ASSESSMENT OF MORPHOLOGICAL AND MOLECULAR DIVERSITY AND GENETICS OF RESISTANCE TO BACTERIAL LEAF BLIGHT AND MOSAIC VIRUS IN COWPEA (*Vigna unguiculata* L. Walp)

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BENGALURU – 560 065

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in

Genetics and PLant Breeding

BENGALURU **JULY, 2011**

Affectionately Dedicated

To

My Beloved Parents ...

DEPARTMENT OF GENETICS AND PLANT BREEDING
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CERTIFICATE

This is to certify that the thesis entitled **"Assessment of morphological and molecular diversity and genetics of resistance to bacterial leaf blight and mosaic virus in cowpea (*Vigna unguiculata* L. Walp)"** submitted by **Mr. HASAN KHAN, ID No. PAK 8036** in partial fulfillment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY** in **GENETICS AND PLANT BREEDING** to the University of Agricultural Sciences, Bengaluru, is a record of research work done by him during the period of his study in this university under my guidance and supervision, and the thesis has not previously been formed the basis of award of any degree, diploma, associateship, fellowship or other similar titles.

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Most Sincerely,

Place: Bengaluru **Hasan Khan**

Date: 14.03.2012

ABSTRACT

An investigation was undertaken with 196 cowpea genotypes to study morphological and molecular diversity using SSR markers and field screening of mosaic virus and Bacterial leaf blight disease for the identification of DNA markers linked to BLB. Analysis of variance revealed significant difference for all the nine quantitative traits. The estimates of PCV and GCV were high for plant height, number of branches plant^{-1}, pods plant^{-1}, pod length, test weight and seed yield plant^{-1}. High heritability and genetic advance was observed for plant height, number of branches plant^{-1}, pods plant^{-1} pod length, seeds pod^{-1}, test weight and seed yield. Genetic divergence study using Mahalanobis D^2 statistic grouped 196 genotypes into 22 clusters. Cluster XXII was the largest comprising of 133 genotypes followed by cluster I with 23 genotypes, and all other clusters had only two genotypes each. Intra cluster distance was highest in the cluster XXII followed by the cluster I and cluster XXI. The genotypes included are found to be very diverse in nature as they showed maximum inter cluster distance (D^2) between the clusters I and XIX, the minimum D^2 value was between the clusters II and VI. Molecular characterization with 60 genotypes revealed at 65 per cent similarity, seven clusters were formed and II cluster had only one genotype *i.e.* EC 472252 and cluster V largest with 28 genotypes. The genotypes found resistant in field screening of 196 entries were subjected to artificial inoculation for both diseases and resistant C-152 susceptible for both diseases and V-16, HC-03-02 were found resistance to BLB and CpMV diseases, respectively. Inheritance studies for CpMV and BLB diseases involving two crosses of susceptible with resistant parents revealed the role of single dominant gene for both the diseases. Only three SSR markers were polymorphic out of 12 markers studied for BLB resistance in the BSA of F_2 cross (C-152 x V-16). Markers CP-641/642 showed significant linkage on the basis of single factor analysis of these three putative markers, which leads to conclude for the possible linkage between the markers and BLB resistance gene.

Hasan Khan

K.P. Viswanatha
(Major Advisor)

"C®¸ÀzÀAiÀÄ CAUÀqÀUÀ¼À ºÁUÀÆ ªÀÄÁ®ÃPÀgÀ ªÀiÁPÀðgï ªÀiÁPÀðgï DzsÁgÀzÀ ªÀiÁ±ÀPÁ»Ã ªÉÆ«zÀvÉ ªÀÄvÀÄÛzÀÄAqÁtÄ CAUÀªÀÄÁj ºÁUÀÆ £ÀÄdÄ gÉÃUÀ ¤gÉÆÃzÀPÀvÉAiÀÄ CzÀåAiÀÄ£À"

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
cM	Centi Morgon
EDTA	Ethylene Diamine Tetra Acetic Acid
GA	Genetic Advance
MAS	Marker Assisted Selection
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SAHN	Sequential Agglomerative Hierarchical Nested
SDS	Sodium Dodecyl Sulphate
SSC	Sodium Saline Citrate
SSR	Simple Sequence Repeats
CpMV	Cowpea Mosaic Virus
BLB	Bacterial Leaf Blight
cm	Centimeter
g	Gram
Viz	Namely
m. t.	Million tonnes
m.ha.	Million hectares
Kg	Kilo gram
mm	Millimeter

Introduction

I. INTRODUCTION

Pulses generally known as food legumes across the world are second most important group of crops next to cereals. They are the main source of protein for vegetarian population around the world, as they contain almost double or three times more protein than cereals. In addition to food they also act as source of nutritious fodder for animals. Pulses are also known to enrich the soil fertility by fixing the atmospheric nitrogen by symbiotic process with *Rhizobium.* Pulses occupy an area of 70.59 million hectare worldwide, with the production of 61.50 million tonnes (Anon., 2010). In India pulses occupy an area of 26.28 million hectare with an annual production of 18.09 million tonnes with an average productivity level of 689 kg per hectare as compared to global productivity of 871 kg per hectare (Anon., 2011). India is running behind of its required pulse production and still depends on imports for domestic consumption (Anon., 2011).

Our country is witnessing a population growth rate of 1.25 per cent per annum while our pulse production growth rate is zero (Bhan *et al.*, 2009). Declining availability of per capita arable land and fragmentation of available agricultural land pose further problems. In this regard, increasing pulse production by increase in area of cultivation is not practically feasible and increase in production through increased productivity is the only solution. Increasing the pulse productivity by means of high yielding varieties with pest and disease resistance is the need of the hour. In this scenario, cowpea, which can be cultivated under tropical to temperate climate conditions, with an ability to better tolerate soils over a wide range of pH, can be cropped alone or intercropped with other crops and has the ability to withstand considerable drought and moderate shade (Timko *et al.*, 2007; Diouf and Hilu, 2005) deserves special attention.

Cowpea (*Vigna unguiculata* L. Walp.), an annual legume among pulses, is one of the most ancient crops known to man. Its origin and subsequent domestication is associated with pearl millet and sorghum in Africa. Cowpea is also commonly referred to as southern pea, blackeye pea, crowder pea and lobia. The largest production is in Africa, with Nigeria and Niger predominating, while Brazil, Haiti, India, Myanmar, Sri Lanka, Australia, U.S., Bosnia, and Herzegovina all have significant production. All parts of the plant in cowpea are used as nutritious food, providing protein, vitamins (notably vitamin B) and minerals. The protein in cowpea seed is rich in the amino acids lysine and tryptophan, compared to cereal grains; however, it is deficient in methionine and cystine when compared to animal proteins. Therefore, cowpea seed is valued as a nutritional supplement to cereals and as an extender of animal proteins. It is now a broadly adapted and highly variable crop, cultivated around the world primarily for seed, but also as a vegetable (for leafy greens, green pods, fresh shelled green peas, and shelled dried peas), a cover crop, and fodder. The cowpea haulm is also a great source of livestock feed, and therefore of great value to farmers. It is also used as a green manure crop, a nitrogen-fixing crop, or for soil erosion control. In India cowpea is mainly cultivated for fodder, green manure and soil improving cover crop. Green pods of cowpea are used as vegetable in Northern Indian States whereas in West Bengal, Tamil Nadu, Andhra Pradesh, Kerala, Karnataka and Maharashtra cowpea is cultivated as a pulse crop.

In India cowpea occupies an area of 3.9 million hectare with an annual production of 2.21 million tonnes with an average productivity level of 570 kg per hectare (Anon., 2011). Cowpea stands fourth in Karnataka among all pulses and is grown as an early *kharif* crop and as regular *kharif* crop. The average yield of cowpea is only about 555 kg/ha as compared to the national average of 570 kg/ha. The area of cowpea

cultivation in Karnataka was 1.90 lakh hectare and production is 0.95 lakh tonnes (Anon. 2011).

The main production constraints of pulses in general and cowpea in particular are biotic and abiotic stresses. Diseases are prominent among biotic stresses know to affect the productivity. Fungi, bacterial and viral diseases are considered as major limiting factors for the production of cowpea in the tropical and subtropical countries (Mali and Thottappilly, 1986). More than 20 viruses are reported from various cowpea growing areas worldwide. Among these viruses, cowpea mosaic virus (CpMV) is the most serious disease of cowpea. It may cause 80-100 % yield reductions (Chant, 1960; Shoyinka, 1974 and Williams, 1977). Cowpea mosaic virus also severely affect vegetative parts of the plant (Bashir *et al.*, 2002). It may cause 14 to 54 % decrease in plant height, 30 to 95 % decrease in dry stem weight of cowpea and mungbean (Ilyas, 1999).

Another disease of cowpea bacterial leaf blight (CoBLB) caused by *Xanthomonas campestris* pv. *Vignicola*, is one of the most devastating diseases on cowpea, especially during *kharif* season which limits the cultivation of the crop. This disease spreads rapidly during heavy rains. The symptoms begin as tiny water soaked spots on leaf surface, which later develop into brown necrotic spots. Heavily infected leaves may abscise and drop off. Sometimes death of the plant may also occur, thus reducing the yield considerably. Watkins (1943) reported 60 per cent mortality in California Black eye variety and Preston (1948) called it as most devastating disease of cowpea.

Chemical control of these diseases is not economically feasible since cowpea is cultivated mostly on marginal land and in rainfed situations. Therefore, development of resistant varieties offer best hope for farmers since they are safe, eco-friendly and efficient means of

combating the diseases. Development of disease resistant crop varieties and study on disease resistance has to receive attention in cowpea research. This calls for screening and evaluating the germplasm available for resistance to diseases in particular as a first step to know the source of resistance. After identifying the resistant source it becomes essential to understand the genetics of resistance that involves the identification of number of genes involved and nature of inheritance. Further, a good understanding of the inheritance of the traits that are correlated with disease is essential for successful disease resistance breeding programme.

Conventional breeding approaches have many drawbacks, especially for the development of disease resistant cultivars. Disease development is seasonal and depends on specific climatic conditions which may lead to chance escape of susceptible genotypes. Further it compels the breeder to wait for favorable climatic conditions. Marker assisted selection helps to overcome these loopholes of conventional breeding. It is possible to identify resistant types through marker assisted selection in seedling stage itself, irrespective of environment, by which breeder can save resources, time and energy. It also prompts the breeder to save generations in marker assisted backcrossing, if susceptibility is dominant, or if a disease is appearing after flowering stage such as rust. Pyramiding of resistant genes, which is the solution for diseases that are controlled by many genes are virtually impossible without marker assisted selection. Identification of markers linked to resistance genes is important in this regard.

Microsatellites or simple sequence repeats (SSR) are DNA sequences with repeat lengths of a few base pairs. Variation in the number of repeats can be detected with PCR by developing primers for the conserved DNA sequence flanking the SSR. As molecular markers,

SSR's combine many desirable marker properties including high levels of polymorphism and information content, unambiguous designation of alleles, even dispersal, selective neutrality, high reproducibility, co-dominance, rapid and simple genotyping assays. Microsatellites have become the molecular markers of choice for a wide range of applications in genetic mapping and genome analysis (Chen *et al.,* 1997; Li *et al.*, 2000), genotype identification and variety protection (Senior *et al.*, 1998), seed purity evaluation and germplasm conservation (Brown *et al.*, 1996), diversity studies (Xiao *et al.*, 1996), paternity determination and pedigree analysis (Ayres *et al.*, 1997), gene and Quantitative Trait Locus analysis (Blair and McCouch, 1997), and marker-assisted breeding (Ayres *et al.*, 1997; Weising *et al.*, 1998). For identification of molecular markers linked to agronomically important genes, SSR's are the best choice when compared to RAPD and AFLP in a more polymorphic information and more cost effective (Lee 1995; Kelly and Miklas 1998; Young 1999). The development and use of molecular marker technologies has also facilitated the subsequent cloning and characterization of disease, insect, and pest resistance genes from a variety of plant species (Kosack and Jones 1997; Ronald 1998; Meyers *et al.* 1999).

Keeping all these in view, present study was conducted with the following objectives.

1. To assess morphological and molecular diversity in cowpea germplasm.
2. To unravel the genetics of resistance to bacterial leaf blight and cowpea mosaic virus.
3. Identification of molecular marker/s for resistance to bacterial leaf blight.

Review of Literature

II. REVIEW OF LITERATURE

The literature available on genetic variability parameters, diversity based on morphological and molecular markers, disease screening, inheritance of disease resistance, and identification of molecular markers linked to disease resistance, are presented here.

Reviews related to the present study are given under following sub-titles

2.1 Genetic variability

2.2 Studies on association of characters

2.3 Genetic divergence studies

2.4 Studies on molecular characterization using different markers

2.5 Evaluation of genotypes for resistance to CpMV and BLB

2.6 Studies on genetic variability, heritability and genetic advance in segregating populations.

2.7 Genetic studies on cowpea mosaic virus and bacterial leaf blight disease resistance

2.8 Identification of DNA markers linked to BLB disease resistance

2.1 Genetic Variability Studies

Plant breeding is known as an art and science of improving genetic pattern of plants in relation to their economic use. In the process of improvement, desirable plants are continuously being selected from variable population. Thus variability is a prerequisite to initiate breeding program in any crop.

In self pollinated crops, the assessment of quantitative variable for genotypic variance, estimates of heritability and genetics advance are important for successful selection programme to evolve promising cultivars.

2.1.1 Genetic Variability

The variation between pure lines is due to both non heritable components and the variance within the pure line is due to environmental factors. The hereditary and environmental components of variation were revealed in the early part of the century (Johanson, 1909).

Genetic variance is separated from total variance using the estimate of environmental variance in non segregating population (Power, 1942). The heritable variation was further divided into additive and non additive components and the later function included dominance and inter allelic interaction (Fisher *et al.*, 1932; Panse and Sukhatme, 1967 and Lush, 1945).

If the heritable variation in the genes controlling a character is purely additive then that character can be fixed by selection and maximum genetic advance can be accomplished by continuous selection and part of the heritable variations is composed of non-additive components (Panse, 1957).

The possibility of achieving improvement in any crop plants primarily depends on the magnitude of genetic variability. Though heritability value of a trait indicates the effectiveness of selection based on phenotypic expression.

The literature on genetic variability for yield and its attributing parameters are considered here.

Many studies conducted so far on cowpea to estimate phenotypic co-efficient of variation (PCV), genotypic co-efficient of variation (GCV), heritability (h^2) and genetic advance as percentage of mean (GA) are presented in Table 1.

It is evident from Table 1 that, high PCV and GCV were recorded in majority of the studies for plant height, primary branches per plant, number of clusters pre plant, pods per plant, test weight and seed yield. However, in few studies, low PCV and GCV were reported for days to fifty per cent flowering and number of seeds per pod.

2.1.2 Heritability

The heritability value of a trait indicates the effectiveness of selection based on phenotypic expression. Quantitative characters are governed by more number of genes and further influenced by environment, of which a proportion observed value is heritable. Estimate of narrow sense heritability is not possible thus; by estimating broad sense heritability along with genetic gain is usually more useful in selecting the best individual (Johnson *et al.*, 1955).

More attribution of the heritable component to the total variation of desirable characters becomes essential. Scientist given more emphasis to improve the yield of a crop by studying the variability and heritability of yield and yield attributing components (Robinson *et al.,* 1949). In 1959, Grafius stated that the major yield components in cowpea were number of pods per plant, seeds per pod and 100 seed weight. Any change in yield has to be brought from a change in one or more of these components.

The proportion of genotypic variance to the phenotypic variance determines the amount of variation that is heritable. The heritability thus obtained is the broad sense heritability (Hanson *et al.*, 1956). For the characteristics with low heritability selection may be ineffective due to masking effect of environment on the genotype. Heritability of characters found to differ depending on the genotypes handed and the prevailing environmental conditions since it is a genetic property of a population.

The review of literature on heritability of different characters in cowpea are presented in Table 1, Which suggested that, day to 50 per cent flowering, days to physiological maturity, plant height, primary branches, number of clusters per plant, pods per plant, pod length, seeds per pod, seed yield and 100 seed weight had high heritability. However, few studies also reported moderate to low heritability for days to physiological maturity, plant height, primary branches, pods per plant, seeds per pod, seed yield and 100 seed weight.

2.1.3 Genetic Advance

The estimates of heritability give no indication of the amount of progress expected from the selection process. The genetic advance is therefore more useful in predicting the expected response to selection (Johnson *et al.,* 1955). GA is generally measured at five per cent selection intensity.

Genetic advance is the measure of improvement that can be achieved by practicing selection in a population. Genetic advance is affected by factors like intensity of selection, heritability and phenotypic variance. High genetic advance coupled with high heritability is an indication of more additive gene action (Panse, 1957).

Table 1 shows the results of studies by different authors on genetic advance in cowpea. It is evident from the Table 1, that the days to 50 per cent flowering, plant height, primary branches, number of clusters per plant, plant height, pods per plant, pod length, seeds per pod, seed yield and 100-seed weight were found to have high genetic advance as per cent of mean (GAM). Few studies also indicated low GA for days to 50 per cent flowering, days to physiological maturity, plant height, primary branches, number of clusters per plant, pods per plant, pod length, seeds per pod, seed yield and 100-seed weight.

Table 1: Review of literature pertaining to phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2) and genetic advance (GA) for different characters in cowpea.

Characters	PCV	GCV	h^2	GA	Reference
Days to 50 per cent Flowering	Med.	Med.	High	Med.	Awopetu *et al.* (2006), Anbu Selvam *et al.* (2000)
	Low	Low	High	High	Tyagi *et al.* (2000), Backiyarani and Nadarajan (1996)
	Low	Low	High	Low	Borah *et al.* (2001), Borah and Fazlullah Khan (1999), Patil and Baviskar (1987)
	Med.	Med.	High	High	Rahul Chauhan *et al.* (2003), Santosh Kumar *et al.* (2002), Shukla *et al.* (1994)
	Low	Low	High	Med.	Venkatesan *et al.* (2003), Sobha and Abdul Vahab (1998).
	High	High	High	Low	Gireesh *et al.* (2006), Vineetha Kumari *et al.* (2003), Sreekumar *et al.* (1996)
	Med.	High	High	High	Roquib and Patnaik (1990)
	High	High	High	High	Selvam *et al.* (2000), Sharma (1999)
	--	--	High	High	Anbumalarmathi *et al.* (2005)
Days to physiological maturity	High	High	--	--	Henry *et al.* (2003)
	Low	Low	High	Low	Marangappanavar (1984)
	--	--	High	Low	Patil and Patil (1986)
	Low	Low	High	Mod.	Apte *et al.* (1987), Patil and Baviskar (1987)
	low	High	Low	Low	Thiyagarajan (1989)
	High	High	--	--	Vineeta kumari *et al.* (2003)

Table 1: Contd…

	High	High	Med.	Low	Anbu Selvam *et al.* (2000), Usha Kumari *et al.* (2000)
	--	--	High	--	Sarvamangala (2004)
	High	Med.	High	High	Shukla *et al.* (1994)
	Med.	Med.	High	High	Venkatesan *et al.* (2003), Kalaiyarasi *et al.* (2000)
	Med.	High	Med.	High	Vineeta -Kumari *et al.* (2003)
	--	--	High	High	Anbumalarmathi *et al.* (2005)
	High	High	High	High	Malarvizhi *et al.* (2005), Pal *et al.* (2003), Borah *et al.* (2001), Sobha and Abdul Vahab (1998), Renganayaki and Rengasamy (1992)
Primary branches per plant	High	High	High	High	Gireesh *et al.* (2006), Malarvizhi *et al.* (2005), Shahid Ahmed *et al.* (2005), Prasanthi. (2004), Nigude *et al.*(2004), Pal *et al.* (2003),Venkatesan *et al.* (2003b), Rahul Chauhan *et al.* (2003), Prakash *et al.* (2003), Anbu Selvam *et al.* (2000), Hazra *et al.* (1999), Sharma (1999), Harsha Vardhana and Savithramma (1998), Sawant (1994), Renganayakii and Rengasamy (1992), Roquib and Patnaik (1990), Lakshmi and Goud (1977)
	--	--	High	High	Anbumalarmathi *et al.* (2005)
	--	High	High	High	Eswaran (2007)
	Low	Low	Mod.	Low	Uma (2001)
	Low	Low	Mod.	Mod	Tharware *et al.* (1991)
	High	High	Med.	Low	Omoigui *et al.* (2006), Prasanthi *et al.* (2004), Singh *et al.* (2002), Anbu Selvam *et al.* (2000), Nehru *et al.* (2001), Tyagi *et al.* (2000)
	Med.	Med.	High	Med.	Santosh Kumar *et al.* (2002), Backiyarani and Nadarajan (1996), Shukla *et al.* (1994)
	Med.	Low	Low	Low	Venkatesan *et al.* (2003)

Table 1: Contd…

	Med.	Med.	High	High	Vineeta Kumari *et al.* (2003), Kalaiyarasi *et al.* (2000), Sobha and Abdul Vahab (1998)
Clusters per plant	High	High	High	High	Henry *et al.* (2003), Sawant (1994)
	--	--	High	High	Anbumalarmathi *et al.* (2005), Suganthi and Murugan (2007),
	High	High	Low	--	Backiyarani and Nadarajan (1996)
	--	Low	Low	--	Rangaiah (1997)
	High	Low	Med.	Low	Selvam *et al.* (2000)
	High	High	High	High	Vineeta -Kumari *et al.* (2003)
	Med.	Med	--	--	Sarvamangala (2004)
Pods per plant	High	High	Med.	Low	Awopetu *et al.* (2006), Singh *et al.* (2002), Anbu Selvam *et al.* (2000)
	High	High	High	Low	Mathura Rai *et al.* (2004), Backiyarani and Nadarajan (1996)
	High	High	High	High	Gireesh *et al.* (2006), Shahid Ahmed *et al.* (2005), Resmi *et al.* (2004), Prasanthi. (2004), Nigude *et al.* (2004), Henry *et al.* (2003), Pal *et al.* (2003), Rahul Chauhan *et al.* (2003), Venkatesan *et al.* (2003), Vineeta Kumari *et al.* (2003), Narayanankutty *et al.* (2003b), Nehru *et al.* (2001), Sobha and Abdul Vahab (1998), Sawant (1994) Renganayaki and Rengasamy (1992), Patil and Baviskar (1987)
	Med.	Med.	High	High	Tyagi *et al.* (2000), Singh and Dabas (1986)

Table 1: Contd…

	--	--	High	High	Anbumalarmathi *et al.* (2005), Suganthi and Murugan (2007)
	High	High	Med.	High	Dharmalingam and Kadambavanasundaram (1989), Lakshmi and Goud (1977), Borida *et al.* (1973), Veeraswamy *et al.* (1973)
Pod length	Med.	Med.	High	Low	Anbu Selvam *et al.* (2000), Backiyarani and Nadarajan (1996)
	Med.	Med.	High	High	Rahul Chauhan *et al.* (2003), Tyagi *et al.* (2000), Kalaiyarasi *et al.* (2000), Lakshmi and Goud (1977)
	High	Med.	High	High	Venkatesan *et al.* (2003), Sreekumar *et al.* (1996), Renganayaki and Rengasamy (1992)
	Med.	Med.	High	Med.	Vineeta -Kumari *et al.* (2003)
	--	--	High	High	Anbumalarmathi *et al.,*(2005)
	High	High	High	Med.	Mathura Rai *et al.* (2004), Sobha and Abdul Vahab (1998), Patil and Baviskar (1987)
	High	High	High	High	Hazra *et al.* (1999)
Seeds per pod	Med.	Med.	Med.	Low	Anbu Selvam *et al.* (2000)
	High	High	Med.	Low	Usha Kumari *et al.* (2000)
	Med.	Med.	High	Med.	Backiyarani and Nadarajan (1996)
	Med.	Med.	High	High	Rahul chauhan *et al.* (2003), Vineeta kumari *et al.* (2003), Kalaiyarasi *et al.* (2000), Lakshmi and Goud (1977)
	--	--	High	High	Anbumalarmathi *et al.,*(2005)
	Med.	Low	Med.	Med.	Tyagi *et al.* (2000)

Table 1: Contd…

Seed yield	Low	Low	Med.	Low	Vineeta kumari *et al.* (2003) Patil and Baviskar (1987)
	High	High	High	High	Resmi *et al.* (2004), Sobha and Abdul Vahab (1998), Sreekumar *et al.* (1996), Renganayaki and Rengasamy (1992)
	Med.	Med.	High	Low	Sharma (1999)
	High	High	Med.	Low	Nehru *et al.* (2001), Anbu Selvam *et al.* (2000)
	High	High	High	Low	Backiyarani and Nadarajan (1996)
	Med.	High	Med.	High	Rahul Chauhan *et al.* (2003) Patil and Baviskar (1987)
	Med.	Med.	High	High	Prakash *et al.* (2003).Kalaiyarasi *et al.* (2000)
	High	High	High	High	Shahid Ahmed *et al.* (2005), Resmi *et al.* (2004), Nigude *et al.* (2004), Henry *et al.* (2003), Venkatesan *et al.* (2003), Vineeta Kumari *et al.* (2003), Tyagi *et al.* (2000), Renganayaki and Rengasamy (1992)
	High	High	Med.	High	Lakshmi and Goud (1977)
	Med.	Med.	Med.	Med.	Venkatesan *et al.* (2003)
	--	--	High	High	Anbumalarmathi *et al.,*(2005), Kumawat *et al.* (2005), Suganthi and Murugan (2007),
	High	High	High	Med.	Sharma (1999)
100-seed weight	Med.	Med.	High	High	Venkatesan *et al.* (2003), Ahmad Neyaz and Bajpai (2002), Tyagi *et al.* (2000), Patil and Baviskar (1987)
	High	High	Low	Low	Singh *et al.* (2002), Anbu Selvam *et al.* (2000)
	High	High	--	--	Henry *et al.* (2003),
	--	--	High	High	Anbumalarmathi *et al.* (2005), Suganthi and Murugan (2007)

Table 1: Contd…

Med.	Med.	Med.	Med.	Venkatesan *et al.* (2003)
High	High	Med.	Med.	Prasanthi *et al.* (2004), Nehru *et al.* (2001)
High	High	High	Low	Omoigui *et al.* (2006), Rocha *et al.* (2003), Backiyarani and Nadarajan (1996)
High	High	High	High	Shahid Ahmed *et al.* (2005), Rahul Chauhan *et al.* (2003), Vineeta Kumari *et al.* (2003), Sobha and Abdul Vahab (1998), Khabiruddin *et al.* (1996), Sawant (1994), Renganayaki and Rengasamy (1992), Lakshmi and Goud (1977)

A comprehensive consideration of the information presented in Table 1 indicated different levels of PCV, GCV, heritability and genetic advance reported by different workers. High PCV and GCV naturally result in higher GAM. Higher PCV coupled with lower GCV lead to low heritability estimates and consequently low GA. In few cases, low PCV and GCV and medium GCV and PCV also resulted in high GA because of high heritability.

2.2 Studies on association of characters

Grain yield in any crop depends on many component characters which influence yield either jointly or single and either directly or indirectly through other related characters. Selection for yield on the basis of *per se* performance alone may not be effective compared to selection based on the component character associated with it, which is biometrically determined by correlation coefficient and path analysis. An understanding of the nature and extent of association of these components with grain yield and amongst themselves is an essential pre-requisite for formulating sound breeding programme.

This concept was given by Fisher (1918) and Wright (1921). The information on correlation is of great importance when simultaneous selection is to be carried out for more than one character to achieve the required improvement in seed yield.

The correlation coefficient analysis measures the mutual relationship between various characters and it determines the component traits on which selection can be relied upon to effect the improvement. The Association of characters can be expressed as phenotypic correlation and genotypic correlation.

Phenotypic correlation is the observable correlation between two variables and includes both genotypic and environmental effects.

Genotypic correlation on the other hand, is the inherent association between two variables may be either due to pleiotropic action of genes or linkage, more likely both or developmentally induced relationships.

A brief review of the nature of correlation of different characters on yield in cowpea investigated by several workers is presented in Table.2.

The literature reviewed indicated that, characters such as days to 50 per cent flowering, days to physiological maturity, plant height, primary branches, number of clusters per plant, pods per plant, pod length, seeds per pod and 100-seed weight were found to be positively correlated with seed yield. Few workers also reported negative correlation of days to 50 per cent flowering, days to physiological maturity, plant height, pods per pod and 100-seed weight with seed yield.

2.3 Genetic Divergence Studies

The magnitude of divergence between two groups under consideration is provided by D^2 statistic developed by Mahalanobis (1936). It considers the variation produced by any character and their consequent effect that it bears on other characters.

The technique in the form of generalized distance was first used by Mahalanobis in an anthropometric survey of the united province in India. Its application was extended later to taxonomic studies. Murthy and Pavate (1962) observed that D^2 analysis could be extended to the situations where overlapping species need to be discriminated and also when the discrimination at sub species level is needed.

Dharmalingam and Kadambavanasundaram (1989) presented a data on 8 yield components measured in 40 genotypes was subjected to multivariate analysis (D^2). Wide genetic diversity was present among the 13 clusters formed. The genotypes Co 2 and G-5, belonging to the 2 most

Table.2: Correlation of different yield attributing characters with seed yield in cowpea

Characters	Nature of correlation	Reference
Days to 50 per cent flowering	Positive	Mittal and Paramjit Singh (2005), Nigude *et al.* (2004), Rahul Chauhan *et al.* (2003), Bezerra *et al.* (2001), Ramesh Kumar *et al.* (2001), Tyagi *et al.* (2000), Sharma and Mishra (1997), Naveen Singh and Singh (1997), Biradar *et al.* (1991)
	Negative	Tewari and Gautam (1989), Oseni *et al.* (1992)
Days to physiological maturity	Positive	Mittal and Paramjit Singh (2005), Nigude *et al.* (2004)
	Negative	Henry *et al.* (2003)
Plant height (cm)	Positive	Nigude *et al* . (2004) Rahul Chauhan *et al.* (2003), Ramesh Kumar *et al.* (2001), Tyagi *et al.* (2000), Kalaiyarasi and Palanisamy (1999), Naveen Singh and singh (1997), Rekha Mathur (1995), Tamilselvam and Vijendra Das (1994), Damarany (1994), Thiyagarajan and Rajasekaran (1989), Gowda (1984), Tyagi *et al.* (1978)
	Negative	Tewari and Gautam (1989)
Primary branches per plant	Positive	Kumawat and Raje (2005), Nigude et al . (2004), Venkatesan *et al.* (2003), Rahul Chauhan *et al.* (2003), Malarvizhi *et al.* (2003), Ramesh Kumar *et al.* (2001), Kalaiyarasi and Palanisamy (1999), Naveen Singh and Singh (1997), Sawant (1994), Tamilselvam and Vijendra Das (1994), Damarany (1994), Shakarad *et al.* (1993), Tewari and Gautam (1989)
Clusters per plant	Positive	Kumawat and Raje (2005), Henry *et al.* (2003), Parmar *et al.* (2003), Nigude *et al* . (2004), Anbumalarmathi *et al.,*(2005)
Pods per plant	Positive	Anbumalarmathi *et al.* (2005), Kumawat and Raje (2005), Nigude *et al.*. (2004), Henry *et al.* (2003), Kutty *et al.* (2003), Venkatesan *et al.* (2003), Rahul Chauhan *et al.* (2003), Parmer *et al.* (2003), Vineetha Kumari *et al.* (2003), Ramesh Kumar *et al.* (2001), Tyagi *et al.* (2000), Kalaiyarasi and Palanisamy (1999), Naveen Singh and Singh (1997), Naidu *et al.* (1996), Sreekumar *et al.* (1996), Rekha Mathur (1995), Damarany (1994), Sawant (1994), Tamilselvam and Vijendra Das (1994),

Table 2: Contd…

		Shakarad *et al.* (1993), Oseni *et al.* (1992), Biradar *et al.* (1991), Thiyagarajan and Rajasekaran (1989), Chikkadevaiah (1985)
Pod length (cm)	Positive	Anbumalarmathi *et al.* (2005), Kutty *et al.* (2003), Rahul Chauhan *et al.* (2003), Ramesh Kumar *et al.* (2001), Tyagi *et al.* (2000),) Harsha Vardhana and Savithramma (1998), Naveen Singh and Singh (1997), Sreekumar *et al.* (1996), Rekha Mathur (1995), Tamilselvam and Vijendra Das (1994), Sawant (1994), Renganayaki and Rengasamy (1992), Oseni *et al.* (1992), Biradar *et al.* (1991), Thiyagarajan and Rajasekaran (1989), Jatasra and Dahiya (1988)
Seeds per pod	Positive	Anbumalarmathi *et al.* (2005), Nigude *et al.* (2004), Rahul Chauhan *et al.* (2003), Malarvizhi *et al.* (2003) Bezerra *et al.* (2001), Naidu *et al.* (1996), Sreekumar *et al.* (1996), Rekha Mathur (1995), Vijendra Das (1995), Tamilselvam and Vijendra Das (1994), Nita Kar *et al.* (1995), Damarany (1994), Sawant (1994), Shakarad *et al.* (1993), Oseni *et al.* (1992), Renganayaki and Rengasamy (1992), Biradar *et al.* (1991), Tewari and Gautam (1989), Thiyagarajan and Rajasekaran (1989), Chikkadevaiah (1985), Rajendran *et al.* (1979)
100-seed weight (g)	Positive	Anbumalarmathi *et al.* (2005), Mittal and Paramjit Singh (2005), Henry *et al.* (2003), Ramesh Kumar *et al.* (2001), Tyagi *et al.* (2000), Naveen Singh and Singh (1997), Biradar *et al.* (1996), Naidu *et al.* (1996), Sawant (1994), Tamilselvam and Vijendra Das (1994), Patil *et al.* (1989), Tewari and Gautam (1989), Chikkadevaiah (1985), Rajendran *et al.* (1979)
	Negative	Rahul Chauhan *et al.* (2003) Shakarad *et al.* (1993), Oseni *et al.* (1992)

divergent clusters, were recommended as suitable for inclusion in heterosis breeding programmes.

Renganayaki and Rangaswamy (1992) analysed the mungbean (*Vigna radiata*), blackgram (*Vigna mungo*) and cowpea (*Vigna unguiculata*) using the Mahalanobis D^2 value and revealed that the genotypes of *Vigna radiata, V. mungo,* and *V. unguiculata* were grouped in seven, five and four clusters, respectively and also concluded that hundred seed weight, pod length and seed yield contributed most towards genetic divergence.

Mahalanobis' D^2 statistic was used (Rewale *et al.* 1996) to estimate genetic divergence of 12 yield-related characters evaluated in 70 genotypes of *V. unguiculata* subsp. cylendria (*V. unguiculata* subsp. *cilíndrica*). Multivariate analysis grouped the genotypes into 19 clusters, of which 11 had only one genotype each. Days to initiation of flowering, 50 per cent flowering and maturity, number of inflorescences and pods/plant, pod length, 100-seed weight, seed yield/plant and harvest index made the largest contribution to total divergence. It is proposed that high yielding genotypes with early maturity and high harvest index could be selected as parents for hybridization.

Santos *et al.* (1997) reported genetic divergence of cowpea under two different environments using Mahalanobis D^2 statistics and the traits length of main branch, hundred seed weight and pod length were the most important characters to affect divergence.

Sharma and Misra (1997) revealed that days to 50 percent flowering, plant height, pods per peduncle, and harvest index contributed the most towards the genetic divergence and seed yield had a high positive phenotypic correlation with pods per peduncle, number of seeds per pod and harvest index.

Backiyarani *et al.* (2000) concluded that genetic divergence for physiological traits like single plant yield, harvest index and earliness in flowering together accounted for 80 % of the total divergence in cowpea.

Ushakumari *et al.* (2000) reported that contribution towards genetic divergence was recorded for plant height (22.69%), seeds per pod (%), number of branches (16.82%), number of pods per cluster (15.27%) and pod length (13.47%) in cowpea.

Borah and Khan (2002) revealed on genetic divergence in fodder cowpea, sixty fodder cowpea cultivars were grouped in to 10 clusters. Dry matter yield, green fodder yield and plant height were recorded the highest contribution to total genetic divergence. These traits could be good criterion for selection of parents in hybridization programme.

Narayanankutty *et al.* (2003) Studied genetic variability and divergence on thirty seven genotypes of vegetable cowpea revealed significant differences for all the characters under study. The thirty-seven genotypes were grouped into eleven clusters using Mahalanobis D^2 statistics. In general, the intercluster distances were higher than intracluster distances. The maximum intercluster distance was between clusters VIII and X, followed by clusters VI and X and clusters VIII and IX, respectively. The intracluster distance was maximum in cluster VII.

Venkatesan *et al.* (2003) reported that Clusters per plant, pods per cluster, pods per plant and seed yield per plant had the maximum contribution towards total divergence.

The nature of magnitude of genetic diversity was studied in a set of 45 cowpea (*Vigna unguiculata*) genotypes from, indigenous and exotic sources (Nigude *et al.* 2004). The genotypes were grouped into five clusters using Mahalanobis D^2 statistics. Cluster-I was the largest with

28 genotypes followed by cluster-II with eleven genotypes and cluster-III with four genotypes. The clusters IV and V were monogenotypic. The maximum inter-cluster distance was observed between clusters III and V followed by distance between clusters II and III. Clusters I and III exhibited the minimum inter-cluster distance. The number of branches per plant, test weight, biomass (dry weight) at harvesting and number of pods per plant had contributed considerably towards divergence.

Kumawat *et al.* (2005) also showed Seed yield per plant had the highest contribution towards the total genetic divergence; followed by seeds per pod, days to 50% flowering, plant height and reproductive period.

Hasan and Abdullah (2007) studied eleven varieties of cowpea (*Vigna unguiculata* (L.) Walp.) for variability in yield and yield component characters. Factor analysis based on principal components (PC) showed that two factors represented 99.13% of the total variation. PC1 accounted for 98.69% of the total variance that was highly correlated with seed and pod size factors. PC2 may be considered as crop cycle and yield/plant. The varieties clustered into two groups by factor and cluster analyses.

Pandey (2007) evaluated forty four grain cowpea for 13 characters to quantify the genetic diversity existing among them by using Mahalanobis D^2 statistics. The genotypes fell into 9 clusters. Cluster strength varied from single genotype (Cluster IV to IX) to 31 genotypes (cluster I). Cluster III had minimum days to first flower opening, days to 50% flowering and stover yield per plant in addition to maximum number of pod per plant and primary branches. Cluster II, V, VII had maximum yield per plant, 100-seed weight; pod length and number of seeds per pod, respectively. Cluster II had minimum days to maturity, while cluster VII showed maximum days to maturity.

Sulnathi *et al.* (2007) assessed genetic divergence in 56 genotypes of cowpea using D^2 statistics for 13 yield contributing characters showed grouping of genotypes into 9 clusters. Cluster I had the maximum number of genotypes. The characters days to maturity, 100-seed weight and days to flowering were the highest contributors to D^2 values.

Naima and Marie (2010) carried out a comparison study between morphological and genetic characterization by using twenty landraces of cowpea scattered throughout Algeria. At the morphological level, for qualitative characters there was no intra-landrace variation and for quantitative characters the variations were low except for landrace NAG2. The AMOVA analysis indicated that the genetic variation was lower within than among agro-ecological regions. A Mantel test, revealed a correlation between the qualitative morphological data and the geographical data ($R = 0.28$; $P = 0.01$), indicating that the degree of morphological change among landraces was roughly proportional to the geographical distances separating them.

The literature reviewed indicate that the major traits *viz.*, pods per plant, 100 seed weight and yield per plant had maximum contribution towards total divergence.

2.4 Studies on molecular characterization using SSR markers.

Simple Sequence Repeats (SSR) also called as microsatellites or short tandem repeats (STR), are in general repetitions of nucleotide motifs of 1-5 bases, widely distributed throughout the genome of plants and animals. It has got particular importance because of their major features that made very popular are abundant, highly polymorphic, reliable, co-dominant, unambiguous designation of allele, even dispersal, selective neutrality, high reproducibility, rapid and simple genotyping assays.

SSR consisting tandemly repeated units of short nucleotides motifs of 1- 6 bp long, di, tri and tetranuleotides are the most common eg. (CA)n, (AAT)n and (GATA)n, respectively. SSR can be further classified into a) pure, b) compound and c) interrupted repeats. Pure SSR consist of uninterrupted repeats of single motifs eg. (AT)n, compound SSR consist of two or more repeats eg. (GT)n (AT)n and interrupted SSR contain interruption in repeats eg. (GT)n GG(GT)n (Perkall *et al.,* 1998).

In recent years, a variety of molecular markers, based on microsatellites or simple sequence repeats (SSRs) have become the markers of choice, thus necessitating their development and use in a variety of plant systems (Gupta and Varshney.,2004). By virtue of their extreme polymorphism, abundance and amount of information derived from such markers, together with the ease by which they can be identified, make them ideal marker (Perkall *et al.,* 1998) for genetic mapping and genome analysis (Chen *et al.,* 1997; Li *et al*., 2000), genotype identification and variety protection (Senior *et al.,* 1998), seed purity evaluation and germplasm conservation (Brown *et al.,* 1996), diversity studies (Xiao *et al.,* 1996), paternity determination and pedigree analysis (Ayres *et al.,* 1997), gene and quantitative trait locus analysis (Blair and McCouch,1997), improvement of the allotetraploid (Ferguson *et al*., 2004) and marker-assisted breeding (Ayres *et al.,*1997). For measuring genetic diversity, assigning lines to heterotic groups and genetic fingerprinting, microsatellites provide power of discrimination equal to or greater than that of RFLP in a more cost effective manner (Smith *et al.,*1997). Furthermore the use of these markers is fundamental both for variety identification and for the characterization and management of genetic resources (Gianfranceschi *et al*., 1998). Charters *et al.* (1996) concluded that anchored SSR-PCR analysis is a highly informative and reproducible method for fingerprinting of populations.

Li *et al.* (2001) used forty-six microsatellite DNA markers to evaluate genetic similarities among 90 cowpea breeding lines developed at IITA. Twenty-seven primer pairs could amplify polymorphic single-locus microsatellites from all of these materials. Two to seven alleles per primer were detected with a polymorphic information content varying from 0.02 to 0.73. By means of only five polymorphic microsatellite primers, 88 of the 90 cowpea lines could be distinguished. A dendrogram based on the microsatellite polymorphisms generally agreed with the pedigree of the cowpea lines.

Diouf and Hilu (2005) estimated the genetic relationships among cowpea breeding lines and local varieties in Senegal using microsatellite and RAPD and this SSR results support the genetic diversification of cowpea in Senegal and underscore their potential in elucidating patterns of germplasm diversity of cowpea in Senegal.

Archana and Jawali (2006) a study was carried by using Microsatellite marker and confirmed that, in the large cowpea gene pool, the cultivated cowpeas (ssp. *unguiculata* var. *unguiculata*) form a genetically coherent group and are closely related to the annual cowpeas (ssp. *unguiculata* var. *spontanea*), which may include the most likely progenitor of cultivated cowpeas.

Adebayo (2008) conducted a genetic diversity among fifty accessions of vegetable cowpea using Simple Sequence Repeat (SSRs) markers. Sixteen SSR primers were used to amplify DNA of the 50 accessions. A total of 63 polymorphic bands were detected and this was used for cluster analysis. A (UPGMA) dendrogram separated the accessions into two main clusters at a little below 0.68% similarity coefficient. While at 0.76% similarity coefficient, 10 clusters were formed with clusters 9 and 10 having more than two-thirds of the total accessions used. There were two pairs of tied accessions showing some

level of similarities in the dendrogram. The distribution of accessions in the Principal Component Analysis (PCA) revealed a high genetic variability of vegetable cowpea with no particular grouping according to continents or origin.

Lee *et al.* (2009) conducted a study on genetic diversity of 492 Korean cowpea landrace accessions that have passport information was estimated using six SSR markers. The mean of Weir's gene diversity was 0.665 from all accessions investigated in the study. Cowpea gene diversity of six local provinces in Korea was ranged from 0.370 in accessions of Gangwon to 0.680 in Jeonra provinces. Low gene diversity of the cowpea gene pool of Gangwon province was probably derived from relatively few introductions. Especially SSR markers VM36 and VM39 seem to be good markers to distinguish the Gangwon accessions from others by occurring at a specific locus with higher than 78% of allele frequency. Except for the Gangwon province with the low genetic diversity, gene diversity of cowpea accessions from other provinces was ranged from 0.600 to 0.680 indicating no big differences among provinces.

Uma *et al.* (2009) carried out a variability study by using eighty three cowpea genotypes were 15 microsatellite markers. Among the total 15 SSR primers used, 12 primers were polymorphic and 3 primers were monomorphic. Genotype specific markers were identified for some genotypes. The clusters constructed based on SSR marker data revealed significant genetic variation among the genotypes. The marker detected significant polymorphism among the local landraces as compared to the cultivated varieties. Genotypes with resistance to rust disease and nutritionally superior ones grouped together in separate clusters.

Tantasawat *et al.* (2010) carried out a study of genetic diversity and relatedness of 23 Yardlong bean (*Vigna unguiculata* spp. *sesquipedalis*)

accessions and 7 hybrid in Thailand were estimated using morphological characters, simple sequence repeat (SSR. Eleven of the sixteen SSR primers yielded clear SSRs, ten of which were polymorphic (90.91% polymorphism), detecting a total of 54 alleles with an average of 4.91 alleles per locus. These 10 polymorphic SSR markers successfully distinguished 28 yardlong bean and dwarf yardlong bean accessions. The polymorphic information content (PIC) among genotypes varied from 0.251 to 0.752 with an average of 0.597.

From the reviews indicates that the molecular marker characterization provides more power of discrimination between the different or same species when compare to biometrical diversity analysis.

2.5 Evaluation of genotypes for resistance to CpMV and BLB

2.5.1. Cowpea mosaic virus

Diseases like cowpea mosaic virus, bacterial blight rust and powdery mildew are the major biotic constraints in the production of cowpea. Developing high yielding varieties with resistance to these diseases would be a great achievement in crop improvement. Brief reviews of available literature related to cowpea mosaic virus and bacterial leaf blight diseases are mentioned below.

Most of the cowpea mosaic viruses infecting legumes in India are transmitted by the Aphids and sap-transmissible, share a very narrow host range within legumes and cause biologically indistinguishable symptoms, making specific identification of the viruses difficult (Nene, 1972). Sap transmissible nature of cowpea mosaic poty virus has also been reported by several workers such as Shankar *et al.* (1973); Khatri and Singh (1974); Diwakar and Mali (1976); Ramachandran and Summanwar (1982) and Patil and Gupta (1986).

Satya Vir *et al.* (1984) screened fifty one cultivars of moth bean against Jassids (*Empoasca kerri*), Whitefly (*B. tabaci*) and Yellow mosaic virus. Twenty two cultivars selected during 1980 were repeated for the second year during kharif 1981. Seven cultivars *viz.*, IPCMO-943, IPCMO-1035, T-16, T-2, Jadia, PLMO-240 and PLMO-216 were isolated to be the least susceptible to the pests and disease. None of the cultivars was found immune to the attack of yellow mosaic virus.

Bhati *et al.* (1987) given description of newly developed cultivar Jwala which is completely field resistant to bean yellow mosaic virus transmitted by *B. tabaci*.

Muniyappa *et al.*, (1987) a scale of 1-9 was devised for estimating the severity of infection of horsegram yellow mosaic virus on horsegram. With its help, coefficients of infection were derived for cultivars with diverse types of disease reaction and the results were compared with estimates based on percentage of infected plants. The rating scale is suggested as the best method for giving uniform results at different locations

Gumedzoe *et al.* (1990) observed some infected samples of cowpea which yellow mosaic virus (cowpea mosaic comovirus) was found in 65% and cowpea mottle virus in 35% of the infected samples. Mixed infections were often observed including other cowpea varieties. A total of 23 cowpea cultivars were screened using an isolate of each virus, resistance was found to one of the two viruses tested but not to the mixed infection. No symptoms were found on cowpea cv. TVX 1850-01E on inoculation with both viruses.

Gubba *et al.,* (1994) observed that by analyzing 109 cowpea leaf samples, which were showing virus symptoms, cowpea aphid borne mosaic virus (CAMV) was detected in 75 samples but none of the

samples tested positive for black eye cowpea mosaic virus (BICMV) where as seven samples were detected for cowpea yellow mosaic virus.

Bashir and Hampton (1996) screened seedlings from 182 cowpea pre-introductions/germplasm accessions from 12 countries were tested under greenhouse conditions for six seed-borne viruses. Twenty-one (13.3%) accessions from eight countries were found to be seed-infected with one of the three following viruses: blackeye cowpea mosaic (BICMV) and cowpea aphid-borne mosaic (CABMV) potyviruses, and cucumber mosaic cucumovirus (CMV). Natural seed transmission incidence of 0–6.9%, 0–13.3%, and 0–2.0% were determined for BICMV, CABMV and CMV respectively.

Umaharan *et al.* (1997) scored 160 germplasm lines in greenhouse for resistance to cowpea severe mosaic virus. Four lines were immune, one line was tolerant, three were resistant, twelve were moderately resistant and others were susceptible. None possessed hypersensitive resistance

Lima *et al.* (1998) carried out a study that involved 44 genotypes, confirmed the immunity of genotypes TVu 379, TVu 382, TVu 966, and TVu 3961 to three strains of CSMV.

Bashir *et al.* (2000) screened several cowpea varieties from IITA and observed that IT86F 2089-5, IT86D-880, IT90K-284-2, IT90K-76, IT86D-1010, and IT87D-611-3 were immune to blackeye cowpea mosaic. Yousafa Ali *et al. (*2002) carried out an experiment at three different locations 0f Punjab with 24 genotypes of cowpea under natural field conditions where yellow mosaic virus disease is a serious problem. The results indicated that genotypes IT-95K-1156-3, IT-94K-137-6, IT-97K-9042-8 and IT-84-552 showed highly resistant to resistant reaction, IT-94K-440-3 and ITK-238-3 gave tolerant to moderately tolerant reaction and II-97K-461-4 showed moderately tolerant to susceptible reaction.

Henry *et al.* (2003) surveys were conducted in the farmer fields in Bikaner, Rajasthan, India, during kharif 2000 and 2001 to determine the spread of yellow mosaic virus (YMV) disease and the extent of damage caused by the disease on moth bean. It was observed that YMV was the most important disease of moth bean in the region during both years. The disease intensity was higher in 2001 than in 2000. The disease was observed mainly on local cultivars. On the other hand, improved cultivars RMO-40, RMO-257, RMO-435 and Jwala, had 0-10 per cent disease intensity.

Ali *et al.* (2004) tested Twenty-six cowpea cultivars were tested for yellow mosaic virus resistance at NIAB Faisalabad. NCPM-1, Elite, IT-84-552, No.44, P-518, S.A. Dandy, IT-97k-350-4, IT-93K-452, IT-97K-1042-8 and IT-97K-497-2 showed resistant reaction to yellow mosaic virus disease.

The viruses causing yellow mosaic diseases of legumes across Southern Asia are identified as 4 distinct bipartite Begomo viruses namely mungbean yellow mosaic virus, horsegram yellow mosaic virus and dolichos yellow mosaic virus (Javaria qazi *et al.*, 2007).

Marappa *et al.*, (2007) screened 430 genotypes of mungbean for MYMV resistance. Eighteen genotypes showed field resistance to MYMV and 12 genotypes viz., CO 4, PMB 43, PBM, OBGG 11, AKM 9911, AKM 8803, BL 849, KM 1883 retained resistance to MYMV even after glass house screening.

Naimuddin *et al.* (2011) the causal virus was identified by PCR using specific primers designed to amplify a segment of DNA A that contained CP gene of four begamo viruses viz., Mungbean yellow mosaic virus (MYMV), Mungbean yellow mosaic virus India virus (MYMIV), Horsegram yellow mosaic virus (HgYMV) and Dolichos yellow mosaic

virus (DoYMV) and a segment of DNA B that contains movement protein gene. Attempts showed that the virus causing yellow mosaic in wild species/sub-species of Vigna grown at Kanpur is caused by MYMIV.

2.5.2. Bacterial leaf blight

Prakash (1980) conducted a bacterial blight screening experiment and revealed that sixteen out of 225 varieties were considered to be resistant; none was immune. Continuous variation in resistance was observed in the F_2 and backcross generations from crossing resistant 779 with four susceptible varieties. The variation in the F_1, F_2 and backcross generations was within the parental limits. Heritability estimates of resistance were moderate to high. Additive and dominance effects were greater than epistasis.

Prakash *et al.* (1982) screened Two hundred and twenty one genotypes of cowpea *[Vigna unguiculata* (L.) Walp.) for bacterial blight *Xanthomonas campestris* pv. *vignicola* (Burkholder) Dye (syn. *X. vignicola)* reaction and were scored on a 0-5 scale. Sixteen genotypes were found to be highly resistant and PLS65/1 was the most resistant line followed by C-20, C-2595, MS-9671, Vita-3, Tvx-201, Tvx-944-02E, P-G, 779, MS-9363, Tvx-7-54, V-11, PLS-35, C-2085, MS-

Adebitan and Olufajo (1998) twelve cowpea varieties were screened for resistance to major diseases under field condition. Varying degrees of susceptibility or resistance were noticed for different diseases. Four varieties were resistant to bacterial blight, while one (IT 81-D-994) showed severe disease infection. Only IAR 7/180-4-5 showed multiple resistances to three diseases.

Bua *et al.* (1998) twenty six cowpea lines were field evaluated for resistance to *Xanthomonas campestris* pv *vignicola* for two seasons in two locations of Uganda following artificial inoculation. During the first rains,

the highest disease incidences (54.96%) and (54.53%) were recorded in the lines IT82E-12 and SLA 59, respectively. During the second rains, however, the highest disease incidence (65.85%) was recorded in the line Ife Brown. Based on AUDPC values, lines were grouped into four categories, i.e. resistant, moderately resistant, moderately susceptible and susceptible. The lines IV 1075 and Icirikukwai were consistently classified as resistant in both seasons.

Okechukwu and Ekpo (2004) they screened 96 cowpea varieties, evaluated earlier in breeders_ fields as resistant to *Xanthomonas campestris* pv. *vignicola,* in field and greenhouse under high inoculums pressure. There were significant differences (P 0.05) in the reactions of cowpea varieties to bacterial blight in terms of disease incidence and severity. Results from the field screening showed that there were 69 susceptible, 25 moderately susceptible, and 2 resistant varieties. In artificial inoculation in the green house IT81D-1228-14, IT82E-16, IT93K-2271-2-2, TVu 1235, and TVu 4630, which were moderately susceptible in the field showed a susceptible reaction. Tvu 12349 and Tvu 15549 gave consistent reactions in the field and in the greenhouse and are therefore good sources of stable resistance to bacterial blight pathogen.

Ajeigbe *et al.* (2009) twenty five cowpea lines including two local land-races were grown at three locations in the first year (2002) and at six locations in the second year (2003) in Nigeria and Niger for field screening for resistance to Bacterial blight. About 44% of the varieties tested were resistant to bacterial blight while 20% showed moderate resistance and others were susceptible. Several improved cowpea breeding lines, IT98K-506-1, IT97K-1113-7, IT97K- 1069-6, IT97K-1092-2, IT97K-1069-5, IT98K-131-2 and IT97K-568-18 produced higher grain and fodder yields than the other varieties and showed combined resistance to the disease.

Okechukwu *et al.* (2010) they carried out a study by using seeds of five cowpea varieties (TVx 12349, IT86D-721, IT82D-889, Ife Brown and TVx 3236) artificially inoculated with three bacterial isolates (Ikenne, Kano and Ibadan), and seeds harvested from infected plants. Results showed that seed to plant transmission caused 6 – 24% post emergence seedling mortality and 26 – 49% incidence of blight in plants raised from infected seeds. These results support seed transmission of *X. campestris* pv. *vignicola* in cowpea and suggest that the distant spread of bacterial blight on cowpea may also be due to seed transmission.

2.6 Studies on genetic variability, heritability and genetic advance in F_2 segregating populations

The possibility of achieving improvement in any crop plants primarily depends on the magnitude of genetic variability. Though heritability value of a trait indicates the effectiveness of selection based on phenotypic expression, though selection is the product of its heritability, phenotypic standard deviation and selection differential (Burton and De vane, 1953).

The brief reviews pertaining to the genetic variability, heritability and genetic advance. In different segregating populations in cowpea is summarized in Table 3.

2.7 Genetic studies on cowpea mosaic virus and Bacterial leaf blight disease resistance

2.7.1 Cowpea mosaic virus

The first step in the study of genetics of viral resistance is to determine whether the resistant response is inherited, if so, the number of genes involved and their mode of inheritance. More than 80% of reported viral resistance is monogenically controlled; the remainder

Table 3: Review of literature on heritability, genetic advance, genotypic coefficient of variation and phenotypic coefficients of variation in segregating populations of cowpea

Character	Materil used	Heritability	Genetic advance	GCV (%)	PCV (%)	References
Plant height (cm)	F_2 population of $_2$ crosses	5.61-27.8	1.7-6.18 -4.64	7.23-1	30.58-4.64	Rangaiah and Nehru (1998)
	F_2, F_3 population of 4 crosses	47.4-79.58	15.14-27.06	10.89-14.72	15.87-15.19	Mehata and Zaveri (1999)
	F_2 population of $_3$ crosses	47.63-59.39	6.14-8.63	14.25-16.86	20.64-21.88	Hadapad (2001)
	F_2, BC_1F_1 and $BC1_2$ F1of three crosses	37.17-58.26	-	10.25-16.79	16.81-21.99	Suma Biradar (2001)
Branches/Plant	F_1s, F2s, BC_1 and BC $_2$ of 2 crosses	57.90-74.40	-	-	-	Biradar *et al.* (1993)
	F_2 population of 2 crosses	37.58-34.03	1.05-1.26	29.07-26.32	47.39-45.12	Rangaiah and Nehru (1998)
	F_2, F_3 enerations of 4 crosses	82.88-88.33	32.00-40.7	17.57-21.68	19.3-23.79	Mehta and Zaveri (1999)
	F_2 population of 3 crosses	37.73-54.86	1.40-2.03	21.61-33.36	34.45-45.04	Hadapad (2001)
Pods per plant	F_2 population	80.33	11.38	41.22	45.99	Gowda *et al.* (1991)
	F_2, F_3 enerations of 4 crosses	76.75-73.5	33.65-39.5	18.64-22.37	21.28-26.03	Mehta and Zaveri (1999)
	F_2, BC_1F_1 and BC_1P_1, F_1 of 3 crosses	70.84	-	39.67-43.43	47.3-51.26	Suma Biradar (2001)
	F_2 populations of 3 crosses	62.76-73	8.53-12.6	36.95-43.5	46.64-50.8	Hadapad (2001
Pod length (cm)	F_2 population of 2 crosses	68.53-96-97	3.17-16.03	14.13-58.23	16.95-59.51	Rangaiah and Nehru(1998)
	F_2, F_3 population of 4 crosses	48.56-69.34	8.99-18.92	6.26-11.03	8.99-13.24	Mehta and Zaveri (1999)

Table 3: continued….

	F_2, BC_1P_1 F_1 and BC_1P_2, F_1 of 3 crosses	33.67-91.05	-	7.49-30.14	11.85-31.59	Suma Biradar (2001)
	F_2 population of 3 crosses	57.46-74.1	1.67-2.30	7.18-8.99	8.56-10.08	Hadapad (2001)
Seeds per pod	3 F_2 population	70.16	3.18	19.24	22.92	Gowda et al. (1991)
	F_2 population of 2 crosses	31.62-48.89	1.39-2.94	11.01-20.08	19.59-20.7	Rangaiah and Nehru(1998)
	F_2, F_3 population of 4 crosses	73.06-78.02	23.19-22.88	13.17-12.57	15.4-14.23	Mehta and Zaveri (1999)
	F_2, BC_1P_1 F_1 and BC_1P_2 F_1 of 3 crosses	56.07-84.74	-	12.41-17.06	16.89-19.59	Suma Biradar (2001)
	F_2 population of 3 crosses	82.40-87.4	2.58-3.45	11.33-14.58	12.49-15.6	Hadapad (2001)
Test weight (g)	3 F2 population	86.00	13.60	21.22	22.99	Gowda et al. (1991)
	F2 population of 2 crosses	14.47-58.5	0.54-2.28	7.05-15.12	18.55-17.75	Rangaiah and Nehru(1998)
	F2, BC11 F1 and BC12 F1 of 3 crosses	11.02-83.96	-	9.29-26.24	22.13-27.97	Suma Biradar (2001)
	F_2 population of 3 crosses	90.63-91.63	2.76-4.26	12.4-19.02	12.6-19.98	Hadapad (2001)
Seed yield /plant	3 F_2 population	85.87	14.31	45.47	49.09	Gowda et al. (1991)
	F_2 population of 2 crosses	70.5-75.28	8.77-14.86	54.93-53.12	65.38-61.2	Rangaiah and Nehru (1998)
	F_2, F_3 population of 4 crosses	77.2-76.9	36.41-41.6	20.11-23.03	28.89-26.26	Mehta and Zaveri (1999)
	F_2, BC1P1, BC1P2, and F1 of 3 crosses	53.97-92.16	-	42.93-62.96	58.43-68.71	Suma Biradar (2001)
	F_2 population of 3 crosses	61.97-73.85	11.26-13.64	33.68-49.40	42.78-57.48	Hadapad (2001)

shows oligogenic or polygenic control. Only slightly more than half of all reported monogenic resistance traits show dominant inheritance.

In most but not all cases, dominance has been reported as complete. The heterozygote may show a clearly different response from that of the homozygote; however this is rarely checked carefully in inheritance studies. Where incomplete dominance is observed, there are important implications for mechanisms that may involve gene dosage effects. The relatively high proportion of recessive viral R genes is in marked contrast to fungal or bacterial resistance where most reported resistance is dominant.

As soon as Mendel's work was rediscovered, Biffen (1905) illustrated that disease resistance may be inherited in accordance with Mendelian laws, and the genetic basic for breeding disease resistant varieties was developed. From that many resistant genes were discovered in a wide range of crops. Many genes resistance to virus diseases were identified in cowpea, such as bean yellow mosaic virus resistance controlled by a single recessive gene (Reeder *et al.*, 1972), cowpea chlorotic mottle virus resistance controlled by a single recessive gene (Rogers *et al.*, 1973), cowpea mottle virus controlled by single dominant gene (Bliss and Robertson, 1971), cowpea severe mosaic virus controlled by a single recessive gene and cucumber mosaic virus resistance controlled by a single dominant gene (Sinclair and Walker,1955). Many other viruses' virulence in cowpea were described in detail with their distinct symptoms and genetic base resistance.

Bliss and Robertson (1971) reported that CYMV caused varied symptoms differ with cowpea variety. Systemic symptoms in susceptible varieties range from an inconspicuous light green mottle to a distinct yellow mosaic, leaf distortion with significantly reduced growth and premature death of plant. The first symptoms of yellow mosaic are

manifested by its damage to the host plant cells causing yellow specks and spots on the leaves (Verma *et al.*, 1991). The leaves emerging from the apex show bright yellow patches interspersed by green areas. Later on the specks coalesce and form bigger spots with yellow area. In severe cases whole leaves become yellow and these symptoms later appear on pods also leading to the formation of shriveled grains. The infected plants also become stunted in growth. The size of the pods and seed reduced.

Lima and Nelson (1977) identified the cultivar Macaibo as having immunity to CSMV and showed that inheritance of this resistance is conditioned by a recessive gene

Thakur *et al.* (1977) studied genetical relationships between reactions to bacterial leaf spot (BLS), yellow mosaic virus (YMV) and *Cercospora* leaf spot (CLS) diseases in mungbean and observed that BLS and CLS were under monogenic dominant gene control where as MYMV was under the control of monogenic recessive gene.

Taiwo *et al.* (1981) made crosses between the resistant cowpea line TVU 2480 obtained from the International Institute of Tropical Agriculture (IITA) and the susceptible domestic cultivar Early Ramshorn were to determine the inheritance of resistance to black eye cowpea mosaic virus (BECMV). Evaluation of F_1, F_2 and reciprocal backcross populations by symptomatology and enzyme-linked immunosorbent assay (ELISA) clearly indicated that a single recessive gene controls the high level of resistance.

Verma and Singh (1986) reported the allelic relation of genes giving resistance to MYMV in two resistant lines of black gram, UPU-2, Pant U 84. F_1, F_2, F_3, populations along with their parents were evaluated during *kharif* season against MYMV. Disease reaction of parents and their F_1's were found to be resistant. All the observation of F_2, F_3

generations confirmed that the two recessive genes were responsible for MYMV resistance.

Kumar *et al.*, (1994) the gene for cowpea yellow mosaic virus resistance was reported controlled by a single dominant gene 'Dixielee' variety is resistant to CYMV due to the dominant gene *Ymr*. In addition, tolerance reaction to CYMV was also reported due to the contribution of three additive loci and the tolerance variety (Alabunch) was probably homozygous for the three genes. The *Ymr* resistance gene segregates independently of the three tolerance genes. The presence of the *Ymr* dominant allele masked the effects of the three additive loci, with tolerant and susceptible plants being seen only when the resistance gene was homozygous recessive (*ymr/ymr*).

Ammavasai *et al.* (2004) studied inheritance of resistance to mungbean yellow mosaic virus in green gram in three susceptible, three resistant parents and resulting P_1, P_2, F_1, BC_1, BC_2, and F_2 generations. The expected susceptible: resistant ratio of 15:1 for three crosses indicated presence of duplicate gene action. Recessive gene was reported to govern the resistance

Pathmanathan Umaharan *et al. (*2004) P_1, P_2, F_1, F_2, BC_1 and BC_2 generations of four resistant × susceptible crosses and three resistant × resistant crosses of cowpea (*Vigna unguiculata* L. Walp) were screened for resistance to cowpea severe mosaic virus (CPSMV), in an insect protected screen house. The segregation ratio, at maturity, showed a 63 susceptible: 1 resistant ratio in the F_2 generation indicating that resistance is governed by three major genes. The backcross tests and the F_3 test confirmed this. The intermediate levels of symptoms observed in the F_1 generation and the progression of symptom expression in the F_2 generation suggested that resistance is gene dosage dependent.

Gupta *et al.* (2005) examined inheritance of MYMV in F_1, F_2 and F_3 populations of inter varietal crosses of blackgram involving eight resistant and six susceptible cultivars. The putative dominant gene is completely dominant in F_1 and segregated 3:1 (resistant: susceptible) in F_2 populations in eight crosses where resistant and susceptible parents were involved.

Marappa *et al.,* (2007) studied inheritance of resistance to MYMV in mungbean crosses involving five resistant genotypes and five susceptible cultivars. Based on the study of parents, F_1, F_2 and F_3, he concluded that MYMV resistance is controlled by single dominant gene.

It is evident from the literature that the resistance to cowpea mosaic virus is governed by single dominant gene. However, in contrast some scientists revealed that the resistance is governed by single recessive gene.

2.7.2 Bacterial leaf blight

Bliss and Robertson (1971) reported single dominant gene for resistance to cowpea mosaic virus, expressed as necrotic local lesions in the inoculated primary leaves.

Singh and Chaudhry (1977) studied the inheritance of resistance to bacterial blight caused *Xanthomonas campestris* pv. *Vignicola* in cowpea using CSV. P-309, P-426, P-910 and Iron as resistant parents along with P-85-1, P-85-2 as susceptible parents. They found that the resistance is governed by a single dominant gene in P-309, P-426 and P-910 and by single recessive gene in Iron genotypes.

Prakash (1980) reported in cowpea that the susceptibility was dominant over resistance. However, the effect of modifying factors and interaction of environment on disease reaction cannot be excluded.

Prakash and Shivashankar (1982) studied inheritance of resistance to bacterial blight of cowpea caused by *Xanthomonas campestris* pv. *vignicola* in the field, crossing a resistant parent '779' with four susceptible cultivars. Susceptibility was dominant over resistance and segregating patterns did not fit into any simple genetic ratios. The resistance appeared to be inherited quantitatively and segregation was affected by genetic background of parents and modifying factors.

The earlier few workers reported that the susceptibility is dominant over the resistance and some scientists revealed that the single dominant gene is responsible for the resistance to the BLB in cowpea.

2.8. Identification of DNA markers linked to BLB disease resistance

DNA molecular markers were defined as DNA sequences that are characteristic of an individual, a group of individuals, of species, even of systematic groups. They are extremely useful for individual and varietal identification, the establishment of phylogenetic relationship, population genetics and for marker assisted selection. Most points on molecular marker based genetic linkage maps are anonymous DNA polymorphisms and do not correspond to any gene of known function. However, some molecular markers (including coding DNA and expressed sequence tag markers, as well as isozyme markers) do pinpoint individual genes. Anonymous DNA markers are generated by a wide variety of techniques, differing in their reliability, difficulty, expense, and nature of polymorphism that they detect. Because of these differences, they also vary greatly in their stability for various uses. DNA markers may be hybridization based (RFLP) or PCR based (RAPD, AFLP, SSRs etc.). DNA markers may detect single locus, oligo-locus, or multiple locus differences and markers detected may be inherited in a presence/ absence, dominant, or co-dominant.

Kelly *et al.* (1998) the cowpea map spans 2670 cM with over 400 markers. In addition to molecular markers, both maps include map locations of defense genes and phenotypic traits for disease and insect resistance, seed size, color and storage proteins, pod color and those traits associated with the domestication syndrome in bean. Since the bean and cowpea maps were developed independently, LGs with the same number probably refer to non-syntenic groups. Map locations of major resistance genes in bean are revealing gene clusters on LGs B1, B4, B7, and B11 for resistance to bean rust, anthracnose, common bacterial blight and white mold.

Matthew *et al.* (2007) developed a co-dominant sequence-characterized amplified region (SCAR) marker, SR2, based on a previously identified random amplified polymorphic DNA (RAPD) marker that is tightly linked to the *bgm*-1 resistance gene and identified the position of the locus in the common bean genome through comparative mapping using two genetic maps for the species. The SR2 marker was mapped relative to *bgm*-1 in a segregating population of recombinant inbred lines developed from the resistant x susceptible cross of DOR476 x SEL1309. Polymorphism was shown to be based on a 37 bp insertion event in the SR2 allele associated with susceptibility compared to the allele associated with resistance and the marker mapped at a distance of 7.8 cM from the resistance gene.

Soumitra Maiti *et al.* (2010) reported that resistance-linked molecular markers were successfully developed from consensus motifs of other resistance (R) gene or R gene homologue sequences. Applying linked marker assisted genotyping, plant breeders can carry out repeated genotyping throughout the growing season in absence of any disease incidence. Two MYMV resistance marker loci, YR4 and CYR1, were identified and of these two CYR1 is completely linked with MYMIV-

resistant germplasms and co-segregating with MYMIV-resistant F_2, F_3 progenies of urdbean.

Agbicodo *et al.* (2010) carried out a study to identify loci with effects on resistance to this pathogen and map QTLs controlling resistance to CoBB, eleven cowpea genotypes were screened for resistance to bacterial blight using 2 virulent Xav18 and Xav19 strains isolated from Kano (Nigeria). Two cowpea genotypes Danila and Tvu7778 were identified to contrast in their responses to foliar disease expression following leaf infection with pathogen. A set of recombinant inbred lines (RILs) comprising 113 individuals derived from Danila (resistant parent) and Tvu7778 (susceptible parent) were infected with CoBB using leaf inoculation method. A single nucleotide polymorphism (SNP) genetic map with 282 SNP markers constructed from the same RIL population was used to perform QTL analysis. Using Kruskall-Wallis and Multiple-QTL model of Map QTL 5, three QTLs, CoBB-1, CoBB-2 and CoBB-3 were identified on linkage group LG3, LG5 and LG9, respectively showing that potential resistance candidate genes co-segregated with CoBB resistance phenotypes.

Material and Methods

III. MATERIAL AND METHODS

The present investigation was initiated during *Kharif* 2009 at Gandhi Krishi Vignana Kendra, University of Agricultural Sciences, Bangalore, which is situated at an altitude of 930 m, latitude of $13^{0}.15'.00$ N and longitude of $77^{0}.32'.00$ E which represents the Eastern Dry Agro-climatic Zone (Zone 5) of Karnataka. The location receives an average annual rainfall of about 930 mm in bimodal distribution with two peaks one being in April-May and other in September-October. The details of the experimental material and methods adopted in the present investigation are described here under.

3.1 Analysis of morphological and molecular diversity in cowpea germplasm

3.1.1 Experimental material

To start with the experimental material for the present study comprised of 196 diverse cowpea genotypes including released varieties maintained at the AICRP on Arid Legumes, Gandhi Krishi Vignana Kendra, Bangalore. The lists of genotypes are presented in the Table 4 and the mean values of morphological parameters are presented in Appendix 1.

3.1.2 Experimental Methods

One hundred and ninety six genotypes of cowpea (Table 4) were sown during *Kharif* 2009 in Simple Lattice Design, in three meter long rows with spacing of 60 cm x 30 cm. Though the recommended row spacing for cowpea is 45 cm, the genotypes were sown in 60 cm row spacing in order to provide an opportunity for all genotypes to express their full genetic potential and also to avoid interlocking of plants. Standard agronomic practices as per the recommendation in package of practices for cowpea were followed.

Table 4. List of cowpea germplasm lines used in the disease screening and morphological diversity analysis

S.N.	Germplasm line	Source	S.N.	Germplasm line	Source	S.N.	Germplasm line	Source	S.N.	Germplasm line	Source
1	EC 458489	NBPGR, New Delhi	31	CPD 31		61	IC 201©	NBPGR, New Delhi	91	ETC 27	-
2	IC 402101	NBPGR, New Delhi	32	EC 394779	NBPGR, New Delhi	62	IC 249588	NBPGR, New Delhi	92	C 24-1	IARI, New Delhi
3	NBC 14	-	33	NBC 42	NBPGR, New Delhi	63	IC 402125	NBPGR, New Delhi	93	CPD 15	-
4	IC 402166	NBPGR, New Delhi	34	IC 330996	NBPGR, New Delhi	64	IC 402098	NBPGR, New Delhi	94	EC 48475	NBPGR, New Delhi
5	V 24O	IARI, New Delhi	35	IC 402166	NBPGR, New Delhi	65	V 578 ©	IARI, New Delhi	95	EC 394779	NBPGR, New Delhi
6	202804(83)	GKVK, Bangalore	36	IC 402164	NBPGR, New Delhi	66	NBC 27	-	96	IC 1071	NBPGR, New Delhi
7	V 585	IARI, New Delhi	37	EC 472250	NBPGR, New Delhi	67	NB 12	-	97	198355(45)	GKVK, Bangalore
8	C 325	IARI, New Delhi	38	IC 402114	NBPGR, New Delhi	68	NBC 7	-	98	EC 458469	NBPGR, New Delhi
9	EC 458506	NBPGR, New Delhi	39	V 585	IARI, New Delhi	69	EC 458417	NBPGR, New Delhi	99	IC 202797(97)	NBPGR, New Delhi
10	IC 249593	NBPGR, New Delhi	40	EC 170584	NBPGR, New Delhi	70	IC 330996	NBPGR, New Delhi	100	C 720	IARI, New Delhi
11	IT 97K 499-38	IITA, Negeria	41	NBC 32	-	71	EC 390287	NBPGR, New Delhi	101	IC 202781	NBPGR, New Delhi
12	IC 402180	NBPGR, New Delhi	42	EC 458402	NBPGR, New Delhi	72	EC 170584-1-1	NBPGR, New Delhi	102	IC 402101	NBPGR, New Delhi
13	202854(97)	GKVK, Bangalore	43	NBC 39	-	73	V 16	IARI, New Delhi	103	IC 402175	NBPGR, New Delhi
14	EC 472257	NBPGR, New Delhi	44	NBC 29	-	74	EC 458440	NBPGR, New Delhi	104	TOME 77-4	-
15	IC 249141	NBPGR, New Delhi	45	EC 458442	NBPGR, New Delhi	75	IC 249593	NBPGR, New Delhi	105	EC 458418	NBPGR, New Delhi
16	EC 170584	NBPGR, New Delhi	46	NBC 33	-	76	EC 170578-1-1	NBPGR, New Delhi	106	NBC 19	-
17	EC 472252	NBPGR, New Delhi	47	EC 458470	NBPGR, New Delhi	77	NBC 40	-	107	GENOTYPE 36	GKVK, Bangalore
18	IC 202867(99)	NBPGR, New Delhi	48	IC 402172	NBPGR, New Delhi	78	IC 202779	NBPGR, New Delhi	108	EC 394779	NBPGR, New Delhi
19	GC 5	Gujarat	49	IC 257428	NBPGR, New Delhi	79	EC 458402	NBPGR, New Delhi	109	EC 472252	NBPGR, New Delhi
20	IC 1071	NBPGR, New Delhi	50	IC 402048	NBPGR, New Delhi	80	202705(49)	GKVK, Bangalore	110	EC 458425	NBPGR, New Delhi
21	EC 458411	NBPGR, New Delhi	51	IT 97499-38	IITA, Negeria	81	NBC 42	-	111	EC 458505	NBPGR, New Delhi
22	IC 402161	NBPGR, New Delhi	52	CPD 19	-	82	IC 402159	NBPGR, New Delhi	112	EC 394708	NBPGR, New Delhi
23	IC 2591054	NBPGR, New Delhi	53	NBC 6	-	83	C 517	-	113	GC 3(C)	Gujarat
24	IC 462099	NBPGR, New Delhi	54	202827(92)	GKVK, Bangalore	84	IC 402162	NBPGR, New Delhi	114	IC 402104	NBPGR, New Delhi
25	IC 58905	NBPGR, New Delhi	55	IC 198326(38)	NBPGR, New Delhi	85	IC 202290	NBPGR, New Delhi	115	NBC 38	-
26	NBC 30	-	56	EC 170585(B9)	NBPGR, New Delhi	86	NBC 41	-	116	IC 2591054	NBPGR, New Delhi
27	EC 458473	NBPGR, New Delhi	57	EC 402159	NBPGR, New Delhi	87	IC 402104	NBPGR, New Delhi	117	IC 202782	NBPGR, New Delhi
28	IC 402182	NBPGR, New Delhi	58	V 578-17	IARI, New Delhi	88	KBC 2	GKVK, Bangalore	118	IC 402174	NBPGR, New Delhi
29	IC 202777	NBPGR, New Delhi	59	IC 249793	NBPGR, New Delhi	89	IC 402159	NBPGR, New Delhi	119	IC 4506	NBPGR, New Delhi
30	IC 170574	NBPGR, New Delhi	60	C 131+CB-2	GKVK, Bangalore	90	V 578	IARI, New Delhi	120	CB 10	

Table: 4. Continued….

S.N.	Germplasm line	Source	S.N.	Germplasm line	Source	S.N.	Germplasm line	Source
121	IC 402098	NBPGR, New Delhi	151	IC 402154	NBPGR, New Delhi	181	EC 472267	NBPGR, New Delhi
122	201095(52)	GKVK, Bangalore	152	EC 458440	NBPGR, New Delhi	182	CB 10	-
123	EC 458480	NBPGR, New Delhi	153	IC 202781	NBPGR, New Delhi	183	IC 402164	NBPGR, New Delhi
124	EC 458483	NBPGR, New Delhi	154	NBC 44	-	184	NBC 40	-
125	C 1071	IARI, New Delhi	155	C 33	IARI, New Delhi	185	NBC 24	-
126	EC 458489	NBPGR, New Delhi	156	27749(25)	GKVK, Bangalore	186	C 152	GKVK, Bangalore
127	EC 458473	NBPGR, New Delhi	157	NBC 48	-	187	TVX 944	IITA, Negeria
128	EC 394839	NBPGR, New Delhi	158	IC 198329(36)	NBPGR, New Delhi	188	KBC 2	GKVK, Bangalore
129	EC 394838	NBPGR, New Delhi	159	NBC 43	-	189	KM 5	GKVK, Bangalore
130	EC 458418	NBPGR, New Delhi	160	V 585©	IARI, New Delhi	190	TC 201	-
131	IC 402159	NBPGR, New Delhi	161	NBC 36	-	191	HC 03-02	Hissar
132	NBC 10	-	162	IC 253251	NBPGR, New Delhi	192	P 695	GKVK, Bangalore
133	IC 1061	NBPGR, New Delhi	163	EC 472250	NBPGR, New Delhi	193	TCM 44-1	-
134	IC 10171	NBPGR, New Delhi	164	EC 458402	NBPGR, New Delhi	194	APC 243-1-865	-
135	EC 458438	NBPGR, New Delhi	165	EC 458441	NBPGR, New Delhi	195	HC 9866	Hissar
136	IC 249593	NBPGR, New Delhi	166	NBC 38	-	196	GC 4	Gujarat
137	IC 402162	NBPGR, New Delhi	167	NBC 18	-			
138	NBC 51	-	168	EC 458480	NBPGR, New Delhi			
139	97767(10)	GKVK, Bangalore	169	EC 394839	NBPGR, New Delhi			
140	IC 249141	NBPGR, New Delhi	170	NB 47	-			
141	NBC 9	-	171	GC 3	Gujarat			
142	IC 206240	NBPGR, New Delhi	172	IC 402090	NBPGR, New Delhi			
143	EC 458430	NBPGR, New Delhi	173	NBC 8	-			
144	V 604-7-29-3	IARI, New Delhi	174	EC 458469	NBPGR, New Delhi			
145	IC 49586	NBPGR, New Delhi	175	C 457	IARI, New Delhi			
146	202827(93)	GKVK, Bangalore	176	NBC 7	-			
147	EC 458490	NBPGR, New Delhi	177	EC 458453	NBPGR, New Delhi			
148	IC 202711(58)	NBPGR, New Delhi	178	IC 402161	NBPGR, New Delhi			
149	C 458492	IARI, New Delhi	179	IC 402106	NBPGR, New Delhi			
150	IC 25105	NBPGR, New Delhi	180	EC 472271	NBPGR, New Delhi			

3.1.3 Observations recorded on quantitative parameters.

The observations on disease reaction and other yield attributing characters were recorded on all 196 cowpea genotypes. Five plants selected at random were tagged from each genotype and observations on following quantitative traits were recorded on these plants.

3.1.3.1 Days to 50 per cent flowering

Number of days from date of sowing to the day on which 50 per cent of the plants in each genotype flowered were counted.

3.1.3.2 Days to maturity

Numbers of days from date of sowing to the day on which all the plants attain physiological maturity were counted.

3.1.3.3 Plant height (cm)

The plant height was measured in centimeters (cm) from ground level to tip of main stem above the ground at the time of maturity.

3.1.3.4 Number of branches per plant

Number of branches were counted in each tagged plant and averaged to get number of branches per plant.

3.1.3.5 Pods per plant

Number of dry pods were counted at the time of maturity on the five randomly selected plants and averaged to get pods per plant.

3.1.3.6 Pod length (cm)

Length of five dry pods from each of the selected plant was recorded and average value for five plants was expressed in centimeters (cm).

3.1.3.7 Seeds per pod

Average number of seeds in five randomly selected pods from each randomly selected plant were counted and later averaged to get number of seeds per pod.

3.1.3.8 Test weight (g)

One hundred seeds were counted from each five randomly selected plants and their weight was recorded as test weight and expressed in grams (g).

3.1.3.9 Seed yield per plant (g)

The weight of seed per plant was recorded in grams and averaged over five randomly selected plants and expressed as mean seed yield per plant in grams (g).

3.1.4 Observations recorded on qualitative characters

3.1.4.1 Flower colour

The colour of the opened flower (petals) was recorded at the time of flowering in the morning between 7 am to 9 am.

3.1.4.2 Plant habit

The plant type was classified into erect, semi-spreading, and spreading plant habits, which were recorded after complete growth of the plant in each genotype.

3.1.4.3 Pod pigmentation

The pigmentation on green pod was recorded at maturity stage before it started drying.

For the sake of comparison between morphological and molecular diversity, only 60 genotypes (Table 5) were selected based on the Mahalanobis D^2 analysis which revealed 22 clusters by using Statistica 2 software. From each cluster randomly selected genotypes were used for the study. The data on selected 60 genotypes from among 196 were used for separate analysis of morphological and molecular analysis.

3.1.5 Molecular characterization

3.1.5.1 Experimental material

Since the primers and other consumables used are costly and molecular characterization of the entire 196 genotypes was cumbersome, only 60 genotypes (Table 5) were selected based on the Mahalanobis D^2 analysis which revealed 22 clusters. From each cluster randomly selected genotypes were used for the study. These 60 genotypes were screened with 42 cowpea specific SSR markers (Table 6) obtained from Krikhouse Trust, UK., for cluster analysis.

3.1.5.2 Isolation of genomic DNA by CTAB method (Sambrook *et al.*, 2001)

Cetyl trimethyl ammonium borate is a detergent which is used along with other reagents to liberate nucleic acids from the cell. This is an efficient method for isolating the plant genomic DNA from leaf tissues. The high molecular weight DNA obtained is purified by phenol: chloroform method to remove proteins and other plant debris.

3.1.5.3 DNA isolation Protocol

1. Leaves were washed in distil water, excess water was removed by using blotting paper and dried properly.
2. One gram of leaf tissues from each individual genotype was weighed separately.

3. The leaf tissue was ground in a pestle and mortar by adding liquid nitrogen with PVP.
4. 5 ml of freshly prepared extraction buffer of 4 per cent CTAB, was added 10 µl 0f 2 per cent mercaptoethanol was added to each sample.
5. Extract was Poured into 2 ml eppendorf tubes in two sets and incubated at 65^{0}C in a water bath for one hour with gentle shaking.
6. Equal volume (5 ml) of 24:1 chloroform iso-amyl alcohol was added and mixed well by inverting the tubes.
7. Samples were centrifuged at 10,000 rpm for 15 minutes at room temperature.
8. To the supernatant equal volume of chilled iso-propanol was added.
9. It was kept at -20^0 C for overnight.
10. Centrifuged for 10 minutes at 5000 rpm and the supernatant was discarded
11. Pellet with 70 per cent ethanol was washed and centrifuged for 10 minutes at 1000 rpm.
12. Pellet was dried and the pellet was dissolved in appropriate amount of TE buffer (Tris-EDTA buffer).
13. DNA was purified by phenol: Chloroform and dissolved in TE buffer. Treated with RNase and stored at -20^{0}C.
14. Physical integrity of DNA was verified by electrophoresis on a 0.8% agarose gel. Finally the DNA was quantified by λ uncut DNA and captured the image in Alpha digidoc machine.

Table 5. List of cowpea germplasm lines used in the molecular diversity analysis

S.N.	Germplasm line	S.N.	Germplasm line
1	EC 458489	31	NBC 6
2	IC 402166	32	202827(92)
3	NBC 14	33	IC 198326(38)
4	202804(83)	34	IC 402125
5	V 585	35	V 578 ©
6	IC 249141	36	NBC 7
7	EC 170584	37	IC 330996
8	EC 472252	38	KBC 2
9	IC 202867(99)	39	V 16
10	GC 5	40	EC 458440
11	IC 1071	41	EC 170584-1-1
12	EC 458411	42	ETC 27
13	IC 402161	43	EC 458473
14	IC 58905	44	IC 249141
15	NBC 30	45	IC 206240
16	IC 402182	46	C 458492
17	EC 458473	47	NBC 44
18	CPD 31	48	C 33
19	NBC 42	49	27749(25)
20	IC 402166	50	C 152
21	IC 402114	51	TVX 944
22	EC 170584	52	KM 5
23	EC 458402	53	GC 3
24	NBC 39	54	HC 03-02
25	NBC 29	55	P 695
26	NBC 33	56	TCM 44-1
27	EC 458470	57	APC 243-1-865
28	IC 257428	58	HC 9866
29	IC 402048	59	GC 4
30	IT 97499-38	60	TC 201

Table 6: List of cowpea specific SSR primers used in the study

S.N.	SSR primer	Annealing temperature	Primer sequence
1	CP 61/62	55°C	F : AACGGGTCCTAAACGAATGA R: ATCCTTGAACTCCGTGTTGC
2	CP 103/104	56°C	F : TGTCCTCAATTTCAATAACAAGTTT R: AACAGTTGGTCGGATACGAAA
3	CP 115/116	55°C	F : GGGAGTGCTCCGGAAAGT R: TTCCCTATGAACTGGGAGATCTAT
4	CP 117/118	55°C	F : GTGGAAGGAATGGGTCCAG R: AGGAAATTTGCATTCCCTTGT
5	CP 121/122	55°C	F : ACCAGGTGCAATGCTTCTCT R: CCTTCCTGTCATCATTTCCAA
6	CP 125/126	56°C	F : AGTACGCACGGCAACCTTA R: GTGCAACCCTAACGCTCTTC
7	CP 131/132	55°C	F : CTCAAGCTTGGTTGAGATGAAA R: ATATCGGGCGCACTTTTGTA
8	CP 137/138	55°C	F : TACAATGAAATGGGCTGCAC R: CACGTTTTCCTTTCCTCACC
9	CP 169/170	55°C	F : TTCCACGAATCATCGACAGA R: TGTTGACTGGCAGAGGTTGA
10	CP 171/172	55°C	F : GTAGGGAGTTGGCCACGATA R: CAACCGATGTAAAAAGTGGACA
11	CP 181/182	55°C	F : TCCATGTGTTTATGACGCAAA R: GGGTGCTTTGCTCACATCTT
12	CP 197/198	56°C	F : GTTGCACTTGGTTGCCCTAT R: TGTAATGGAGCAACTTCTTGGA
13	CP 201/202	56°C	F : GGTTTCCTAGTTGGGAAGGAA R: ATTATGCCATGGAGGGTTCA
14	CP 215/216	55°C	F : CAGAAGCGGTGAAAATTGAAC R: GCATGTTGCTTTGACAATGG
15	CP 221/222	56°C	F : GCAAAGGGATCACCAAACAT R: TCGTTCAGTTGAGCCAC
16	CP 239/240	55°C	F: CACCCCCGTACACACACAC R: CACTTAAATTTTCACCAGGCATT
17	CP 273/274	55°C	F : TGGTGCTTGTAAGAAAAACAGAA R: GGAGAGCAGAAGATGAAGTGAA
18	CP 289/290	56°C	F : AAGCTGATTGTGGAACCATTG R: TGGAGGCATAAAAATGACACCT
19	CP 315/316	55°C	F : TGGAGGCATAAAAATGACACCT R: TGAAGCTGATTGTGGAACCAT
20	CP 341/342	55°C	F : ACGAAACGATGTTAATGCTGATT R:AAAAAGATTTGATGTGATCTATGATGTT
21	CP 349/350	55°C	F : TGGTGCTCAACTTCCTCACTT R: GGCACTCCTCCAGGTGACTA

Table 6: continued…

S.N.	SSR primer	Annealing temperature	Primer sequence
22	CP 379/380	55°C	F:AAACATCAAAATTAAGGATAATCAATG R: AGTAGCGTGGGTGGAATTTG
23	CP 395/396	55°C	B : GTTGTGAGCTTCCCCAGATG R: AATTTTGAACCCACCACCAG
24	CP 397/398	55°C	F : TCATGGGTTAAATTTGCTTCAA R: AAACCATGTGGTTGTTGCAC
25	CP 399/400	56°C	F : CGAAAATTCACAGAGATGCAG R: CAGTCTAACGAAGAACTGGGCTA
26	CP 427/428	55°C	F : TTCCATGTTGGTGATGCCTA R: ATCGCGGAGTTACAAGGTGT
27	CP 501/502	55°C	F : ATCTCCACCACCCCTTTTCT R: TTTAATAAAAAGTTATTCCAACTCTC
28	CP 573/574	55°C	F : GCAGAATCCTTGTGAACCTG R: TTTCGCAATATGCCCTTTTC
29	CP 605/606	55°C	F : AAAGAGATACACATGCCTAACA R: GACCAACAGCGACTTTGAGC
30	CP 641/642	55°C	F : CATAATGTCACAGAGGTGGAAAA R: TCTTTCCTTCCTTTTCACCAA
31	MS 3/4	55°C	F : GGAATTGAAATTGATCTAATG R: GTATTTAAGTGGCTTATGAGGTTG
32	MS 20/21	56°C	F : TTGTTGGTCATGTTGGGATG R: AATAGATTGTTAGGGAAAC
33	MS 24/25	56°C	F : GTTGGCTTCTGTTGTGGCAT R: GTTACACCAATGCCAAAAAC
34	MS 31/32	55°C	F : GTGACTACAATGGCGGAACT R: GGAGGTACCGAAAAGAAAG
35	MS 38/39	56°C	F : GTACCTAACAAGTATGATGAA R: AAACTTCTCCATTATTGAGT
36	MS 43/44	55°C	F : AAGTTTTTAGGGGCTATGGC R: CATTTTTTAATCCATTTTTATC
37	MS 50/51	56°C	F : CCTAAACGAATTCTACCTGG R: TTTAAAATGGTCCCTCCCGT
38	MS 53/54	56°C	F : AAGAGTGACAAGAAAGATTT R: GAAAGTATATGTTGTTAACTCT
39	MS 62/63	56°C	F : TTGAGAGGAAGGAAAGCATG R: GGTTTTTGTTTACTGTGCTA
40	MS 98/99	56°C	F : GATAAAGAGGAAAATAGACA R: AAAATGTGGCAGATAAGGAA
41	MS 99/100	56°C	F : AAACTGACACTTGAACACGA R: CTCATGCAGAGTTCAAGATC
42	MS 113/114	56°C	F : GTTAAAGTTTTCTTCATCAT R: ATCTTGATCCAGAAAATGTTT

3.1.5.4 Amplification of DNA using Polymerase chain reaction (PCR)

The PCR profile consisting of 34 cycles, and amplification condition for SSR consisted of Initial denaturation at 94^0 for 1 min, denaturation at 94^0 for 30 seconds, primer annealing vary with each primer listed in the Table 6, final extension at 72^0 for10 min, hold at 4^0 for 10 min.

Table 7: Preparation of master mix

Sl. No.	Reaction	Volume
1.	DNA template (25 ng/ µl)	2.0 µl
2.	*Taq* polymerase (5u/µl)	0.3 µl
3.	*Taq* buffer (10X)	1.2 µl
4.	dNTPs (1mM)	2.0 µl
5.	Forward Primer (10pm)	1.0 µl
6.	Reverse Primer (10pm)	1.0 µl
7.	Sterile Distilled water	4.5 µl
	Total	12.0 µl

3.1.5.5 Agarose Gel Electrophoresis (AGE)

Reagents used :

Agarose : 3 % (3g agarose in 100ml of 1X TBE)

Running Buffer : 1X TBE Buffer (54 g Tris, 27.5 g boric acid, 20 ml of 0.5M EDTA pH 8.0 in one liter sterile water)

Ethidium Bromide : 0.5 µg/ml (10 mg/ml stock)

Loading buffer : 0.25% bromophenol blue in 30% glycerol

Amplification products were resolved on 3% agarose gel containing Ethidium bromide (0.5 µg/ml) using 1X TBE (Sambrook *et al.,* 2001). 10 µl of PCR products were mixed with 4 µl of loading buffer and 6 µl was loaded in the wells in agarose gel. Electrophoresis was carried out at a constant voltage of 100V for 2 hours. The gels were visualized under UV light and documented by using gel doc unit in the computer.

3.1.5.6 Scoring of SSR generated bands

Each amplified loci considered as a unit character and was scored as '0' and '1' for different level of amplification obtained for each SSR marker.

3.1.5.7 Statistical analysis

NTSYS –pc was used to know the genetic similarity among the divese cowpea genotypes. The similarity coefficient was computed using Dice coefficient (Dice., 1945) to create similarity matrix. Dice coefficient was computed by

$$S_D = \frac{2n_{ik}}{n_i + n_k}$$

Where,

n_{ik} : The number of identical bands present in both variety i and variety k

n_i : The number of bands present in variety i but absent in variety k

n_k : Number of bands present in variety k but absent in variety i.

Genotypes were clustered using SAHN method (Sequential, Agglomerative, Hierarchical and Nested clustering) based on the similarity matrix.

Genotypes were also clustered using correlation coefficient $r_{(i,,j)}$. $r_{(i,,j)}$ was computed by using the formula,

$$r_{(i,j)} = \frac{n \, \Sigma \, Xi \, Xj - \Sigma \, Xi \, \Sigma \, Xj}{[\, n \, \Sigma \, Xi^2 - n \, (\Sigma \, Xi)^2 \,]^{0.5} \; [\, n \, \Sigma \, Xj^2 - n \, (\Sigma \, Xj)^2 \,]^{0.5}}$$

Where,

$r_{(i,j)}$: Correlation coefficient between variety i and variety j

n : The number of studied traits

x_i : The value of variety i

x_j : The value of variety j.

3.2 Field screening of cowpea genotypes for resistance to CpMV and BLB

The screening of these germplasm was undertaken at appropriate stages for the two important diseases *viz.,* cowpea mosaic virus and bacterial leaf blight. The per cent leaf area infected by these diseases has been recorded. The genotypes were grouped into different categories using disease scoring scale suggested by Muniyappa *et al.,* 1987 (Table 8) for cowpea mosaic virus and James *et al.* (1971) scoring scale for bacterial leaf blight (Table 9).

3.2.1 Cowpea mosaic virus disease scoring (CpMV)

One hundred and ninety six cowpea genotypes were screened against cowpea mosaic virus disease under field condition during September 2009. The percent disease severity on each genotype was assessed regularly at 15 days intervals. The disease severity was recorded on five trifoliate leaves on each plant. The genotypes were grouped into different categories using disease scoring scale suggested by Muniyappa *et al.,* 1987 (Table 8).

3.2.2 Bacterial leaf blight disease scoring

Disease severity observations were recorded (by using scale of James *et al.*, 1971) on five randomly chosen plants from each genotype. The mean disease scores (MDS) was calculated by adding the individual scores and divided by the number of plants observed.

The disease was scored on all the genotypes after the symptoms were seen under natural field conditions. The genotypes were grouped as highly resistant, resistant, moderately susceptible, susceptible, highly

Table 8. Disease scoring scale for cowpea mosaic virus disease (Muniyappa *et al.*, 1987)

Rating	Per cent foliage affected	Reaction
1	No visible symptoms on leaves	Resistant
2	Small necrotic yellow specks with restricted spread covering 0.1-5 per cent leaf area	
3	Mottling of leaves, covering 5.1-10 per cent leaf area	Moderately resistant
4	Yellow mottling of leaves, covering 10-15 per cent leaf area	
5	Yellow mottling and discolorations of leaves, covering 15.1-30 per cent leaf area	Moderately susceptible
6	Yellow discolorations of 30-50 per cent of leaves	Susceptible
7	Pronounced yellow mottling and discoloration of leaves reduction in leaf size and stunting of plants, and covering 50-75 per cent of foliage	
8	Severe yellow discoloration of leaves (75.1-85 per cent of foliage), stunting of plants, reduction in pod size.	Highly susceptible
9	Severe yellowing of entire leaves, stunting of plants and covering 85-100 per cent of foliage	

Table 9. Disease scoring scale for cowpea bacterial leaf blight disease (James *et al.*, 1971)

Rating	Per cent foliage affected	Reaction
0	No spots on leaves, completely free of disease	Highly resistant
1	Less than 2 per cent leaf the area of plant affected.	Resistant
2	2 to 10 per cent leaf area of plant affected.	Moderately Resistant
3	10 to 25 per cent leaf area of plant affected.	susceptible
4	25 to 40 per cent leaf area of plant affected.	Moderately Susceptible
5	More than 40 per cent leaf area of plant affected and plant dying as a result.	Very Highly Susceptible

susceptible and very highly susceptible according to their mean disease scores.

3.3 Screening of parental lines under glass house conditions

Six resistant entries *viz.*, V-16, CS-88, HC-9866 (resistant to bacterial leaf blight), HC-03-02, GC-3, GC-5 (resistant to cowpea mosaic virus) and a susceptible genotype (C-152) which was selected based on phenotypic observations under natural field conditions were subjected for confirmation under controlled conditions with artificial inoculations of both the diseases. The experiment was conducted in *Rabi* 2009 at glass house of Department of Plant Pathology, GKVK, Bangalore.

3.3.1 Cowpea mosaic virus

3.3.1.1 Preparation of inoculums for sap transmission

Young leaves of 15-20 days old, showing characteristic mosaic symptoms were collected from infected cowpea plants, washed in tap water to removed the dust particles adhering to them and dried between folds of blotting paper. The leaves were then macerated in chilled mortar and pestle using potassium phosphate buffer (pH 7.0, 0.05M) at the rate of 1ml/gm of leaf tissue. The resultant pulp was squeezed through double layered muslin cloth and the extract thus obtained was used as standard inoculum. The extracted sap was maintained in an ice cold conditioned stage (ice box). The fresh sap prepared was used on the same day for inoculation.

3.3.1.2 Method of inoculation

To the standard extract, celite (600 mesh) was added at the rate of 0.025 g\ml of the extract. The inoculum was applied gently on the upper surface of the leaves with a small piece of absorbent cotton wool. The leaves inoculated unidirectionally from petiole to tip only once by keeping

the palm beneath the leaf as a support. The 10 plants were maintained to test mechanical inoculation and symptom expression. The inoculated leaves were washed 1-2 minutes after inoculation to remove the excess of inoculum with a fine jet of distilled water from a squeeze bottle and plants were kept under observation for 15-20 days in the glass house. A sap transmissible cowpea aphid borne mosaic virus (CABMV) which produced mosaic symptoms eight to twelve days after inoculation.

3.3.2 Bacterial Blight

3.3.2.1 Isolation of Bacteria

The bacterial blight on cowpea was noticed during the *Rabi* (2009-10) in the Zonal Agricultural Research Station, GKVK, Bangalore. During field screening a typical bacterial leaf blight leaves of cowpea were collected from C-152 variety which is highly susceptible for the isolation of Bacteria [Plate 1(A)]. The causal agent was confirmed as bacteria by conducting ooze test. The bacterium was isolated by the ooze and tissue isolation methods.

3.3.2.2 Chemical composition of Nutrient broth used for bacterial growth

Chemical	Quantity
Peptone	5.00gm
Beef extract	3.00gm
NaCl	5.0g
Agar	18g
Distilled water	1000ml
pH	6.8 -7.0

3.3.2.3 Maintenance of Culture

Culture of the isolate was maintained on nutrient agar slants and sub-culture were preserved by incubating for 2 days at 30 ± 1^0 C and

then held at 4-6^0 C [Plate 1(B)]. The culture was renewed by sub-culturing once in fortnight and no variation in culture characteristics were observed even after several generations.

3.3.2.4 Isolation of the bacterial suspension for inoculation

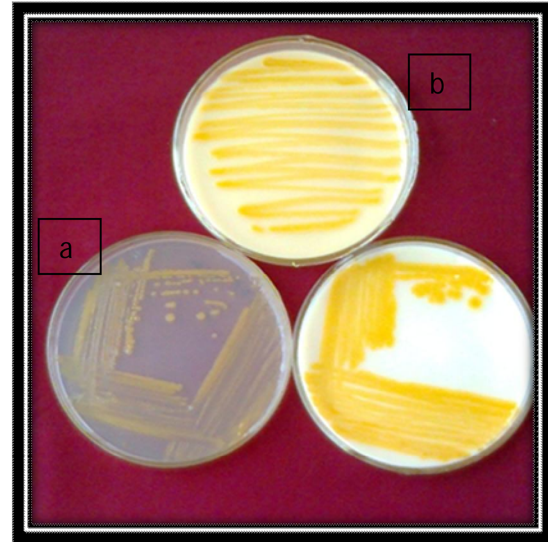
Bacteria was multiplied on nutrient agar at 10±1^0 C for 48 hours, in petriplates and then colonies were separated and suspended in sterile water and thus a suspension was prepared. The plants were grown under humid chamber for effective disease expression. The plants to be sprayed were injured by pricking method using dry thorny plant. Alcohol sterilization was done before wounding. After pricking, the bacterial suspension was sprayed in the evening hours using a hand sprayer. The plants were watered 24 hours before and after inoculation to create sufficient humidity for the development of disease. The disease appeared 8-10 days after inoculation with brownish water soaked minute spots appearing on leaf and gradually increased in size [Plate 1(C)]. The disease develops completely in 20-25 days. Plants were scored for disease on 30 days after sowing on individual plants of all the genotypes by the same scoring method adopted in the field study. Later plants were grouped according to their mean disease scores.

3.4 Genetic studies on bacterial leaf blight and cowpea mosaic virus resistance

3.4.1 Material

The material used for the study includes three parental lines *viz*. C-152 (agronomically superior but susceptible for both the diseases CpMV and BLB), V-16 (resistant to bacterial leaf blight disease) and HC-03-02 (resistant to cowpea mosaic virus disease). They were selected based on the results of evaluation under both natural field conditions and in glass house screening and used for hybridization. The experiment

(A)



(B)

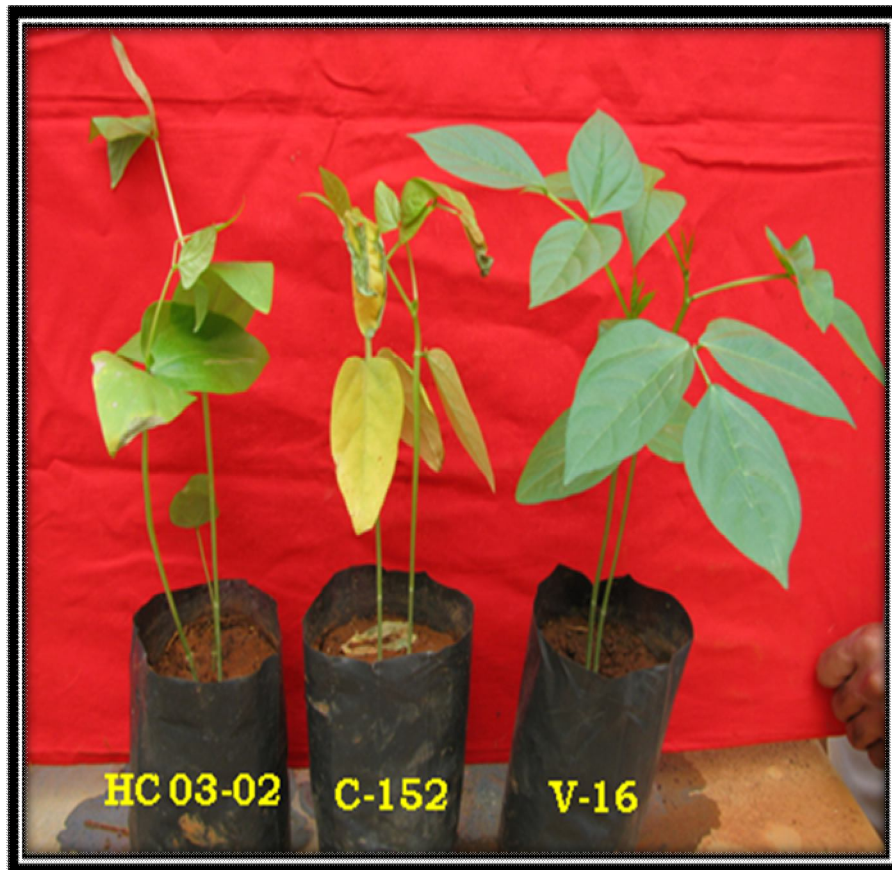


Plate 1: Cowpea leaf affected with BLB (A), Typical colonies of *Xanthomonas campestris pv. vignicola* on Nutrient Agar (a) YDCA media (b) and Cowpea genotypes with different disease reaction against *Xanthomonas campestris pv. vignicola* under artificial inoculation (C).

was carried out in summer, 2010.The salient features of the parents were presented in Table 10. They were sown in the field for the purpose of hybridization.

3.4.2 Hybridization

3.4.2.1 Emasculation: Emasculation was done on unopened flower buds during previous evening between 4-6 PM prior to day of the flower opening. An incision was made in the middle of the bud. Anthers were removed along with part of petals without damaging the gynoecium. Then the emasculated flower buds were covered with butter paper bag.

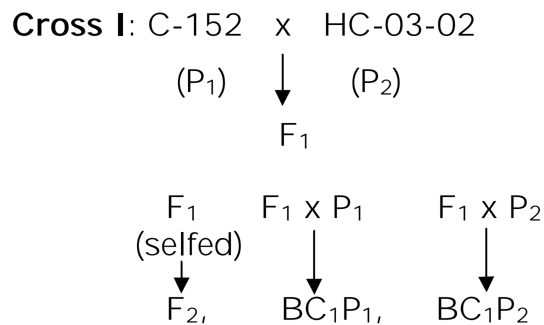
Table 10: The salient features of the parental lines

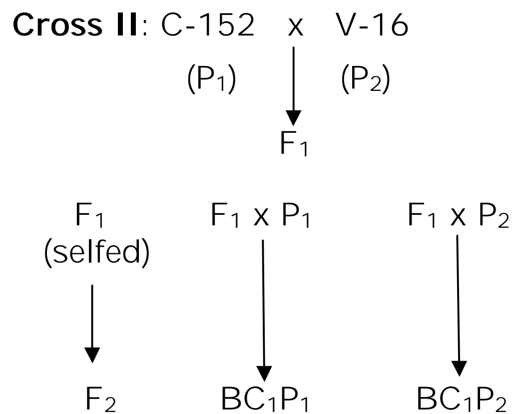
Parents	Year of release	Features
C-152	1985	Highly stable variety with high yielding, matures in 90-100 days, suitable for mixed cropping, and as a cover crop in plantation crops. Released for southern zone of India. Susceptible to both CpMV and BLB diseases.
V-16	1981	Resistant to Bacterial leaf blight and tolerant to fungal diseases, red seeded, adapted to whole country.
HC-03-02		High yielding and resistant to cowpea mosaic virus disease.

3.4.2.2 Pollination: The next day morning between 7.00 to 9.00 AM, pollens from the male parent were collected and dusted on the stigma of emasculated flowers. They were labeled with appropriate male parents for proper identification of the crossed pods. After the pod set, crossed pods were harvested, dried, threshed and stored with care to protect from storage pests and to maintain viability.

3.4.3 Raising of F_1 generation and production of back cross seeds

The F_1 hybrids were sown along with their parents during *kharif* 2010, and true crosses were confirmed based on the characters of male parents in F_1. Dominant morphological characters *viz,* pigmentation on peduncle in V-16 and spreading growth habit of HC-03-02, which were not present on female parent C-152 were used to identify the true F_1s of respective crosses in F_1 generation. Hybridization was done again on identified true F_1s with both the parents (P_1 and P_2) to produce back cross generations as indicated in the following diagram.





3.4.4 Raising and evaluation of F_2 generation

Seeds from true F_1 plants from both the crosses were collected and used for raising F_2 generation during *Rabi* 2010. The morphological observations and disease scoring for both diseases in respective crosses

were recorded. Seeds obtained from each individual F_2 plants were collected and forwarded to F_3 generation.

3.4.5 Raising of F_3 generation

The F_2 seeds were divided into two parts. Part of the F_2s were grown under nylon nets during summer 2011, for rapid generation of F_3s and rest were planted during *rabi* 2010 in controlled conditions for screening them against CpMV and BLB, to understand the nature of its inheritance and to identify markers linked to CpMV and BLB resistance. The morphological observations and disease scoring for both diseases in respective crosses were recorded.

3.4.6 Growing of segregating populations, F_1's and parents for study of inheritance

The experiment comprising six populations (*viz.*, P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2) of the two cross C-152 x HC-03-02 and C-152 x V-16 were laid out during *rabi* 2010 in Randomized complete block design with two replications. The non-segregating populations (P_1, P_2 and F_1s) were grown in two row plots of 3.5 m length, while the segregating populations *viz.*, F_2, BC_1P_1 and BC_1P_2 were grown in two row plots in each replication, while F_2 were grown in six row plots in separate blocks. The entries were sown in plots of either two row or six rows spaced 60 cm apart and with a plant spacing of 15 cm in each row. Thinning was done to retain one healthy plant per hill at 15 and 25 days after sowing. All the recommended package of practices was followed to raise a good and healthy crop.

3.4.7 Chi- square test

The inheritance pattern was studied in both the crosses *viz.,* C-152 x HC-03-02 (for CpMV) and C-152 x V-16 (for BLB).The disease resistance was classified into two groups, those with no apparent

symptoms as resistant and those with visual expression of symptoms as susceptible. The reactions for both the diseases were recorded as resistant and susceptible on all individual plants of F2 and back cross generations of both the crosses. The recorded observations were subjected to chi-square test based on expected ratios. The goodness of fit between expected and observed segregation ratios were worked out. The chi-square was calculated by using the following formula.

$$x^2 = \sum \frac{(O - E)^2}{E}$$

Where,

O = Observed frequency

E = Expected frequency

The significance of Chi-square value was tested by comparing the calculated Chi-square value with table value at 5 % and 1 % level of significance at appropriate degrees of freedom (n-1, where, n = number of classes of trait under consideration).

3.5 Biometrical analysis

The biometrical analysis of the data on quantitative parameters was carried out using computer packages like MSTATC, Statistica 2, NTSYS and Genres, at the Department of Genetics and Plant Breeding and Computer Center, College of Agriculture, University of Agricultural Sciences, Bangalore following the methods explained below.

3.5.1 Genetic variability

3.5.1.1 Analysis of Variance (ANOVA)

The analysis of variance for different characters was carried out using simple lattice design as suggested by Cochran and Cox (1957)

Table 11. Schematic representation of ANOVA table of simple lattice design

Source of variation	Degrees of freedom (df)	Sum of squares (SS)	Mean square	Computed *F*	F ratio
Replication	r - 1	*SSR*	*MSR*	$\frac{MSR}{MSE}$	$\frac{MST\text{(unadj.)}}{MSE}$ $\frac{MSB\text{(adj.)}}{MSE}$
Genotypes (unadj.)	k^2 - 1	SSg (unadj.)	*MSg* (unadj.)	$\frac{MST\text{(unadj.)}}{MSE}$	
Blocks within replication (adj.)	$r(k-1)$	*SSB* (adj.)	*MSB* (adj.)	$\frac{MSB\text{(adj.)}}{MSE}$	
Intra-block error	$(k-1)(rk-k-1)$	*SSE*	*MSE*		
Total	rk^2 - 1	*SSTO*			

Where, r = Number of replications, k^2 Number of treatments (genotypes)

The adjusted variable mean differences were tested for significance as follows

Source of variation	df	Sum of squares	Mean squaree	F ratio
Genotypes (adjusted)	k^2-1	SSg	G	G/Ee
Error (intra block)	(k-1) (rk-k-1)	SSE	Ee	

The computed F value was compared with the table F value at (k^2-1) and (k-1) (rk-k-1) degrees of freedom for 5% and 1% level of significance.

To test significance of the differences between any two adjusted genotypic means, the standard error of mean was computed using the formula

$$S.E= [2Ee/r\ (1+2ku/k+1)]^{0.5}$$

Where, S.E = Standard error of mean

Ee = Mean sum of squares for error (intra block)

r = Number of replications

k = Number of genotypes in each sub-block

u = Weightage factor computed

3.5.1.2 Population means and variances

Means and variances in respect of eight quantitative traits were calculated for each population irrespective of replication using data recorded on individual plants as detailed below.

$$\text{Mean} = \Sigma x_i / N$$

$$\text{Variance} = \frac{\Sigma x_i^2 - (\Sigma x)^2/n}{n-1}$$

Variance of sample mean = Variance/n

$$\text{Standard error (SE)} = \sqrt{\text{Variance of population} / n}$$

Where, x_i = i^{th} observation of a population

n = Number of observations

3.5.1.3 Estimation of genetic variability parameters

In order to assess and quantify the genotypic variability among the genotypes for the characters under study, the following parameters were estimated as given below.

A. Estimation of variance components

Phenotypic and genotypic components of variance estimated by applying the formula as suggested by Cochran and Cox (1957).

$$\text{Genotypic variance}\left(\sigma_g^{\ 2}\right) = \frac{\text{MS due to genotypes - MS due to error}}{\text{r}}$$

Phenotypic variance (σ_p^2) = $\sigma_g^2 + \sigma_e^2$ (MS due to error)

Where, r= number of replications.

B. Phenotypic (PCV) and genotypic (GCV) coefficients of variations

Phenotypic and genotypic coefficients of variability were computed as per the method suggested by Burton (1953).

$$PCV(\%) = \frac{\sqrt{\text{Phenotypic variance}}}{\text{General mean}} \times 100$$

$$GCV(\%) = \frac{\sqrt{\text{Genotypic variance}}}{\text{General mean}} \times 100$$

PCV and GCV were classified as per Robinson *et al.* (1949)

0 – 10%	- Low
10-20	- Moderate
20% and above	- High

C. Heritability

The broad sense heritability (h^2_{bs}) was estimated for all the characters as the ratio of genotypic variance to the total variance as suggested by Hanson *et al.* (1956) as indicated below.

$$h^2_{bs} = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

Where, σ^2_p is the phenotypic variance and σ^2_g is the genotypic variance of respective characters.

Heritability percentage was categorized as per Robinson *et al.* (1949)

0-30%	- Low
30-60%	- Moderate
60% and above	- High

D. Genetic advance (GA)

It was predicted by using the formula provided by Johnson *et al.* (1955).

$$GA = h^2_{(bs)} \times \sigma_p \times k$$

Where,

$h^2_{(bs)}$ = Heritability in broad sense

σp = Phenotypic standard deviation of the trait

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

E. Genetic advance as per cent mean (GAM)

It was computed by the formula.

$$GAM\ (\%) = \frac{\text{Genetic advance}}{\text{General mean of the character}} \times 100$$

The genetic advance as per cent mean was categorized as suggested by Johnson *et al.* (1955).

0- 10% - Low

10-20% - Moderate

20% and above - High

3.5.1.4 Correlation analysis

The correlation coefficients among all possible character combinations at phenotypic (rp) and genotypic (rg) level were estimated (Al-Jibouri *et al.*, 1958).

$$\text{Genotypic correlation} = r_{xy}(g) = \frac{Cov_{(xy)}(g)}{\sqrt{\sigma^2(x)_g \times \sigma^2(y)_g}}$$

$$\text{Phenotypic correlation} = r_{xy}(p) = \frac{Cov_{(xy)}(p)}{\sqrt{\sigma^2(x)p \times \sigma^2(y)p}}$$

Where,

COV_{xy} (p) and COV_{xy} (g) are phenotypic and genotypic covariances between x and y characters, while V_x (p) and V_x (g) represent variances of x character and V_y (p) and V_y (g) denote variances of y character at phenotypic and genotypic level, respectively.

The test of significance for association between characters was done by comparing table 'r' values at n-2 error degrees of freedom for phenotypic and genotypic correlations with estimated values, respectively.

3.5.2 Genetic divergence analysis

A. Mahalanobis D^2 analysis

Mahalanobis's (1936) D^2 statistic was used for assessing the genetic divergence between different populations. Mahalanobis generalized distance (D^2) between any two populations is given by the formula.

$$D^2 = ij \; r_{ij}$$

Where, D^2 = Square of generalized distance

r_{ij} = Reciprocal of the common dispersal matrix

i = $(\mu_{i1} - \mu_{i2})$

j = $(\mu_{j1} - \mu_{j2})$

μ = General mean

The formula for estimation of distance D^2 for the samples.

$$D^2p = d^1 S^{-1} d$$

Where, D^2p = Square of distance considering 'p' variables

D = Vector observed differences of the mean values of all the characters $(x_{i1} – x_{i2})$

x_{i1} = Vector of the mean values of all the characters

S^{-1} = inverse of variance and covariance matrix

Since inversing the matrix is complicated the original correlation variable (x_i) were transformed to non-correlated variables (y_i). The computation of D^2 values reduce to simple summation of the squares of the difference between the values of transformed variables of the two populations.

This transformation was done by Pivotal condensation method. These newly transformed uncorrelated variables were used to calculate the square of distance using the formula

$$D^2 = (Y_{i1} - Y_{i2})^2$$

Where, Y = Vector of transformed mean values

The square of these D^2 values gives the general distance between the two populations. The D^2 values were arranged in a matrix form.

The significance of D^2 values between any two populations is tested using the formula.

$$T^2 = \frac{N_1 \times N_2}{N_1 + N_2} \times D^2$$

Using T^2, the F values was calculated

$$F = \frac{N_1 + N_2 - P\text{-}1}{(N_1 + N_2 - 2)\ P} \times T^2$$

This computed 'F' value was compared with the table 'F' value at five per cent and one per cent level of probability at P and ($N_1 + N_2 - P\text{-}1$) degrees of freedom.

B. Clustering of D^2 values

All then n (n -1)/2 D^2 values were clustered using Tocher's method.

C. Intra cluster distance

The intra cluster distances were calculated by the formula given by Singh and Chaudhary (1977).

$$\text{Square of intra cluster distance} = \Sigma Di^2 / n$$

Where, ΣDi^2 = sum of distance between all possible combinations.

n = Number of all possible combinations

D. Inter cluster distance

The inter cluster distance were calculated by the formulae described by Singh and Chaudhary (1977).

$$\text{Square of inter cluster distance} = \Sigma Di^2 / n_i \, n_j$$

Where,

ΣDi^2 is the sum of distances between all possible combinations ($n_i n_j$) of the entries included in the cluster study.

n_i = Number of entries in cluster i

n_j = Number of entries in cluster j

E. Contribution of individual characters towards genetic divergence

The character contribution towards diversity was calculated by the method of Singh and Choudhary (1977). In all combinations, each character is ranked on the basis of di = y_i^j -y_i^k values

Where,

di = Mean deviation

y_i^j =Mean value of the jth genotype for the ith character and

y_i^j =Mean value of kth genotype for I^{th} character.

Rank 'I' is given to the highest mean difference and Rank p is given to the lowest mean difference

Where, P is the total number of characters.

3.6 Identification of molecular markers linked to bacterial leaf blight disease

3.6.1 Parental polymorphism studies for BLB using SSR markers

The protocol for isolation of parental, F_1 and F_2 DNA and other steps were same as that of germplasm lines (section 3.1.2). The parental polymorphism is assessed between the resistant parents (V-16) and susceptible parent (C-152) by screening with 42 cowpea specific SSR markers (Table 6) obtained from Krikhouse Trust, UK.

3.6.2 Bulk Segregant Analysis of C-152 X V-16 for BLB using SSR

Bulk segregant analysis was carried out with polymorphic SSR primers obtained from screening of the two parental lines. The F_2 individuals of the cross C-152 x V-16 were used for bulk segregant analysis by following the methods of Michelmore *et al.*, 1991.

3.6.3 Marker Segregation and Linkage Analysis

The putative linked SSR marker from the BSA was used for linkage analysis of 144 F_2 individuals of the cross C-152 x V-16. Chi-square test analysis was performed to examine the goodness of fit between the expected Mendelian ratios for the segregation data of two linked SSR markers (Agbicodo *et al.* 2010). Single marker analysis was done based on the single factor ANOVA.

Experimental Results

IV. EXPERIMENTAL RESULTS

The results obtained in the present investigation of 'Assessment of morphological and molecular diversity and genetics of resistance to bacterial leaf blight and cowpea mosaic virus in cowpea' are presented under the following headings.

4.1 Analysis of variance.

4.2 Genetic variability parameters.

4.3 Genetic diversity analysis.

4.4 Cluster analysis using biometrical and SSR data.

4.5 Disease reaction of cowpea germplasm to BLB and CpMV.

4.6 Screening of selected lines for BLB and CpMV resistance in controlled conditions.

4.7 Study of inheritance of BLB and CpMV disease resistance.

4.8 Genetic variability parameters for yield and its attributing traits in F_2 populations of cowpea.

4.9 Identification of molecular markers linked to BLB resistance.

4.1 Analysis of variance (ANOVA)

The analysis of variance was carried out for nine quantitative characters including yield and yield attributing characters in one hundred and ninety six genotypes, which were evaluated during *Kharif* 2009. The results obtained are presented in the Table 12. The results revealed significant genotypic differences for all the traits.

4.2 Genetic variability parameters

The variability for yield and other related characters are given in Table 13, which includes mean, range, phenotypic coefficient of variation

Table 12: Analysis of variance for nine quantitative characters in 196 cowpea genotypes during *Kharif* - 2009

Sources of variation	df	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9
Replications	1	0.09	40.84	8.62	1.75	60.67	10.14	0.21	0.43	60722.39
Genotypes (Unadjusted)	195	19.93*	72.73*	6.00*	37.08*	163.16*	4.07*	26.66*	16.81*	65691.40*
Blocks within Replications (adj.)	26	0.92	7.51	0.54	0.76	7.48	0.60	1.13	0.18	32457.05
Error(intra block)	169	1.80	46.35	3.25	5.24	28.32	1.53	17.30	8.35	70815.48

***, ** - indicate significance at 5% and 1% level respectively**

Where,

X_1 - Days 50 % Flowering
X_2 - Number of pods per plant
X_3 - Number of seeds per pod
X_4 - Pod length (cm)
X_5 - Plant height (cm)
X_6 - Number of branches per plant
X_7 - Test weight (gm)
X_8 - Days to physiological maturity
X_9 - Seed yield per plant (gm)

(PCV), genotypic co-efficient of variation (GCV), heritability in broad sense (h^2) and genetic advance as per cent of mean (GAM).

In general, PCV values were higher than GCV for all the nine characters studied. Among the characters studied, number of pods per plant, pod length, and test weight had comparatively wide difference between PCV and GCV. The variability parameters are presented below character wise and graphically depicted in Fig. 1.

4.2.1 Days to 50 per cent flowering

The variation in the germplasm for days to 50 per cent flowering ranged from 48 (C 325 genotype) to 62 days (IT 97499-38, C 1071) with an overall mean of 56 days. The estimates of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) for days to fifty per cent flowering are same i.e., 5.83 with high heritability of 99.88 per cent followed by moderate genetic advance as per cent of mean (11.98).

4.2.2 Days to physiological maturity

Range values for this trait were from 84 days (IC 402048) to 107 days (IC 202777, IC 402162, ETC 27) with an overall mean of 88.81. Low magnitude of PCV and GCV were found for this trait (3.89 and 3.88 per cent, respectively) and the trait has registered high heritability (99.66 %) with low GAM of 7.99 per cent.

4.2.3 Number of pods per plant

Number of pods showed a wide range from 7.25 to 52.33 and the mean was 21.00. The lowest number of pods was observed in the genotype EC 390287 (7.25), whereas IC249141 (52.33) had maximum number of pods per plant. Character showed high PCV (34.88 %) and

GCV (33.09 %). The heritability was high (89.98 %) and also the trait showed high GAM 64.66 per cent.

4.2.4 Number of seeds per pod

The values for this character ranged from 6.00 to 18.00 with an overall mean performance of 11.00. The genotype EC 170578-1-1 (18.00) showed maximum number of seeds, while, NB 47 and EC 458490 moderate showed minimum number of seeds per plant (6.00). It showed medium estimates of PCV (18.44) and GCV (16.58). Heritability was high with 80.86 per cent followed by high GAM 30.72 per cent.

4.2.5 Pod length (cm)

Pod length ranged from 5.00 cm to 24.22 cm with an overall mean of 10.64 cm. The genotype CPD 15 had short pod (5.0cm), while the genotype NB 12 had long pod (24.22 cm). High PCV and GCV 42.13 per cent and 42.59 per cent, respectively were recorded for this trait. The estimate of heritability (97.50 per cent) and the Genetic advance as per cent of mean was high (86.63 per cent).

4.2.6 Plant height (cm)

A wide range of variation was recorded for plant height, with an overall mean of 28.02 cm. The genotype IC 202290 was the shortest with 12 cm and IC 458440 was the tallest with 57.75 cm height. The PCV and GCV magnitudes observed were high for plant height *i.e.,* 34.78 and 34.32 per cent, respectively. Heritability for plant height was high (97.40 %) with high GAM (69.78 %).

4.2.7 Number of branches per plant

The genotypes differed in the number of primary branches which varied from 3.00 to 13.00 with a grand mean of 7.00. The genotype NBC 41 and V-16 (13.00) had more number of primary branches and the

genotypes IC 198325, EC 472250 and NBC 18 (3.00) had least number of primary branches. While, the PCV (24.47 per cent) and GCV (22.59 per cent) estimates were high. Heritability was high with 85.25 per cent and genetic advance as per cent of mean was 42.97 per cent.

4.2.8 Test weight (g)

Test weight of 100 seeds exhibited the range values from 7.20 to 31.50 g and its overall mean was 14.70. The lowest seed weight was found in the genotype CB 10 (7.20 g), while the genotype EC 458442 had the highest seed weight (31.50 g). PCV and GCV estimates were found to be high for this trait with 31.12 per cent and 31.08 per cent, respectively. Heritability was high at 99.71 per cent and the character showed high GAM at 63.93 per cent.

4.2.9 Seed yield per plant (g)

A wide range of variation (9.60 g to 92.05 g) was recorded for seed yield per plant with an overall mean of 34.11 g. The genotype IC 402182 showed the lowest yield (9.60 g), while IC 249141 had the highest seed yield per plant (92.05 g). GCV and PCV for this trait were high with 44.62 and 41.64 per cent, respectively. Seed yield per plant also exhibited high heritability (87.09 per cent). Genetic advance as per cent of mean was very high at 80.05 per cent.

4.3. Genetic diversity and cluster analysis

Genetic diversity present in the available germplasm has immense value in crop improvement for character of interest. From the point of selecting the parents for hybridization, which are divergent enough for the trait of interest, estimation of the genetic distance is most important. This genetic divergence can be estimated by using an effective statistical tool, Mahalanobis D^2 statistic which gives clear idea about the diverse nature of the population.

Table 13: Estimation of mean and genetic variability parameters for nine characters in 196 cowpea genotypes

S.NO	Characters	Mean±SE	Range	PCV (%)	GCV (%)	h^2 (%)	GA as% mean
1	Days to 50% flowering	55.79 ±0.23	47.00-62.00	5.83	5.82	99.88	11.98
2	Days to physiological maturity	88.81±0.24	84.00-107.00	3.89	3.88	99.66	7.99
3	Number of pods per plant	20.67±0.46	7.25-52.33	34.88	33.09	89.98	64.66
4	Number of seeds per pod	11.46± 0.13	6.00-17.66	18.44	16.58	80.86	30.72
5	Pod length (cm)	10.64 ±0.32	5.00-24.22	43.13	42.59	97.50	86.63
6	Plant height (cm)	28.02 ±0.66	12.00-52.75	34.78	34.32	97.40	69.78
7	Number of branches per plant	6.74 ±0.10	3.00 -13.33	24.47	22.59	85.25	42.97
8	Test weight (g)	14.70 ±0.32	7.20-31.50	31.12	31.08	99.71	63.93
9	Seed yield per plant (g)	34.11 ±1.00	9.60-92.05	44.62	41.64	87.09	80.05

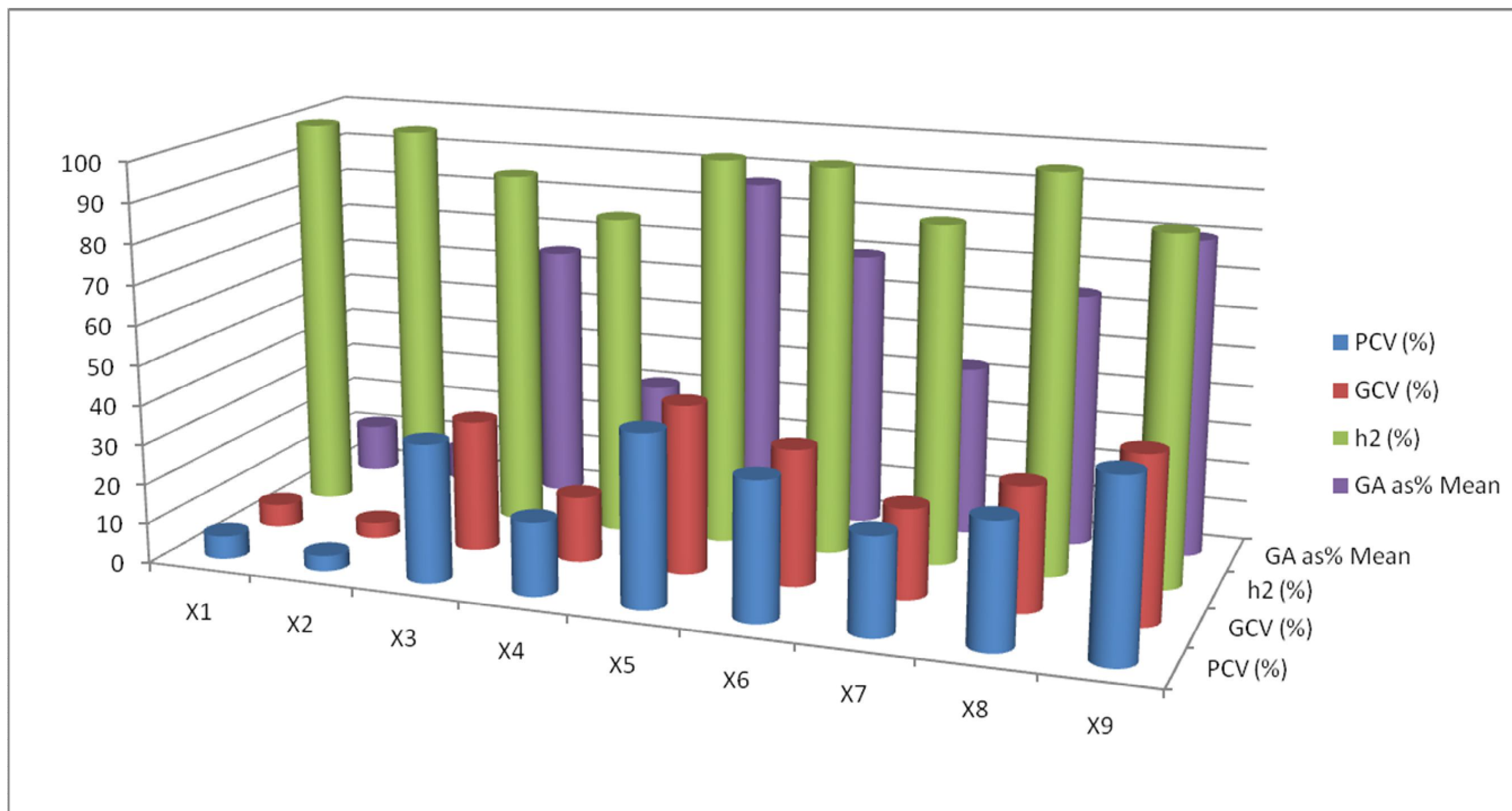


Fig. 1: Genetic variability parameters for nine characters in 196 cowpea genotypes

4.3.1 Group Constellation

Based on D^2 values the genotypes were grouped into 22 clusters using Tocher's methods given by Rao (1952). Clustering of genotypes is presented in table 14. Of the 22 clusters, cluster XXII was the largest comprising of 133 genotypes followed by cluster I with 23 genotypes, and all other clusters had only two genotypes each.

4.3.2 Inter Cluster Distance

Inter cluster D^2 values are represented in the Appendix 2 and the nearest and farthest clusters from each cluster based on D^2 value are given in the Table 15. The genotypes included are found to be very diverse in nature as they showed maximum inter cluster distance (D^2) of 4343.52 between the clusters I and XIX, the minimum D^2 value was between the clusters II and VI (34.60).

Cluster I showed maximum inter cluster distance with the cluster XIX (4343.52) and it had minimum distance with the cluster III (852.00). Cluster II has maximum inter cluster distance with the cluster XXII (2710.87) and is closer to the cluster VI (34.66).Cluster III is more distant from the cluster XIX (3479.87) and is closer to the cluster X (146.65). Cluster IV has more D^2 distance with the cluster XXII (2624.81) and is closer to the cluster XVI (73.43). Cluster V has more D^2 distance with the cluster XXII (2400.23) and less D^2 distance with the cluster XVI (85.17). Cluster VI is genetically more distant with the cluster XIX (3028.46), while it is closer to the cluster XX (308.31).

Cluster VII exhibited maximum distance from the cluster XXII (2223.36) cluster IX (148.13) reported smaller D^2 distance with cluster VII. Cluster VIII has more D^2 distance with the cluster I (2491.62) and less D^2 distance with the cluster XVIII (154.82). Cluster IX is more distanced from the cluster I (2242.70) and is closer to the cluster XVIII

Table 14 : Clustering pattern of 196 cowpea germplasm lines

Clusters	No. of genotypes	Genotypes
I	23	EC 458489,IC 402101,NBC 14,IC 402166, V 24O,202804(83,V 585,C 325,EC 458506,IC 249593,IT 97K 499-38,IC 402180,202854(97),EC 472257,IC 249141,EC 170584,EC 472252,IC 202867(99),KM 5,IC 1071,EC 45841,NBC 29,EC 170584-1-1
II	2	NBC 33,EC 458417
III	2	IC 1071,EC 394779
IV	2	IC 249593,HC 9866
V	2	TC 201,CPD 15
VI	2	V 578-17,IC 402172
VII	2	GENOTYPE 36,EC 458418
VIII	2	NBC 51,NBC 10
IX	2	NBC 30,97767(10)
X	2	NBC 9,NBC 38
XI	2	27749(25),IC 402182
XII	2	IC 402175,EC 394779
XIII	2	EC 458430,IC 402098
XIV	2	NB 12,EC 458402
XV	2	EC 458505,NBC 43
XVI	2	EC 458402,EC 394708
XVII	2	IC 402159,TOME 77-4
XVIII	2	IC 402159,IC 10171
XIX	2	NBC 8,EC 458441
XX	2	CB 10,EC 394839
XXI	2	IC 249588,NBC 27
XXII	133	IC 402161,IC 2591054,IC 462099,IC 58905,EC 458473,IC 202777,IC 170574,CPD 31,EC 394779,NBC 4,IC 330996,IC 402166,IC 402164,EC 472250,IC 402114,V 585,EC 170584,NBC 32,NBC 39,EC 458442,EC 458470,IC 257428,IC 402048,IT 97499-38,CPD 19,NBC 6,202827(92),IC 198326(38),EC 170585(B9),EC 402159,IC 249793,C 131+CB-2,IC 201©,IC 402125,IC 402098,V 578 ©,NBC 7,IC 330996,EC 390287,V-16,EC 458440,IC 249593,EC 170578-1,NBC 40,IC 202779,EC 458402,202705(49),NBC 42,IC 402159,C 517,IC 402162,IC 202290,NBC 41,IC 402104,KBC 2,V 578,ETC 27,C 24-1,C 48475,198355(45),EC 458469,IC 202797(97),C 720,IC 202781,IC 402101,NBC 19,EC 472252,EC 458425,GC 3(C),IC 402104,NBC 38,IC 2591054,IC 202782,IC 402174,IC 4506,201095(52),EC 458480,EC 458483,C 1071,EC 458489,EC 458473,EC 394838,EC 458418,IC 1061,EC 458438,IC 402162,IC 249141,IC 206240,V 604-7-29-3,IC 25105,202827(93),EC 458490,IC 202711(58), C 458492,IC 49586,IC 402154,EC 458440,IC 202781,NBC 44,C 33,NBC 48,IC 198329(36),V 585©,NBC 36,IC 253251,EC 472250,EC 458402,NBC 18,EC 458480,EC 394839,NB 47,GC 3,IC 402090,EC 458469,C 457,NBC 7,EC 458453,IC 402161,IC 402106,EC 472271,EC 472267,CB 10,IC 402164,NBC 40,NBC24,C-152,TVX 944,KBC 2,km 5,HC 03-02,P 695,APC 243-1-865,TCM 44-1

(54.23). Cluster X has more D^2 distance with the cluster I (2960.44) and is closer to the cluster VII (106.61). Cluster XI has more D^2 distance with the cluster XXII (3324.46) and less D^2 distance with the cluster XX (250.02). Cluster XII exhibited maximum distance from the cluster XIX (2454.60), cluster III (146.65) reported smaller D^2 distance with cluster XII. Cluster XIII has more D^2 distance with the cluster XXII (2004.65) and less D^2 distance with the cluster VIII (200.89). Cluster XIV is the farthest from the cluster XX (2765.81) and nearer to the cluster XIII (248.37). Cluster XV is the farthest from the cluster I (3457.90) and nearer to the cluster XIX (106.24).

Cluster XVI showed maximum inter cluster distance with the cluster XXII (2389.66) and it has minimum distance with the cluster XXI (157.27). Cluster XVII has maximum inter cluster distance with the cluster XI (2590.42) and is closer to the cluster VIII (142.55). Cluster XVIII exhibited maximum distance from the cluster XXII (2113.07), cluster IX (54.23) reported smaller D^2 distance with cluster XVII. Cluster XIX has more D^2 distance with the cluster XX (3470.99) and less D^2 distance with the cluster XVI (106.26). Cluster XX is more distanced from the cluster XIX (3470.99) and is closer to the cluster XI (250.02). Similarly the Cluster XXI has more D^2 distance with the cluster I (1626.56) and is nearer to the cluster X (164.93) and cluster XXII has more D^2 distance with the cluster I (3760.82) and less D^2 distance with the cluster XIII (2004.65), respectively.

4.3.3. Intra cluster distance

All the clusters showed more intra cluster distances. Intra cluster D^2 is presented in the Table 15. All the clusters constituted more than one genotype. Intra cluster distance was the highest in the cluster XXII (2767.30) followed by the cluster I (1689.36) and cluster XXI (52.4).

4.3.4 Cluster Mean Analysis

The cluster means in respect of eight characters and overall character wise score across the 22 clusters are presented in table 16. Cluster I included genotypes which are early flowering (52.82) whereas cluster XIX (59.00) comprised of late flowering genotypes. For days to physiological maturity, genotypes of cluster IV showed characteristic early maturing habit with mean number of days for physiological maturity being 86 days, while genotypes of cluster XVII had late maturing habit with 91.00 days. Cluster VI included taller genotypes (48.65 cm) compared to the cluster III which included dwarf (17.43 cm) genotypes. With respect to cluster mean for number of branches, the variation was very small across the clusters and among which cluster II showed the highest number of branches (7.68) per plant while, cluster XV showed the least number of branches per plant (4.00).

Genotypes with the highest number of pods per plant were grouped under cluster VII (28.99) followed by cluster XVIII with mean number of 28.73 pods per plant. Cluster XV had the genotypes showing least number of 12.75 pods per plant.

The cluster XIV included genotypes with more number of seeds per pod (14.08), followed by cluster XIII (13.25). Less number of seeds was observed in the cluster XV (8.45). Genotypes with test weight of as low as 9.85 g were noticed in cluster XI while, genotypes with maximum mean test weight of 18.65 g was observed in cluster XV. For seed yield per plant, genotypes of cluster XVII showed the highest mean seed yield of 47.99 g followed by cluster XIII with a mean value of 44.50 g. The genotypes of cluster XI recorded the least mean seed yield of 14.04 g per plant.

Table 15. The nearest and farthest clusters from each cluster based on D^2 values in 196 cowpea genotypes

Cluster	Intra cluster	Nearest cluster	Farthest cluster
I	1689.36	III (852.01)	XIX (4343.52)
II	4.19	VI (34.66)	XXII (2710.87)
III	5.75	XXII (146.65)	XIX (3479.87)
IV	9.20	XVI (73.43)	XXII (2624.81)
V	17.00	XVI (85.17)	XXII (2400.23)
VI	19.40	XX (308.31)	XIX (3028.40)
VII	20.40	IX (148.13)	XXII (2223.36)
VIII	22.70	XVIII (154.82)	I (2491.62)
IX	26.60	XVIII (54.23)	I (2242.70)
X	30.80	VII (106.61)	I (2960.44)
XI	30.90	XX (250.02)	XXII (3324.46)
XII	34.10	III (146.65)	XIX (2454.56)
XIII	35.00	VIII (200.89)	XXII (2004.65)
XIV	35.50	XIII (248.37)	XX (2765.81)
XV	37.20	XIX (106.24)	I (3457.90)
XVI	38.20	XXI (157.27)	XXII (2389.66)
XVII	41.50	VIII (142.55)	XI (2590.42)
XVIII	44.10	IX (54.23)	XXII (2113.07)
XIX	45.70	XV (106.249)	XX (3470.99)
XX	48.80	XI (250.02)	XIX (3470.99)
XXI	52.40	X (164.93)	I (1626.56)
XXII	2767.30	XIII (2004.65)	I (3760.82)

Table 16: Cluster mean values for eight quantitative parameters in cowpea genotypes

Clusters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	Overall score	Rank
I	52.82	18.83	12.02	26.36	7.00	14.59	33.08	87.63	96	12
II	54.00	23.88	10.70	47.51	7.68	12.35	31.48	88.00	80	10
III	53.00	19.12	12.22	17.43	6.57	13.95	32.64	88.00	108	17
IV	56.00	16.66	12.32	27.25	7.00	10.82	22.14	86.00	117	19
V	56.00	30.41	10.58	17.66	6.75	11.05	35.37	88.00	103	14
VI	54.00	21.00	10.68	48.65	6.76	13.52	29.93	88.00	89	11
VII	58.00	28.99	11.33	32.41	6.58	13.15	43.05	89.00	58	2
VIII	57.00	16.65	10.70	27.96	7.12	17.07	30.05	89.00	71	7
IX	57.00	17.74	13.00	24.66	6.97	12.67	29.39	89.00	77	8
X	58.00	28.29	11.99	22.70	6.41	12.45	42.16	88.00	80	9
XI	54.00	16.75	8.54	24.12	6.50	9.85	14.04	86.00	148	22
XII	54.00	13.66	11.20	23.12	4.91	14.85	22.76	88.50	123	20
XIII	56.00	21.08	13.25	28.00	6.17	15.82	44.50	87.75	70	6
XIV	57.00	18.25	14.08	45.41	7.24	14.77	38.21	88.00	56	1
XV	58.00	12.75	8.45	20.04	4.00	18.65	20.48	86.50	131	21
XVI	56.00	20.33	12.28	25.99	6.50	10.85	27.13	87.50	107	16
XVII	57.00	22.91	12.58	22.50	5.66	16.52	47.99	91.00	61	3
XVIII	57.00	28.73	12.07	21.74	7.40	12.70	43.52	88.75	64	5
XIX	59.00	15.62	8.75	22.55	4.87	18.50	24.95	86.50	116	18
XX	54.00	20.25	11.45	29.49	6.80	10.62	24.70	88.50	101	13
XXI	56.00	15.91	10.68	29.12	7.18	13.42	23.23	87.50	105	15
XXII	56.23	21.06	11.39	28.33	6.79	15.03	35.11	89.26	63	4

Where,

X1 - Days to 50% flowering
X2 - Number of pods per plant
X3 - Number of seeds per pod
X4 - Plant height (cm)
X5 - Number of branches per plant
X6 - Test weight (g)
X7 - seed yield per plant (g)
X8- Days to physiological maturity

4.3.5 Contribution of different characters towards divergence

The genetic diversity among 196 genotypes was measured by employing D^2 statistic. The contribution of each character towards total genetic diversity is presented in the Table 17**.** Out of nine characters studied, seed yield contributed maximum towards the total diversity with the value 35.82 per cent, followed by test weight (26.99 per cent), days to 50 per cent flowering (12.36 per cent), days to physiological maturity (8.76 per cent), plant height (7.20 per cent), pod length (7.01 per cent), number of branches per plant (1.13 per cent), number of seeds per pod (0.35) and number of pods per plant (0.34).

While, characters wise scoring the cluster means, the most desirable magnitude of the trait is given score 1. Hence, the cluster with least overall score across eight characters assigned with rank 1 and that cluster with the highest score gets the 22th rank (Table 16).The cluster XIV with overall score of 56 across the nine characters get I rank followed by cluster VII and XVIII. Most promising genotypes and including local checks were grouped in the cluster XIV.

4.3.6. Characterization of germplasm for qualitative parameters.

Germplasm were characterized based on the leaf shape, plant habit, flower colour and pod pigmentation. The variations for these characters are presented in the Table 18. About 93 genotypes showed erect plant habit, followed by semi-erect and spreading with genotypes 53 and 50, respectively [Plate 6(A)]. Most of the germplasm lines (171) had mave pink flowers and others were varying in flower colour [Plate 6(C)]. Majority of the germplasm (172) did not show special pod pigmentation (Plate 7). Numbers of genotypes with light green, purple and light purple pigmented pods were three, twelve and nine, respectively. With respect to the parameter leaf shape, about 190 genotypes had ovate shape and only six genotypes had lanceolate shape.

Table 17: Relative contribution of nine characters towards divergence in cowpea genotypes

S.NO	Character	Percent contribution
1	Days to 50% flowering	12.36
2	Days to physiological maturity	8.76
3	Number of pods per plant	0.34
4	Number of seeds per pod	0.35
5	Pod length (cm)	7.01
6	Plant height (cm)	7.20
7	Number of branches per plant	1.13
8	Test weight (g)	26.99
9	Seed yield per plant (g)	35.82
	Total	100

Table 18: Variation for qualitative characters in cowpea genotype

S. No.	Character		Number of genotypes	% of genotypes
1	Flower colour			
	A	Mave pink	171	87.24
	B	Violet	16	8.16
	C	Purple yellowish	7	3.57
	D	White yellowish	2	1.02
2	Plant habit			
	A	Erect	93	47.44
	B	Semi-erect	53	27.04
	C	Spreading	50	25.51
3	Leaf Shape			
	A	Lanceolate	6	3.06
	B	Ovate	190	96.93
4	Pod pigmentation			
	A	None (green)	172	87.75
	B	Light green	3	1.53
	C	Purple	12	6.12
	D	Light Purple	9	4.59

4.4 Cluster analysis using biometrical and SSR data

4.4.1 Cluster analysis – Using Biometrical data

Clustering of the genotypes based on the biometrical data was carried out using D^2 values for all the nine traits (days to 50 per cent flowering, days to physiological maturity, plant height, number of branches, number of pods per plant, pod length, number of seeds per pod, test weight and seed yield per plant) studied adopting the Statistica 2 programme. From the results it can be observed that, similarity among the genotypes varied from 82 to 100 per cent (Fig. 2).

At 92 per cent similarity, 60 genotypes formed seven distinct clusters, I, V and VI were solitary with a genotype IT 97499-38-1, C 33 and EC 170584, respectively. While, II cluster had two genotypes *viz.,* NBC 30 and IC 202867-(92). In the clusters III, IV and VII the number of genotypes were 12, 10 and 32, respectively (Table 19). At 90 per cent similarity, there were five clusters, II cluster had only single genotype (C 33), five genotypes were observed in cluster V. Whereas, IV cluster consists of 12 genotypes and the major cluster I had rest of the genotypes.

4.4.2 Molecular diversity: SSR marker analysis

The study aims at determining the genetic variability among 60 cowpea genotypes selected from the set of 196 germplasm lines. 42 cowpea specific microsatellite primers (SSR markers) were used to amplify the repeat regions in the cowpea genotypes. Out of 42 primers, 34 primers were successful in amplifying microsatellite repeats in the sample.

4.4.2.1 SSR polymorphism in cowpea germplasm

Twenty three out of the 42 cowpea specific primer pairs used in the study produced polymorphisms among the 60 cowpea genotypes used

(Plate 2-5). The 11 primers produced monomorphic bands. Unambiguous polymorphic bands obtained from these primers were utilized to analyze 60 cowpea genotypes. On an average, two bands were produced by each polymorphic primer pairs.

4.4.2.2 Cluster analysis of molecular marker data

The diversity among the various genotypes was estimated by cluster analysis using genotype data from both 23 polymorphic and 11 monomorphic primers with the help of NTSYS-pc 2.1 programme. The similarity matrix representing the Jaccard's coefficient was used to cluster the data using the Unweighted Paired Group Method Average (UPGMA) algorithm. The UPGMA based dendrogram was obtained from the binary data deduced from the DNA profiles of the samples analysed. The 60 diverse cowpea genotypes were clustered by the Jaccard's coefficient of Psimilarity from 45 to 100 per cent (Fig. 3). The clustering was started at the Jaccard's similarity coefficient of 57 per cent and the genotype EC 472252 exhibited 41 per cent dissimilarity with the other genotypes studied. At 65 per cent similarity, seven clusters are formed and II cluster had only one genotype *i.e.* EC 472252, VII cluster had only two genotypes (IC 402048 and EC 458473), while the III and IV cluster had three genotypes in each. The VI and I cluster had 14 and 9 genotypes, respectively. Whereas, the cluster V had remaining all other genotypes (Table 20).

At the Jaccard's similarity coefficient of 65 per cent, twelve main groups were clearly clustered based on data of 23 primers used in the study. The eighth, ninth and tenth cluster had one genotype each (*viz.,* V 585, EC 472257, V 582 respectively), first and seventh clusters had two genotypes each. The cluster second, third, fourth, fifth, sixth and twelfth had 14, four, four, 19, three and six genotypes, respectively. The genotypes V 578 and NBC 7 showed 100 per cent similarity based on

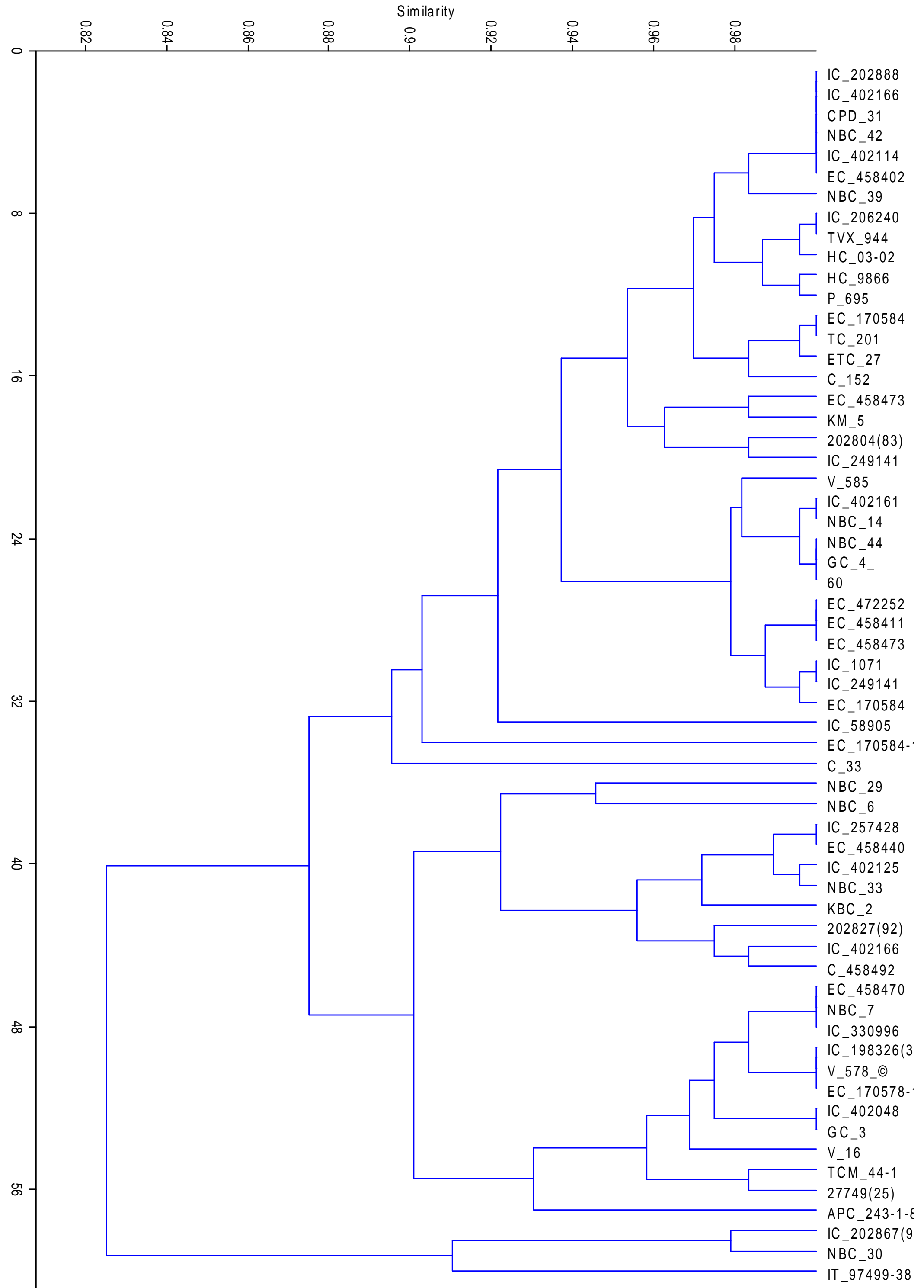


Fig. 2: Dendrogram of 60 cowpea genotypes based on phenotypic data

Table 19. Clustering of 60 cowpea genotypes based on phenotypic data (at 92 per cent similarity)

Clusters	No. of genotypes	Genotypes
I	1	IT-97499-38-1
II	2	NBC 2, IC 202867-(92)
III	12	APC-243-1-1,27749(25),TCM-44-1,V-16,GC-3,IC-402048,EC-170578, V-578,IC-330996,IC-198326(38),IC-330996,NBC-7,EC-458470
IV	10	C-458492,IC-402166,202827(92),KBC-2,NBC-33,IC-402125, EC-458440,IC-257428,NBC-6,NBC-29
V	1	C-33
VI	1	EC-170584
VII	33	EC-170584,IC-249141,IC-1071,EC-458473,EC-458411,EC-472252, TC-201,GC-4,NBC-44,NBC-14,IC-402161,V-585,IC-249141, 202804(83,KM-5,EC-458473,C-152,ETC-27,TC-201,EC-170584, P-695,HC-9866,TVX-944,IC-206240,NBC-39,EC-402114,NBC-42, CPD-31,IC-402166,IC-202888)

Table 20. Clustering of 60 cowpea genotypes based on molecular data (at 65 per cent similarity

Clusters	No. of genotypes	Genotypes
I	9	GC 5,202804(83),IC 402166,EC 458489, EC 170584,IC 202867(99), NBC 14, IC 249141,V 578
II	1	EC-472252
III	3	IC 1071,EC 458411,NBC 29
IV	3	IC 402161,P 695,IC 402166
V	28	IC 58905,NBC 30,27749(25), NBC 33,EC 458470,HC 03-02, IC 257428, TCM 44-1, IC 330996,EC 458440,EC 170584-1-1, NBC 6,V 16,KBC 2, 202827(92),IC 198326(38),NBC 44,C 458492, ETC-27, IT 97499-38, C 152,GC 3,TVX 944, KM 5,APC 243-1-865,TC 201,GC 4, HC 9866
VI	14	IC 402182,EC 458473,EC 458402, EC 170584,IC 402125,V 578 ©,NBC 7,NBC 44,IC 249141, IC 206240,CPD 31,NBC 42,NBC 39,IC 402114
VII	2	IC-402048,EC-458473

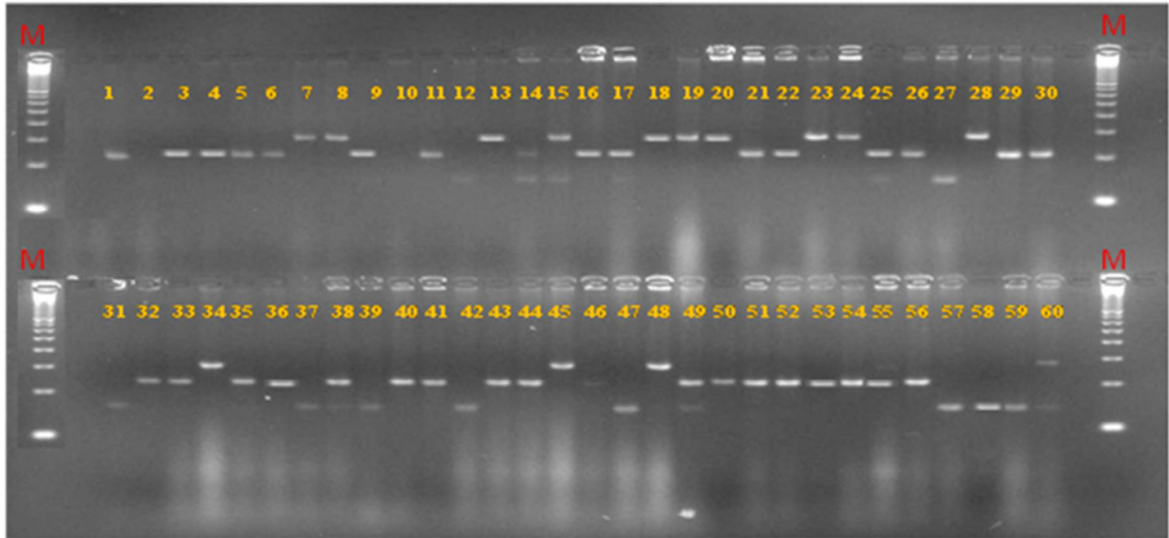


Plate 2: SSR profile of 60 cowpea genotypes from CP 115/116 marker on Agarose gel

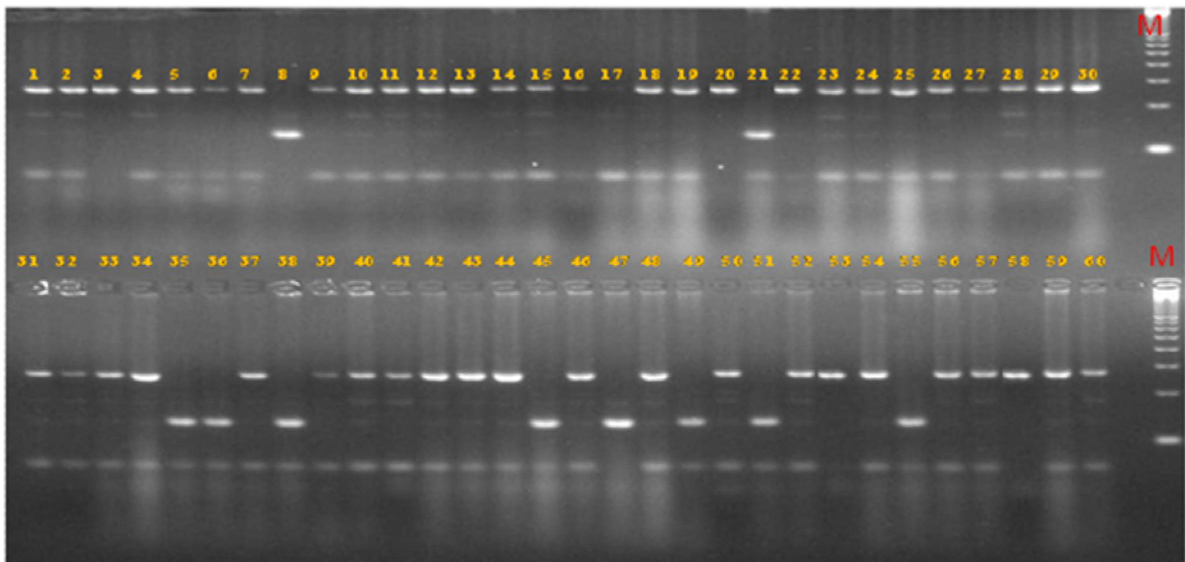


Plate 3: SSR profile of 60 cowpea genotypes from CP 117/118 marker on Agarose gel

Note: M = Marker, L1 to L60 represents the 60 cowpea genotypes (Table 7) in order

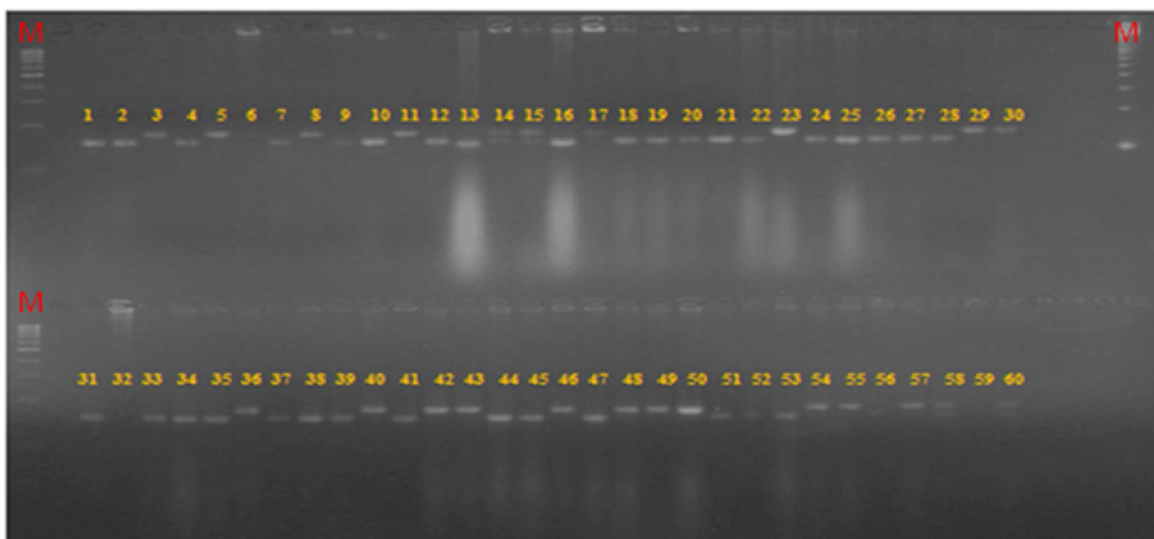


Plate 4: SSR profile of 60 cowpea genotypes from CP 171/172 marker on Agarose gel electrophoresis

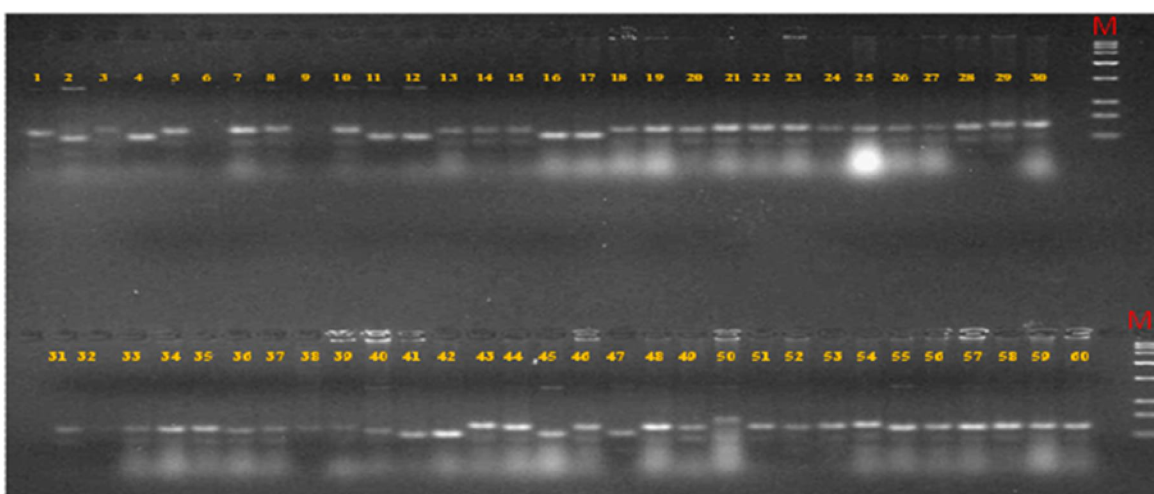


Plate 5: SSR profile of 60 cowpea genotypes from CP 605/606 marker on Agarose gel electrophoresis

Note: M = Marker, L1 to L60 represents the 60 cowpea genotypes (Table 7) in order

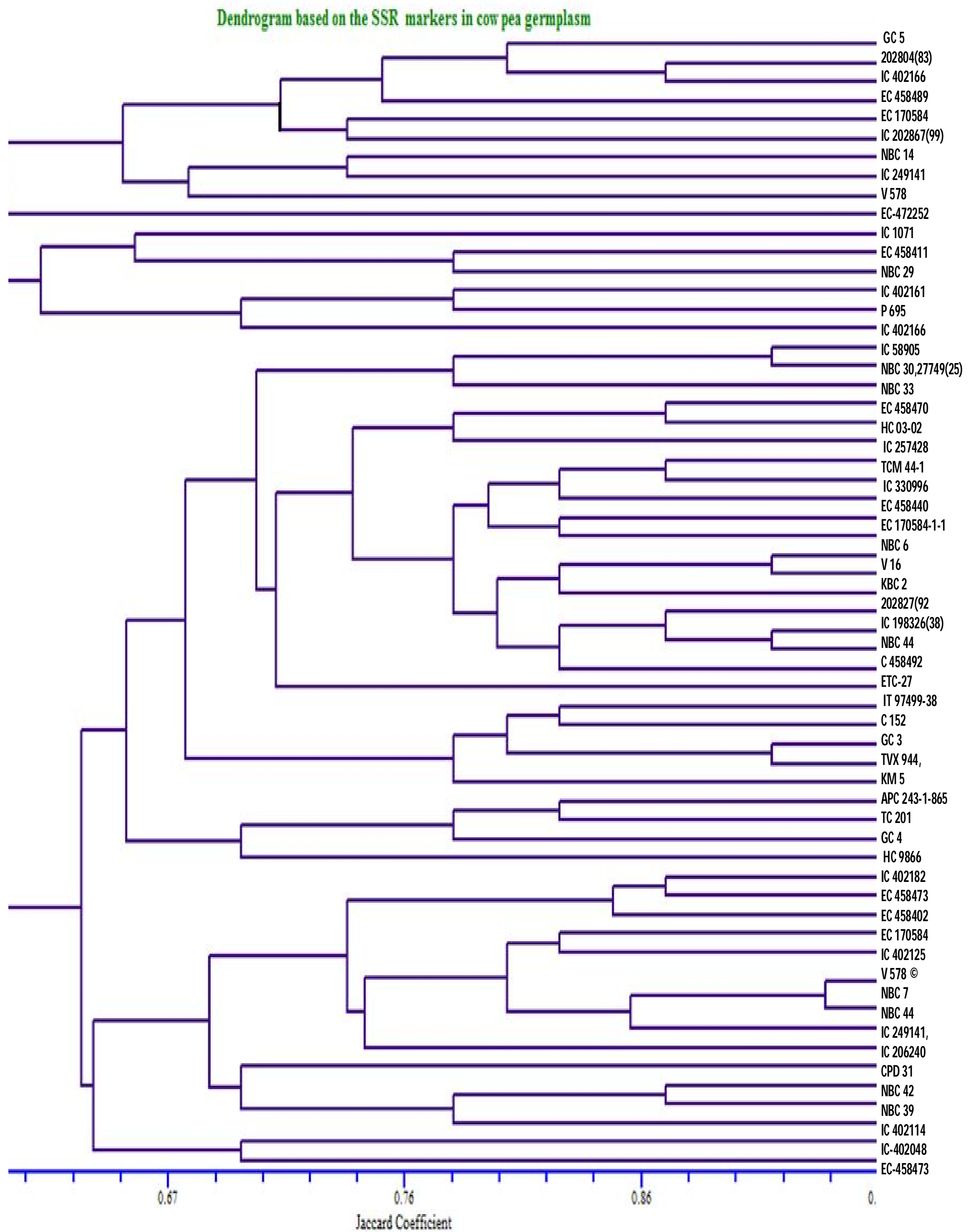


Fig. 3: Denrogram of 60 cowpea genotypes based on SSR marker data

(A)

(B)

(C)

Plate 6: Different types of growth habits (A and B) and Variation for flower color among genotypes (C)

(A) **(B)**

(C)

Plate 7: Variation in pod size (A), color (B) and for seed color and size (C) in cowpea

genotypic data. The genotypes from last two clusters mentioned above were found to differ from rest of the genotypes grouped in remaining other clusters at 59 per cent the similarity level. Since, these genotypes showing more diversity they can be used as parents for hybridization programme in crop improvement.

4.5 Disease reaction of cowpea germplasm to BLB and CpMV

Disease is the most important biotic stress in any of the crop, which causes high reduction in the productivity. Screening for disease resistance under field condition would be effective to identify most adoptable variety through any of the breeding programme. 196 genotypes (Table 4) were screened under field condition for two major diseases *viz.*, cowpea mosaic virus disease and bacterial leaf blight during *Kharif* 2009.

4.5.1 Screening for cowpea mosaic virus resistance

The reaction of one hundred and ninety six cowpea genotypes screened under field condition against cowpea mosaic disease is presented in Table 21. Among the 196 genotypes screened, 110 genotypes showed resistant reaction (scale 0 and 1), forty two genotypes showed moderately resistant reaction (scale 3 and 4), eighteen genotypes showed moderately susceptible reaction (scale 5), twenty one genotypes showed susceptible reaction (scale 6 and 7) and remaining only five genotypes showed highly susceptible reaction (scale 8 and 9).

4.5.2 Screening for bacterial leaf blight resistance

Disease scoring results are presented in Table 22. The leaf spot severity was ranged from 0-6 disease scale. The reaction of one hundred and ninety six cowpea genotypes was screened under field condition against cowpea bacterial leaf blight disease. Among 196 genotypes, 30 genotypes showed highly resistant reaction (scale 0), 120 genotypes showed resistant reaction (scale 0-1), 28 genotypes showed moderately

Table 21: Grouping of cowpea genotypes/ varieties based on their reaction against cowpea mosaic virus under field condition

Rating	Per cent disease severity	Reaction	Genotypes/varieties
0 - 1	No visible symptoms on leaves and Small necrotic yellow specks with restricted spread covering 0.1-5 per cent leaf area s	Resistant (110)	EC-458489, IC-402101, NBC-14, V-240, V-585, IC-249593, IC-402180, IC-462099, IC-58905, IC-402182, IC-202777, IC-170574,EC-394779, NBC-42, IC-402166, EC-472250, IC-402114, EC-170584, NBC-29, NBC-33, EC-458470, IC-402172, IC-402048,CPD-19, NBC-6, V-578-17, IC-249793, C-131+CS-2, IC-201, IC-249588, IC-402125, IC-402098, IC-330996, EC-170584, V-16, EC-458440, IC-249593, IC-402162, IC-202290, HBC-41, KBC-2, V-578, ETC-27, C-24-1, C-PD-15, IC-1071, 198355 (45), EC-458469, TOME-77-4, EC-458418, genotype-36, EC-394779, EC-472252, EC-458505, NBC-38, IC-2591054, IC-202781, CB-10, IC-402098, EC-458480, C-1071, EC-394839, EC-394838, EC-458418, IC-402159, NBC-10, IC-1061, IC-10171, IC-249593, IC-402162, HBC-51, 97767(10), IC-249141, IC-206240, EC-458430, V-604-7-29-3, 202827(93), EC-458490, IC-25105, IC-202781, HBC-44, C-33, IC-198329(36), NBC-43, V-585 (C), EC-458402, EC-458441, EC-4580480, HB-47, HBC-8, EC-45846, C-457, NBC-7, EC-458453, IC-402161, IC-402106, CB-10, IC-402164, NBC-40, NBC-24, KM-5, TC-201, P-695, TCM-44, APC-81, HC-9566 , HC-03-02
3 - 4	Mottling of leaves, covering 5.1-10 per cent leaf area and Yellow mottling of leaves, covering 10-15 per cent leaf area	Moderately resistant (42)	IC-402166, IC-249141, KM-5, IC-1071, IC-402161, IC-2591054, CPD-31, IC-330996, IC-402164, V-585, EC-458442, 202827(92), IC-198326, EC-170585(B-9), EC-402159, V-578CC, NBC-7, EC-170578, NBC-40, IC202777, EC-458402, 232705(49), NBC-42, C-157, IC-402104, IC-402159, EC-48475, EC-394779, C-720, IC-402175, EC-394708, GC-3(C), IC-402174, EC-458489, EC-458438, IC-49586, IC-402154, NBC-43, EC-472250, EC-394839, 4C3(C), EC472267
5	Yellow mottling and discoloration of leaves, covering 15.1-30 per cent leaf area	Moderately susceptible (18)	EC-458506, EC-472257, EC-170584 B-9, EC-472252, EC-458411, NBC-39, IC-202797(78), IC-402101, IC-4506, IC-201095(52), EC-458473, IC-458492, EC-458440, IC-253251, NBC-38, NBC-18, IC-402090
6 - 7	Yellow discoloration of 30-50 per cent of leaves and Pronounced yellow mottling and discoloration of leaves, and reduction in leaf size and stunting of plants covering 50-75 per cent of foliage	Susceptible (21)	202804(83), C-325, IT-97K5499-38, 202854(97), IC-202867(99), NBC-30, EC-458473, NBC-32, EC-458402, IC-257428, NB-12, EC-458417, EC-120584-1-4, IC-402159, IC-202781, IC-402104, EC-458483, NBC-148, EC-472271, TVX-944
8- 9	Sever yellow discoloration of leaves, stunting of plants, reduction in pod size, covering 75.1-85 per cent of foliage and Sever yellowing of entire leaves, stunting of plants and covering 85-100 per cent of foliage	Highly susceptible (5)	HBC-9, IC-202711, 27749(25), C-152, GC 4,

Table 22: Grouping of cowpea genotypes/ varieties based on their reaction against cowpea bacterial leaf blight under field condition

Rating	Per cent disease severity	Reaction	Genotypes/varieties
0	No spots on leaves, completely free of disease	Highly resistant (30)	V-16, EC-458440, IC-249593, IC-402162, IC-202290, HBC-41, KBC-2, V-578, ETC-27, C-24-1, C-PD-15, IC-1071, 198355 (45), EC-458469, IC-2591054, IC-202781, CB-10, IC-402098, EC-458480, C-1071, CB-10, IC-402164, NBC-40, NBC-24, KM-5, TC-201, P-695, TCM-44, APC-81, HC-9566
1	Less than 2 per cent leaf the area of plant affected.	Resistant (120)	EC-394839, EC-394838, EC-458418, IC-402159, NBC-10, IC-1061, IC-10171, IC-249593, IC-402162, HBC-51, 97767(10), IC-249141, IC-206240, EC-458430, V-604-7-29-3, 202827(93), EC-458490, IC-25105, IC-202781, HBC-44, C-33, IC-198329(36), NBC-43, V-585 (C), EC-458402, EC-458441, EC-4580480, HB-47, HBC-8, EC-45846, C-457, NBC-7, EC-458453, IC-402161, EC-458489, IC-402101, NBC-14, V-240, V-585, IC-249593, IC-402180, IC-462099, IC-58905, IC-402182, IC-202777, IC-170574,EC-394779, NBC-42, IC-402166, EC-472250, IC-402114, EC-170584, NBC-29, NBC-33, EC-458470, IC-402172, IC-402048,CPD-19, NBC-6, V-578-17, IC-249793, C-131+CS-2, IC-201, IC-249588, IC-402125, IC-402098, IC-330996, EC-170584, IC-402166, IC-249141, KM-5, IC-1071, IC-402161, IC-2591054, CPD-31, IC-330996, IC-402164, V-585, EC-458442, 202827(92), IC-198326, EC-170585(B-9), EC-402159, V-578CC, NBC-7, EC-170578, NBC-40, IC202777, EC-458402, 232705(49), NBC-42, C-157, IC-402104, IC-402159, EC-48475, EC-394779, C-720, IC-402175, EC-394708, GC-3(C), IC-402174, EC-458489, EC-458438, IC-49586, IC-402154, NBC-43, EC-472250, EC-394839, 4C3(C), EC472267
2	2 to 10 per cent leaf the area of plant affected.	Moderately resistant (28)	HBC-9, IC-202711, 27749(25), NBC-32, EC-458402, IC-257428, NB-12, EC-458417, EC-120584-1-4, IC-402159, IC-202781, IC-402104, EC-458483, NBC-148, EC-472271, TVX-944, HC-03-02, NBC-32, EC-458402, IC-257428 EC-458506, EC-472257, EC-170584 B-9, EC-472252, EC-458411, NBC-39, IC-202797(78), IC-402101
3	10 to 25 per cent leaf the area of plant affected.	Susceptible (7)	202804(83), C-325, IT-97K5499-38, 202854(97), IC-202867(99), NBC-30, EC-458473
4	25 to 40 per cent leaf the area of plant affected.	Moderately susceptible (9)	IC-4506, IC-201095(52), EC-458473, IC-458492, EC-458440, IC-253251, NBC-38, NBC-18, IC-402090
5	More than 40 per cent leaf the area of plant affected and plant dying as a result.	Very highly susceptible (2)	C-152, GC 4

resistant reaction, seven genotypes showed susceptible reaction, nine genotypes showed moderately susceptible reaction and remaining only two genotypes showed highly susceptible reaction.

4.6 Screening of selected lines for BLB and CpMV resistance in controlled conditions

The resistant entries for bacterial leaf blight (V-16, CS-88, HC-9866) and cowpea mosaic (HC-03-02, GC-3, GC-5) and C-152 (the susceptible genotype for both diseases) were selected based on field resistance, and subjected to artificial inoculation for confirmation. From each test entries, two sets of 10 seeds were sownd in polythene bags and inoculated for both diseases separately and results obtained were presented in the Table 23 and Table 24. Among these entries, C-152 showed susceptible reaction for both the diseases under controlled conditions. The lines V-16 and HC-03-02 showed resistant reaction to BLB and CpMV diseases. These entries were selected and used for hybridization work.

4.7 Inheritance of resistance to bacterial leaf blight and cowpea mosaic disease

This experiment was essentially designed to study the genetics of disease resistance in cowpea. The experimental material consists of two crosses of cowpea involving two highly resistant lines *viz.,* HC-03-02 (resistant to cowpea mosaic disease) and V-16 (resistant to bacterial leaf blight disease) and C-152 as highly susceptible line to both CpMV and BLB diseases. The inheritance of disease resistance was studied during 2010 by growing F_1, F_2, BC_1P_1, BC_1P_2, P_1 and P_2 generations. Data collected from individual plants in various generations were subjected to goodness of fit with the expected genetic ratio using chi-square test. The results are presented for both diseases separately.

Table 23: Disease reaction of cowpea cultivars to cowpea mosaic virus disease under controlled conditions (Muniyappa *et al*., 1987)

Cultivars	Percentage foliage affected	Disease reaction
C-152	85	HS
HC-03-02	0.00	HR
GC-3	2.50	R
GC-5	5.20	MR

Table 24: Disease reaction of cowpea cultivars to bacterial leaf blight under controlled conditions (James *et al*., 1971)

Cultivars	Per cent leaves infected	Per cent leaf area affected	Disease reaction
C-152	42.50	36.00	HS
V-16	0.00	0.00	HR
CS-88	11.00	15.00	MS
HC-9866	11.20	17.00	MS

Where,

HS= Highly susceptible, MR= Moderately resistant, S=Susceptible, R=Resistant, MS= Moderately susceptible

4.7.1 Inheritance of resistance to CpMV disease resistance

The inheritance pattern was studied in the cross C-152 x HC-03-02. Per cent disease incidence was considered for forming frequency distribution in F_1 and other segregating generations, *viz.*, F_2, F_3, BC_1P_1 and BC_1P_2 (Table 25). The two parents involved in this cross showed distinctly different pattern for disease resistance incidence. This was used to classify plants as resistant (0-1 scale) and susceptible (3-9 scale) by using scoring method proposed by Muniyappa *et al*. (1971)

The segregation for disease resistance and susceptibility in different generations of this cross (C-152 x HC-03-02) is given in the Table 25. It was evident from the data that disease resistance was dominant over susceptibility. In F_2, the segregation pattern of 3:1 (3 resistant: 1 susceptible) was observed and showed very good fit with the expected ratio and chi-square values were non-significant at n-1 degrees of freedom ($n_=$ number of classes).

4.7.2 Inheritance of resistance to BLB disease resistance

The inheritance pattern was studied in the cross C-152 x V-16 using per cent disease incidence in F_1 and other segregating generations, *viz.*, F_2, F_3, BC_1P_1 and BC_1P_2 (Table 26). The two parents involved in this cross showed distinctly different pattern for disease resistance incidence. The plants were classified as resistant (0-1 scale) and susceptible (3-5 scale) by using scoring method of James *et al*. (1971).

The segregation for disease resistance and susceptibility in different generations of this cross (C-152 x V-16) is given in the Table 26. The analysis indicated that resistance was dominant over susceptibility. In F_2 the segregation pattern of 3:1 (3 resistant: 1 susceptible) was observed which showed very good fit with the expected ratio.

Table 25: Segregation for CpMV disease resistance and susceptibility in different generations of the cross C-152 x HC-03-02

Crosses/ generations	No. of plants								Test ratio	Chi-square value	Table value
	Observed				Expected						
	Resistant (R)	Segregants	Susceptible (S)	Total	Resistant (R)	Segregants	Susceptible (S)	Total	R:S		
P_1	0	-	38	38	-	-	-	38	-	-	-
P_2	43	-	0	43	-	-	-	43	-	-	-
F_1	61	-	0	61	-	-	-	61	-	-	-
F_2	388	-	138	526	394	-	131	526	3:1	0.428	3.841
F_3	55	83	42	180	45	90	25	180	1:2:1	2.96	5.991
BC_1P_1	27	-	30	57	28	-	28	57	1:1	0.157	3.841
BC_1P_2	56	-	3	59	59	-	0	59	1:0	0.305	3.841

(A) **(B)**

(C)

Plate 8: Parental line C 152 (A), Overall view of hybridization block (B), F1 (C) and F2 experimental plots

Table 26: Segregation for BLB disease resistance and susceptibility in different generations of the cross C-152 x V-16.

Crosses/ generations	No. of plants								Test ratio	Chi-square value	Table value
	Observed				Expected						
	Resistant (R)	Segregants	Susceptible (S)	Total	Resistant (R)	Segregants	Susceptible (S)	Total	R:S		
P_1	0	-	49	49	-	-	-	49	-	-	-
P_2	53	-	0	53	-	-	-	53	-	-	-
F_1	60	-	0	60	-	-	-	60	-	-	-
F_2	242	-	91	333	249	-	83	333	3:1	0.961	3.841
F_3	52	92	36	180	45	90	45	180	1:2:1	2.92	5.991
BC_1P_1	22	-	27	49	24	-	24	49	1:1	0.510	3.841
BC_1P_2	52	-	2	54	54	-	0	54	1:0	0.148	3.841

4.8 Genetic variability parameters for yield and its attributing traits in both F_2 populations of cowpea

The variability parameters *viz.,* mean, range, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (h^2) and genetic advance as per cent mean with respect to plant height (cm), number of branches per plant, number of seeds per pods, number of pods per plant, test weight (g) and seed yield per plant (g) in F_2 populations of both the crosses are presented below.

4.8.1 Cross C-152 x HC-03-02

The F_2 population of this cross exhibited wide variations for all the traits studied presented in Table 27 and graphically depicted in Fig.4. PCV estimate was high for number of branches per plant (34.75) followed by seed yield per plant (31.22), number of pods per plant (27.82), plant height (21.89), number of seeds per pod (21.72) and it was moderate in test weight (11.40). GCV estimates were high for plant height (20.19) and moderate for number of branches per plant (18.71), number of pods per plant (13.81), test weight (11.40), number of seeds per pod (11.37) and very low in seed yield per plant (5.78).

Heritability in broad sense was high for test weight (97.71) and plant height (92.23), moderate in parameters like number of branches per plant (53.84), numbers of seeds per pod (52.34) and number of pods per plant (49.64). Whereas, it was low for seed yield per plant (18.51). Genetic advance as per cent mean was high for all the parameters like plan height (41.6), number of branches per plant (38.54), number of pods per plant (28.45), number of seeds per plant (23.41), test weight (22.96) and seed yield per plant (20.53).

4.8.2 Cross C-152 x V-16

The F_2 populations of this cross exhibited wide variations for all the traits studied (Table 28) and graphically depicted in Fig. 5. For number of pods per plant (52.49), seed yield per plant (37.87), number of seeds per pod (33.13), number of branches per plant (34.75), plant height (23.67) PCV was high and it was moderate in case of test weight (19.20). GCV estimates were high for seed yield (37.87) and number of pods per plant (28.36). It is moderate for the traits like number of seeds per plant (19.92), test weight (18.99) and plant height (11.01). Whereas, it is low for the traits number of branches per plant (8.80)

High heritability was observed for test weight (98.90), number of pods per plant (80.25) and seed yield per plant (77.55). Number of pods per plant (54.02) and Plant height (46.51) recorded moderate heritability. Whereas, number of branches plant (26.57) recorded low heritability. For the parameters like seed yield per plant (78.01), number of pods per plant (58.41), number of seeds per plant (41.04), test weight (39.10), number of branches per plant (38.54) and plant height (22.62) genetic advance as per cent mean (GAM) was high. Whereas, in case of numbers of branches per plant (18.13) it was moderate.

4.8.3 Correlation studies in F_2 generations of both the crosses

The simple correlations were estimated among six important quantitative characters to determine the nature of relationship in both F_2 population and the results are presented below.

4.8.3.1 Cross C-152 x HC-03-02

The simple correlations estimated in the cross C-152 x HC-03-02 among six characters are presented in Table 29. Seed yield per plant exhibited significant positive association with number of pods per plant (0.701), number of seeds per pod (0.674) and test weight (0.350). While,

Table 27: Estimates of mean and genetic variability parameters for six characters in F_2 generation of the cross C-152 x HC-03-02 of cowpea

S.No	Characters	Mean	Range	PCV (%)	GCV (%)	h^2 (%)	GA as% mean
1	Plant height (cm)	69.28	46-100.5	21.89	20.19	92.233	41.6
2	Number of pods per plant	18.28	12-28	27.82	13.81	49.64	28.45
3	Number of seeds per pod	13.28	10-17	21.72	11.37	52.34	23.41
4	Number of branches per plant	4.14	3-7	34.75	18.71	53.84	38.54
5	Test weight (g)	16.42	15-18	11.4	11.14	97.71	22.96
6	Seed yield per plant (g)	44.72	19.2-194.94	31.22	5.78	18.51	20.53

Table 28: Estimates of mean and genetic variability parameters for six characters in F_2 generation of the cross C-152 x V-16 of cowpea

S.No	Characters	Mean	Range	PCV (%)	GCV (%)	h^2 (%)	GA as% mean
1	Plant height (cm)	57.27	26.5-88	23.67	11.01	46.51	22.62
2	Number of pods per plant	17.99	4-55	52.49	28.36	54.02	58.41
3	Number of seeds per pod	11.12	4-16	22.82	19.92	80.25	41.04
4	Number of branches per plant	5.40	6-11	33.12	8.8	26.57	18.13
5	Test weight (g)	16.81	7.5-21	19.20	18.99	98.90	39.10
6	Seed yield per plant (g)	34.16	7.28-138.6	48.83	37.87	77.55	78.01

it had positive non- significant association with plant height (0.049) and number of branches per plant (0.050).

4.8.3.1 Cross C-152 x V-16

The simple correlations estimated in the cross C-152 x V-16 among six characters are presented in Table 30. Seed yield per plant exhibited significant positive association with number of pods per plant (0.863) and number of seeds per pod (0.395). But it had positive non-significant association with test weight (0.156) and negative non-significant association with plant height (-0.033) and number of branches per plant (-0.035).

4.9. Identification of molecular markers linked to BLB resistance.

4.9.1 Screening of parental genotypes using SSR markers for bacterial leaf blight (BLB)

Forty two cowpea specific SSR genomic markers obtained from the Kirkhouse Trust, UK, were used for screening parental genotypes of the cross C-152 X V-16. A total 12 primers were polymorphic between the parents for the cross C-152 x V-16 (Table 31).

4.9.2 Bulk Segregant Analysis (BSA) in F_2 population of the cross C-152 x V-16 using SSR markers for Bacterial leaf blight

The SSR primers polymorphic between the parents of the mapping population of cross C-152 x V-16 were subjected to BSA to identify putative linked markers for bacterial leaf blight disease resistance. Out of 12 SSR primers that were polymorphic between the parents, three SSR primers were found to be polymorphic between the resistant and susceptible F_2 bulk, indicating that these markers are putatively linked to BLB resistant gene (Table 31).

Table 29: Simple correlations among seed yield and its attributing characters in F_2 generation of the cross C-152 x HC-03-02 of cowpea

	X_1	X_2	X_3	X_4	X_5	X_6
X_1	**1.000**	0.116	-0.042	-0.082	-0.088	0.049
X_2		**1.000**	0.105	-1.017	0.051	0.701*
X_3			**1.000**	0.050	-0.017	0.674*
X_4				**1.000**	0.113	0.050
X_5					**1.000**	0.350*
X_6						**1.000**

Table 30: Simple correlations among seed yield and its attributing characters in F_2 generation of the cross C-152 x V-16 of cowpea

	X_1	X_2	X_3	X_4	X_5	X_6
X_1	**1.000**	-0.003	-0.040	-0.214	0.001	-0.033
X_2		**1.000**	0.029	0.028	-0.124	0.863*
X_3			**1.000**	-0.086	0.071	0.395*
X_4				**1.000**	-0.098	-0.035
X_5					**1.000**	0.156
X_6						**1.000**

* & ** indicates significant at 5 %and 1 % level respectively

X_1 - Plant height (cm)

X_2 - Number of pods per plant

X_3 - Number of seeds per pod

X_4 - Number of branches per plant

X_5 - Test weight (g)

X_6 - Seed yield per plant (g)

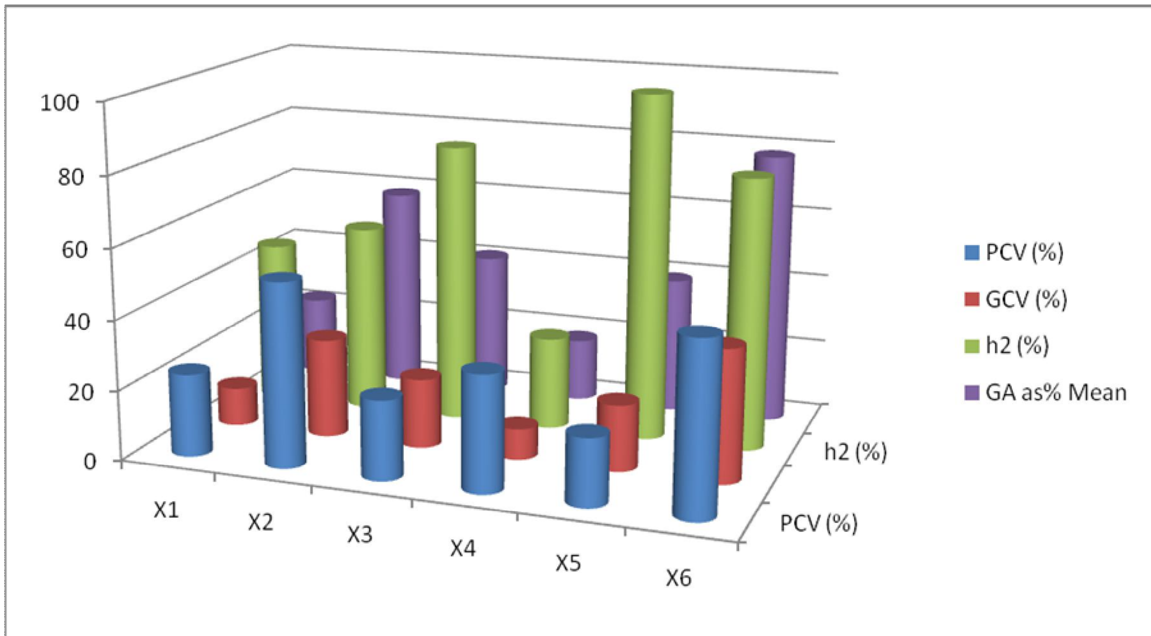


Fig. 4: Estimates of mean and Genetic variability parameters for six characters in F_2 generation of the cross C 152 x HC 03 02 of cowpea

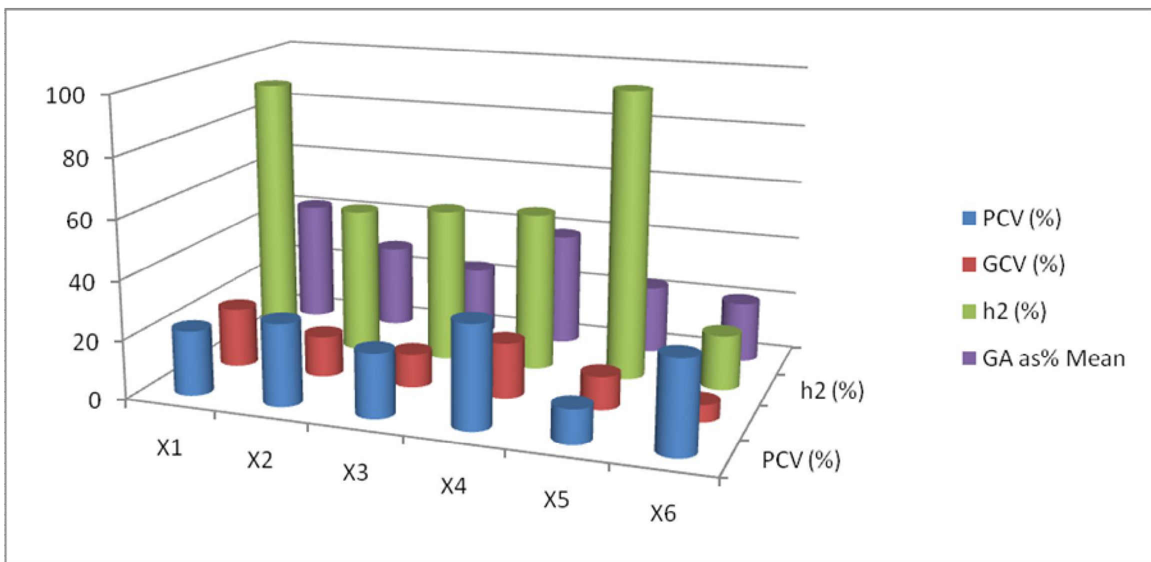


Fig. 5: Estimates of mean and Genetic variability parameters for six characters in F_2 generation of the cross C 152 x V 16 of cowpea

Table 31: Details of the polymorphic SSR markers detected in parents and bulks in the F_2 of the cross C-152 x V-16

S. No	SSR primers	Approx. Product Size	Polymorphic Details	
			In parents (C-152 and V-16)	R and S Bulks of F_2 cross (C-152 x V-16)
1	CP 121/122	200	Polymorphic	Monomorphic
2	CP 137/138	200	Polymorphic	Monomorphic
3	CP 171/178	200	Polymorphic	Monomorphic
4	CP 215/216	250	Polymorphic	Monomorphic
5	CP 273/274	200	Polymorphic	Monomorphic
6	CP 395/396	200	Polymorphic	Monomorphic
7	CP 641/642	200	Polymorphic	Polymorphic
8	MS 20/21	250	Polymorphic	Monomorphic
9	MS 24/25	200	Polymorphic	Monomorphic
10	MS 50/51	200	Polymorphic	Polymorphic
11	MS 99/100	200	Polymorphic	Polymorphic
12	MS 113/114	200	Polymorphic	Monomorphic

4.9.3 Linkage analysis between BLB resistant gene and SSR marker in F_2 population of cross C 152 x V 16

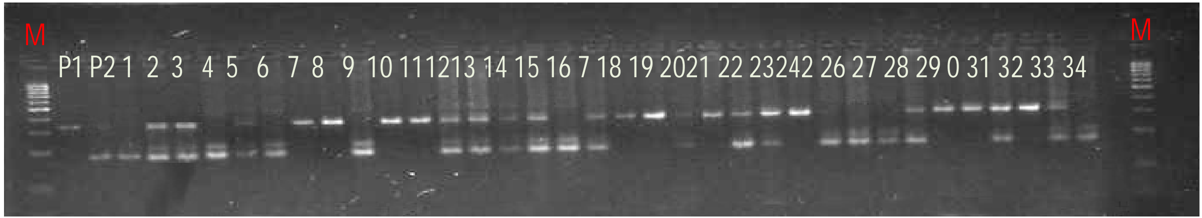
Band information of three SSR markers that showed Mendelian segregation ratio (Table 32) was compared with disease reaction. The data from individual marker analysis on F_2 population along with disease reaction were subjected to Single Marker Analysis (SMA). SMA is based on the idea that if there is an association between a marker genotype and a trait value, it is likely that a trait is close to that marker locus. The results of SMA are presented in the Table 33. From the ANOVA of single factor analysis (Table 33) of these three markers, only marker CP 641/642 showed the significant difference at 5 % level of significance, as the calculated F value (4.364) is more than Table F value (3.0872), and hence we predict that there could be a linkage between the marker and the BLB disease resistance gene. Whereas, in the markers MS 99/100 and CP 215/216 there is no significance difference between the marker classes at 5 % level of significance. Therefore we assume that there is no linkage between marker and BLB resistance gene.

Table 33. SMA-analysis of locus CP 641/642 for BLB disease reaction

Source of Variation	df	MS	P-value
Between SSR markers	2	1385.02	0.015
Within marker classes	100	317.36	
Total	102		

Table 32: Segregation behaviour of SSR Markers in F_2 generation C-152 x V-16

S.N.	SSR primers	Total no. of F_2 plants	Observed Polymorphic Bands			Expected polymorphic bands			Test ratio	Chi-square (cal)	Chi-square (tab.)
			M1M1	M1M2	M2M2	M1M1	M1M2	M2M2			
1	CP 641/642	102	24	51	27	25.5	51	25.5	1:2:1	0.098	5.991
2	MS 99/100	96	26	48	22	24	48	24	1:2:1	0.541	5.991
3	CP 215/216	102	33	43	26	25.5	51	25.5	1:2:1	4.119	5.991



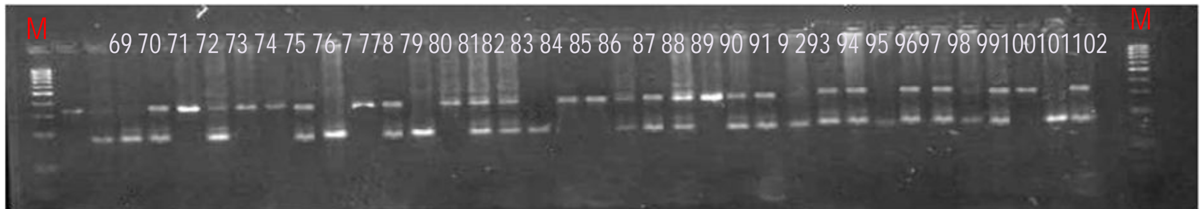


Plate 9: Segregation pattern of SSR marker CP 641/642 in F2 population on Agarose gel.

****Lane P1=C-152, Lane P2 =V-16, L1 to L102 = F_2 segregating populations and M= Marker (100bp)**

Plate 10: Segregation pattern of SSR marker MS 99/100 in F2 population on Agarose gel.

****Lane P1=C-152, Lane P2 =V-16, L1 to L96 = F_2 segregating populations and M= Marker (100bp)**

Discussion

V. DISCUSSION

The production and productivity of pulses in general and cowpea in particular is affected by biotic and abiotic stresses. Since, majority of the pulses including cowpea are being grown under rain fed situations with minimum inputs, breeding for biotic stresses, particularly disease gains paramount importance in crop improvement programme.

Before initiating crop improvement programme in any crop, breeder should thoroughly evaluate, screen and understand the genetic architecture of the germplasm he is handling. Estimation of genetic variability parameters is the foremost step to be adopted in the source population if the breeding programme is aimed at improving economically important traits. The success of a crop improvement programme depends on the ability of the breeder to define and assemble the required genetic variability and select for yield indirectly through yield associated and highly heritable characters after eliminating the environmental component of phenotypic variation (Mather, 1983).

Variability is the key factor for any selection programme, which can be generated through various ways. To achieve or create variability, addition of some more diverse genotypes with the available collection is necessary or creation of new variability by other means is very much needed. Since the productivity of cowpea is very low due to lack of high yielding varieties with resistance to biotic stresses such as diseases which is the major hindrance in achieving potential yield of cowpea. Among many cowpea diseases, rust, bacterial blight and cowpea mosaic virus are considered as major diseases. Therefore, it is very essential to identify resistant genotypes for each of these diseases. Host plant resistance is the most common and efficient method of disease control. For developing high yielding disease resistant varieties (for both BLB and

CpMV) of cowpea, knowledge of inheritance of resistance to these diseases is of prime importance in cowpea breeding programme.

The morphological observations recorded in the field usually will be the sum total of genotypic as well as environmental effects. Hence, the diversity obtained from the field data should be verified to ensure that the variability present is at genotypic level. Analysis of these genotypes at molecular level using markers which are free of environmental influence can be made use for this purpose. Among these, SSR markers are more crops specific and very useful in identifying markers which are linked to any desirable trait of our interest like bacterial leaf blight disease resistance. In addition to this, SSR markers give a clear picture regarding the diversity at molecular level.

The results obtained in the present study are discussed in the light of available literature and presented under the following headings.

5.1 Analysis of variance

One hundred and ninety six cowpea genotypes were assessed for variability of quantitative parameters. The genotypes recorded highly significant variation for all the characters such as days 50 per cent flowering, days to physiological maturity, plant height, number of branches per plant, number of pods per plant, pod length, number of seeds per pod, test weight and seed yield per plant. It indicated the presence of sufficient variability for these characters, thus there is a lot of scope for selection. Sarvamangala (2004) also reported the existence of good amount of variability in cowpea.

5.2 Range and mean performance of genotypes

One of the ways of assessing the variability is through examining the range of variation. The range in the values reflects the extent of

phenotypic variability in respect of the character, which includes genotypic, environmental and genotype environmental interaction components. In the present study the genotypes exhibited considerable amount of variation for nine characters *viz.,* days 50 per cent flowering, days to physiological maturity, plant height, number of branches per plant, number of pods per plant, pod length, number of seeds per pod, test weight and seed yield per plant. Sawant (1994) recorded higher range for these characters, which was in accordance to the present study. The high range of values indicated the good scope for selection of suitable basic material for breeders for further improvement.

Lower mean values for days to 50 per cent flowering and days to physiological maturity enabled identification of several short duration genotypes. The lower mean values for days to 50 per cent flowering were observed in C 325, IC 402180 and IC 458411. Whereas, lower mean values for days to physiological maturity was observed in the genotypes 202804 (83), IC 462099, IC 402048, IC 2021190 and NBC 10. These genotypes can be used in niche areas where early varieties are needed or as parents in hybridization for the development of early duration and high yielding varieties.

Though plant height is one of the important quantitative parameter, taller plants are not suitable from the point of high yield, especially in cowpea, since higher the plants yields more the biomass. Cowpea is a crop with a tendency to high biomass, when excess moisture and nitrogen is provided. The mean values with wide range in the present investigation are more, indicating scope for selection for desired plant height. Sarvamangala (2004) suggested that plant height should not be extremely high or low which may be resulting in either more vegetative growth or lodging condition respectively.

Genotypes showed lower mean value for number of pods per plant over the check KBC 2 and KM 5. Among various quantitative parameters, the contribution of each parameter towards yield varies from crop to crop. In cowpea, number of pods/plant, pod length, seeds/pod and test weights are more important. However, Tamilselvan and Vijendra Das (1994) suggested that number of clusters per plant, number of pods per plant, and test weight should be used as a selection criterion in the development of high yielding genotypes of cowpea. Whereas, Marappa *et al.*, (2007) suggested number of seeds per pod and seed yield per plant should be used as selection criterion in yield improvement breeding programmes.

The mean values for hundred seed weight in this study showed wide variation among the genotypes. The genotypes EC 458442, NBC 14, EC 394779, NBC 42, IC 330996, IT 97499-38, IC 249793, IC 202290, IC 202781, C 33, V 585 were exhibited higher test weight over the check KBC 2, indicating the scope for selecting bold seeded cowpea varieties.

5.3 Genetic variability

Genetic variability is a basic information needed for the breeders to improve the crops by adopting appropriate method of selection based on variability that exist in the material. In this regard, it is necessary to partition the total variability into heritable and non-heritable components *viz.,* genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) and further to compute heritability and genetic advances for various metric traits.

5.3.1 Phenotypic and genotypic coefficient of variation

Comparison of variability between two traits is possible with coefficient of variation as it is free of units. As expected, the PCV values were greater than the GCV values for all the characters indicating

considerable influence of environment on the expression of these characters under field conditions. The difference between PCV and GCV was more for seed yield per plant, pod length, number of branches per plant and number of pods per plant indicating the major role of environment on these characters. Earlier reports on cowpea by Anbu Selvam *et al.* (2000) and Awopetu *et al.* (2006) are in conformation with these results.

In general, the PCV and GCV were quite high for number of branches per plant, number pods per plant, pod length, plant height, test weight and seed yield per plant indicating greater scope for improvement of these characters by simple selection. However, number of seeds per pod showed moderate PCV and GCV values, while days to 50 per cent flowering and days to physiological maturity exhibited low values. Several earlier workers also reported high PCV and GCV for number of branches per plant (Marappa *et al.* 2007; Borah and Khan., 2001; Malarvizhi *et al.*, 2005), number of pods per plant (Prasanthi., 2004; Resmi *et al.*, 2004; Awopetu *et al.*, 2006; Girish *et al.*, 2006), pod length (Shobha and Abdul Vahab., 1998; Mathura Rai *et al.*, 2004), plant hieght (Marappa *et al. 2007*; Omoigui *et al.*, 2006, Prashanthi *et al.*, 2004, Singh *et al.*, 2002), test wieght (Marappa *et al.* 2007; Omoigui *et al.*, 2006, Prashanthi *et al.*, 2004, Rocha *et al.*, 2003) and seed yield (Tyagi *et al.*, 2000; Venkatesan *et al.*, 2003; Vineeta Kumari *et al.*, 2003; Resmi *et al.*, 2004).

Low PCV and GCV values were also reported for days to physiological maturity (Marangappanavar, 1984; Patil and Baviskar, 1987; Thiyagarajan, 1989), days to 50 per cent flowering (Marangappanavar, 1984; Apte *et al.*, 1987; Patil and Baviskar, 1987), while moderate PCV and GCV values were reported for number for seeds per pod (Lakshmi and Goud,1977; Backiyarani and Nadarajan,1996; Sharma,1999; Kalaiyarasi *et al.*, 2000; Rahul Chauhan *et al.*, 2003;

Vineeta Kumari *et al.,* 2003). But contradicting the present results, moderate to low PCV and GCV values were reported for plant height (Venkatesan *et al.,* 2003; Vineeta Kumari *et al.,* 2003), branches per plant (Venkatesan *et al.,* 2003), pods per plant (Tyagi *et al.*, 2000), pod length (Rahul Chauhan *et al.,* 2003), and seed yield per plant (Rahul Chauhan *et al.*, 2003). High PCV and GCV were recorded for days to 50 per cent flowerig and days to physiological maturity by Henry *et al.* (2003) and Veneeta kumari *et al.* (2003).

There may be any one of the three situations in any study *viz.*, high PCV and GCV; high PCV low GCV and low PCV and GCV. A treatment having low PCV with high GCV is a desirable for plant breedrs to have effective selection. The contradictory reports for similar traits from different studies might be due to various factors like genotypes and another environmental variations such as soil fertility, season, moisture availabilty etc.

5.3.3 Heritability and genetic advance as per cent of mean

The effectiveness of selection for any yield component depends not only on the amount of variability but also how much of it can be carried forward to next generations. The coefficient of variation indicates only the extent of variation and does not discriminate the variability into heritable and non-heritable components. Genotypic coefficient of variation together with the heritability estimates would give the best information of the amount of genetic advance to be expected from each selection (Burton, 1952; Panse and Sukhatme, 1967). The estimates of genetic advance when expressed as a per cent of mean provide actual genetic gain that is expected to be attained in relation to *per se* performance (Burton, 1952). In the present investigation, genetic advance estimates were medium to high (11.98 per cent to 86.63 per

cent) for majority of the characters studied and low in case of days to physiological maturity (7.99 per cent).

The characters like test weight, plant height, number of branches, number of pods per plant, pod length, seeds per pod and seed yield per plant exhibited high heritability along with high genetic advance. Days to 50 per cent flowering recorded maximum heritability (99.88) compared to other traits. Plant height exhibited maximum genetic advance (86.63 per cent) compared to the other characters. Days to 50 per cent flowering exhibited high heritability coupled with moderate genetic advance as per cent of mean. Whereas days to physiological maturity expressed high heritability coupled with low genetic advance as per cent of mean. Several earlier workers have also reported high heritability coupled with high genetic advance for plant height (Gireesh *et al.*, 2006; Shahid ahemed *et al.*, 2005; Nigude *et al.*, 2004; Prashanthi *et al.*, 2004; Pal *et al.*, 2003; Prakash *et al.*, 2003), seed yield per plant (Shahid ahemad *et al.*, 2005; Reshmi *et al.*, 2004; Henry *et al.*, 2003; Tyagi *et al.*, 2000; Vineeta kumara *et al.*, 2003; Anbumalaramathi *et al.*, 2005; Sughanthi and Muragan, 2007), number of branches per plant (Renganayaki and Rengasamy,1992; Shobha and Abdul Vahab,1998, Borah *et al.*, 2001; Malarvizhi *et al.*, 2005), number of pods per plant (Nehru *et al.*, 2000; Venkatesan *et al.*, 2003; Rahul Chauhan *et al.*, 2003; Vineeta Kumari *et al.*, 2003; Prasanthi, 2004; Resmi *et al.*, 2004; Gireesh *et al.*, 2006), pod length (Kalaiyarasi *et al.*, 2000; Tyagi *et al.*, 2000; Rahul Chauhan *et al.*, 2003), test weight (Tyagi *et al.*, 2000; Ahmad Neyaz and Bajpai, 2002; Venkatesan *et al.*, 2003), seeds per pod (Kalaiyarasi *et al.*, 2000; Rahul Chauhan *et al.*, 2003; Vineeta Kumari *et al.*, 2003) and seed yield (Tyagi *et al.*, 2000; Venkatesan *et al.*, 2003; Vineeta Kumari *et al.*, 2003; Resmi *et al.*, 2004). Some reports indicated high heritability and medium genetic advance for days to 50 per cent flowering (Awopetu *et al.*, 2006; Anbusevam *et al.*, 2000; Venkatesan *et al.*, 2003; Gireesh *et al.*, 2006;

Sree kumar *et al.*, 1996). High heritability and low genetic advance were reported for days to physiological maturity (Marangappanavar, 1984; Patil and Patil, 1986; Sarvamangala *et al.*, 2004).

High heritability estimate indicates less influence of environment on respective characters. Hence, direct selection can be followed to improve early maturing genotypes. Low heritability (broad sense) indicates predominance of non-additive gene action indicating the scope for breeding. High estimates of GA coupled with substantial amount of heritability indicate that selection for such characters would result in the improvement of characters in the desired direction as the character is governed by additive genes. High heritability coupled with low genetic advance indicates non-additive gene action. The heritability exhibited due to favorable influence of environment rather than genotypes and selection for such traits may not be rewarding. If, low heritability coupled with low genetic advance indicates such character was highly influenced by environment and selection would be ineffective for those traits.

5.4 Genetic diversity

5.4.1 Mahalanobis Generalized Distance

The concept of genetic distance has been of vital utility in many contexts and more so in differentiating well defined populations. Quantification of genetic diversity existing within and between groups of germplasm is important and particularly useful in proper choice of parents for realizing higher heterosis and obtaining useful recombinants. Several methods have been advocated by various workers to estimate the genetic divergence in crop plants. Of the several methods available, Mahalanobis generalized distance estimated by D^2 statistic is a unique tool for discriminating populations considering a set of parameters together rather than inferring from indices based upon morphological similarities, eco-geographical diversity and phylogenetic relationships.

In the present study on 196 cowpea genotypes, all the characters studied contributed to the total genetic divergence. The highest contribution was made by seed yield per plant followed by test weight, days to 50 per cent flowering, days to physiological maturity, plant height, pod length, number of branches per plant, number of seeds per pod and the lowest contribution was from number of pods per plant. Rewale *et al.* (1996) reported maximum contribution towards the total diversity by days to 50% flowering and maturity, number of pods per plant, pod length, 100-seed weight, and seed yield per plant. Similar results were also made by Backiyarani *et al.* (2004), Borah *et al* (2002), Chikkdevaiah *et al.* (1985), Nigude *et al.* (2004), Narayankuttuy et al. (2003), Pandey *et al.* (2007), Santos *et al.* (1997) and Sulnathi *et al.* (2007). While Venkatesan, *et al.* (2004) reported clusters per plant, pods per cluster, pods per plant and seed yield per plant contributing maximum towards total divergence. Similarly, Kumawat and Raje (2005) also reported that seed yield per plant contributed the highest towards the total genetic divergence; followed by days to 50 per cent flowering, seeds per pod and plant height.

In the present study based on D^2 values, the 196 cowpea genotypes were grouped into 22 clusters. The maximum number of genotypes fell in the cluster XXII (133 genotypes) followed by the cluster I (23 genotypes), and remaining all clusters had two genotypes each.

The genotypes falling in a particular cluster will have close genetic background with smaller intra-cluster distance between the genotypes within a cluster. The genotypes between the clusters have more D^2 value with more genetic distance. Further, genotypes belonging to more distanced clusters will serve as good sources of divergent genes which is very much required for breeding to exploit heterosis as reported by Gill *et al.* (1982).

Maximum intercluster distance was observed between the clusters I and XIX indicating genotypes included in these clusters are highly divergent which indicated large differences between the cluster means for many characters like days to 50 per cent flowering, number of seeds per pod, number of branches per plant and test weight. For other characters like number of pods per plant, plant height, and seed yield per plant the differences were not substantial.

Minimum inter cluster distance observed between the clusters II and VI and same was reflected in the cluster means for different characters showed small divergence between the clusters II and VI. Cluster XXII showed more D^2 distance with other clusters indicating that genotypes in this cluster are more divergent from genotypes of other clusters. Intra-cluster D^2 value was small in the cluster II with only two genotypes whereas cluster XXII has recorded maximum intra-cluster D^2 value indicating that, 133 genotypes in the cluster XXII were not closely related compared to the genotypes in the cluster II. When we select the genotypes for hybridization it is desirable to select the genotypes from the clusters with maximum intercluster distance.

5.4.2 Cluster Mean Analysis

All the accessions spread over 22 clusters and means were scored across the clusters for eight characters.

The highest cluster mean was given the first rank and next cluster possessing next best means were given 2nd, 3rd and so on up to 22nd rank for all the traits. Based on the overall score across eight traits, the clusters were ranked. The lowest scoring cluster was given first rank and next cluster possessing the score above the previous one were given 2nd, 3rd and so on up to 22nd rank. Accordingly cluster XIV with overall score of 56 across eight characters received first rank indicating that cluster

XIV possess the genotypes with high overall performance followed by cluster VIII, XVII and XXII, indicating presence of most promising genotypes in them and can be extensively used for further breeding programme to generate new material.

5.4.3 Variation in qualitative characters

Qualitative characters are useful for characterization of any genotype which is associated with the yield and its contributing characters. Association of any qualitative character with yield and its components, resistance to diseases which can serve as a marker for selection process. In the present study the variability for four qualitative characters in cowpea such as leaf shape, plant habit, flower colour and immature pigmentation on green pods have been studied as it is essential to characterize the local germplasm collections for future use.

Among 196 genotypes including exotic genotypes, majority (190) of the genotypes showed only two types of leaf shapes ovate and six genotypes with lanceolate leaf shape. Plant habit is a very important character for the breeders which can be used as a selection criterion in plant breeding. The genotypes showed higher variability for plant habit with erect (93), semi-erect (53) and spreading (50). The semi-erect types are much better for further improvement of cowpea compared to other two plant types. Flower colours of the genotypes were grouped into four categories *viz.,* violet (16), mave pink (171), purple yellowish (7) and white yellowish (2) which had higher variability. In contrast, Naima *et al.* (2010) reported that absence of intra-land race variation for qualitative characters and presence of low variation for quantitative characters at morphological level.

Pigmentation on green pod (Plate 8) is highly visible trait for characterization of genotypes. The genotypes showed higher variability

for immature pod pigmentation. However, most of the genotypes did not show special pigmentation (172) and remaining genotypes exhibited purple (12), light purple (9) and light green pigmentation (3).

5.5 Molecular characterization

DNA markers have been proved to be useful for assessment of genetic variation in any germplasm. Over the last two decades, molecular biology provided well defined set of tools to estimate the genetic divergence of an organism. The threat of genetic erosion has led to a significant interest in the assessment of genetic diversity in germplasm.

Simple sequence repeats (SSRs), also known as microsatellites, are very informative and excellent markers for a wide range of applications including genetic diversity studies. Microsatellite markers have shown high levels of polymorphism in many important crops including rice (Chen *et al.,* 1997), oat (Li *et al.,* 2000), maize (Senior *et al.,* 1998), soybean (Akkaya *et al.* 1992), chickpea (Winter *et al.* 1999), Yardlong bean (Tantasawat *et al.* 2010) and cowpea (Archana *et al*. 2006, Gupta et al. 2004, Li *et al.,* 2001, Perkall et al., 1998, Uma *et al.*, 2009). Genetic diversity in the available gene pool is the basic pre-requisite for all plant improvement programmes.

Since, the molecular study involves lot of cost, a representative sample of a core set of 60 genotypes were selected from 196 genotypes for molecular study with 42 cowpea specific SSR markers. The average number of alleles amplified per primer pair was two. Twenty Three out of 42 primers used in the study were showing polymorphism which was much less compared to many other crops. Li *et al.* (2001) also proved that the polymorphism in cowpea lines was less (three to seven) compared to the other crops. The probable reasons for less allelic difference in each specific study could be due to the inclusion of limited

number of genotypes with limited number of primers. The resolution can definitely be improved by increasing the number of genotypes and number of primers in each study and probably one can conclude on these by exhaustive study of the entire germplasm with maximum possible number of primers.

Sixty cowpea genotypes were clustered based on band information of both polymorphic and monomorphic primers by the Jaccard's coefficient of similarity from 57 to 100 per cent (Fig 3). Cluster analysis was used to group the varieties and to construct a dendrogram. The similarity matrix representing the Jaccard's coefficient was used to cluster the data using the UPGMA algorithm. The UPGMA based dendrogram obtained from the binary data deduced from the DNA profiles of the samples analysed adds a new dimension to the genetic similarity perspectives generated.

The clustering started at the Jaccard's similarity coefficient of 57 per cent in which the genotype EC 472252 differed from rest of the genotypes. The diverse genotypes were divided into many subgroups with increase in the Jaccard's similarity coefficient.

At the Jaccard's similarity coefficient of 65 per cent, seven clusters were clearly formed based on data of 23 primers used in the study (Fig 2 and Table 20). The II cluster had single genotype EC 472252, VII had two (IC 402048, EC 458473), III and IV cluster had three genotypes, while VI and I cluster had 14 and nine genotypes, respectively. The V cluster was the biggest cluster with remaining all other genotypes. A similar study by Adebayo *et al.*, 2008, only two major groups were formed at 68 per cent similarity coefficient in 50 cowpea genotypes.

5.5.1 Comparison of molecular diversity with that of biometrical diversity

All these years, breeders used to depend on morphological data by growing all germplasm in the field in the suitable environment and analyzing the recorded observations on all morphological characters in order to assess diversity which involves space, time and cost. Since molecular tools also provide a more precise result of the diversity among genotypes within the germplasm. An attempt in the present investigation was made to compare the morphological diversity with that of molecular diversity.

Present study indicated a similarity coefficient ranging from 57 to 100 per cent for marker data (Fig 3), while for morphological data, the dendrogram showed similarity from 82 to 100 per cent (Fig 2 and Table 19), suggesting higher resolution for diversity from molecular data than morphological data. Looking into the literature and also based on the results of the present investigation, one can conclude that molecular diversity will definitely be a better tool in getting a clear picture of genetic diversity with high resolution at DNA level, since role of environment can be totally eliminated in this case. Such results will provide the breeder a closer look of the genotypes at the genetic or DNA level.

The Genotypes NBC 30, V 16, GC 3, KBC 2, NBC 6, NBC 29, C 33, GC 4, KM 5, C 152, HC 03 02, EC 458402, exhibited more diversity than the rest of the genotypes at both molecular and morphological and level these can be used as parents for hybridization programme to generate desired variability and better recombinants.

5.6 Screening for Diseases resistance

Among production constraints of any crop, biotic stresses play a major role. Diseases play a dominant role among biotic stresses. Though,

chemical control measures are available to check these diseases, the importance of identifying and developing resistant varieties against diseases especially for rain fed situations cannot be disputed. Hence, an attempt was made in the present investigation to identify genotypes resistant or tolerant from the 196 cowpea genotypes against major diseases such as cowpea mosaic virus and bacterial leaf blight under prevailing environmental conditions.

5.6.1 Cowpea Mosaic virus

In the experiment conducted during *Kharif* 2009, it was found that 110 genotypes were resistant, 42 genotypes moderately resistant, 18 genotypes moderate susceptible, 21 genotypes susceptible, while remaining seven genotypes were highly susceptible for cowpea mosaic virus in cowpea. The entry IC 249141 was resistant to the mosaic virus and in addition it has recorded the highest seed yield per plant and more number of pods per plant. Such high yielding and promising genotypes with resistant can directly be evaluated in yield trials for consideration to release as variety, only after reconfirmation of its resistance under controlled epiphytotic conditions. The genotype IC 202781, KBC 2 and HC 03 02 recorded resistance but their seed yields were moderate. Sahoo *et al.* (1991), Bashir *et al.* (1996, 2000 and 2002), Bhati *et al.*, (1987), Gubba *et al.*, (1994), Gumedzoe *et al.*, (1990), Naimuddin *et al.* (2011), Satya Vir *et al*. (1997) and Yousaf et al., (2002) reported resistance to cowpea mosaic virus in cowpea.

5.6.2 Bacterial leaf blight

Performance of cowpea genotypes against bacterial leaf blight resistance revealed that 30 genotypes were highly resistant, 120 genotypes resistant, 28 genotypes moderately resistant, seven genotypes susceptible, nine genotypes showed moderately susceptible, whereas only two genotypes such as C 152 and GC 4 were highly susceptible.

Similarly, Uma (2001) reported that C 152, was highly susceptible to BLB. Another study carried out by Ajeigbel *et al.* (2008) over three different locations reported that about 40 per cent of the genotypes tested were resistant, while 20 per cent showed moderate resistance and others were susceptible. Young *et al.* (1999) observed the relationship between disease severity and duration of saturated humidity. It is always desirable and essential to confirm the resistance with artificial inoculation under controlled conditions. We confess the limitations of present investigation that screening for both the diseases were restricted to only selected genotypes under controlled conditions.

The ultimate aim of any breeder is to identify and develop crop varieties with disease resistance coupled with high yield potential. The resistance from these resistant sources can be transferred to agronomically superior susceptible varieties by back cross or any other breeding method.

5.7 Genetics of resistance to cowpea mosaic virus

Cowpea yield is low mainly due to its susceptibility to most devastating disease, CpMV. Plant resistance is the most common and efficient method of tackling the CpMV disease. For developing high yielding and CpMV resistant varieties of cowpea, knowledge on inheritance of resistance to cowpea CpMV is essential. Hence, in the present study an attempt was made to investigate the genetics of resistance to cowpea CpMV under field condition in the cross C 152 x HC 03 02.

In F_2 generation of the cross C 152 x HC 03 02, a good fit of 3:1 (Resistant: Susceptible) ratio was an indication of major dominant gene controlling resistance to CpMV disease in cowpea (Table 23).

The expression of resistance in F_1 generation is an indication of dominant gene controlling resistance of CpMV. The role of single dominant gene in controlling CpMV was evident from the F_2 segregating ratio of 3 resistant and 1 susceptible. The same was confirmed by the segregating behaviour of 1:2:1 ratio (Resistant: Segregating: Susceptible) in F_3 population and test ratio back cross generations BC1P1 (1 Resistant: 1 Susceptible) and BC1P2 (1 Resistant: 0 Susceptible). The segregation pattern in different generations proved the resistance was under the control of single dominant gene (Table 23). Similar findings were made by Bliss and Robertson (1971), Sinclair and Walker (1955), Kumar *et al.* (2004) in cowpea. In mungbean Marappa *et al.* (2007) and Dahiya *et al.*, (1977); Reddy and Singh, (1993); Gupta *et al.*, (2005) in blackgram. On the contrary, Thakur *et al.*, (1977), Reeder *et al.* (1972), Rogers *et al.* (1973), Lima and Nelson (1977), Taiwo *et al.*, (1981), they observed single recessive controlling CpMV resistance/tolerance. But Singh, (1986) and Ammavasai *et al.* (2004) claimed that MYMV resistance was due to duplicate recessive genes. Solanki *et al.*, (1982) and Verma and Singh, (1986), Reddy and Singh (1993) reported that MYMV resistance was due to one dominant and one recessive gene may be due to the differential behavior of parental confirmations in their study.

The simple genetics of resistance to CpMV in cowpea will aid the breeders in the development of high yielding resistant genotypes through simple selection in the segregating generations of crosses between resistant and susceptible genotypes.

5.7.2 Genetics of resistance to BLB in cowpea

There is considerable loss of yield reported in cowpea by bacterial leaf blight in India. Due to the low yield of the crop and neglected conditions under which it is grown especially under rainfed situation, plant protection measures are uneconomical. Therefore, resistant

breeding appears to be the most important approach in disease management. For developing high yielding BLB resistant varieties of cowpea, knowledge of inheritance of resistance to BLB is of prime importance in cowpea breeding programmes. Hence, in the present study an attempt was made to investigate mode of genetic control of resistance to cowpea BLB under field condition in the cross C 152 x V 16.

The expression of resistance reaction in F_1 generation is an indication of the role of dominant gene in controlling BLB in cowpea.

Based on classification of plants into resistant and susceptible in F_2 segregating generation of the cross C 152 x V16, a good fit of 3:1 (Resistant: Susceptible) ratio indicated the role of major dominant gene for field resistance which is evident from Table 22.

The F3 population of this cross showed 1:2:1 ratio (Resistant: Segregating: Susceptible) and back cross generations BC_1P_1 and BC_1P_2 segregated in 1:1 (Resistant: Susceptible) and 1:0 (Resistant: Susceptible) test ratio respectively. The segregation pattern in different generations confirmed of the role of single dominant gene in controlling resistance to cowpea bacterial leaf blight (Table 22). Bliss and Robertson (1971) and Singh and Chaudhry (1977) also reported dominance of resistance reaction to bacterial leaf blight in cowpea. Hence, the results of present investigation on the inheritance of disease resistance are in conformity to the earlier reports. However, Singh and Chaudhry (1977) and Prakash (1980) reported dominance of susceptible reaction over resistance in bacterial leaf blight. Since, the parents used in the present investigation and in earlier studies are different, there is a possibility that the gene in question may be varying for that locus across genotypes.

5.8 Genetic variability parameters for seed yield and its attributing traits in F_2 segregating population

The improvement of character in a population is a function of variability existing in the population. Hence, formulation of objectives in breeding programme should be essentially accompanied with the assessment of existing variability in the segregating populations.

The variability quantified by range includes influence of environment and genotype x environmental components of variation. Since, all these variations are not heritable it is appropriate to partition the phenotype variation into heritable (genetic) and non-heritable (environmental) components, and thus, true breeding value of the genotype can be precisely estimated by separating genetic variance from environmental variance. In this direction, the components of variance such as PCV and GCV, heritability and predicted genetic advance as per cent mean were computed for six quantitative characters studied in F_2 population of crosses C-152 x V-16 and C-152 x HC-03-02.

Evaluation of F_2 segregating populations for six biometrical traits exhibited wide range of variation for all the traits studied in both the crosses. This variation indicated that there is substantial scope for selection of genotypes for these traits. Rangaiah and Nehru (1998) also reported high range of variation for above mentioned characters in F2 segregating populations of two crosses of cowpea, which is in confirmity with the results of present investigation.

In the F_2 segregating population of the cross C-152 x V-16 high phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) estimates were recorded for number of pods per plant and seed yield per plant. This result is in conformity with findings of Gowda *et al.* (1991) and Hadpad (2001). This indicates greater scope for

selection to improve upon these characters in the crosses studied. Whereas, high PCV and moderate GCV estimates were recorded for traits *viz.,* plant height, number of seeds per pod and in case of number of branches per plant, high PCV and low GCV were recorded. While, for the parameter test weight moderate PCV and GCV were recorded. Similar results were obtained by Rangaiah and Nehru (1998) and Hadpad (2001).

In the F_2 segregating population of the cross C-152 x HC-03-02, high PCV and GCV were estimated for the trait plant height. Similar results were obtained by Suma Biradar *et al.* (2001), Mehta and Zavari (1999). Whereas high PCV and moderate GCV were observed for the traits *viz.,* number of branches per plant, number of pods per plant, number of seeds per pod. These results are in conformity with earlier findings of Gowda *et al.* (1991). While, moderate PCV and GCV were estimated in test weight and high PCV and low GCV was obtained in the parameter seed yield per plant.

Genotypic coefficient of variation (GCV) would be more useful for the assessment of variability than the phenotypic coefficient of variation (PCV) since, it depends upon the heritable portion of the total variability (Allard, 1970). Higher the proportion of GCV more will be the chance for exploitation of that particular character. Many practical decisions in breeding programs are based on the magnitude of heritable variation.

In comparing PCV and GCV estimates it was evident that the influence of environment on the expression of most of the characters was low indicating the greater role of genetic factors causing variability in these characters. These observations indicated ample scope for improvement of the above traits by selection from their phenotypic values.

In the F_2 segregating population of the cross C-152 x V-16, heritability (broad sense) was high for the traits *viz.,* number of seeds/pod, test weight and seed yield per plant. While, it recorded moderate heritability in the traits like plant height, number of pods per plant and number of branches per plant. Similarly in the F_2 segregating population of the cross C-152 x HC-03-02, high heritability was estimated in plant height and test weight. While, moderate heritability in the traits *viz.,* number of pods per plant, number of seeds per pod and number of branches per plant. The trait seed yield per plant recorded low heritability.

Heritability values cannot provide sufficient information on the amount of genetic progress that would result from selecting the best individuals since their scope is limited by their interaction with environment. Burton *et al.*, (1952) reported that heritability estimates along with genetic gain would be more useful than the former alone, in predicting the effectiveness of selecting the best individuals from segregating populations. Therefore, it is essential to consider the predicted genetic advance along with heritability estimates as a tool in the selection programme for better efficiency in the selection.

High heritability coupled with high GAM for the traits like number of seeds per pod, test weight and seed yield per plant was observed in the F_2 population of cross C-152 x V-16. Similar results were earlier obtained by Rangaiah and Nehru (1998), Gowda *et al.* (1991). Moderate heritability and high GAM showed for plant height and number of pods per plant. These results were in accordance with work of Hadpad (2001) and Mehta and Zaveri (1998).While moderate heritability and GAM for the trait number of branches per plant.

High heritability with high GAM was observed for plant height and test weight in the F_2 population of the cross C-152 x HC-03-02, whereas

moderate heritability and high GAM was observed for number of pods per plant, number of seeds per pod and number of branches per plant. Low heritability and high GAM were observed for seeds yield per plant. Similar results were obtained earlier by Hadpad (2001).

High heritability coupled with high genetic advance as per cent mean for the above said traits indicated that these traits are under the control of additive gene action and directional phenotypic selection for these traits in genetically diverse genotypes could be effective for desired genetic improvement. These traits could further be improved by applying pedigree selection. Moderate heritability coupled with moderate genetic advance which is being observed in the present study indicated that considerable influence of environment apart from non-additive gene action. Therefore, simple selection may not be effective in improvement of these traits.

The straight selection on the basis of phenotypic performance alone would not, therefore be rewarding in the improvement of these traits indicating non-additive gene action.

5.9 Correlation studies

Correlation of characters serves as a measure and forms the basis of selection as it gives strength and direction of a relationship between the characters studied. Selection for yield is effective, only when it is based on yield components rather than relying on yield alone. In addition to the variability analysis, correlation coefficient helps the breeders in determining the direction of selection and number of characters to be considered in improving the yield and quality attributing traits.

In the present study the quantitative traits *viz.,* number of pods per plant, number of seeds per pod and test weight showed strong relationship with seed yield per plant in the cross C 152 x HC 03 02. In

the cross C 152 x V 16, number of pods per plant and number of seed per pod showed strong relationship with seed yield. Therefore, it can be suggested that individual plant selections may be practiced for plants with above mentioned characters which ultimately lead to improvement in seed yield in the later generations. Similar, kind of results were earlier reported by Reddy *et al.*, (1994), Gill *et al.*, (1995) and Anbumalarmathi *et al.,* (2005). However, plant height and number of branches per plant in the cross C 152 x V 16 recorded negative non significant relationship with seed yield per plant, indicating selection for these traits would not improve seed yield in the segregating generations.

Overall, the important yield attributing traits in the present study *viz.*, number of pods per plant and number of seeds per pod had significant positive correlation among themselves in both the crosses studied. It may be inferred that these traits have strong influence on seed yield per plant. Since, the characters are inter-correlated among themselves, selection for any one of the traits will result in the improvement of other trait thereby, resulting in increased seed yield.

5.10 Parental polymorphism, BSA and identification of markers linked to BLB disease resistance gene

5.10.1 Parental polymorphism and BSA in the cross C-152 x V-16

Parental polymorphism using 42 cowpea specific primers were carried out with resistant (V 16) and susceptible (C 152) parents. Out of 42, only 12 SSR primers were polymorphic between the parents C 152 and V 16. The good amount of polymorphism may be due to specificity of SSR primers, as we used cowpea specific instead of other legume specific primers. In similar studies, Venkatesh *et al.* (2007) reported low level of polymorphism in field bean using legume specific markers of *Medicago*, cowpea, *Vigna radiata* and *Phaseolus vulgaris*. This may be due to cross species nature of SSR markers used from different legumes.

Bulkd segregant analysis (BSA) is a rapid method of identifying markers that are linked to genes of interest in specific regions of the genome (Michelmore *et al.*, 1991). In the present study 12 SSR markers which showed polymorphism between the parents of the cross C 152 x V 16 were subjected to BSA analysis to identify putative markers linked to BLB resistant gene. Three SSR primers which were polymorphic between parents of the above cross were also found polymorphic between resistant and susceptible bulk, indicating these markers were putatively linked to BLB resistant gene. Several reports by Agbicodo *et al.*, (2010) also identified markers linked to bacterial leaf blight disease resistance through BSA approach.

5.10.2 Identification of SSR markers linked to BLB resistant gene

Marker assisted selection (MAS) is a powerful tool for breeding programs, since it provides significant advantages over traditional phenotypic screening. Marker assisted selection is rapid, reliable and relatively inexpensive which is not hampered by pathogen non-availability. There are no environmental limitations, since MAS can be performed during off-season and it allows simultaneous screening for many diseases. The combination of BSA and highly polymorphic PCR based markers permits the identification and mapping of useful molecular markers for breeding programmes. Linkage analysis of the 102 F_2 individuals using putatively linked SSR markers from bulk segregant analysis of the cross C 152 x V 16 revealed that only one SSR marker out of three polymorphic SSR makers was co-segregating with resistant gene for bacterial leaf blight disease. Hence, the marker CP 641/642 marker can be used for effective indirect selection. Identification of homozygotes for the BLB resistant gene is of critical importance in early stage of plant development and in early segregating populations, especially when such homozygotes are used to be forwarded during back cross breeding.

However, there is a need to saturate the said locus with more markers to obtain tightly linked markers for BLB disease resistance gene.

Further in future, identification of markers linked to BLB resistance gene would prove useful for initiating the cloning and characterization of BLB resistant gene using one or more of the currently based methods for map based cloning. The study described above represents the first step towards reaching the goal for improvement of cowpea for BLB diseases using DNA marker.

5.11 Suggested future line of work

1. Since the results of our present study with 196 genotypes of cowpea represents part of the cowpea germplasm available, it is suggested to include a study with exhaustive germplasm from different geographical origin.
2. In the present study for both BLB and CpMV, screening was done under field conditions due to limitations. However, it is always advisible to confirm the resistance under glass house conditions with artificial inoculation to avoid escape mechanism usually occurs in field conditions.
3. SSR marker linked to BLB disease resistant gene in the present investigation can be validated with other genotypes and other cross combinations. Which will enable the breeders to use that marker in molecular assisted breeding.
4. F_2 mapping population of both the crosses can be forwarded by single seed descent method to develop RIL's for further studies.
5. The linkage between marker and the trait could be strengthened by adding more number of gene specific SSR markers for getting better resolution of the gene of interest.

Summary

VI. SUMMARY

It is needless to stress the importance of pulses in human diet especially for the vegetarian population. Among many tropical legumes in the world which are considered as food legumes, cowpea occupied significant importance in Asia and Africa. Out of more than a dozen pulse crops that are grown in India, cowpea ranks fifth position next to chickpea, pigeon pea, mungbean and urd, which is being grown and utilized throughout the country. The production and productivity of cowpea both at national and state (Karnataka) level is hindered due to many constraints including abiotic and biotic stresses. Diseases occupied prominence position among biotic stresses. Breeding for disease resistance coupled with yield improvement is of prime importance in pulses in general and so also in cowpea.

Profound knowledge on variability of genetic material would be of immense importance to any plant breeding programmes. Continuous breeding will lead to narrowing down the variability among the genotypes, which will further hinder the yield in any crop. One of the ways to increase the variability is by introduction of genetically diverse new genotypes. The present investigation was carried out at Gandhi Krishi Vignana Kendra, University of Agricultural Sciences, Bangalore involving a germplasm set of 196 cowpea genotypes obtained from different institutes in India and international centers. The experiment was laid out in simple lattice square design during *kharif* 2009 and observations were recorded on seed yield and its nine attributing characters namely days to 50 per cent flowering, days to physiological maturity, plant height, number of branches per plant, number of pods per plant, pod length, number of seeds per pod, test weight and seed yield per plant. Analysis of variance revealed significant differences among the genotypes tested for all the nine characters, justifying the

selection of genotypes for the study. PCV values were of higher magnitude than GCV for all the characters under study. The estimates of PCV and GCV were high for number of pods per plant, pod length, plant height, number of branches per plant, test weight and seed yield per plant.

All the characters showed high heritability and high genetic advance as per cent of mean was observed for number of pods per plant, number of seeds per pod, pod length, plant height, number of branches per plant, test weight and seed yield per plant, while moderate GAM was noticed for days to 50 per cent flowering and low GAM for days to physiological maturity.

The phenotypic and genotypic correlations were computed among nine characters. There was a narrow difference between the genotypic and phenotypic correlation coefficients for pairs of characters indicating less influence of environment and high heritable nature of the characters. Association analysis revealed positive and significant correlations of seed yield with days to physiological maturity, number of pods per plant, number of branches per plant, number of seeds per plant and test weight. Significant correlations among the other yield attributing characters were also observed.

The genotypes were also grouped for four qualitative characters such as flower colour, growth habit, leaf shape and immature pod pigmentation. Majority of the genotypes exhibited ovate leaf shape (96.93 per cent), mave pink (87.24 per cent), erect growth habit (47.44 per cent) and pod pigmentation (87.75 per cent).

Genetic diversity study by using Mahalanobis D^2 statistics for 196 genotypes revealed that maximum contribution towards diversity was by the character seed yield per plant followed by test weight. The 196

genotypes were classified into 22 clusters based on Tocher's method. Maximum genotypes were observed in cluster XXII followed by cluster I and remaining all clusters consisted of two genotypes each.

Sixty representative genotypes belonging to different clusters were used for molecular diversity analysis using SSR markers. Clustering of the genotypes was carried out using Jaccard's coefficient for the pooled SSR data and using correlation coefficient for the morphological data. The analysis of the morphological data using correlation coefficient revealed 82 to 100 per cent similarity in the genotypes under study. Sixty diverse cowpea genotypes exhibited similarity from 57 to 100 per cent. At 59 per cent similarity, the genotype EC 472252 differed from the other genotypes.

The amount of diversity in sixty diverse cowpea genotypes obtained from molecular marker analysis was higher than the one obtained from the biometrical data analysis.

The same sets of genotypes were also evaluated for two major diseases *viz.*, cowpea mosaic virus and bacterial leaf blight under field condition. Out of the 196 lines screened for these diseases, 108 and 150 lines were found resistant to CpMV and BLB diseases in the field condition, respectively.

The genotypes IC 249141, IC 202781 were found superior to the checks (KBC 2 and KM 5) with high seed yield along with resistant to diseases. These genotypes are recommended for further confirmation of resistance under artificial inoculation. Diverse genotypes identified through molecular characterization can be utilized as parents in hybridization programme.

To know the inheritance of genes responsible for resistance to both the diseases (BLB and CpMV), highly susceptible variety for both the diseases (C-152), which is agronomically superior was used to hybridize with highly resistant varieties V-16 (BLB resistant) and HC-03-02 (CpMV resistant). The F_1, F_2 and F_3 generation of both the crosses (C-152 x V-16, C-152 x HC-03-02), were evaluated and screened against respective diseases. Based on F_2 segregating ratio and confirmation with F_3, BC_1P_1 and BC_1P_2 revealed the role of single dominant gene for both the diseases separately. Hence, it was suggested to transfer resistant gene to susceptible varieties either simple back cross breeding or with molecular assisted selection.

In F_2 generation of both the crosses were used for variability and correlation analysis. In the F_2 population of C-152 x V-16 cross, high PCV estimates were observed for plant height, number of branches per plant, number of seeds per pod, seed yield per plant and number of pods per plant. But GCV estimate was high for the traits like number of pods per plant and seed yield per plant. Heritability in broad sense was high for test weight, number of branches per plant and seed yield. The GAM estimates were high for all the traits studied except number of branches per plant. Similarly, in another cross C-152 x HC-03-02, high PCV values were estimated for plant height, number of branches per plant, seeds per pod, seed yield and number of pods per plant. Whereas, GCV and heritability were high for plant height and test weight and GAM was high for all the traits studied.

Correlation studies in both F_2 populations revealed significant positive correlation of pods per plant, number of seeds per pod and test weight, inferring these traits strongly influenced seed yield per plant.

The SSR markers CP 641/642, MS99/100 and CP 215/216 exhibited Mendelian segregation of 1:2:1 (1RR:2Rr:1rr for BLB) which is

typical ratio of co-dominant markers. Linkage analysis revealed that one SSR marker (CP 641/642) was linked to resistant alleles for BLB disease.

The present investigation is an attempt towards understanding genetic diversity based on both morphological and molecular marker analysis and also in identifying the sources of resistance along with nature of inheritance for two important diseases in cowpea. Though there are many limitations in the study, it is a sincere attempt in the use of molecular marker technology for disease resistance breeding in cowpea. Considering the limitations and draw backs of the study, several points are highlighted in the future line of work.

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* Original not seen.

Appendices

Appendix 1. Mean values for twelve characters in 196 genotypes of cowpea

S.NO	G lines	DFF	NPP	NSd	PL	PH	N Br	TW	SY	DM
1	EC 458489	51	25.00	14.00	5.25	26.00	8.50	18.90	66.15	87
2	IC 402101	50	25.33	12.00	8.00	25.50	6.33	20.00	60.79	90
3	NBC 14	51	17.00	8.50	6.25	22.70	7.50	22.30	32.22	89
4	IC 402166	49	31.66	13.33	7.33	13.80	8.00	15.40	64.99	91
5	V 24O	53	13.33	12.00	6.75	20.00	6.25	14.50	23.19	88
6	202804(83)	53	14.00	10.00	6.00	25.00	4.00	12.00	16.80	84
7	V 585	51	32.33	15.33	6.00	23.50	5.70	11.30	56.00	86
8	C 325	48	35.00	13.00	7.25	13.50	7.00	13.70	62.34	87
9	EC 458506	52	23.00	8.60	6.33	22.25	8.60	12.80	25.32	88
10	IC 249593	53	22.66	14.33	7.33	19.50	7.33	11.60	37.67	89
11	IT 97K 499-38	59	17.00	8.00	6.25	18.50	7.00	18.30	24.89	87
12	IC 402180	49	13.00	7.00	6.33	26.75	5.00	13.70	12.47	86
13	202854(97)	54	9.33	13.33	7.33	22.50	6.30	12.40	15.42	86
14	EC 472257	54	13.66	17.33	7.75	27.25	4.66	19.10	45.22	88
15	IC 249141	53	14.66	14.00	7.00	22.50	7.33	12.00	24.63	88
16	EC 170584	55	19.33	10.66	6.75	23.50	8.20	13.10	26.99	86
17	EC 472252	52	8.66	14.00	9.00	28.00	8.20	14.00	16.97	88
18	IC 202867(99)	53	17.66	11.33	6.12	27.00	7.20	16.80	33.61	86
19	KM 5	54	23.66	14.33	6.80	39.50	8.66	11.80	40.01	88
20	IC 1071	57	15.33	9.33	5.75	21.20	8.50	11.80	16.88	88
21	EC 458411	57	16.66	15.00	8.33	28.16	9.50	11.40	28.49	89
22	IC 402161	26	22.33	12.00	9.00	26.13	9.50	14.40	38.59	87
23	IC 2591054	54	22.00	11.00	8.00	25.00	10.00	15.00	36.30	86
24	IC 462099	59	11.00	10.00	11.00	12.90	9.00	16.00	17.60	84

25	IC 58905	58	10.33	13.00	11.60	29.50	6.33	13.10	17.59	86
26	NBC 30	57	16.66	12.00	6.00	22.00	8.20	12.60	25.19	89
27	EC 458473	59	19.66	12.25	5.50	19.00	8.40	16.00	38.53	88
28	IC 402182	54	14.00	6.66	5.00	24.60	5.00	10.30	9.60	86
29	IC 202777	59	30.33	14.25	6.50	24.00	8.50	11.40	49.27	107
30	IC 170574	59	18.00	8.50	5.50	29.50	4.50	16.90	25.86	90
31	CPD 31	55	19.66	13.00	6.50	19.30	9.33	14.30	36.55	88
32	EC 394779	55	20.33	12.66	6.50	25.30	8.66	21.80	56.11	88
33	NBC 42	56	14.33	10.33	7.00	28.75	4.70	20.30	30.05	88
34	IC 330996	52	14.66	13.00	8.66	34.70	6.00	24.50	46.69	87
35	IC 402166	53	24.66	12.00	7.66	36.30	8.33	17.00	50.31	89
36	IC 402164	53	20.00	16.00	9.33	27.00	6.00	16.40	52.48	88
37	EC 472250	55	19.33	15.00	9.00	26.00	9.00	18.30	53.06	87
38	IC 402114	55	30.66	13.00	6.50	27.00	8.33	8.90	35.47	86
39	V 585	55	36.66	11.25	5.50	30.00	8.66	11.40	47.02	88
40	EC 170584	56	22.00	11.00	6.00	24.00	7.00	14.20	34.36	89
41	NBC 32	58	17.00	6.50	5.50	23.50	7.00	12.70	14.03	89
42	EC 458402	56	23.33	13.33	5.75	23.66	6.66	10.60	32.96	88
43	NBC 39	52	15.20	9.00	20.00	28.80	7.20	15.00	20.52	86
44	NBC 29	54	15.60	9.60	12.80	38.60	6.80	14.10	21.12	88
45	EC 458442	53	10.60	13.25	12.80	54.80	6.60	31.50	44.24	103
46	NBC 33	54	24.20	10.80	17.60	48.90	8.40	12.30	32.15	88
47	EC 458470	55	16.00	10.25	13.75	36.00	6.20	16.80	27.55	87
48	IC 402172	54	24.25	9.75	15.75	47.37	5.80	13.40	31.68	88
49	IC 257428	56	15.50	10.20	14.20	40.25	5.80	8.90	14.07	89
50	IC 402048	56	9.50	15.00	17.66	28.50	4.00	9.50	13.54	84

51	IT 97499-38	62	14.00	12.00	16.00	32.16	6.00	28.56	47.98	104
52	CPD 19	58	15.00	11.00	15.30	29.00	5.00	21.00	34.65	90
53	NBC 6	59	31.50	12.33	15.66	46.00	7.33	17.10	66.42	91
54	202827(92)	59	46.00	13.66	19.75	36.43	6.33	10.60	66.61	91
55	IC 198326(38)	59	18.66	10.00	14.00	35.33	5.00	9.10	16.98	89
56	EC 170585(B9)	60	16.33	9.66	11.33	36.00	5.00	13.40	21.14	87
57	EC 402159	55	20.20	13.75	19.32	50.40	9.75	15.40	42.77	87
58	V 578-17	54	14.75	12.00	15.55	49.87	6.25	13.30	23.54	88
59	IC 249793	53	12.75	9.00	13.12	30.50	6.50	29.80	34.20	89
60	C 131+CB-2	56	20.25	13.00	12.75	51.12	8.25	11.30	29.75	88
61	IC 201©	54	16.75	11.75	17.37	44.12	8.75	16.10	31.69	91
62	IC 249588	56	18.66	11.75	14.87	27.00	6.25	13.40	29.38	87
63	IC 402125	58	21.00	10.00	15.12	49.50	7.25	11.70	24.57	89
64	IC 402098	56	24.66	13.66	18.00	43.33	8.50	11.10	37.39	91
65	V 578 ©	59	22.00	8.66	13.66	33.00	7.00	14.90	28.39	89
66	NBC 27	56	12.00	9.00	13.75	31.50	7.50	13.10	14.15	88
67	NB 12	57	17.00	17.33	24.22	45.66	7.33	14.90	43.90	88
68	NBC 7	58	19.00	9.33	14.00	41.33	6.00	13.60	24.11	87
69	EC 458417	54	26.33	10.00	15.66	48.16	7.33	12.30	32.39	88
70	IC 330996	54	23.66	13.00	17.83	52.03	6.66	15.10	46.44	92
71	EC 390287	52	7.25	12.75	20.00	25.25	7.25	12.70	11.74	89
72	EC 170584-1-1	54	14.00	10.75	15.00	38.25	6.50	14.30	21.52	88
73	V 16	55	24.00	10.66	12.00	50.50	13.25	9.80	25.07	92
74	EC 458440	56	21.00	9.75	15.75	57.75	8.25	20.30	41.56	86
75	IC 249593	57	14.33	10.50	14.50	42.66	4.00	10.80	16.25	89
76	EC 170578-1-1	55	19.25	18.00	9.75	53.00	8.50	14.50	50.24	86

77	NBC 40	55	8.00	10.00	17.00	43.00	7.00	23.30	18.64	86
78	IC 202779	56	18.00	9.00	10.00	33.00	6.00	7.90	12.80	88
79	EC 458402	57	19.00	11.00	16.33	46.00	7.66	14.60	30.51	88
80	202705(49)	56	25.00	11.33	14.00	50.00	8.66	16.00	45.32	87
81	NBC 42	54	27.00	9.20	13.33	51.66	10.00	7.80	19.38	88
82	IC 402159	56	22.33	10.30	13.33	57.33	9.00	12.00	27.60	86
83	C 517	57	13.33	8.66	11.50	48.00	6.00	8.80	10.16	87
84	IC 402162	56	20.66	15.33	21.00	48.33	8.00	11.80	37.37	107
85	IC 202290	57	16.50	10.75	7.87	12.00	6.50	30.40	53.92	85
86	NBC 41	53	14.50	8.00	5.75	15.00	13.33	17.00	19.72	88
87	IC 402104	53	15.33	9.66	6.50	13.00	6.66	17.50	25.92	88
88	KBC 2	53	26.33	11.33	6.75	20.00	8.60	12.70	37.89	88
89	IC 402159	57	27.33	13.30	6.50	18.16	6.60	12.40	45.07	89
90	V 578	56	25.33	11.33	5.50	16.33	9.30	13.20	37.88	89
91	ETC 27	55	43.33	11.33	5.75	20.00	9.30	12.10	59.40	107
92	C 24-1	59	23.33	11.33	6.75	13.33	10.00	17.60	46.52	85
93	CPD 15	56	33.33	9.33	5.00	15.00	6.00	10.80	33.58	88
94	EC 48475	53	26.33	15.00	7.50	15.66	6.80	12.70	50.16	89
95	EC 394779	53	15.50	12.25	6.30	15.75	5.50	13.90	26.39	88
96	IC 1071	53	19.00	11.66	6.66	18.50	6.80	14.10	31.23	88
97	198355(45)	52	20.66	12.00	7.00	18.00	8.00	13.90	34.46	88
98	EC 458469	52	39.00	15.20	7.75	18.00	6.80	12.40	73.50	87
99	IC 202797(97)	58	14.33	11.33	13.50	28.50	6.00	8.90	14.44	86
100	C 720	56	26.00	16.33	14.66	23.50	8.00	15.80	67.08	93
101	IC 202781	55	12.00	14.66	21.66	36.50	5.50	30.70	54.00	92
102	IC 402101	55	16.80	12.00	16.66	32.00	5.00	13.90	27.78	88

103	IC 402175	54	12.00	10.50	14.50	22.50	5.50	14.90	18.97	89
104	TOME 77-4	57	22.00	11.66	13.50	27.00	4.66	16.60	42.58	91
105	EC 458418	58	31.66	11.00	14.50	31.00	6.66	13.30	46.31	89
106	NBC 19	57	18.50	10.00	15.00	25.50	4.00	17.70	30.97	89
107	GENOTYPE 36	58	28.33	11.33	16.50	33.66	5.66	13.00	41.72	89
108	EC 394779	54	11.66	11.33	16.00	24.00	3.66	14.80	19.55	88
109	EC 472252	55	27.00	11.33	17.00	21.66	5.66	18.30	55.98	88
110	EC 458425	58	21.00	10.00	13.00	33.00	5.00	18.40	38.64	89
111	EC 458505	58	10.00	6.50	12.00	17.50	4.00	18.40	11.96	87
112	EC 394708	56	17.00	11.80	15.66	28.33	4.66	11.10	22.00	87
113	GC 3(C)	59	32.00	11.66	15.00	19.00	4.00	11.30	42.16	89
114	IC 402104	57	19.33	10.33	14.00	31.00	6.00	10.80	21.56	88
115	NBC 38	56	15.50	9.00	13.66	30.33	4.50	13.00	18.13	91
116	IC 2591054	56	18.33	11.00	13.66	26.00	5.00	9.70	19.55	90
117	IC 202782	55	13.00	12.33	21.66	29.00	6.33	28.90	46.32	105
118	IC 402174	58	22.66	9.00	13.33	37.00	6.80	11.00	22.43	92
119	IC 4506	54	19.33	7.33	23.66	18.66	10.00	12.80	18.13	90
120	CB 10	54	16.00	11.00	15.00	29.66	5.20	10.70	18.83	89
121	IC 402098	56	19.33	12.00	16.30	27.00	4.70	11.22	19.00	88
122	201095(52)	55	23.33	12.33	18.66	27.33	4.00	16.00	46.03	89
123	EC 458480	59	16.00	10.66	15.50	32.00	5.33	12.10	20.63	91
124	EC 458483	58	12.66	7.66	13.20	23.00	4.50	14.00	13.57	92
125	C 1071	62	24.00	10.00	12.75	32.00	8.00	12.60	30.24	88
126	EC 458489	58	21.00	8.33	14.66	25.33	3.66	16.80	29.38	93
127	EC 458473	53	41.60	11.00	5.50	26.00	10.20	9.00	41.18	88
128	EC 394839	54	23.00	11.80	5.50	29.33	8.00	10.40	28.22	88

129	EC 394838	56	32.66	13.66	6.55	32.33	7.20	13.30	59.33	88
130	EC 458418	56	8.66	7.00	5.50	25.66	4.30	16.90	10.24	89
131	IC 402159	57	26.66	15.00	8.82	18.00	6.00	16.10	64.38	91
132	NBC 10	57	19.82	9.20	6.00	27.20	6.50	16.80	30.63	89
133	IC 1061	57	14.00	10.66	7.00	15.20	6.20	18.10	27.01	91
134	IC 10171	57	32.60	11.00	6.00	23.80	8.00	13.00	46.61	88
135	EC 458438	59	9.00	12.66	6.00	17.00	8.30	18.80	67.41	89
136	ZIC 249593	56	17.00	13.00	7.83	27.82	7.00	11.00	12.53	86
137	IC 402162	57	15.00	13.00	6.50	18.66	6.00	13.10	28.95	90
138	NBC 51	57	13.80	11.60	7.00	27.66	7.00	17.70	34.51	89
139	97767(10)	57	22.66	14.00	5.80	22.66	5.70	12.70	20.17	89
140	IC 249141	57	52.33	11.66	7.50	24.30	8.50	17.00	89.60	89
141	NBC 9	58	34.50	11.33	6.66	19.80	6.66	12.80	50.03	88
142	IC 206240	54	15.00	16.00	9.00	27.00	6.20	18.30	44.60	88
143	EC 458430	56	23.00	14.00	11.00	28.00	7.00	16.00	51.52	87
144	V 604-7-29-3	59	30.33	13.00	6.75	18.00	7.33	10.70	42.18	88
145	IC 49586	54	28.25	12.66	6.66	26.50	5.25	11.00	39.15	87
146	202827(93)	54	28.33	14.00	6.75	19.33	4.17	12.50	49.57	89
147	EC 458490	54	10.00	6.00	8.50	24.00	4.50	21.80	13.08	87
148	IC 202711(58)	54	14.00	10.80	8.50	18.00	6.00	10.80	16.32	89
149	C 458492	54	18.66	7.33	8.50	21.00	9.50	16.80	22.97	89
150	IC 25105	59	20.00	13.20	10.00	27.00	5.20	10.60	27.98	88
151	IC 402154	59	19.66	11.00	11.00	21.50	6.30	12.90	27.89	90
152	EC 458440	57	25.33	8.00	12.00	21.00	7.00	21.60	43.77	89
153	IC 202781	57	21.00	14.50	11.00	20.25	7.00	29.90	92.05	91
154	NBC 44	57	12.33	6.50	10.00	18.00	5.00	17.10	13.70	87

155	C 33	58	34.00	7.33	11.33	29.33	5.40	13.30	32.39	88
156	27749(25)	54	21.00	9.00	11.25	23.00	7.00	9.40	17.76	86
157	NBC 48	56	26.00	10.66	14.20	27.33	3.66	8.90	24.66	87
158	IC 198329(36)	59	23.00	10.66	16.80	28.66	3.00	11.20	27.46	86
159	NBC 43	58	10.00	9.33	16.50	21.66	4.00	18.90	17.63	86
160	V 585©	58	13.33	15.33	22.33	31.00	4.20	29.30	59.87	92
161	NBC 36	57	17.66	11.00	14.16	24.66	3.50	12.10	23.50	86
162	IC 253251	57	20.00	9.66	13.00	32.66	3.50	13.20	25.50	87
163	EC 472250	57	18.33	9.33	14.00	30.00	3.00	9.20	15.73	88
164	EC 458402	57	13.00	8.33	14.16	30.33	3.40	14.40	15.59	91
165	EC 458441	59	12.00	9.00	12.80	18.00	5.00	18.40	19.87	87
166	NBC 38	58	27.66	13.33	14.30	26.00	6.00	12.10	44.61	88
167	NBC 18	58	19.33	10.00	13.50	16.66	3.00	16.00	30.92	88
168	EC 458480	57	32.50	9.50	12.75	19.33	4.50	11.80	36.43	89
169	EC 394839	56	32.00	13.00	6.70	15.00	6.00	12.20	12.20	91
170	NB 47	56	13.00	6.00	5.50	25.66	7.00	16.60	16.60	89
171	GC 3	56	21.00	10.80	6.20	17.00	7.20	11.60	11.60	89
172	IC 402090	59	20.00	14.00	6.20	16.66	4.50	11.50	11.50	89
173	NBC 8	59	17.50	7.00	5.50	21.20	4.50	18.60	18.60	86
174	EC 458469	54	12.00	10.50	5.80	25.00	4.50	11.50	11.50	91
175	C 457	57	23.00	13.20	8.00	25.50	7.20	20.90	20.90	92
176	NBC 7	57	22.00	11.00	7.00	33.00	6.00	22.00	53.24	88
177	EC 458453	60	38.33	10.00	6.50	23.00	6.66	12.00	45.99	89
178	IC 402161	60	14.33	11.66	7.20	18.00	6.00	16.50	28.88	89
179	IC 402106	60	23.00	11.00	5.00	19.00	6.00	9.30	23.53	88
180	EC 472271	59	21.00	10.80	5.50	18.00	5.00	8.70	19.73	88

181	EC 472267	59	38.00	15.00	6.60	26.00	6.80	10.00	57.00	86
182	CB 10	59	25.00	13.00	5.00	15.50	5.20	7.20	23.40	85
183	IC 402164	58	23.20	14.20	8.00	22.20	6.00	15.70	50.00	89
184	NBC 40	58	9.00	11.00	5.50	15.00	5.80	20.70	20.49	91
185	NBC 24	57	22.00	12.00	6.00	22.00	7.00	15.00	39.60	89
186	C 152	55	23.66	13.00	6.50	21.00	7.00	12.70	39.06	88
187	TVX 944	56	22.20	7.00	5.00	16.33	6.50	12.70	19.73	86
188	KBC 2	57	43.00	9.00	5.80	18.80	7.20	18.40	71.20	89
189	km 5	58	25.00	12.50	6.80	18.00	6.20	12.00	37.50	89
190	TC 201	56	28.66	11.00	6.20	18.66	6.00	11.20	36.58	88
191	HC 03-02	57	23.00	12.20	6.70	18.20	4.75	17.00	47.70	86
192	P 695	59	22.00	9.50	5.70	18.00	7.50	11.50	24.03	93
193	TCM 44-1	60	15.00	7.00	8.50	26.00	6.20	9.80	10.29	86
194	APC 243-1-865	57	28.66	8.50	6.50	22.33	10.00	18.60	45.31	89
195	HC 9866	56	17.66	11.70	7.20	26.20	6.00	10.30	21.28	86
196	GC 4	55	16.00	12.00	7.10	21.00	7.00	11.00	20.00	86

Where,

1 DFF - Days to 50% flowering
2 NPP- Number of pods/plant
3 NSd – Number of seeds/pod
4 PL - Pod length(cm)
5 PH - Plant height (cm)
6 N Br - Number of branches/plant
7 TW - Test weight (g)
8 SY - Seed yield/ plant (g)
9 DM - Days to maturity

Appendix 2 : Average intra and inter cluster D^2 values

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII
I	1689.36	1179.4	852.01	1858.0	1805.2	1145.9	2993.7	2491.6	2242.7	2960.4	1402.0	962.2	1696.7	2381.1	3457.9	1760.1	2717.9	2261.0	4343.5	1162.6	1126.5	3760.8
II		4.19	498.9	718.7	754.1	34.6	1468.7	1463.4	1008.0	1604.1	567.9	403.2	808.9	943.6	2614.1	592.2	1703.8	1064.1	3274.6	199.38	560.76	2710.8
III			5.75	958.9	824.0	495.5	2107.8	1683.9	1323.0	1992.4	512.6	146.65	884.3	1656.6	26023.5	843.15	1840.1	1314.9	3479.4	314.11	788.21	2929.4
IV				9.25	198.1	817.2	807.1	1190.8	435.1	562.9	386.7	766.3	589.1	746.2	1587.9	73.43	1552.6	439.4	1990.9	513.2	229.9	2624.8
V					17.07	862.9	607.6	981.6	289.9	418.6	561.1	638.0	514.9	798.7	1554.4	85.17	1105.7	208.9	1955.6	449.5	245.0	2400.2
VI						19.47	1441.4	1275.7	1001.4	1621.9	726.7	330.3	688.7	836.3	2366.3	683.5	1526.0	1080.9	3028.4	308.3	539.2	2614.9
VII							20.45	361.1	148.1	106.6	1984.1	1373.6	464.2	250.7	778.9	592.8	439.0	154.8	823.7	1526.1	433.5	2223.3
VIII								22.77	359.6	540.0	2238.8	936.1	200.8	262.9	366.6	964.2	142.5	418.4	570.1	1672.6	453.8	2062.8
IX									25.67	148.7	1274.9	788.7	257.1	265.2	879.3	254.3	438.5	54.23	1105.6	906.6	164.9	2073.6
X										30.83	1678.6	1377.5	501.9	446.5	756.5	439.5	676.0	113.54	827.4	1463.0	399.5	2352.7
XI											30.98	698.7	1247.5	1736.4	2962.4	465.5	2590.4	1252.0	3695.4	250.0	750.6	3324.4
XII												34.15	405.5	956.3	1737.1	608.3	1074.5	835.1	2454.5	360.3	410.6	2393.3
XIII													35.05	248.3	604.0	465.1	403.5	289.1	978.4	907.0	140.6	2004.6
XIV														35.51	722.2	610.3	504.2	371.2	955.6	1258.3	288.0	2128.8
XV															37.22	1544.9	713.1	896.0	106.2	2765.8	949.4	2676.4
XVI																38.67	1170.6	256.5	1942.9	382.8	157.2	2389.6
XVII																	41.51	490.4	921.5	1800.8	705.2	2138.9
XVIII																		44.13	1116.6	927.7	201.4	2113.0
XIX																			45.70	3470.9	1331.0	3005.4
XX																				48.89	553.3	2799.9
XXI																					52.46	2090.3
XXII																						2767.33