ± SE_m	σp^2	σg^2	σe^2	PCV (%)	GCV (%)
14.	Pod length (cm)	12.35-76.08	29.86±1.42	226.00	222.01	4.05	50.35	49.90
15.	Pod girth (cm)	1.99-4.43	2.72±0.13	0.24	0.20	0.03	17.88	16.61
16.	Pod weight (g)	2.42-43.60	10.21±0.72	66.32	65.29	1.05	79.74	79.12
17.	Pods per plant	6.87-102.59	48.04±8.91	533.33	376.78	158.97	48.07	40.40
18.	Seeds per pod	7.80-21.34	16.66±0.86	6.49	5.02	1.50	15.29	13.45
19.	100 – seed weight (g)	4.51-20.77	11.73±0.58	12.90	12.23	0.68	30.62	29.81
20.	Seed length (mm)	5.08-13.03	8.76±0.39	4.77	4.47	0.30	24.92	24.15
21.	Seed width (mm)	3.57-6.75	5.47±0.18	0.46	0.40	0.07	12.42	11.50
22.	Seed thickness (mm)	2.93-5.10	3.89±0.17	0.19	0.13	0.06	11.09	9.29
23.	Number of harvests	2.50-8.00	4.73±0.53	2.24	1.69	0.56	31.66	27.47
24.	Yield per plant (g)	72.50-1136.89	340.14±56.31	44948.33	38702.09	0.23	62.33	57.84
25.	Keeping quality (days)	2.00-5.50	3.36±0.35	0.80	0.60	0.05	26.72	22.47
26.	Pod protein (%)	3.50-8.75	6.27±0.16	1.37	1.32	0.02	18.71	18.35
27.	Pod fibre (%)	1.95-3.72	2.53±0.11	0.14	0.11	6342.43	14.65	13.25

1 - Days to seedling emergence
2 - Vine length
3 - Collar girth
4 - Primary branches per plant
5 - Petiole length
6 - Terminal leaflet length
7 - Terminal leaflet width
8 - Lateral leaflet length
9 - Lateral leaflet width
10 - Root : shoot ratio
11 - Days to first flowering
12 - Pollen viability
13 - Peduncle length
14 - Pod length
15 - Pod girth
16 - Pod weight
17 - Pods per plant
18 - Seeds per pod
19 - 100-seed weight
20 - Seed length
21 - Seed width
22 - Seed thickness
23 - Number of harvests
24 - Yield per plant
25 - Keeping quality
26 - Pod protein
27 - Pod fibre

Fig. 1. Phenotypic and genotypic coefficients of variation for 27 characters in vegetable cowpea

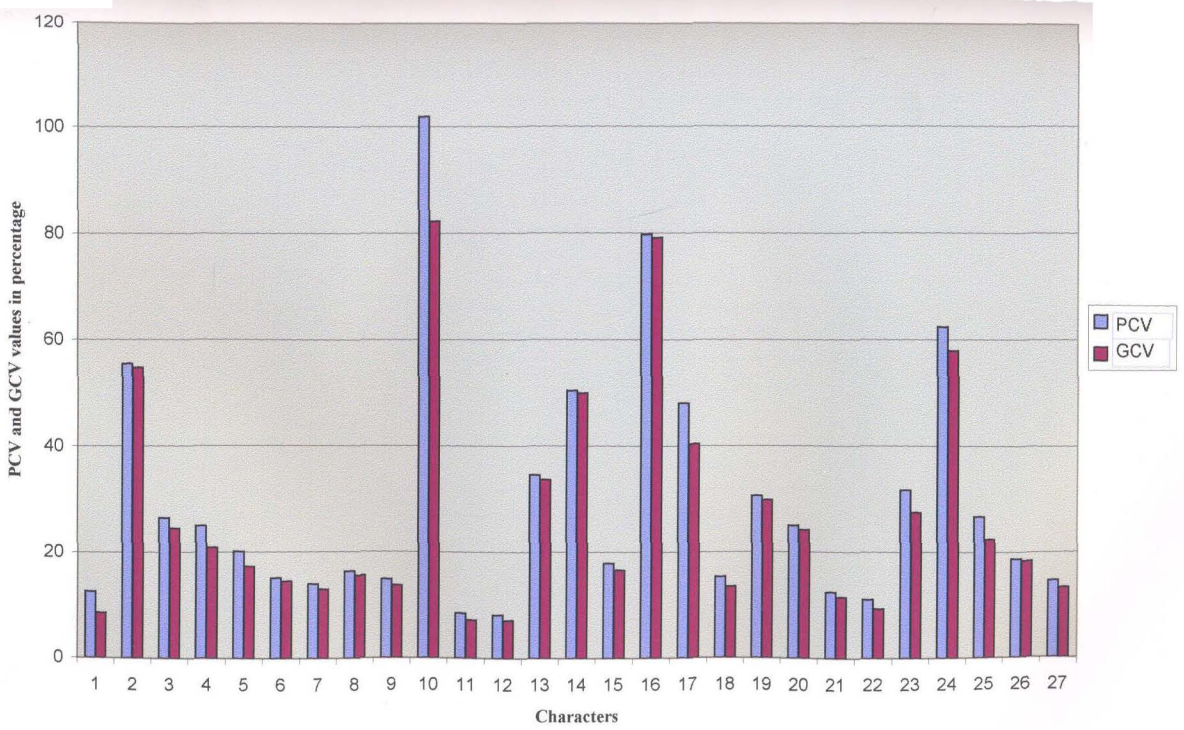
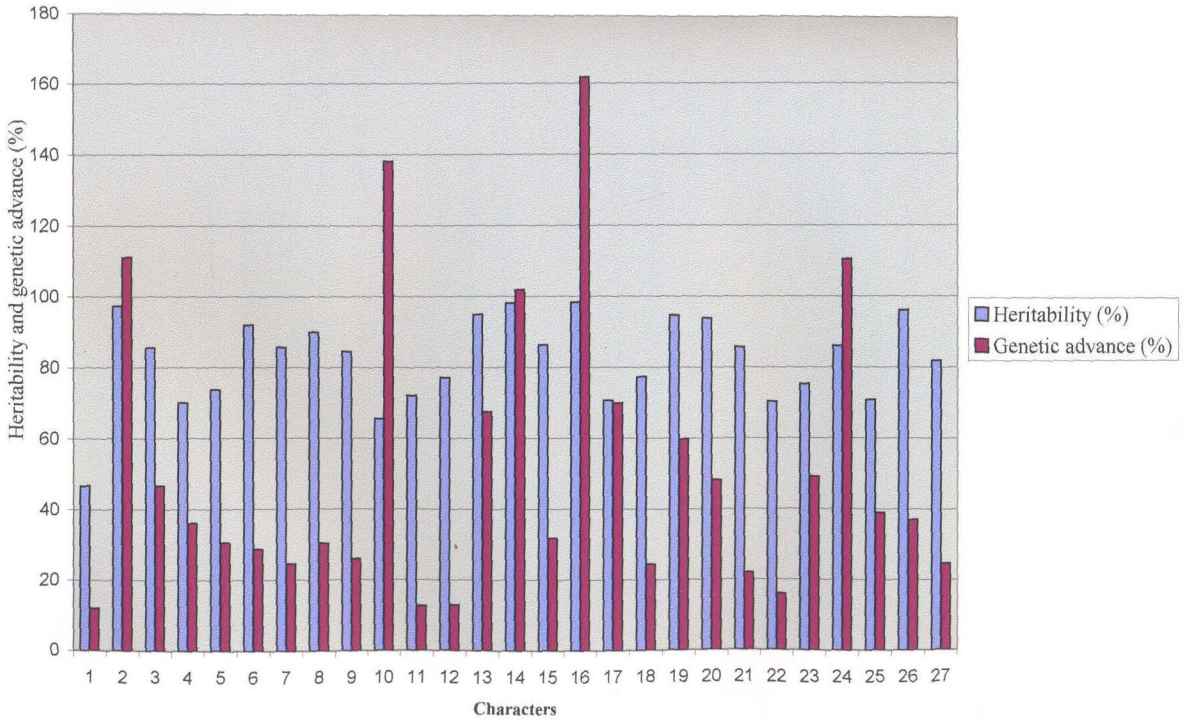


***Table 9. Heritability* and genetic advance for different characters in vegetable cowpea**

Sl. No.	Character	Heritability (%)	Genetic advance (%)
1.	Days to seedling emergence	46.67	12.08
2.	Vine length at final harvest (m)	97.42	111.19
3.	Collar girth (cm)	85.48	46.56
4.	Primary branches	70.10	36.11
5.	Petiole length (cm)	73.68	30.59
6.	Terminal leaflet length (cm)	92.03	28.61
7.	Terminal leaflet width (cm)	85.69	24.60
8.	Lateral leaflet length (cm)	89.95	30.26
9.	Lateral leaflet width (cm)	84.54	25.92
10.	Root : shoot ratio	65.50	138.14
11.	Days to first flowering	72.11	12.64
12.	Pollen viability (%)	77.06	12.96
13.	Peduncle length (cm)	94.96	67.52
14.	Pod length (cm)	98.23	101.88
15.	Pod girth (cm)	86.24	31.82
16.	Pod weight (g)	98.44	161.75
17.	Pods per plant	70.65	69.95
18.	Seeds per pod	77.32	24.36
19.	100 – seed weight (g)	94.79	59.78
20.	Seed length (mm)	93.85	48.19
21.	Seed width (mm)	85.78	21.94
22.	Seed thickness (mm)	70.27	16.03
23.	Number of harvests	75.27	49.07
24.	Yield per plant (g)	86.10	110.56
25.	Keeping quality (days)	70.74	38.91
26.	Pod protein (%)	96.22	37.06
27.	Pod fibre (%)	81.75	24.72

1 - Days to seedling emergence
2 - Vine length
3 - Collar girth
4 - Primary branches per plant
5 - Petiole length
6 - Terminal leaflet length
7 - Terminal leaflet width
8 - Lateral leaflet length
9 - Lateral leaflet width
10 - Root : shoot ratio
11 - Days to first flowering
12 - Pollen viability
13 - Peduncle length
14 - Pod length
15 - Pod girth
16 - Pod weight
17 - Pods per plant
18 - Seeds per pod
19 - 100-seed weight
20 - Seed length
21 - Seed width
22 - Seed thickness
23 - Number of harvests
24 - Yield per plant
25 - Keeping quality
26 - Pod protein
27 - Pod fibre

Fig. 2. Heritability and genetic advance for 27 characters in vegetable cowpea



4.2.4 Correlation analysis

The phenotypic, genotypic and environmental correlation coefficients were estimated for 27 characters (Tables 10,11 and 12).

(A) Phenotypic correlation

(i) Correlation between yield and other characters

Yield per plant showed high positive correlation with vine length (0.532), collar girth (0.422), pod length (0.536), pod girth (0.438), pod weight (0.503), pods per plant (0.508), seeds per pod (0.394), 100-seed weight (0.376), seed length (0.469), number of harvests (0.306) and keeping quality (0.246). Peduncle length was found to be negatively correlated with yield per plant (-0.467).

(ii) Correlation among yield component characters

Days to seedling emergence was found to be uncorrelated with any other character. On the other hand, vine length exhibited high positive correlation with several yield components like collar girth (0.540), terminal leaflet length (0.344), lateral leaflet length (0.263), days to first flowering (0.495), pod length (0.827), pod girth (0.612), pod weight (0.756), seeds per pod (0.557), 100-seed weight (0.650), seed length (0.702), seed width (0.434) and keeping quality (0.322). Root : shoot ratio and peduncle length were negatively correlated with vine length (-0.473 and -0.573 respectively). The same set of characters which had high correlation with vine length excepting leaf dimensions were also significantly correlated with collar girth.

Primary branches per plant showed no high correlation with other yield components. Petiole length and keeping quality were positively correlated with each other (0.307).

High positive correlation was observed among the leaf dimensions. Terminal leaflet length was positively correlated with pod length (0.416), pod girth (0.381), pod weight (0.314), seeds per pod (0.277), 100-seed weight (0.545), seed length (0.604), seed width (0.531), seed thickness (0.321) and keeping quality (0.341). Root : shoot ratio and pods per plant were negatively correlated with terminal leaflet length

X1 - Days to seedling emergence

X2 - Vine length

X3 - Collar girth

X4 - Primary branches per plant

X5 - Petiole length

X6 - Terminal leaflet length

X7 - Terminal leaflet width

X8 - Lateral leaflet length

X9 - Lateral leaflet width

X10 - Root : shoot ratio

X11 - Days to first flowering

X12 - Pollen viability

X13 - Peduncle length

X14 - Pod length

X15 - Pod girth

X16 - Pod weight

X17 - Pods per plant

X18 - Seeds per pod

X19 - 100-seed weight

X20 - Seed length

X21 - Seed width

X22 - Seed thickness

X23 - Number of harvests

X24 - Keeping quality

X25 - Pod protein

X26 - Pod fibre

X27 - Yield per plant

Table 10. Phenotypic correlation coefficients among yield and its components

Character	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19	X20	X21	X22	X23	X24	X25	X26	X27
X1	1.000																										
X2	0.008	1.000																									
X3	-0.151	0.540**	1.000																								
X4	-0.120	-0.074	0.145	1.000																							
X5	-0.017	-0.173	-0.204	-0.171	1.000																						
X6	-0.115	0.344**	0.212	-0.084	-0.120	1.000																					
X7	-0.124	-0.029	0.042	-0.010	-0.034	0.691**	1.000																				
X8	-0.119	0.263*	0.242	-0.118	-0.094	0.896**	0.701**	1.000																			
X9	-0.194	0.026	0.150	-0.027	-0.002	0.721**	0.872**	0.840**	1.000																		
X10	-0.044	-0.473**	-0.397**	0.024	0.049	-0.268	0.064	-0.261*	-0.076	1.000																	
X11	0.087	0.495**	0.033	-0.129	-0.101	0.080	-0.080	-0.028	-0.112	-0.138	1.000																
X12	-0.227	-0.047	-0.015	0.022	0.204	-0.058	-0.019	-0.058	0.003	0.110	-0.218	1.000															
X13	-0.080	-0.573**	-0.246*	0.193	0.123	-0.136	0.221	-0.058	0.153	0.235	-0.287*	-0.043	1.000														
X14	-0.095	0.827**	0.585**	-0.059	-0.167	0.416**	-0.009	0.399**	0.122	-0.464**	0.301	-0.017	-0.470**	1.000													
X15	-0.149	0.612**	0.447**	0.029	-0.077	0.381**	0.105	0.309*	0.195	-0.239	0.147	0.013	-0.347**	0.766**	1.000												
X16	-0.103	0.756**	0.547**	-0.054	-0.102	0.314*	-0.008	0.259*	0.088	-0.409**	0.242	-0.049	-0.396**	0.915**	0.808**	1.000											
X17	0.084	-0.149	-0.084	-0.016	0.010	-0.275*	-0.196	-0.241	-0.220	0.187	-0.277*	0.007	-0.138	-0.271*	-0.285*	-0.294*	1.000										
X18	-0.224	0.557**	0.346**	-0.021	-0.017	0.277*	-0.063	0.216	0.021	-0.273*	0.290*	0.008	-0.392**	0.631**	0.457**	0.554**	-0.136	1.000									
X19	-0.020	0.650**	0.593**	-0.109	-0.127	0.545**	0.252*	0.521**	0.353**	-0.398**	0.249*	-0.115	-0.337*	0.753**	0.661**	0.752**	-0.337*	0.540**	1.000								
X20	-0.032	0.702**	0.617**	-0.027	-0.208	0.604**	0.169	0.560**	0.324*	-0.512**	0.211	-0.071	-0.412**	0.831**	0.686**	0.740**	-0.314*	0.518**	0.830**	1.000							
X21	-0.025	0.434**	0.397**	-0.118	-0.046	0.531**	0.314*	0.519**	0.391**	-0.239	0.194	-0.010	-0.310*	0.496**	0.424**	0.409**	-0.261*	0.446**	0.714**	0.648**	1.000						
X22	-0.064	-0.050	0.045	-0.092	-0.104	0.321*	0.319*	0.367**	0.318*	-0.036	0.035	0.066	-0.010	0.038	0.033	0.024	-0.226	0.143	0.338*	0.165	0.499**	1.000					
X23	0.052	-0.176	-0.012	-0.074	0.127	-0.243	-0.159	-0.170	-0.114	0.107	-0.390**	-0.033	-0.193	-0.125	-0.066	-0.085	0.508**	0.026	-0.081	-0.236	-0.022	0.042	1.000				
X24	-0.160	0.322*	0.531**	0.137	-0.307*	0.341*	0.218	0.389**	0.291*	-0.294*	-0.018	-0.072	-0.092	0.386**	0.263*	0.336*	-0.097	0.255*	0.479**	0.453**	0.329*	0.248*	-0.066	1.000			
X25	0.036	0.140	0.164	-0.076	0.189	0.119	0.101	0.139	0.165	-0.075	0.135	0.025	-0.184	0.139	0.095	0.137	0.010	0.140	0.242	0.161	0.355	-0.016	0.040	-0.055	1.000		
X26	-0.055	0.068	0.057	-0.127	0.164	-0.014	0.053	-0.012	0.009	-0.037	-0.059	0.076	0.094	0.242	0.244	0.342*	0.044	0.055	0.169	0.010	0.017	0.052	0.163	0.235	-0.030	1.000	
X27	-0.050	0.531**	0.422**	0.012	0.128	0.189	-0.049	0.182	0.076	-0.201	-0.008	-0.070	-0.467**	0.536**	0.438**	0.503**	0.508**	0.397**	0.376**	0.469**	0.239	-0.169	0.306*	0.246*	0.214	0.119	1.000

* Significant at 5 per cent level ** Significant at 1 per cent level

11. Genotypic correlation coefficients among yield and its components

Character	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19	X20	X21	X22	X23	X24	X25	X26	X27
X1	1.000																										
X2	0.002	1.000																									
X3	-0.259*	0.582**	1.000																								
X4	-0.372**	-0.098	0.164	1.000																							
X5	-0.134	-0.192	-0.239	-0.259*	1.000																						
X6	-0.150	0.370**	0.238	-0.113	-0.161	1.000																					
X7	-0.206	-0.080	0.007	-0.083	-0.063	0.726**	1.000																				
X8	-0.192	0.285*	0.251*	-0.161	-0.159	0.949**	0.738**	1.000																			
X9	-0.282*	0.089	0.154	-0.029	-0.036	0.792**	0.915**	0.873**	1.000																		
X10	-0.164	-0.564**	-0.484**	0.153	0.104	-0.393**	0.089	-0.358**	-0.136	1.000																	
X11	0.196	0.565**	0.067	-0.164	-0.068	0.133	-0.041	-0.010	-0.135	-0.202	1.000																
X12	-0.329*	-0.057	-0.077	-0.006	0.272*	-0.074	-0.016	-0.058	0.039	0.163	-0.210	1.000															
X13	-0.088	-0.599**	-0.291*	0.237	0.177	-0.151	0.245*	-0.066	0.177	0.290*	-0.351**	-0.033	1.000														
X14	-0.113	0.838**	0.640**	-0.082	-0.193	0.439**	-0.015	0.357**	0.130	-0.573**	0.363**	-0.022	-0.488**	1.000													
X15	-0.279*	0.663**	0.497**	0.048	-0.081	0.424**	0.097	0.349**	0.218	-0.375**	0.208	-0.003	-0.395**	0.811**	1.000												
X16	-0.131	0.771**	0.607**	-0.046	-0.116	0.335**	-0.015	0.278*	0.094	-0.509*	0.261*	-0.049	-0.410**	0.928**	0.878**	1.000											
X17	0.111	-0.183	-0.118	-0.023	-0.057	-0.337**	-0.258*	-0.304*	-0.258*	0.282*	-0.364**	-0.024	-0.147	-0.313*	-0.311*	-0.335**	1.000										
X18	-0.261*	0.628**	0.445**	-0.068	-0.061	0.326**	-0.041	0.272*	0.072	-0.324*	0.374**	-0.061	-0.458**	0.702**	0.539**	0.638**	-0.151	1.000									
X19	-0.018	0.679**	0.645**	-0.121	-0.170	0.576**	0.272*	0.548**	0.389**	-0.518**	0.310*	-0.126	-0.354*	0.785**	0.747**	0.780**	-0.419**	0.629**	1.000								
X20	-0.061	0.731**	0.661**	-0.052	-0.262*	0.649**	0.199	0.606**	0.369**	-0.615**	0.268*	-0.102	-0.431**	0.867**	0.779**	0.777**	-0.422**	0.605**	0.879**	1.000							
X21	0.089	0.472**	0.432**	-0.105	-0.046	0.571**	0.362**	0.570**	0.431**	-0.317*	0.271*	-0.017	-0.352**	0.536**	0.449**	0.451**	-0.328*	0.522**	0.780**	0.706**	1.000						
X22	-0.098	-0.053	0.077	-0.151	-0.114	0.438**	0.441**	0.449**	0.432**	-0.082	0.006	0.069	-0.010	0.054	0.037	0.024	-0.297*	0.187	0.426**	0.216	0.659**	1.000					
X23	0.042	-0.189	-0.022	-0.061	0.123	-0.274*	-0.201	-0.188	-0.106	0.169	-0.366**	-0.089	-0.215	-0.149	-0.102	-0.047	0.561**	0.026	-0.090	-0.284	0.003	0.017	1.000				
X24	-0.250*	0.372**	0.658**	0.118	-0.382**	0.435**	0.269*	0.498**	0.377**	-0.366**	-0.082	-0.099	-0.113	0.451**	0.304*	0.389**	-0.007	0.299*	0.567**	0.582**	0.399**	0.316*	-0.036	1.000			
X25	0.038	0.150	0.190	-0.102	0.209	0.134	0.104	0.139	0.170	-0.089	0.176	0.069	-0.196	0.144	0.116	0.139	0.038	0.176	0.245*	0.179	0.399**	-0.024	0.046	-0.075	1.000		
X26	-0.099	0.071	0.030	-0.241	0.183	-0.033	0.020	-0.016	-0.025	-0.059	-0.079	0.139	0.088	0.264*	0.288*	0.375**	0.062	0.085	0.163	0.033	-0.010	0.064	0.203	0.221	-0.062	1.000	
X27	-0.125	0.586**	0.478**	-0.021	-0.209	0.215	-0.056	0.206	0.097	-0.270*	0.045	-0.109	-0.499**	0.593**	0.544**	0.560**	0.444**	0.515**	0.416**	0.489**	0.287*	-0.224	0.279*	0.397**	0.252*	0.139	1.000

* Significant at 5 per cent level ** Significant at 1 per cent level

Table 12. Environmental correlation coefficients among yield and its components

Character	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19	X20	X21	X22	X23	X24	X25	X26	X27
X1	1.000																										
X2	0.018	1.000																									
X3	0.047	0.142	1.000																								
X4	0.233	0.086	0.075	1.000																							
X5	0.163	-0.123	-0.076	0.056	1.000																						
X6	-0.080	-0.131	0.007	0.045	0.087	1.000																					
X7	0.022	-0.021	0.250*	0.073	0.081	0.436**	1.000																				
X8	0.026	-0.078	0.181	0.058	0.217	0.371**	0.438**	1.000																			
X9	-0.060	-0.151	0.128	-0.022	0.131	0.202	0.626**	0.628**	1.000																		
X10	0.110	-0.239	-0.155	-0.248*	-0.075	0.225	0.157	0.077	0.111	1.000																	
X11	-0.068	0.255*	-0.095	-0.042	-0.189	-0.191	0.010	-0.119	-0.081	0.004	1.000																
X12	-0.083	0.022	0.260*	0.099	-0.001	0.028	-0.084	-0.065	-0.165	-0.020	-0.243	1.000															
X13	-0.081	0.081	0.188	-0.001	-0.211	0.081	0.003	-0.093	-0.067	0.048	0.033	-0.137	1.000														
X14	-0.186	0.361**	-0.040	0.123	-0.045	-0.028	0.090	0.086	0.079	-0.047	-0.062	0.035	0.022	1.000													
X15	0.102	0.082	0.139	-0.043	-0.065	0.027	0.158	0.014	0.057	0.198	-0.086	0.084	0.128	0.397**	1.000												
X16	-0.155	0.020	-0.203	-0.235	-0.056	-0.128	0.123	-0.059	0.055	-0.004	0.336*	-0.109	0.023	0.147	-0.014	1.000											
X17	0.052	0.025	0.038	0.001	0.184	-0.019	0.022	0.006	-0.096	-0.016	-0.060	0.071	-0.147	-0.152	-0.212	-0.216	1.000										
X18	-0.193	0.160	-0.089	0.111	0.118	0.015	-0.164	-0.071	-0.197	-0.152	0.043	0.241	-0.001	0.300*	0.097	-0.043	-0.095	1.000									
X19	-0.048	-0.065	0.145	-0.085	0.124	0.118	0.081	0.198	0.051	0.073	-0.061	-0.068	-0.034	-0.134	-0.175	-0.061	0.045	0.019	1.000								
X20	0.045	0.077	0.257*	0.113	0.075	0.010	-0.097	0.041	-0.045	-0.207	-0.075	0.136	-0.099	-0.035	-0.163	-0.233	0.211	0.020	0.011	1.000							
X21	-0.227	0.042	0.187	-0.177	-0.046	0.227	0.026	0.155	0.162	-0.007	-0.097	-0.023	0.084	0.082	0.270*	-0.113	-0.027	0.116	0.128	0.155	1.000						
X22	-0.020	-0.057	-0.074	0.047	-0.078	-0.203	-0.111	0.055	-0.069	0.063	0.105	0.056	-0.015	-0.086	0.021	0.063	-0.056	0.019	-0.073	-0.079	-0.061	1.000					
X23	0.076	-0.172	0.027	-0.110	0.139	-0.106	0.012	-0.098	-0.148	-0.041	-0.458**	0.145	-0.099	0.052	0.087	0.076	0.368**	0.026	-0.045	0.024	-0.116	0.112	1.000				
X24	-0.040	0.148	0.095	0.182	-0.115	-0.062	0.040	-0.047	-0.003	-0.140	0.143	0.005	-0.001	0.135	0.124	0.164	-0.315*	0.133	0.124	-0.155	0.092	0.084	-0.147	1.000			
X25	0.076	-0.166	-0.113	0.073	0.132	-0.120	0.092	0.157	0.149	-0.089	-0.113	-0.370**	0.071	-0.013	-0.149	0.089	-0.205	-0.122	0.171	-0.175	-0.098	0.035	0.007	0.067	1.000		
X26	0.022	0.065	0.198	0.236	0.101	0.123	0.225	0.014	0.173	0.055	0.004	-0.167	0.168	0.096	0.013	0.100	-0.015	-0.061	0.260*	-0.176	0.156	0.013	0.020	0.289*	0.298*	1.000	
X27	0.105	-0.090	0.086	0.137	0.203	-0.024	-0.006	0.010	-0.045	0.006	-0.218	0.105	-0.185	-0.202	-0.224	-0.274*	0.803**	-0.147	-0.003	0.317*	-0.054	0.028	0.440**	-0.316*	-0.212	0.013	1.000

* Significant at 5 per cent level ** Significant at 1 per cent level

(-0.268 and -0.275 respectively). Lateral leaflet length also exhibited a similar trend except for pods per plant and seeds per pod. Width of terminal and lateral leaflets also showed high positive correlation with seed dimensions.

Root : shoot ratio recorded negative correlation with most of the characters especially with pod length (-0.464), pod weight (-0.409), seeds per pod (-0.273), 100-seed weight (-0.398), seed length (-0.512) and keeping quality (-0.294). Days to first flowering was found to be positively associated with pod length (0.301), seeds per pod (0.290) and 100-seed weight (0.249), where as peduncle length, pods per plant and keeping quality were negatively correlated (-0.287, -0.277 and -0.390 respectively).

Pollen viability had no correlation with other characters. High negative correlation was observed for peduncle length with pod length (-0.470), pod girth (-0.347), pod weight (-0.396), seeds per pod (-0.392), 100-seed weight (-0.337) and seed length (-0.412).

Pod characters *viz.*, pod length, pod girth and pod weight were positively correlated among each other (0.766, 0.915 and 0.808 respectively) and negatively correlated with pods per plant (-0.271, -0.285 and -0.294 respectively). Seeds per pod, 100-seed weight, seed length, seed width and keeping quality were positively correlated with pod characters. High negative correlation was also recorded by pods per plant with 100-seed weight (-0.337), seed length (-0.314) and seed width (-0.261).

Seeds per pod had high positive association with 100-seed weight (0.540), seed length (0.518), seed width (0.446) and keeping quality (0.255). The same set of characters along with seed thickness were also strongly correlated with 100-seed weight.

Seed length was positively correlated with seed width (0.648), which was in turn correlated with seed thickness (0.499). All the seed dimensions were positively correlated with keeping quality (0.453, 0.329 and 0.248 respectively).

Pod protein and pod fibre had no correlation with other yield characters, except between pod fibre and pod weight (0.342).

(B) Genotypic correlation

Genotypic correlation coefficients were in general higher than phenotypic coefficients.

(i) Correlation between yield and other component characters

Yield per plant was positively correlated with vine length (0.586), collar girth (0.478), pod length (0.593), pod girth (0.544), pod weight (0.560), pods per plant (0.444), seeds per pod (0.515), 100-seed weight (0.416), seed length (0.489), seed width (0.287), number of harvests (0.279), keeping quality (0.397) and pod protein (0.252), where as root : shoot ratio and peduncle length were negatively correlated (-0.270 and -0.499 respectively).

(ii) Correlation among yield component characters

Correlation among yield component characters observed a similar trend as in the phenotypic level. Those characters which had no high correlation phenotypically recorded some amount of association at the genotypic level. Days to seedling emergence observed negative correlation with collar girth (-0.259), primary branches per plant (-0.372), lateral leaflet width (-0.282), pollen viability (-0.329), pod width (-0.279), seeds per pod (-0.261) and keeping quality (-0.250). Primary branches per plant was negatively associated with petiole length (-0.259). Petiole length was found positively correlated with pollen viability (0.272) and negatively with seed length (-0.262). Pod protein was positively correlated with 100-seed weight (0.245) and seed width (0.399), where as pod fibre was positively associated with pod length (0.264), pod girth (0.288) and pod weight (0.375).

(C) Environmental correlation

These were found to be negligible among most of the characters with few exceptions indicating that the influence of environment was less in the expression of characters. Correlation between leaf dimensions were comparatively high. Similarly

pod weight, pods per plant, seed length, number of harvests and keeping quality had high environmental correlation with yield.

4.2.5 Path analysis

In path coefficient analysis, the genotypic correlation coefficients among yield and its component characters were partitioned into direct and indirect contribution of each character to pod yield (Table 13). Vine length, collar girth, root : shoot ratio, peduncle length, pod length, pod girth, pod weight, pods per plant, seeds per pod, 100-seed weight, seed length, seed width, number of harvests, keeping quality and pod protein were selected for path coefficient analysis.

Pods per plant recorded the highest positive direct effect on yield (0.6709), followed by seed length (0.4368), pod weight (0.2372), vine length (0.1779), pod protein (0.1247), pod girth (0.1091) and number of harvests (0.1033). 100-seed weight exerted a negative direct effect on yield (-0.1529). The direct effects of collar girth, root : shoot ratio, peduncle length, pod length, seeds per pod, seed width and keeping quality were negligible.

Indirect effects through seed length were consistently high signifying the importance of the character, followed by pod weight and vine length. Thus in the case of collar girth, pod length, seeds per pod, 100-seed weight, seed width and keeping quality, high positive correlation with yield was mainly due to their positive indirect effects through seed length, pod weight and vine length. Similarly high negative correlation of root : shoot ratio and peduncle length on yield was due to high negative indirect effects through seed length (-0.2259 and -0.1792 respectively). In the case of vine length, pod girth and pod weight, the correlation was mainly built by the direct as well as indirect effects *via* seed length, pod weight and vine length.

4.2.6 Selection index

A discriminant function analysis was carried out for identifying superior accessions. Selection indices were worked out involving the characters vine length (X_1), collar girth (X_2), root : shoot ratio (X_3), peduncle length (X_4), pod length (X_5),

Table 13. Direct and indirect effect of selected yield components on pod yield in vegetable cowpea

Character	Vine length	Collar girth	Root:shoot ratio	Peduncle length	Pod length	Pod girth	Pod weight	Pods per plant	Seeds per pod	100-seed weight	Seed length	Seed width	Number of harvests	Keeping quality	Pod protein	Correlation with yield
Vine length	**0.1779**	-0.0043	-0.0330	-0.0257	0.0368	0.0667	0.1793	-0.1000	0.0359	-0.0993	0.3050	-0.0249	-0.0181	0.0171	0.0174	0.5858
Collar girth	0.0960	**-0.0079**	-0.0276	-0.0110	0.0260	0.0487	0.1297	-0.0558	0.0222	-0.0905	0.2672	-0.0227	-0.0014	0.0283	0.0203	0.4775
Root:shoot ratio	-0.0834	0.0031	**0.0704**	0.0105	-0.0204	-0.0254	-0.0958	0.1250	-0.0176	0.0610	-0.2259	0.0142	0.0098	-0.0143	-0.0095	-0.2695
Peduncle length	-0.1019	0.0019	0.0164	**0.0449**	-0.0209	-0.0378	-0.0939	-0.0926	-0.0253	0.0515	-0.1792	0.0178	-0.0199	-0.0049	-0.0230	-0.4994
Pod length	0.1471	-0.0046	-0.0323	-0.0211	**0.0445**	0.0835	0.2170	-0.1817	0.0406	-0.1150	0.3607	-0.0284	-0.0129	0.0206	0.0174	0.5933
Pod girth	0.1088	-0.0035	-0.0164	-0.0156	0.0341	**0.1091**	0.1916	-0.1910	0.0294	-0.1006	0.2966	-0.0241	-0.0070	0.0142	0.0117	0.5439
Pod weight	0.1344	-0.0043	-0.0284	-0.0178	0.0407	0.0881	**0.2372**	-0.1969	0.0357	-0.1147	0.3208	-0.0234	-0.0038	0.0179	0.0171	0.5601
Pods per plant	-0.0265	0.0007	0.0131	-0.0062	-0.0121	-0.0311	-0.0696	**0.6709**	-0.0088	0.0515	-0.1366	0.0150	0.0521	-0.0051	0.0012	0.4436
Seeds per pod	0.0990	-0.0027	-0.0192	-0.0176	0.0280	0.0497	0.1312	-0.0913	**0.0645**	-0.0826	0.2256	-0.0256	0.0028	0.0134	0.0175	0.5145
100-seed weight	0.1155	-0.0047	-0.0281	-0.0151	0.0335	0.0718	0.1780	-0.2260	0.0348	**-0.1529**	0.3619	-0.0411	-0.0080	0.0250	0.0301	0.4160
Seed length	0.1242	-0.0048	-0.0364	-0.0184	0.0368	0.0741	0.1742	-0.2098	0.0333	-0.1267	**0.4368**	-0.0374	-0.0232	0.0229	0.0202	0.4890
Seed width	0.0769	-0.0031	-0.0173	-0.0139	0.0220	0.0457	0.0962	-0.1749	0.0287	-0.1092	0.2842	**-0.0576**	-0.0014	0.0165	0.0442	0.2870
Number of harvests	-0.0311	0.0001	0.0067	-0.0086	-0.0056	-0.0074	-0.0086	0.3386	0.0018	0.0119	-0.0981	0.0008	**0.1033**	-0.0044	0.0051	0.2792
Keeping quality	0.0562	-0.0041	-0.0186	-0.0040	0.0169	0.0287	0.0787	-0.0627	0.0159	-0.0708	0.1850	-0.0176	-0.0084	**0.0540**	-0.0070	0.3966
Pod protein	0.0249	-0.0013	-0.0054	-0.0083	0.0062	0.0102	0.0325	0.0064	0.0091	-0.0370	0.0706	-0.0204	0.0042	-0.0030	**0.1247**	0.2517

Residue = 0.1830 Direct effects-diagonal elements Indirect effects-off diagonal elements

pod girth (X_6), pod weight (X_7), pods per plant (X_8), seeds per pod (X_9), 100-seed weight (X_{10}), seed length (X_{11}), seed width (X_{12}), number of harvests (X_{13}), keeping quality (X_{14}), pod protein (X_{15}) and yield per plant (X_{16}).

The selection indices worked out are as follows :

$$I = 17.95906\ X_1 - 7.522001\ X_2 + 42.79663X_3 + 0.2617779\ X_4 + 2.42933\ X_5 + 94.36029\ X_6 + 6.623003\ X_7 + 4.597428\ X_8 + 6.739223\ X_9 - 10.75113\ X_{10} + 7.947279\ X_{11} - 7.880835\ X_{12} + 9.492894\ X_{13} + 51.53454\ X_{14} + 30.77027\ X_{15} - 71.14921\ X_{16}$$

The scores obtained for the accessions based on the selection index are given in Table 14.

Based on the selection index, VS 27 ranked first (1301.08), followed by VS 8 (1296.02) and VS 19 (1183.16) (Plates 5, 6 and 7). The lowest scores were obtained for VS 42 (609.16), followed by VS 69 (615.21).

4.2.7 Mahalanobi's D^2 analysis

Following Mahalanobi's D^2 statistic, the 66 accessions were subjected to cluster analysis based on sixteen characters *viz.*, vine length, collar girth, root : shoot ratio, peduncle length, pod length, pod girth, pod weight, pods per plant, seeds per pod, 100-seed weight, seed length, seed width, number of harvests, keeping quality, pod protein and yield per plant.

The 66 accessions fell under ten clusters. The clustering pattern is furnished in Table 15. Cluster I was the largest with 18 accessions, followed by clusters II and III with eight accessions each. Seven accessions were grouped under cluster IV, where as clusters V and VI comprised of six accessions each. Cluster VII had five accessions, followed by clusters VIII and IX with three accessions each. The smallest cluster was cluster X with two accessions.

The cluster means of the sixteen characters are presented in Table 16. Cluster X comprising VS 4 and VS 19 observed the highest values for vine length (6.13 m), collar girth (5.81 cm), pod length (71.86 cm), pod girth (4.17 cm), pod weight (43.19 g),

Table 14. Selection indices arranged in descending order

Rank	Accessions	Selection index
1	VS 27	1301.08
2	VS 8	1296.02
3	VS 19	1183.16
4	VS 30	1177.84
5	VS 4	1174.51
6	VS 29	1158.88
7	VS 22	1126.82
8	VS 17	1106.30
9	VS 25	1102.17
10	VS 26	1096.47
11	VS 18	1078.15
12	VS 39	1054.98
13	VS 24	1053.63
14	VS 31	1049.18
15	VS 49	1045.95
16	VS 9	1011.87
17	VS 20	1011.74
18	VS 28	1000.63
19	VS 2	995.05
20	VS 13	982.05
21	VS 21	978.65
22	VS 14	976.20
23	VS 3	961.70
24	VS 16	950.87
25	VS 53	942.32
26	VS 57	937.99
27	VS 32	933.92
28	VS 52	932.42
29	VS 10	920.05
30	VS 64	894.77
31	VS 65	875.54
32	VS 5	866.66
33	VS 44	863.23

(Contd...)

Table 14. Continued...

Rank	Accessions	Selection index
34	VS 63	862.86
35	VS 15	862.85
36	VS 56	845.96
37	VS 38	845.53
38	VS 50	839.48
39	VS 66	835.78
40	VS 45	824.33
41	VS 58	823.51
42	VS 41	822.31
43	VS 35	817.84
44	VS 60	815.46
45	VS 62	812.33
46	VS 34	812.26
47	VS 54	809.65
48	VS 68	799.99
49	VS 59	798.74
50	VS 23	797.06
51	VS 11	778.65
52	VS 61	774.75
53	VS 1	758.94
54	VS 12	748.86
55	VS 7	742.96
56	VS 36	739.50
57	VS 40	733.46
58	VS 43	726.88
59	VS 37	711.35
60	VS 55	701.18
61	VS 48	662.56
62	VS 46	642.50
63	VS 33	628.24
64	VS 6	623.95
65	VS 69	615.21
66	VS 42	609.16

Plate 5. VS 27 – an accession ranked first based on selection index

Plate 6. VS 8– an accession ranked second based on selection index

Plate 7. VS 19 – an accession ranked third based on selection index

Plate 5

Plate 6

Plate 7

Table 15. Clustering pattern of 66 accessions of vegetable cowpea

Cluster No.	Number of accessions	Accessions
I	18	VS 33, VS 37, VS 40, VS 43, VS 45, VS 48, VS 49, VS 50, VS 52, VS 53, VS 55, VS 57, VS 61, VS 62, VS 63, VS 64, VS 65, VS 69
II	8	VS 2, VS 3, VS 9, VS 13, VS 15, VS 16, VS 20, VS 21
III	8	VS 36, VS 39, VS 44, VS 56, VS 58, VS 59, VS 60, VS 66
IV	7	VS 22, VS 25, VS 26, VS 27, VS 29, VS 30, VS 31
V	6	VS 23, VS 24, VS 28, VS 32, VS 34, VS 35
VI	6	VS 6, VS 38, VS 41, VS 42, VS 46, VS 68
VII	5	VS 5, VS 7, VS11, VS 12, VS 54
VIII	3	VS 1, VS 10, VS 18
IX	3	VS 8, VS 14, VS 17
X	2	VS 4, VS 19

Table 16. Cluster means of sixteen biometric characters

Cluster	Vine length (m)	Collar girth (cm)	Root : shoot ratio	Peduncle length (cm)	Pod length (cm)	Pod girth (cm)	Pod weight (g)	Pods per plant	Seeds per pod	100-seed weight (g)	Seed length (mm)	Seed width (mm)	Number of harvests	Keeping quality (days)	Pod protein (%)	Yield per plant (g)
I	1.73	3.76	0.25	26.12	18.92	2.46	4.70	53.43	15.78	9.19	6.96	5.10	5.28	2.81	6.11	251.14
II	4.60	4.81	0.08	14.17	45.02	3.07	16.10	35.77	18.66	13.91	10.72	5.85	4.19	3.70	5.74	336.06
III	2.23	3.70	0.40	20.69	17.95	2.43	4.72	55.19	15.60	9.90	6.87	5.64	5.63	2.79	7.46	267.06
IV	5.10	5.47	0.08	15.95	47.88	2.92	16.95	54.48	18.72	15.36	11.13	5.99	4.71	4.17	7.80	612.51
V	3.89	4.64	0.08	20.46	31.33	2.93	11.71	34.70	18.02	14.49	10.37	5.95	3.67	3.27	7.28	337.46
VI	2.34	3.25	0.28	28.74	16.15	2.24	3.98	45.12	13.47	7.49	6.33	4.55	4.00	2.82	4.56	170.25
VII	0.63	5.03	0.22	37.40	25.02	2.69	7.62	37.73	15.08	11.93	9.52	5.42	3.80	4.33	5.81	225.48
VIII	4.14	5.14	0.07	14.77	35.18	2.88	11.88	44.84	17.45	13.26	9.97	5.47	5.00	4.54	4.23	397.85
IX	4.76	4.75	0.10	21.06	43.97	3.28	16.69	61.54	17.99	13.14	10.84	5.74	4.83	3.76	5.82	740.68
X	6.13	5.81	0.07	20.30	71.86	4.17	43.19	23.20	20.05	20.08	12.03	5.83	5.50	3.48	6.68	613.59

seeds per pod (20.05), 100-seed weight (20.08 g), seed length (12.03 mm), number of harvests (5.50) and lowest for root : shoot ratio (0.07) and pods per plant (23.20). Cluster VI had the lowest values for most of the characters *viz.*, collar girth (3.25 cm), pod length (16.15 cm), pod girth (2.24 cm), pod weight (3.98 g), seeds per pod (13.47), 100-seed weight (7.49 g), seed length (6.33 mm), seed width (4.55 mm) and yield per plant (170.25 g). Cluster VII had the shortest accessions with a vine length of 0.63 m. The highest pods per plant (61.54) and yield per plant (740.68 g) were recorded in cluster IX.

The average inter and intracluster distances are given in Table 17. The cluster diagram is shown in Fig.3.

Cluster VIII had the highest intracluster distance (293.71), followed by cluster IX (288.77), where as the lowest intracluster distance was observed by cluster X (90.19). Cluster X maintained high intercluster distances with all other clusters, the highest being with cluster VI (4127.49), followed by cluster I (3585.56) and cluster III (3504.53). The same clusters I and III had the lowest intercluster distance of 231.90 showing a close relationship among each other.

4.3 Screening for pest and disease resistance

Among the various pests and diseases reported in vegetable cowpea, incidence of legume pod borer, *Maruca vitrata* and fungal wilt caused by *Fusarium* spp. were observed during the study. The scoring method employed for both are furnished in materials and methods. Incidence of other major pests and diseases like aphids, pod bug, mosaic etc. were negligible owing to the high intensity of rainfall received during the cropping season. The scoring done for *Fusarium* wilt is given along with cataloguing, while that for legume pod borer is furnished below.

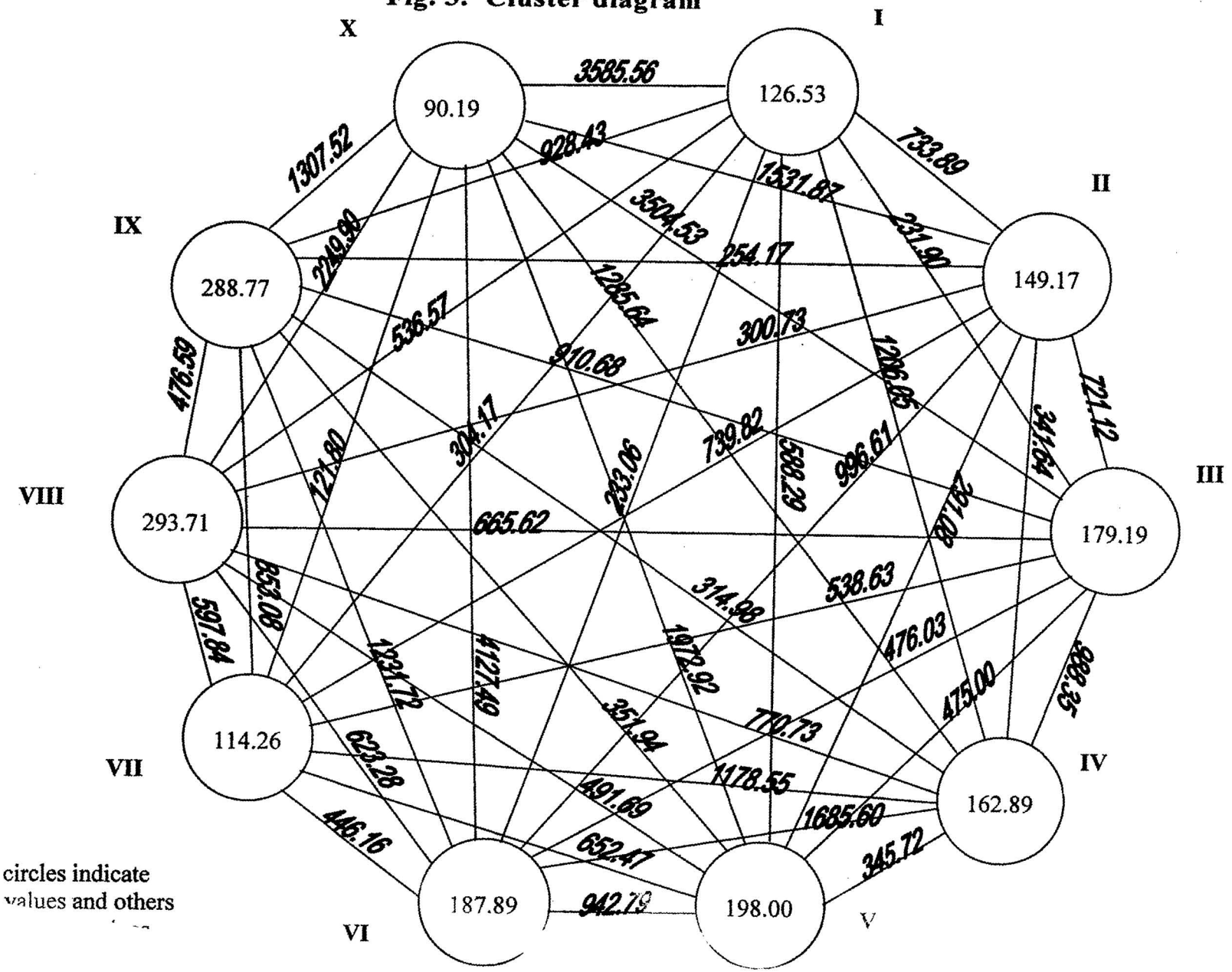
Legume pod borer (*Maruca vitrata*)

The major feeding sites of legume pod borer larvae are the flowers, developing pods and seeds. Screening of accessions for tolerance to pod borer based on the extent of damage to flowers, pods and seeds was attempted in the study.

Table 17. Average inter and intracluster distances

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X
I	126.53									
II	733.89	149.17								
III	231.90	721.12	179.19							
IV	1206.05	341.64	988.35	162.89						
V	588.29	291.08	475.00	345.72	198.00					
VI	233.06	996.61	476.03	1685.60	942.79	187.89				
VII	304.17	739.82	538.63	1178.55	652.47	446.16	114.26			
VIII	536.57	300.73	665.62	770.73	491.69	623.28	597.84	293.71		
IX	928.43	254.17	910.68	314.98	351.94	1231.72	853.08	476.59	288.77	
X	3585.56	1531.87	3504.53	1285.64	1972.92	4127.49	3121.80	2249.90	1307.53	90.19

Fig. 3. Cluster diagram



e values in circles indicate
D values and others

Damage parameters and resistance evaluation

The legume pod borer damage parameters and overall plant susceptibility index (Ips) relating to 66 accessions are presented in Table 18. Analysis of variance revealed significant differences among the accessions for all the damage parameters and plant susceptibility index (Table 19).

The criteria employed for assessment of flower damage was number of larvae in 25 flowers. Percentage infestation of pods, pod damage severity and seed damage index were recorded to assess the damage on pods and seeds.

VS 49 had the highest flower damage with 36.38 larvae per 25 flowers, while VS 19 recorded the lowest (0.49 larvae per 25 flowers). In the case of pod and seed damage, VS 42 was found to have the highest values for percentage infestation of pods (73.34), pod damage severity (1.73) and seed damage index (182.22), where as VS 19 had the lowest (4.52, 0.03 and 6.71 respectively). The overall plant susceptibility index (Ips) also showed the same pattern. VS 42 was found to be the most susceptible accession with an Ips value of 65.86 and VS 19 as the most tolerant (Ips = 2.40).

Correlation among damage parameters

Correlation among the different parameters for the assessment of legume pod borer damage to flowers, pods and seeds were estimated and presented in Table 20. All the damage parameters *viz.*, number of larvae per 25 flowers, percentage infestation of pods, pod damage severity and seed damage index showed high positive association among each other. This means that as larval count increases in the flowers it increases pod infestation and finally resulting in higher seed damage.

Anatomical and biochemical characters and legume pod borer resistance

Based on Ips values, ten susceptible and ten tolerant accessions were selected to study the influence of anatomical and biochemical characters on legume pod borer resistance. The parameters studied included glandular, non-glandular and total trichome density, stomatal density, vascular bundle thickness, cuticle thickness,

Table 18. Damage parameters and plant susceptibility indices of legume pod borer

Accession	Number of larvae per 25 flowers	Percentage infestation of pods	Pod damage severity	Seed damage index	Plant susceptibility index
VS 1	4.45	35.56	0.27	44.45	21.48
VS 2	2.95	23.58	0.19	28.29	14.05
VS 3	1.32	10.56	0.08	13.57	6.44
VS 4	1.26	9.30	0.09	11.20	5.59
VS 5	10.71	13.93	0.21	23.42	13.90
VS 6	15.94	24.45	0.28	32.25	19.30
VS 7	24.65	47.22	0.89	96.35	44.12
VS 8	9.59	22.52	0.23	30.35	17.36
VS 9	1.97	15.75	0.13	19.00	9.41
VS 10	14.13	10.60	0.18	21.26	13.55
VS 11	13.40	30.00	0.48	53.57	27.36
VS 12	18.75	35.10	0.42	48.61	28.00
VS 13	1.34	9.99	0.12	13.26	7.53
VS 14	1.56	12.41	0.11	13.89	7.23
VS 15	4.79	20.09	0.33	39.28	15.64
VS 16	0.91	7.40	0.03	7.32	4.27
VS 17	1.95	15.89	0.13	25.71	11.15
VS 18	11.77	19.17	0.32	34.64	18.05
VS 19	0.49	4.52	0.03	6.71	2.40
VS 20	1.22	9.60	0.08	11.26	5.54
VS 21	3.71	29.63	0.24	35.65	17.67
VS 22	1.27	10.15	0.08	12.26	6.06
VS 23	19.73	36.68	0.50	49.91	29.18
VS 24	1.70	13.14	0.10	15.99	8.09
VS 25	1.23	9.06	0.08	10.70	5.36
VS 26	0.68	5.59	0.04	7.42	3.29
VS 27	1.19	9.51	0.08	10.95	5.59
VS 28	18.13	32.38	0.51	62.72	30.31
VS 29	0.64	5.12	0.29	7.22	3.20
VS 30	1.19	9.48	0.08	10.93	5.58
VS 31	2.76	20.72	0.19	24.25	15.79
VS 32	0.84	5.89	0.05	7.84	3.62
VS 33	17.16	67.92	1.31	142.48	54.16
CD (5%)	14.11	22.69	0.56	55.75	20.04

(Contd...)

Table 18. Continued...

Accession	Number of larvae per 25 flowers	Percentage infestation of pods	Pod damage severity	Seed damage index	Plant susceptibility index
VS 34	19.95	37.43	0.51	50.35	29.76
VS 35	23.67	42.89	0.79	86.00	39.68
VS 36	17.18	25.77	0.31	35.92	22.52
VS 37	4.12	32.81	0.32	32.29	17.85
VS 38	5.66	28.30	0.39	42.14	19.24
VS 39	8.01	32.59	0.63	65.46	25.84
VS 40	10.63	39.24	0.76	80.12	32.20
VS 41	3.05	15.95	0.16	24.58	10.65
VS 42	22.09	73.34	1.73	182.22	65.86
VS 43	20.25	40.08	0.47	49.49	31.74
VS 44	20.67	55.73	0.77	82.05	44.24
VS 45	15.77	56.63	0.50	55.54	33.14
VS 46	18.82	65.30	1.46	159.84	58.11
VS 48	14.13	58.62	0.38	49.12	34.61
VS 49	36.38	51.34	1.50	151.53	60.11
VS 50	23.97	23.53	0.45	41.20	29.15
VS 52	16.51	41.48	0.82	88.45	36.82
VS 53	12.97	39.26	0.55	108.21	37.50
VS 54	21.61	41.24	1.02	107.40	42.45
VS 55	10.24	28.50	0.35	41.08	21.01
VS 56	8.11	30.95	0.54	64.88	25.19
VS 57	12.32	51.43	0.82	98.57	39.73
VS 58	6.94	33.84	0.36	43.09	24.81
VS 59	34.72	45.13	1.21	129.96	54.73
VS 60	15.52	28.76	0.60	65.27	28.23
VS 61	4.45	24.04	0.32	35.58	16.17
VS 62	25.21	37.72	0.96	109.04	43.35
VS 63	12.15	30.36	0.50	50.99	24.70
VS 64	15.00	34.95	0.44	56.90	28.63
VS 65	14.82	37.01	0.58	69.48	31.33
VS 66	11.83	43.14	0.84	94.60	31.29
VS 68	25.55	53.54	1.33	142.04	54.30
VS 69	7.74	38.40	0.54	61.91	26.99
CD (5%)	14.11	22.69	0.56	55.75	20.04

Table 19. Analysis of variance for damage parameters and plant susceptibility index (Mean squares)

Source	df	Number of larvae per 25 flowers	Percentage infestation of pods	Pod damage severity	Seed damage index	Plant susceptibility index
Replication	1	0.20	783.81*	0.32	2824.44	304.65
Genotype	65	161.25**	572.69**	0.32**	3561.67**	509.28**
Error	65	49.76	128.73	0.08	777.15	100.45

*Significant at 5 per cent level ** Significant at 1 per cent level

Table 20. Correlation between various parameters of pod borer damage

Damage parameters	Number of larvae per 25 flowers	Percentage infestation of pods	Pod damage severity	Seed damage index
Number of larvae per 25 flowers	1.0000			
Percentage infestation of pods	0.6302**	1.0000		
Pod damage severity	0.7429**	0.8329**	1.0000	
Seed damage index	0.7272**	0.8579**	0.9741**	1.0000

** Significant at 1 per cent level

phenol, proline and chlorophyll contents. The mean values as well as their analysis of variance are given in Tables 21 and 22 respectively. Significant differences were noticed among the accessions for all the anatomical and biochemical characters except chlorophyll content.

Correlation among anatomical and biochemical characters and plant susceptibility index

Correlation among anatomical and biochemical characters and plant susceptibility index was worked out to study the role of these characters to legume pod borer resistance (Table 23). Plant susceptibility index observed high negative correlation with glandular, non-glandular and total trichome density (-0.8007, -0.9124 and -0.9010 respectively) (Plates 8 and 9), as well as with phenol content (-0.7213). Phenol content in turn was positively associated with glandular, non-glandular and total trichome density (0.7123, 0.7478 and 0.7684 respectively), vascular bundle thickness (0.3503) and proline content (0.3208). High negative correlation was recorded between proline content and stomatal density (-0.4012), where as vascular bundle thickness and cuticle thickness were positively associated among each other (0.7063).

4.4 Organoleptic analysis

The organoleptic quality of 66 accessions were evaluated separately for vegetable types and dual purpose / grain types with respect to appearance / colour, doneness, flavour, taste, texture and overall acceptability by a four point scale (Table 24).

Among the vegetable types, the highest overall acceptability of 3.60 was scored by VS 43, while the lowest acceptability was recorded by VS 10 (2.00). Dual purpose and typical grain types at their vegetable maturity stage showed an overall acceptability range from 2.40 in VS 46, VS 52 and VS 62 to 3.60 in VS 58.

Table 21. Mean values of anatomical and biochemical characters of vegetable cowpea

	Accession	Glandular trichome density (mm^{-2})	Non-glandular trichome density (mm^{-2})	Total trichome density (mm^{-2})	Stomatal density (mm^{-2})	Vascular bundle thickness (µm)	Cuticle thickness (µm)	Phenol (mg/g)	Proline (µmoles per g)	Chlorophyll (mg/g)
Tolerant	VS 4	5.29	4.66	9.95	3.98	595.12	27.21	8.61	3.22	1.65
	VS 7	2.86	3.27	6.13	4.78	759.80	42.19	5.02	1.49	1.57
	VS 16	4.51	5.19	9.70	4.91	673.24	32.79	6.81	2.76	1.50
	VS 19	4.84	6.03	10.87	3.46	788.50	44.81	9.46	3.18	1.31
	VS 22	6.17	4.45	10.62	4.16	636.76	33.50	9.01	2.44	1.47
	VS 25	5.38	5.01	10.38	4.03	642.10	26.50	7.62	2.23	1.44
	VS 26	6.90	5.31	12.21	5.72	548.50	30.09	6.82	1.97	1.60
	VS 27	4.84	4.57	9.41	4.83	578.50	36.23	7.08	1.91	1.67
	VS 29	6.84	5.46	12.30	3.82	593.90	32.91	8.15	2.51	1.85
	VS 30	5.04	4.76	9.80	5.20	557.02	26.77	6.51	2.26	1.64
Susceptible	VS 32	5.29	5.24	10.52	4.94	464.50	24.00	7.04	2.51	1.48
	VS 33	3.38	2.19	5.57	4.00	554.98	30.20	4.14	2.96	1.62
	VS 42	2.53	1.87	4.40	4.56	520.11	21.00	3.39	2.12	1.48
	VS 44	3.60	2.91	6.51	4.13	608.89	32.80	5.32	2.45	1.46
	VS 46	2.42	2.14	4.56	4.16	585.50	27.50	5.32	2.20	1.84
	VS 49	2.80	1.97	4.77	3.59	615.20	42.00	6.28	2.59	1.38
	VS 54	3.17	3.38	6.55	4.30	792.06	38.50	6.64	2.72	1.36
	VS 59	4.51	2.17	6.68	3.90	471.80	21.40	6.62	2.41	1.36
	VS 62	3.58	3.32	6.90	3.42	436.94	24.00	5.07	2.49	1.26
	VS 68	2.43	2.09	4.51	4.58	467.50	30.50	5.57	2.84	1.37

Table 22. Analysis of variance for anatomical and biochemical characters in vegetable cowpea

Source	df	Glandular trichome density	Non-glandular trichome density	Total trichome density	Stomatal density	Vascular bundle thickness	Cuticle thickness	Phenol	Proline	Chlorophyll
Replication	1	1.13*	0.44**	2.98**	1.00	0.01	3.60	0.03	0.06	0.03
Genotype	19	4.07**	3.96**	14.63**	0.73*	20912.58**	95.49**	5.00**	0.36**	0.05
Error	19	0.14	0.05	0.19	0.32	531.58	30.13	0.06	0.04	0.03

*Significant at 5 per cent level ** Significant at 1 per cent level

Table 23. Correlation between anatomical and biochemical characters and pod borer resistance index

Character	Plant susceptibility index	Glandular trichome density	Non-glandular trichome density	Total trichome density	Stomatal density	Vascular bundle thickness	Cuticle thickness	Phenol	Proline	Chlorophyll
Plant susceptibility index	1.0000									
Glandular trichome density	-0.8007**	1.0000								
Non-glandular trichome density	-0.9124**	0.8041**	1.0000							
Total trichome density	-0.9010**	0.9513**	0.9482**	1.0000						
Stomatal density	-0.3036	0.1589	0.2017	0.1895	1.0000					
Vascular bundle thickness	-0.2031	0.0074	0.2920	0.1554	-0.0889	1.0000				
Cuticle thickness	-0.1102	-0.0252	0.1721	0.0758	-0.1080	0.7063**	1.0000			
Phenol	-0.7213**	0.7123**	0.7478**	0.7684**	-0.1278	0.3503**	0.2722	1.0000		
Proline	-0.0608	0.0279	0.1028	0.0682	-0.4012**	0.0364	0.0560	0.3208**	1.0000	
Chlorophyll	-0.1402	0.1945	0.1315	0.1721	0.1392	-0.0300	-0.0900	0.0451	-0.2628	1.0000

** Significant at 1 per cent level

Plate 8. Larvae of legume pod borer (*Maruca vitrata*)

Plate 9. Glandular and non-glandular trichomes on cowpea pods

Plate 8

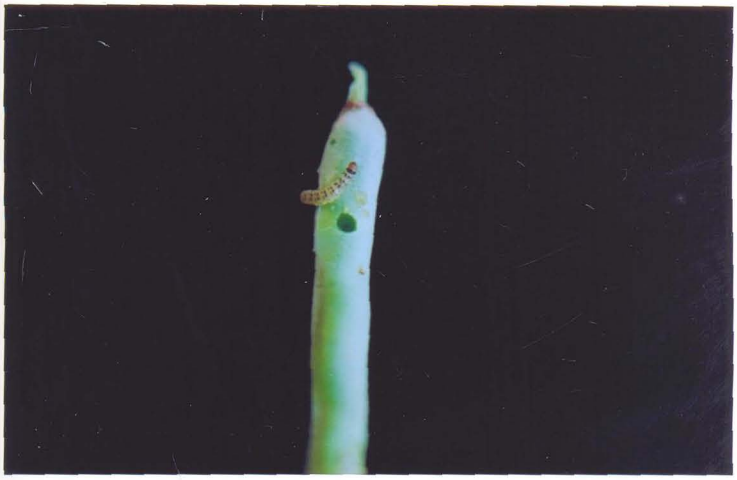


Plate 9

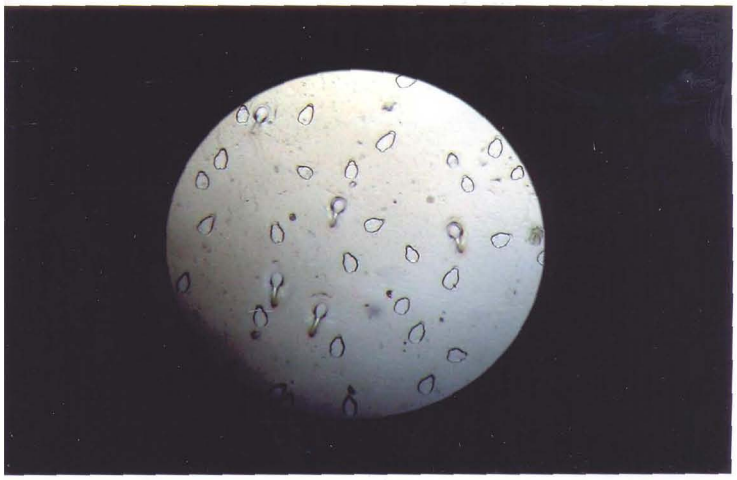


Table 24. Organoleptic evaluation and overall acceptability of vegetable cowpea

Accession	Appearance / Colour	Doneness	Flavour	Taste	Texture	Overall acceptability
Vegetable types						
VS 1	2.40	3.60	3.00	3.40	3.40	3.20
VS 2	3.00	2.80	3.20	3.00	2.80	3.00
VS 3	3.20	3.40	3.20	3.00	3.60	3.20
VS 4	3.60	2.80	2.40	2.80	2.80	2.80
VS 5	3.20	3.00	2.40	2.40	2.80	2.80
VS 7	3.40	2.80	3.20	3.00	2.00	2.80
VS 8	2.40	3.40	3.00	3.20	3.40	3.00
VS 9	3.00	3.20	3.20	2.80	2.80	3.00
VS 10	1.80	2.60	2.20	2.00	2.00	2.00
VS 11	3.40	2.80	3.40	3.20	3.00	3.20
VS 12	3.00	2.60	3.00	2.60	2.80	2.80
VS 13	3.60	3.40	2.80	2.60	2.80	3.00
VS 14	3.00	2.60	2.20	2.40	2.80	2.60
VS 15	2.00	2.80	2.00	2.40	2.40	2.40
VS 16	2.20	3.00	2.00	2.00	2.00	2.20
VS 17	3.00	3.40	3.20	3.20	3.20	3.20
VS 18	3.00	2.80	2.80	3.00	2.00	2.80
VS 19	3.00	2.80	1.80	1.60	2.00	2.20
VS 20	3.20	3.40	2.60	2.60	3.00	3.00
VS 21	3.20	3.20	2.20	2.00	2.60	2.60
VS 22	3.20	2.20	2.00	2.40	2.20	2.40
VS 24	2.40	2.60	2.40	2.00	2.00	2.40
VS 25	3.00	2.80	2.40	2.40	3.00	2.80
VS 26	2.20	2.60	2.40	2.60	2.60	2.40
VS 27	3.20	2.80	2.80	3.00	3.20	3.00
VS 28	3.60	3.00	3.20	2.60	3.00	3.00
VS 29	2.80	2.00	2.20	2.00	2.20	2.20
VS 30	3.20	3.40	2.80	2.60	2.80	3.00
VS 31	3.20	3.40	3.40	3.40	3.20	3.00
VS 32	3.00	3.60	2.80	3.40	3.40	3.40
VS 33	3.60	3.00	3.00	3.00	3.20	3.20
VS 34	3.80	3.00	3.20	3.00	2.80	3.20
VS 43	3.40	3.60	3.60	3.40	3.60	3.60

Contd...

Table 24. Continued...

Accession	Appearance / Colour	Doneness	Flavour	Taste	Texture	Overall acceptability
VS 44	1.60	2.80	2.20	2.00	2.00	2.20
VS 54	3.40	3.60	3.00	2.80	3.00	3.20
Dual purpose/grain types						
VS 6	3.40	2.60	2.20	2.40	2.00	2.50
VS 23	3.80	2.40	2.40	2.20	2.20	2.60
VS 35	2.50	3.60	3.60	3.60	3.40	3.40
VS 36	2.60	3.00	3.20	2.80	2.00	2.80
VS 37	2.50	2.80	2.40	2.40	1.60	2.40
VS 38	3.40	3.20	3.20	3.00	3.20	3.20
VS 39	3.60	3.60	3.40	3.20	3.20	3.40
VS 40	2.20	2.40	2.20	2.20	2.80	2.40
VS 41	3.00	3.20	3.60	3.60	2.60	3.20
VS 42	2.00	2.20	2.80	2.60	1.80	2.40
VS 45	3.00	3.40	3.60	3.00	3.00	3.20
VS 46	2.80	2.40	2.80	2.00	2.20	2.40
VS 48	3.40	3.00	3.40	3.00	3.00	3.20
VS 49	3.40	3.00	3.00	3.00	2.60	3.00
VS 50	3.00	2.80	2.80	2.60	2.00	2.60
VS 52	2.20	2.60	1.80	3.00	1.80	2.40
VS 53	2.20	2.60	3.00	2.80	2.80	2.80
VS 55	3.20	3.20	3.00	3.00	2.60	3.00
VS 56	3.40	3.20	3.40	3.00	3.20	3.20
VS 57	3.60	2.20	2.40	2.40	2.00	2.60
VS 58	3.60	3.60	3.60	3.20	3.60	3.60
VS 59	3.60	3.40	3.20	3.40	3.40	3.40
VS 60	3.20	3.00	3.20	2.80	3.00	3.00
VS 61	3.40	3.00	3.00	2.80	2.80	3.00
VS 62	3.00	2.00	3.00	2.60	2.00	2.60
VS 63	3.00	2.00	2.00	2.20	2.20	2.40
VS 64	3.40	3.20	3.00	3.00	2.80	3.00
VS 65	3.00	2.60	3.00	2.60	2.60	2.80
VS 66	3.00	2.80	2.80	3.00	2.40	2.80
VS 68	3.20	3.00	2.00	2.40	2.00	2.60
VS 69	3.00	2.80	2.60	3.00	2.40	2.80

4.5 Seed protein electrophoresis

Variation in total seed protein among 66 accessions of cowpea was investigated and analysed by means of SDS-PAGE. There was difference in the number of bands in all the accessions. The banding pattern given in Plates 10 and 11 shows the presence of 15 polypeptide bands dispersed over a molecular weight range of 20 to 97.4 kDa. The major bands with a molecular weight around 97.4 kDa, two bands found jointly at 43 kDa and another band around 29 kDa were present in almost all accessions excepting VS 41 and VS 42, which makes them quite distinct from other accessions.

Among yard long beans, polymorphism was less even in the minor bands. But marked variation was noticed when compared to accessions from VS 33 to VS 69 which were primarily comprised of dual purpose and grain type cowpea. The variation was observed in the absence of some minor bands lying in the molecular weight zone of 68 to 97.4 kDa and 29 to 43 kDa. Within the same group, accessions from VS 43 to VS 46 as well as from VS 58 to VS 60 stands apart for their distinct banding pattern. The rest of the accessions shows only slight differences among each other.

4.6 Molecular characterization based on RAPD

Gel electrophoresis

Genomic DNA was observed as a single crisp band showing its unsheared nature. The yield of DNA obtained from various accessions ranged from 120 to 5730 µg/ml with a purity range from 1.50 to 1.98 (Table 25).

Polymerase chain reaction

Polymerase chain reaction was done on 50 selected accessions of vegetable cowpea. Eight decamer primers which were reported to give sufficient amplification in cowpea by Pandey *et al.* (2004) were screened for their efficiency using the DNA isolated from VS 14 as the representative sample. All of the eight primers screened yielded amplification products with the DNA. The nucleotide sequence, total number

Plate 10. Seed protein banding pattern –Accessions VS 1 to VS 40

M	Marker
Lane No.	**Accession**
1	VS 1
2	VS 2
3	VS 3
4	VS 4
5	VS 5
6	VS 6
7	VS 7
8	VS 8
9	VS 9
10	VS 10
11	VS 11
12	VS 12
13	VS 13
14	VS 14
15	VS 15
16	VS 16
17	VS 17
18	VS 18
19	VS 19
20	VS 20
21	VS 21
22	VS 22
23	VS 23
24	VS 24
25	VS 25
26	VS 26
27	VS 27
28	VS 28
29	VS 29
30	VS 30
31	VS 31
32	VS 32
33	VS 33
34	VS 34
35	VS 35
36	VS 36
37	VS 37
38	VS 38
39	VS 39
40	VS 40

Plate 10

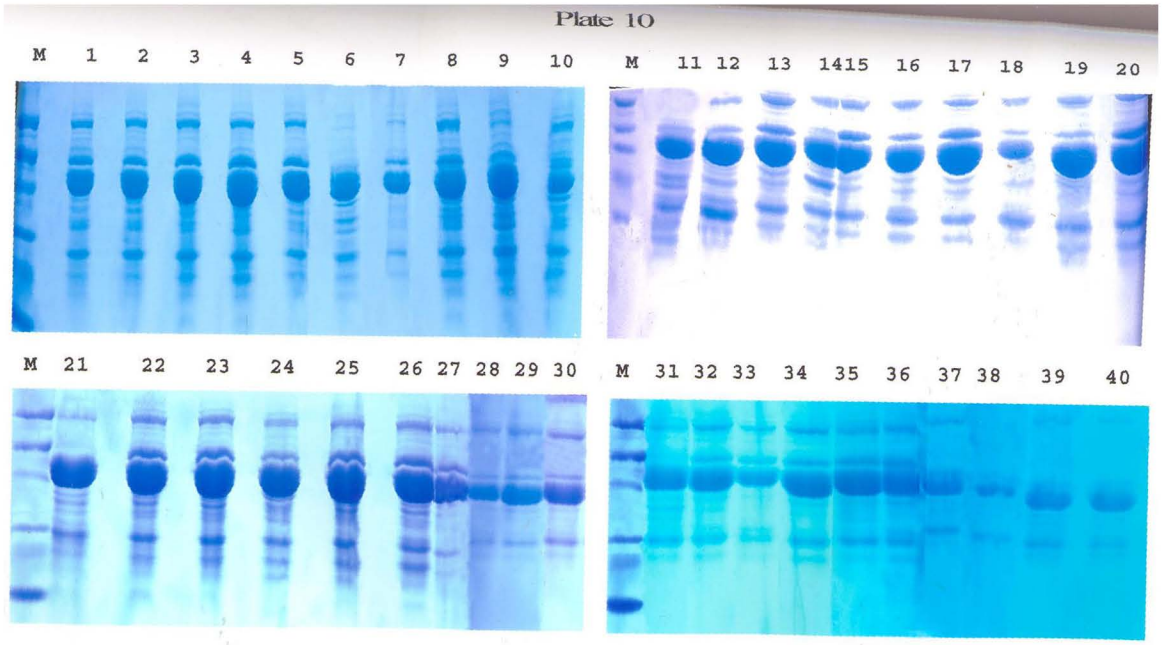

M 1 2 3 4 5 6 7 8 9 10
M 11 12 13 1415 16 17 18 19 20
M 21 22 23 24 25 26 27 28 29 30
M 31 32 33 34 35 36 37 38 39 40

Plate 11. Seed protein banding pattern –Accessions VS 41 to VS 69

M Lane No.	Marker Accession
41	VS 41
42	VS 42
43	VS 43
44	VS 44
45	VS 45
46	VS 46
47	VS 48
48	VS 49
49	VS 50
50	VS 52
51	VS 53
52	VS 54
53	VS 55
54	VS 56
55	VS 57
56	VS 58
57	VS 59
58	VS 60
59	VS 61
60	VS 62
61	VS 63
62	VS 64
63	VS 65
64	VS 66
65	VS 68
66	VS 69

Plate 11

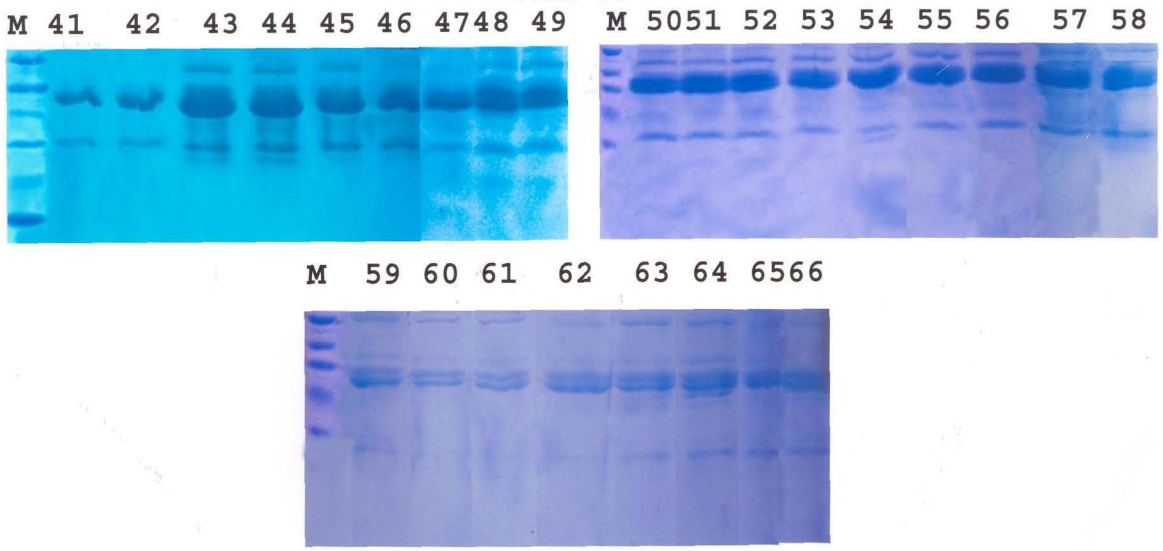

M 41 42 43 44 45 46 4748 49
M 5051 52 53 54 55 56 57 58
M 59 60 61 62 63 64 6566

Table 25. Quality and quantity of DNA isolated from 50 accessions of vegetable cowpea

Sl. No.	Accession	Absorbance at 260 nm ($A_{260\ nm}$)	Absorbance at 280 nm ($A_{280\ nm}$)	Purity of DNA (A_{260} / A_{280})	Quantity (yield) of DNA (µg/ml)
1.	VS 1	0.149	0.090	1.65	4470
2.	VS 5	0.003	0.002	1.50	120
3.	VS 7	0.079	0.044	1.81	2370
4.	VS 8	0.108	0.068	1.59	3240
5.	VS 9	0.105	0.059	1.77	3150
6.	VS 10	0.129	0.072	1.78	3870
7.	VS 12	0.038	0.020	1.90	1140
8.	VS 13	0.064	0.038	1.69	1920
9.	VS 14	0.141	0.083	1.70	4230
10.	VS 15	0.076	0.044	1.72	2280
11.	VS 16	0.024	0.013	1.81	720
12.	VS 17	0.079	0.044	1.78	2370
13.	VS 18	0.025	0.015	1.64	750
14.	VS 19	0.079	0.044	1.80	2370
15.	VS 20	0.073	0.041	1.77	2190
16.	VS 22	0.005	0.003	1.70	150
17.	VS 23	0.011	0.006	1.83	330
18.	VS 24	0.012	0.006	1.98	360
19.	VS 25	0.012	0.007	1.74	360
20.	VS 26	0.021	0.013	1.62	630
21.	VS 27	0.033	0.019	1.74	990
22.	VS 28	0.038	0.022	1.70	1140
23.	VS 29	0.025	0.015	1.63	750
24.	VS 32	0.017	0.010	1.71	510
25.	VS 39	0.078	0.044	1.76	2340
26.	VS 40	0.032	0.017	1.87	960
27.	VS 41	0.098	0.057	1.71	2940
28.	VS 42	0.077	0.044	1.76	2310
29.	VS 44	0.067	0.040	1.68	2010
30.	VS 45	0.075	0.042	1.77	2250
31.	VS 46	0.103	0.061	1.69	3090
32.	VS 48	0.050	0.028	1.78	1500
33.	VS 49	0.173	0.091	1.90	5190
34.	VS 50	0.191	0.113	1.69	5730
35.	VS 52	0.106	0.063	1.67	3180
36.	VS 53	0.092	0.049	1.86	2760
37.	VS 54	0.084	0.047	1.80	2520
38.	VS 55	0.091	0.048	1.88	2730
39.	VS 56	0.024	0.013	1.82	720
40.	VS 57	0.013	0.008	1.70	390
41.	VS 58	0.024	0.013	1.84	720
42.	VS 59	0.044	0.024	1.82	1320
43.	VS 60	0.013	0.007	1.84	390
44.	VS 61	0.007	0.004	1.86	210
45.	VS 62	0.018	0.010	1.83	540
46.	VS 63	0.050	0.027	1.88	1500
47.	VS 64	0.024	0.014	1.75	720
48.	VS 65	0.034	0.019	1.81	1020
49.	VS 66	0.023	0.013	1.76	690
50.	VS 68	0.021	0.012	1.80	630

produced by the primers are presented in Table 26. A total of about 55 amplification products or bands were generated by eight decamer primers, of which 46 (83.64 per cent) were polymorphic. The number of bands for various primers ranged from five (OPK 7 and OPL 8) to ten (OPL 12), with an average of 6.88 bands per primer, while the number of polymorphic bands ranged from four (OPH 5, OPH 19 and OPK 7) to ten (OPL 12), with an average of 5.88 bands per primer.

Five primers namely, OPH 17, OPH 18, OPK 7, OPL 12 and OPL 13 were selected for amplifying DNA from the 50 accessions. The RAPD profile generated by the five selected primers were shown in Plates 12 to 16 and Figures 4 to 8.

A total of 58 scorable bands (average of 11.6 bands per primer) were produced of which, three were monomorphic and the remaining 55 were polymorphic (94.8 per cent). The highest number of scorable bands was given by OPL 13 (14 bands), in which all were polymorphic, followed by primers OPH 18, OPL 12, OPH 17 and OPK 7 with 13, 12, 10 and 9 bands each. While several polymorphic bands were obtained, some bands were present or absent in particular accessions. For instance, VS 32 was found to be distinct from the rest of the accessions due to the presence of a unique band of around 3000 bp.

Data analysis

RAPD marker data were subjected to cluster analysis using NTSYS program to estimate similarity indices and genetic relatedness among the accessions. The reproducible bands were scored for their presence (+) or absence (-) for all the accessions studied. A genetic similarity matrix was constructed using the Jaccard's coefficient method (Table 27).

A dendrogram was generated by UPGMA cluster analysis based on similarity coefficient values (Fig. 9). The similarity coefficient among the accessions varied from 0.20 (between VS 27 and VS 44) to 0.97 (between VS 1 and VS 68 as well as VS 65 and VS 66).

Cluster analysis revealed that at about 66 per cent similarity index, the accessions were grouped into eleven clusters. Out of them, nine clusters contained only one accession each *viz.*, VS 23, VS 26, VS 25, VS 32, VS 29, VS 28, VS 27, VS 24

Table 26. Primer associated banding pattern with the DNA of VS 14

Sl. No.	Primer	Primer sequence (5′ to 3′)	Number of faint bands	Number of intense bands	Total number of bands	Number of polymorphic bands
1.	OPH 05	AGTCGTCCCC	3	3	6	4
2.	OPH 17	CACTCTCCTC	0	9	9	8
3.	OPH 18	GAATCGGCCA	1	5	6	5
4.	OPH 19	CTGACCAGCC	4	3	7	4
5.	OPK 07	AGCGAGCAAG	0	5	5	4
6.	OPL 08	AGCAGGTGGA	1	4	5	5
7.	OPL 12	GGGCGGTACT	1	9	10	10
8.	OPL 13	ACCGCCTGCT	2	5	7	6

Plate 12. RAPD profile of 50 accessions of vegetable cowpea using OPL 13

M Lane No.	Marker Accession	Lane No.	Accession
1	VS 1	26	VS 40
2	VS 5	27	VS 41
3	VS 7	28	VS 42
4	VS 8	29	VS 44
5	VS 9	30	VS 45
6	VS 10	31	VS 46
7	VS 12	32	VS 48
8	VS 13	33	VS 49
9	VS 14	34	VS 50
10	VS 15	35	VS 52
11	VS 16	36	VS 53
12	VS 17	37	VS 54
13	VS 18	38	VS 55
14	VS 19	39	VS 56
15	VS 20	40	VS 57
16	VS 22	41	VS 58
17	VS 23	42	VS 59
18	VS 24	43	VS 60
19	VS 25	44	VS 61
20	VS 26	45	VS 62
21	VS 27	46	VS 63
22	VS 28	47	VS 64
23	VS 29	48	VS 65
24	VS 32	49	VS 66
25	VS 39	50	VS 68

Plate 12

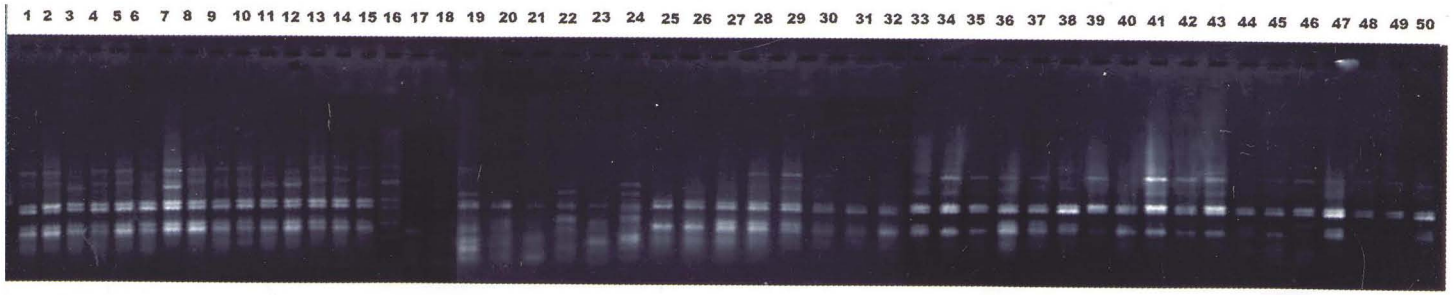

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50

Plate 13. RAPD profile of 50 accessions of vegetable cowpea using OPK 7

M Lane No.	Marker Accession
1	VS 1
2	VS 5
3	VS 7
4	VS 8
5	VS 9
6	VS 10
7	VS 12
8	VS 13
9	VS 14
10	VS 15
11	VS 16
12	VS 17
13	VS 18
14	VS 19
15	VS 20
16	VS 22
17	VS 23
18	VS 24
19	VS 25
20	VS 26
21	VS 27
22	VS 28
23	VS 29
24	VS 32
25	VS 39
26	VS 40
27	VS 41
28	VS 42
29	VS 44
30	VS 45
31	VS 46
32	VS 48
33	VS 49
34	VS 50
35	VS 52
36	VS 53
37	VS 54
38	VS 55
39	VS 56
40	VS 57
41	VS 58
42	VS 59
43	VS 60
44	VS 61
45	VS 62
46	VS 63
47	VS 64
48	VS 65
49	VS 66
50	VS 68

Lane No.	Accession

Plate 13

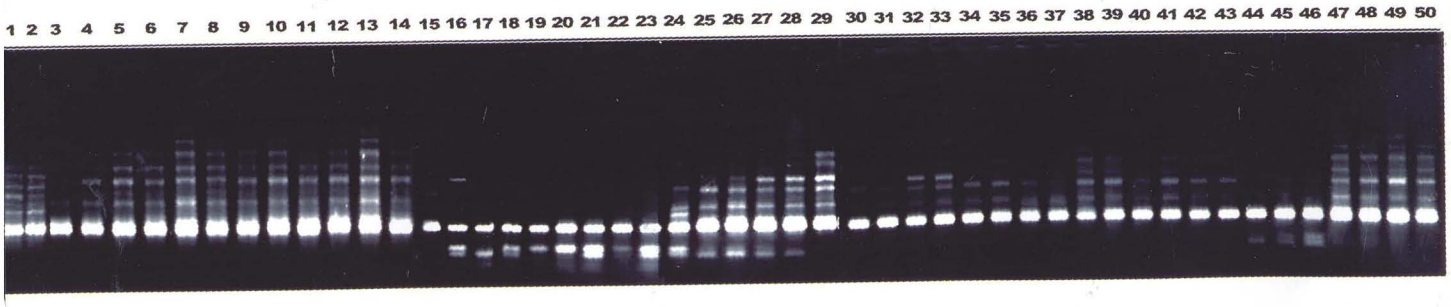

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50

Plate 14. RAPD profile of 50 accessions of vegetable cowpea using OPH 18

M Lane No.	Marker Accession
1	VS 1
2	VS 5
3	VS 7
4	VS 8
5	VS 9
6	VS 10
7	VS 12
8	VS 13
9	VS 14
10	VS 15
11	VS 16
12	VS 17
13	VS 18
14	VS 19
15	VS 20
16	VS 22
17	VS 23
18	VS 24
19	VS 25
20	VS 26
21	VS 27
22	VS 28
23	VS 29
24	VS 32
25	VS 39
26	VS 40
27	VS 41
28	VS 42
29	VS 44
30	VS 45
31	VS 46
32	VS 48
33	VS 49
34	VS 50
35	VS 52
36	VS 53
37	VS 54
38	VS 55
39	VS 56
40	VS 57
41	VS 58
42	VS 59
43	VS 60
44	VS 61
45	VS 62
46	VS 63
47	VS 64
48	VS 65
49	VS 66
50	VS 68

Plate 14

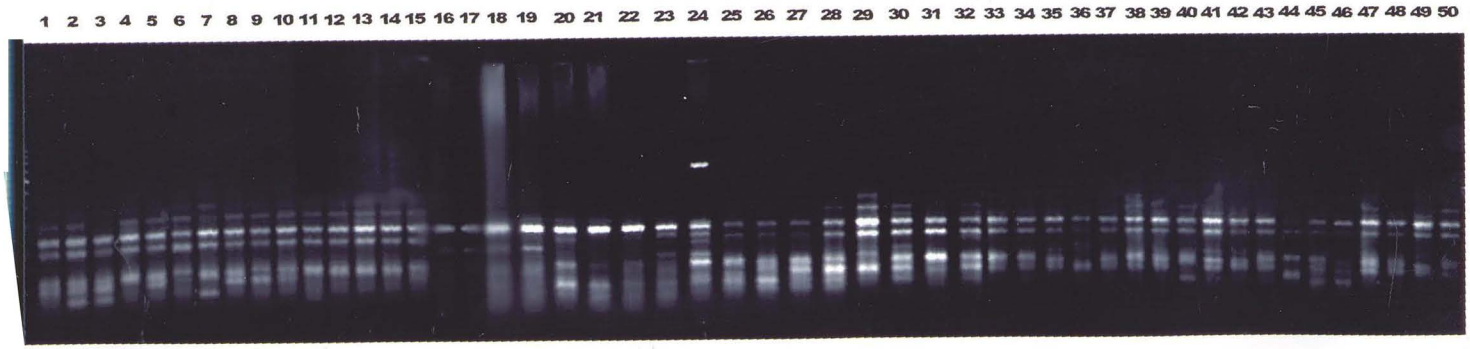

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50

Plate 15. RAPD profile of 50 accessions of vegetable cowpea using OPH 17

M Lane No.	Marker Accession
1	VS 1
2	VS 5
3	VS 7
4	VS 8
5	VS 9
6	VS 10
7	VS 12
8	VS 13
9	VS 14
10	VS 15
11	VS 16
12	VS 17
13	VS 18
14	VS 19
15	VS 20
16	VS 22
17	VS 23
18	VS 24
19	VS 25
20	VS 26
21	VS 27
22	VS 28
23	VS 29
24	VS 32
25	VS 39
26	VS 40
27	VS 41
28	VS 42
29	VS 44
30	VS 45
31	VS 46
32	VS 48
33	VS 49
34	VS 50
35	VS 52
36	VS 53
37	VS 54
38	VS 55
39	VS 56
40	VS 57
41	VS 58
42	VS 59
43	VS 60
44	VS 61
45	VS 62
46	VS 63
47	VS 64
48	VS 65
49	VS 66
50	VS 68

Plate 15

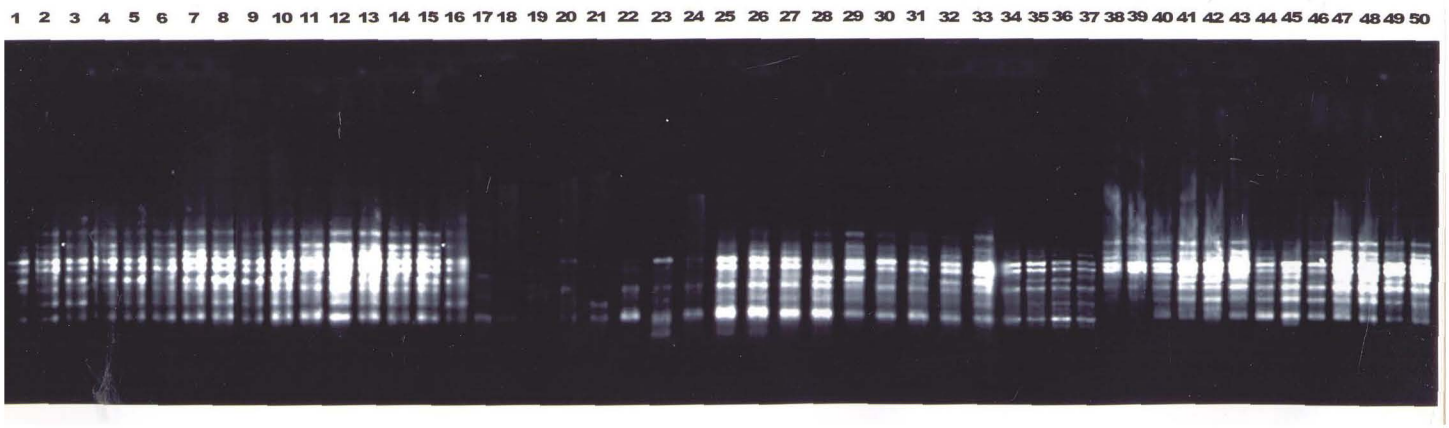
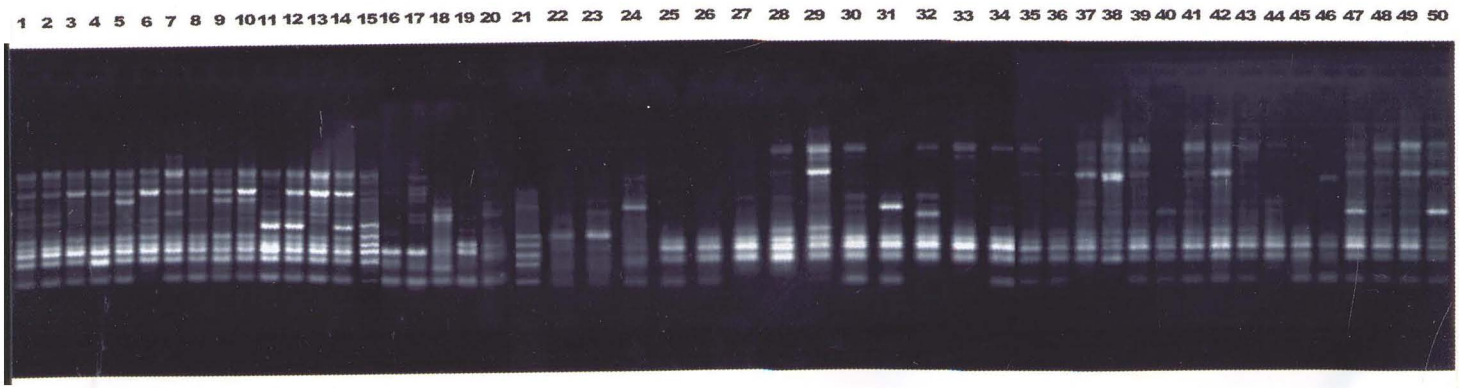

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50

Plate 16. RAPD profile of 50 accessions of vegetable cowpea using OPL 12

M Lane No.	Marker Accession
1	VS 1
2	VS 5
3	VS 7
4	VS 8
5	VS 9
6	VS 10
7	VS 12
8	VS 13
9	VS 14
10	VS 15
11	VS 16
12	VS 17
13	VS 18
14	VS 19
15	VS 20
16	VS 22
17	VS 23
18	VS 24
19	VS 25
20	VS 26
21	VS 27
22	VS 28
23	VS 29
24	VS 32
25	VS 39
26	VS 40
27	VS 41
28	VS 42
29	VS 44
30	VS 45
31	VS 46
32	VS 48
33	VS 49
34	VS 50
35	VS 52
36	VS 53
37	VS 54
38	VS 55
39	VS 56
40	VS 57
41	VS 58
42	VS 59
43	VS 60
44	VS 61
45	VS 62
46	VS 63
47	VS 64
48	VS 65
49	VS 66
50	VS 68

Plate 16


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50

2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
+	-	-	+	-	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	+
+	-	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	+	-	-	+	+	-	+	-	-	-	-	-	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	+	+	-	+	-	-	+	+	-	+	-	-	-	-	-	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-

. 4. Representation of amplification profile of DNA using primer OPK 7

2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	-	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	+	-	-	+	-	-	-
-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	+	+	+	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+
+	+	-	+	+	-	+	+	+	-	-	-	-	+	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-

. 5. Representation of amplification profile of DNA using primer OPH 18

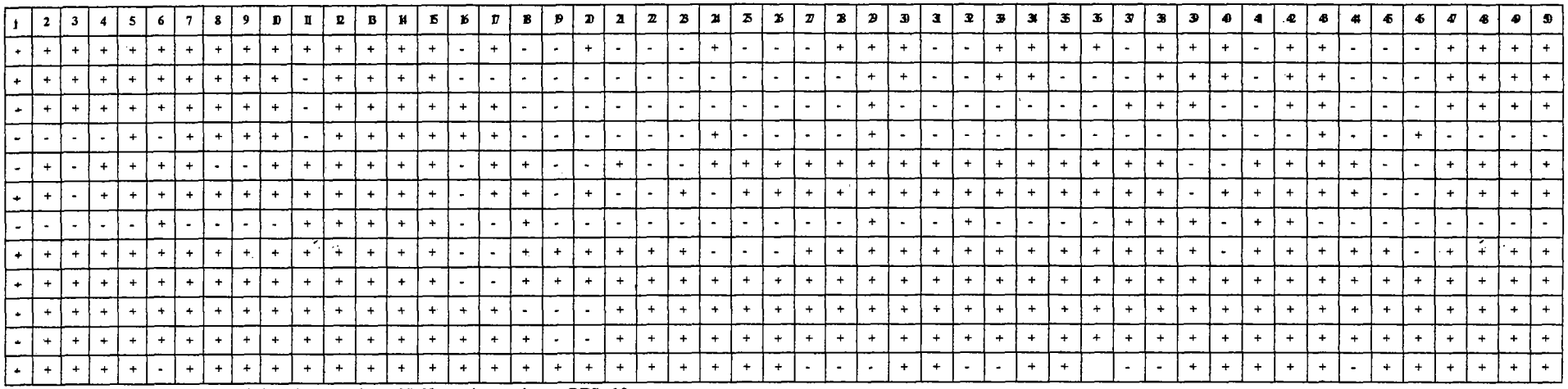


Fig. 6. Representation of amplification profile of DNA using primer OPL 12

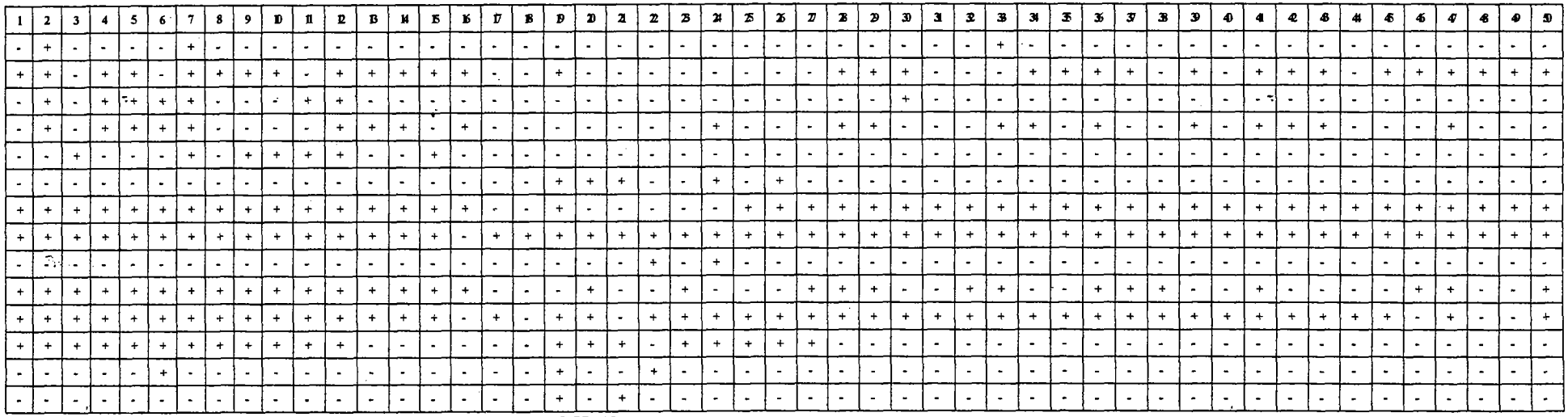


Fig. 7. Representation of amplification profile of DNA using primer OPL 13

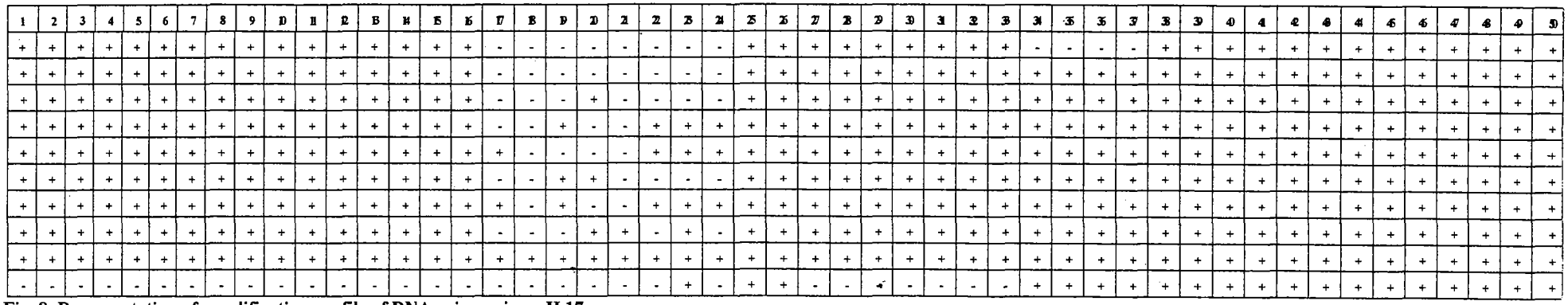


Fig. 8. Representation of amplification profile of DNA using primer H 17

Table 27. Similarity matrix of 50 accessions of cowpea

Sl. No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	1.00																							
2	0.88	1.00																						
3	0.76	0.71	1.00																					
4	0.80	0.88	0.73	1.00																				
5	0.83	0.91	0.71	0.88	1.00																			
6	0.76	0.84	0.73	0.85	0.84	1.00																		
7	0.81	0.93	0.70	0.86	0.89	0.78	1.00																	
8	0.85	0.84	0.77	0.81	0.93	0.77	0.82	1.00																
9	0.87	0.81	0.84	0.79	0.86	0.74	0.84	0.92	1.00															
10	0.88	0.86	0.79	0.83	0.90	0.79	0.88	0.88	0.95	1.00														
11	0.73	0.72	0.78	0.78	0.72	0.78	0.74	0.69	0.75	0.76	1.00													
12	0.79	0.86	0.71	0.88	0.91	0.84	0.93	0.84	0.86	0.90	0.80	1.00												
13	0.81	0.84	0.65	0.86	0.84	0.77	0.86	0.81	0.79	0.84	0.70	0.88	1.00											
14	0.80	0.84	0.68	0.85	0.88	0.81	0.86	0.85	0.83	0.88	0.73	0.93	0.95	1.00										
15	0.73	0.73	0.74	0.74	0.77	0.74	0.75	0.74	0.80	0.85	0.74	0.81	0.79	0.83	1.00									
16	0.44	0.42	0.46	0.45	0.45	0.39	0.44	0.49	0.46	0.44	0.40	0.45	0.47	0.49	0.45	1.00								
17	0.33	0.30	0.31	0.32	0.33	0.29	0.32	0.32	0.33	0.34	0.32	0.33	0.33	0.35	0.39	0.39	1.00							
18	0.24	0.24	0.24	0.26	0.24	0.26	0.24	0.23	0.24	0.26	0.33	0.27	0.28	0.29	0.32	0.23	0.41	1.00						
19	0.34	0.30	0.35	0.33	0.30	0.30	0.30	0.33	0.33	0.32	0.33	0.30	0.28	0.30	0.32	0.31	0.25	0.36	1.00					
20	0.38	0.36	0.43	0.39	0.36	0.36	0.36	0.39	0.40	0.38	0.44	0.36	0.34	0.36	0.38	0.24	0.25	0.42	0.46	1.00				
21	0.27	0.27	0.31	0.29	0.27	0.26	0.26	0.26	0.26	0.28	0.32	0.27	0.24	0.26	0.28	0.26	0.33	0.55	0.40	0.46	1.00			
22	0.35	0.34	0.44	0.33	0.34	0.37	0.30	0.37	0.38	0.36	0.38	0.31	0.32	0.33	0.39	0.28	0.36	0.43	0.48	0.37	0.42	1.00		
23	0.40	0.39	0.46	0.39	0.39	0.39	0.35	0.42	0.43	0.41	0.44	0.36	0.34	0.36	0.41	0.35	0.39	0.41	0.35	0.50	0.50	0.58	1.00	
24	0.39	0.41	0.44	0.43	0.44	0.40	0.43	0.43	0.41	0.43	0.45	0.44	0.42	0.43	0.40	0.31	0.42	0.34	0.34	0.38	0.42	0.48	0.50	1.00
25	0.63	0.59	0.67	0.60	0.59	0.60	0.54	0.60	0.61	0.62	0.68	0.56	0.53	0.56	0.60	0.44	0.36	0.38	0.37	0.41	0.41	0.47	0.58	0.54
26	0.63	0.60	0.68	0.60	0.60	0.60	0.55	0.60	0.62	0.63	0.68	0.57	0.54	0.57	0.57	0.46	0.34	0.35	0.39	0.43	0.42	0.44	0.54	0.55
27	0.69	0.65	0.74	0.66	0.65	0.70	0.60	0.66	0.68	0.68	0.75	0.61	0.59	0.62	0.63	0.47	0.31	0.36	0.36	0.44	0.39	0.45	0.56	0.49
28	0.77	0.76	0.73	0.78	0.76	0.78	0.70	0.78	0.75	0.76	0.78	0.72	0.70	0.73	0.70	0.47	0.32	0.30	0.33	0.41	0.32	0.38	0.47	0.49
29	0.83	0.81	0.66	0.79	0.86	0.83	0.80	0.83	0.80	0.85	0.67	0.86	0.88	0.92	0.80	0.46	0.33	0.24	0.27	0.30	0.20	0.31	0.33	0.38
30	0.73	0.72	0.68	0.78	0.72	0.69	0.67	0.69	0.70	0.71	0.74	0.68	0.70	0.69	0.74	0.37	0.32	0.30	0.33	0.37	0.29	0.42	0.44	0.45
31	0.70	0.62	0.66	0.67	0.62	0.63	0.57	0.63	0.64	0.65	0.71	0.58	0.63	0.63	0.68	0.39	0.34	0.31	0.35	0.35	0.30	0.45	0.43	0.41
32	0.69	0.65	0.69	0.70	0.65	0.74	0.60	0.66	0.68	0.68	0.80	0.65	0.71	0.70	0.71	0.39	0.28	0.32	0.29	0.36	0.28	0.41	0.43	0.38
33	0.77	0.76	0.68	0.73	0.72	0.73	0.70	0.73	0.71	0.71	0.65	0.68	0.74	0.73	0.62	0.40	0.29	0.23	0.27	0.33	0.23	0.34	0.37	0.39
34	0.71	0.67	0.67	0.72	0.67	0.63	0.61	0.68	0.65	0.66	0.63	0.63	0.68	0.68	0.68	0.41	0.36	0.29	0.37	0.37	0.29	0.42	0.41	0.46
35	0.78	0.68	0.69	0.69	0.68	0.65	0.63	0.69	0.71	0.72	0.69	0.64	0.66	0.69	0.66	0.42	0.38	0.30	0.38	0.38	0.29	0.44	0.42	0.44
36	0.68	0.64	0.64	0.69	0.64	0.61	0.63	0.65	0.63	0.63	0.69	0.64	0.62	0.65	0.62	0.46	0.38	0.26	0.38	0.42	0.33	0.35	0.50	0.51
37	0.70	0.62	0.66	0.63	0.62	0.67	0.57	0.63	0.64	0.65	0.67	0.62	0.63	0.67	0.72	0.43	0.34	0.31	0.35	0.35	0.26	0.41	0.43	0.38
38	0.82	0.77	0.69	0.78	0.77	0.83	0.71	0.74	0.76	0.80	0.70	0.77	0.83	0.83	0.75	0.38	0.31	0.25	0.26	0.32	0.21	0.33	0.35	0.34
39	0.77	0.72	0.73	0.78	0.72	0.73	0.67	0.78	0.75	0.71	0.65	0.72	0.78	0.78	0.70	0.47	0.29	0.23	0.33	0.30	0.23	0.38	0.33	0.39
40	0.68	0.63	0.73	0.68	0.63	0.64	0.62	0.68	0.70	0.67	0.74	0.63	0.65	0.68	0.69	0.40	0.35	0.28	0.32	0.41	0.27	0.42	0.40	0.46
41	0.73	0.68	0.64	0.73	0.68	0.69	0.63	0.69	0.67	0.67	0.69	0.68	0.74	0.73	0.66	0.47	0.29	0.30	0.33	0.33	0.26	0.38	0.44	0.42
42	0.76	0.71	0.72	0.73	0.71	0.73	0.66	0.73	0.70	0.71	0.68	0.71	0.73	0.77	0.78	0.46	0.38	0.31	0.35	0.35	0.27	0.40	0.38	0.44
43	0.74	0.74	0.75	0.75	0.78	0.71	0.72	0.79	0.77	0.78	0.67	0.74	0.76	0.79	0.81	0.49	0.41	0.31	0.34	0.38	0.30	0.43	0.41	0.50
44	0.54	0.48	0.53	0.51	0.48	0.51	0.47	0.48	0.49	0.50	0.58	0.48	0.49	0.51	0.55	0.47	0.38	0.39	0.39	0.39	0.38	0.45	0.47	0.41
45	0.62	0.55	0.66	0.55	0.55	0.51	0.50	0.59	0.60	0.57	0.58	0.51	0.52	0.55	0.59	0.52	0.30	0.31	0.44	0.35	0.34	0.50	0.47	0.45
46	0.59	0.52	0.63	0.52	0.56	0.49	0.51	0.60	0.62	0.59	0.55	0.52	0.53	0.56	0.61	0.63	0.31	0.28	0.36	0.32	0.31	0.42	0.44	0.46
47	0.90	0.84	0.68	0.81	0.80	0.73	0.78	0.81	0.79	0.79	0.65	0.76	0.86	0.81	0.70	0.45	0.32	0.23	0.30	0.33	0.23	0.33	0.39	0.40
48	0.86	0.76	0.68	0.73	0.72	0.65	0.70	0.73	0.75	0.76	0.61	0.68	0.78	0.73	0.66	0.44	0.32	0.26	0.30	0.30	0.26	0.34	0.33	0.36
49	0.84	0.74	0.67	0.71	0.74	0.64	0.73	0.76	0.78	0.78	0.60	0.70	0.80	0.76	0.68	0.46	0.35	0.26	0.29	0.29	0.25	0.33	0.33	0.38
50	0.97	0.85	0.74	0.78	0.81	0.74	0.79	0.83	0.85	0.85	0.70	0.77	0.83	0.83	0.75	0.45	0.34	0.25	0.32	0.35	0.24	0.36	0.38	0.37

Contd..

Table 27. Continued...

Sl. No.	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
1																										
2																										
3																										
4																										
5																										
6																										
7																										
8																										
9																										
10																										
11																										
12																										
13																										
14																										
15																										
16																										
17																										
18																										
19																										
20																										
21																										
22																										
23																										
24																										
25	1.00																									
26	0.94	1.00																								
27	0.84	0.85	1.00																							
28	0.72	0.73	0.85	1.00																						
29	0.53	0.55	0.63	0.75	1.00																					
30	0.68	0.64	0.66	0.74	0.67	1.00																				
31	0.75	0.71	0.73	0.71	0.64	0.82	1.00																			
32	0.69	0.69	0.82	0.80	0.72	0.75	0.84	1.00																		
33	0.59	0.60	0.70	0.74	0.79	0.69	0.71	0.75	1.00																	
34	0.66	0.62	0.64	0.72	0.69	0.82	0.81	0.69	0.77	1.00																
35	0.68	0.69	0.71	0.74	0.71	0.74	0.77	0.71	0.74	0.84	1.00															
36	0.63	0.59	0.66	0.74	0.63	0.69	0.67	0.61	0.65	0.78	0.75	1.00														
37	0.65	0.61	0.73	0.71	0.73	0.67	0.74	0.78	0.67	0.75	0.77	0.72	1.00													
38	0.56	0.57	0.67	0.70	0.89	0.70	0.72	0.81	0.84	0.68	0.70	0.58	0.72	1.00												
39	0.55	0.56	0.58	0.69	0.79	0.74	0.71	0.70	0.74	0.77	0.74	0.61	0.67	0.79	1.00											
40	0.72	0.68	0.65	0.69	0.62	0.79	0.77	0.70	0.64	0.72	0.69	0.64	0.61	0.65	0.69	1.00										
41	0.63	0.64	0.70	0.74	0.75	0.74	0.76	0.80	0.78	0.77	0.79	0.74	0.76	0.74	0.78	0.59	1.00									
42	0.67	0.63	0.66	0.73	0.79	0.78	0.76	0.69	0.73	0.88	0.79	0.74	0.81	0.74	0.83	0.73	0.78	1.00								
43	0.69	0.66	0.68	0.76	0.77	0.81	0.74	0.68	0.71	0.85	0.76	0.71	0.74	0.68	0.76	0.76	0.71	0.91	1.00							
44	0.71	0.67	0.74	0.63	0.53	0.58	0.70	0.64	0.58	0.61	0.63	0.58	0.65	0.55	0.50	0.61	0.58	0.62	0.60	1.00						
45	0.75	0.71	0.73	0.67	0.56	0.67	0.74	0.63	0.58	0.70	0.72	0.62	0.69	0.55	0.67	0.66	0.67	0.71	0.69	0.76	1.00					
46	0.72	0.68	0.70	0.64	0.58	0.59	0.66	0.60	0.55	0.62	0.64	0.59	0.66	0.53	0.59	0.63	0.64	0.63	0.66	0.61	0.83	1.00				
47	0.56	0.57	0.62	0.73	0.83	0.78	0.71	0.70	0.82	0.76	0.74	0.69	0.67	0.83	0.82	0.64	0.82	0.77	0.75	0.51	0.59	0.56	1.00			
48	0.59	0.60	0.62	0.65	0.75	0.74	0.71	0.66	0.78	0.77	0.79	0.61	0.67	0.79	0.78	0.64	0.74	0.78	0.76	0.54	0.62	0.59	0.87	1.00		
49	0.58	0.59	0.60	0.63	0.78	0.72	0.69	0.64	0.76	0.75	0.77	0.59	0.65	0.77	0.76	0.62	0.72	0.76	0.78	0.53	0.61	0.62	0.84	0.97	1.00	
50	0.60	0.61	0.67	0.74	0.85	0.74	0.72	0.71	0.79	0.73	0.80	0.66	0.72	0.84	0.79	0.69	0.74	0.78	0.76	0.55	0.63	0.61	0.92	0.89	0.86	1.00

Sl. No.	Accession
1	VS 1
2	VS 5
3	VS 7
4	VS 8
5	VS 9
6	VS 10
7	VS 12
8	VS 13
9	VS 14
10	VS 15
11	VS 16
12	VS 17
13	VS 18
14	VS 19
15	VS 20
16	VS 22
17	VS 23
18	VS 24
19	VS 25
20	VS 26
21	VS 27
22	VS 28
23	VS 29
24	VS 32
25	VS 39

Sl. No.	Accession
26	VS 40
27	VS 41
28	VS 42
29	VS 44
30	VS 45
31	VS 46
32	VS 48
33	VS 49
34	VS 50
35	VS 52
36	VS 53
37	VS 54
38	VS 55
39	VS 56
40	VS 57
41	VS 58
42	VS 59
43	VS 60
44	VS 61
45	VS 62
46	VS 63
47	VS 64
48	VS 65
49	VS 66
50	VS 68

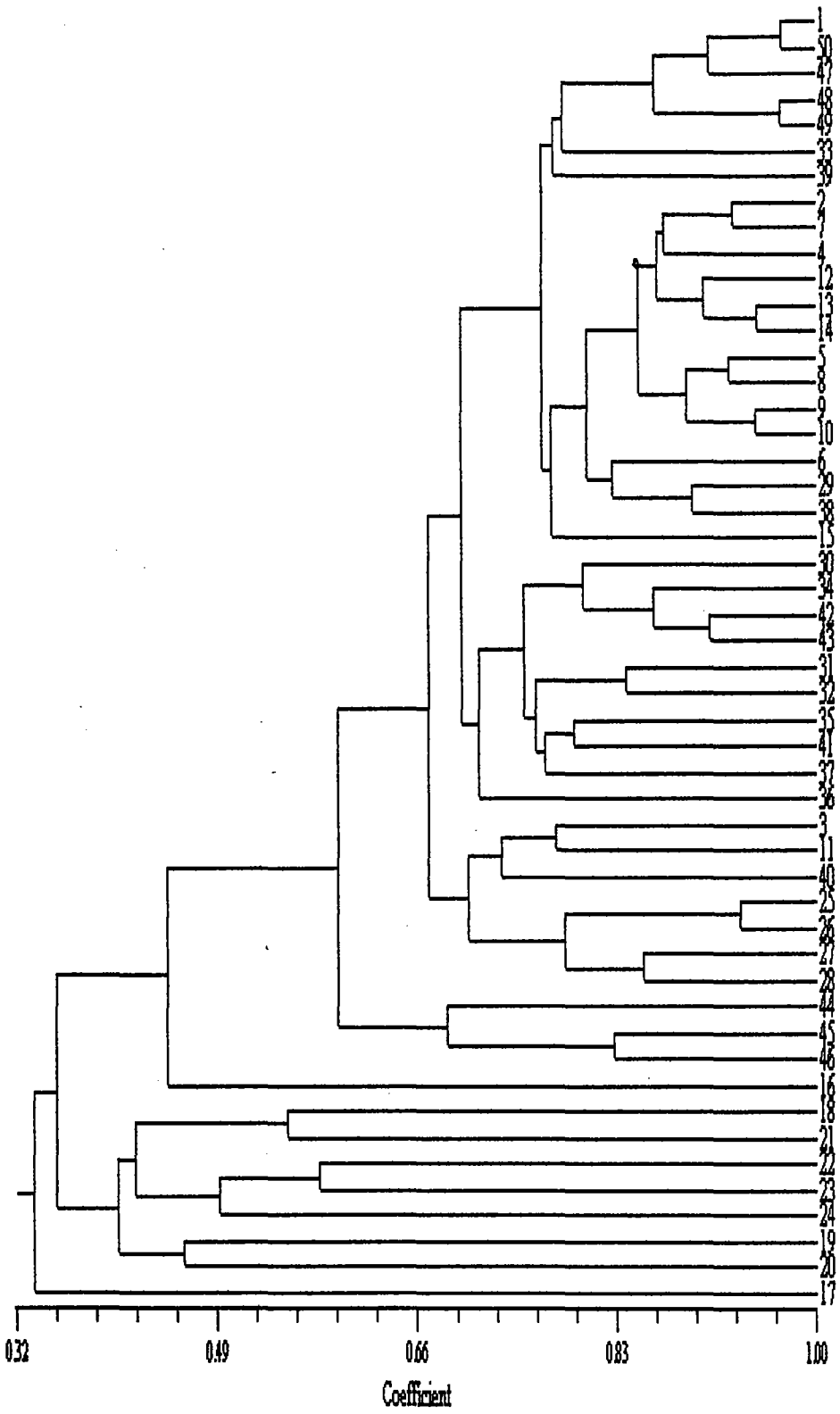


Fig. 9. Dendrogram of 50 accessions of cowpea

and VS 22. At 38.8 per cent similarity, all these accessions excepting VS 22 fell into a single cluster.

At 66 per cent similarity, the tenth cluster included three accessions namely, VS 61, VS 62 and VS 63. The rest of the 38 accessions formed a single large cluster. At 72.8 per cent similarity, this single cluster may be further split up into five small clusters. At higher similarity index, these may be even broken down into simpler groups. The understanding of the genetic relationships among the accessions, together with analysis of their morphology and agronomic performance may help in their further utilization in breeding programmes.

Discussion

5. DISCUSSION

Cowpea (*Vigna unguiculata* (L.) Walp.), the most popular and traditional leguminous vegetable is a rich and cheap source of vegetable protein. Kerala is blessed with diverse climatic and soil conditions which have helped in the development of different landraces of vegetable cowpea having high variability. These landraces, the products of natural selection maintain genetic heterogeneity in balance over time. The exploitation of this heterogeneity can help in the improvement of the crop.

The genetic improvement of any crop aims at increasing the production potential and quality by altering the genetic makeup of the existing varieties. To achieve this goal, a plant breeder requires information on certain genetic parameters like variability, heritability, genetic advance and association between characters. For the development of superior varieties, studies on variability is a basic necessity, which is lacking in vegetable cowpea.

Hence a study was undertaken to collect and catalogue the available landraces of vegetable cowpea for various morphological characters and to assess the magnitude of genetic variability for identifying superior types based on yield, quality and pest and disease tolerance.

Recently, with the development of molecular marker techniques, a direct method to estimate genetic diversity within and among populations has become possible. Hence an attempt has been made to characterize the available germplasm of vegetable cowpea based on protein as well as RAPD based DNA markers. The results obtained in the study are discussed below.

5.1 Genetic cataloguing

Genetic cataloguing based on standard descriptors helps to describe the morphological features of an accession easily and thus helps in the exchange of information about new accessions in a more clear way.

The 66 accessions upon cataloguing showed distinct variation among each other with respect to vegetative, inflorescence, fruit and seed characters. Most of the accessions were climbing with indeterminate growth pattern and pronounced twining tendency. Leaf colour was intermediate to dark green having varying plant and inflorescence pigmentation. The accessions with moderate to intermediate pigmentation on vegetative parts had more pigmented flowers. Nature of flowering was mostly synchronous, bearing straight pendant pods throughout the canopy. Pod pigmentation was absent in many accessions while few of them with fully pigmented valves or pigmented tips. Seed shape varied from kidney to rhomboid with smooth, rough or smooth to rough testa texture. The variation observed in seed colour was remarkable. Seed colour ranged from light brown to black with varying levels in between. It should be mentioned that deeply pigmented vegetative parts and completely pigmented flowers especially calyx may be correlated to pigmented pods and dark brown seeds, while pods with pigmented tips was found associated with lightly pigmented calyx and black seeds. Cataloguing of vegetable cowpea has also been attempted by Resmi (1998) and Gopalakrishnan (2004).

5.2 Variability

An insight into the magnitude of variability present in a crop species is of utmost importance as it provides a basis for effective selèction. The observed variability in the population is the sum total of the variations that arise due to genotypic and environmental effects. Hence, a knowledge on the nature and magnitude of genetic variation contributing to gain under selection is essential.

In the present investigation, analysis of variance revealed significant differences among the 66 accessions for all the characters coming under growth, flowering, pod, yield and quality. Such variations indicated the scope for improving the population for these characters as reported earlier by Sobha and Vahab (1998), Kumar and Sangwan (2000) and Venkatesan *et al.* (2003).

Primary branches per plant was found to vary from 2.00 to 6.75. Similar results were also reported by Borah and Khan (2000). Ample variation was also observed for days to seedling emergence. VS 5 was the earliest to emerge (3.50 days). In the case of vine length and collar girth VS 19 and VS 1 recorded the highest value (6.17 m and 6.48 cm respectively), while VS 58 had the lowest (0.42 m and 2.20 cm respectively). Vidya (2000) reported wide variation for length of main stem in yard long bean.

Wide range of variation was observed for petiole length, length and width of terminal leaflets, length and width of lateral leaflets and root : shoot ratio. Resmi (1998) recorded significant differences among the genotypes for leaf characters, while Ogbonnaya *et al.* (2003) reported variation in root : shoot ratio.

Days to first flowering exhibited a range of 34.67 to 51.17, while pollen viability had an overall mean of 91.80 per cent. Wide variation was found in the case of peduncle length, with VS 5 having the longest (40.50 cm) and VS 2 having the shortest (10.38 cm) peduncles. High genetic variability was observed for peduncle length by several workers (Trehan *et al.*, 1970 ; Panicker, 2000).

Considerable variation was observed for pod length, pod girth and pod weight. VS 19 recorded the highest pod length and pod girth (76.08 cm and 4.43 cm respectively). Among the accessions, pod weight was found to range from 2.42 to 43.60 g, showing ample variability and scope for improvement. VS 4 had the highest pod weight of 43.60 g. High genetic variability for pod length was reported by Bapna and Joshi (1973), while Sobha (1994) and Ajith (2001) observed the same for pod girth and pod weight respectively.

Pods per plant and yield per plant exhibited high variability. Among the accessions evaluated, pods per plant and yield were highest in VS 8 (CHCP-1). In the case of other biometric characters especially pod characters, the accession had only an average value. Hence for VS 8, the high yield is mainly contributed by

high pods per plant. Wide range of variability was reported for pods per plant and yield per plant by Kutty *et al.* (2003).

Significant differences among accessions were observed for seeds per pod, 100-seed weight, seed length, seed width, seed thickness and number of harvests. VS 4 had the highest number of seeds per pod, while VS 19 had the highest 100-seed weight and seed length. The same accession was also noted for its high pod length and pod girth which may be contributing to increased seed weight and seed length. Significant variation among accessions was also reported for seed length and seed width by Jalajakumari (1981), while Sobha (1994) and Kumari *et al.* (2003) observed high variability in seeds per pod and 100-seed weight respectively. It was also observed that most long poded varieties or yard long beans have dark brown to black seed colour with few exceptions and have more than 10 mm seed length. Hence, by looking into the seed characteristics, one can predict the overall nature of the accession.

Among the quality characters, wide variation was recorded for keeping quality, pod protein and pod fibre. Similar results were also reported by Resmi (1998).

Closer values of phenotypic and genotypic variances obtained in the study suggests the predominant influence of genotypic component over the environmental effect on its genotype. Coefficient of variation, phenotypic (PCV) and genotypic (GCV) are another means of expressing variability. It is a better index for comparison of characters with different units of measurement, than estimates of quantitative variation like range and variation around them. In the present study, PCV ranged from 8.16 to 101.83. Root : shoot ratio had the highest PCV, followed by pod weight and yield per plant. Since phenotypic value constitute both genotypic effect and environmental influence, crop improvement programme cannot be undertaken solely on phenotypic performance. GCV provides a more precise measure of genetic variability. It ranged from 7.17 to 82.30. As in the case of PCV, GCV was also highest for root : shoot ratio, followed by pod weight and yield per plant. High PCV

and GCV were reported for pod weight by Rangaiah (2000) and for yield by Kumari *et al.* (2003). The high magnitude of GCV for most of the characters revealed high amount of variability for these characters, thereby suggesting good scope for improvement through selection. Further the closer values of PCV and GCV indicated that selection on phenotypic basis will be effective.

5.3 Heritability and genetic advance

The total variability existing in a population is a sum of heritable and non-heritable components, and it is necessary to apportion these components, since the magnitude of heritable variability is an important aspect of genetic constitution of the breeding material.

The present investigation revealed high values of heritability for most of the characters. Heritability estimate was highest (> 90%) for pod weight, followed by pod length and vine length. This result is in agreement with the findings of Roquib and Patnaik (1990), Umaharan *et al.* (1997) and Vidya (2000). High heritability estimates indicate the presence of large number of fixable additive factors and hence these traits can be improved by selection.

High heritability estimates does not necessarily mean a high genetic advance for a particular character. The effectiveness of selection depends upon the heritability and genetic advance of the character selected. High heritability coupled with high genetic advance was observed in the present investigation for several characters including yield per plant, pods per plant, pod length, pod weight, root : shoot ratio and vine length. Kumar and Sangwan (2000) also reported high heritability and genetic advance for yield per plant and pods per plant.

Days to seedling emergence recorded a medium or moderate heritability, while days to first flowering, pollen viability and seed thickness had high heritability and low genetic advance, indicating the action of non-additive genes for the expression of these characters. Sreekumar *et al.* (1996) also reported high heritability and low genetic advance for days to first flowering.

On the basis of the present study, it can be concluded that simultaneous selection based on multiple characters having high estimates of heritability and genetic advance might be of appreciable use in this crop.

5.4 Correlation studies

Correlation studies provide information on the nature and extent of relationship between all pairs of characters. A study of correlation among yield and its components will be of great value in planning and evaluating breeding programmes. In the present study, the genotypic correlation coefficients were in general higher than phenotypic coefficients. At the genotypic level, yield per plant observed high positive correlation with vine length, collar girth, pod length, pod girth, pod weight, pods per plant, seeds per pod, 100-seed weight, seed length, seed width, number of harvests, keeping quality and pod protein. On the other hand, root : shoot ratio and peduncle length were negatively correlated with yield and yield component characters. Trehan *et al.* (1970) also observed high positive correlation between yield and pod characters, while Jalajakumari (1981) observed correlation between yield and seed width. Correlation of pods per plant and seeds per pod with yield was also reported by Subbiah *et al.* (2003).

Vine length as well as collar girth exhibited a similar pattern of association with yield component characters. Vine length also had high positive correlation with leaf dimensions like length of terminal and lateral leaflets. Leaf dimensions in turn are positively correlated among each other. Length of leaflets (terminal and lateral) were positively associated with pod characters, seed characters and keeping quality, whereas width of leaflets (terminal and lateral) observed high positive correlation with seed dimensions. This suggests that, eventhough leaf dimensions are not directly correlated with yield, it can contribute to yield improvement by means of its positive correlation with yield component characters. Moreover, they can also be taken easily as a morphological marker in selection procedure.

The present investigation revealed that pod characters and seed characters are positively correlated among each other, while both are negatively correlated with pods per plant. This means that, when we go for selection of increased pod length, pod girth or pod weight, it will lead to an improvement of seeds per pod, 100-seed weight, seed length and seed width, but will reduce the number of pods. Even then there will be some amount of yield improvement, as pod characters and seed characters are positively correlated with yield. It was observed that large sized pods having more number of seeds, high seed weight and seed length are dark green in colour with thick pod walls as in the case of the accessions VS 4 (Kanjikuzhi Payar) and VS 19 (local variety from Aryanad, Thiruvananthapuram). These accessions were also characterized by low number of pods per plant. But since the consumer preference is for light green coloured pods, with medium size, selection of accessions with medium sized light green pods with more number of pods per plant is to be done. Moreover, it was found that pod weight is positively correlated with pod fibre. Hence selection of large sized pods will lead to inceased pod fibre content, ultimately reducing consumer acceptability. Therefore, medium sized pods are to be preferred for selection.

Seeds per pod as well as 100-seed weight were positively associated among each other and also with seed dimensions. All the seed dimensions were positively correlated among each other as well as with keeping quality.

On the basis of the present study, it is evident that selection based on growth characters like vine length and collar girth, leaf dimensions, pod characters and seed characters may be done for yield improvement.

5.5 Path coefficient analysis

Yield is a complex quantitative character governed by a large number of genes and it is greatly influenced by environmental factors. Apart from yield per plant, vine length, collar girth, root : shoot ratio, peduncle length, pod length, pod girth, pod weight, pods per plant, seeds per pod, 100-seed weight, seed length, seed width,

number of harvests, keeping quality and pod protein were selected for path coefficient analysis. The study provided information on the nature and association of several characters contributing to yield, by means of untangling the direct and indirect contribution of various characters in building up a complex correlation. As evidenced from correlation studies, path coefficient analysis also signifies the importance of the character pods per plant which exhibited the highest positive direct effect on yield. Sobha (1994) reported pod weight and pod girth as the most important yield components in vegetable cowpea, while Kutty *et al.* (2003) found pods per plant exerted the highest direct effect on yield. In the present study, 100-seed weight exerted a negative direct effect on yield. The indirect effects through seed length were consistently high signifying the importance of the character, followed by pod length and vine length. Hence, the high correlation observed between yield and its component characters is mainly attributed by the high indirect effects through seed length, pod weight and vine length.

In the present study, the residual effect noticed was only 0.1830 indicating that the variation in pod yield was highly attributable to the factors selected for analysis.

5.6 Selection index

Selection index provides information on yield components and thus aids in indirect selection for the improvement of yield. It involves discriminant function analysis which is meant for isolating superior genotypes based on the phenotypic and genotypic correlations. Identification of superior accessions of vegetable cowpea based on discriminant function analysis was also done by Resmi (1998). A model involving the same set of characters which was used for path coefficient analysis was selected for ranking the accessions. Upon ranking the selection index scores, the accession VS 27 (Pattom, Thiruvananthapuram) ranked first, followed by VS 8 (CHCP-1) and VS 19 (Aryanad, Thiruvananthapuram). These accessions with high

yield and quality may be subjected to screening for pest and disease resistance and multilocational testing before recommending as elite types for cultivation.

5.7 Mahalanobi's D^2 analysis

Breeding crop plants adopting hybridization as a tool is one of the most important crop improvement methods. The success of hybridization programme is mainly dependent on the genetic diversity of the parents chosen for the purpose. Crosses between genetically diverse parents are likely to produce high heterotic effects. Mahalanobi's D^2 statistic is one of the potent techniques for measuring genetic divergence at both intra and intercluster levels and thus provides a basis for selection of genetically diverse parents in hybridization programmes. In vegetable cowpea, genetic divergence and clustering pattern was studied by several workers (Sobha, 1994 ; Resmi, 1998 ; Vidya, 2000).

In the present study, based on Mahalanobi's D^2 analysis, the 66 accessions were grouped into ten gene constellations. The highest number of accessions (18) were included in cluster I, followed by clusters II and III with eight accessions each. The smallest cluster was cluster X with 2 accessions. Almost all the dual purpose and grain type cowpea were grouped under clusters I, III, V and VI, whereas typical bush type vegetable cowpea constitutes cluster VII. The yard long bean types is split up among clusters II, IV, VIII, IX and X. Thus it is evident that, clustering based on Mahalanobi's D^2 statistic agrees with simple grouping based on morphological characters.

Considering the cluster means for the various characters studied, cluster X comprising the accessions VS 4 and VS 19 observed the highest values for most of the biometric characters, whereas cluster VI had lowest values for several characters. Cluster VII which are bush types had the shortest vine length. Pods per plant and yield per plant was highest in cluster IX, which is mainly due to the presence of the accession VS 8, the top yielder among all the 66 accessions. VS 27 which ranked first in discriminant function analysis is under cluster IV comprising yard long bean types

with light green medium sized fruits (excepting VS 31 with purple fruits) closely followed cluster X for the cluster means of several biometric characters studied.

The average intracluster distance was highest in cluster IX and lowest in cluster X. This means that the accessions in cluster X namely, VS 4 and VS 19 are close to each other which is in conformity with the morphological observations. On the other hand, the accessions in cluster IX needs further grouping.

Cluster X maintained high intercluster distance with all other clusters, showing the unique characteristics of the accessions included, with the highest intercluster distance with cluster VI. This suggests that clusters VI and X are the most extreme clusters with the highest genetic distance between them. The lowest intercluster distance was between clusters I and III, both consisting of dual purpose and grain types of cowpea.

5.8 Screening for legume pod borer resistance (*Maruca vitrata*)

Legume pod borer, *Maruca vitrata* (Fab.), which is one of the most important post-flowering pests of cowpea in the tropics is a major limiting factor in cowpea cultivation in all seasons. In high rainfall areas, the crop loss due to the pest goes even up to 80 per cent (Jackai and Adalla, 1997).

Application of host plant resistance as a major aspect of pest management is currently gaining importance. In this respect, breeding for resistance to the pest assumes utmost importance, both in terms of environmental safety and checking the cost of cultivation. Pest resistance is often found in unimproved or traditional germplasm. Hence development and standardization of screening techniques for traditional and local germplasm is a basic requirement for breeding for host plant resistance. Even crop varieties with moderate levels of resistance or partial resistance to the concerned pest can substantially reduce the use of insecticides for pest control. Such varieties suffer less damage than susceptible varieties, since they reduce the viability of the pest and enhance the activity of natural enemies. Low levels of pesticide residues should be ensured in the harvested produce in a crop like cowpea to

increase the suitability of consumption and to meet the market specifications. In the present investigation, an attempt has been made to screen the local germplasm of vegetable cowpea for legume pod borer resistance. The data on flower and pod damage indicated that adequate pest population was developed in the experimental field. A variety that suffers lesser insect attack or lesser damage in the event of comparable pest population can be considered as partially resistant (Dent, 1995).

Tingey (1986) suggested that assessment of plant resistance through measurements of insect damage should be made employing damage criteria closely associated with ultimate loss in crop yield and quality. The field screening technique involving computation of overall plant susceptibility index (Ips) based on flower, pod and seed damage parameters was employed in the present study.

Analysis of variance revealed significant differences among accessions for all the damage parameters as well as plant susceptibility index. Among the 66 accessions screened, VS 49 had the highest flower damage. VS 42 which had the highest pod and seed damage was found to be the most susceptible accession showing the highest Ips. On the other hand, VS 19 was the most tolerant with the lowest flower, pod and seed damage and plant susceptibility index.

Correlation among damage parameters revealed that flower, pod and seed damage parameters are positively associated among each other. It is logical to conclude that, when larval count in flower increases, it leads to increased pod damage, finally resulting in higher seed damage and plant susceptibility index.

Role of plant characters in host plant resistance

Discernment of morphological characters of plants conferring resistance to insect pests is important in breeding for resistance. Morphological basis of resistance include factors such as colour and shape of plant that influence orientation of the pest towards the plant. Singh (1978) reported that cowpea varieties with long upright peduncles that hold pods away from the canopy as well as from each other suffers less damage by legume pod borer. Oghiakhe *et al.* (1992) also observed a reduction in

pod damage in varieties with wide pod angle. This means that varieties with viny growth habit, especially yard long bean types having short peduncles and closely placed pods that are held within the canopy should suffer more damage than bush types. But in the present investigation, comparatively yard long bean types suffered less damage, whereas accessions with acute erect, erect, semi-erect, semi-prostrate, prostrate as well as climbing types with less foliage and shorter pods exposing them to pest attack were found to be more susceptible. This may be because yard long beans are native to the humid tropical climate of Kerala, while the short poded dual / grain types as well as bush vegetable cowpeas were introduced ones. It is a generally accepted fact that traditional landraces offer more resistance to pests and diseases than introduced genotypes.

It was also observed that most of the bush types, dual purpose and grain types started flowering a few days before yard long beans. Moreover the pod set in yard long beans is comparatively lesser especially in rainy season. Hence, by the time yard long beans start flowering and bear pods, oviposition and pest build up might have occurred in the other group. As a result, pest population will be more concentrated in this group and the yard long beans escape from heavy pest incidence. It was also observed that webbing together of pods, a typical symptom of heavy incidence of pod borer was absent among yard long beans. Hence it is suggested that screening for pod borer resistance is to be conducted as a separate experiment, with yard long bean and other types raised separately in distant plots, so that pest incidence in one does not lead to escapism of the other.

Role of anatomical and biochemical characters in legume pod borer resistance

Anatomical characters that influence pod borer resistance include presence or absence of pubescence and type of cuticle waxes that affect oviposition, locomotion or feeding by insects, tissue toughness that influence feeding and such other characters that impede host feeding and / or utilization by insect pests. Pubescence on plant surfaces is made up of individual trichomes or hairs. When pubescence is

present, the mechanism of resistance may depend upon one or more of the four characteristics of trichomes namely, their density, erectness, length and shape. Moreover, some trichomes also possess glands (glandular trichomes), the exudates of which contain phenol and alkaloids which can enhance the biochemical defence against insects (Oghiakhe *et al.*, 1992).

Biochemical characters that can influence legume pod borer resistance include phenolic content and pigments like chlorophyll content of leaves.

In the present study, an attempt has been made to evaluate the role of various anatomical and biochemical characters on legume pod borer resistance. Anatomical characters studied include glandular, non-glandular and total trichome density, stomatal density, vascular bundle thickness and cuticle thickness, whereas biochemical characters include phenol, proline and chlorophyll contents. Analysis of variance revealed significant differences among the accessions for all characters, except chlorophyll content.

Correlation studies revealed that both trichome density (glandular, non-glandular and total) as well as phenol content were negatively correlated with plant susceptibility index. Similar findings were also reported by Oghiakhe *et al.* (1992). Pubescence (trichomes) on cowpea pods affect oviposition, mobility and food consumption by the borer (Oghiakhe, 1995). Veeranna (1998) recorded higher phenol content in legume pod borer tolerant genotypes of cowpea than susceptible ones.

Phenol content in turn was positively associated with trichome density (glandular, non-glandular and total), vascular bundle thickness and proline content. Correlation between phenolics and trichome density may be due to high concentration of phenolics produced from the glandular trichomes. High negative association was observed between proline content and stomatal density. Proline concentration in plants varies in response to stress. As leaf stomatal density increases, the plant will be more exposed to environmental stress, leading to increased proline production.

5.9 Organoleptic analysis

A preliminary study was conducted to assess the organoleptic quality of the 66 accessions at vegetable maturity stage or at harvest with respect to appearance / colour, doneness, flavour, taste, texture and overall acceptability by a four point scale.

The overall acceptability ranged from 2.00 to 3.60 in vegetable types and from 2.40 to 3.60 in dual purpose and grain types, with only slight difference among accessions. Organoleptic analysis of vegetable cowpea were also done earlier by several workers (Umaharan *et al.*, 1997 ; Negri *et al.*, 2001).

But flavour, taste, nutrient content as well as overall acceptability of cowpea pods changes with stage of harvest, as reported by Omueti *et al.* (1986). Hence based on organoleptic analysis, the stage of harvest of pods is to be standardized first for each accession separately and then comparison may be done between accessions.

5.10 Seed protein electrophoresis

Frequently, morphological variation between cultivars within a plant species are so unclear that it is difficult to distinguish different cultivars. Morphological description of plant cultivars often pose problems in clear cut identification, because the phenotypic difference between species may be too minute to discriminate (Wilkinson and Beard, 1972). Differences can be measured by comparing the product of gene activity, i.e., by using proteins as genotype markers.

In cowpea, a number of landraces and cultivars are available with almost same characteristics. Therefore it becomes important to develop electrophoretic techniques to distinguish closely related cultivars / landraces. The ability to characterize cowpea seed proteins of various landraces and to select the most diverse types for breeding purpose may be useful for cowpea breeders. Hence a study was conducted to characterize seed proteins of 66 accessions of cowpea using SDS-PAGE. Similar studies were also done by Kalloo *et al.* (2001).

The study revealed the presence of 15 polypeptide bands over a molecular weight range of 20 to 97.4 kDa. There was difference in the number of bands

between accessions. Three major bands of 97.4 kDa, 43 kDa and 29 kDa were common to almost all accessions excepting VS 41 and VS 42. Polymorphism among yard long beans was very much limited when compared to dual purpose and grain type cowpea. While all the polypeptide bands were present in yard long beans, some minor bands were absent in dual purpose and grain types. The variation was observed in the absence of a few minor bands lying in the zone above 97.4 kDa and 43 kDa. The result shows that discrimination of single variety from others will be quite difficult because of the presence of large number of polypeptides and variation in major bands is very much limited. But by using a combination of band number and band location, a satisfactory discrimination of accessions may be done. Sobha (1994) reported a trend of changing pod length to number of protein bands and a least number of two bands were observed for shorter pods in bush type vegetable cowpea.

The variation in electrophoretic pattern suggested that molecular traits regardless of morphological characters might have been influenced by evolutionary process particularly by random drift during domestication and subsequent dispersion. Identification of seed proteins as well as their genetic variability may prove useful in breeding for improved protein quality and quantity in cowpea.

5.11 Molecular characterization based on RAPD

Morphological characters especially quantitative characters are subjected to environmental variation. This results in unreproducible phenotypic expression of polygenic traits. In order to obtain stable data, it is often needed to conduct multilocational trial over environment and years. The development of molecular marker technique has provided a direct method to estimate genetic diversity within and among populations.

Molecular marker possess ideal characteristics, since they analyze genetic diversity at the DNA level, and are available in an almost unlimited number. Random amplified polymorphic DNA (RAPD) is generated by polymerase chain reaction (PCR) with single short oligonucleotides of arbitrary sequence and provides genetic

information at the DNA level with relative ease. It can be used to evaluate intraspecific variation (Williams *et al.*, 1990). In the present investigation, RAPD based DNA fingerprinting of selected 50 accessions of cowpea was carried out for precise identification, and polymorphism observed was analyzed to assess the genetic variability among them.

DNA fingerprinting

A total of 55 amplification products or DNA bands were generated by eight decamer primers, of which 46 were polymorphic. The number of bands for the various primers ranged from five (OPK 7 and OPK 8) to ten (OPL 12). Five primers namely, OPH 17, OPH 18, OPK 7, OPL 12 and OPL 13 were used for amplifying DNA from all the accessions. Nine to fourteen scorable bands (total 58 RAPD) were produced, of which 94.8 per cent (55 RAPD) were polymorphic. While several polymorphic bands were observed among the accessions, some of them were specific to certain accessions. The variety Malika (VS 32) produced a specific marker of about 3000 bp using OPH 18. These specific markers will aid in unambiguous identification of the accessions and to maintain their seed purity. It was observed that among the accessions, vegetable types shared more number of monomorphic bands compared to dual and grain types. This shows lesser genetic divergence within the former group.

The genetic similarity analysis showed considerable variability among the accessions. Analysis of genetic diversity in cowpea by RAPD technique was also done by Shim *et al.* (2001) and Fall *et al.* (2003), who suggested that RAPD technique can be used to reorganize the national germplasm, eliminating the putative duplicates and to identify elite varieties. In the present study, similarity coefficient ranged from 0.20 to 0.97. This means that no two accessions are exactly identical. The most divergent accessions were VS 27 and VS 44 (0.20), and were also morphologically different. VS 27 is a climbing type with light green long pods, while VS 44 is also climbing, but with short purple pods. The most similar accessions, VS 1

and VS 68 (0.97) are morphologically different, but they are quite similar being very low yielders. In cowpea, Nkongolo (2003) also reported a general lack of agreement between clustering based on RAPD and morphological features.

At 38.8 per cent similarity, the 50 accessions could be grouped into two large clusters. The first cluster includes VS 23, VS 26, VS 25, VS 32, VS 29, VS 28, VS 27 and VS 24, while the rest of the accessions grouped into the second cluster. The first cluster is similar to morphological clustering where the eight accessions come together in two separate clusters. At 66 per cent similarity, these eight accessions along with VS 22 got split up into nine different clusters. This means that even though the accessions are morphologically similar, they show genetic variation at the molecular level.

At 66 per cent similarity, the 50 accessions were grouped into eleven clusters. The first nine clusters contain single accession each as mentioned earlier, while the tenth cluster includes the accessions VS 61, VS 62 and VS 63. These three accessions were also grouped together in morphological clustering. The rest of the accessions form one large cluster, i.e., the eleventh cluster. The accessions coming under it are morphologically quite different from each other, suggesting the need to include more primers to discriminate different morphotypes among each other. It should be noted that all the dual purpose and grain types come under it just as in the case of clustering based on D^2 analysis. The cluster includes yard long beans, bush type vegetable cowpea, dual purpose and grain types. This shows that all these types are having a common genetic background, and during the process of evolution and domestication, slight changes might have occurred in their genetic make up which finally resulted in morphologically divergent genotypes. In the eleventh cluster itself, smaller groups can be noticed among the accessions, with most of the yard long beans coming together and the dual purpose and grain types forming separate groups. For example, the accessions VS 5 and VS 12 which are bush type vegetable cowpea form one separate cluster.

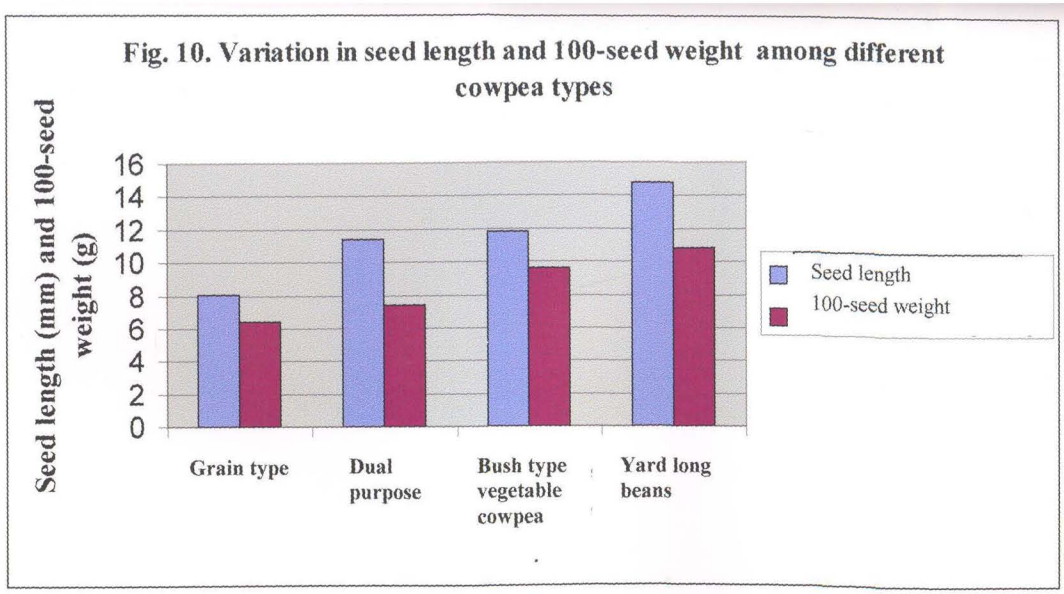
Based on Mahalanobi's D^2 statistic, cluster X which included VS 19 was found to be the most divergent one showing the highest value of intercluster distance. Even though morphologically distinct from others, it was grouped along with other yard long beans at the molecular level. Considering the similarity index values, VS 24 and VS 27 were found to be the most divergent from the rest of the accessions. Both are comparatively good yielders, but VS 27 stands first in discriminant function analysis. Based on the information of genetic diversity observed in the present investigation, selection of parents may be done for crossing programme. More precise data could be obtained by further detailed investigation using RAPD or other molecular markers.

The RAPD technique used in the study has been useful in finding out the genetic relationship between various accessions of cultivated cowpea. The RAPD profile can be used for the identification of these landraces and to supplement traditional methods of classification at the species and subspecies level. The method may also serve as a useful technique to help parental selection in plant breeding.

To recapitulate the discussion in terms of origin, evolution and adaptation of different landraces of edible cowpea, seed length and 100-seed weight deserve special mention (Fig. 10). The present study confirmed that seed length and 100-seed weight was found to be lowest among grain types (8.01 mm and 6.43g respectively), and reached the highest value (14.47 mm and 10.33 g respectively) in yard long beans, through a gradual increase *via* dual purpose and bush type vegetable cowpeas. Steele (1979) reported small seed size and seed weight as primitive characters in cowpea. This gives an indication that grain type cowpea was domesticated first and yard long beans, the advanced form through human selection and adaptation. The dual purpose and bush type vegetable cowpea may be considered as intermediate forms.

The theory of origin and spread of cowpea also pointed out that the subsp. *unguiculata* evolved in Africa and distributed to Asia, especially India, where the subspecies *cylindrica* and *sesquipedalis* got established through conscious human

Fig. 10. Variation in seed length and 100-seed weight among different cowpea types



selection (Steele, 1979). Neema (1986) reported a change in chromosome number from 2n = 22 in *unguiculata* to 2n = 24 in *sesquipedalis*, which also supports the advanced nature of *sesquipedalis*. Further, the RAPD profile obtained in the present study showed more number of common bands and less variability in yard long beans compared to dual / grain types, indicating that the subspecies is a more advanced one.

It is also suggested that these observations need to be supplemented with detailed karyotype analysis supported by further confirmation using more reliable molecular markers like RFLP, AFLP, microsatellites etc.

Summary

6. SUMMARY

The present investigation on "Characterization of vegetable cowpea (*Vigna unguiculata* (L.) Walp.)" was conducted at the Department of Olericulture and Department of Plant Biotechnology, College of Agriculture, Vellayani, during the period 2002 to 2005.

The study envisaged genetic cataloguing of the available germplasm of vegetable cowpea in Kerala, assessment of genetic variability at morphological and molecular levels. Based on morphology, association among the characters including direct and indirect effects of various characters on yield and formulation of a selection index for identifying suitable lines have been attempted. Molecular characterization included studying the variability based on protein markers as well as RAPD based DNA markers.

The experimental material which consisted of 66 accessions of vegetable cowpea collected from different parts of Kerala was laid out in randomized block design with two replications. The accessions were genetically catalogued based on the descriptor list for cowpea proposed by IPGRI. The results revealed distinct variations among the accessions with respect to vegetative, inflorescence, fruit and seed characters.

Significant differences were observed among the accessions for all the biometric characters studied *viz.*, growth, flowering, pod, yield and quality characters. Among the accessions evaluated, VS 8 (CHCP-1) had the highest yield (1136.89 g) and pods per plant (102.59), while VS 19 (Aryanad, Thiruvananthapuram) was noted for its extremely long pods (76.08 cm), pod girth (4.43 cm), vine length (6.17 m), 100-seed weight (20.77 g), and seed length (13.03 mm). The highest pod weight (43.60 g) was observed by VS 4 (Kanjikuzhi Payar) which also recorded the highest number of seeds per pod (21.34).

Considering the genetic parameters, high phenotypic (PCV) and genotypic (GCV) coefficients of variation were observed for most of the characters. Root :

shoot ratio had the highest PCV and GCV, followed by pod weight and yield per plant. The lowest PCV and GCV were exhibited by pollen viability and days to first flowering. High heritability coupled with high genetic advance was observed for yield per plant, pods per plant, pod length and pod weight, indicating scope for improvement of these characters through selection.

Correlation studies revealed that characters like vine length, collar girth, pod length, pod girth, pod weight, pods per plant, seeds per pod, 100-seed weight, seed length, seed width, number of harvests, keeping quality and pod protein observed high positive correlation with yield. On the other hand, root : shoot ratio and peduncle length were negatively correlated with yield and yield component characters.

Path coefficient analysis, selection index and Mahalanobi's D^2 analysis were worked out based on the characters vine length, collar girth, root : shoot ratio, peduncle length, pod length, pod girth, pod weight, pods per plant, seeds per pod, 100-seed weight, seed length, seed width, number of harvests, keeping quality, pod protein and yield per plant. Path coefficient analysis indicated that pods per plant exerted the highest positive direct effect on yield, followed by seed length, pod weight and vine length. The indirect effects through seed length, pod weight and vine length were also high signifying the importance of these characters.

A discriminant function analysis was carried out for isolating superior accessions of vegetable cowpea based on high yield and quality. Upon ranking the selection index scores obtained, the accession VS 27 (Pattom, Thiruvananthapuram) ranked first, followed by VS 8 (CHCP-1) and VS 19 (Aryanad, Thiruvananthapuram).

Based on Mahalanobi's D^2 statistic, the 66 accessions were grouped into ten clusters. Cluster I was the largest containing 18 accessions, while cluster X was the smallest with two accessions. Considering the cluster means, cluster X comprising VS 4 and VS 19 performed better for most of the biometric characters maintaining high intercluster distance with all other clusters, the highest being with cluster VI.

Screening of all the 66 accessions for legume pod borer resistance was done by working out plant susceptibility indices based on flower, pod and seed damage parameters. VS 19 (Aryanad, Thiruvananthapuram) was the most tolerant with least damage to flowers, pods and seeds, while VS 42 (Pilicode, Kasargode) was the most susceptible. Correlation studies revealed that all the damage parameters were correlated among each other. Moreover, pod trichome density and phenol content were negatively correlated with plant susceptibility index.

On comparing the accessions for various characters, VS 27, VS 8 and VS 19 were found to be promising based on their superiority in yield, quality and tolerance to legume pod borer and hence they may be utilized for further crop improvement programmes.

A study was conducted to assess the organoleptic quality and overall acceptability of all the accessions based on appearance / colour, doneness, taste, flavour and texture. The overall acceptability ranged from 2.00 to 3.60 among vegetable types and 2.40 to 3.60 among dual purpose / grain types. Short poded accessions at their vegetable maturity stage had better overall accepatility.

Characterization of vegetable cowpea based on seed protein banding pattern revealed the presence of three major bands common to all the accessions and variation in minor bands which can be utilized to discriminate accessions among each other. Molecular characterization based on RAPD signified the existence of variability far beyond what was observed morphologically. Similarity coefficient ranged from 0.20 to 0.97, based on which a dendrogram was constructed. At 66 per cent similarity, the selected 50 accessions were grouped into eleven clusters. The technique has been useful to understand the relationship among the various accessions and can serve as a tool for DNA fingerprinting as well as parental selection for further crop improvement in vegetable cowpea.

The study also gives an indication that grain type cowpeas were the first which was domesticated from its wild ancestor and the yard long beans the most advanced, with the dual purpose and bush type vegetable cowpea as intermediate forms.

References

7. REFERENCES

Afzal, M. A., Haque, M. M. and Shanmugasundaram, S. 2004. Random amplified polymorphic DNA (RAPD) analysis of selected mung bean (*Vigna radiata* (L.) Wiczek) cultivars. *Asian J. Pl. Sci.* 3 : 20-24

Aghora, T. S., Mohan, N. and Somkumar, R. G. 1994. Evaluation of vegetable cowpea (*Vigna unguiculata* L. Walp.) for earliness, protein content and green pod yield. *Leg. Res.* 17 : 138-140

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, p.64

Allard, R.W. 1960. *Principles of Plant Breeding*. John Wiley and Sons, Inc., New York, p.485

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

Anbuselvam, Y., Manivannan, N., Saravanan, K. and Ganesan, J. 2000. Studies on genetic divergence in cowpea (*Vigna unguiculata* L. Walp.). *Madras agric. J.* 87 : 343-345

Angadi, S. P., Subramani, A. and Kulkarni, R. S. 1978. Genetic variability for some quantitative traits in cowpea. *Agric. Res. J. Kerala* 16 : 60-62

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

Asante, I. K., Dapaah, A. H. and Addison, P. 2004. Seed weight, protein and tannin contents of 32 cowpea accessions in Ghana. *Trop. Sci.* 44 : 77-79

Astley, D. 1992. Preservation of genetic diversity and accession integrity. *Fld Crops Res.* 219 : 205-224

Awolumate, E. O. 1983. Accumulation and quality of storage protein in developing cowpea, mung bean and soyabean seeds. *J. Sci. Fd Agric.* 34 : 1351-1357

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 : 539-550

Backiyarani, S., Nadarajan, S., Rajendran, C. and Shanthi, S. 2000. Genetic divergence for physiological traits in cowpea (*Vigna unguiculata* (L.) Walp.). *Leg. Res.* 23 : 114-117

Bapna, C. S. and Joshi, S. N. 1973. A study on variability following hybridization in *Vigna sinensis* (L.) Savi. *Madras agric. J.* 60 : 1369-1372

Borah, H. K. and Khan, A. K. F. 2000. Variability, heritability and genetic advance in fodder cowpea. *Madras agric. J.* 87 : 165-166

Borah, H. K. and Khan, A. K. F. 2001. Genetic divergence in fodder cowpea (*Vigna unguiculata* (L.) Walp.). *Madras agric. J.* 88 : 625-628

Bretting, P. K. and Widerlechner, M. P. 1995. Genetic markers and horticultural germplasm management. *HortScience* 30 : 1349-1356

Chikkadyavaiah, A. 1985. Genetic divergence in cowpea (*Vigna unguiculata* (L.) Walp.). *Mysore J. agric. Sci.* 19 : 131-132

Choulwar, S. B. and Borikar, S. T. 1987. Path analysis in M_3 generation of cowpea. *J. Maharashtra agric. Univ.* 12 : 74-75

DeMooy, B. E. 1985. Variability of different characteristics of Botswana cowpea germplasm. *Trop. Grain Leg. Bull.* 31 : 1-4

Dent, D. R. 1995. *Integrated Pest Management.* Chapman and Hall, London, p.356

Ebong, U. U. 1970. A classification of cowpea varieties (*Vigna sinensis* Endl.) in Nigeria into subspecies and groups. *Nigerian agric. J.* 7 : 5-18

Erlich, H. A., Gelfand, D. and Srinsky, G. 1991. Recent advances in the polymerase chain reaction. *Science* 252 : 1643-1651

Fall, L., Diouf, D., Ndiaye, F., Badiane, F. A. and Gueye, M. 2003. Genetic diversity in cowpea (*Vigna unguiculata* (L.) Walp.) varieties determined by ARA and RAPD techniques. *Afr. J. Biotech.* 2 : 48-50

Fisher, R.H. 1936. The use of multiple measurement in taxonomic problems. *Ann. Eugen.* 7 : 179-188

Geetha, V. 2004. Shade response and nutrient requirement of common rainfed intercrops of coconut. Ph. D. thesis, Kerala Agricultural University, Thrissur, p.221

Ghimiray, T. S. and Das, P. K. 1996. Stomatal characters and diurnal variation in two cultigroups of cowpea (*Vigna unguiculata* (L.) Walp.). *Environ. Ecol.* 14 : 86-88

Gopalakrishnan, T. R. 2004. *Three Decades of Vegetable Research in Kerala Agricultural University.* Kerala Agricultural University, Vellanikkara, Thrissur, p.152

Gupta, R. S. P. C. and Pradhan, K. 1974. Variability in protein, cell-wall constituents and in vitro dry matter and cell-wall digestibility of some promising cowpea strains (*Vigna sinensis*). *Haryana agric. Univ. J. Res.* 4 : 309-313

Hanchinal, R. R., Habib, A. F. and Goud, J. V. 1979. Correlation and path analysis in cowpea (*Vigna unguiculata* (L.) Walp.). *Mysore J. agric. Sci.* 8 : 253-257

Hanson, C.H., Robinson, H.F. and Comstock, R.E. 1956. Biometrical studies on yield in segregating population of Korean lespedesa. *Agron. J.* 48 : 268-272

Hazra, P., Bhattacharya, C., Mandal, A. R. and Som, M. G. 1988. Heterosis in stomatal characters in three subspecies of cultivated cowpea (*Vigna unguiculata* (L.) Walp.). *Exp. Genet.* 4 : 28-34

Hazra, P., Das, P. K. and Som, M. G. 1993. Characterization of the three cultigroups of cowpea (*Vigna unguiculata* (L.) Walp). *Crop Res.* 6 : 465-474

Hazra, P., Das, P. K. and Som, M. G. 1996. Variability in stomatal frequency, length and breadth in cowpea (*Vigna unguiculata* (L.) Walp.). *Crop Res.* 11 : 78-83

Hiscox, J. D. and Israelstam, G. F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration using dimethyl sulfoxide. *Can. J. Bot.* 57 : 1332-1334

Hussain, A., Ramirez, H., Bushok, W. and Roca, W. 1986. Field bean (*Phaseolus vulgaris* L.) cultivar identification by electrophoregrams of cotyledon storage proteins. *Euphytica* 35 : 729-732

Hussain, A., Ramirez, H., Roca, W. and Bushok, W. 1988. Interaccession variation of electrophoregrams of cotyledon proteins of field beans (*Phaseolus vulgaris* L.). *Euphytica* 39 : 109-111

IBPGR. 1983. *Descriptors for cowpea.* International Plant Genetic Resources Institute, IPGRI Secretariat, Rome, p.82

Iqbal, M. S., Ghafoor, A., Qureshi, A. S. and Ahmad, Z. 2003. Genetic dissimilarities in cowpea (*Vigna unguiculata* (L.) Walp.) for protein peptides and their significance for quantitative trait loci. *Pakist. J. Bot.* 35 : 377-386

Iwasaki, T., Shibuya, N., Suzuki, T. and Chikubu, S. 1982. Gel filtration and electrophoresis of soluble rice protein extracted from long medium and short-grain varieties. *Cereal Chem.* 59 : 192-195

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. *Advances in Cowpea Research.* (eds. Singh, B. B., Mohanraj, D. R., Dashiell, K. E. and Jackai, L. E. N.). IITA, Nigeria, pp.240-258

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

Jackai, L. E. N., Padulosi, S. and Ng, Q. 1996. Resistance to the legume pod norer, *Maruca vitrata* Fabricius, and the probable modalities involved in wild *Vigna*. *Crop Prot.* 15 : 753-761

Jalajakumari, M. B. 1981. Variability studies in cowpea. M. Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, p.98

Johnson, H.W., Robinson, H.F. and Comstock, R.E. 1955. Estimation of genetic and environmental variability in soybeans. *Agron. J.* 47 : 314-318

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

Kalaiyarasi, R. and Palanisamy, G. A. 2001. A study on character association and path analysis in F_4 generation of cowpea (*Vigna unguiculata* (L.) Walp.). *Leg. Res.* 24 : 36-39

Kalloo, G., Singh, M., Chakraborty, S., Singh, P. M., Singh, A. K. and Banerjee, M. K. 2001. Seed protein electrophoresis for varietal identification of cowpea (*Vigna unguiculata* (L.) Walp.). *Seed Res.* 29 : 1-6

Kapoor, A., Sahoo, M. S. and Beri, S. M. 2000. Divergence in cowpea (*Vigna unguiculata* (L.) Walp.). *Crop Improv.* 27 : 105-108

Kar, N., Dasgupta, T., Hazra, P. and Som, M. G. 1995. Association of pod yield and its components in vegetable cowpea. *Indian Agricst* 39 : 231-238

Kempthorne, O. 1977. *An Introduction to Genetic Statistics.* Jagmander Book Agency, New Delhi, p.221

Kerala Agricultural University. 2002. *Package of Practices Recommendations : Crops.* 12th edition. Kerala Agricultural University, Thrissur, p.278

Kochhar, N., Walker, A. F. and Pike, D. J. 1988. Effect of variety on protein content, amino acid composition and trypsin inhibitor activity of cowpeas. *Fd Chem.* 29 : 65-78

Kumar, A., Misra, S. N. and Verma, J. S. 1982. Studies on genetic diversity in cowpea. *Crop Improv.* 9 : 160-163

Kumar, A., Misra, S. N. and Verma, J. S. 1983. Correlation and path analysis in cowpea. *Crop Improv.* 10 : 36-39

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

Kumar, R., Sangwan, R. S. and Luthra, Y. P. 2002. Variability, heritability, genetic advance and association analysis for biochemical traits in cowpea (*Vigna unguiculata* (L.) Walp.). *Nat. J. Pl. Improv.* 4 : 69-70

Kumari, V., Arora, R, N. and Singh, J. V. 2003. Variability and path analysis in grain cowpea. *Proceedings of National Symposium on Arid Legumes for Food, Nutrition Security and Promotion of Trade, May 15-16, 2002* (eds. Henrya, A. and Kumar, D.). Scientific Publishers, Jodhpur, pp. 59-62

Kutty, C. N., Mili, R. and Jaikumaran, U. 2003. Correlation and path coefficient analysis in vegetable cowpea (*Vigna unguiculata* (L.) Walp.). *Indian J. Hort.* 60 : 257-261

Kutty, C. N., Mili, R. and Jaikumaran, U. 2003. Variability and genetic advance in vegetable cowpea. *J. Maharashtra agric. Univ.* 28 : 26-29

Ladizinsky, G. and Hymowitz, T. 1979. Seed protein electrophoresis in taxonomic and evolutionary studies. *Theor. appl. Genet.* 54 : 145-151

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature* 227 : 680-685

Lakshmi, P. V. and Goud, J. V. 1977. Variability in cowpea (*Vigna sinensis* L.). *Mysore J. agric. Sci.* 11 : 144-147

Lanham, P. G., Forster, B. P., McNicol, J. P. and Powell, M. W. 1994. Seed storage protein variation in Arachis species. *Genome* 37 : 487-496

Lee, S. J., Shin, J. S., Park, K. W. and Hong, Y. P. 1996. Detection of genetic diversity using RAPD – PCR and sugar analysis in watermelon (*Citrullus lanatus* (Thunb.) Mansf.) germplasm. *Theor appl. Genet.* 92 : 719-725

Lovely, B. 2005. Genetic analysis of yield and mosaic resistance in yard long bean [*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdc.]. Ph. D thesis, Kerala Agricultural University, Thrissur, p.147

Lush, J.L. 1949. *Animal Breeding Plans.* Iowa State University Press, Annes, p. 473

Macfoy, C. 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. *Insect Sci. Appl.* 4 : 147-152

Magoon, M. L., Singh, A. and Mehra, K. L. 1973. Gene pool helps in improving fodder crops. *Indian Fmg* 23 : 5-7

Matysik, J., Alia, Bhalu, B. and Mohanty, P. 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr. Sci.* 82 : 525-532

McDaniel, R. G. 1970. Electrophoretic characterization of proteins in Hordeum. *J. Hered.* 61 : 243-247

Mignouna, H. D., Ng, N. Q., Ikea, J. and Thottapilly, G. 1998. Genetic diversity in cowpea as revealed by random amplified polymorphic DNA. *J. Genet. Breed.* 52 : 151-159

Miller, P.A., Williams, V.C., Robinson, H.P. and Comstock, R.E. 1958. Estimation of genotypic and environmental variances and covariance in upland cotton and their implication in selection. *Agron. J.* 5 : 126-131

Mimura, M., Yasuda, K. and Yamaguchi, H. 2000. RAPD variation in wild, weedy and cultivated azuki beans in Asia. *Genet. Resour. Crop Evol.* 47 : 603-610

Misra, N. M. and Kar, R. K. 1990. Regulation of storage proteindegradation in cotyledons of germinating cowpea. *Indian J. Pl. Physiol.* 33 : 333-339

Moustakas, M., Symeonidis, L. and Coucoli, H. 1986. Seed protein electrophoresis in *Agropyron junceum* (L.) P. B. Complex. *Ann. Bot.* 57 : 35-40

Murray, M. and Thompson, W. 1980. The isolation of high molecular weight plant DNA. *Nucl. Acids Res.* 8 : 4321-4325

Neema, V. P. 1986. Genomic relationship in *Vigna* sp. M. Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, p.61

Neema, V. P. and Palanisamy, G. A. 2003. Character association in F_2 population of cowpea (*Vigna unguiculata* (L.) Walp.). *Leg. Res.* 26 : 146-148

Negri, V., Floridi, S. and Montanari, L. 2001. Organoleptic and chemical evaluation of Italian cowpea (*Vigna unguiculata* subsp. *unguiculata* cv gr. *unguiculata* (L.) Walp.) landraces from a restricted area. *Italian J. Fd Sci.* 13 : 383-390

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

Nkongolo, K. K. 2003. Genetic characterization of Malawian cowpea (*Vigna unguiculata* (L.) Walp) landraces : diversity and gene flow among accessions. *Euphytica* 129 : 219-228

Odeigah, P. G. C. and Osanyinpeju, A. O. 1996. Seed protein electrophoresis of cowpea (*Vigna unguiculata*) germplasm from IITA gene bank. *Genetic Resour. Crop Evol.* 43 : 485-491

Ogbonnaya, C. I., Sarr, B., Brou, C., Diouf, O., Diop, N. N. and Macauley, H. R. 2003. Selection of cowpea genotypes in hydroponics, pots and field for drought tolerance. *Crop Sci.* 43 : 1114-1120

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. 1997. Trichomes and resistance to major insect pests in cowpea (*Vigna unguiculata* (L.) Walp.) : a review. *Discov. Innovat.* 9 : 173-178

Oghiakhe, S., Cook, R. J. and Jackai, L. E. N. 1993. The characterization of cultivars of cowpeas *Vigna unguiculata* (L.) Walp and *Vigna vexillata* Benth by electrophoresis of seed proteins. *Trop. Agric.* 70 : 190-192

Oghiakhe, S., Jackai, L. E. N. and Makanjuola, W. A. 1991. Cowpea plant architecture in relation to infestation and damage by legume pod borer, *Maruca testulalis* Geyer (Lepidoptera : Pyralidae) : 1. Effect of canopy structure and pod position. *Insect Sci. Appl.* 12 : 193-200

Oghiakhe, S., Jackai, L. E. N. and Makanjuola, W. A. 1992a. Cowpea plant architecture in relation to infestation and damage by legume pod borer, *Maruca testulalis* Geyer (Lepidoptera : Pyralidae) : 2. Effect of pod angle. *Insect Sci. Appl.* 13 : 339-344

Oghiakhe, S., Jackai, L. E. N. and Makanjuola, W. A. 1992b. Pod wall toughness has no effect on cowpea resistance to the legume pod borer *Maruca testulalis* Geyer (Lepidoptera : Pyralidae). *Insect Sci. Appl.* 13 : 345-349

Oghiakhe, S., Jackai, L. E. N., Hodgson, C. J. and Ng, Q. 1993. Anatomical and biochemical parameters of resistance of the wild cowpea, *Vigna vexillata* Benth (Acc. TVNu 72) to *Maruca testulalis* Geyer (Lepidoptera : Pyralidae). *Int. J. Pest Mgmt* 39 : 261-264

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

Oghiakhe, S., Makanjuola, W. A. and Jackai, L. E. N. 1993. 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 Mgmt* 39 : 261-264

Omueti, O., Ojomo, O. A., Ogunyanwo, O. and Olafare, S. 1986. Biochemical components and other characteristics of maturing pods of vegetable cowpea (*Vigna unguiculata*). *Exp. Agric.* 22 : 25-32

Osanyinpeju, A. O. and Odeigah, P. G. C. 1998. Variation in seed proteins from mutagen-treated cultivars and selected lines of *Vigna unguiculata* L. Walp. *Pl. Breed.* 117 : 361-365

Padi, F. A. 2003. Genetic analysis of pigmentation in cowpea. *Pakist. J. Biol. Sci.* 6 : 1655-1659

Pal, A. K., Maurya, A. N., Singh, B., Ram, D. and Kumar, S. 2003. Genetic variability, heritability and genetic advance in cowpea (*Vigna unguiculata* (L.) Walp). *Orissa J. Hort.* 31 : 94-97

Pandey, R. N., Dhanasekar, P. and Souframanien, J. 2004. RAPD based DNA fingerprinting and analysis of genetic diversity in radiation induced mutants of cowpea (*Vigna unguiculata* (L.) Walp.). *Indian J. Genet.* 64 : 97-101

Pandita, M. L., Vashistha, R. N., Bhutani, R. D. and Batra, B. R. 1982. Genetic variability studies in cowpea (*Vigna sinensis* L. Savi) under dry farming conditions. *Haryana agric. Univ. J. Res.* 12 : 241-245

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, p.92

Panse, V.G. and Sukhatme, P.V. 1967. *Statistical Methods for Agricultural Workers.* Indian Council of Agricultural Research, New Delhi, p.347

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

Patil, R. B. and Bhapkar, D. G. 1987. Correlation studies in cowpea. *J. Maharashtra agric. Univ.* 12 : 56-59

Patil, S. B. and Patil, R. B. 1987. Genetics of some quantitative characters in cowpea. *Curr. Res. Rep.* 3 : 5-10

Pekoen, E. and Artuk, C. 2004. Comparison of some cowpea (*Vigna unguiculata* (L.) Walp.) genotypes from Turkey for seed yield and yield related characters. *J. Agron.* 3 : 137-140

Peksen, A., Peksen, E. and Bozoglu, H. 2002. Effects of sowing dates on yield and quality of cowpea (*Vigna unguiculata* L. Walp.) genotypes grown in greenhouse. *Acta Hort.* 579 : 351-354

Philip, M. C. A. 2004. Genetic analysis of legume pod borer (*Maruca vitrata* (Fab.)) resistance and yield in cowpea (*Vigna unguiculata* (L.) Walp). Ph. D. thesis, Kerala Agricultural University, Thrissur, p.171

Prasad, D. T., Umapathy, N. S. and Veeranna, R. 1996. Genotypic variation in cowpea (*Vigna unguiculata*) cultivars in relation to insect resistance. *J. Pl. Biochem. Biotech.* 5 : 47-49

Purseglove, J. W. 1974. *Tropical Crops Dicotyledons*. Longman Group Ltd., Essex, p. 719

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

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

Rangaiah, S. and Mahadevu, P. 2000. Genetic variability, correlation and path co-efficient analysis in cowpea (*Vigna unguiculata* (L.) Walp.). *Madras agric. J.* 86 : 381-384

Rao, C.R. 1952. *Advanced Statistical Methods in Biometric Research.* John Wiley and Sons, New York, p.390

Rao, R., Vaglio, D., Del, M., Urzo, P. D. and Monti, L. 1992. Identification of *Vigna* spp. through specific seed storage polypeptides. *Euphytica* 62 : 39-43

Resmi, P. S. 1998. Genetic variability of yard long bean *Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt). M. Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, p. 93

Robinson, H.F., Comstock, R.E. and Harvey, P.H. 1949. Estimation of heritability and the degree of dominance in corn. *Agron. J.* 14 : 352-359

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

Roychoudhury, B., Ghosh, S., Mandal, N. and Dasgupta, T. 2005. Genetic diversity in some wild and cultivated germplasms in *Vigna* by RAPD marker. *Proceedings of National Symposium on Biotechnological Interventions for Improvement of Horticultural Crops : Issues and Strategies,* January 10-12, 2005 (eds. Rao, G. S. L. H. V. P., Nazeem, P. A., Girija, D., Kesavachandran, R., Joseph, L. and John, P. S.). Kerala Agricultural University, Thrissur, pp. 239-242

Sadasivam, S. and Manickam, A. 1992. *Biochemical Methods for Agricultural Sciences.* Wiley Eastern Limited, New Delhi, p.246

Shim, J. H., Chung, J. I. and Go, M. S. 2001. Construction of molecular genetic linkage map using RAPD markers in cowpea. *Korean J. Crop Sci.* 46 : 341-343

Sindhu, A. R. 2001. Biochemical basis of resistance against black eye cowpea mosaic virus in cowpea (*Vigna unguiculata* (L.) Walp.). M. Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, p.65

Singh, B. B. and Dapaah, H. K. A. 1998. A partial male sterile mutant in cowpea. *Afr. Crop Sci. J.* 6 : 97-101

Singh, H. P., Singh, P. V. and Saxena, R. P. 1991. Identification of chick pea cultivar using PAGE of storage seed proteins. *Ann. Biol.* 72 : 167-175

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, R., Joshi, B. S. and Singh, S. 1982. Correlation studies in cowpea (*Vigna unguiculata* (L.) Walp). *Trop. Grain Leg. Bull.* 26 : 3-5

Singh, R.K. and Choudhary, B.D. 1985. *Biometrical Methods in Quantitative Genetic Analysis*. Kalyani Publishers, New Delhi, p.318

Singh, S. R. 1978. Resistance of pests of cowpea in Nigeria. *Pests of Grain Legumes : Ecology and Control*. (eds. Singh, S. R., van Embden, H. F. and Taylor, T. A.). Academic Press, London, pp. 267-280

Singh, S. R. and Jackai, L. E. N. 1988. The legume pod-borer, *Maruca testulalis* (Geyer) : past, present and future research. *Insect Sci. Appl.* 9 : 1-5

Smith, F.H. 1936. A discriminant function for plant selection. *Ann. Eugen.* 7 : 240-250

Sobha, P. P. 1994. Variability and heterosis in bush type vegetable cowpea. M. Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, p.123

Sobha, P. P. and Vahab, M. A. 1998. Genetic variability, heritability and genetic advance in cowpea (*Vigna unguiculata* (L.) Walp). *J. trop. Agri.* 36 : 21-23

Soller, M. and Beckmann, J. S. 1983.Genetic polymorphism in varietal identification and genetic improvement. *Theor appl. Genet.* 67 : 25-33

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

Sreelathakumary, I. 2000. Genetic analysis of shade tolerance in chilli (*Capsicum* spp.). Ph. D. thesis, Kerala Agricultural University, Thrissur, p.153

Staub, J. E., Robbins, M. D., Chung, S. and Lopez-Sese, A. I. 2004. Molecular methodologies for improved genetic diversity in cucumber and melon. *Acta Hort.* 637 : 41-47

Steele, W. M. 1979. Cowpeas. *Evolution of Crop Plants* (eds. Simmonds, N. W.). Longman Group Ltd., London, pp. 183-185

Subbiah, A., Anbu, S., Selvi, B. and Rajankam, J. 2003. Studies on the cause and effect relationship among the quantitative traits of vegetable cowpea (*Vigna unguiculata* (L.) Walp.). *Leg. Res.* 26 : 32-35

Swaminathan, M. 1974. *Diet and Nutrition in India : Essentials of Food and Nutrition Applied Aspects*. Ganesh and Company, Madras, p.367

Tayo, T. O. 1988. Flower and pod development in three cowpea (*Vigna unguiculata* (L.) Walp.) varieties with varying susceptibility to the pod-borer, *Maruca testulalis* (Geyer). *Insect Sci. Appl.* 9 : 249-253

Tikka, S. B. S., Jaimini, S. N., Asawa, B. M. and Mathur, J. R. 1977. Genetic variability, interrelationship and discriminant function analysis in cowpea (*Vigna unguiculata* (L.) Walp.). *Indian J. Hered.* 9 : 1-9

Tingey, W. M. 1986. Techniques for evaluating plant resistance to insects. *Insect –Plant Interactions* (eds. Miller, J. A. and Miller, T. A.). Springer-Verlag, New York, pp. 251-284

Trehan, K. B., Bagrecha, L. R. and Srivastava, V. K. 1970. Genetic variability and correlations in cowpea *Vigna sinensis* L. Savi under rainfed conditions. *Indian J. Hered.* 2 : 39-43

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

Uguru, M. I. 1996. A note on the Nigerian vegetable cowpea. *Genet. Resour. Crop Evol.* 43 : 125-128

Umaharan, P., Ariyanayagam, R. P. and Haque, S. Q. 1997. Genetic analysis of yield and its components in vegetable cowpea (*Vigna unguiculata* (L.) Walp.). *Euphytica* 96 : 207-213

Ushakumari, R., Backiyarani, S. and Dhanakodi, C. V. 2000. Character contribution to diversity in cowpea. *Leg. Res.* 23 : 122-125

Vaillancourt, R. E., Wedden, W. F. and Barnard, J. 1993. Isozyme diversity in the cowpea species complex. *Crop Sci.* 33 : 606-613

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

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

Veeraswamy, R., Palaniswamy, G. A. and Rathnaswamy, R. 1973. Genetic variability in some quantitative characters of *Vigna sinensis* (L.) Savi. *Madras agric. J.* 60 : 1359-1360

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

Venkatesan, M., Veeramani, N., Thangavel, P. and Ganesan, J. 2004. Genetic divergence in cowpea (*Vigna unguiculata* (L.) Walp.). *Leg. Res.* 27 : 223-225

Verdcourt, B. 1970. Studies on the Leguminosae – Papilionidae for the flora of East Tropical Africa. *Kew Bull.* 24 : 507-569

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

Welsh, J. and McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucl. Acids Res.* 18 : 7213-7218

Wilkinson, J. F. and Beard, J. B. 1972. Electrophoretic identification of *Agrostis palustris* and *Poa pratensis* cultivars. *Crop Sci.* 12 : 833-834

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

Wright, S. 1954. The interpretation of multivariate systems. *Statistics and Mathematics in Biology* (eds. Kempthorne, O., Bancroft, T.A., Gowen, J.W. and Lush, J.L.). State University Press, Iowa, pp. 11-13

Yap, T. C. 1983. Genetic studies and improvement of long bean (*Vigna unguiculata* (L.) Walp.) in Malaysia. *Crop Improv.* 10 : 295-302

Youn, S. J. and Chung, H. D. 1998. Genetic relationship among the local varieties of the Korean native squashes (*Cucurbita moschata*) using RAPD technique. *J. Korean Soc. hort. Sci.* 39 : 517-521

Appendices

APPENDIX-1

Score card for the organoleptic evaluation of cooked cowpea fruits

Sl. No.	Quality attributes	Subdivisions of attributes	Score of each attribute	Accessions 1	2	3	4	Overall acceptability
1.	Appearance/ Colour	Natural colour well preserved	4					
		Slightly discoloured	3					
		Moderately discoloured	2					
		Highly discoloured	1					
2.	Doneness	Highly acceptable	4					
		Moderately acceptable	3					
		Slightly acceptable	2					
		Least acceptable	1					
3.	Flavour	Very pleasant	4					
		Pleasant	3					
		Moderately pleasant	2					
		Unpleasant	1					
4.	Taste	Very good	4					
		Good	3					
		Fair	2					
		Poor	1					
5.	Texture	Very good	4					
		Good	3					
		Fair	2					
		Poor	1					

CHARACTERIZATION OF VEGETABLE COWPEA
(*Vigna unguiculata* (L.) Walp.)

MANJU P. R.

Abstract of the thesis
submitted in partial fulfillment of the requirement
for the degree of

Doctor of Philosophy in Horticulture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2006

Department of Olericulture
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM - 695 522

ABSTRACT

The research project "Characterization of vegetable cowpea (*Vigna unguiculata* (L.) Walp.)" was conducted at the Department of Olericulture and Department of Plant Biotechnology, College of Agriculture, Vellayani, during 2002 to 2005. The objective of the study was to genetically catalogue the accessions based on IPGRI descriptor for cowpea, to estimate the genetic parameters for different traits in the germplasm as well as to characterize them based on morphological, anatomical, biochemical and molecular parameters.

Sixty six accessions of vegetable cowpea collected from various sources upon cataloguing pointed out wide variation for several morphological characters. Analysis of variance revealed significant differences among the accessions for all the characters studied coming under growth, flowering, pod, yield and quality.

Among the accessions evaluated, VS 8 (CHCP-1) had the highest yield (1136.89 g) and pods per plant (102.59), while VS 19 (Aryanad, Thiruvananthapuram) and VS 4 (Kanjikuzhi Payar) were noted for their high pod length, pod weight, pod girth, seeds per pod, 100-seed weight and vine length.

Root : shoot ratio had the highest phenotypic and genotypic coefficients of variation, followed by pod weight and yield per plant. High heritability coupled with high genetic advance was observed for yield per plant, pods per plant, pod length and pod weight.

Correlation studies revealed that characters like vine length, collar girth, pod length, pod girth, pod weight, pods per plant, seeds per pod, 100-seed weight, seed length, seed width, number of harvests, keeping quality and pod protein observed high positive correlation with yield, whereas root : shoot ratio and peduncle length were negatively correlated with yield.

Path coefficient analysis indicated that pods per plant exerted the highest positive direct effect on yield, while seed length, pod weight and vine length had high indirect effects on pod yield.

In discriminant function analysis, the accession VS 27 (Pattom, Thiruvananthapuram) ranked first, followed by VS 8 (CHCP-1) and VS 19 (Aryanad, Thiruvananthapuram).

Based on Mahalanobi's D^2 statistic, the 66 accessions were grouped into ten clusters. Cluster I was the largest containing 18 accessions, while cluster X was the smallest with two accessions. Cluster X performed better most of the biometric characters, with the highest intercluster distance observed between clusters VI and X.

On screening the accessions for legume pod borer resistance, VS 19 (Aryanad, Thiruvananthapuram) was found to be the most tolerant, while VS 42 (Pilicode, Kasargode) was the most susceptible. Pod trichome density as well as phenol content were negatively correlated with plant susceptibility index.

On the basis of the present study, VS 27, VS 8 and VS 19 were found to be promising based on their superiority in yield, quality and tolerance to legume pod borer and hence they may be utilized for further crop improvement programmes.

The organoleptic quality and overall acceptability of all the accessions was also assessed based on appearance / colour, doneness, taste, flavour and texture. The overall acceptability ranged from 2.00 to 3.60 and 2.40 to 3.60 in vegetable and dual purpose / grain types respectively.

Characterization of vegetable cowpea based on seed protein banding pattern as well as RAPD revealed the presence of wide variability among the accessions. Similarity coefficient values ranged from 0.20 to 0.97. At 66 per cent similarity, the selected 50 accessions were grouped into eleven clusters. It may be concluded that molecular characterization may be used as a tool for DNA fingerprinting as well as parental selection for further crop improvement in vegetable cowpea. The study also highlighted the probable development of yard long beans from grain type cowpeas with the dual purpose and bush types as intermediate forms.