

**GENETIC VARIABILITY STUDIES IN MINI-CORE  
COLLECTION SET OF PIGEONPEA (*Cajanus cajan* (L.)  
Millsp.) AND MOLECULAR CHARACTERIZATION IN  
SPECIAL RELATION TO STERILITY MOSAIC DISEASE,  
*FUSARIUM* WILT AND PROTEIN CONTENT**

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# INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the most versatile grain legume crop of rainfed agriculture in the semi-arid tropics. It is the only cultivated food crop of the *Cajaninae* sub-tribe and has a diploid genome with 11 pairs of chromosomes ( $2n = 2x = 22$ ) and a genome size estimated to be 858 M bp (Greilhuber and Obermayer, 1998). The genus *Cajanus* comprises 32 species, most of which are found in India, Australia and one species is native to West Africa. Pigeonpea is cultivated in more than 25 tropical and sub-tropical countries, either as sole crop or as an inter crop with finger millet, sorghum, pearl millet, maize or with short duration legumes. It plays an important role in food security, balanced diet and alleviation of poverty because of its diverse usages as a food, fodder and fuel (Rao *et al.*, 2002).

In India, pigeonpea is grown on an area of 4.37 million hectare with a production of 2.86 million tonnes and productivity of 655 kg per ha (Anon., 2011). In Karnataka, it is grown in an area of 5.97 lakh ha with an annual production of 3.14 lakh tonnes and productivity of 467 kg per ha (Anon., 2011) and is mainly grown in Gulbarga, Bidar, Bijapur, Raichur and Koppal districts in the northern region popularly known as the 'Pigeonpea Bowl' contributing to 82 per cent of the total pigeonpea production in Karnataka

The Indian sub continent alone contributes nearly 92 per cent of the total world production. Major Pigeonpea growing states are Maharashtra, Karnataka, Madhya Pradesh, Andhra Pradesh, Uttar Pradesh and Gujarat together contributes about 90 per cent of area and 93 per cent of production. Although, India leads the world both in area and production of pigeonpea, its productivity is lower than the world average. This is attributed to factors such as biotic (*Fusarium* wilt, sterility mosaic and pod borers) and abiotic (e.g., drought, salinity and water-logging) stresses. Among the biotic stresses, *Fusarium* wilt and sterility mosaic diseases are considered to be the most important diseases of pigeonpea in India.

The plant genetic resources are essential components to meet future food security needs of the world. The germplasm of crop diversity contributes to develop improved crop cultivars aimed at increasing crop productivity. The large size of germplasm collections coupled with non availability of detailed data and information, has resulted in low use (<1%) of germplasm leading to a narrow genetic base in many crops. The miniaturization of crop collections with almost full representation of genetic diversity in the form of mini core (~1 per cent of the entire collection) approach is an effective methodology to enrich and enhance crop improvement programmes. The mini core provides a means for accessing the larger collections for further exploration and also helps in proper assessment of genetic diversity, population structure, association mapping and targeted gene mining. Use of mini core approach will lead to greater utilization of diverse germplasm for developing broad-based cultivars, especially in the context of climate change.

Yield being a complex trait is collectively influenced by various component characters, which are polygenically inherited and highly influenced by the environmental variations. So the observed variability for these characters is the sum total of hereditary effects and influence of the environment. Hence it becomes necessary to partition the observed variability into heritable and non-heritable components measured as genotypic and phenotypic coefficient of variations, heritability and genetic advance. Higher genotypic coefficient of variance is more important than the phenotypic coefficient of variance because the heritable portion of the character depends upon the proportion of genotypic variance present in the total phenotypic variance.

In plant breeding, correlation studies help us to know the association prevailing between highly heritable characters and give a better understanding of the contribution of each trait in the genetic makeup of the crop. Further, the method of path coefficient analysis measures the direct influence of one variable upon the other and permits the separation of correlation coefficient into components of direct and indirect effects. The present study was also conducted to obtain this information for important characters in the pigeonpea accessions.

Divergence studies are also important to know the genetically divergent genotypes. Considering the choice of diverse parental material in the study, lot of diversity is expected to be generated for various characters. Genetic diversity analysis is therefore used to identify the divergent genotypes and to utilize these genotypes to exploit heterosis. The genetically diverse parents are likely to produce not only heterotic effect but also desirable segregants to be selected.

*Fusarium* wilt (FW) is an important disease of pigeonpea incited by a soil borne fungus *Fusarium udum*. Butler. It can survive up to 5 years on infected plant debris. The germ tubes of the pathogen generally penetrate through the delicate root tips of pigeonpea seedlings. This is followed by a rapid mycelia growth through xylem tissues that block the vascular system in the plants resulting in partial or complete wilting of branches and main stem. The disease symptoms usually appear when plants are at the pre-flowering and podding stage (100 per cent loss), at maturity (67 per cent loss) and at pre-harvest stage (30 per cent loss) but sometimes symptoms also appear in 1-2 month-old plants. In India alone, the annual yield loss due to this disease has been estimated at 71 million (Kannaiyan *et al.*, 1984; Reddy *et al.*, 1993)

Resistance to fusarium wilt is controlled by multiple factors (Pal, 1934). Resistance is conditioned by two complementary genes (Shaw, 1936) and those genes determine the resistance (Pathak, 1970). It is also indicated that resistance to wilt is controlled by a single dominant gene (Joshi *et al.*, 1957).

Sterility mosaic disease (SMD), considered as the “green plague of pigeonpea” caused by pigeonpea sterility mosaic virus (PPSMV) (Kumar *et al.*, 2003) and transmitted by eriophyid mite (*Aceria cajani* Channabasavanna), leads to heavy yield losses and can cause yield loss upto 95 per cent under favourable condition (Kannaiyan *et al.*, 1984). SMD infection at an early stage (<45-days-old plants) results in 95-100 per cent loss in yield (Reddy *et al.*, 1990), yield losses due to SMD were estimated at 205, 000 t of grain valued at US\$ 76 million annually (Kannaiyan *et al.*, 1984) in India and Nepal in 1993, losses were US\$ 280 million (Reddy *et al.*, 1993). This disease was first reported from Pusa, Bihar (Mitra, 1931). The disease is characterized by the symptoms like bushy and pale green appearance of plants followed by reduction in leaf size and mosaic mottling of leaves and finally partial or complete cessation of reproductive structures.

Resistance to sterility mosaic virus in pigeonpea appears complex. Resistance is controlled by four independent non-allelic genes (Singh *et al.*, 1983 and Sharma *et al.*, 1984). Of these, two were found to be dominant and the remaining two recessive; the presence of at least one dominant and one recessive gene is essential for imparting resistance to this disease. The allele responsible for tolerance is dominant over the other three alleles.

Control of sterility mosaic disease (SMD) and *fusarium* wilt (FW) by chemical method though effective is not feasible economically and non eco-friendly (Nene *et al.*, 1977). Breeding resistant varieties is considered to be one of the most effective and economic methods of reducing crop losses and has received top priority.

It is observed that pigeon pea is economically and nutritionally important legume as major source of proteins in poor communities of many tropical and subtropical regions of the world (Singh *et al.*, 1984). Legumes represent a major source of nutrients, including valuable but incompletely balance protein, particularly in vegetarian’ diet (Ghadge *et al.*, 2008). It has been estimated that the total production of legumes provide almost as much protein (20-30 %) to the world as wheat and over 50 per cent more than rice or corn (Rockland *et al.*, 1981 and Gopalan *et al.*, 1985). In addition, legumes supply significant amount of energy through carbohydrates (60-70%), lipids (1-7%), dietary fibers and minerals (2-5%), also the legume oilseeds contains reasonable levels of thiamine, riboflavin and niacin (Bressani *et al.*, 1974 and Arora, 1977).

Pigeonpea is an important source of protein. Pigeonpea seed is highly nutritive with an average protein content of 20–22 per cent. Wild relatives of pigeonpea, such as *C. scarabaeoides*, *C. sericeus* and *C. albicans*, have protein content as high as 32–34 per cent (Saxena *et al.*, 1987). Similarly, the range of seed protein in the minicore collection set of pigeonpea at ICRISAT is from 12.4 to 29.5 per cent. There is a need to improve the protein content along with the yield in the cultivated pigeonpea. The present investigation was carried out to know the presence of genetic diversity existing among the selected accessions of pigeonpea for seed protein content in minicore collection set.

Breeding for resistance to sterility mosaic disease (SMD) is a difficult proposal as the disease caused by virus and transmitted by mite. *Fusarium* wilt (FW) is a caused by soil borne pathogen and needs the creation of sick plot at a large scale demanding huge investment of resources. This humongous task of developing resistant varieties is further complicated because of the gene plasticity of the pathogen which has warranted the use of strain specific sources of resistance in crop improvement. Though conventional plant breeding in pigeonpea has facilitated greatly to the develop method improved varieties with enhanced resistance to biotic/abiotic stresses (Varshney *et al.*, 2006), selection process is tedious and time consuming. Therefore, it is necessary to tackle these problems

at molecular level by developing cultivars which are resistant to biotic stresses and have greater recovery from the damage. Genomic tools especially molecular markers have facilitated breeding in many crops leading to development of several improved cultivars/varieties with enhanced resistance/tolerance to biotic stresses (Varshney *et al.*, 2006).

Among different kinds of molecular markers available at present, simple sequence repeat (SSR) markers have proven as the markers of choice in practical breeding (Gupta and Varshney, 2000; Varshney *et al.*, 2005) because they are more reliable and reproducible as compared to RAPD markers and less cumbersome and time consuming than RFLPs and AFLP techniques (Vos *et al.*, 1995). In pigeonpea, availability of number of molecular markers is meagre and but the level of polymorphism is low (Yang *et al.*, 2006, Odeny *et al.*, 2007). However, recent reports indicate that many SSR markers are available in pigeonpea. Recently, Ganapathy *et al.* (2010) and Gnanesh *et al.* (2011) have identified few linked markers for SMD resistance in pigeonpea. Thus availability of more number of SSRs in pigeonpea would enable the breeders to know the location of specific genes and QTLs making it possible to improve the efficiency of breeding through marker assisted selection (MAS). Use of molecular markers in precise mapping of genes in the genome will be pivotal for MAS in breeding programmes. Hence, there is a need to integrate SSR markers to construct genetic maps and to identify the markers linked to SMD resistance to enhance the marker density on genetic maps. For integrating the SSR markers into genetic maps, the markers should detect polymorphism between parental genotypes and mapping populations. Hence, past information on marker polymorphism in parental genotypes and mapping populations is essential. With this background the present study was undertaken with the following objectives:

1. To screen the genotypes for Fusarium wilt and Sterility mosaic disease in pigeon pea collection.
2. To assess the genetic variability in the minicore collection set and to estimate the correlation coefficient and path coefficient analysis among various yield and yield parameters.
3. To identify the alleles associated in different categories of the mini core collection set such as; resistant, moderately resistant, susceptible, highly susceptible pigeon pea (*Cajanus cajan*) genotypes in special relation to SMD and FW and also to identify the motifs present.

# REVIEW OF LITERATURE

The progress in the improvement of plant type by the plant breeders will be determined by the variability existing in the populations. Thus, for effective selection and utilization of genotypes in breeding programme a thorough study on genetic variability, heritability and genetic advance is essential. Genotypic coefficient of variability (GCV), which indicates the relative magnitude of genetic diversity present in the material, will help to study the genetic variability present for different characters.

Germplasm serves as a great reservoir for most of the desirable traits like early maturity, resistance to biotic and abiotic stress as well as for some nutritional aspects like high protein content. Genetic variability is the gift of nature and its fruitful utilization in any crop species requires systematic collection, evaluation, description and grouping based on economic descriptors. Utilization of exotic germplasm resources in breeding programmes is required to enhance the diversity of cultivars, but lack of proper characterization and evaluation of germplasm was one of the bottlenecks in its utilization (Knauft and Gorbet, 1989). To overcome these constraints, it is essential to recognize a smaller subset or 'core' collection that probably represents the most of the genetic variation in whole collection. A careful understanding of the genetic diversity, extent of variation and genetic architecture of the plant among these genotypes would help in developing sound plant improvement programme.

The literature relevant to the objectives of the present study is reviewed under the following headings.

- 2.1 Introduction
- 2.2 Mini-core collection
- 2.3 Genetic variability parameters for yield and its attributing traits
- 2.4 Correlation coefficients
- 2.5 Path analysis
- 2.6 Molecular marker studies in pigeonpea
- 2.7 Screening techniques for fusarium wilt and sterility mosaic diseases resistance.

## 2.1 Introduction

Among legumes, pigeonpea [*Cajanus cajan* (L.) Millsp.] occupies an important place in rainfed agriculture. The crop is known to be grown in 22 countries but it is cultivated in large areas only in a few countries. In Asia, Myanmar (560,000 ha), China (150,000 ha) and Nepal (20,000 ha) are the other major pigeonpea growing countries besides India. In the African continent, Kenya, Malawi, Uganda, Mozambique and Tanzania produce considerable amounts of pigeonpea. The Caribbean islands and some South American countries also have reasonable areas under pigeonpea cultivation. In India, de-hulled split cotyledons of pigeonpea are cooked as a thick spicy soup, locally called *dal*, which is eaten with bread and rice, while in southern and eastern Africa and southern America both whole dry and immature seeds are used as vegetable. Its nutritious broken seeds, husks and pod walls are fed to cattle; while its dry stems make an important household fuel wood.

**Origin:** For a long time, the origin of pigeonpea has remained unclear. A perusal of early literature on this subject indicates that the crop originated either in Africa (Zanzibar or Guinea) or India. The presence of pigeonpea seeds in historical tombs indicated that it was cultivated in Egypt around 2000 BC. The availability of high diversity among germplasm made Vavilov (1939) concluded that India is the primary center of origin of the cultivated pigeonpea. De (1974) and Van der Maesen (1980) also reported that the cultivated pigeonpea originated in India and from here it was taken to Africa around 2000 BC.

**Taxonomy and distribution:** Van der Maesen (1986) reported that the first scientific name of pigeonpea was given by Bauhin and Cherler during 1650-1651 and they called it *Arbor trifolia indica* (*Thora Paerou*), which means 'common *dal*' in the Malayalam language of India. Linnaeus (1753) gave pigeonpea its first binomial nomenclature – *Cytisus cajan*. Van der Maesen (1986) has written an excellent monograph on this aspect and at present the following taxonomical classification is globally accepted. Based on various morphological, cytological, chemical and hybridization data, Van der Maesen (1986) merged genus *Atylosia*, the nearest wild relative of pigeonpea, with genus

*Cajanus*. Consequently, genus *Cajanus* now has 32 species and pigeonpea (*Cajanus cajan*) is the lone cultivated species of the *Cajaninae* sub-tribe.

Khan and Rachie (1972) evaluated 5,400 germplasm lines collected from India, the Caribbean and the Philippines and identified single promising plants for progeny row evaluation and selection. Based on the plant types, spreading and compact type progenies were selected for low and high density cropping systems. The first pigeonpea breeding program in South and East African region began in 1968 at Makerere University in Uganda with the main objective of breeding grain type varieties with early maturity.

The first scientific breeding effort in India was made by Shaw (1933) who described morphological and agronomic traits of 86 elite field collections and observed that some of the accessions were resistant to fusarium wilt.

Mahate and Dave (1931) identified a few elite genotypes which were early and late maturing with high yield but significant impact on productivity was not noticed. Hence, by considering importance of pigeonpea in India, Indian Council of Agricultural Research (ICAR) started an All India Coordinated Pigeonpea Improvement Project in 1965. The major objectives were to assemble pigeonpea germplasm, identify sources of resistance to pest and disease and to develop high yielding varieties.

Van der Maesen (1980) based on the vast natural genetic variability in local germplasm and wild relatives, concluded that India is probably its primary centre of origin for pigeonpea. Kumar *et al.* (1983) and Ae *et al.* (1990) stated that pigeonpea is mainly grown for its de-hulled peas, immature pods, fodder, *etc.* In a cropping season it fixes 40 kg per ha of nitrogen and adds valuable organic matter to the soil through fallen leaves.

Van der Maesen (1990) stated that the genus *Cajanus* has 32 species, 18 of which were endemic to Asia, 13 to Australia and one to Africa. ICRISAT conserves 555 *Cajanus* accessions. The introgression from the secondary gene pool by crossing *C. acutifolius* with *C. cajan* and one or two applications of growth hormones to pollinated buds improves fertilization and embryo development (Mallikarjuna and Saxena, 2002).

The successful examples of using crossable wild species in pigeon pea breeding include the development of genetic dwarfs (Saxena and Sharma, 1995), high protein lines (Saxena *et al.*, 2002), cytoplasmic male sterile (CMS) lines (Saxena *et al.*, 2006) and highly cleistogamous line (Saxena, 2008).

Sunitha *et al.* (2008) screened the six promising short duration pigeonpea genotypes against *Maruca vitrata* (Geyer) under field, laboratory and greenhouse conditions. The field and greenhouse experiments showed that significantly lower pod damage by *Maruca* was noticed in ICPL 98003 and ICPL 98008 as compared to the susceptible genotype ICPL 88034. In addition, greenhouse and laboratory studies showed less consumption of food and reduced larval and pupal weights of *M. vitrata* when reared on resistant genotypes like ICPL98003 and ICPL98008.

Singh and Van Emden (1979) reported that *Maruca vitrata* is distributed throughout tropical and subtropical regions. Atachi and Djihou (1994) reported that pest has a wide host range but is restricted to legumes. *M. vitrata* is a serious pest of pigeonpea in India, Sri Lanka and Africa (Lateef and Reed (1990). Gopali *et al.* (2010) noticed that incidence of spotted pod borer was high in early (140-150 days) and late maturing (190-200 days) varieties, moderate in medium duration (170-180 days).

## 2.2 Mini-core collection

Reddy *et al.* (2005) described the development of core collection from 12,370 pigeonpea accessions collected from 53 countries maintained at ICRISAT, Patancheru, India. The germplasm accessions from 56 countries were placed under 14 clusters based primarily on geographic origin.

Upadhyaya *et al.* (2006) developed a mini-core collection of pigeonpea, comprising of 146 accessions (about 10 per cent of core collection and 1 per cent of entire collection) representing almost the entire spectrum of diversity. The mini-core of 146 accessions of pigeonpea was developed based on evaluation of 18 qualitative traits and 16 quantitative traits of core collection of the 1,290 accessions of pigeonpea at ICRISAT, Patancheru, India. The conservation of crop germplasm diversity involves the establishment of *in situ* and *ex situ* gene banks. The major activities for *ex situ* gene banks include assembling, conserving, characterizing and providing easy access to germplasm

for scientists. He mainly focused his research on germplasm diversity assessment, developing core and mini-core collections and using molecular characterization approach to enhance the utilization of germplasm in research and improve the efficiency of germplasm management.

Manyasa *et al.* (2007) collected a total of 123 pigeonpea landraces from four major pigeonpea production areas in Tanzania and the accessions grown were characterized for diversity using 16 qualitative and 14 quantitative traits. They identified significant polymorphism in qualitative traits for base flower colour, pod colour, flowering pattern, streak pattern, seed colour. The principal component and cluster analysis separated the variability in the germplasm based on days to flowering, days to maturity, plant height, number of primary and secondary branches and number of racemes per plant. Overall, they identified two distinct diversity clusters with coastal, eastern and southern accessions in one cluster and northern highlands accessions in another cluster.

Singh and Eggum (1984) studied the important factors affecting the protein quality in pigeonpea and noticed large variation in the levels of protease inhibitors of pigeonpea varieties. The concentrations of the inhibitors were significantly higher in some of the wild relatives of pigeonpea. The protein digestibility of cooked pigeonpea meal remained low which could be due to absence of trypsin inhibitors. Through the investigation, they also found that the pigeonpea polyphenolic compounds adversely affected the activity of digestive enzymes and that would affect the protein quality of pigeonpea. They found that the protein quality of pigeonpea was greatly influenced by storage and processing practices.

## 2.2.1 Genetic divergence

Katiyar *et al.* (2004) evaluated 221 accessions of early maturing pigeonpea genotypes (*Cajanus cajan*) of diverse eco-geographical origin. Data were recorded for days to 50 per cent flowering, plant height, days to maturity, number of primary and secondary branches, number of racemes, pod length, number of seeds per pod, 100-seed weight and yield per plant. The accessions were grouped into 14 clusters. The maximum number of genotypes (36 accessions) was observed in cluster I, while the minimum number was observed in cluster IV (3 genotypes). Highest average distance of cluster members from cluster centroids was recorded in cluster X which ranged from 1.593 to 2.590. As far as inter-cluster distance is concerned, clusters IV and XIV were the farthest (5.714) from each other, followed by clusters VII and XIV (5.624). The genetic diversity was not found related to geographical diversity. The selection of parents in hybridization programme based on such analysis is expected to be helpful in development of early maturing pigeonpea.

Chattopadhyay and Dhiman (2005) evaluated hundred accession of pigeonpea, which were found all tall, late maturing, indeterminate types and almost free from *Heliothis* infestation, whereas, variable in other characters like days to fifty per cent flowering, days to seventy five per cent maturity, plant height, test weight, number of seeds per pod, plant number per hectare and plant yield (kg/ha) including seed coat colour. But, when their diversity was studied, genotypes were grouped under three clusters. The Cluster I was the largest and cluster II contained two germplasm which gave around 3500 kg per ha seed yield in around 270 days. The cluster III contained one germplasm. Very high inter and intra cluster distances suggested huge range of variability among the germplasm studied.

Gohil (2006) conducted a field experiment to study the genetic diversity among 39 pigeonpea cultivars. Data were recorded on grain yield per plant, days to fifty per cent flowering, days to maturity, number of pods per cluster, number of branches per plant, number of clusters per plant, number of pods per plant, number of seeds per pod, pod length, 100-seed weight, harvest index, plant height and protein content. The cultivars were grouped into 5 clusters, which indicated the presence of a large amount of diversity in the population. A total of 35 genotypes were grouped in cluster I while the remaining clusters contained a single genotype. The value of intra-cluster distance for cluster I was 39.98. The maximum inter-cluster distance was between cluster I and IV followed by between cluster IV and II. There was a minimum distance between clusters II and III.

Singh *et al.* (2010b) evaluated thirty-one genotypes of pigeonpea for days to fifty per cent flowering, days to maturity, plant height, number of primary branches, number of secondary branches, pods per plant, pod length, seeds per pod, 100-seed weight and seed yield per plant. The analysis of variance revealed significant mean square estimates for all the characters studied indicating sufficient diversity among the genotypes. Thirty-one genotypes were placed in 5 different clusters. The average cluster means of 10 characters revealed that cluster II comprising of 12 genotypes recorded highest seed yield per plant (43.33 g), pods per plant (154.73), number of primary branches (14.31) and

number of secondary branches (15.67). Pod length showed negligible contribution (0.79%) whereas, seed yield per plant contributed maximum (40.71%) towards the genetic diversity.

Bhadru (2011) evaluated one hundred and twenty pigeonpea accessions to study their variability and genetic divergence. The number of pods, seed yield per plant, primary and secondary branches per plant, raceme length, test weight and plant spread had the higher genotypic and phenotypic coefficient of variation, heritability and genetic advance as per cent of mean. The  $D^2$  values were significant among the 120 genotypes, which were grouped into 12 clusters. The traits number of pods per plant, plant height, seed yield per plant, days to maturity and days to 50 per cent flowering contributed most towards genetic divergence.

Narendra *et al.* (2011) evaluated one hundred twenty germplasm accessions to assess the extent of genetic diversity present in pigeonpea for 11 quantitative traits. The wide range of diversity was observed among the genotypes of different attributes. All the genotypes were grouped into six different non-overlapping clusters. The maximum inter cluster values (6.534) were observed between cluster V and cluster II, followed by 6.019 in between cluster V and IV. The cluster means indicated that none of the cluster was superior for all characters studied.

Rekha *et al.* (2011) evaluated 49 genotypes of pigeonpea to assess the genetic divergence which belonged to different eco-geographical regions was studied by using Mahalanobis  $D^2$  statistics. They were grouped into 6 clusters and clustering pattern of genotypes did not follow geographical origin, suggesting that geographical isolation may not be the only factor causing genetic diversity. Hence, selection of parents for hybridization should be more based on genetic diversity rather than geographic diversity. Phenol content contributed maximum towards genetic divergence followed by 100 seed weight, number of pods per plant and pod length.

## 2.3 Genetic variability parameters for yield and its attributing traits in pigeonpea

The progress in the improvement of plant type by the plant breeders will be determined by the variability existing in the populations. Thus, for effective selection and utilization of genotypes in breeding programme a thorough study on genetic variability, heritability and genetic advance is essential. Genotypic Coefficient of variation (GCV), which indicates the relative magnitude of genetic diversity present in the material, will help to study the genetic variability present for different characters.

Bashiruddin and Sreeramulu (1981) observed high GCV for 100 seed weight, cluster per plant, pods per plant and branches per plant.

Jagashoram (1983) studied 100 genotypes of pigeonpea for two years and reported high magnitude of phenotypic variability for all the characters except for seeds per pod. High estimates of GCV and heritability accompanied by moderate to high genetic advance for pods per plant, days to maturity, plant height and days to flowering were observed.

Premsagar and Jatasra (1984) reported high GCV for branches per plant, followed by pods per plant. High heritability was observed for days to maturity, seed yield, pods per plant, days to flowering and plant height. The genetic advance was high for pods per plant followed by seed yield and branches per plant.

In a study of traits in 12 genotypes, Balyan and Sudhakar (1985) reported high estimates of PCV and GCV, heritability and expected genotypic advance for primary and secondary branches, pods per plant, 100 seed weight and seed yield.

Sindhu *et al.* (1985) found high variability for pods per plant. The heritability estimates were high for all the traits studied except seed yield and seeds per pod. Genetic advance was high for pods per plant while, higher values of heritability and genetic advance for days to maturity, days to flowering and plant height were reported by Kanwan and Hazarika (1988). Tikka (1986) reported high estimates of heritability for days to flowering, branches per plant, plant height, pods per plant, pod length, pod weight and 100 seed weight. High GCV was observed for pod number followed by cluster number and seed yield while, it was lowest for seeds per pod. High heritability and high genetic advance were observed for pod number, cluster number and seed yield (Natarajan *et al.*, 1990).

Holkar *et al.* (1991) evaluated 27 pigeonpea genotypes and reported high GCV and PCV estimates for pods per plant and seed yield and high heritability and genetic advance for days to flowering, maturity and pods per plant.

Khapre and Nerker (1992) reported high genetic variability and high genetic advance for seed yield per plant and high heritability for all important yield attributes studied except primary branches per plant.

Ghodke *et al.* (1994) reported high estimates of heritability for number of pods, days to flowering and days to maturity. Gamber *et al.* (1996) reported moderate PCV and GCV estimates for days to fifty per cent flowering, pod length and 100 seed weight at both genotypic and phenotypic level.

Aher *et al.* (1998) observed wide genetic variability for plant height, number of secondary branches per plant and days to flowering. High heritability accompanied by high genetic advance was observed for primary and secondary branches per plant, followed by seed yield per plant, days to flowering and plant height.

Pansuriya *et al.* (1998) reported information on variability, heritability and correlation coefficients from the 20 early maturing pigeonpea genotypes. Plant height and pods per plant showed a wide range of phenotypic variation. The PCV and GCV were highest for dry matter per plant, harvest index, pods per plant and seed yield per plant. Heritability estimates were high for all the characters studied. However, high genetic advance was obtained only for dry matter per plant followed by pods per plant and plant height.

Takalkar *et al.* (1998) reported the highest level of variability for pods per plant followed by straw yield per plant and plant height. The high heritability estimates were observed for all the characters under study except, straw yield per plant. The expected genetic advance was high for pods per plant, plant height, straw yield per plant and days to maturity. Low genetic advance was observed for branches per plant, seeds per pod and 100 seed weight.

Vikas and Singh (1998) reported high estimates of GCV for number of pigeonpea pods per plant, seed yield and plant height. High heritability estimates were observed for plant height and 100 seed weight. The genetic advance was high for plant height and seed yield.

Jagdish Singh (1999) reported that genotypic and phenotypic coefficients of variations were high for seed yield per plant, pods per plant and branches per plant. These characters also exhibited high heritability coupled with high genetic advance.

Srinivas *et al.* (1999) reported higher values of genetic variability for number of pods and lower values for seeds per pod. Heritability estimates were high for all the traits, except seeds per pod.

Basavarajaiah *et al.* (2000) reported high PCV and GCV values for days to flowering, pods per plant, seed yield per plant and length of pod bearing branches. High heritability coupled with high genetic advance was observed for days to fifty per cent flowering, yield per plant, length of pod bearing branches and 100 seed weight.

Deshmukh *et al.* (2000) reported that secondary branches per plant recorded the highest genetic variation. Heritability estimates were high for days to 50 per cent flowering, days to maturity, 100 grain weight and primary and secondary branches per plant.

Venkateswarlu (2001) reported maximum variability for number of pods per plant and plant height. High heritability estimates were observed for number of secondary branches per plant, seed yield per plant, days to maturity and number of primary branches per plant. The expected genetic advance was high for plant height, number of pods per plant, grain yield per plant and days to maturity.

Ahmad Neyaz and Bajpai (2002) reported high heritability and genetic advance values for 100 seed weight. Firoz Mahamad (2003) reported high estimates of GCV and PCV values for branches per plant, pods per plant and low values for days to flowering, days to maturity, pod length and pod width.

Singh *et al.* (2003) reported that heritability and genetic advance was high for seed yield per plant, seeds per pod, 100 seed weight and length of pod bearing branch.

Ram Dhari *et al.* (2004) reported that branches and pigeonpea pods per plant exhibited high GCV and PCV.

Chattopadyaya and Dhiman (2005) reported high GCV and PCV for pigeonpea seeds per pod and days to maturity and high heritability and genetic advance for seed yield, plant height, branches per plant, days to maturity and seeds per pod.

Sathish Kumar *et al.* (2006) reported high PCV and GCV values for pods per plant, primary and secondary branches per plant and low for days to maturity. High heritability in broad sense coupled with high genetic advance as per cent mean were recorded for pods per plant, seed yield per plant and plant height. Moderate heritability and low genetic advance for days to maturity, days to fifty per cent flowering and seeds per pod.

Anantharaju and Muthiah (2008) reported low PCV and GCV for traits like days to maturity, days to first flowering and 100 seed weight and moderate to high PCV and GCV for plant height and 100 seed weight in F<sub>2</sub> populations of pigeonpea genotypes. High heritability estimates were recorded for traits *viz.*, days to first flowering, days to maturity, plant height and seeds per pod while, moderate to high heritability estimates for primary branches, pods per plant, 100 seed weight and seeds per pod. Genetic advance as per cent of mean was high for traits *viz.*, seeds per pod, seed yield per plant and moderate to high for days to first flowering, plant height, primary branches per plant, pods per plant and 100 seed weight.

Ganapathy (2009) reported high PCV and GCV values for pods per plant, secondary branches, length of the pod bearing inflorescence and seed yield per plant. High heritability coupled with high predictable genetic advance for secondary branches per plant, plant height and pods per plant indicated that these characters are under additive gene action.

Vange and Egbe (2009) reported high PCV and GCV values for pigeonpea pods per plant, pod weight, grain yield and numbers of primary branches: moderate for days to 50 per cent flowering, maturity and plant height. Heritability (broad sense) was high for all the traits studied with exception of seeds per pod. High expected genetic advance was obtained for number of branches per plant (58.26%), number of pods per plants (161.84%), pod weight (68.48%) and grain yield (82.65%). High heritability and expected genetic advance in these traits indicated the presence of additive gene effects. Ajay (2010) reported high PCV and GCV values for secondary branches per plant, Primary branches per plant, pods per plant, pod yield and seed yield. Whereas moderate to low values for plant height, shelling per cent, 100 seed weight, Seeds per pod had moderate PCV and low GCV. High heritability with high genetic advance for primary branches, secondary branches, pods per plant and plant height.

Bhadru (2010) reported moderate to high PCV and GCV for number of pods, seed yield per plant, plant height and plant spread. High heritability with high genetic advance for number of pods, primary and secondary branches per plant, test weight, plant height and plant spread. Days to 50 per cent flowering, plant spread, primary and secondary branches per plant, number of pods and raceme length had moderate to low direct effect and its true relationship of these traits with grain yield. Hence, direct selection for these traits would be rewarding for yield improvement in pigeonpea.

Singh *et al.* (2010a) studied seven genotypes of pigeonpea, comprising three cultivars, four wild relatives and 10 interspecific crosses for genetic variability and character association. The high estimates of PCV and GCV were exhibited for all the characters except days to 50 per cent flowering and days to maturity. In pigeonpea high heritability accompanied with high genetic advance was observed for primary branches/plant, pod length, number of chambers/pod, 100-seed weight and seed yield/plant, whereas days to 50 per cent flowering showed high heritability and low genetic advance as per cent of mean. Seed yield/plant had significant and positive correlation coefficient with plant height. Similar correlation was noticed between numbers of chambers/pod with number of seeds/pod.

Hamid *et al.* (2011) reported high PCV and GCV for primary branches, plant height, shelling percentage and seed yield per plant. In pigeonpea general heritability (broad sense) was high for all the traits except days to maturity and days to fifty per cent flowering.

Upadhyaya *et al.* (2013) studied a total of 198 accessions representing 18 species of the genus *Cajanus*. It was characterized for 27 morpho-agronomic traits at ICRISAT farm, Patancheru, India. Newman-Keuls test of significance for mean values indicated significant differences among the species for one or more traits under study. Mean diversity for all traits was maximum in *C. scarabaeoides* ( $H' = 0.590 \pm 0.010$ ). First three principal components (PCs) captured 84.3 per cent of total variation among all species. Cluster analysis resulted in three clusters. *C. albicans* and *C. mollis* formed Cluster 1; *C. cajanifolius*, *C. crassus* and *C. platycarpus* formed Cluster 2 and *C. acutifolius*, *C. scarabaeoides*, *C. lineatus* and *C. sericeus* formed Cluster 3. *C. platycarpus* for extra early flowering (34–40 days); *C. scarabaeoides* for early flowering (51–118 days); *C. albicans* for broad pods; *C. mollis*, *C. albicans*, *C. cinereus* for more seeds per pod (>6) and *C. crassus*, *C. cajanifolius*, *C. mollis*, *C. platycarpus* and *C. albicans* for high seed protein (>30 %) were found as promising

sources. Long duration perennial species such as *C. crassus*, *C. mollis* and *C. albicans* are good sources for forage. Five accessions (ICP 15661, ICP 15664, ICP 15666, ICP 15668 and ICP 15671) of *C. platycarpus*, two accessions (ICP 15653 and ICP 15658) of *C. mollis* and one accession each of *C. acutifolius* (ICP 15611), *C. albicans* (ICP 15620), *C. cajanifolius* (ICP 15632), *C. crassus* (ICP 15768), *C. lineatus* (ICP 15646), *C. scarabaeoides* (ICP 15922) and *C. sericeus* (ICP 15760), found as promising for multiple trait combinations.

## 2.4 Correlation coefficients

Salunke *et al.* (1995) studied fifty-four genotypes of early pigeonpea which revealed that grain yield per plant was significantly and positively associated with pods per plant, primary and secondary branches, plant spread, plant height and test weight. They also noticed strong negative association with seeds per pod. The yield components like days to 50 per cent flowering, days to maturity, plant height, plant spread, primary branches, secondary branches and test weight were positively and significantly associated among themselves. The pods per plant were found positively and significantly associated with primary branches, secondary branches, plant height and plant spread.

Musaan and Nahdy (1998) in a diallel study involving eight pigeonpea (*Cajanus cajan* (L.) Millsp.) inbreds evaluated and recorded the observation on yield and yield components in parents, F<sub>1</sub> and F<sub>2</sub> populations. The estimated phenotypic and genotypic correlations showed that plant height, pods per plants, seeds per pods, seed weight and number of primary branch were the primary component determinants of yield in pigeonpea.

Aher *et al.* (1998) studied the nature of association for ten traits in sixty-four genotypes of pigeonpea (*Cajanus cajan* (L.) Millsp.) and found that all the characters studied were significantly and positively correlated with grain yield and also among them.

Vikas and Singh (1998) studied association of characters in thirty-six early and extra early genotypes of pigeonpea brought from ICRISAT. The studies revealed that the days to 75 per cent flowering with number of pods per plant; test weight with days to maturity and plant height in early indeterminate group and days to 75 per cent flowering with test weight and seed yield; days to maturity with number of pods per plant; and number of pods per plant with seed yield in extra early and semi determinate group were found significantly correlated.

Chauhan *et al.* (2002) carried out correlation studies in pigeonpea to know how photoperiod responses of the Minnesota lines (MN) are different from lines bred in tropics. The Five ICPL lines and three MN lines were grown under artificially extended day length (ED) simulating 45°N and normal day length (ND) at Patancheru, India (17°N) during rainy seasons. The Six ICPL lines and two MN lines were tested at Suwan (37°N), Korea. The line × photoperiod integration was found highly significant for grain yield, total dry matter, harvest index, days to flowering and maturity, under ND. The yield for MN lines was significantly correlated with total dry matter, days to flowering and maturity. But, yield of Korea was found to be negatively correlated with days to flowering and maturity. Finally, they suggested that the selection for high harvest index potential and early flowering in MN lines for potential in ICPL lines to shorter days should be carried out.

Baskaran and Muthiah (2006) evaluated twenty- seven genotypes (Eighteen hybrids, nine parents) of pigeonpea for 11 characters. The correlation studies indicated that seed yield per plant had significant positive relationship with number of pods per plant, number of clusters per plant, plant height, days to maturity and days to 50 per cent flowering.

Sodavadiya *et al.* (2009) found that the estimated genotypic correlation coefficients were higher than their corresponding phenotypic correlation coefficients which indicated strong inherent association among the traits. The seed yield per plant had significant and positive association with days to 50 per cent flowering, days to maturity, number of branches per plant, number of pods per plant and test weight at both genotypic as well as phenotypic levels of pigeonpea genotypes.

Bhadru (2010a) carried out an investigation on pigeonpea to understand the association among the yield components and indicated that the number of pods, secondary branches and primary branches per plant and plant spread showed significant and positive correlation with seed yield at genotypic and phenotypic levels.

The correlation studies of Thanki and Sawargaonkar (2010) revealed that correlations of number of pods per plant and harvest index were found significant and positive association with respect to yield in pigeonpea.

Sreelakshmi *et al.* (2010) studied that, most of the yield contributing characters in pigeonpea genotypes like days to maturity, number of pods per plant and plant height showed significant positive correlation whereas, number of primary branches per plant showed significant negative correlation with seed yield.

## 2.5 Path analysis

Salunke *et al.* (1995) studied path coefficient analysis in 54 diverse genotypes of pigeonpea which revealed that pods per plant, seeds per pod and test weight had high direct positive effect on yield. The pods per plant and test weight had also exhibited high positive indirect effect through most of the characters. Therefore, the characters pods per plant, seeds per pod and test weight can be used as a selection criteria for descend important in early pigeonpea.

Dhahat *et al.* (1995) carried out path analysis in 30 genotypes of Rahuri indicating direct contribution of number of primary branches and secondary branches and number of pod per plant on yield both in sole crop as well as in intercrop. The plant height and number of seeds per pod in intercrop also showed direct contribution towards grain yield.

Chattopadhyay and Dhiman (2005) subjected hundred pigeonpea accessions for path studies and noticed plant height and seed per pod exhibited direct and positive effects whereas, contribution of test weight was negative towards the seed yield.

The path analysis studies on conducted by Baskaran and Muthiah (2007) on twenty-seven genotypes (Eighteen hybrids, nine parents) of pigeonpea revealed that pods per plant, test weight and plant height exhibited major direct effect on seed yield.

In a path analysis study conducted by Sodavadiya *et al.* (2009) reported that the characters *viz.*, test weight, days to maturity and pod length exerted high direct effects on seed yield of pigeonpea. The test weight and days to maturity also contributed indirectly towards the seed yield per plant through most of the characters studied.

Bhadru (2010b) studied path coefficient analysis involving 55 white seed coated pigeonpea lines which revealed that the number of pods, secondary branches and primary branches per plant were found most important character which can be strategically used to improve yield in pigeonpea.

Thanki and Sawargaonkar (2010) studied path coefficient analysis in 28 different promising genotypes of pigeonpea revealed that number of pods per plant, test weight and harvest index exerted maximum direct effect towards seed yield per plant. In addition, number of branches per plant and plant height also contributed indirectly via number of pods per plant towards seed yield per plant.

Sreelakshmi *et al.* (2010) studied 23 drought tolerant genotypes of pigeonpea and reported that, days to maturity had maximum direct effect on seed yield followed by number of pods per plant and plant height and number of secondary branches showed negative direct effect on seed yield. The number of secondary branches per plant, number of pods per plant and days to maturity were identified as important yield components of pigeonpea improvement.

## 2.6 Molecular marker studies in pigeonpea

Conventional plant breeding have had limited success in enhancing genetic resistance against diseases due to lack of genetic information and complexity of genome. Genetic studies on SMD revealed that resistance is mostly controlled by recessive genes hence necessitating more generations and large population to identify resistant segregants. Transfer of resistance to SMD from land races and wild relatives to cultivated background is difficult due to linkage drag *viz.*, undesirable traits, low yield, poor adaptability and long duration associated with resistance. Under these circumstances, newly emerging biotechnological tools like marker assisted selection can play crucial role in the success of disease resistance breeding. Molecular markers are useful in disease resistance breeding as they can substitute phenotypic screening in the early phase of breeding program and to identify resistant lines at juvenile stage to save time and cost of screening. It helps in easy identification and transfer of recessive genes and to monitor alien gene introgression, reduces the linkage drag and aids in eliminating undesirable traits in much shorter time frame than those expected through conventional breeding programs. It facilitates map-based cloning of disease resistance genes and pyramiding of genes for multiple disease resistance in a single cultivar, faster recovery of the recurrent parent genome in the backcross breeding programme (Tanksley *et al.*, 1989). It could also reduce the need for phenotypic selection that may be inappropriate in identifying

genotypic differences and in selection of rare recombinants between tightly linked resistance genes. Molecular markers offer great scope for improving the efficiency of conventional plant breeding.

The essential requirements for developing MAS system are (i) availability of germplasm with substantially contrasting phenotypes for the traits of interest (ii) highly accurate and precise screening techniques for phenotyping mapping population for the trait of interest (iii) identification of flanking markers closely associated with the loci of interest and the flanking region on either side and (iv) simple and robust DNA marker technology to facilitate rapid and cost-effective screening of large population (Paterson *et al.*, 2004).

### 2.6.1 Molecular diversity studies in pigeonpea

Varietal identification is important for the documentation of genetic resources. Traditional techniques like morphometric traits observation and biochemical techniques based on protein and isozyme polymorphism have been used. But for differentiation and characterization of varieties at molecular level, fingerprinting of crop varieties using DNA markers is very useful and this is found to be more reliable than traditional markers.

Vasconcelos *et al.* (1996) reported that microsatellite or simple sequence repeat markers are short tandem repetitive DNA sequences with a repeat length of a few (1-5) base pairs (Litt and Luty, 1989). Microsatellite markers have been increasingly used to assess the genetic diversity and population structure among plants (Li *et al.*, 2000, Pillen *et al.*, 2000). The high variability of repeat numbers among individuals has led to the use of microsatellite markers for the development of genome specific DNA fingerprints in pigeonpea (Weising *et al.*, 1992; Zavadra *et al.*, 2000).

The amplified fragment length polymorphic (AFLP) marker (Vos *et al.*, 1995) is one of the important techniques that have been used for genetic characterization of plant pathogens. AFLP techniques were more efficient in detecting polymorphism among closely related cultivars that could not be detected by other marker systems. AFLP markers have been proved as more reliable and reproducible as compared to RAPD markers and less cumbersome and time consuming than the RFLPs (Okori *et al.*, 2003 and Panguluri *et al.*, 2005).

AFLP technique initially developed for fingerprinting plant genomes (Vos *et al.*, 1995) has emerged as an important technique for genome mapping (Becker *et al.*, 1995; Maheshwaran *et al.*, 1997), gene tagging (Maksem *et al.*, 1995), assessment of genetic diversity (Paul *et al.*, 1997; Zhu *et al.*, 1998; Aggarwal *et al.*, 2002; Bensnard *et al.*, 2002), phylogenetic analysis of closely related plant species (Hill *et al.*, 1996; Sharma *et al.*, 1996; Aggarwal *et al.*, 1999) and to assess somaclonal variation of pigeonpea (Polanco and Ruiz, 2002).

Restriction fragment length polymorphism (RFLPs) has been used in pigeonpea, to overcome the problems associated with phylogenic grouping such as inconsistencies in taxonomic relationships based on data from morphology, cytology and crossability. RFLP analysis has revealed that accessions of cultivated species *Cajanus cajan* shared more DNA fragments with *Cajanus scarabaeoides* than with *C. cajanifolius* (Nadimpalli *et al.*, 1992).

RFLP markers have been utilized to study the cytoplasmic variation in the lines of pigeonpea developed by interspecific crosses using four probes from maize mitochondrial DNA- atp  $\alpha$ , atp  $\beta$ , cox -I and cox- II (Sivaramakrishnan *et al.*, 1996).

Rathnaparkhe *et al.* (1995) reported high levels of polymorphism among the wild species using RAPD markers, while little polymorphism was found within cultivated *Cajanus cajan* accessions.

RAPD markers were used for investigating quantitative trait loci (QTLs) in two strains of pigeonpea and in the F<sub>1</sub> and F<sub>2</sub> progeny (Tyagi, 1997). However, the level of polymorphism among parents was very low. The F<sub>1</sub> hybrid was intermediate between two parents, but F<sub>2</sub> showed little variation, indicating that both parents were different morphologically, but with little genetic variation at DNA level.

The somaclonal variants of pigeonpea line, ICPL 87 were distinguished at the molecular level by RAPD analysis using specific arbitrary sequences of 19 decamer primers. A high level of polymorphism was evident with the primer OPA-20. Whereas, a low level was observed with the primer OPA-07 which served as molecular markers for specific somaclonal variants thereby, providing a method for selecting somaclones with better agronomic performance.

Prasannalatha *et al.* (1999) Burns *et al.* (2001) reported a set of 10 simple sequence repeat (SSR) markers in pigeonpea. Ten loci exhibited polymorphism when 20 primer pairs were screened across 12 diverse pigeonpea accessions.

Lohithaswa *et al.* (2003) studied the genetic divergence in 11 pigeonpea genotypes using RAPD markers. Decamer oligo-nucleotides primers were initially screened to identify the most promising primers for detecting polymorphism. Eight primers were selected for screening and 52 bands were detected. Of the 52 bands, 33 (63.46 %) bands were polymorphic between the genotypes. The genotype ICPL 87, TS 3, GS 1 and GS 3 had high genetic diversity between them. The primer OPB 15 produced unique banding pattern specific to different varieties, whereas the primer OPB 19 produced specific banding pattern profiles in ICP 8863 and GS 1.

Souframanien *et al.* (2003) used RAPD markers for identification of two pigeonpea cytoplasmic male sterile (CMS) lines derived from crosses between the wild (*Cajanus scarabaeoides* and *C. sericeus*) and the cultivated species (*Cajanus cajan*). The male sterile (A) line and its maintainer (B) line could be easily differentiated with certain random primers. Amplification product of 600 bp amplified by primer OPC-11 was observed in both the cytoplasmic male sterile lines (288 A and 67 A), which was absent in the maintainer lines (288 B and 67 B) and the putative R-line (TRR 5 and TRR 6). Dendrogram constructed based on the similarity index showed that considerable genetic variation exists between CMS lines, two putative R lines and wild species studied.

Panguluri *et al.* (2005) detected DNA polymorphism in the cultivated pigeonpea and two of its wild relatives *Cajanus volubilis* and *Rhynchosia bracteata* using amplified fragment length polymorphism (AFLP) fingerprinting. The two wild species shared only 7.15 per cent bands with the pigeonpea cultivars, whereas 86.71 per cent common bands were seen among cultivated cultivars. Similarly, 62.08 per cent bands were polymorphic between *C. volubilis* and pigeonpea cultivars in comparison to 63.33 per cent polymorphic bands between *R. bracteata* and pigeonpea cultivars and 13.28 per cent polymorphic bands among pigeonpea cultivars. The cluster analysis revealed low polymorphism among pigeonpea cultivars and very high polymorphism between cultivated pigeonpea and its wild relatives.

Wasike *et al.* (2005) used AFLP markers to study the genetic relatedness between Asia and African pigeonpea cultivars and recorded limited genetic variability among the genotypes used for the study. ANOVA at continent wide hierarchical level, revealed significantly weak population structure but when treating the cultivars as samples from a panmictic population revealed a stronger genetic structure. Estimates of average gene diversity were higher for Indian genotypes suggesting East African pigeonpea cultivars are less genetically diverse than Indian cultivars.

The study also demonstrated AFLP markers as a suitable tool for DNA fingerprinting and genetic studies in pigeonpea. Dendrogram constructed by combined RAPD and SSR data depicted that the SMD susceptible genotypes TTB 7 and ICP 8863 clustered together while, the resistant genotypes Hy 3c and BRG 3 sub clustered with ICP 7035 indicating ICP 7035, BRG 3, HY 3C are differing at molecular level from the SMD susceptible genotypes TTB 7 and ICP 8863 (Gangadhara, 2006).

Diversity array technology (DArT) markers revealed low level of genetic diversity in cultivated pigeonpea as compared to wild relatives. Most of the diversity was among the wild relatives of pigeonpea or between the wild and the cultivated species (Yang *et al.*, 2006).

Datta *et al.* (2007) studied genetic relatedness among 16 varieties representing eight different pulse crops namely, pea, lentil, lathyrus, chickpea, pigeonpea, frenchbean, urdbean and mungbean using 40 RAPD markers. From the clustering analysis, chickpea and lentil grouped together whereas, pea and lathyrus were close to each other. The two crops of the genus vigna (mungbean and urdbean) grouped together with their varieties forming subclusters. Frenchbean and pigeonpea were grouped into distinct clusters showing relative divergence of these crops from other pulse crops.

Choudhury *et al.* (2007) identified 21 RAPD markers for identification of specific genotypes and assessment of genetic relatedness among the pigeonpea cultivars. Among these, 16 primers were found to be unique, producing 40 genotype specific bands in 16 different genotypes. An average of 12.6 bands per primer was obtained with 89.4 per cent polymorphism.

Odeny *et al.* (2007) identified 19 SSR primers to be polymorphic among 15 cultivated and nine wild pigeonpea accessions providing evidence for cross species transferability within the genus *Cajanus*. A total of 98 alleles were detected at the 19 polymorphic loci with an average of 4.9 alleles per locus. Less allelic variation (31 alleles) was observed within the cultivated species than across

the wild species (92 alleles) and suggested for development of more microsatellite markers for future genomic studies in pigeonpea. 108 RAPD markers were screened to identify CMS lines derived from crosses between wild (*Cajanus scarabaeoides* and *C. sericeus*) and cultivated pigeonpea (Choudhury *et al.*, 2008). A set of RAPD primers were identified that could distinguish the CMS systems of GT 288 A/B and 67 A/B. Moreover, specific primers differentiating the CMS lines (GT 288/67A), maintainers (GT288B/67B) and putative restorers (ICP 41 and DPPA 85-7) were identified for use in heterosis breeding.

Singh *et al.* (2008) used 21 SSR markers obtained from different crop species to assess polymorphism in 16 cultivated pigeonpea genotypes. Based on SSR fingerprinting, 16 genotypes were grouped into two groups as early and late duration genotypes indicating that SSR markers could be used as a good choice to classify the pigeonpea genotypes.

Odeny *et al.* (2009) used 113 pigeonpea genomic SSRs, 73 of which amplified interpretable bands. Thirty-five of the primers revealed polymorphism among 24 pigeonpea breeding lines. The number of alleles detected ranged from 2 to 6 with a total of 110 alleles and an average of 3.1 alleles per locus. GT/CA and GAA class of repeats were the most abundant dinucleotide and tri-nucleotide repeats respectively. Additionally, 220 soybean primers were tested in pigeonpea, 39 of which amplified interpretable bands.

Saxena *et al.* (2009a) identified 13 polymorphic SSR markers to be polymorphic amongst 32 cultivated and eight wild pigeonpea genotypes representing six *Cajanus* species. These markers amplified a total of 72 alleles ranging from two to eight alleles with an average of 5.5 alleles per locus. The polymorphic information content for these markers ranged from 0.05 to 0.55 with an average of 0.32 per marker. These markers should be useful for genome mapping, trait mapping, diversity studies and assessment of gene flow between populations in pigeonpea.

In order to maximize polymorphism in the mapping populations for mapping loci for fusarium wilt (FW) and sterility mosaic disease (SMD) resistance in pigeonpea, a set of 32 pigeonpea lines were screened using 30 SSR markers by Saxena *et al.* (2009b). A total of 23 marker loci showed polymorphism with 2-4 alleles and the PIC for these markers ranged from 0.12 to 0.65 with an average of 0.43 per marker.

Ganapathy *et al.* (2010) identified SSR and AFLP markers associated with the sterility mosaic disease in the F<sub>2</sub> population of the cross TTB 7 (susceptible) and BRG 3 (resistance). A total of 156 SSRs and 16 AFLP primer pairs were surveyed for identification of polymorphic markers between the parents and DNA bulks of susceptible and resistance F<sub>2</sub> individuals. Out of 10 polymorphic SSR's identified between the parents, none of them were polymorphic between the DNA bulks. From 13 polymorphic AFLP primer combinations between the parents, two AFLP primer pairs generated 4 markers which were polymorphic between the resistant and susceptible bulks indicating that these markers are linked to SMD.

Saxena *et al.* (2010a) reported 36 microsatellite loci from a SSR enriched genomic library. Primer pairs were designed for 23 SSR loci, of which 16 yielded amplicons of expected size. Thirteen SSR markers were polymorphic amongst 32 cultivated and eight wild pigeonpea genotypes representing six *Cajanus* species. These markers amplified a total of 72 alleles ranging from two to eight alleles with an average of 5.5 alleles per locus. The PCV for these markers ranged from 0.05 to 0.55 with an average of 0.32 per marker.

In order to maximize polymorphism in the mapping populations for mapping loci for *Fusarium* wilt (FW) and sterility mosaic disease (SMD) resistance in pigeonpea, a set of 32 pigeonpea lines were screened for polymorphism with 30 microsatellite markers. A total of 23 marker loci showed polymorphism with 2-4 alleles and the polymorphism information content (PIC) for these markers ranged from 0.12 to 0.65 with an average of 0.43 per marker. Based on the genetic diversity five parental combinations were identified and populations were developed. Of these crosses, one cross segregate for *Fusarium* wilt resistance, two for SMD resistance and the remaining two crosses segregate for resistance to both (Saxena *et al.*, 2010b).

Datta *et al.* (2010) revealed a significant transferability (46%) of chickpea microsatellites to *Cajanus*. In cultivated pigeonpea, chickpea specific SSRs showed 38–39 per cent transferability, while among wild *Cajanus* species, it ranged from 26 per cent in *Cajanus sericeus* ICP 15760 to 40 per cent in *C. sericeus* ICP 15761. The transferable primers exhibited extensive polymorphism in *Cajanus* with an average number of 4.11 alleles per marker. High level of polymorphism exhibited by

chickpea microsatellite markers in the present study indicates their usefulness in diversity analysis, mapping agronomically important traits and marker-assisted breeding in pigeonpea.

Metkar *et al.* (2010) reported the SSR based molecular characterization of CMS lines in twenty five pigeonpea genotypes. Totally 16 SSR primers were used for detection of polymorphism, out of which four primers *viz.*, CCB-5, CCB-8, CCB-9, CCB-10, were found polymorphic. A total of 13 alleles were detected on an average 3.25 alleles.

Raju *et al.* (2010) generated 908 EST's which are available in public domain. At the time of analysis, a set of 5,085 unigenes were used for identification of molecular markers in pigeonpea. For instance, 3,583 simple sequence repeat (SSR) motifs were identified in 1,365 unigenes and 383 primer pairs were designed. Assessment of a set of 84 primer pairs on 40 elite pigeonpea lines showed polymorphism (28.8%) with an average of four alleles per marker with an average PIC value of 0.40. Datta *et al.* (2011) reported that 550 validated genic- SSR markers in pigeonpea using deep transcriptome sequencing. From these, 20 highly polymorphic markers were used to evaluate the genetic relationship among species of the genus *Cajanus*. A comprehensive set of genic-SSR markers were developed as an important genomic resource for diversity analysis and genetic mapping in pigeonpea.

Gnanesh *et al.* (2011) used microsatellite markers to screen F<sub>2</sub> population of cross ICP 8863×ICPL 20097 (segregating for Patancheru SMD isolate) and TTB7× ICP7035 (segregating for both Patancheru and Bangalore SMD isolates) and identified QTL's linked to SMD. After screening over 3000 SSR markers on parental genotypes of each mapping population, Intra-specific genetic maps comprising of 11 linkage groups and 120 and 78 SSR loci were developed for ICP 8863 × ICPL 20097 and TTB 7 × ICP 7035 populations, respectively. Composite Interval Mapping (CIM) based QTL analysis by using genetic mapping and phenotyping data provided four QTL's for Patancheru SMD isolate and two QTL's for Bangalore SMD isolate. One QTL namely qSMD4 identified within an interval of 2.8 cM on LG7 explaining 24.72 per cent of phenotypic variance.

## 2.6.2 Identification of trait specific molecular markers

Till date very little literature pertaining to identification of DNA markers linked to pigeonpea sterility mosaic disease is reported. Very few reports regarding identification of trait specific markers in pigeonpea are available. Hence, literature pertaining to identification of trait specific markers in related crops is also reviewed.

The use of DNA marker systems, such as Randomly Amplified polymorphic DNAs (Williams *et al.*, 1990), amplified fragment length polymorphisms (Vos *et al.*, 1995) and simple sequence repeats (Akkaya *et al.*, 1992), has contributed greatly to the development of genetic linkage maps for many important crop species including cowpea (Fatokun *et al.*, 1993; Waugh *et al.*, 1997).

In combination with the bulked segregant analysis (BSA) method (Michelmore and Meyers, 1998) the use of RAPDs, AFLPs and SSRs has made it possible to rapidly identify molecular markers linked to genes of agronomic importance (Lee 1995; Young, 1999).

The development and use of molecular marker technologies has also facilitated the subsequent cloning and characterization of disease and pest resistance genes from a variety of plant species (Meyers *et al.*, 1999; Hammond and Jones, 1997).

Tiwari *et al.* (1998) identified coupling and repulsion phase RAPD markers linked to powdery mildew resistant gene *er-1* in pea using bulk segregant analysis of F<sub>3</sub> individuals. Marker OPO-18 was found to be linked in coupling phase while, the markers OPE 16 and OPL 6 were in repulsion phase to resistant gene *er-1*.

Quedraogo *et al.* (2001) identified three AFLP markers (E-AAC/MCAA300 (2.6 cM), E-ACT/M-CAA524 (0.9 cM) and E-ACA/M-CAT140/150 (0.9 cM), tightly linked to *Rsg2-1* which appears to be co-dominant. Segregation analysis of a different F<sub>2</sub> population resulting from a cross of the striga susceptible line IT84S-2246-4 with Tvu 14676, a *S. gesnerioides* race 3 resistant line, showed that resistance to *S. gesnerioides* race 3 was controlled by a single dominant gene, designated as *Rsg4-3*. The identification of AFLP markers linked to striga resistance provides a stepping stone for a marker assisted selection program and the eventual cloning and characterization of the gene(s) encoding resistance to this noxious parasitic weed.

Quedraogo *et al.* (2002) identified seven AFLP markers linked to *Striga gesnerioides* gene *Rsg3* from the F<sub>2</sub> population of cross Gorom x Tvx 3236 using bulk segregant analysis. From the

linkage analysis the distance between the marker and Rsg3 locus ranged from 9.9 to 2.5 cM, with two markers E-AGA/MCAG300 and E-AGA/ M-CTA460 flanking the Rsg3 locus at 2.5 and 2.6 cM respectively.

Kotresh *et al.* (2006) identified RAPD markers associated with pigeonpea wilt using F<sub>2</sub> population derived from contrasting parents GS I (susceptible), ICPL 87119 (resistant) and ICP 8863 (resistant). PCR testing revealed presence of two amplicons at 704 bp and 500 bp linked with susceptibility. Analysis of individual F<sub>2</sub> plants showed a segregation ratio of 3:1 for the presence: absence of amplicons in the crosses.

Selvi *et al.* (2006) identified three RAPD markers in mungbean *viz.*, OPT16, OPS7 and OPAK 19 specific to MYMV resistant parent and resistant bulk but absent in MYMV susceptible parent and susceptible bulk. From linkage analysis, one RAPD marker OPS7900 was identified to be associated with mungbean yellow mosaic virus resistance.

Blair *et al.* (2007) developed a co-dominant SCAR marker SR2, tightly linked to bgm-1 resistance gene using common bean RIL population derived from the cross DOR 476 x Sel 1309. The polymorphism between the resistant and the susceptible genotype was based on 37 bp insertion event in the SR2 allele associated with susceptibility.

Ganapathy *et al.* (2009) used two AFLP primer pairs generating 4 markers (E-CAA/M-GTG150, E-CAA/M-GTG60, E-CAG/M-GCC120 and ECAG/M-GCC150) which were polymorphic between the resistant and susceptible bulks indicating these markers are linked to SMD and located at a map distance of 5.7, 4.8, 5.2 and 20.7 cM respectively. The markers E-CAA/MGTG150, E-CAA/M-GTG60 were linked in coupling phase to the susceptible dominant allele amplifying only in susceptible individuals, which can be effectively used for marker assisted selection in pigeonpea.

Yang *et al.* (2011) developed a genetic map in pigeonpea (*Cajanus spp.*) using diversity arrays technology (DArT) markers and a total of 554 DArT markers showed polymorphism in pigeonpea F<sub>2</sub> mapping population of 72 progenies derived from an interspecific cross of ICP 28 (*Cajanus cajan*) and ICPW 94 (*Cajanus scarabaeoides*). The two groups of genetic maps were generated using DArT markers.

Mir *et al.* (2012) used 11 determinate (DT) and 83 indeterminate (IDT) lines for genotyping with DArT arrays (with 6144 features) and 768 SNP markers using Golden Gate assay. The PIC for these markers varied from 0.02 to 0.50. Association analysis on marker genotyping and phenotyping data showed a significant association ( $P \leq 0.01$ ) of determinacy with 19 SNP and 6 DArT markers explaining 8.05–8.58 per cent and 7.26–14.53 per cent phenotypic variation, respectively. Clustering based on entire DArT and SNP markers could not discriminate DT lines from IDT lines; however, analysis with associated markers discriminated DT lines from the IDT lines.

## 2.7 Screening techniques for sterility mosaic disease resistance

Three methods are being used for evaluating resistance to SMD. An effective technique called “Leaf stapling technique” for screening pigeonpea germplasm and breeding material for resistance to SMD was developed by Nene and Reddy (1977). It is the most commonly used method under field and glass house conditions. This technique involves stapling of SMD infected pigeonpea leaves on the healthy seedlings at 2-4 leaf stage. Mites infected with virus from the stapled leaf migrate and transmit the virus to the test plants. This technique was shown to facilitate inoculation at primary leaf stage and to express disease symptoms rapidly.

### 2.7.1 Resistant source material

Rangaswamy *et al.* (2005) evaluated ICP 7035, along with the two local varieties, TTB 7 and Hy 3c, in SMD nursery at the Gandhi Krishi Vignana Kendra (GKVK), Bangalore. Average SMD incidence in pigeonpea susceptible cultivars ranged from less than 2.0 to 90.3 per cent during various years, but ICP 7035 remained free from SMD. Among 79 genotypes, screened against SMD, seven genotypes *viz.*, ICP 7035, BAD 2001-6, NDA 98-8, Hy 3c, MAL 24, MAL 23 and BRG 3 showed moderately resistant reaction with 11- 30 per cent SMD incidence while, the remaining genotypes including the susceptible check TTB 7 were found susceptible with disease incidence more than 50 per cent (Saifulla *et al.*, 2005).

Four genotypes of pigeonpea *viz.*, BRG 3, ICP 7035, Hy 3c and ICP 8863 were screened against SMD for three consecutive years from 2002-03 to 2005-06. BRG 3 and ICP 7035 recorded

resistant reaction while, the genotype HY 3C recorded moderate resistant reaction to SMD. The susceptible check ICP 8863 recorded 100 per cent disease incidence (Saifulla *et al.*, 2006).

Ganapathy (2009) has confirmed the resistant levels of four genotypes *viz.*, BRG 3, ICP 7035, TTB 7 and ICP 8863 before using them as parents in his crossing programme. BRG 3 and ICP 7035 showed 100 per cent resistance with no mosaic symptoms while, the susceptible genotypes TTB 7 and ICP 8863 showed 100 per cent susceptibility with severe mosaic symptoms.

Singh *et al.* (2011) evaluated Four long duration pigeonpea genotypes, *viz.*, IPA 16 F, IPA 8 F, IPA 9 F and IPA 12 F possessing acceptable yield levels were evaluated for their reaction to wilt disease in wilt sick plots continuously for three to five years at hot spots in north east plain zone, central zone and south zone of the country. All the four genotypes showed resistant to moderately resistant reaction to wilt over the years at all the hot spots.

Sharma *et al.* (2012) evaluated the pigeonpea mini-core set to identify resistance to FW and SMD under artificial field epiphytotic conditions. Six accessions originated from India and Italy out of 146 accessions developed from a core collection of 1290 accessions from 53 countries were found resistant to FW (<10 per cent mean disease incidence). High level of resistance to SMD was noticed in 24 accessions (mean incidence <10%) originated from India, Italy, Kenya, Nepal, Nigeria, Philippines and United Kingdom. However, Combined resistance to both SMD and FW was noticed in five accessions.

## 2.7.2 Inheritance studies for SMD resistance

Singh *et al.* (1983) in a study involving inheritance of resistance to SMD in 15 crosses, of pigeonpea genotypes involving five resistant and three susceptible genotypes reported that the resistance was controlled by four independent non-allelic genes. The symbols Sv1, Sv2, Sv3 and Sv4 were assigned to four resistant genes and Sv1 and Sv2 genes were reported to exhibit duplicate dominant epistasis while, Sv3 and Sv4 exhibited duplicate recessive epistasis. He concluded that presence of at least one dominant allele at locus 1 or 2 and homozygous recessive genes at locus 3 or 4 were essential for resistant reaction.

Sharma *et al.* (1984) reported both 9 (resistant): 7 (susceptibility) and 1 (resistant): 3 (susceptible) segregation ratios in different pigeonpea crosses and explained basis of inheritance of SMD controlling two genes and more than two alleles per locus.

Reddy *et al.* (1995) reported that in the SMD affected genotypes of pigeonpea, leaf cuticle and the epidermal cell wall thickness were found less compared to resistant genotypes. Thick leaf cuticle and epidermal cell wall of resistant genotypes prevents the mites to feed on them which might confer resistance against the disease. Resistance is therefore attributed to the thick cuticle of the resistant lines through which the mite vectors cannot penetrate into the living epidermal cells to transmit the SMD pathogen.

Murugesan *et al.* (1997) reported monogenic inheritance of resistance to SMD of pigeonpea. They studied F<sub>1</sub> and F<sub>2</sub> generation of Vamban 1 (resistant) x Gulbarga 1 (susceptible). The F<sub>1</sub> hybrid was resistant indicating resistance being dominant over susceptibility. The F<sub>2</sub> population segregated in 3 resistant: 1 susceptible ratio indicating monogenic control.

Srinivas *et al.* (1997) studied the inheritance of resistance and allelic relationship in three resistant pigeonpea sources for strain 2 of sterility mosaic pathogen. The resistant genotypes ICP 7035, ICP 7349 and ICP 8850 were crossed with susceptible genotypes BDN 1 and LRG 30 to determine the inheritance of resistance. The resistant and susceptible genotypes were crossed among themselves to obtain information on their allelic relationship. Parents, F<sub>1</sub> and F<sub>2</sub> generations were sown in pots and screened using "Infector hedge row technique". Observations obtained from parents, F<sub>1</sub> and F<sub>2</sub> generations, indicated dominance of resistance in certain crosses and the dominance of susceptibility in others.

Nagaraj *et al.* (2004) studied the inheritance to Bangalore strain of sterility mosaic virus in pigeonpea crosses involving two resistant lines (ICP 7035 and MAL 14) with no apparent symptoms and susceptible lines (TTB 7, ICP 8863 and BDN 1) with severe mosaic symptoms. The F<sub>1</sub>, F<sub>2</sub>, BC1 and BC2 generations were sown in the field and screened following infector hedge and leaf stapling techniques to study the inheritance pattern. They opined that resistance was recessive and appeared to be governed by two independent non-allelic genes exhibiting complementary epistasis. The presence of at least one allele in homozygous recessive condition was found necessary to express resistant phenotype.

One hundred and fifteen wild *Cajanus* accessions from six species (*C. albicans*, *C. platycarpus*, *C. cajanifolius*, *C. lineatus*, *C. scarabaeoides* and *C. sericeus*) were evaluated against three SMD isolates prevailing in peninsular India. Evaluations were done under greenhouse conditions in endemic locations of each isolate through mite-mediated virus inoculation and graft inoculation techniques. Fifteen wild accessions showed resistance to all three isolates of SMD. Most of the wild accessions did not support mite multiplication. The majority of the accessions resistant to SMD following inoculations with viruliferous mites were susceptible by graft inoculation, suggesting that vector resistance is conferring resistance to infection with PPSMV (Kumar *et al.*, 2005).

Ganapathy *et al.* (2009) reported  $F_1$ 's of the pigeonpea resistant x susceptible cross were susceptible indicating susceptibility to be dominant over resistance. A digenic ratio of 7 resistant: 9 susceptible was obtained in the  $F_2$  population of cross ICP 8863 (S) x ICP 7035 (R) indicating complementary nature of two genes for resistance. In contrast, a monogenic ratio of 1 resistant: 3 susceptible was obtained for the cross TTB 7 (S) x BRG 3 (R) indicating single gene control.

Karimi *et al.* (2010) reported  $F_1$ ,  $F_2$  and backcross populations of the crossing resistant accessions (ICEAP 00554, ICEAP 00557) with susceptible pigeonpea accessions (KAT 60/8, ICP 7035). The Parents,  $F_1$ ,  $F_2$ , backcrosses (BC1 $F_1$  and BC2 $F_1$ ) populations were evaluated for Fusarium wilt resistance.  $F_2$  populations derived from ICEAP 00554 x KAT 60/8, ICEAP 00557 x KAT 60/8, ICEAP 00554 x ICP 7035, ICEAP 00557 x ICP 7035 crosses exhibited a 3:1 ratio which indicated that resistance to Fusarium wilt was under the control of major gene, however, a recessive gene was detected from ICP 7035 x KAT 60/8 cross.

Sreelakshmi *et al.* (2011) reported  $F_2$  population of crosses LRG 30 x ICPL 87119, MRG 66 x ICPL 87119 and ICPL 85063 x ICPL 87119 segregated into a 13 R: 3 S. When back crossed with resistant parent ICPL 87119, it segregates in a 3R: 1S phenotypic ratio and with susceptible parent, it segregated into a phenotypic ratio of 1R: 1S. LRG 41 x ICPL 87119 and Nallakandi x ICPL 87119,  $F_2$  population segregated into a ratio of 9R: 7S. The back crosses population B1 to the susceptible parent in both the crosses segregated in the phenotypic ratio of 1 R: 3S and to the resistant parent segregated in the ratio of 3R: 1S. Inheritance studies on *Fusarium* wilt resistance affirmed the dominance of resistance to susceptibility and the role of two gene interactions *viz.*, inhibitory (13:3) and complementary (9:7) types.

Gnanesh *et al.* (2011) determine the inheritance of resistance to Bangalore and Patancheru isolates of the SMD involving a resistant (ICP7035) and susceptible (TTB 7) pigeonpea genotypes. Observations in parents,  $F_1$  indicated dominance of susceptibility over resistance. The disease reaction of the individual  $F_2$  plant derived  $F_3$  families for Patancheru isolate was controlled by two genes with dominance epistasis and for Bangalore isolate, absence of resistant plants indicate action of two or more genes in controlling resistance to SMD.

Ganapathy *et al.* (2012) studied the nature of inheritance of SMD in resistant (BRG 3 and ICP 7035) and susceptible (ICP 8863 and TTB 7) genotypes of pigeonpea. SMD incidence observed in parents,  $F_1$  and  $F_2$  generations indicated resistance to be controlled by recessive gene and appeared to be monogenic in cross TTB 7 x BRG 3 and governed by two independent non-allelic genes exhibiting complementary epistasis in cross ICP 8863 x ICP 7035. Expression of at least one SMD gene in homozygous recessive condition was necessary for resistant phenotype in the above mentioned crosses. Resistant x resistant and susceptible x susceptible  $F_2$  individuals showed no segregation indicating function of same loci/ linked loci to govern resistance and susceptibility in the parents studied.

# MATERIAL AND METHODS

The details of materials and methods adopted in the present investigation are described in this chapter under the following headings;

- 3.1 Assessment of genetic variability in mini core-collection of pigeonpea (*Cajanus cajan*) for yield and yield attributes.
- 3.2 Screening of selected genotypes for sterility mosaic disease (SMD), fusarium wilt (FW) and protein content genotypes using markers.
- 3.3 Genetic diversity studies using simple sequence repeat (SSR) markers.

The present investigation was carried out with an assessment of genetic variability among 192 pigeonpea accessions (mini-core collection set and accessions collected from Agricultural Research Station, Gulbarga). The mini-core collection set was obtained from International Crop Research Institute for Semi-arid Tropics (ICRISAT), Patancheru, Andhra Pradesh. The genotypes were evaluated along with 4 popular varieties as checks. The list of genotypes and checks used in the present investigation are mentioned in Table 1. The present study concentrated on the characterization of subset of pigeonpea minicore collection. A minicore collection (from different countries) set was characterized for the reactions to diseases (SMD and FW). They have been grouped them into resistant, moderately resistant, susceptible, highly susceptible genotypes based on morphological observations (Jaggal, 2012).

The list of disease resistant and susceptible pigeonpea accessions are mentioned in Table 2 and 3. The present study aims to identify existence of different alleles for the resistant and susceptible groups. Also efforts have been done to identify the different alleles for high and low protein genotypes (Timmaraju, 2012). The list of genotypes used for molecular characterization are provided in Table 4.

## 3.1 Assessment of genetic variability in mini-core collection of pigeonpea (*Cajanus cajan*) for yield and yield attributes

### 3.1.1 Experimental layout

The experiment was conducted at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. A total of 192 germplasm lines were grown during *kharif* 2012-13 following augmented experimental design. The experimental plot was divided in to 5 blocks. In each block contained 38 genotypes and 4 checks, where all the lines and checks were raised at spacing of 60 cm between rows and 30 cm between plants in a 6 meter row. Checks were replicated in each block while genotypes were un-replicated. Standard package of practices was followed for raising a good and healthy crop. The monthly meteorological data obtained from Meteorological department from Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, with regard to temperature, relative humidity, rainfall and number of rainy days during the course of investigations is presented in Appendix I.

### 3.1.2 Collection of data

The following observations were recorded on five randomly selected plants per genotype. Quantitative characters

#### 3.1.2.1 Days to 50 per cent flowering

The number of days from sowing to the opening of flowers in fifty per cent of plants in each genotype was recorded.

#### 3.1.2.2 Plant height

The height of plant was measured in centimeter from ground level to the tip of the main stem at the time of harvest.

#### 3.1.2.3 Number of primary branches

Number of branches on each randomly selected five plants was counted at the time of harvest.



**Plate 1. Field view of experimental plot**

**Table 1: List of the pigeonpea genotypes with accession number along with their country of origin used in the present study**

Sl. No.	Accession No.	Source/Country of origin	Sl. No.	Accession No.	Source/Country of origin
1	ICP - 348	India	35	ICP - 7223	India
2	ICP - 655	India	36	ICP - 7260	India
3	ICP - 772	India	37	ICP - 7314	India
4	ICP - 939	India	38	ICP - 7366	India
5	ICP - 995	India	39	ICP - 7426	India
6	ICP - 1071	India	40	ICP - 7507	India
7	ICP - 1156	India	41	ICP - 7803	India
8	ICP - 2577	Myanmar	42	ICP - 7869	India
9	ICP - 2698	India	43	ICP - 8012	India
10	ICP - 2746	India	44	ICP - 8255	India
11	ICP - 3046	India	45	ICP - 8266	India
12	ICP - 3049	India	46	ICP - 8384	India
13	ICP - 3451	India	47	ICP - 8602	India
14	ICP - 3576	India	48	ICP - 8757	India
15	ICP - 4029	India	49	ICP - 8840	India
16	ICP - 4167	India	50	ICP - 8860	ICRISAT
17	ICP - 4317	India	51	ICP - 8921	India
18	ICP - 4392	India	52	ICP - 8949	India
19	ICP - 5142	India	53	ICP - 9045	India
20	ICP - 5863	India	54	ICP - 9414	India
21	ICP - 6049	India	55	ICP - 9655	India
22	ICP - 6123	India	56	ICP - 9691	India
23	ICP - 6128	Bangladesh	57	ICP - 9750	India
24	ICP - 6668	India	58	ICP - 10094	India
25	ICP - 6739	India	59	ICP - 10228	India
26	ICP - 6815	India	60	ICP - 10397	India
27	ICP - 6859	India	61	ICP - 10447	India
28	ICP - 6929	Trinidad and Tobago	62	ICP - 10503	India
29	ICP - 6971	unknown	63	ICP - 10559	India
30	ICP - 6992	India	64	ICP - 10654	India
31	ICP - 7057	India	65	ICP - 11015	ICRISAT
32	ICP - 7076	India	66	ICP - 11059	ICRISAT
33	ICP - 7148	Sri Lanka	67	ICP - 11230	ICRISAT
34	ICP - 7221	unknown	68	ICP - 11281	ICRISAT

*Contd.....*

Sl. No.	Accession No.	Source/Country of origin	Sl. No.	Accession No.	Source/Country of origin
69	ICP - 11320	Nepal	104	ICP - 14294	Italy
70	ICP - 11477	Unknown	105	ICP - 14368	Venezuela
71	ICP - 11627	ICRISAT	106	ICP - 14444	ICRISAT
72	ICP - 11690	ICRISAT	107	ICP - 14471	ICRISAT
73	ICP - 11823	India	108	ICP - 14569	India
74	ICP - 11833	India	109	ICP - 14638	ICRISAT
75	ICP - 11910	India	110	ICP - 14701	ICRISAT
76	ICP - 11946	India	111	ICP - 14722	ICRISAT
77	ICP - 12105	Tanzania	112	ICP - 14801	ICRISAT
78	ICP - 12123	Tanzania	113	ICP - 14819	ICRISAT
79	ICP - 12142	Tanzania	114	ICP - 14832	ICRISAT
80	ICP - 12298	India	115	ICP - 14900	ICRISAT
81	ICP - 12410	India	116	ICP - 14976	ICRISAT
82	ICP - 12515	India	117	ICP - 15049	ICRISAT
83	ICP - 12596	India	118	ICP - 15068	ICRISAT
84	ICP - 12654	India	119	ICP - 15161	ICRISAT
85	ICP - 12680	India	120	ICP - 15185	Nigeria
86	ICP - 13139	Kenya	121	ICP - 15382	Uganda
87	ICP - 13167	Kenya	122	ICP - 16264	ICRISAT
88	ICP - 13191	Australia	123	ICP - 4575	India
89	ICP - 13244	Kenya	124	BRG-7-3	India
90	ICP - 13270	Kenya	125	BRG-6-12	India
91	ICP - 13304	Italy	126	ICP-1730	India
92	ICP - 13359	Malawi	127	ICP-4019	India
93	ICP - 13431	Malawi	128	BRG-9-2	India
94	ICP - 13571	Nigeria	129	BRG-207	India
95	ICP - 13575	Sierra Leone	130	TTB-7	India
96	ICP - 13577	China	131	TM224	India
97	ICP - 13579	Philippines	132	ICP-1224	India
98	ICP - 13633	Nigeria	133	BRG-7-5	India
99	ICP - 13662	India	134	TM-26	India
100	ICP - 13884	Puerto Rico	135	ICP-5641	India
101	ICP - 14116	Jamaica	136	ICP-12460	India
102	ICP - 14147	Brazil	137	ICP-1550	India
103	ICP - 14229	Zambia	138	DPD-2-38	India

Contd.....

Sl. No.	Accession No.	Source/Country of origin	Sl. No.	Accession No.	Source/Country of origin
139	ICP-12333	India	168	ICPL-87	India
140	ICP-10804	India	169	ICP-12692	India
141	ICP-12674	India	170	Jamadhar local	India
142	Gullyal local	India	171	WRP-1	India
143	ICP-4878	India	172	Bidar local	India
144	PUSA992	India	173	UPAS-120	India
145	ICP-6528	India	174	JKE-144	India
146	Gullyal red	India	175	PUSA-92	India
147	HY-3-C	India	176	ICP-8863	India
148	ICP-12266	India	177	PG-12	India
149	ICP-9903	India	178	MA-29	India
150	ICP-1366	India	179	Karithogari	India
151	ICP-4395	India	180	ICPB 2091	India
152	UPAS-120	India	181	CORG-295	India
153	BRG-9-2	India	182	Chaple	India
154	TS-3®	India	183	Gulyal white	India
155	DM-155	India	184	CORG-9701	India
156	TM-389	India	185	WRP-1 (W)	India
157	BRG-7-2	India	186	Rudrawadi local	India
158	GC-11-39	India	187	JKM-197	India
159	ICP-12301	India	188	CORG-2004-01	India
160	BRG-7-4	India	189	PJT-501	India
161	GRG-2-95	India	190	GRG-801	India
162	ICP-12674	India	191	Bennur local	India
163	ICP-4275	India	192	GRG-206	India
164	ICP-12282	India			
165	ICP-13270	India			
166	BRG-7-6	India			
167	DM-158	India			

Sl. No.	Checks	Source/Country of origin
1	BSMR-736	India
2	MARUTI	India
3	ASHA	India
4	GS-1	India

#### 3.1.2.4 Number of pods per plant

The average number of pods present on the main branches in five randomly selected clusters in each plant was recorded at the time of harvest.

#### 3.1.2.5 Pod length

Pod length in centimeters was obtained by averaging length of randomly selected pods per plant at the time of harvest.

#### 3.1.2.6 Number of seeds per pod

Number of seeds per pod was obtained as average number of seeds in five randomly selected pods per plant.

#### 3.1.2.7 Test weight

Weight of randomly selected 100 seeds in grams was recorded as test weight.

#### 3.1.2.8 Seed yield per plant

Weight of seed from each plant was recorded at the time of harvest.

#### 3.1.2.9 Straw yield per plant

The weight of the straw per plant after sun drying to a constant weight was recorded in grams.

#### 3.1.2.10 Specific Gravity

Ratio of test weight to the volume of 100 seeds was worked out and recommended as specific gravity.

#### 3.1.2.11 Harvest Index

Harvest Index was computed as suggested by Donald (1962);

$$\text{Harvest Index} = \frac{\text{Economic Yield}}{\text{Biological Yield}} \times 100$$

#### 3.1.2.12 Days to maturity

Number of days from sowing to physiological maturity of the plant was observed and recorded in number of days.

#### 3.1.2.13 Duration of flower initiation to maturity

Number of days from the day of initiation of flower to physiological maturity was observed and recorded in number of days.

#### 3.1.2.14 Pest load

##### Pod borer (*Helicoverpa armigera*)

Pest observation was taken at the maturity stage because *Helicoverpa* was noticed after pod set. Larval count on 10 randomly selected plants in each plot was taken. When pest incidence crossed ETL (Economic Threshold Level), *i.e.* three larvae per plant, protection measures were taken by spraying Avaunt @ 0.3 ml/lit.

##### Spotted pod borer (*Maruca vitrata*)

Pigeonpea genotypes were evaluated for the incidence of spotted pod borer. Pest incidence was noticed in the initial period (one month old plant). The larvae feed by remaining inside the webbed mass of leaves, flowers and pods. Observations were recorded for crop damage from ten randomly selected plants from each plot. The plant protection measures were taken up when pest incidence crossed ETL, *i.e.* at 2 to 3 larvae per plant.

### 3.1.3 Statistical Analyses for Quantitative characters

Five plants were selected randomly and data on individual mean for each trait was subjected to statistical analysis. The data was analysed using INDOSTAT software programme. For the analysis of the data the following statistical methods were employed.

### 3.1.3.1 Analysis of variance (ANOVA)

The data on mean values for all the characters were analyzed for the variance following augmented analysis as per the outline presented below.

Source of variation	Degrees of freedom	Sum of squares	M.S.S	“F” ratio
Blocks	(b-1)	bSS	bMSS	bMSS/EMS
Entries	(e-1)	eSS	eMSS	eMSS /EMS
Checks	(c-1)	cSS	cMSS	cMSS/EMS
Varieties	(v-1)	vSS	vMSS	vMSS/EMS
Checks vs. Varieties	1	cvSS	cvMSS	cvMSS/EMS
Error	(c-1)(b-1)	ESS	EMSS	
Total	N-1	TSS		

Where,

b = Number of blocks.

v = Number of genotypes

e = number of entries.

c = number of checks.

The significance of “F” value was tested by comparing the computed value with the table value as given by Fisher. Further, standard error of means (S. Em±), critical difference (C.D) and coefficient of variation (C.V) were worked out using appropriate formulae to facilitate the comparison of means of the genotypes.

### 3.1.3.2 Estimation of Genetic parameters

In order to identify and ascertain the genetic variability among the genotypes and also to confirm the presence of environmental effect on various characteristics of genotypes, different genetic parameters were estimated by adopting appropriate formulae.

#### 3.1.3.2.1 Estimation of variance component

Genotypic and phenotypic components of variance were estimated by the following formulae.

$$\text{MSS (treatment) – MSS (error)}$$

$$\text{Genotypic variance } (\sigma^2g) = \frac{\text{MSS (treatment) – MSS (error)}}{\text{Number of replications}}$$

$$\text{Number of replications}$$

$$\text{Phenotypic variance } (\sigma^2p) = \sigma^2g + \text{MSS (error)}$$

#### 3.1.3.2.2 Co-efficient of variability

Both genotypic and phenotypic coefficients were computed for each character as per the method suggested by Burton and Davane (1953).

$$\sigma g$$

$$\text{Genotypic coefficient of variability (GCV)} = \frac{\sigma g}{\bar{X}} \times 100$$

$$\sigma p$$

$$\text{Phenotypic coefficient of variability (PCV)} = \frac{\sigma p}{\bar{X}} \times 100$$

Where,

$\sigma_g$  = Genotypic standard deviation

$\sigma_p$  = Phenotypic standard deviation

$\bar{X}$  = General mean of character

GCV and PCV values were categorized as low, moderate and high values as suggested by Sivasubramanian and Menon (1973), which is as below.

1-10 per cent : Low

10.1-20 per cent : Moderate

20.1 per cent and above : High

### 3.1.3.2.3 Heritability ( $h^2$ )

Heritability in broad sense was computed as the ratio of the total phenotypic variance as suggested by Hanson *et al.* (1956) and expressed as percentage.

$$\text{Heritability } (h^2) = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

Where,

$\sigma^2_g$  = Genotypic variance

$\sigma^2_p$  = Phenotypic variance

The heritability percentage was low, moderate and high as given by Robinson *et al.* (1949).

0-30 per cent : Low

30.1-60 per cent : Moderate

60.1 per cent and above : High

### 3.1.3.2.4 Genetic advance (GA)

Genetic advance was estimated by using the formula given Johnson *et al.* (1955).

$$GA = h^2 k \sigma_p$$

Where,

$h^2$  = Heritability in broad sense

$k$  = Selection differential which is equal to 2.06 at 5 per cent intensity of selection (Lush, 1949)

$\sigma_p$  = Phenotypic standard deviation

### 3.1.3.2.5 Genetic advance as per cent of mean (GAM)

$$GAM = \frac{GA}{\bar{X}} \times 100$$

Where,

GA = Genetic advance

$\bar{X}$  = General mean of the character

Genetic advance as per cent mean was categorized as low, moderate and high as given by Johnson *et al.* (1955).

It is as follows,

0-10 per cent	: Low
10.1-20 per cent	: Moderate
20.1 per cent and above	: High

### 3.1.4 Correlation analysis

The correlation coefficients were worked out to determine the degree of association of a character with yield and also among the yield components.

Genotypic and phenotypic correlations were computed by using the formula given by Weber and Morthy (1952).

$$r = \frac{\text{Cov}(xy)_p}{\sigma_{p_x} \times \sigma_{p_y}} \times 100$$

$$r = \frac{\text{Cov}(xy)_g}{\sigma_{g_x} \times \sigma_{g_y}} \times 100$$

Where,

r = Correlation coefficient

Cov (xy) = Covariance between the characters 'x' and 'y'

$\sigma_{p_x}$  and  $\sigma_{p_y}$  = Phenotypic variance of the character 'x' and 'y' respectively

$\sigma_{g_x}$  and  $\sigma_{g_y}$  = Genotypic variance of the character 'x' and 'y' respectively

Correlation coefficients were compared against 'r' values given in Fisher and Yates (1963) Table at (n-2) degrees of freedom at the probability levels of 0.05 and 0.01 to test their significance.

### 3.1.5 Path analysis

Path analysis was carried out by using both phenotypic and genotypic correlation coefficients to know the direct and indirect effects of the components on yield as suggested by Wright (1921) and illustrated by Dewey and Lu (1959).

Path coefficients were obtained by solving the simultaneous equations, which express the basic relationship between correlations and path coefficients. The equations are as follows:

$$r_{1y} = a + r_{12}b + r_{13}c + \dots + r_{1l}l_i$$

$$r_{2y} = r_{21}a + b + r_{23}c + \dots + r_{2l}l_i$$

$$r_{3y} = r_{31}a + r_{32}b + c + \dots + r_{3l}l_i$$

$$r_{ly} = r_{l1}a + r_{l2}b + r_{l3}c + \dots + l_i$$

Where,

$r_{1y}$  to  $l_{1y}$  = Coefficient of correlation between causal factors 1 to l with dependent characters y.

$r_{12}$  to  $r_{l1}$  = Coefficient of correlation among causal factors.

a, b, c...l = Direct effects of characters 'a' to 'l' on the dependent characters 'y'

Residual effect is computed as follows,

$$\text{Residual effect (R)} = 1 - \sqrt{a^2 + b^2 + c^2 + \dots + l_i^2 + 2abr_{12} + 2acr_{13} + \dots}$$

### 3.1.6 Genetic diversity analysis

Multivariate analysis using  $D^2$  statistics

Mahalanobis (1936)  $D^2$  - statistic was used for assessing of the genetic divergence between genotypes.

The generalized distance between any two populations is defined as,

$$D^2 = Y_{ij} \beta_i \beta_j$$

Where,

$Y_{ij}$  = The reciprocal matrix to the common dispersion matrix

$\beta_i$  = The difference between the two mean values of the two populations for  $i^{\text{th}}$  character ( $\mu_{i1} - \mu_{i2}$ )

$\beta_j$  = The difference between the mean values of the two populations for the  $j^{\text{th}}$  character ( $\mu_{j1} - \mu_{j2}$ )

$\mu$  = Vector mean values for all the characters

The formula for the estimation of distance,  $D^2$  from samples

$$D^2 p = d_1 (S-1) d$$

Where,

$D^2 p$  = Square of the distance considering P values.

$$d_1 = (X_{i1} - X_{i2})$$

X = Vector for mean values of all the characters

S - 1 = inverse of variance covariance matrix

Formula for computation of D values, which requires inversion of the matrix, becomes complicated especially when the numbers of variables under consideration are large.

Therefore, the original correlated un-standardized variables ( $X_i$ ) were transformed to standardized uncorrelated variables ( $Y_i$ ) so that the computation of  $D^2$  values reduce to simple summation of squares of the differences between values of transformed variables of the two population *i.e.*,  $D^2_i$ .

From the newly transformed uncorrelated variables, the square of the distance was computed using the following formula,

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

Where,

$Y_{i1}$  = Vector of transformed mean values, for first genotype

$Y_{i2}$  = vector of transformed mean values, for second genotype

The square root of the  $D^2$  values gives the generalized distance (D) between the two populations. The  $D^2$  values were arranged in a matrix form. The significance of  $D^2$  values between any populations was tested using the following formula

$$F = \frac{(n_1 + n_2 - p - 1) (n_1 n_2) D^2}{(n_1 + n_2 - 2) P (n_1 + n_2)}$$

This computed F values was compared with table F value at 5 per cent and 1 per cent levels of significance with P (number of characters) and  $(n_1 + n_2 - p - 1)$  degrees of freedom.

### 3.2 Screening of selected genotypes for fusarium wilt and sterility mosaic disease using markers

#### 3.2.1 *Fusarium* wilt

The number of dead plants due to fusarium wilt were counted among the total number of plants present per genotype and the per cent disease was estimated.

Recoding observations

The checks were sown in alternate rows. Per cent mortality was determined as the ratio of dead /or wilted plants at maturity divided by germination count.

$$\text{Per cent disease incidence} = \frac{\text{Number of plants Infected in a Row}}{\text{Total Number of plants in a Row}} \times 100$$

Disease rating was done on a 1-9 scale (Nene *et al.*, 1981).

Scale	Description	Category
1	Genotypes exhibiting <1 per cent infection.	Resistant
3	1-10 per cent infection.	Moderately resistant
5	11-20 per cent infection.	Moderately susceptible
7	21-50 per cent infection.	Susceptible
9	50-100 per cent infection.	Highly susceptible

#### 3.2.2 Sterility mosaic disease

Experimental layout for screening of sterility mosaic disease was laid out at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. Screening of SMD was done by following the standard method "Leaf stapling technique" (Nene and Reddy 1977) during *kharif* 2012-13. For infection of SMD from diseased plants to the test seedlings, mite infected leaves were collected from the SMD infected plants and stapled at 2-3 leaf stage of plants to be screened. As the stapled leaflets from the infected plants gets dried, mites from the infected leaves migrate to healthy leaf and inoculates the virus. After transmission of virus from the infected plants to healthy seedlings, seedlings were scored for SMD incidence at 15 days interval up to 75 days. To enhance the viral transmission, infector rows of Maruti variety (HS) was sown intermittently and also around the experimental block. The plant considered as 'susceptible' which showed mosaic symptoms while the other will be considered as 'resistant'.

Recording observation

The magnitude of the occurrence of disease was taken at later stages of disease development. The number of diseased plants and the total number of plants in each line was counted.

$$\text{Per cent disease incidence} = \frac{\text{Number of plants infected in a row}}{\text{Total number of plants in a row}} \times 100$$

The genotypes were later grouped into different categories on a 1-9 scale as given by Mayee and Datar (1986).

Disease scale used for scoring:

Scale	Description	Category
1	Genotypes exhibiting <1 per cent infection.	Resistant
3	1-10 per cent infection.	Moderately resistant
5	11-20 per cent infection.	Moderately susceptible
7	21-50 per cent infection.	Susceptible
9	50-100 per cent infection.	Highly susceptible



ICP 8863



SMD symptom



PJT-501

Plate 2. Sterility mosaic disease

**Table 2: Resistant and susceptible pigeonpea (*C. cajan*) accessions considered for molecular characterization of sterility mosaic disease based on per cent disease incidence**

Resistance		Susceptible	
Sl. No.	Genotype	Sl. No.	Genotype
1	ICP772	1	ICP7260
2	ICP995	2	ICP4392
3	ICP1071	3	ICP10397
4	ICP1126	4	ICP11946
5	ICP1273	5	ICP13575
6	ICP6859	6	ICP15403
7	ICP6929	7	ICP6123
8	ICP7076	8	ICP6815
9	ICP7148	9	ICP8921
10	ICP7803	10	ICP11690
11	ICP11059	11	ICP12515
12	ICP11230	12	ICP12680
13	ICP11281	13	ICP14094
14	ICP11627	14	ICP14229
15	ICP11910	15	ICP14900
16	ICP13662	16	ICP15382
17	ICP13270	17	ICP8863
18	ICP14116	18	ICP7314
19	ICP14569	19	ICP8384
20	ICP14155	20	ICP8757
21	ICP7	21	ICP9691
22	ICP655	22	ICP10503
23	ICP7057	23	ICP11320
24	ICP10654	24	ICP12298
25	ICP14444	25	ICP14368

**Table 3: Resistant and susceptible pigeonpea (*C. cajan*) accessions considered for molecular characterization of fusarium wilt based on per cent disease incidence**

Resistance		Susceptible	
Sl. No.	Genotypes	Sl. No.	Genotypes
1	ICP348	1	ICP655
2	ICP1071	2	ICP772
3	ICP2577	3	ICP995
4	ICP4575	4	ICP1126
5	ICP6815	5	ICP1156
6	ICP6859	6	ICP3049
7	ICP7057	7	ICP4167
8	ICP8860	8	ICP4392
9	ICP9750	9	ICP6128
10	ICP11230	10	ICP7148
11	ICP11281	11	ICP7223
12	ICP11690	12	ICP8255
13	ICP11823	13	ICP9414
14	ICP12680	14	ICP9691
15	ICP13304	15	ICP10503
16	ICP13633	16	ICP10559
17	ICP13662	17	ICP10654
18	ICP14444	18	ICP11059
19	ICP14569	19	ICP13579
20	ICp14545	20	ICP14368
21	ICP14638	21	ICP14801
22	ICP14701	22	ICP16309
23	ICP14722	23	ICP12123
24	ICP14832	24	ICP7318

### 3.3 Genetic diversity studies using SSR markers

The laboratory work on molecular diversity of pigeonpea was carried out at the research laboratory, Department of Genetics and Plant Breeding, UAS, Dharwad. In the present study 150 SSR primers (Table 1) available in public domain (Saxena *et al.*, 2010a; Saxena *et al.*, 2010b and Metkar *et al.*, 2010) were used to screen the selected set of genotypes of pigeonpea. The list of the primers used in the present investigation and the respective T<sub>m</sub> values along with the expected product size are provided in Table 4.

#### 3.3.1 Plant material

The material for the study comprised of 25 SMD resistance and 25 susceptible pigeonpea genotypes (Table 2), 24 Fusarium wilt resistance and 24 susceptible pigeonpea genotypes (Table 3). Also 23 high protein content and 23 low protein content genotypes (Table 4) were considered for molecular characterization.

#### 3.3.2 Buffers and solutions used for DNA extraction

##### I. 1M TRIS HCl pH 8.0

1. 121.1 g of TRIS [Tris-(Hydroxymethyl) Aminomethane]
2. Dissolve in about 650 ml of millipore water
3. Bring pH down to 8.0 by adding concentrated HCl
4. Bring total volume to 1 liter with millipore water
5. Sterilize/autoclave the stock solution

##### II. 0.5M EDTA

1. 186.12 g of EDTA [Ethylene Diamine Tetra Acetic acid]
2. Add about 650 ml of millipore water
3. Adjust the pH to 8.0 by adding 16-18 g of NaOH pellet. (EDTA will not be dissolve until the pH is near 8.0)
4. Bring total volume to 1 liter with millipore water
5. Sterilize/autoclave the stock solution

##### III. 5M NaCl

1. 292.2 g of NaCl (Sodium Chloride)
2. Dissolve in about 650 ml of millipore water (Note: do not add NaCl all at once because it will never go into solution)
3. Bring the total volume to 1 liter with millipore water
4. Sterilize/autoclave the stock solution

##### IV. TE buffer

1. 10 ml of 1M TRIS HCL pH 8.0
2. 2 ml of 0.5M EDTA
3. Bring the total volume to 1 liter with millipore water

##### V. DNA Extraction CTAB working buffer

1. 100 ml of 1M TRIS HCL pH 8.0
2. 40 ml of 0.5M EDTA
3. 280 ml of 5M NaCl
4. 20-25 g of CTAB (N, N, N, N-Cetyl Trimethyl Ammonium Bromide)
5. Make up the volume to 1 liter with sterilized millipore water



**Plate 3. *Fusarium* wilt affected plants**

**Table 4: Selected pigeonpea accessions for molecular characterization of high and low protein content genotypes**

High protein content			Low protein content		
Sl. No.	Genotypes	Protein content (%)	Sl. No.	Genotypes	Protein content (%)
1	Maruti	22.00	1	ICP12266	12.25
2	10397	20.13	2	2678	12.08
3	BSMR-736	20.00	3	2746	13.61
4	11627	20.13	4	3049	14.00
5	11690	19.60	5	6668	13.13
6	11946	19.25	6	6992	14.00
7	13662	21.00	7	7148	13.83
8	4575	21.00	8	7221	12.25
9	1730	21.53	9	7260	14.00
10	4019	20.30	10	8255	7.53
11	BRG-9-2	19.60	11	10654	13.83
12	5641	19.25	12	11059	13.48
13	DM-155	19.60	13	11910	14.00
14	GC-11-39	19.25	14	12142	14.00
15	Jamadhar Local	21.53	15	12515	13.13
16	WRP-1	21.53	16	13244	14.00
17	PUSA-92	19.25	17	13579	13.13
18	PG-12	21.53	18	PIJ-501	14.00
19	CORG-9701	20.48	19	14368	13.13
20	CORG-206	22.05	20	14801	13.83
21	ICPB 2091	20.13	21	14976	14.00
22	Chaple	19.78	22	Ulliyal Local	14.00
23	Gullal white	19.43	23	Gullyal Red	13.13

### 3.3.3 DNA extraction protocol

Total genomic DNA was extracted from fully expanded first trifoliate leaves by following CTAB method as per Murray and Thompson (1980) with slight modifications.

1. About 2 gram of leaves were taken and ground with liquid nitrogen to fine powder.
2. Then add 5 ml pre-heated extraction buffer and 50  $\mu$ l of  $\beta$ - mercaptaethanol.
3. Then contents were shaken briefly and the tubes were placed on hot water bath at 65  $^{\circ}$ C for 15 minutes.
4. Then the contents of the tube were shaken gently and equal volume of 24:1 chloroform/isoamyl alcohol was added and centrifuged at 13000 rpm for 15 minutes.
5. The upper aqueous phase (aliquot) was taken into fresh labeled tube and equal volume of 24:1 chloroform: isoamyl alcohol was added and centrifuged at 13000 rpm for 15 minutes.
6. The upper aqueous phase was taken into fresh labeled tube and adds 1/10th of 5M NaCl + equal volume of 100 per cent Isopropanol and kept at -20 $^{\circ}$ C for 2 hr, then spin at 13,000 rpm for 15 minutes.
7. Decant the supernatant and add 500  $\mu$ l of 70 per cent chilled ethanol and spin at 13000 rpm for 3 minutes.
8. Discard the supernatant and air dry the pellets.
9. Finally 50-100  $\mu$ l of T<sub>10</sub> E<sub>0.1</sub> buffer was added to dissolve DNA pellets.

### 3.3.4 Quantification of genomic DNA

The DNA was quantified by electrophoresis on 0.8 per cent Agarose gel using 1X TBE electrode buffer and Ethidium Bromide (5  $\mu$ l /100 ml of Agarose) as staining chemical. Then 2  $\mu$ l of DNA sample from each genotype was mixed with 2  $\mu$ l of loading dye (50 per cent sucrose + 5  $\mu$ l of bromophenol blue and xylene cyanone) and loaded in to the gel. 100 bp ladder (50 ng/ $\mu$ l) was also loaded as reference to quantify the DNA. The electrophoresis was carried out at 70 volts for one hour. The gel was viewed under UV trans-illuminator (Cambridge gel documentation system) to ascertain the quality of DNA.

### 3.3.5 Simple Sequence Repeats (SSR) analysis

SSR analysis was carried out for the molecular studies on fusarium wilt (FW), sterility mosaic disease (SMD) and protein content of genotypes of pigeonpea and characterize the selected set of genotypes of pigeonpea. The genomic DNA sample obtained from selected genotypes was diluted using T<sub>10</sub>E<sub>1</sub> buffer for the required concentration of 15 ng/  $\mu$ l and used to screen by employing 150 SSR primers.

#### 3.3.5.1 SSR reaction mixture

Polymerase Chain Reactions (PCR) were performed by using Touch - Down PCR. Then DNA was amplified in 5  $\mu$ l reaction mixture using mastercycler (model eppendorf gradient PCR system 9700). The cocktail of reaction mixture is given below.

SSR reaction mixture 1 X ( $\mu$ l), Template DNA 1 $\mu$ l (5 ng), Primer mix 0.25  $\mu$ l each of forward and reverse (2pmols), dNTP mix 0.5 $\mu$ l (2mM), 0.7 M *Taq* buffer (10X), *Taq* polymerase 0.02  $\mu$ l (5U/ $\mu$ l) (Bangalore Genei), Sterile water 2.53  $\mu$ l.

#### 3.3.5.2 Amplification programme

A Touch - Down PCR programme was used to amplify the DNA fragments. The primers used in the study were varied with the annealing temperature. Hence, three different touch-down annealing temperatures *viz.*, 55-45 $^{\circ}$ C, 60-55 $^{\circ}$ C and 65-60 $^{\circ}$ C were used for PCR amplification.

Amplification was carried out in 5  $\mu$ l reaction mixture containing 1  $\mu$ l (5 ng/ $\mu$ l) of DNA, 0.675  $\mu$ l (10X) *Taq* Buffer, 0.25  $\mu$ l (2 mM) dNTP's, 0.25  $\mu$ l each of forward and reverse primer (2 pmols), 0.075  $\mu$ l (5 units) of *Taq* polymerase (Bangalore Genei) and 3.00  $\mu$ l of double distilled water using an Eppendorf Thermo cyclor. PCR was processed with the following program: a first cycle of pre-denaturation at 95 $^{\circ}$ C for 3 min, followed by 35 cycles of denaturation for 20 sec at 94 $^{\circ}$ C, 20 sec at the

annealing temperature of 60°C, followed by 30 sec extension at 72°C and a final extension of 20 min at 72°C.

#### 3.3.5.3 Gel electrophoresis

The amplified PCR products were fractionated on agarose gel to detect polymorphism among the selected genotypes.

##### Agarose gel electrophoresis

The PCR amplified products were fractionated on 3.0 per cent agarose horizontal gel electrophoresis. The gel was prepared in 1 x TBE buffer. The solution was added with Ethidium bromide (5 µl/100 ml). Then the gel was poured into the gel casting platform after placing the comb in the trough. While pouring, sufficient care was taken to ensure that there are no air bubbles trapped inside the gel. The comb was removed after the gel solidification. Then the solidified gel was transferred into the electrophoresis unit containing sufficient buffer so as to cover the wells completely. The amplified PCR products of 2 µl volume with 2 µl of loading dye were carefully transferred into the wells. The electrophoresis conditions were held at 100 V for 4 h at room temperature. After the completion of electrophoresis, the DNA profile was documented using UV transilluminator (Cambridge gel documentation system)

##### Recording of the observations

The products of PCR were scored visually by comparing with the standard marker of size 100 bp (Bangalore genie). The amplicons obtained with regard to the expected size were scored by denoting '0' (absent) and '1' (present), each of which was treated as an independent character regardless of its intensity, thus producing a binary data matrix. The binary data were thus obtained was used as an input for further estimations.

#### 3.3.5.5 Statistical Analyses

Allelic variation was calculated from the frequencies of genotypes at each locus as the polymorphic information content (PIC). Genetic parameters namely major allele frequency, genotype frequency and Polymorphic information content (PIC) were estimated using the software program Power Marker version 3.25 (Liu and Muse, 2005). Phylogenetic trees were constructed using the Neighbourhood-joining algorithm using the program DARwin 5.0 (Perrier *et al.*, 2003).

**Table 5: List of primers used in the present investigation**

SI No.	Marker	Tandem repeats	Sequence	Product Size (bp)
1	CcM0008F	(TA)19	CGGTGAAAAGGGTCAATGAG	182
	CcM0008R		CAAATTAAGCCTACTTATTTTACGA	
2	CcM0021F	(TTA)10	TGAATGTTTTCCAGGATTTTACA	280
	CcM0021R		GCGCAAATATAAGAGCCAG	
3	CcM0030F	(AAT)8	GCAATATCAATTCATGGTGGGA	218
	CcM0030R		TGACAGATGCACTCTCTCGTTT	
4	CcM0039F	(AT)37	AGGAATAATGTTTGCTGCGG	261
	CcM0039R		TTGGTATGTGGAACGATTGC	
5	CcM0047F	(A)12n (TC)5	TGTCTTTTGGATGAAAGTAGGGA	160
	CcM0047R		GTTGGGGATGGGAAGAGAAT	
6	CcM0051F	(AT)8	ACCTTTATTTTGAGCAGGAAAA	269
	CcM0051R		TGAATCATTTTCTGTTGAAGGG	
7	CcM0057F	(AAAT)6	CAATGTTGGCATAGGAACCA	269
	CcM0057R		GCTTAAACTTGTGGGGCAA	
8	CcM0080F	(AT)13 (AG)11n (AG)8	TGCATGTTGTATGTTGGTTGG	173
	CcM0080R		GTAATGCATCTCAATAATTTCAACA	
9	CcM0082F	(AT)16n (TA)7	TGTCAATGTCATGTTGGGCT	245
	CcM0082R		CATGCCATCTTCCTTTCTCC	
10	CcM0093F	(AT)27	TCATTGACCCCTCTGGAAAT	268
	CcM0093R		ACAATTGAAAAATAAGTGAGTGAT	
11	CcM0095F	(TC)9	ATAATTAGTGGGCTGGGCCT	208
	CcM0095R		TGCCTCATAATCATGTTGCTTC	
12	CcM0112F	(TA)7	GCAGGCATGCAAGCTTATTA	148
	CcM0112R		GCGCGCCATCTTAATATCAT	
13	CcM0121F	(TA)17	AGAAATTGGAGGCTTGGTCA	273
	CcM0121R		GGTATAAGGCTCAAACCCGA	
14	CcM0126F	(TAT)21	TGGTCCATGTTTCTCACTCA	218
	CcM0126R		CCAATGAAAATGAGAACCTTCA	
15	CcM0133F	(TA)9	GTTGTCCCATTTTGACCTCC	176
	CcM0133R		CCATAATCCAATCCAAATCCA	
16	CcM0134F	(AT)12	CTCTGCCCGATGTCATATT	240
	CcM0134R		TTGGGATGTGAAGATGATGAA	
17	CcM0137F	(ATA)8	GGGATGATCCAAAAACGAGA	225
	CcM0137R		TCACCGATGACTTGTTCAT	
18	CcM0173F	(TAAA)6	TTGTCAATGGGACCTCAACA	278
	CcM0173R		CTATCCCATGCCATCAACCT	
19	CcM0176F	(GA)10	GAAATTAAGAAGCGGCGATG	235
	CcM0176R		AACCTTATTGCCACGCAAAC	

Contd....

SI No.	Marker	Tandem repeats	Sequence	Product Size (bp)
20	CcM0179F	(TTA)6 (TA)17	GCAAAATTGCACTAAAATTTGTTT	189
	CcM0179R		CCATCTTCGCCTGTCGTATT	
21	CcM0181F	(G)13n (TG)5	TAGTTCACCGCCTGTCCTTC	278
	CcM0181R		TGCAGACGATAAACATTCCGG	
22	CcM0183F	(AT)10n (TA)8	GCCCATTTTGTTCATCCCTAA	236
	CcM0183R		TTCAACAGTTGGATCGTTCA	
23	CcM0185F	(TA)8	TTGATCATGACTTATGCCTTTGA	232
	CcM0185R		GGCTTGCTTTGAGTTCCTTG	
24	CcM0193F	(TA)20	TAAATCACCACCCTTGAGGC	190
	CcM0193R		TGCAAAAACACATCCTGGAA	
25	CcM0195F	(AT)11	CAACAATAAAGCATAAACCACCA	223
	CcM0195R		TGACGTAGATTGGGTAGTTAGGA	
26	CcM0207F	(TA)15	TTTTGGCGGTCATTTTAACC	235
	CcM0207R		TTAGTCGGGAGCAACTGA	
27	CcM0208F	(TAT)10n (TTA)7	GCATCTAAATACAATTAATATTGTGGG	122
	CcM0208R		ATAGGGTGGATCTCTGGTGC	
28	CcM0246F	(AT)16	ATGGAGCCAAAGTGTCCAAG	226
	CcM0246R		ATGAAAAGCAACTACGCGCT	
29	CcM0248F	(AT)6	TGGAATTTGACTCATTTAGAATAGGA	279
	CcM0248R		CCCACAGACAGCATATCAACA	
30	CcM0252F	(AT)23	CATAGAAGCCCACCTTCCAA	234
	CcM0252R		CTGCATGCAAAACGAAGAAG	
31	CcM0257F	(AG)7 (TG)15	GCCGTTACGAGGGTAATGAA	241
	CcM0257R		CTGTCTCAAAGGGACCCTGA	
32	CcM0268F	(AT)24 (GT)10	CCTTTTGGGTTAGGGTATCCA	210
	CcM0268R		CCCCTAACGTAGCCTGTCAA	
33	CcM0271F	(A)23 (AT)25	TGCTTCGCATTCTCTTTTT	268
	CcM0271R		AGGAAAATGCTGCTTTGCAC	
34	CcM0273F	(CTT)5	CAGGTATACGCTGCTCACCA	264
	CcM0273R		GCAGTTGCTGATGGAGTTGA	
35	CcM0293F	(TA)11	GTTCCCGGCTACCAAATGTA	231
	CcM0293R		AAAAACACAAAAGATAAACATACATGC	
36	CcM0303F	(TA)7nt (GC)6	CAAGCTTTTGTAGGTTGACA	205
	CcM0303R		TCACGCAAGAAATTCACAGC	
37	CcM0306F	(AAT)5	TCATTGTCTCTTTCTTTTCCATTT	280
	CcM0306R		GGCATGCTAACATGGCATT	
38	CcM0317F	(AT)26	GGGTTTGTAGACTATTGGAGGG	171
	CcM0317R		ATGAAATTGGGCGTGAAGAG	
39	CcM0322F	(AT)21	AAAGACCACGATATGGGCAA	276
	CcM0322R		GAGTTGAACTTTTATTTTCATCGAAT	

Contd....

SI No.	Marker	Tandem repeats	Sequence	Product Size (bp)
40	CcM0353F	(AT)14	GATTCGCAAGTTGCTCCTTC	189
	CcM0353R		TTGTGATCACTTATCATCATTTTGG	
41	CcM0361F	(TA)9	TCTTCCTGTCCTCATCCTCG	172
	CcM0361R		TGGAACCAAAGTTGTGCAT	
42	CcM0366F	(AT)8	CCTATGCTCCACAACAAGAAAA	170
	CcM0366R		GCGATAGAAACATATTGCACACA	
43	CcM0374F	(TA)11	GAACCGTCTTAAAATTTCTCATTT	161
	CcM0374R		CAATGGCACATTGTCAAAAA	
44	CcM0381F	(TA)21	CGATCCCTGCTTCAAATCAT	267
	CcM0381R		GGTTCAAGCGATGCACTACA	
45	CcM0392F	(AT)7	TGCTCCCTAATTTGAGACATTG	143
	CcM0392R		CAACATGAATCCATCATCATCA	
46	CcM0399F	(TA)16	AATTATAAACGGTTGAATTGAAAAA	247
	CcM0399R		TCGGCCTAGTCAGTCACCAT	
47	CcM0402F	(TGA)6n (AG)5	CAGCATTTGAAGGAGAAGCC	179
	CcM0402R		GCAGATCCCTAACTCCTCCC	
48	CcM0407F	(TA)7 (TG)14	TCAAGTGGTTGGGCCTTTAG	190
	CcM0407R		AAAGAAAATTCAAATAAATGGATACC	
49	CcM0413F	(T)10 (CT)6	ATGCACTAAGCTTTGCCGTT	256
	CcM0413R		GGTTGAGCCTTCTTGTTGGA	
50	CcM0431F	(CATA)5 (AT)9	CCATAATCCAATCCAAATCCA	126
	CcM0431R		TCACTGTAACGCCATCGAAA	
51	CcM0438F	(ACA)5	TAGAGTCGACCTGCAGGCAT	234
	CcM0438R		ATCGATTTGCTCATGCACAC	
52	CcM0443F	(TA)17n (AT)5	TGACAAAATAATGCGGTCACA	261
	CcM0443R		CAAGCCAAAGTTTGTGTTGAACT	
53	CcM0444F	(TA)7	TGTCATGAGTGGCTGATCCT	184
	CcM0444R		TCAACCAAAATCCAAACCAA	
54	CcM0445F	(TC)5n (CT)5	AAAGAGACAAAGGAAAGTAGGGAA	231
	CcM0445R		TATGGAAGGGGAGAGAGAGG	
55	CcM0450F	(AT)7	GCCAAGTTGAGCTGAAAAG	156
	CcM0450R		GGTCGATTCATGTTTGGAGG	
56	CcM0468F	(TAT)5	ATAAAAATATCCGCAACCGC	183
	CcM0468R		CGAAAGCAATGTCAAAGCAA	
57	CcM0471F	(AT)12	AAAATTTTTATCCACCCACTAAAA	273
	CcM0471R		TTTATGGCATTAAATTGATTACACTTT	
58	CcM0476F	(AT)8	TGGCTTCTTGTTGTTGCTTG	237
	CcM0476R		AAAAATATGAGAGAGATCTCAACTGG	
59	CcM0484F	(T)12n (ATT)5n (AT)5	TGGAATTAACACCATGAAACA	248
	CcM0484R		TGCATGCTACCAAGGAATTG	

Contd....

SI No.	Marker	Tandem repeats	Sequence	Product Size (bp)
60	CcM0492F	(AT)21	AAAATTTACGAGCACTAAAATGAAAAA	271
	CcM0492R		TCAACAATAAATTGTCATATGTCTGG	
61	CcM0494F	(AT)21	ACGTGAAAAATCCGCAACTT	117
	CcM0494R		GCTTGTGTTTCAAATCCAACCTT	
62	CcM0502F	(TAA)19	CATCCTGCAAGACACGTGAA	240
	CcM0502R		TTGTACGACTTGCACCTTT	
63	CcM0516F	(TC)14	ATTGATGGTGTGTGGCAGA	191
	CcM0516R		TTCGTGACACTCACTGGTCC	
64	CcM0522F	(TA)17	TTGTCTGTGGGTTTCATGTGAG	190
	CcM0522R		AGAGGCACTCACAAATTCTCAA	
65	CcM0529F	(AT)7 (AG)5	TGATTTTTCTGTTTTGCCCTG	151
	CcM0529R		AAAAATATGATTGTTTGACGGTG	
66	CcM0537F	(CTC)5	TATACTCGAGCTCCGCCAAC	244
	CcM0537R		GATCCTGATAACCCTGCCAA	
67	CcM0542F	(AT)7	CCCAGTGAGCATTCAAAGGT	264
	CcM0542R		GGACCTACTGGTGGTAGGCA	
68	CcM0553F	(AG)6n (AG)11n (A)11	TTTCTCCAACCTCCACCATC	279
	CcM0553R		TTGGAAGCACTCCTAGCTTTG	
69	CcM0583F	(TA)21	AGTTGGAAGCGATTGGATAAA	245
	CcM0583R		ATCCCTAAAATAGGTTCGATTAGATT	
70	CcM0588F	(ATA)19	AAAACAATTATTGCGTAAGATTATCA	266
	CcM0588R		ACGTTAGGAGCAAAGCGTGT	
71	CcM0591F	(AT)20	TGGCATGCAAATATATCAATCA	222
	CcM0591R		CCTCTTGATCTTTCACACATGA	
72	CcM0594F	(GA)9n (TC)9	GGCTTGTTCTTTCTTGGTG	185
	CcM0594R		AAGTCCCTGACTTTCCCCAT	
73	CcM0596F	(A)13n (TA)6	GAAGTCATTGAATACAACATGCAA	235
	CcM0596R		TTGGGTGTTTAGGGATTGAGA	
74	CcM0601F	(TG)8	AGGAAGAAGCTCGTGAAGCA	279
	CcM0601R		AGACGGAACCACACTCGTT	
75	CcM0602F	(AT)14	TTTGCTCTATAACAAGGGATTCA	216
	CcM0602R		TGCTCTAATTCATGTCAAACCC	
76	CcM0603F	(A)21 (AG)9	TGAGAGAGGATGTGTGGTGC	232
	CcM0603R		GTTGCACACACTGGCAAATC	
77	CcM0611F	(TA)15	AAGTGATGTCCAAATACATGATGG	259
	CcM0611R		GACCCCATCACACTTTC	
78	CcM0613F	(TA)9	TCTCGCAATTCACAATCACA	227
	CcM0613R	(TAT)10n (ATT)12n	ACGACTCCCCAACTTTTC	
79	CcM0624F	(ATT)10n (TTA)5n (TTA)14	TTGAAAAGACAAATAGTGTGCCTC	263
	CcM0624R		ACCTATCTTCCCCTCGCTTT	

Contd....

SI No.	Marker	Tandem repeats	Sequence	Product Size (bp)
80	CcM0627F	(TC)10	AGCGAAAAGTTGCCCAAAC	278
	CcM0627R		ACGCCCTAAATTGGTACCCT	
81	CcM0635F	(AT)10	CAGTAGCAGCACCAGTCCAA	240
	CcM0635R		GAAGCAAAAATTTTACAAGAACCAA	
82	CcM0637F	(AT)11	CCATCCACGTGGGTATTCTC	160
	CcM0637R		GGAGGCGTGAATCTGAAAAA	
83	CcM0641F	(AAG)5	CTTGGAAAGGAGGAGGTTCC	172
	CcM0641R		GACTCCCCGTCAGGTCAATA	
84	CcM0657F	(TTC)6	ACCTCTTTTGTGGCTTGGTC	143
	CcM0657R		TCGTCCAATCTTGCTCTTGA	
85	CcM0673F	(AT)6 (AG)9	TGACCACCAACCATTACCAA	272
	CcM0673R		CATGCACCAGACCAGAATCA	
86	CcM0679F	(TA)17	CACCTTACAACATTCGCCCT	252
	CcM0679R		AAGACATCTCTCCTATTGAGCCC	
87	CcM0705F	(TA)8n (AG)6	TCTTCGTCTACACCCCTTGG	218
	CcM0705R		CGGTTGAATTGTTAAAATTTGATG	
88	CcM0710F	(TTA)15	TTTTATTAGGCATATCAAGCTATTTTT	276
	CcM0710R		AACAACAACCACAAAATAAGAGGA	
89	CcM0721F	(AT)19	ATCCAACCACGTGTTTCACA	169
	CcM0721R		TTTGAAATGGTATCGATGATTA	
90	CcM0724F	(ATT)10	AGTTTTCCAATATACCTCAAAGC	275
	CcM0724R		CAGTGC GGATTTGGATTTTT	
91	CcM0727F	(AT)9	TTGATTATATGGACATATGTGTTGTGA	237
	CcM0727R		GAAAAGGAGGTTTCATCAGTTCAA	
92	CcM0737F	(AT)17	TGTTGTTCCCAACTCTTCTTCA	173
	CcM0737R		ATAGCGGCTTATGGCAGATG	
93	CcM0743F	(AT)12	TCAAAAATGACTTTTCTATGACCG	251
	CcM0743R		CGAACAACCTCGCTCGTGTA	
94	CcM0752F	(AT)10	TGAAGCCGGGATATCAAAC	227
	CcM0752R		CATAGTACGCCAATTAGAATGTTCA	
95	CcM0754F	(ATA)6n (AT)5	AGTATCGGGGTACGCAATGA	144
	CcM0754R		CAGCCAACTAACAAACGGGT	
96	CcM0772F	(AT)15n (A)13	TCCAATTTAGAGTTTGGGAC	264
	CcM0772R		GTGGGTGTGGCTAGTGGAAAT	
97	CcM0775F	(ATT)10	GTGGGTTTTGCATTGTGATG	219
	CcM0775R		CAATCCCTCTCATTCTCCCA	
98	CcM0785F	(AT)9	GCATGTGTTTTACTTGAGTCGTC	277
	CcM0785R		TGGAGGCGATCTCTTTCTTG	
99	CcM0805F	(AT)12	CGACAATGGTGGGTTGAAAT	266
	CcM0805R		CATTTTCCTTGTGCAGACGA	
100	CcM0810F	(AT)19n (TA)6	TTTTTCCGGTCATTAACCATA	202
	CcM0810R		TTGGTCGGTCCTAAAACAGAA	

Contd....

Sl. No.	Markers	Sequence	Tm <sup>0</sup>
101	Cc1-F	CCTGTGACTCAACTCAATCTC	58.9
	Cc1-R	TGGAATACCGAATTAATGCTC	60.4
102	Cc2-F	GTCCAACATGACAATTACAAAA	58.9
	Cc2-R	CTACTTGATTGTCACAAATTTTC	57.0
103	Cc3-F	TGCTTATGGTAACATATGAAGT	55.4
	Cc3-R	AGGATATAATGGTCTAACAAATG	55.5
104	Cc4-F	TCAATCACCCCTAACATCTTCA	59.6
	Cc4-R	AACTCCAATACGATTTTCTCA	57.8
105	Cc5-F	GATAAGACCTTTTCACAAGCA	58.4
	Cc5-R	TGGGGGATAATTTACTTTGAC	59.2
106	Cc6-F	CAAGATAACGACCAAAAATGT	58.1
	Cc6-R	CGCTTATCGTTATGATTTTAC	58.7
107	Cc7-F	TGTACAAGTGCAAATAAAGCA	58.5
	Cc7-R	CTTCATAACCACCATCACCTA	58.9
108	Cc8-F	TTTTCATGAGAAGAAGCATACA	59.0
	Cc8-R	CCTTCTCTAACCCCTGAACCTA	58.9
109	Cc9-F	GGACTGTAAAAAGCTTTAGGAA	58.2
	Cc9-R	CAATCCTCATTGCATATACCT	58.0
110	Cc10-F	TTTTAACATCAAGGTTCCGTA	58.6
	Cc10-R	AATTCTTCTAAACATGCACCT	56.9
111	Cc11-F	ATTTTGGAGGATACAGCATT	59.0
	Cc11-R	ACCATCACGAGTATATCTCCA	58.5
112	Cc12-F	GGGGTCATGTTATTTTCTCTT	58.5
	Cc12-R	AGGTAGTTTGGAGGGTAATCT	57.2
113	Cc13-F	CAAACATGACCAACCATACT	58.8
	Cc13-R	GCAGATTTGATGCTCTACAGT	58.6
114	Cc14-F	TCCATCCAACATAACAATCTT	58.4
	Cc14-R	CATTTACGCTGATTGAATCTT	58.3
115	Cc15-F	ATTATTAAGGCACGAGGTGT	59.1
	Cc15-R	GTTATTGCACAAATCGGTATC	58.5
116	Cc16-F	ATAATTTGGGGTGAATTGTTT	59.1
	Cc16-R	AACTTGTGAAGGAATGTATGG	58.0
117	Cc17-F	TGAAACCCTTACATCAACATT	58.4
	Cc17-R	TCCTGTTCTGTCCAGAAGTTA	58.9
118	Cc18-F	CGTATCCAAAGAGAAGTGAAA	58.4
	Cc18-R	GAACCACCTAGGTACAACACA	59.0
119	Cc19-F	GCAACCACACTATTATTCCAC	58.4
	Cc19-R	CGGAAAGGTTAGTTCAAATTAT	57.8
120	Cc20-F	AATGTTCTATTGTTTTACGAGTG	56.6
	Cc20-R	AATTTCTCGTGTGATTGTGAT	58.5
121	Cc21-F	GCAGGTCAGTTGCTTAACATA	59.4
	Cc21-R	AAGGCCAGAAATAACTTCTTC	58.0
122	Cc22-F	TCACAAACACAACAACAAT	59.0
	Cc22-R	CTCTCTGCAGTTCTGGAATAC	58.1
123	Cc23-F	AAAGCTATGGAGCAATAGAAGA	58.7
	Cc23-R	TAATGGGTTGACCAGAAATTA	58.4
124	Cc24-F	GGAATGTGGAATTATAACCAA	57.8
	Cc24-R	GGTTGTTAAAACGAATTTTG	56.3
125	Cc25-F	AATGGTCATAGGGGATTAGAA	58.8
			59.2
	Cc25-R	GCAATGTCAATGTTGAGAACT	

Contd....

Sl. No.	Markers	Sequence	Tm <sup>0</sup>
126	Cc26-F	AACCTTCAAACACTTGCATAA	58.8
	Cc26-R	CCATGTTCTCCTCACTCAAATA	59.1
127	Cc27-F	CTTTGGGAATTTATACGAACA	58.1
	Cc27-R	TTTGTGTTAAACGATGTGAGA	58.3
128	Cc28-F	GATTTGAAACTCTCTATGTTGAA	56.6
	Cc28-R	TAACAACCTTCACCTTGCATA	59.7
129	Cc29-F	AGAACACACAAAATGTAAAAGG	57.3
	Cc29-R	CTAGTGTATGGCATCACCATC	59.5
130	Cc30-F	CTCCCGCAAATCATGTA	59.6
	Cc30-R	GATTGTCAAATGAAGAATGAGA	58.2
131	Cc31-F	ACATCTGAGATTTTTCACTCG	58.4
	Cc31-R	ATAGAGATTTGCAGCGTCTTT	59.6
132	Cc32-F	CTTTGCGTCTTCTCAGAATTA	58.7
	Cc32-R	GCATACATAGTTATCGAATCCA	58.1
133	Cc33-F	TGGAAAGTCACTCTTTTTAATAC	55.8
	Cc33-R	TCTCACTCATCTATTCACAATTT	56.9
134	Cc34-F	TCTTGAGTAAGTGAACATTCAA	58.0
	Cc34-R	GGTGAAACTCAACTCAACACT	58.3
135	Cc35-F	TGTGAGTCCAAACAGTAAACA	58.2
	Cc35-R	AAGAAAATTAGGCTGAGGTTG	58.9
136	Cc36-F	GCCCTTTATTTGGTAGCTTAG	58.5
	Cc36-R	TTAATTGCATTTTCACTCCTC	58.3
137	Cc37-F	TCAAACTCCTCAGGTAATAAAA	58.4
	Cc37-R	ACTGCATTTAGTTTTGGACAG	58.4
138	Cc38-F	CACGTATAACAAATTGAAACCA	58.9
	Cc38-R	GATGTGAGACAACGAGATGTT	59.2
139	Cc39-F	CCAAAACACAAAACCTTGTTAC	57.7
	Cc39-R	TATCTATGATCCACCCAGTCA	59.4
140	Cc40-F	GTCGCAAAAACACACTAATCT	58.4
	Cc40-R	GCATTACATCCCACAATTTAC	58.3
141	Cc41-F	GTACGATACATGGTTAAATTACA	54.9
	Cc41-R	AAAAATGGCCCTCTTAAAAA	59.1
142	Cc42-F	TTTCAATATGTTGCCTATATACC	56.4
	Cc42-R	CCAGATCCAAATTAAGAATG	57.7
143	Cc43-F	GGCTTTAGGTTAGTAGTCAATTT	56.4
	Cc43-R	GCATTCTTAATGAGTGTTTTGA	58.4
144	Cc44-F	ACTGAACCCAATAATGTTTGA	58.4
	Cc44-R	AAATGGGGGTACTCGTATTAG	58.4
145	Cc45-F	GGTGGAAGGAATCTTAACTT	57.7
	Cc45-R	ACTTTCCTTTGATGCTTTTTTC	58.9
146	Cc46-F	CATCGAGGTTGTTATTTTGT	58.1
	Cc46-R	TTTAAGTTGGTCCGTCGATA	59.6
147	Cc47-F	TGATGTGATTGGTAAACACTTT	58.5
	Cc47-R	GCTATTTGGTAACAACCTTTGG	57.8
148	Cc48-F	TCATGTCTATGCACTTAATCG	57.8
	Cc48-R	ACCATACCACGACTGTCATAC	58.8
149	Cc49-F	GATATTGCGAGCATATTTTTG	58.7
	Cc49-R	AATGGGAAAAGAGAAGGAAG	58.8
150	Cc50-F	AGTTTTTGTGTTTTCAACCTG	58.3
	Cc50-R	GAGCAAATAATCATTCAAACAC	57.7

# EXPERIMENTAL RESULTS

The data on genetic variability studies among 192 pigeonpea accessions, which included 124 accessions of mini core collection set obtained from International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh and 68 accessions collected from ARS Gulbarga was subjected to statistical analysis. The genotypes which were found resistant to Fusarium wilt and sterility mosaic disease (Jaggal *et al.*, 2012) along with susceptible entries were characterized by employing 150 SSR primers. A set of genotypes with high protein and low protein content genotypes (Thimmaraju. 2012) were also characterized using 150 SSR primers in order to enrich the list of primers for marker assisted selection. The results pertaining to characterization of the pigeonpea accessions in relation to fusarium wilt, sterility mosaic disease and seed protein content was also subjected to statistical analysis (DARwin 5.0 and Power marker Version 3.25). The results obtained are presented here under.

- 4.1 Analysis of variance
- 4.2 Variability studies
- 4.3 Evaluation of genotypes for their productivity
- 4.4 Correlation studies
- 4.5 Path coefficient analysis for seed yield
- 4.6 Genetic diversity
- 4.7 Evaluation of genotypes for *Fusarium* wilt reaction and *Helicoverpa armigera* incidence
- 4.8 Screening for sterility mosaic disease
- 4.9 Molecular characterization studies
- 4.10 Molecular identification of alleles

## 4.1 Analysis of variance

The analysis of variance for yield and yield attributing characters of 192 pigeonpea accessions is presented in Table 6. Out of 14 quantitative characters studied, 10 characters exhibited significant values for entries (ignoring Blocks) which indicated that the variability among the genotypes selected was adequate. As evident from the Table 6, traits *viz.* plant height (523.24), pod length (0.43), days to fifty per cent flowering (296.39), days to maturity (385.37), test weight (2.65), straw yield (2410.58) harvest index (404.19), seed yield per plant (286.69), specific gravity (0.03) grain yield per ha (8846.91) were found significant for entries (ignoring Blocks) while, characters such as number of primary branches per plant, days from flowering to maturity pod length and pods per plant, were found non-significant.

## 4.2 Variability studies

Assessment of variability was carried out for 192 pigeonpea genotypes. The mean values obtained for various traits included in the present study with respect to all the genotypes are provided in Appendix II. The variability parameters *viz.*, range, mean, phenotypic and genotypic variances, heritability estimates and the genetic advance as per cent of mean in respect of the characters considered in the present investigation are provided in Table 7 and depicted in Fig. 1 and 2. The results obtained in respect of these variability parameters are presented here under.

### 4.2.1 Days to fifty per cent flowering

The days to fifty per cent flowering ranged from 77 to 169 days with mean value of 123.39 days. The highest and lowest days to fifty per cent flowering were recorded for the accessions ICP13191 (169.00) and ICP14368 (77.00) respectively.

The days to fifty per cent flowering had a genotype variance of 262.04 and phenotypic variance of 276.52 with genotypic and phenotypic coefficient of variation of 13.10 and 13.46 per cent respectively. The heritability estimated was 94.76 per cent with genetic advance as per cent mean of 26.28 per cent for the trait considered.

**Table 6: ANOVA for yield and yield contributing traits of pigeonpea genotypes (*Cajanus cajan*)**

Source of variation	DF	Plant ht (cm)	Number of Primary branches	Pods per plant	Pod length (cm)	seeds per pod	Yield per plant (g)	Test weight (g)	Number of Days to 50% flowering	Number of days taken from flowering to maturity	Number of days to maturity	Harvest index (%)	Specific gravity (g)	straw yield (g)/plant	Yield (kg/ha)
Blocks (eliminating Checks +Var)	4	229.10	0.514	833.09	0.054	0.35	6.76	1.46	27.56	97.53	2.56	8.481	0.011	283.87	208.37
Entries (ignoring Blocks)	193	523.24**	0.383	1902.77	0.43	0.24*	286.69***	2.65*	296.39 ***	73.47	385.37***	404.19***	0.03**	2410.58*	8846.91***
Checks	3	162.285	0.110	5063.76*	0.11	0.03	2314.24***	0.16	210.98 ***	34.22	430.98***	3789.44***	0.03 *	8553.54**	71412.20***
Varieties	189	506.616**	0.389	1856.48	0.44	0.24*	205.73 ***	2.68*	298.70 ***	74.33	386.59***	330.16***	0.03 **	2318.25*	6348.70***
Checks vs. Varieties	1	4749.06***	0.081	1168.02	0.02	1.32**	9505.36***	3.88	116.01 *	28.23	18.24 ***	4240.81***	0.000	1434.68*	293312.20** *
Error	12	134.31	0.366	951.59	0.25	0.076	6.07	1.048	14.47	56.46	1.477	30.48	0.007	905.45	187.43

\* = Significant at 5 per cent  
 \*\* = Significant at 1 per cent  
 \*\*\*=significant at 0.1per cent

#### 4.2.2 Days from flowering to maturity

The number of days taken by the accessions for the expression of flowering to maturity ranged from 34.7 to 85.50 days with a mean of 58.87 days. The highest and lowest days from flowering to maturity was registered by accessions ICP 13270 (85.50) and ICP 2746 (34.70) respectively.

The trait exhibited a genotypic and phenotypic variance of 52.24 and 72.94 respectively and the genotypic and phenotypic coefficient of variation was 6.89 and 14.49 per cent respectively. The heritability estimated for the trait was 22.60 per cent with a genetic advance as per cent of mean of 6.74.

#### 4.2.3 Days to maturity

The average number of days to maturity recorded was 181.39 days. The values for the trait ranged from 100 to 235.05 days. The highest and lowest days to maturity were registered by the accessions ICP 14819 (235.05) and ICP 14819 (100) respectively.

The observed genotypic variance was 355.05 and the phenotypic variance was 356.53. The genotypic and phenotypic coefficient of variation recorded for the trait was 10.37 and 10.40 per cent respectively. The genetic advance as per cent of mean was 21.33 per cent with a heritability estimate of 99.50 per cent.

#### 4.2.4 Plant height

The plant height ranged from 200 cm to 323 cm with a mean value of 242.71 cm. The genotype, ICP 13431 recorded maximum height (323 cm) while, the genotype ICP 14368 recorded the least plant height (200 cm).

The genotypic co-efficient of variation of 7.62 per cent and a phenotypic co-efficient of variation of 8.99 per cent were recorded for the trait. The genotypic and phenotypic variance values were 343.24 and 477.55 respectively for the trait. A heritability estimate of 71.87 per cent was recorded for the trait while, the genetic advance was 32.32 per cent and genetic advance as per cent of mean was 13.32.

#### 4.2.5 Number of primary branches

The number of branches for the genotypes ranged from 4.2 to 8 with a mean of 5.25. The maximum number of branches was found in the genotype ICP 9750 (8) while minimum numbers of branches were found in ICP 7366 (4.2).

A genotypic co-efficient of variation of 51.66 per cent and a phenotypic co-efficient of variation of 73.24 per cent were registered for the trait. A heritability value of 51 per cent and genetic advance as per cent of mean of 84.82 was noticed for the trait.

#### 4.2.6 Number of pods per plant

The number of pods ranged from 12.60 to 279.20 with an average of 119.99 for the mini core collection set evaluated. The highest number of pods was found in the accession, ICP 4575 (279.20). The lowest number of pods were found in the genotype, ICP 13633 (12.60).

The genotypic and phenotypic co-efficient of variation recorded for the trait were 24.03 and 35.15 per cent respectively. The genotypic variability and phenotypic variability values were 834.26 and 1785.85 respectively for the trait. The heritability estimate recorded for the trait was 46.70 per cent while the genetic advance as per cent of mean was 33.83 for the trait.

#### 4.2.7 Number of seeds per pod

The average number of seeds per pod recorded was 4.03 for the character. The number of seeds per pod ranged from 3.00 to 4.80 for the trait. The highest number of seeds per pod was recorded by the accession, ICP 6992 (5.80) while, the lowest number of seeds per pod was registered by two accessions 3451 (3.00) and 3576 (3.00).

The genotypic and phenotypic coefficients of variation for number of seeds per pod recorded were 9.72 and 11.88 per cent respectively for this trait. The heritability value recorded for the trait was 67.02 per cent while, genetic advance as per cent of mean was 16.40 per cent for this trait.

#### 4.2.8 Pod length (cm)

The pod length values ranged from 3.80 to 7.90 cm with an average pod length of 5.16 cm. The highest pod length was recorded in the accession ICP 14229 (7.90 cm). The lowest pod length was registered by the accession ICP 11320 (3.80 cm).

The estimated genotypic variance was 0.17 and phenotypic variance was 0.42 respectively. The genotypic and phenotypic coefficients of variation for pod length were 8.21 and 12.69 per cent respectively for the trait. The genetic advance as per cent of mean was 10.95 per cent with a heritability estimate of 41.00 per cent.

#### 4.2.9 Seed yield per plant (g)

The maximum and minimum seed yield per plant was recorded for the accessions PG-12 (95.03g) and ICP 11015 (12.89g) respectively with a mean value of 32.26 g.

The genotypic and phenotypic variance for this trait was 184.08 and 190.15 respectively. The genotypic and phenotypic coefficient of variation for seed yield was 42.19 and 42.88 per cent respectively. A heritability estimate of 96.80 per cent and the genetic advance as per cent of mean was 85.52 per cent.

#### 4.2.10 Seed yield per ha (kg)

The average gain yield per ha was 179.20 (kg), the values ranged from 71.60 to 527.91 kg per ha. The maximum seed yield obtained from the accession PG-12 (527.91 kg) and the minimum seed yield observed for the accession ICP 11015 (71.60 kg).

The trait exhibits a genotypic and phenotypic variance of 5680.39 and 5867.82 respectively. The genotypic and phenotypic coefficient of variation was 42.19 and 42.88 per cent respectively for the trait. The heritability estimated was 96.00 per cent for seed yield per ha with a genetic advance as per cent of mean 85.52 per cent.

#### 4.2.11 Straw yield per plant (g)

The straw yield value ranged from 20.93 to 330.00 per plant to g per plant with mean value of 150.00g per plant. The maximum straw yield was recorded for the accession was Karithogari (330 g per plant) and minimum straw yield was recorded by the accession ICP 4019 (20.93 g per plant).

The straw yield recorded for a genotypic and phenotypic variance was 1302.50 to 2207.99. The genotypic and phenotypic variance coefficient of variation was 23.98 and 31.23 per cent respectively. The estimated genetic advance per cent of mean for the character was 37.95 per cent while the heritability estimated was 58.00 per cent.

#### 4.2.12 Test weight (g)

The average test weight recorded was 13.06 g. The test weight ranged from 8.60 g to 17.20 g. The highest and lowest test weights were recorded for the accessions viz., ICP 14444 (13.06) and ICP 15068 (8.60) respectively.

The trait exhibited a genotypic and phenotypic variance of 1.54 and 2.55 respectively for the trait. The genotypic and phenotypic coefficient of variation recorded was 9.42 and 12.26 per cent respectively. The test weight exhibited genetic advance as per cent of mean of 14.91 and with a heritability estimate of 59.00 per cent.

#### 4.2.13 Specific gravity (g per ml)

The specific gravity ranged from 0.53 to 1.56 g per ml with an average of 0.99 g per ml. The maximum specific gravity was recorded by the accession ICP 14444 (1.56) and the minimum specific gravity was registered by the accession ICP 15068 (0.53).

The estimated genotypic and phenotypic variance was 0.02 to 0.03 for the trait. The genotypic and phenotypic co-efficient of variation recorded for the trait were 15.68 and 17.85 per cent respectively. The heritability estimate recorded for the trait was 77.19 per cent while, the predicted genetic advance 0.28 and as per cent of mean was 28.39 per cent.

**Table 7: Estimates of genetic parameters for 14 quantitative characters studied in 192 genotypes pigeonpea (*Cajanus cajan*)**

Sl. No.	Characters	Mean	Range		Variance		Coefficient of variance		Heritability (h <sup>2</sup> (bs) %)	Genetic advance as per cent of mean (%)
			Min	Max	Phenotypic	Genotypic	Phenotypic (%)	Genotypic (%)		
1	Plant height (cm)	242.71	200	323	477.55	343.24	8.99	7.62	71.87	13.32
2	Number of primary branches	5.25	4.2	8	11.84	7.00	73.24	51.66	51	84.82
3	Pods per plant	119.99	12.60	279.20	1785.85	834.26	35.15	24.03	46.70	33.83
4	Pod length (cm)	5.16	3.80	7.90	0.42	0.17	12.69	8.21	41.00	10.95
5	Seeds per pod	4.03	3.00	4.80	0.22	0.15	11.88	9.72	67.02	16.40
6	Yield per plant (g)	32.26	12.89	95.03	190.15	184.08	42.88	42.19	96.8	85.52
7	Test weight (g)	13.06	8.60	17.20	2.55	1.54	12.26	9.42	59.00	14.91
8	Number of days to 50 per cent flowering	123.39	77	169	276.52	262.04	13.46	13.10	94.76	26.28
9	Number of days taken from flowering to maturity	58.87	34.7	85.50	72.94	52.24	14.49	6.89	22.60	6.74
10	Number of days to maturity	181.39	100.00	235.05	356.53	355.05	10.40	10.37	99.50	21.33
11	Straw yield per plant	150.00	20.93	330	2207.99	1302.50	31.23	23.98	58.00	37.95
12	Harvest index (%)	24.67	6.65	134.76	14.77	14.28	15.98	15.36	90.00	32.53
13	Specific gravity (g)	0.99	0.53	1.56	0.031	0.024	17.85	15.68	77.19	28.39
14	Yield (kg/ha)	179.20	71.60	527.91	5867.82	5680.39	42.88	42.19	96.00	85.52

#### 4.2.14 Harvest index (%)

The average harvest index estimated was 24.67 per cent and the values for the trait which ranged from 6.65 to 134.76 per cent. The minimum and maximum harvest index was found in the accessions ICP 13579 (6.65%) and ICP 4019 (134.76%) respectively.

The trait showed a genotypic variance of 14.28 and phenotypic variance of 14.77. The genotypic and phenotypic coefficient of variation with respect to harvest index was 15.36 and 15.98 per cent respectively. The genetic advance as per cent of mean was 32.53 per cent with an estimated heritability of 90.00 per cent.

### 4.3 Evaluation of genotypes

One hundred and ninety-two pigeonpea genotypes were evaluated with four checks for their yield and yield attributes. Mean values of the yield and yield related traits of the pigeonpea genotypes are presented in Appendix II.

#### 4.3.1 Number of days to 50 per cent flowering

The genotypes viz. ICP348 (110.00), ICP 655 (108.00), ICP 995 (101.00), ICP 1071 (105.00), ICP 1156 (115.00), ICP 2577 (108.00), ICP 2746 (115.00), ICP 3576 (100.00), ICP 4317 (102.00), ICP 6049(113.00), ICP 6128 (102.30), ICP 6815(116.00), ICP 6992 (103.00), ICP 7148 (100.50), ICP 7221 (109.50), ICP 7260 (114.00), ICP 7366 (99.00), ICP10094 (110.00), ICP 10447 (91.00), ICP 11015 (108.00), ICP 12298 (108.00), ICP 12515 (100.00), ICP 12596 (110.00), ICP 14368 (77.00), ICP 14444 (83.00), ICP 14819 (80.00), ICP 14832 (90.00), ICP 15049 (87), BRG-7-3 (104.00), ICP 1730 (102.00), ICP 4019 (114.00), BRG-9-2 (112.00), BRG-207 (114.00), BRG-7-5 (99.00), ICP 1550 (114.00), ICP 12333 (100.00), ICP 10804 (108.00), ICP 12674 (103.00), ICP 4878(81.00), PUSA992 (114.00), ICP 6528(112.00), HY-3-C (114.00) ICP 12266 (103.00), ICP 4395 (91.00), BRG-9-2 (114.00), BRG-7-2 (90.00), GC-11-39 (114.00) ICP 12282 (114), DM-158 (77), ICP12692(110.00), Jamadhar local (115.00), Bidar local (91.00), UPAS-120 (107.00), JKE-144 (94.00), Chaple (109.00), Gullyal white (104.00), WRP-1 (W) (102.00), JKM-197 (113.00), CORG-2004-01 (114.00), PJT-501 (115.00), GRG-801 (100.00), Bennur local (116.00) and GRG-206 (107) recorded significantly less number of days to 50 per cent flowering as compared to the best check Maruti (117.00 days) at 5 per cent level of critical difference.

#### 4.3.2 Number of Days to maturity

The genotypes viz. ICP 655 (163.40), ICP 995 (157.00), ICP 1071 (160.00), ICP 2577 (165.00), ICP 6128 (160.20), ICP 6815 (165.00), ICP 6992 (156.00), ICP 7148 (164.50), ICP 12515 (165.60), ICP 13167 (100.00), ICP 14819 (115.70), ICP 14832 (143.65), BRG-7-3 (165.00), ICP 1730 (157.00), ICP 12333 (158.00), ICP 12674 (160.00), ICP 4878 (146.00), ICP6528 (155.00), ICP12266 (156.00), ICP4395 (135.00), BRG-7-2 (135.00), Jamadhar local (160.00), Bidar local (135.00), JKE-144 (154.00), Chaple (159.00), WRP-1 (165), JKM-197 (155) and GRG-801 (155.00) Bennur local (164.00) were significantly early maturing genotypes than the best check Maruti (168.60 days) at 5 per cent level of critical difference.

#### 4.3.3 Number of primary branches

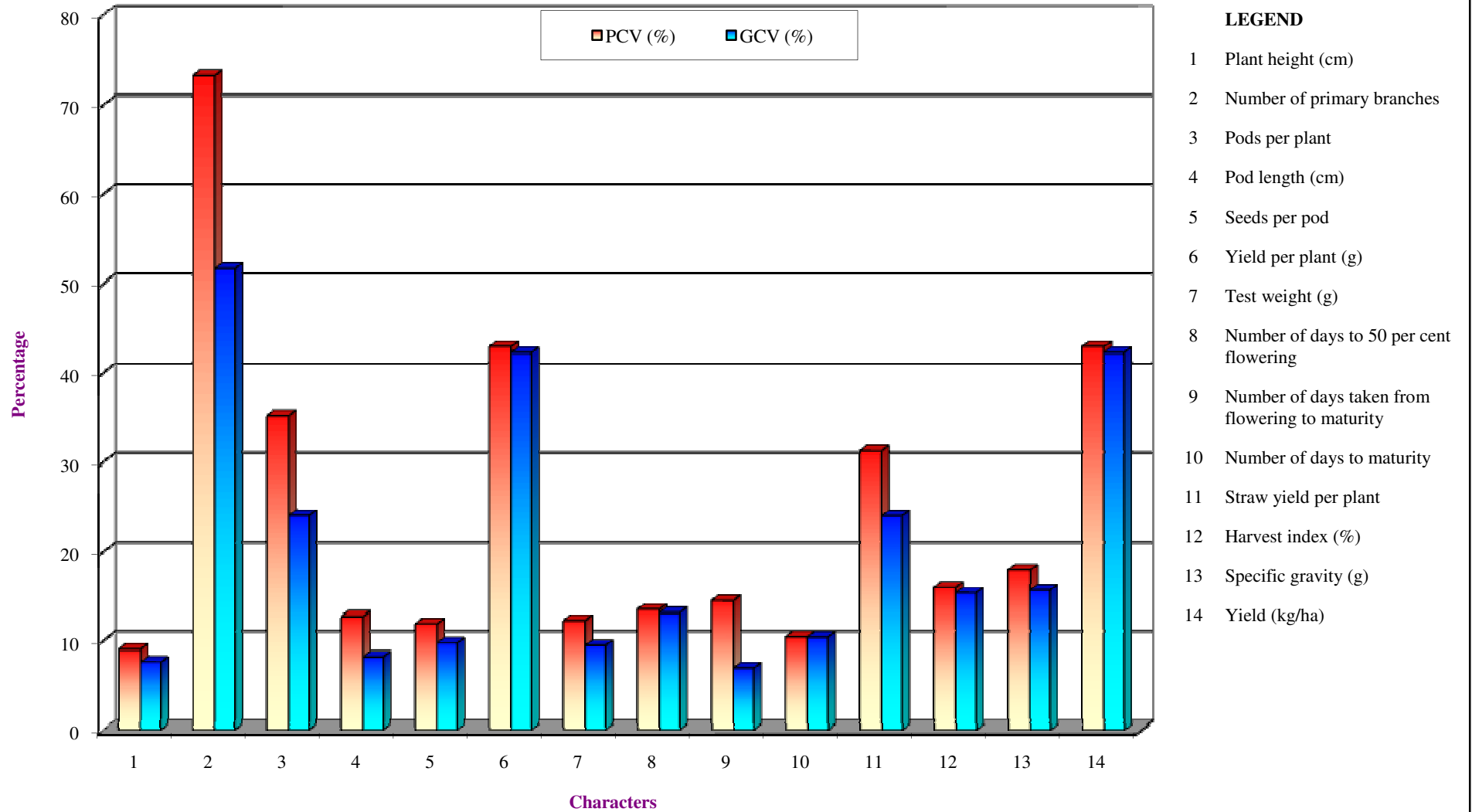
The genotypes viz. ICP 3451 (6.90g), ICP 7071 (7.20g), ICP 9750 (8.0g), ICP 11910 (6.56g), ICP 12654 (7.0g) and HY-3 (6.43g) exhibited significantly higher number of primary branches per plant in comparison to best check Maruti (5.48) at 5 per cent level of critical difference.

#### 4.3.4 Number of pods per plant

The genotypes viz. ICP 772 (223.60), ICP 6859 (215.50), ICP 7507 (210.60), ICP 4575 (279.20), ICP 14832 (185.80), CORG-295 (190.24) and ICP 14671 (201.80) exhibited significantly higher number of pods per plant compared to best check BSMR 736 (170.28) at 5 per cent level of critical difference.

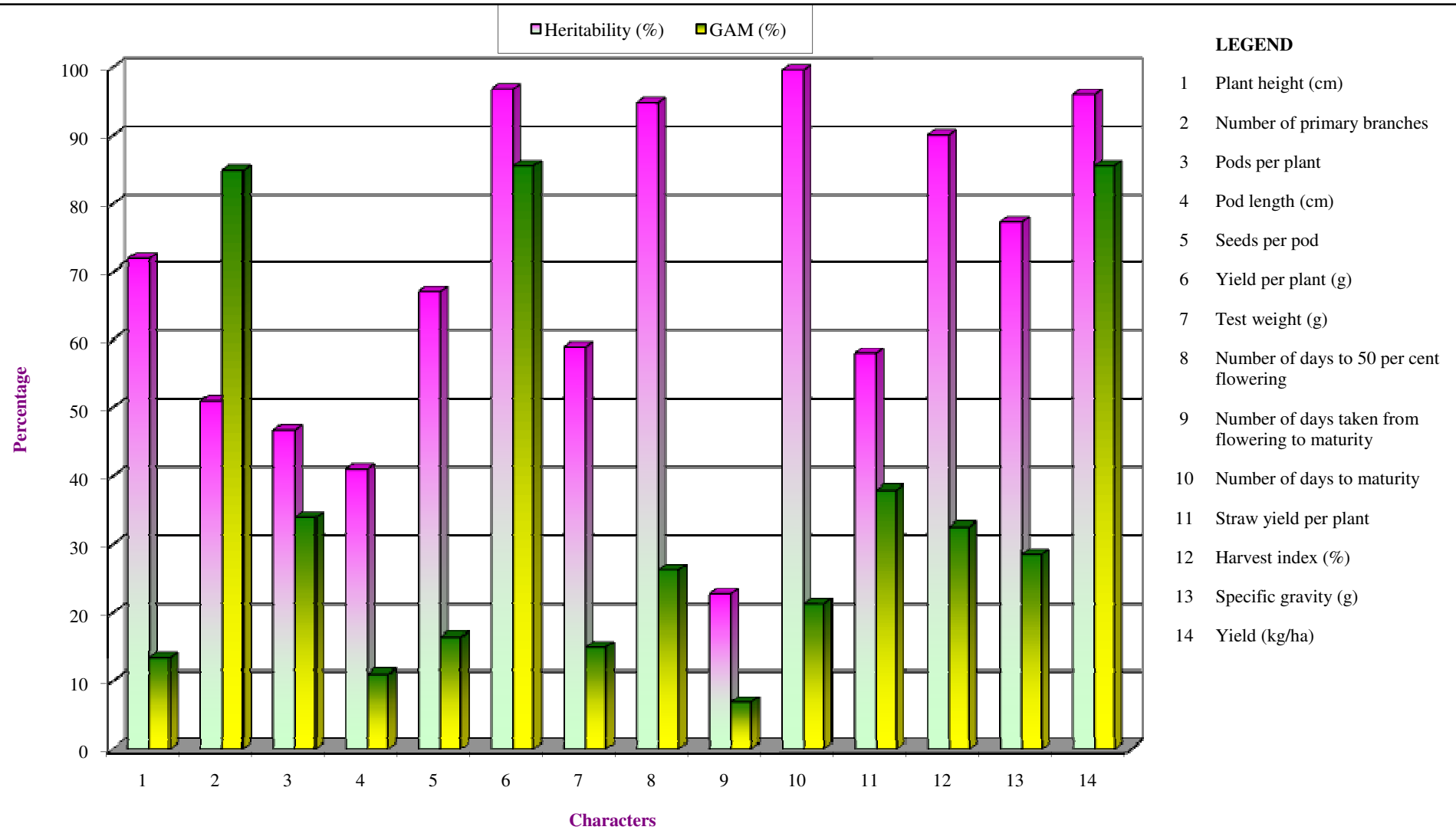
#### 4.3.5 Seed yield per plant

The genotypes viz. ICP 655 (47.80g), ICP 3046 (45g), ICP 3049 (84.40g), ICP 3451 (49.60g), ICP 3576 (47.60g), ICP 4167 (48.40g), ICP4392 (58.40g), ICP 6128 (56.20g), ICP 6971 (55.91g), ICP 7223 (45.68g), ICP 14638 (51.23g), ICP 14071 (55g), ICP 14722 (45.55g), ICP 14801 (55.55g), ICP 14976 (46.27g), ICP 10804 (45.20g), ICP 4878 (45.41g), ICP 4275 (52.61g), ICP 12282 (57.88g), ICP



**Fig. 1: Estimates of PCV and GCV parameters for 14 quantitative characters studied in 192 genotypes of pigeonpea**





**Fig. 2: The genetic parameters estimated for fourteen characters studied in 192 genotypes of pigeonpea**

13270 (91.33g), BRG-7-6 (58.33g), DM-158 (49g), ICPL-87 (52.83g), Jamadhar local (56.22g), Bidar local (51.26g), Jke-144 (57.04g), PUSA-92 (62.28g), PG-12 (95.03g), MA-29(62.62g), Karithogari (58.27g), ICPB2091 (53.80g), CORG-295 (59.16g), Chaple (55g), Gullyal white (46.80g), CORG-970 (53.60g), CORG-2004-01 (47.55), PJT-501 (48.30g) and Bennur local (45.80) recorded significantly highest seed yield per plant as compared to the best check GS-1 (43.97g) at 5 per cent level of critical difference.

#### 4.3.6 Test weight

In the present study the genotypes *viz.* ICP 6128 (15.80g), ICP 6668 (15g), ICP 7057 (15.92g), ICP 7223 (15g), ICP 7260 (15.60g), ICP7507 (14.60g), ICP7869 (14.60g), ICP 8921 (14.40g), ICP 9045 (14g), ICP 10397 (14.60g), ICP 11015 (14.60g), ICP 13270 (14.60g), ICP 13579 (14.80g), ICP 14229 (15.60g), ICP14444 (17.20g), ICP 14638 (15g), ICP 14071 (14.60g), ICP 14722 (14g), ICP 14832 (14.76g), ICP 15161 (14.40g), ICP 15382 (15.80g), ICP 4575 (15.80g), ICP 4019 (17g), BRG-9-2 (14.40g), BRG-207 (15.77g), ICP 1224 (15.60g), BRG-7-5 (15g), ICP 1550 (14.80g), ICP 12674 (15.40g), ICP 12266 (16.20g), UPAS-120 (15.60g), GC-11-39 (15.75g), ICP 4275 (14.40g), ICP 12282 (15g), BRG-7-6 (14.60g), ICP 12692 (14.60g), Jamadhar local (14.80g), PG-12 (15.40g), Chaple (16.60g) and Bennur local (16.20g), recorded significantly higher test weight as compared to the best check Asha (14.34g) at 5 per cent level of critical difference.

### 4.4 Correlation analysis

Improvement in the seed yield per plant is an important activity of plant breeding; the ultimate seed yield is a complex trait that will be affected by many other genetic and non-genetic factors. In order to know the magnitude of the association between the seed yield with other yield influencing traits, correlation analysis is an effective tool. The correlation studies provide us an idea about how the traits are associated with each other as well as with the seed yield. The results of the association studies (Table 8) are presented hereunder.

#### 4.4.1 Days to fifty per cent flowering

The days to fifty per cent flowering exhibited positive significant correlation with plant height (0.4028) and days to maturity (0.8358) at phenotypic level. However, the number of primary branches (0.0414), number of pods per plant (0.1019), pod length (0.0135), flowering to maturity (0.1053) and straw yield per hectare (0.0598) exhibited positive association with days to fifty per cent flowering. Seed yield per hectare (-0.1772) showed negative significant association. Harvest index (-0.0189), test weight (-0.0270), seeds per pod (-0.0814) and specific gravity (-0.0326) showed negative and non-significant association with days to 50 per cent flowering.

#### 4.4.2 Days from flowering to maturity

The days from flowering to maturity exhibited significant positive association with the days to maturity (0.3891) at phenotypic level. The days from flowering to maturity had positive non-significant association with plant height (0.0945), days to 50 per cent flowering (0.1053), straw yield per ha (0.1222), test weight (0.0386) and harvest index (0.0512) at phenotypic level. The days from flowering to maturity exhibited negative non-significant association with number of primary branches (-0.0348), pods per plant (-0.1001), pod length (-0.0556), seeds per pod (-0.1187), specific gravity (-0.0353) and seed yield per hectare (-0.1044) at the phenotypic level.

#### 4.4.3 Days to maturity

The days to maturity exhibited positive and significant association with the plant height (0.3102), days to fifty per cent flowering (0.8358) and days from flowering to maturity (0.3891) at the phenotypic level. However, the days to maturity exhibited non-significant negative association with seed yield per hectare (-0.1757) pod length (-0.0621), harvest index (-0.0205), specific gravity (-0.0046) and test weight (-0.0167). Also, days to maturity showed positive but non-significant association with pods per plant (0.0654), seeds per pod (-0.1510) and straw yield (0.0242) at phenotypic level.

#### 4.4.4 Number of primary branches

The number of primary branches showed positive significant association with number of seeds per pod (0.15) at the phenotypic level. However, the number of branches showed negative association with seed yield per hectare (-0.109), harvest index (-0.02), number of days from flowering to maturity (-0.03) and straw yield per ha (-0.02) at phenotypic level. Positive but non-significant

association was observed for the trait like plant height (0.0480), days to 50 per cent flowering (0.0414), pod length (0.02), number of pods per plant (0.4), test weight (0.09) and specific gravity (0.01), at phenotypic level in the present study.

#### 4.4.5 Plant height

The plant height exhibited positive and significant association with days to fifty per cent flowering (0.40) and days to maturity (0.31) at phenotypic level. It showed positive association with number of primary branches (0.04), number of pods per plant (0.06), test weight (0.01), straw yield per ha (0.003), specific gravity (0.0735) and days from flowering to maturity (0.09) at phenotypic level. However, the plant height had negative and significant association with seed yield per ha (-0.27). The plant height had negative association with pod length (-0.07), number of seed per pod (-0.07) and harvest index (-0.03) at the phenotypic level.

#### 4.4.6 Number of pods per plant

The number of pods showed positive association with plant height (0.0628), seed yield per hectare (0.0035), number of primary branches (0.0403), test weight (0.0069), days to 50 per cent flowering (0.1019), days to maturity (0.0654), specific gravity (0.1317) at phenotypic level. The number of pods exhibited negative significant association with number of seeds per pod (-0.1551) at the phenotypic level. Positive association was observed for the trait like plant height (0.0628). Negative association was observed for the traits like days from flowering to maturity (-0.1001), straw yield per ha (-0.0163), pod length (-0.1248) and harvest index (-0.0262) at phenotypic level.

#### 4.4.7 Number of seeds per pod

The number of seeds per pod exhibited positive significant association with number of primary branches (0.1565), pod length (0.1532) and test weight (0.1549) at phenotypic level. The trait showed positive non-significant association with harvest index (0.0344) at phenotypic level, Whereas, number of seeds per pod showed negative significant association with the traits like days to maturity (-0.1510), pods per plant (-0.01551). Negative association was observed for the traits like days to 50 per cent flowering (-0.0814), straw yield per plant (-0.0178), specific gravity (-0.0122) and seed yield per hectare (-0.0286) at phenotypic level.

#### 4.4.8 Pod length

The pod length exhibited positive and significant association with number of seeds per pod (0.1532). Positive association with number of primary branches (0.0226), days to 50 per cent flowering (0.0135), straw yield per ha (0.0684), test weight (0.0801), harvest index (0.0066), specific gravity (0.0204) and yield per hectare (0.0522) at phenotypic level. However, number of days from flowering to maturity (-0.0556), plant height (-0.0702), pods per plant (-0.1248) and days to maturity (-0.0621) had negative association with pod length at the phenotypic level.

#### 4.4.9 Straw yield per hectare

The straw yield per ha showed positive and significant association with harvest index (0.5912) at the phenotypic level. The straw yield per ha showed positive association with plant height (0.0033), pod length (0.0684), specific gravity (0.0037), days to maturity (0.0242), days to flowering to maturity (0.12222) and days to 50 per cent flowering (0.0598).Whereas, negative non significant association was registered with number of primary branches (-0.0155), pods per plant (-0.0163), seeds per pod (-0.0178), test weight (-0.0329) and seed yield per hectare (-0.1299) at phenotypic level.

#### 4.4.10 Seed yield per hectare

The seed yield recorded high significant and positive association with harvest index (0.4179). Pods per plant (0.0035), pod length (0.0522), test weight (0.0413) recorded positive association with seed yield at the phenotypic level. The traits, pods per plant (0.0035), pod length (0.0522) and test weight (0.0413) registered positive association with seed yield per hectare at the phenotypic level. Number of primary branches (-0.1091), seeds per pod (-0.0286), days taken flowering to maturity (-0.1044), straw yield per hectare (-0.1299) and specific gravity (-0.0207) recorded negative association with seed yield per hectare at the phenotypic level.

**Table 8: Phenotypic correlation coefficients among 13 different quantitative characters in 192 genotypes of pigeonpea (*Cajanus cajan*)**

	PHT	BRH	PPP	PL	SPP	DFF	DFM	DM	SY	TW	HI	SG	SYPH
PHT	<b>1.0000</b>	0.0480	0.0628	-0.0702	-0.0705	0.4028***	0.0945	0.3102***	0.0033	0.0119	-0.0393	0.0735	-0.276**
BRH		<b>1.0000</b>	0.0403	0.0226	0.1565 *	0.0414	-0.0348	0.0443	-0.0155	0.0954	-0.0229	0.0146	-0.1091
PPP			<b>1.0000</b>	-0.1248	-0.1551 *	0.1019	-0.1001	0.0654	-0.0163	0.0069	-0.0262	0.1317	0.0035
PL				<b>1.0000</b>	0.1532 *	0.0135	-0.0556	-0.0621	0.0684	0.0801	0.0066	0.0204	0.0522
SPP					<b>1.0000</b>	-0.0814	-0.1187	-0.1510 *	-0.0178	0.1549 *	0.0344	-0.0122	-0.0286
DFF						<b>1.0000</b>	0.1053	0.8358***	0.0598	-0.0270	-0.0189	-0.0326	-0.1772*
DFM							<b>1.0000</b>	0.3891***	0.1222	0.0386	0.0512	-0.0353	-0.1044
DM								<b>1.0000</b>	0.0242	-0.0167	-0.0205	-0.0046	-0.1757*
SY									<b>1.0000</b>	-0.0329	0.5912***	0.0037	-0.1299
TW										<b>1.0000</b>	0.1563 *	0.6622***	0.0413
HI											<b>1.0000</b>	0.1017	0.4179**
SG												<b>1.0000</b>	-0.0207
													<b>1.0000</b>

\* - Significant at 5 per cent  
 \*\* - Significant at 1 per cent  
 \*\*\* - Significant at 0.1 per cent

PHT – Plant height  
 SPP – Seeds per pod  
 DFM – Days flowering to maturity  
 SG – Specific gravity

BRH – Number of primary branches  
 TW – Test weight,  
 DM – Days to maturity  
 SY PH– Seed yield per ha

PPP – Pods per plant PL – Pod length  
 DFF – Days to 50 per cent flowering  
 HI – Harvest index  
 SY-straw yield

#### 4.4.11 Test weight

The test weight exhibited significant and positive association with number of seeds per pod (0.1549), harvest index (0.1563), specific gravity (0.6622). The test weight showed positive and non-significant association with plant height (0.0119), primary branches (0.0954), number of pods per plant (0.0069), pod length (0.0801) and seed yield per hectare (0.0413) at the phenotypic level. The trait, days to maturity (-0.0205), days to flowering to maturity (-0.0167), days to 50 per cent flowering (-0.0270) and straw yield per hectare (-0.0329) registered negative association and non-significant with test weight.

#### 4.4.12 Specific gravity

The specific gravity exhibited significant positive association with test weight (0.6622) at phenotypic level. The traits like plant height (0.0735), number of primary branches (0.0146), pods per plant (0.1317), pod length (0.0204), straw yield per hectare (0.0037) and harvest index (0.1017) showed positive non-significant correlation with specific gravity. The specific gravity exhibited negative association with seeds per pod (-0.0122), days to maturity (-0.0046), days from flowering to maturity (-0.0353), days to 50 per cent flowering (-0.0326) seed yield per hectare (-0.0207) at phenotypic level.

#### 4.4.13 Harvest index

The harvest index recorded positive significant association with seed yield per hectare (0.4179), straw yield per hectare (0.5912) and test weight (0.1563) at phenotypic level. However, pod length (0.0066), seeds per pod (0.0344), flowering to maturity (0.0512) and specific gravity (0.1017) registered non-significant positive association. The harvest index recorded negative association with plant height (-0.0393), number of primary branches (-0.0229), pods per plant (-0.0262), days to 50 per cent flowering (-0.0189) and days to maturity (-0.0205) at the phenotypic level.

### 4.5 Phenotypic path analysis for seed yield

The path coefficient analysis was worked out to partition the correlation coefficient into direct and indirect effects. The genotypic and phenotypic correlation coefficients of various characters with seed yield were subjected to path coefficient analysis to estimate the direct and indirect effects of component traits on seed yield considered as dependent variable for the analyses. The direct and indirect effects of various traits with respect to yield are presented in Table 9.

#### 4.5.1 Days to fifty per cent flowering

The days to fifty per cent flowering recorded positive direct effect (0.0124) for yield at phenotypic level.

The negative indirect effect was exhibited by days to fifty per cent flowering via, plant height (-0.0936), days to maturity (-0.0884) and harvest index (-0.0077) at phenotypic level.

#### 4.5.2 Days to maturity

The days to maturity showed direct negative effect (-0.1057) for yield at the phenotypic level. The days to maturity showed positive indirect effect for yield via, days to fifty per cent flowering (0.0104) at phenotypic level. It also exhibited negative indirect effect for yield via, plant height (-0.0721) and harvest index (-0.0083) at phenotypic level.

#### 4.5.3 Plant height

The plant height recorded negative direct effect (-0.2324) for yield at phenotypic level. The plant height showed positive indirect effect for yield via days to fifty per cent flowering (0.005). The negative indirect effect was noticed for seed yield by plant height via days to maturity (-0.0328) and harvest index (-0.016) at the phenotypic level.

#### 4.5.4 Harvest index

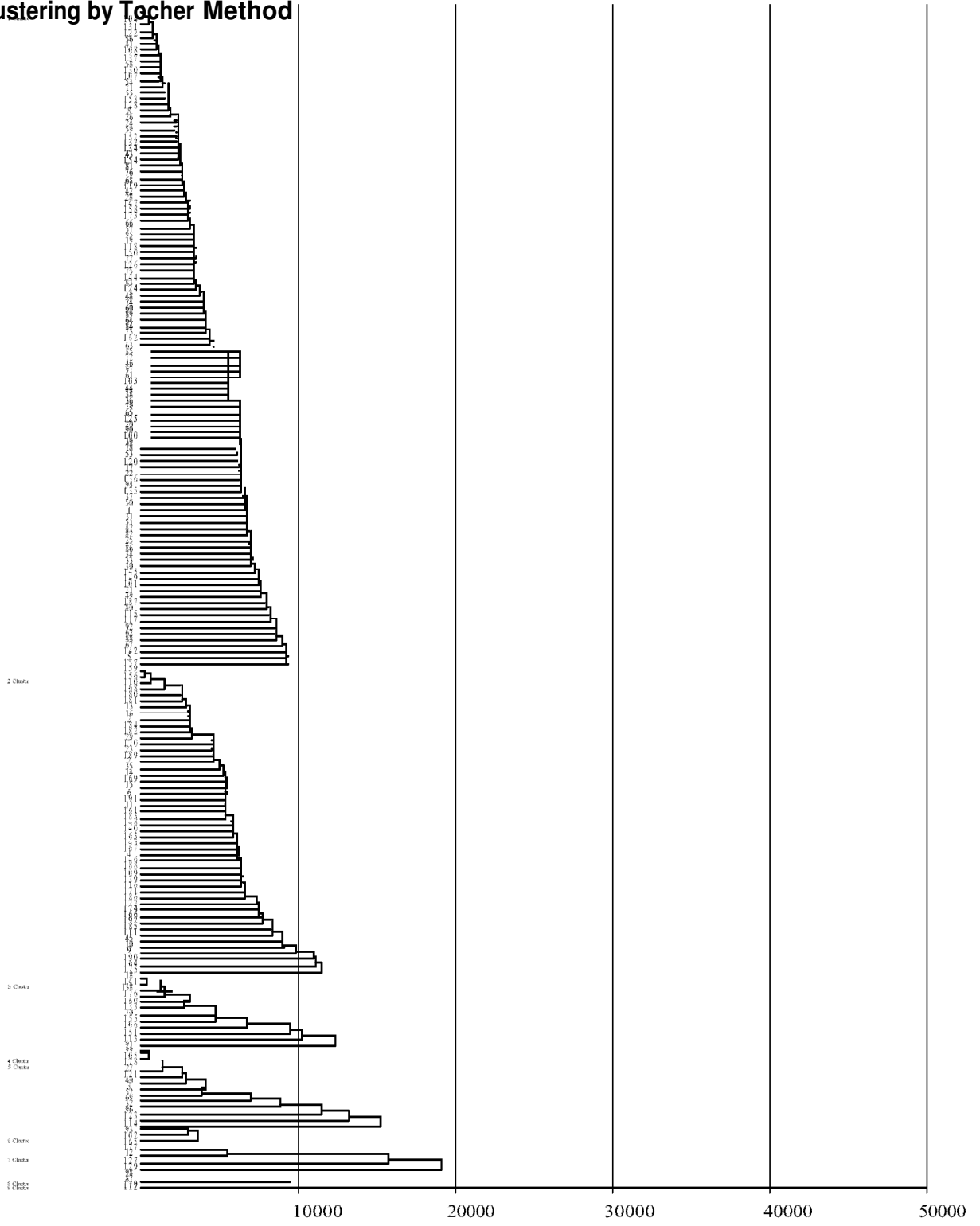
The harvest index exerted positive direct effect (0.4069) for yield at the phenotypic level. The harvest index also exerted negative indirect effect for seed yield via, days to fifty per cent flowering (-0.0002) at phenotypic level. The harvest index recorded positive indirect effect for seed yield via, plant height (0.0091), days to maturity (0.0022) at phenotypic level.

**Table 9: Phenotypic path coefficient analysis using seed yield as a dependent character in pigeonpea (*Cajanus cajan*)**

	<b>Plant height</b>	<b>Days to 50 per cent flowering</b>	<b>Days to maturity</b>	<b>Harvest index</b>	<b>Seed yield per ha (r)</b>
Plant height	<b>-0.2324</b>	0.005	-0.0328	-0.016	-0.2761**
Days to 50 per cent flowering	-0.0936	<b>0.0124</b>	-0.0884	-0.0077	-0.1772*
Days to maturity	-0.0721	0.0104	<b>-0.1057</b>	-0.0083	-0.1757*
Harvest index	0.0091	-0.0002	0.0022	<b>0.4069</b>	0.4179**

**Residual effect = 0.8657**

### Clustering by Tócher Method



**Fig 3: Dendrogram showing clustering of 192 pigeonpea genotypes by Tócher method**

## 4.6 Genetic diversity

### 4.6.1 Mahalanobis generalized distances ( $D^2$ )

The genetic diversity among 192 genotypes was measured by employing  $D^2$  statistic, as from the evident of Table 10. Out of 13 character studied seed yield per hectare contributed maximum to the diversity (55.12%) followed by number of pods per plant (28.59%), plant height (7.34%), number of days to maturity (4.35%), number of days to 50 per cent flowering (2.14%) and harvest index (2.11%).

Based on  $D^2$  values the genotypes were grouped into 9 clusters using Tocher's method given by Rao (1952). The dendrogram of 192 accessions are presented in Table 11 and represented in Fig. 3. Of the 9 clusters, cluster I was the largest cluster comprising 108 genotypes followed by cluster II and III comprising 50 and 12 genotypes, cluster V comprised 12 genotypes, clusters IV, VIII and IX were solitary with single genotypes each.

#### Inter cluster distance

The inter cluster  $D^2$  values were maximum (385.01) between I and VI clusters followed by VI and VII clusters (383.46). The minimum distance observed was 107.41 between I and III clusters followed by II and IV (109.17).

#### Intra cluster distance

The intra cluster  $D^2$  values are given in Table 10. The intra cluster distance was observed only in cluster I, II, III, V, VI and VII. The other remaining three clusters viz., IV, VIII and IX contained only one genotype. The highest intra cluster distance was observed in cluster VII (146.77) followed by V (100.1), III (89.53), II (85.59), I (77.46) and VI (68.34) (Table 12).

#### Cluster mean analysis

Comparison of cluster means for different characters as provided in the Table 13, indicated considerable difference between clusters for all the characters. Maximum yield for plant height (285.65cm) was observed in the cluster VII, followed by cluster IX (271.00 cm) while minimum mean (223.00cm) was observed in the cluster VIII. Maximum mean (5.50) for number of primary branches was observed in cluster V, followed by cluster VII (5.47), while the minimum mean for number of primary branches was observed in cluster VIII (4.2). The maximum mean for number of pods per plant (274.95) was observed in cluster VIII followed by cluster V (195.13), the minimum mean for number of pods per plant (61.4) was observed in cluster VII. The maximum mean for pod length was observed in cluster IX (6.20 cm) followed by cluster VIII and cluster IV (5.80). while, the minimum mean was observed in cluster V (5.02)

The cluster mean for number of seeds per pod was highest in cluster VII (4.45) followed by cluster III (4.43). While, the minimum was observed in cluster VI (3.93). Maximum mean for seed yield per plant was observed in cluster VI (90.25 g) followed by cluster IV (62.62 g), while minimum mean was observed in cluster I (23.62 g). The highest cluster mean for test weight was observed in cluster VII (14.55 g) followed by cluster VIII (14.20 g), while the minimum mean was observed in cluster IV (12.80 g). Days to 50 per cent flowering showed highest cluster mean with the cluster IX (167.00) followed by cluster VIII (163.00), while the minimum mean was observed in cluster III (112.33). Highest cluster mean for number of days from flowering to maturity was associated with cluster IX (68.05.00) followed by cluster VII (63.34) while the minimum mean was observed in cluster VIII (50.00). Highest cluster mean for number of days to maturity was observed in cluster IX (235.05) followed by cluster VIII (213.00) while the minimum mean was observed in cluster VII (162.59). The highest cluster mean for harvest index was observed in cluster IX (129.34 %) followed by cluster VII (67.45 %), while the minimum was observed in cluster I (17.52 %). The highest cluster mean for seed yield per ha was observed in cluster VI (501.37 kg) followed by cluster IV (347.85 kg) while the minimum was observed in cluster I (131.22 kg). The maximum cluster mean for specific gravity was observed in clusters V III (1.13 g/ml) followed by clusters V and VI (1.06 g/ml), while the minimum mean was observed in the cluster IX (0.91 g/ml). The genotypes evaluated in the present study were collected from 22 different countries (Australia, Bangladesh, Brazil, China, Italy, Jamaica, Kenya, Malawi, Myanmar, Nepal, Nigeria, Philippines, Sri Lanka, Sierra Leone, Tanzania, Trinidad and Tobago, Puerto Rico, Uganda, Venezuela, Zambia,) including India. Grouping of genotypes by employing  $D^2$  statistic revealed that, genetic diversity was not found to be related to geographical diversity.

**Table 10: Per cent contribution of 13 characters towards divergence in 192 pigeonpea genotypes**

<b>Sl. No.</b>	<b>Characters</b>	<b>Times ranked 1<sup>st</sup></b>	<b>Contribution %</b>
1	Plant height (cm)	1346	7.34
2	Number of primary branches	00	0.00
3	Number of pods per plant	5242	28.59
4	Pod length (cm)	00	0.00
5	Number of seeds per pod	00	0.00
6	Yield per plant (g)	00	0.00
7	Number of days to 50 per cent flowering	392	2.14
8	Number of days from flowering to maturity	66	0.36
9	Number of days to maturity	797	4.35
10	Test weight (g)	00	0.00
11	Harvest index (%)	387	2.11
12	Specific gravity (g)	00	0.00
13	Seed yield per ha (kg)	10106	55.12

**Table 11: Clustering pattern of 192 genotypes of pigeonpea based on D<sup>2</sup> analysis**

Cluster group	Number of genotypes	Genotypes
I	108	ICP 14294, TM 224, ICP 16264, ICP 9691, ICP 7803, ICP 14569, ICP 1550, ICP 10095, TTB-7, ICP 14471, ICP 9414, ICP 11627, ICP 9655, BRG-9-2, BRG-9-2, ICP 2577, ICP 6815, ICP 6668, ICP 10228 , UPAS-120, ICP 1224, TM-26, ICP 8012, TS-3®, ICP 12410, ICP 11946, ICP11281, ICP 15161, ICP 7869, ICP 6929, HY-3-C, GC-11-39, UPAS-120, ICP 11059, ICP 9750, ICP 13575, ICP 5142, ICP 15068, ICP 1366, ICP 12105, ICP 1730, ICP 11910, PUSA 992, ICP 12596, BRG-7-3, ICP 8757, ICP 11833, ICP 10397, ICP 13244, ICP 10654, ICP 12654, ICP 11823, ICP 12674, ICP 10559, ICP 12680, ICP 11690, ICP 8384, ICP 13579, ICP 10447, ICP 14229, ICP 8255, ICP 7366, ICP 7260, ICP 12142, ICP 11015, BRG-6-12, ICP 5863, ICP 13270, ICP 13884, ICP 7426, ICP 12123, ICP 9045, ICP 15185, ICP 4317, ICP 6123, ICP 12460, ICP 13571, ICP 5641, ICP1550, ICP 8860, ICP 348, ICP 7057, ICP 8921, ICP 8602, ICP 12515, ICP 6739, ICP 13139, ICP 7221, ICP 7148, ICP 6992, ICP 6528, ICP 9903, ICP 14116, ICP 6049, ICP 8840, JKM-197, ICP 12298, ICP14900, ICP 15049, ICP 13359, ICP 10503, ICP 13191, ICP 11230, GULLYAL LOCAL, ICP 995, BRG-7-2, ICP 12301, TM-389.
II	50	ICP 14701, ICPL-87, ICPB 2091, CORG-295, ICP 3451, ICP 4167, ICP 655, CORG-9701, CHAPLE, ICP 6971, JAMADHAR LOCAL, ICP 6128, PJT-501, ICP 1156, ICP 7223, ICP 3576, ICP 12692, ICP 4029, ICP 1071, BENNUR LOCAL, ICP 3046, GRG-2-95, GULLYAL WHITE, ICP 12266, ICP 10804, ICP 4275, ICP 4878, DM-158, ICP 939, GULLYAL RED, CORG-2004-01, ICP 14638, ICP 12333, ICP 14976, WRP-1, RUDRAWADI LOAL, BIDAR LOCAL, JKE-144, BRG-7-6, GRG-206, WRP-1 (W), ICP 14722, ICP 8266, ICP 2746, ICP 2698, GRG-801, ICP 12282, PUSA-92, ICP 4392, ICP 12675.
III	12	DPD-2-38, ICP 8863, BRG-7-4, BRG-7-5, ICP 11477, DM-155, ICP 14444, ICP 4395, ICP 14819, ICP 13304, ICP 13662, ICP 14368.
IV	1	MA-29
V	12	ICP 6859, ICP 15382, ICP 7507, ICP 6992, ICP 8949, ICP 11320, ICP 7076, ICP 13577, ICP 4575, ICP 14832, ICP 13431, ICP 14147.
VI	3	ICP 13270, PG-12, ICP 3049.
VII	4	ICP 4019, BRG-207, ICP 13633, ICP 13167.
VIII	1	KARITHOGARI
IX	1	ICP 14801.



**Table 13: Cluster mean for 13 quantitative characters of pigeonpea genotypes**

	<b>PHT(cm)</b>	<b>BRH</b>	<b>PPP</b>	<b>PL (cm)</b>	<b>SPPD</b>	<b>SYPP (g)</b>	<b>DFF</b>	<b>DFM</b>	<b>DM</b>	<b>TW (g)</b>	<b>HI (%)</b>	<b>SG (g/ml)</b>	<b>SY/ha (kg)</b>
I	244.76	5.25	117.67	5.09	4.00	23.62	126.06	59.93	185.65	12.83	17.52	0.98	131.22
II	235.12	5.20	123.10	5.32	3.98	47.86	116.30	57.17	173.91	13.25	33.66	1.00	265.88
III	227.91	5.27	67.43	5.11	4.43	30.25	112.33	54.25	164.25	13.16	19.78	0.99	168.02
IV	235.00	4.80	93.40	5.80	4.00	62.62	123.00	53.00	176.00	12.80	54.67	1.00	347.85
V	259.30	5.50	195.13	5.02	4.00	27.97	132.38	58.83	189.68	13.50	22.34	1.06	155.34
VI	231.07	5.19	106.97	5.35	3.93	90.25	123.00	63.27	186.27	13.93	61.48	1.06	501.37
VII	285.65	5.47	61.40	5.18	4.45	25.95	126.00	63.34	162.59	14.55	67.45	1.01	144.15
VIII	223.00	4.20	274.95	5.80	4.20	58.27	163.00	50.00	213.00	14.20	17.66	1.13	323.68
IX	271.00	5.40	89.00	6.20	4.40	55.55	167.00	68.05	235.05	13.40	129.34	0.91	308.58

PHT – Plant height  
 SPP – Seeds per pod  
 DFM – Days flowering to maturity  
 SG – Specific gravity  
 BRH – Number of primary branches  
 SYPP – Seed yield per plant  
 DM – Days to maturity  
 SY – Seed yield per ha  
 PPP – Pods per plant  
 TW – Test weight,  
 HI – Harvest index  
 PL – Pod length  
 DFF – Days to 50 per cent flowering

## 4.7 Evaluation of genotypes for diseases and pest incidence

In the present investigation an effort was made to screen the genotypes for *Fusarium* wilt and *Helicoverpa armigera* incidence under natural conditions. The *Fusarium* wilt incidence was from 0 to 100 per cent while, *Helicoverpa* incidence observed was up to 68.79 per cent whereas, the incidence of *Maruca vitrata* was not observed in the present study.

## 4.8 Screening for sterility mosaic disease (SMD)

All the 192 pigeonpea accessions were studied for their field resistance to sterility mosaic disease under sick plot technique/leaf stapling technique (Lavakumar, 2002) during 2012. The per cent disease incidence among 192 accessions ranged from 0 to 100 per cent. Based on per cent disease, the genotypes were grouped into different classes as indicated in Table 14. The data revealed that a total of 121, 14, 4, 37 and 16 accessions fell into various categories *viz.*, resistant, moderately resistant, susceptible and highly susceptible respectively (Table 15.)

Sixteen accessions recorded highly susceptible reaction for the sterility mosaic with a disease incidence ranging from 52.94 to 83.33 per cent while, one hundred and twenty one accessions recorded zero per cent disease and were found resistant to sterility mosaic disease reaction. Fourteen accessions recorded moderately resistant reaction for the sterility mosaic with a disease incidence ranging from 1 to 10 per cent. The pigeonpea accessions ICP13579, ICP14147, ICP14900 and ICP12333 showed moderately susceptible reaction with a disease incidence ranging from 11 to 20 per cent followed by 37 accessions (21 to 50 per cent disease incidence) (Plate 4 and 5).

## 4.9 Molecular characterizations

A total of 150 crop specific simple sequence repeat (SSR) markers were used for molecular characterization of selected accessions. The genotypes were selected based on per cent disease incidence (PDI) of sterility mosaic disease and fusarium wilt. Among them Twenty five resistant and twenty five susceptible accessions selected for molecular characterization of sterility mosaic disease (Table 2). Similarly, the Table 3 consisted of twenty four resistant and twenty four susceptible accessions for molecular characterization of fusarium wilt. Based on per cent protein content the genotypes were grouped as a high protein content and low protein content genotypes contrasting genotypes for the trait were selected for molecular characterization (Table 4).

The number of alleles, major allele frequency, gene diversity and polymorphic information content (PIC) for pigeonpea genotypes were estimated using Power marker Version 3.25 (Liu and Muse, 2005) and are presented in Tables 16, 17 and 18.

In order to understand the relationships among the accessions, genetic similarity matrix was estimated through unweighted pair group method with arithmetic mean (UPGMA) using DARwin V5.0.128 software. The cluster analysis based on UPGMA-radial dendrogram, revealed three major clusters which were further divided into sub clusters (Fig. 4, 5 and 6). The sub clusters contained both resistant and susceptible genotypes for sterility mosaic diseases, fusarium wilt and also for high and low protein content (Table 17, 18 and 19). Higher resolution in such clusters may be possible if sufficiently large numbers of markers are used for the study.

### 4.9.1 Molecular characterization of sterility mosaic disease

Out of 150 SSR markers, 62 markers generated amplicons in the accessions selected for sterility mosaic disease studies (Plate 1). Out of 62 amplified markers, 58 markers generated polymorphic amplicons. Markers CcM438, CcM207, CcM195 and CcM257 generated monomorphic amplicons. A total of 314 alleles were generated; with an average number of 5.06 alleles per marker in the accessions evaluated. Majority of markers amplified six alleles per marker. However, twelve alleles were observed for marker *viz.*, Cc15 and eight alleles were observed for markers *viz.*, Cc21, Cc26, Cc27 and Cc48. The major allele frequency (0.26) was least for the marker Cc27, Cc31 and Cc40. Gene diversity varied between 0 to 0.82 (Cc32). The Polymorphic Information Content value (PIC) ranged from 0.00 to 0.812 (Cc20) with an average PIC value of 0.53 per marker (Table 16).

The cluster analyses which considered the marker data for sterility mosaic disease clustered the genotypes into three major clusters A, B and C as provided in the Table19. Among the three major clusters, sub cluster I-A with 11 accessions, Cluster II-A with 5 accessions and cluster III-A with 9 accessions were found resistant. Similarly the sub cluster I-B which contained 10accessions,

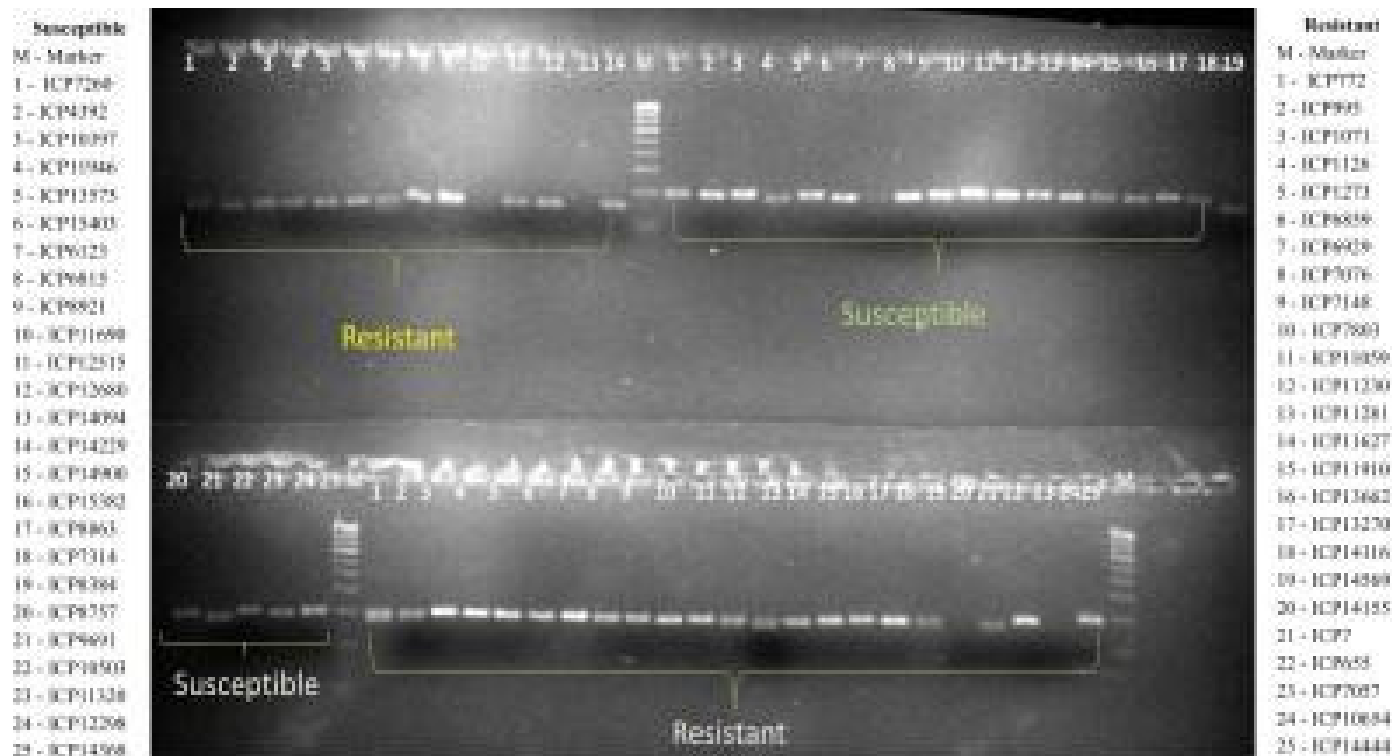


Plate 4. Gel Picture indicating polymorphism between SMD resistance and susceptible genotypes of pigeonpea (Primer Cc26)

**Table 14: Disease reaction of pigeonpea (*C.cajan*) accessions to sterility mosaic disease**

Sl. No.	ICP NO.	Score	Grade
1	348	MR	3
2	655	MR	3
3	772	S	7
4	939	R	1
5	995	R	1
6	1071	S	7
7	1156	R	1
8	2577	S	7
9	2698	R	1
10	2746	S	7
11	3046	R	1
12	3049	R	1
13	3451	HS	9
14	3576	R	1
15	4029	HS	9
16	4167	HS	9
17	4317	S	7
18	4392	R	1
19	5142	R	1
20	5863	HS	9
21	6049	HS	9
22	6123	HS	9
23	6128	HS	9
24	6668	HS	9
25	6739	R	1
26	6815	R	1
27	6859	HS	9
28	6929	R	1
29	6971	R	1
30	6992	S	7
31	7057	HS	9
32	7076	S	7
33	7148	R	1
34	7221	S	7
35	7223	S	7
36	7260	R	1
37	7314	S	7
38	7366	R	1
39	7426	S	7
40	7507	R	1
41	7803	HS	9
42	7869	S	7
43	8012	S	7
44	8255	R	1
45	8266	R	1
46	8384	MR	3

Contd.....

<b>Sl. No.</b>	<b>ICP NO.</b>	<b>Score</b>	<b>Grade</b>
47	8602	R	1
48	8757	MR	3
49	8840	R	1
50	8860	S	7
51	8921	R	1
52	8949	R	1
53	9045	R	1
54	9414	HS	9
55	9655	R	1
56	9691	R	1
57	9750	R	1
58	10094	HS	9
59	10228	R	1
60	10397	R	1
61	10447	R	1
62	10503	R	1
63	10559	HS	9
64	10654	S	7
65	11015	R	1
66	11059	R	1
67	11230	R	1
68	11281	HS	9
69	11320	S	7
70	11477	R	1
71	11627	MR	3
72	11690	R	1
73	11823	R	1
74	11833	HS	9
75	11910	R	1
76	11946	R	1
77	12105	R	1
78	12123	MR	3
79	12142	R	1
80	12298	R	1
81	12410	R	1
82	12515	R	1
83	12596	S	7
84	12654	R	1
85	12680	R	1
86	13139	R	1
87	13167	R	1
88	13191	S	7
89	13244	R	1
90	13270	R	1
91	13304	R	1
92	13359	R	1
93	13431	R	1
94	13571	MR	3
95	13575	MS	5
96	13577	R	1

*Contd.....*

SI. No.	ICP NO.	Score	Grade
97	13579	MS	5
98	13633	R	1
99	13662	R	1
100	13884	S	7
101	14116	R	1
102	14147	MS	5
103	14229	S	7
104	14294	R	1
105	14368	R	1
106	14444	R	1
107	14471	R	1
108	14569	MR	3
109	14638	R	1
110	14701	R	1
111	14722	S	7
112	14801	R	1
113	14819	S	7
114	14832	S	7
115	14900	MS	5
116	14976	R	1
117	15049	R	1
118	15068	R	1
119	15161	R	1
120	15185	R	1
121	15382	R	1
122	16264	R	1
123	4575	MR	3
124	BRG-7-3	R	1
125	BRG-6-12	R	1
126	1730	MR	3
127	4019	MR	3
128	BRG-9-2	R	1
129	BRG-207	R	1
130	TTB-7	R	1
131	TM224	R	1
132	1224	R	1
133	BRG-7-5	R	1
134	TM-26	R	1
135	5641	R	1
136	12460	R	1
137	1550	R	1
138	DPD-2-38	R	1
139	12333	MS	5
140	10804	R	1
141	12674	S	7
142	Gullyal local	R	1
143	4878	S	7
144	PUSA992	R	1
145	6528	R	1
146	Gullyal red	S	7

Contd.....

Sl. No.	ICP NO.	Score	Grade
147	HY-3-C	S	7
148	12266	R	1
149	9903	R	1
150	1366	R	1
151	4395	S	7
152	UPAS-120	R	1
153	BRG-9-2	R	1
154	TS-3@	S	7
155	DM-155	S	7
156	TM-389	R	1
157	BRG-7-2	S	7
158	GC-11-39	R	1
159	12301	S	7
160	BRG-7-4	R	1
161	GRG-2-95	R	1
162	12674	R	1
163	4275	R	1
164	12282	MR	3
165	13270	R	1
166	BRG-7-6	R	1
167	DM-158	R	1
168	ICPL-87	R	1
169	12692	R	1
170	Jamadhar local	R	1
171	WRP-1	S	7
172	Bidar local	R	1
173	UPAS-120	R	1
174	JKE-144	R	1
175	PUSA-92	R	1
176	8863	S	7
177	PG-12	S	7
178	MA-29	R	1
179	Karithogari	R	1
180	ICPB 2091	R	1
181	CORG-295	R	1
182	Chaple	R	1
183	Gulyal white	S	7
184	CORG-9701	R	1
185	WRP-1 (W)	R	1
186	Rudrawadi local	R	1
187	JKM-197	R	1
188	CORG-2004-01	R	1
189	PJT-501	HS	9
190	GRG-801	R	1
191	Bennur local	MR	3
192	GRG-206	MR	3

**Table 15: Disease reaction of 192 Pigeon pea accessions to Sterility mosaic disease**

Sl. No.	Disease reaction	Disease grade	Number of accessions	Accessions list
1	Resistant (0 per cent)	1	121	ICP939, ICP995, ICP1156, ICP2698, ICP3046, ICP3049, ICP3576, ICP4392, ICP5142, ICP6739, ICP6815, ICP6929, ICP6971, ICP7148, ICP7260, ICP7366, ICP7507, ICP8255, ICP8266, ICP8602, ICP8840, ICP8921, ICP8949, ICP9045, ICP9655, ICP9691, ICP9750, ICP10228, ICP10397, ICP10447, ICP10503, ICP11015, ICP11059, ICP11230, ICP11477, ICP11690, ICP11823, ICP11910, ICP11946, ICP12105, ICP12142, ICP12298, ICP12410, ICP12515, ICP12654, ICP12680, ICP13139, ICP13167, ICP13244, ICP13270, ICP13304, ICP13359, ICP13431, ICP13577, ICP13633, ICP13662, ICP14116, ICP14294, ICP14368, ICP14444, ICP14471, ICP14638, ICP14701, ICP14801, ICP14976, ICP15049, ICP15068, ICP15161, ICP15185, ICP15382, ICP16264, BRG-7-3, BRG-6-12, BRG-9-2, BRG-207, TTB-7, TM224, ICP1224, BRG-75, TM-26, ICP5641, ICP12460, ICP1550, DPD-2-38, ICP10804, GULLYAL LOCAL, ICP6528, ICP12266, ICP9903, ICP1366, UPAS-120, TM-389, GC-11-39, BRG-7-4, GRG-2-95, ICP12674, ICP4275, ICP13270, BRG-7-6, DM-158, ICPL-87, ICP12692, JAMADHAR LOCAL, BIDABR LOCAL, JKE-144, PUSA-92, MA-29, KARITHOGARI, ICPB2091, CORG-295, CHAPLE, CORG-9701, WRP-1 (W), RUDRAWADI LOCAL, JKM-197, CORG-2004-01, GRG-801
2	Moderately resistant (0-10 per cent)	3	14	ICP348, ICP655, ICP8384, ICP8757, ICP11627, ICP12123, ICP13571, ICP14569, ICP4575, ICP1730, ICP4019, ICP12282, BENNUR LOCAL, GRG-206
3	Moderately susceptible (10-20 per cent)	5	4	ICP13579, ICP14147, ICP14900, ICP12333
4	Susceptible (20-50 per cent)	7	37	ICP772, ICP1071, ICP2577, ICP2746, ICP4317, ICP6992, ICP7076, ICP7221, ICP7223, ICP7314, ICP7426, ICP7869, ICP8012, ICP8860, ICP10654, ICP11320, ICP12596, ICP13191, ICP13884, ICP14229, ICP14722, ICP14819, ICP14832, ICP12674, ICP4878, PUSA992, GULLYAL RED, HY-3-C, ICP4395, TS-3, DM-155, BRG-7-2, ICP12301, WRP-1, ICP8863, PG-12, GULLYAL WHITE
5	Highly susceptible (More Than 50 per cent)	9	16	ICP3451, ICP4029, ICP4167, ICP5863, ICP6049, ICP6123, ICP6128, ICP6668, ICP6859, ICP7057, ICP7803, ICP9414, ICP10094, ICP10559, ICP11281, ICP11833

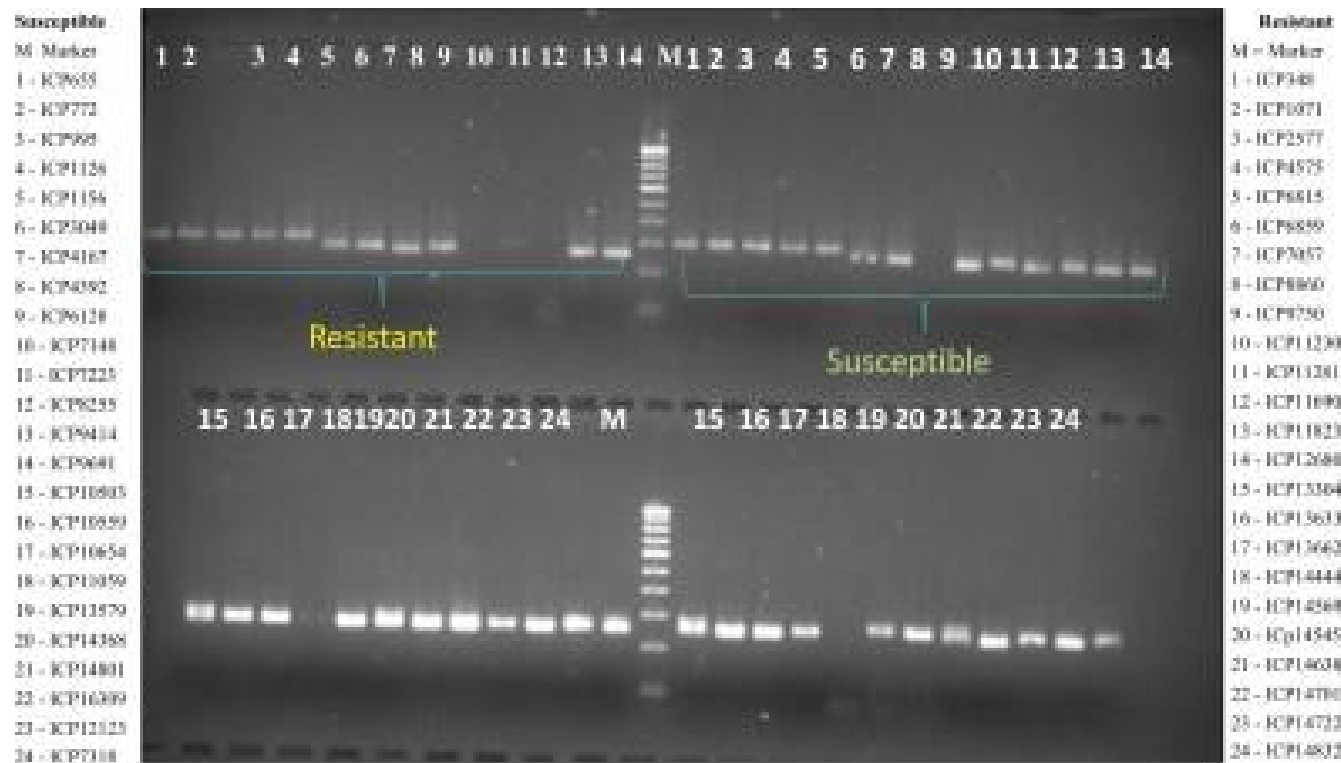


Plate 5. Gel Picture indicating polymorphism between FW resistance and susceptible genotypes of pigeonpea (primer Cc22)

cluster II-B which contained 3 accessions and cluster III-B which contained 7 accessions were found susceptible. The major cluster C which diverged to form a separate cluster consisted of 5 accessions which also were found susceptible.

#### 4.9.2 Molecular characterization of fusarium wilt

Out of 150 SSR markers, 41 markers generated amplicons in the 48 selected accessions for fusarium wilt studies (Plate 2). 37 out of 41 markers generated amplified products which were polymorphic. CcM627, CcM137, Cc42 and Cc44 generated monomorphic amplicons. A total of, 163 alleles were generated from the markers; with an average number of 3.97 alleles per marker in the accessions evaluated. However, 11 alleles were observed for marker *viz.*, Cc15 and eight alleles were observed for markers *viz.*, Cc12, Cc40 and CcM185. Majority of the markers amplified three alleles per marker. The major allele frequency (0.31) was least for the marker Cc40. Gene diversity varied from 0 to 0.79 (Cc40). The Polymorphic Information Content value (PIC) ranged from 0.00 to 0.77 (Cc40) with an average PIC value of 0.43 per marker (Table 17).

The cluster analysis based on the marker data for fusarium wilt clustered the genotypes into three major clusters A, B and C as provided in the Table 20. Among the three major clusters, sub cluster I-A with 15 accessions, Cluster II-A with 9 accessions and cluster III-A with 1 accessions were found resistant. Similarly the sub cluster I-B which contained 9 accessions, cluster II-B which contained 11 accessions and cluster III-B which contained 2 accessions were also found susceptible. The major cluster C which diverged to form a separate cluster consisted of a lone accession which was found susceptible.

#### 4.9.3 Molecular characterization of protein content

The results revealed that out of 150 SSR markers selected for the present study, 46 markers generated amplification for the 46 genotypes selected for protein content studies (Plate 3). 45 out of 46 markers generated polymorphic amplicons, while marker CcM445 generated monomorphic amplicons. These polymorphic markers amplified a total of 213 alleles with an average of 4.63 alleles per marker in the accessions evaluated. Majority of the markers amplified five alleles per marker. However, 12 alleles were observed for marker *viz.*, Cc15 and 8 alleles were observed for marker Cc13. Major allelic frequency was least (0.24) for Cc31. Maximum gene diversity (0.81) generated by the marker Cc12. The Polymorphic Information Content value (PIC) value ranged from 0.00 to 0.78 (Cc12 & Cc27) with an average PIC value of 0.53 per marker (Table 18).

The cluster analysis which took into consideration the alleles generated for protein content clustered the genotypes into three major clusters A, B and C as provided in the Table.21. The first cluster A, subdivided into three sub clusters *viz.*, sub cluster I-A consisted of 8 accessions, cluster II-A which consisted of 3 accessions along with 10 accessions of cluster III A had low protein content. The clusters I-B (13 accessions), II-B (2 accessions), I-C (5 accessions) and II –C (3 accessions) recorded high protein content. The molecular characterization for protein content could differentiate the accessions with high protein and low protein content into divergent groups (Fig. 6.)

### 4.10 Molecular identification of alleles

In the present study molecular investigation was undertaken to identify the existence of alleles associated with resistant and susceptible pigeonpea genotypes with respect to SMD and FW and also high and low protein content genotypes. PCR amplified products of the primers were subjected to gel electrophoresis using 3 per cent agarose gel in 1X TAE buffer. The fragment sizes which ranged from 150 to 200 were detected by comparing the amplicons with a 100 bp DNA ladder. The ethidium bromide stained gels were documented using UV Trans-illuminator (Cambridge gel documentation system). The amplified products were analyzed on agarose gel and the expected sizes of amplicons were selected.

#### 4.10.1 Alleles identified for sterility mosaic disease

Out of 150 SSR markers used, six markers generated alleles which were specific for sterility mosaic disease resistance and susceptibility (Table 22). The markers Cc15 and CcM181 generated alleles of size at 180 bp (resistant) and 200 bp (susceptible) respectively (Fig. 7).

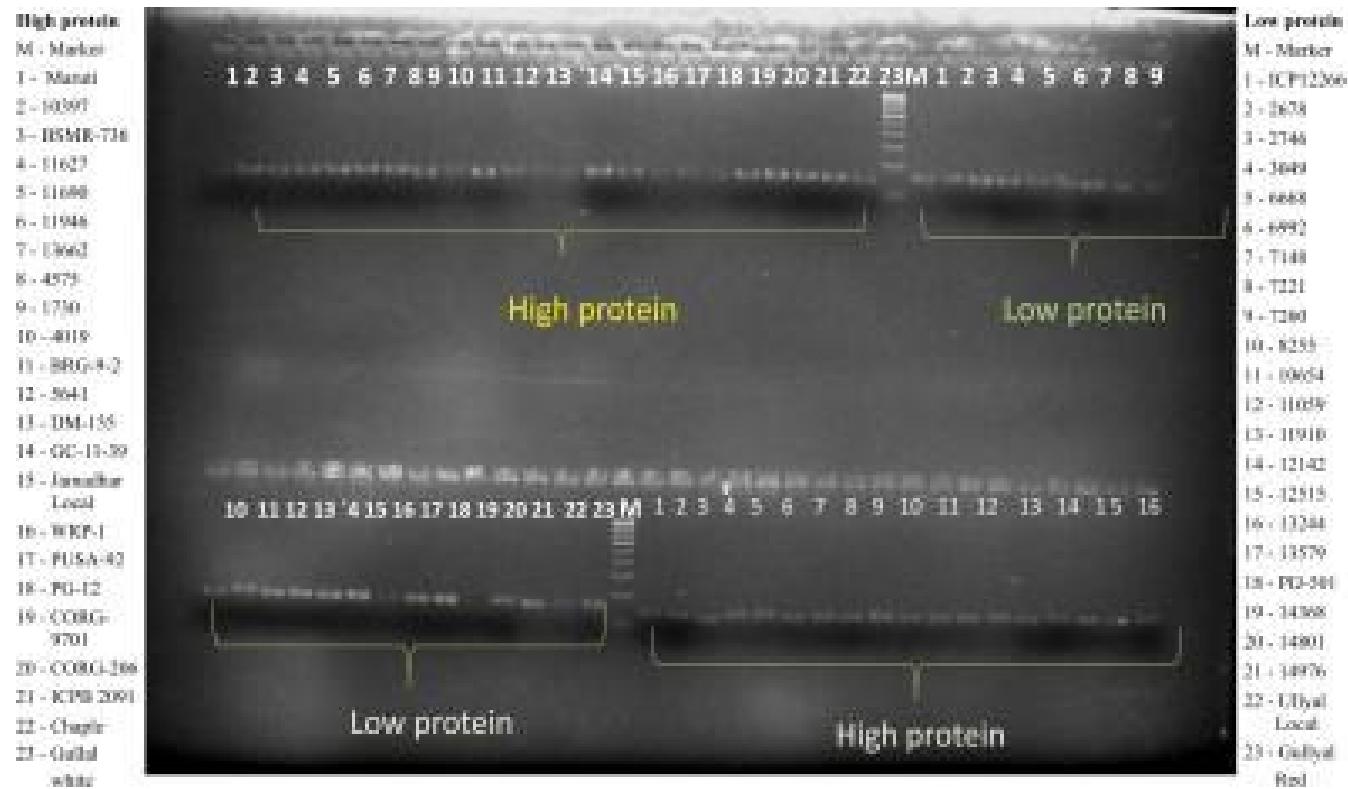


Plate 6. Gel Picture indicating polymorphism among the high and low protein content of pigeonpea (primer Cc22)

**Table 16: Major allele frequency, gene diversity and Polymorphic Information Content of 50 genotypes of pigeonpea (*Cajanus cajan*) for sterility mosaic disease**

Marker	Major Allele Frequency	Sample size	Allele No.	Gene Diversity	PIC
Cc7	0.84	50	3	0.27	0.25
Cc8	0.46	50	5	0.68	0.63
Cc9	0.56	50	3	0.57	0.49
Cc10	0.34	50	6	0.76	0.73
Cc11	0.46	50	4	0.60	0.52
Cc12	0.28	50	9	0.82	0.80
Cc13	0.48	50	9	0.72	0.70
Cc14	0.62	50	6	0.58	0.55
Cc15	0.44	50	12	0.77	0.75
Cc16	0.56	50	3	0.52	0.42
Cc17	0.60	50	3	0.56	0.50
Cc18	0.58	50	6	0.62	0.58
Cc19	0.42	50	6	0.69	0.64
Cc20	0.28	50	9	0.83	0.81
Cc21	0.48	50	8	0.71	0.68
Cc22	0.56	50	6	0.63	0.59
Cc23	0.32	50	5	0.76	0.72
Cc24	0.40	50	4	0.66	0.59
Cc25	0.40	50	6	0.72	0.68
Cc26	0.36	50	8	0.79	0.77
Cc27	0.26	50	8	0.81	0.78
Cc28	0.36	50	7	0.77	0.73
Cc29	0.52	50	7	0.67	0.64
Cc30	0.46	50	6	0.70	0.66
Cc31	0.26	50	7	0.82	0.80
Cc32	0.36	50	6	0.71	0.66
Cc34	0.34	50	7	0.78	0.75
Cc35	0.30	50	7	0.80	0.77
cc36	0.76	50	3	0.37	0.32
Cc37	0.42	50	6	0.74	0.70
Cc38	0.52	50	4	0.54	0.43
Cc39	0.48	50	6	0.66	0.61
Cc40	0.26	50	9	0.83	0.80

Contd....

<b>Marker</b>	<b>Major. Allele. Frquency</b>	<b>Sample size</b>	<b>Allele No.</b>	<b>Gene Diversity</b>	<b>PIC</b>
Cc41	0.44	50	6	0.66	0.60
Cc42	0.84	50	4	0.28	0.27
Cc43	0.52	50	6	0.64	0.58
Cc44	0.64	50	4	0.55	0.51
Cc45	0.38	50	7	0.76	0.72
Cc46	0.40	50	5	0.68	0.62
Cc47	0.36	50	6	0.74	0.70
Cc48	0.34	50	8	0.79	0.77
Cc49	0.30	50	6	0.76	0.73
Cc50	0.42	50	4	0.66	0.59
CcM181	0.36	50	7	0.76	0.73
CcM183	0.58	50	5	0.58	0.52
CcM185	0.40	50	7	0.76	0.73
CcM121	0.68	50	5	0.49	0.44
CcM173	0.70	50	2	0.42	0.33
CcM176	0.60	50	2	0.48	0.36
CcM407	0.42	50	4	0.65	0.57
CcM413	0.62	50	2	0.47	0.36
CcM438	1.00	50	1	0.00	0.00
CcM468	0.60	50	2	0.48	0.36
CcM445	0.54	50	2	0.50	0.37
CcM727	0.50	50	4	0.57	0.48
CcM095	0.84	50	2	0.27	0.23
CcM207	1.00	50	1	0.00	0.00
CcM195	1.00	50	1	0.00	0.00
CcM257	1.00	50	1	0.00	0.00
CcM273	0.90	50	2	0.18	0.16
CcM271	0.96	50	2	0.08	0.07
CcM293	0.98	50	2	0.04	0.04
<b>Mean</b>	<b>0.53</b>	<b>50</b>	<b>5.06</b>	<b>0.58</b>	<b>0.53</b>

**Table 17: Major allele frequency, gene diversity and Polymorphic Information Content of 48 genotypes of pigeonpea (*Cajanus cajan*) for Fusarium wilt**

Marker	Major. Allele. Frquency	SampleSize	Allele No.	Gene Diversity	PIC
Cc7	0.85	48	3	0.26	0.24
Cc8	0.60	48	4	0.56	0.49
Cc9	0.52	48	3	0.57	0.47
Cc10	0.36	48	5	0.74	0.70
Cc11	0.46	48	4	0.59	0.50
Cc12	0.35	48	8	0.79	0.76
Cc13	0.50	48	9	0.71	0.68
Cc14	0.69	48	6	0.50	0.48
Cc15	0.48	48	11	0.74	0.72
Cc16	0.52	48	3	0.52	0.40
Cc20	0.58	48	7	0.62	0.59
Cc21	0.60	48	6	0.60	0.58
Cc25	0.79	48	4	0.36	0.33
Cc29	0.75	48	4	0.41	0.39
Cc31	0.60	48	3	0.51	0.42
Cc32	0.38	48	3	0.66	0.59
Cc34	0.74	48	2	0.39	0.31
Cc35	0.33	48	5	0.72	0.66
Cc37	0.49	48	5	0.66	0.60
Cc38	0.83	48	3	0.29	0.27
Cc39	0.44	48	5	0.65	0.58
Cc40	0.31	48	8	0.80	0.77
Cc41	0.51	48	3	0.55	0.46
Cc42	1.00	48	1	0.00	0.00
Cc43	0.59	48	4	0.57	0.52
Cc44	1.00	48	1	0.00	0.00
Cc45	0.57	48	3	0.58	0.52
Cc46	0.96	48	2	0.08	0.08
Cc47	0.64	48	3	0.52	0.46
Cc48	0.82	48	2	0.29	0.25
Cc50	0.45	48	3	0.64	0.56
CcM21	0.96	48	2	0.07	0.07
CcM47	0.64	48	3	0.52	0.46
CcM095	0.53	48	2	0.50	0.37
CcM133	0.51	48	3	0.52	0.41
CcM134	0.75	48	3	0.41	0.37
CcM137	1.00	48	1	0.00	0.00
CcM179	0.36	48	5	0.73	0.69
CcM181	0.88	48	2	0.21	0.19
CcM185	0.38	48	8	0.77	0.74
CcM627	1.00	48	1	0.00	0.00
<b>Mean</b>	<b>0.63</b>	<b>48.00</b>	<b>3.98</b>	<b>0.48</b>	<b>0.43</b>

**Table 18: Major allele frequency, gene diversity and Polymorphic Information Content of 46 genotypes of pigeonpea (*Cajanus cajan*) for Protein content**

Marker	Major Allele Frquency	Sample size	Allele No.	Gene Diversity	PIC
Cc4	0.39	46	4	0.67	0.61
Cc5	0.39	46	5	0.68	0.61
Cc6	0.41	46	4	0.66	0.59
Cc7	0.84	46	2	0.26	0.23
Cc8	0.60	46	4	0.55	0.49
Cc9	0.54	46	3	0.57	0.49
Cc10	0.35	46	5	0.75	0.70
Cc11	0.46	46	3	0.58	0.48
Cc12	0.30	46	9	0.81	0.79
Cc13	0.48	46	8	0.71	0.68
Cc14	0.67	46	6	0.51	0.48
Cc15	0.48	46	12	0.74	0.72
Cc16	0.57	46	3	0.53	0.43
Cc17	0.57	46	3	0.59	0.52
Cc18	0.46	46	5	0.69	0.64
Cc19	0.46	46	5	0.66	0.60
Cc20	0.43	46	7	0.74	0.70
Cc21	0.40	46	6	0.76	0.72
Cc22	0.33	46	5	0.78	0.74
Cc24	0.44	46	5	0.70	0.66
Cc26	0.38	46	6	0.75	0.72
Cc27	0.26	46	7	0.81	0.78
Cc28	0.39	46	6	0.75	0.72
Cc29	0.54	46	6	0.66	0.62
Cc30	0.54	46	5	0.63	0.58
Cc31	0.24	46	6	0.81	0.78
Cc32	0.38	46	6	0.74	0.69
Cc34	0.92	46	2	0.15	0.14
Cc37	0.56	46	5	0.63	0.59
Cc40	0.80	46	3	0.34	0.31
Cc41	0.49	46	5	0.64	0.58
Cc42	0.95	46	2	0.09	0.09
Cc43	0.46	46	5	0.64	0.57
Cc44	0.77	46	3	0.38	0.35
Cc45	0.35	46	7	0.76	0.72
Cc46	0.54	46	4	0.56	0.47
Cc47	0.44	46	5	0.70	0.65
Cc48	0.32	46	7	0.78	0.74
Cc49	0.38	46	5	0.68	0.62
Cc50	0.61	46	3	0.52	0.44
CcM444	0.95	46	2	0.10	0.09
CcM445	1.00	46	1	0.00	0.00
CcM516	0.73	46	2	0.39	0.31
CcM529	0.86	46	2	0.24	0.21
CcM601	0.78	46	2	0.35	0.29
CcM657	0.77	46	2	0.36	0.29
<b>Mean</b>	<b>0.54</b>	<b>46</b>	<b>4.63</b>	<b>0.57</b>	<b>0.53</b>

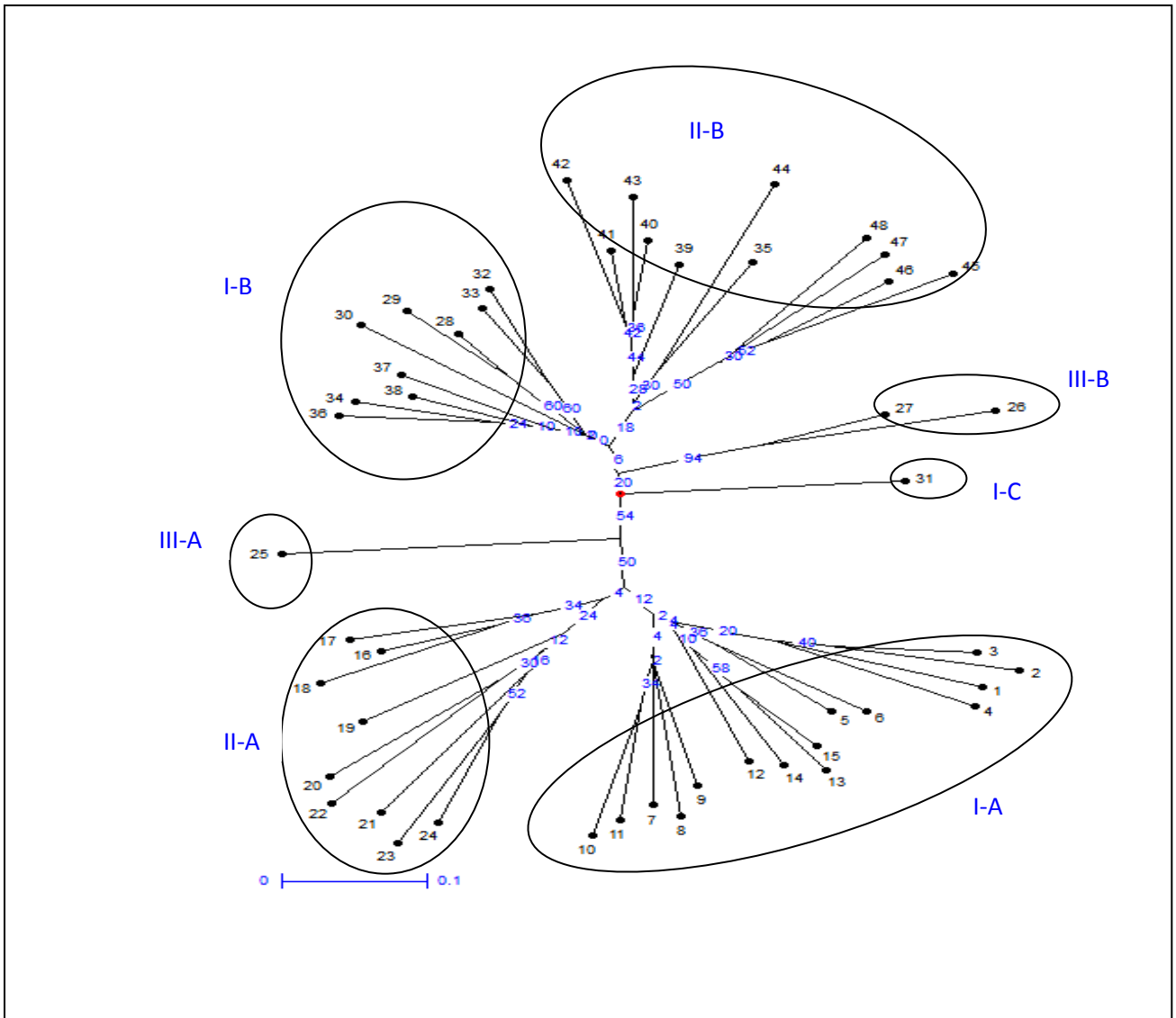
**Table 19: Cluster distribution of 50 pigeonpea (*Cajanus cajan.*) accessions based on molecular characterization for sterility mosaic disease**

Major cluster	Sub cluster	No. of genotypes	Cluster composition
A Resistant genotypes	I-A	11	ICP6859, ICP1273, ICP995, ICP772, ICP6929, ICP7148, ICP1071, ICP1126, ICP11230, ICP7803, ICP7076
	II-A	5	ICP13662, ICP11910, ICP11627, ICP11281, ICP11059
	III-A	9	ICP7057, ICP7, ICP14515, ICP14444, ICP10654, ICP655, ICP14569, ICP14116, ICP13270
B Susceptible genotypes	I-B	10	ICP7314, ICP8863, ICP15382, ICP14900, ICP14229, ICP11690, ICP6815, ICP8921, ICP14094, ICP094
	II-B	3	ICP6123, ICP15403, ICP13575
	III-B	7	ICP12298, ICP10503, ICP14368, ICP11320, ICP9691, ICP8757, ICP8384
C Susceptible genotypes	I-C	5	ICP4392, ICP7260, ICP10397, ICP12680, ICP11946



**Table 20: Cluster distribution of 48 pigeonpea (*Cajanus cajan.*) accessions based on molecular characterization for Fusarium wilt**

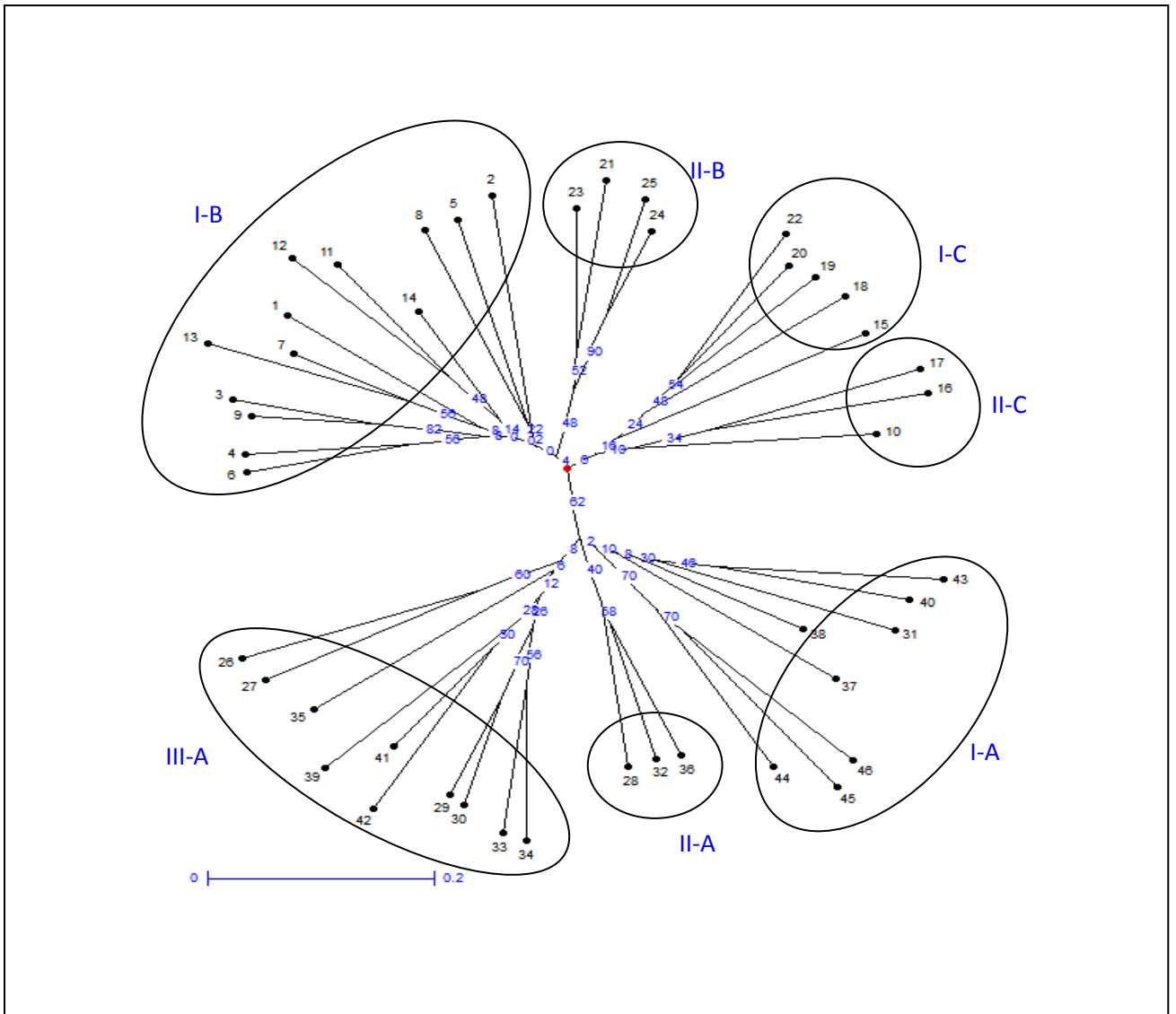
Major cluster	Sub cluster	No. of genotypes	Cluster composition
A Resistance genotypes	I-A	15	ICP2577, ICP1071, ICP348, ICP4575, ICP6859, ICP6815, ICP11823, ICP12515, ICP11690, ICP9750, ICP8860, ICP7057, ICP11281, ICP11230, ICP14801,
	II-A	9	ICP14832, ICP14722, ICP14638, ICP14701, ICP14545, ICP14569, ICP14444, ICP13633, ICP13662
	III-A	1	ICP655
B Susceptible genotypes	I-B	9	ICP9691, ICP7148, ICP9691, ICP9414, ICP3049, ICP1156, ICP1126, ICP6128, ICP4392,
	II-B	11	11059, ICP10654, ICP13579, ICP10397, ICP10503, ICP14368, ICP7223, ICP7314, ICP12123, ICP16309, ICP14801
	II-B	2	ICP995, ICP772
C Susceptible genotypes	I-C	1	ICP 4167



**Fig. 5: Radial-Dendrogram generated for fusarium wilt disease using 48 genotypes of minicore collection set of pigeonpea (*C. cajan*)**

**Table 21: Cluster distribution of 46 pigeonpea (*Cajanus cajan.*) accessions based on molecular characterization for Seed Protein content**

Major cluster	Sub cluster	No. of genotypes	Cluster composition
A Low protein	I-A	8	Gullyal local, ICP14368, ICP7260, ICP13244, ICP12515, PJT-501, ICP12266, Gullyal red,
	II-A	3	ICP12142, ICP8255, ICP6992
	III-A	10	ICP11059, ICP10654, ICP7221, ICP7148, ICP14976, ICP14801, ICP13579, ICP11910, ICP6668, ICP3049
B High Protein	I-B	13	GC-11-39, BRG-9-2, PUSA-92, ICP1730, CORG-9701, jamadhar local, maruti, gullyal white, chaple, CORG-206, WRP-1, ICP5641, BSMR-736
	II-B	4	ICP4575, ICP11996, ICP2746, ICP2678
C High Protein	I-C	5	ICP13662, ICP11690, ICP11627, ICP10397, ICP4019
	II-C	3	ICPB2091, DM-155, PG-12



**Fig. 6:** Radial-Dendrogram generated for seed protein using 46 genotypes of mini core collection set of pigeonpea (*C. cajan*)

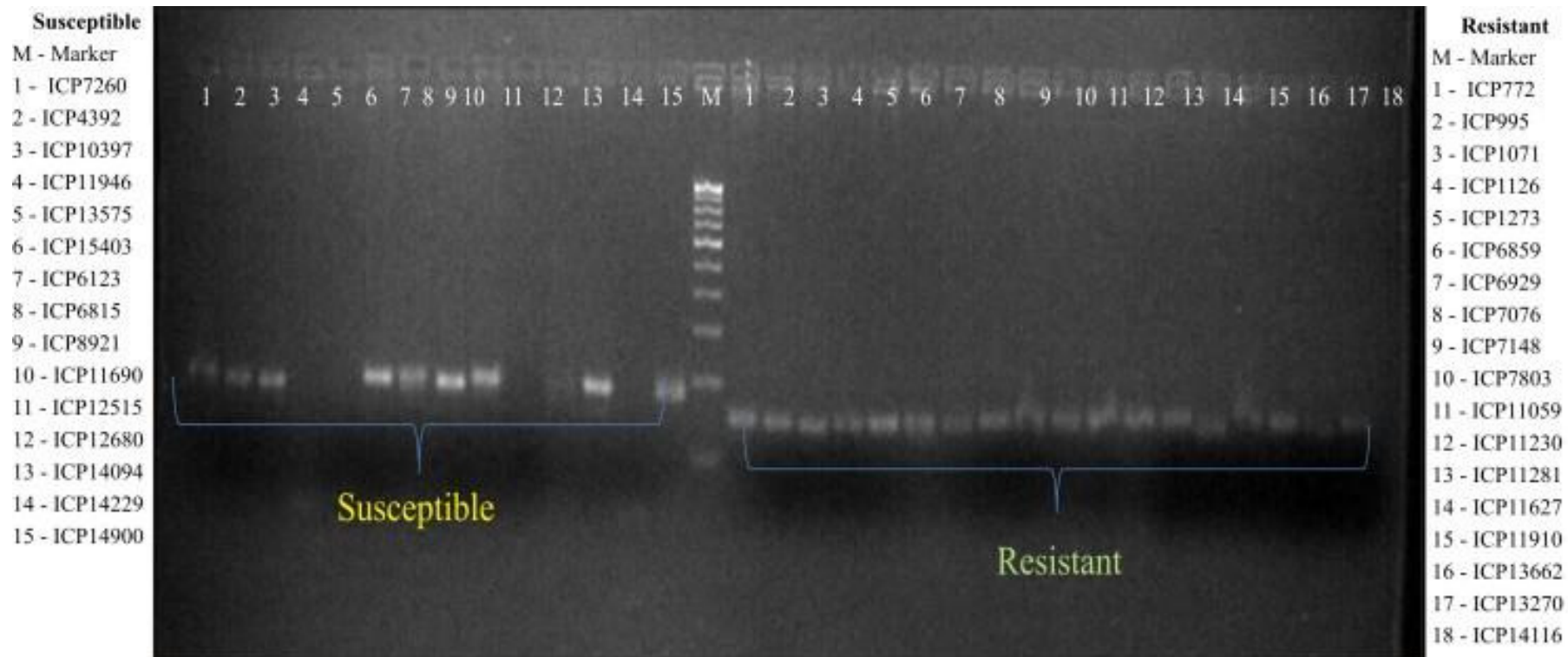


Fig 7. The markers Cc15 generated allele's size at 180 bp (resistant) and 200 bp (susceptible) for sterility mosaic disease

**Table 22: Alleles identified for Sterility mosaic disease**

Sl. No.	Marker	Resistant	Susceptible
		Allele size (bp)	
1	Cc12	160	190
2	Cc13	160	200
3	Cc14	150	180
4	Cc15	180	200
5	CcM181	180	200
6	CcM185	180	190

**Table 23: Alleles identified for Fusarium wilt**

Sl. No.	Marker	Resistant	Susceptible
		Allele size (bp)	
1	Cc9	150	180
2	Cc12	160	190
3	Cc13	160	200
4	Cc14	180	200
5	CcM181	160	200
6	CcM185	180	190

**Table 24: Alleles identified for Protein content**

Sl No.	Marker	Low protein	High protein
		Allele size (bp)	
1	Cc9	180	200
2	Cc10	180	200
3	Cc12	160	190
4	Cc13	160	200
5	Cc14	170	200
6	Cc15	150	180
7	Cc26	190	200

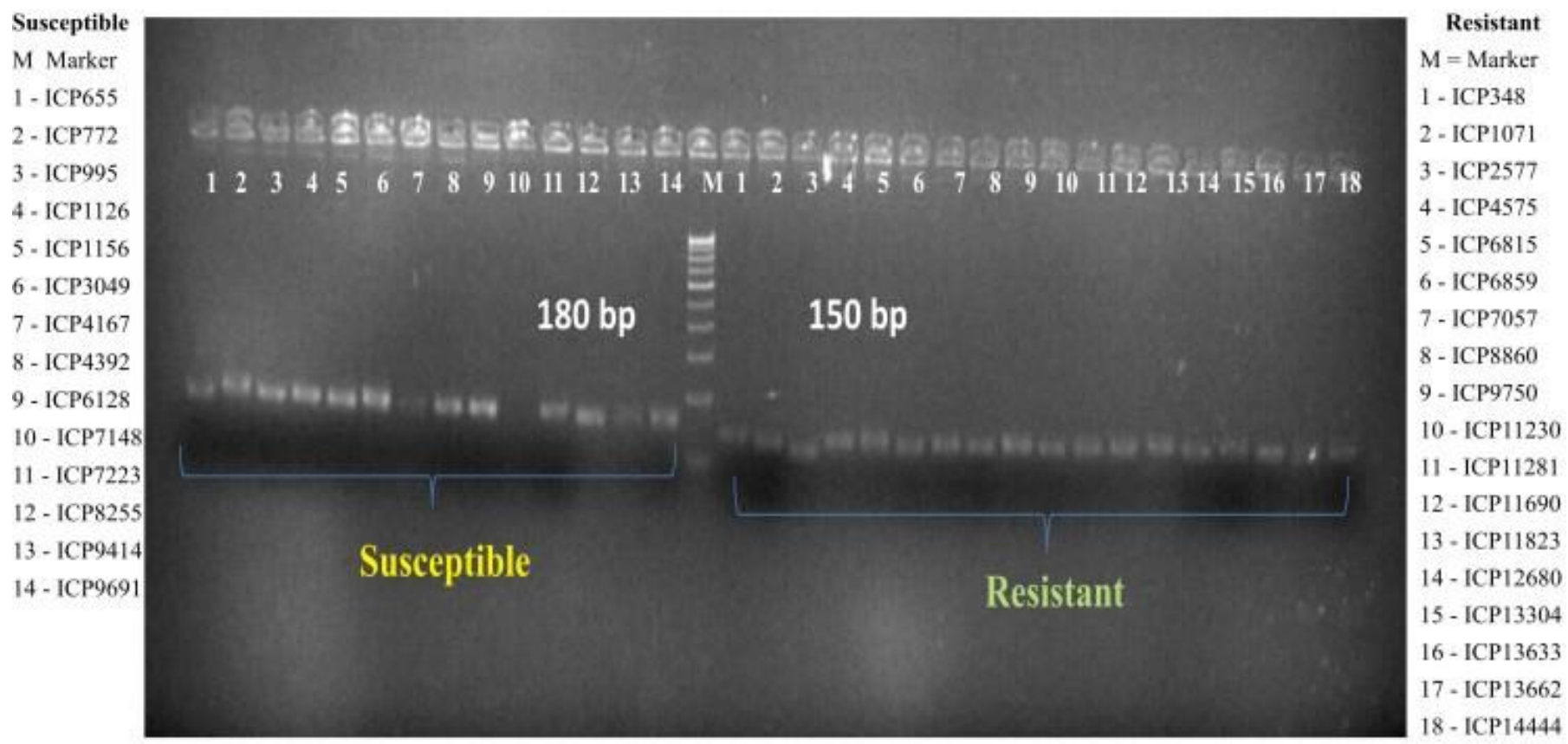


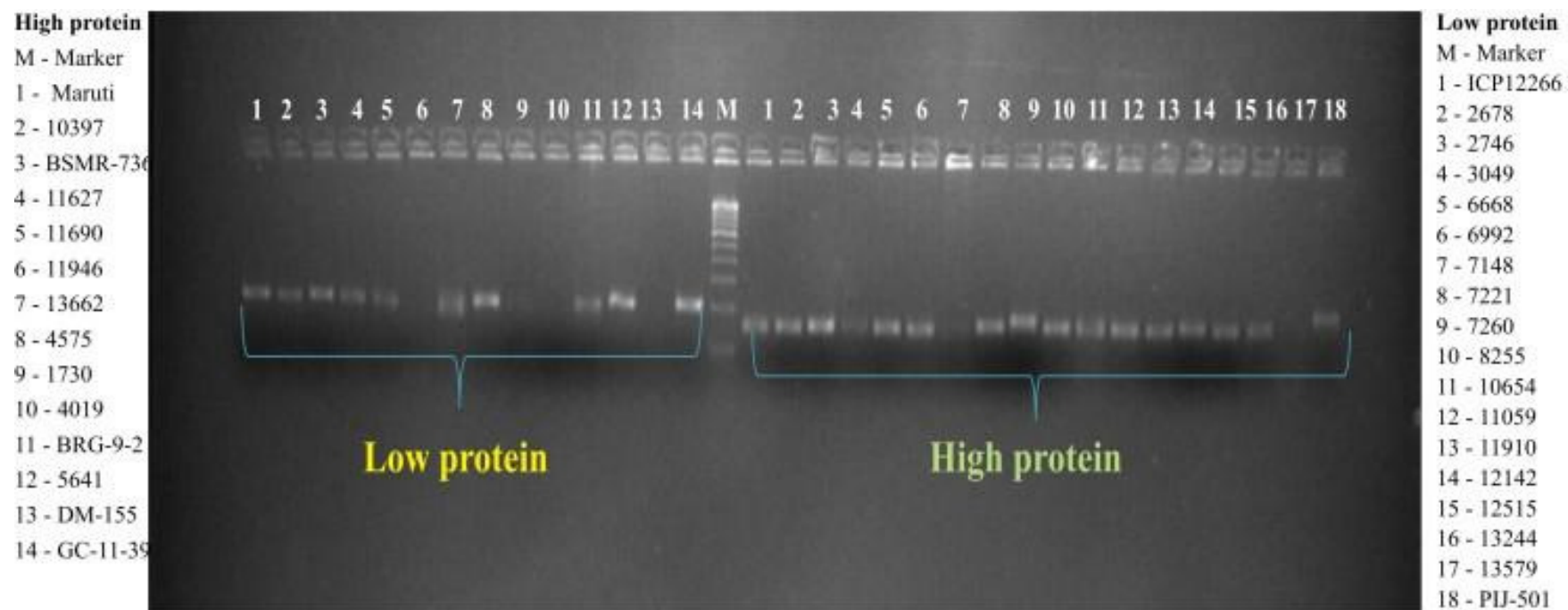
Fig 8. The marker Cc9 generated allele's size at 150 bp (resistant) and 180 bp (susceptible) for *fusarium* wilt

#### 4.10.2 Alleles identified for fusarium wilt

Out of 150 SSR markers, six markers yielded alleles which were associated with fusarium wilt resistant and susceptible accessions (Table 23). Cc9 generated alleles of size at 150 bp (resistant) and 180 bp (susceptible) (Fig. 8).

#### 4.10.3 Alleles identified for protein content

The results revealed that out of 150 SSR markers selected for the present study, seven markers generated specific alleles for the 46 genotypes selected for protein content studies (Table 24). Two markers viz., Cc9 and Cc15 generated polymorphic allele sizes at 180 bp (high protein) and 200 bp (low protein) respectively (Fig. 9). Maximum gene diversity (0.81) was generated by the marker Cc12. The maximum Polymorphic Information Content value (PIC) was observed for the marker Cc12.



**Fig 9. The marker Cc15 generated polymorphic alleles size at 180 bp (high protein) and 200 bp (low protein) for protein content**

## DISCUSSION

The aim of plant breeding programme is to improve the plant traits for agronomic and commercial superiority. Enhancement in productivity of any crop rests on the availability of variable material or germplasm for exploiting them in the breeding programmes and successful determination of breeding value. Phenotypic variance measures the degree of variation arising out of differences in phenotypic values while, the genotypic variance measures the magnitude of inherent variation due to differences in genotypic value.

Heritability assessments aid in determining the relative amount of heritable percentage of variation. Heritability value itself does not indicate the amount of genetic progress that would result from selecting the best individuals. Ramanujam and Thirumalachar (1967), while studying the genetic variability in red pepper, discussed the boundaries of assessing the heritability in broad sense as it includes both additive and non-additive gene effects. They concluded that heritability estimates in broad sense accompanied with genetic advance would be more reliable.

Yield is a complex character and is a function of numerous contributory characters and their interaction with environment. Direct selection based on yield alone will not be very effective in crop improvement programmes. Grafius (1964) pointed out that structure of yield probed through its components and their inter relationship along with yield and their direct and indirect contribution to yield is of immense importance. Falconer (1981) said that it assists in selection procedure to strike a balance when two opposite desirable characters affecting the principal character are being selected. It also helps to improve direct characters simultaneously.

Path analysis by Dewey and Lu (1959) partitions the cause and effect relationship into direct and indirect effects, which make up correlation coefficient.

In the present investigation, an attempt was made to assess the amount of variability, broad sense heritability, environmental, genotypic and phenotypic variances, predicted genetic advance, GCV and PCV with respect to 14 characters. The association among these characters and their direct and indirect effect on seed yield has been studied. Also Mahalanobis'  $D^2$  analysis to assess the genetic diversity among the 192 pigeonpea accessions is attempted. An attempt to screen the genotypes for *Helicoverpa armigera* and *Fusarium* wilt under natural conditions has also been made.

Selecting the genetically detached parents for hybridization is a basic need for synthesis of genotypes with desirable characters. Genetic diversity between genotypes indicates difference in the gene frequencies. Mahalanobis' generalized distance is the most widely used technique in plant breeding to know the distance between the genotypes. This statistical tool has been employed widely to resolve genetic divergence at inter varietal and sub species level in classifying crop plants (Rao, 1960). This is possible by clustering the entries based on  $D^2$  values, as it represents the index of genetic diversity among genotypes and clusters.

Knowledge on nature and extent of genetic variation, diversity available in the germplasm helps the breeder for planning sound breeding programmes. Thus, germplasm collection from diverse eco-geographical source and evaluation under uniform agro-ecological conditions for effective utilization is a pre-requisite and hence, this present investigation was undertaken as it includes the germplasm accessions from as many as 21 countries. A basic knowledge of number of genes governing the traits is essential for efficient selection. There are conflicting reports about the genetics of resistance to Sterility mosaic disease claiming both susceptibility and resistance to be dominant. However in most cases, susceptibility was shown to be dominant and resistance to be under the control of recessive genes (Singh *et al.*, 1983). The task of developing resistant varieties has been complicated in view of the reported genetic variability of the pathogen. This dynamic nature of the SMD has warranted the identification and use of isolate specific sources of resistance in the crop improvement programmes.

Although pigeonpea is drought tolerant, it is mainly affected by biotic stresses like fusarium wilt and sterility mosaic disease. The productivity can be enhanced by controlling these diseases to major extent. Since, both the diseases are difficult to control either physically or chemically. The development of resistant cultivars is only the best option to increase the productivity (Saxena *et al.*, 2010). Plants contain and employ hundreds of different defense genes, which act upon pathogen attack (Zhau *et al.*, 2004). Since plants and pathogens co evolve, every time a pathogen changes its mode of attack, the plant has to overcome the imposed challenge by developing a new mode of defense.

One of the means to improve yield is through exploitation of germplasm which might carry a resistant gene for various disease. Upadhyaya *et al.* (2010) carried an experiment, where among 192 accessions of pigeonpea mini core collection, 98 accessions showed resistance to fusarium wilt and 121 accessions showed resistance to sterility mosaic disease. It is essential to breed for a range of resistances to pathogenic organisms in order to reduce the need for chemical controls to a minimum and thus lower production costs, increase the nutritional value of agricultural products and improve the environment (Borojevic, 1990).

An extensive characterization of plant genetic resources provides an opportunity for structural dissection to mine the allelic variations and identify diverse accessions for crop improvement. These mini core collections can be used for molecular characterization to analyze genetic diversity at DNA level and to select distinct parents with maximizing diversity. The molecular characterization provides information related to rare alleles from cultivated and wild species accessions which could be used to select specific accessions for allele mining (Upadhyaya *et al.*, 2010).

Conventional breeding of pigeonpea has continued entirely without the aid of molecular methods and made limited use of germplasm resources, resulting in a very narrow genetic base in the domesticated species. As a consequence, pigeonpea genetic improvement programme have made relatively little progress in addressing the primary constraints to crop production, which includes a range of biotic (SMD, *Fusarium* wilt and pod boring insects) and abiotic stresses (drought, salinity and water logging). With the advent of next generation sequencing technologies several crop legumes have recently been subjected to intensive analysis, making marker assisted breeding a reality (Varshney *et al.*, 2006).

Pigeonpea is abundant in protein; making it an ideal supplement to traditional cereal, banana and tuber based diets for most people's which are generally protein deficient. Supplement of cereals with protein rich legumes is considered as one of the best solutions to protein calorie malnutrition in the developing world (Chitra *et al.*, 1996). Protein is an important part of human diet. Nitrogen that is used by the young seedling during germination is stored in the seed in the form of storage proteins. Seeds contain 16 to 50 per cent of protein and provide one third of all dietary protein nitrogen (Graham and Vance, 2003). Anticipating the increasing demand for protein food sources, the Protein Advisory Group of the United Nations has identified the improvement of legumes as a critically important area of research. The protein-rich legumes as a complement to cereals make one of the best solutions to protein-calorie malnutrition, particularly in developing countries.

Among the molecular markers, simple sequence repeats are one of the most promising markers used for validation. Microsatellites (Tautz and Rentz 1984), also known as Simple Sequence Repeat (SSR) markers, are DNA-based molecular markers that offer several advantages because they are reproducible, polymorphic, polymerase chain reaction (PCR)-based and readily portable within a species (Edwards *et al.*, 1996). Only 20 SSRs have been previously reported in pigeonpea, of which only half were polymorphic in cultivated pigeonpea germplasm (Burns *et al.*, 2001).

The results of the present investigations are discussed under the following subheads.

- 5.1 Analysis of variance
- 5.2 Genetic parameters
- 5.3 Evaluation of genotypes
- 5.4 Correlation studies
- 5.5 Path coefficient analysis for seed yield per ha
- 5.6 Genetic diversity
- 5.7 Evaluation of genotypes for *Fusarium* wilt and *Helicoverpa armigera*
- 5.8 Screening for sterility mosaic disease
- 5.9 Molecular characterization
- 5.10 Molecular identification of alleles

## 5.1 Analysis of variance

In the present investigation, significant variation was observed for seed yield and its yield attributing characters. This variation indicated substantial variation in the genotypes selected for the present investigation.

The degree of variability for many measurable traits including the seed yield accessible to breeder regulates the achievement that can be realized in the genetic enhancement of that species. Significant variation as indicated by the analysis of variance (Table 6) for entries (Ignoring Blocks) suggested the existence of adequate variability for all the phenological and productivity traits studied except for number of days taken from flowering to maturity, pods per plant, pod length number of primary branches per plant. The existence of appropriate variability for the characters *viz.*, plant height, seed yield per plant, days to fifty per cent flowering, days to maturity, test weight, specific gravity, straw yield, harvest index and seed yield per ha has shown that the choice of these traits will have significant bearing on crop improvement.

A unique means of evaluating the variability is through simple method of examining range of variation. Variability was maximum in seed yield per ha, straw yield, plant height, number of pods per plant, days to maturity, seed yield per plant, harvest index and days to 50 per cent flowering. A similar finding was reported for plant height, number of pods per plant, days to maturity, seed yield per plant, days to 50 per cent flowering, by Gohil (2006); Manyasa *et al.* (2008) and Shivani *et al.* (2010). Moderate variability was seen in number of primary branches, pod length, seeds per pod, test weight, number of days taken for flowering to maturity and specific gravity. Reports of Dhamelia and Pathak (1994) indicated moderate variability with respect to characters like days to 50 per cent flowering, days to maturity, plant height, pod length, number of seeds per pod and test weight in ten short duration pigeonpea varieties.

The existence of variability provides abundant chances to a breeder to pick up the genotypes according to his necessities or type of breeding programme. In the current study, the plant height recorded was of higher magnitude than the earlier studies conducted by Kimani *et al.* (1994); Vange and Egbe. (2009). In contrast, the reports of Sreelakshmi *et al.* (2010) and Kimani *et al.* (1994) indicated that the range of variation for plant height was of lower magnitude than the present study.

The mean values for the plant height, days to maturity, straw yield were of higher magnitude than reported by Kimani *et al.* (1994) and Sreelakshmi *et al.* (2010) but, were of lower magnitude than reported by Vange and Egbe. (2009). The mean value for number of branches, number of pods, days from flowering to maturity, test weight, harvest index, specific gravity were of moderate magnitude while, lower magnitude means were recorded for seed protein and seed yield. Mean values recorded by Bhadru (2010), were found similar to the results obtained for days to fifty per cent flowering (120.90) but, in the present investigation the mean values of pod length, number of seeds per pod and test weight was found higher than Bhadru (2010) studies. Similar significant genetic variations for the yield and yield attributing characters were reported by Deshmukh, *et al.* (2000); Venkateswarulu (2001); Anantharaju and Muthiah (2008); Bhadru (2010) and Hamid *et al.* (2011) in pigeonpea.

## 5.2 Genetic parameters

The awareness of phenotypic coefficient of variation and genotypic coefficient of variation is crucial to get an idea of heritability of the trait. The evidence on phenotypic coefficient of variation and heritability will be useful for estimation of the possible genetic advance by selection for the character. The heritability estimate is the measure of phenotypic variance attributable to genetic causes which has a good predictive purpose of breeding crops.

The genotypic coefficient of variation and genetic advance help to partition the total variability into heritable and non-heritable components. Usually, genetic enhancement for measurable traits can be realized through a strong understanding of the nature and extent of variability existing in the material and also extent to which the desirable traits are heritable (Venkateswarlu, 2001).

The degree of variability as quantified by genotypic coefficient of variation and phenotypic coefficient of variation also gives evidence concerning the relative amount of variation in different characters. A narrow variance between phenotypic coefficient of variation and genotypic coefficient of variation designate the insignificant impact of extraneous factor on the trait and hence the selection practised would result in superior genetic gain.

In the present study, 192 diverse genotypes of pigeonpea were studied to assess their genetic potential. All the genotypes exhibited substantial amount of differences in their mean performance with respect to all the characters studied except number of primary branches per plant and seeds per plant. This is evident by highly significant values for entries (ignoring blocks) and for the varieties, which specified that the accessions under study were genetically diverse and appropriately chosen.

#### Days to 50 per cent flowering

In the present study, moderate phenotypic and genotypic coefficients of variability were recorded for this trait, the gap between phenotypic and genotypic variance observed was also low, which indicated that there was less influence of environmental factors. High heritability coupled with high genetic advance as per cent of mean observed for this character is in agreement with the findings of earlier workers Vange and Egbe. (2009). It indicated that the trait was under the influence of additive gene action.

#### Number of days from flowering to maturity

Number of days from flowering to maturity recorded low phenotypic and genotypic coefficient of variance, the difference between phenotypic and genotypic variance observed was high, which indicated more influence of non-genetic factors. The broad sense heritability was moderate coupled with low genetic advance as per cent of mean indicated that trait was predominantly influenced by non additive gene action.

#### Days to maturity

Days to maturity registered moderate phenotypic and genotypic coefficient of variability for the trait, the close correspondence between phenotypic and genotypic variance indicated less influence of environmental factors. Higher heritability accompanied by higher genetic advance was observed for the trait. Which is suggestive of high response to appropriate selection procedures as indicated by additive gene action predominance and thus selection for this trait will result in maximum genetic gain.

Similar findings were recorded by Vange and Egbe who observed moderate phenotypic and genotypic coefficient of variability coupled with high heritability and moderate genetic advance. In contrast Venkateshwaralu (2001); Manyasa *et al.* (2008) observed high GCV, PCV observed for the trait.

#### Number of primary branches per plant

The genotypic and phenotypic coefficients of variability recorded were high but the observed difference for both phenotypic and genotypic variance was high this indicated that the trait was high influenced by environmental changes. The moderate heritability along with high genetic advance recorded for the trait indicated that number of primary branches was influenced by non additive gene action.

#### Plant height

Plant height showed low genotypic and phenotypic coefficients of variability. The difference between phenotypic and genotypic variance observed was high, which indicated more influence of non-genetic factors. High heritability coupled with high genetic advance as per cent of mean recorded for the trait indicated that plant height is reliable for selection as there was predominance of additive gene action. These results are in conformity with Bhadru (2010); Vange and Egbe (2009). In contrast, Vikas and Singh (1998) showed high GCV and PCV for plant height.

#### Number of pods per plant

Number of pods per plant showed high PCV and GCV. The difference between these variances was high which indicated that the trait was influenced by environmental factors. Moderate heritability coupled with high genetic advance was suggestive of influence of non additive gene action. The research results are in agreement with previous studies of Vikas and Singh (1998); Gohil (2006); Bhadru (2008); Bhadru (2010) and Udensi *et al.* (2011).

#### Pod length

The phenotypic and genotypic coefficients of variability observed for pod length were low. The observed difference for both phenotypic and genotypic variance was maximum, which indicated more influence of non-genetic factors. The results are in agreement with reports of Singh *et al.* (2010) and

Gohil (2006). In the present study non additive gene action was indicated as heritability estimate for the trait was high and genetic advance as per cent of mean was moderate for the character which is in conformity with Udensi *et al.*, studies (2011).

#### Number of seeds per pod

The phenotypic and genotypic coefficients of variability recorded for the number of seeds per pod was low. There was an indication of more influence of environmental factors as there was no close correspondence between the phenotypic and genotypic variance. The results were in agreement with earlier studies of Vange and Edge (2009) and Gohil (2006). Low heritability along with moderate genetic advance as per cent of mean was evident in the present study indicative of influence of non additive gene action.

#### Seed yield per plant

In the present study, GCV (42.19) and PCV (42.88) estimate were high for the seed yield per plant. The observed difference for both phenotypic and genotypic variance was very less which indicated that the trait was less influenced by environmental factors. A high heritability (96.8) in broad sense along with a high genetic advance as per cent of mean (85.52) were recorded in the present study. The trait, seed yield per plant was less influenced by environmental changes and improvement in the trait would be more effective through selection owing to their additive gene action. This is in agreement with Aher *et al.* (1998); Vikas and Singh (1998); Vange and Egbe (2009); Badhru (2011) and Udensi (2011). In contrast to this Manyasa *et al.* (2008) reported low heritability and genetic advance as per cent of mean. Venkateshwaralu (2001) reported low variability for seed yield per plant.

#### Seed yield per ha

Seed yield per ha registered a moderate PCV and GCV and the difference between the GCV and PCV were less which indicated lesser magnitude of environmental influence. The heritability was very high coupled with high genetic advance as per cent of mean. This is suggestive of high response to appropriate selection procedures as indicated by additive gene action predominance and thus selection for this trait will result in maximum genetic gain.

#### Straw yield per plant

The PCV and GCV estimates were moderate for straw yield per plant. Also, there was less difference between genotypic and phenotypic coefficient of variance which indicated presence of lesser magnitude of environmental influence. Moderate heritability coupled with high genetic advance as per cent of mean implicated non additive gene action for the trait.

#### Test weight

In present study low GCV and PCV values were recorded for the test weight. There was high difference between genotypic and phenotypic coefficient of variance which indicated more influence of non-genetic factors. Low heritability coupled with moderate genetic advance as per cent of mean indicated that the trait was predominantly influenced by non additive gene action. The result are in accordance with findings of Udensi *et al.* (2011) who observed low PCV GCV, broad sense heritability coupled with low genetic advance as per cent of mean.

#### Harvest index

Moderate GCV and PCV were observed for the harvest index and there was narrow difference between genotypic and phenotypic coefficient of variance which indicated that the trait was less influenced by environmental changes. High heritability and high genetic advance as per cent of mean was indicative of additive gene action.

#### Specific gravity

Specific gravity exhibited a moderate level of PCV and GCV in the present study. The difference between the genotypic and phenotypic coefficient of variance was narrow which indicated low environmental influence; the broad sense heritability was higher in magnitude associated with the higher genetic advance as per cent of mean suggestive of additive gene action. Simple selection procedures would lead to improvement in the trait.

### 5.3 Superior genotypes with multiple traits in the pigeonpea minicore collection set

In the present investigation of evaluation of 192 genotypes for yield and yield attributes, a good number of genotypes performed better than the best check for productive traits (Table.25) viz., number of pods per pant, test weight, seed yield per plant, number of primary branches, number of days to maturity. The genotypes viz., PG-12, ICP13270, ICP3049, PUSA-92 and MA-29 exhibited significantly high seed yield per plant at 5 per cent level of critical difference in comparison with best check (Maruti).

However, the accessions ICP13270 and ICP3049 had the desirable character like test weight as evident in the Table 18. The accession, ICP4575 had highest number of pods per plant (270.20) and seed yield per plant (52.61g) compared to all the genotypes studied. Out of 192 genotypes, 38 genotypes exhibited significantly higher seed yield per plant than the best check (Maruti).

### 5.4 Phenotypic correlation coefficients

The ultimate aim of a plant breeder is to alter the genetic makeup of the plant in order to improve the seed yield. Seed yield is a quantitative trait and is a multiplicative product of yield components. For a notable selection for higher yields, a thorough understanding of yield contributing traits, inter-relationship among yield and yield attributes is essential. An adjustment in one of the component may instantaneously bring alteration in the other character. If the association is positive, enhancements in one character will simultaneously bring about improvement in the other (Sidramappa, 2003). The correlation is the powerful tool to study the character, association and is therefore, very beneficial to facilitate selection for important characters without sacrificing the gain in other (Sidramappa, 2003).

The knowledge of correlation was presented by Galton (1889) and was later expanded by Fisher (1918) and Wright (1921); Lush (1940) and Hazel (1943) applied genetic correlations in animal breeding.

The plant height recorded positive and significant association with days to fifty per cent flowering and days to maturity. Similarly, days to fifty per cent flowering and days to maturity were also positively and significantly associated among themselves. Similar results of positive and significant association of days to fifty per cent flowering, days to maturity and plant height were reported by Aher *et al.* (1998). Selection of these characters together will be helpful in crop improvement. The plant height showed negative and significant association with seed yield per plant. Similar results were reported by Musaan and Nahdy (1998).

Number of primary branches recorded positive and significant association with seeds per pod at the phenotypic level. The results of the present study were similar to the reports of Chandirakala *et al.* (2010) and Thanki *et al.* (2010) except for the pod length.

Number of pods per plant was positive and non-significantly associated with the seed yield per plant. The results obtained in the present study are in conformity with the reports of Salunke *et al.* (1995); Vikas and Singh (1998); Baskaran and Muthiah (2006); Sodavadiya *et al.* (2009); Sawant *et al.* (2009). The number of pods per plant was negatively and significantly associated with seeds per pod.

The number of seeds per pod had positive and significant association with test weight at phenotypic level. The results are in conformity with the findings of Chandirakala *et al.* (2010).

In the present study the number of seeds per pod exhibited positive and significant association with number of primary branches, pod length and test weight at phenotypic level. The results obtained by Chandirakala *et al.* (2010); Musaan and Nahdy (1998) reported positive and significant association between number of seeds per pod and seed yield. Therefore stressing upon these traits during the process of selection will make genetic gain for the trait.

The days to fifty per cent flowering showed a positive and significant association with days to maturity and plant height. Similar results for days to fifty per cent flowering exhibiting positive and significant association with plant height were reported by Dhamelia and Pathak (1994). Bhadr (2010) reported positive and significant association of days to fifty per cent flowering with days to maturity. Aher *et al.* (1998) and Salunke *et al.* (1995) reported that, the days to maturity, plant height and test weight exhibited significant and positive association with days to fifty per cent flowering.

**Table 25: Promising pigeonpea genotypes for productive traits**

Sl. No.	Character	Number of genotypes
1	Number of pods per plant	ICP772 (223.60), ICP6859 (215.50), ICP7507 (210.60), ICP4575 (279.20), ICP14832 (185.80), CORG-295 (190.24), and ICP14671 (201.80)
2	Test weight (g)	ICP6128 (15.80g), ICP6668 (15g), ICP7057 (15.92g), ICP7223 (15g), ICP7260 (15.60g), ICP7507 (14.60g), ICP7869 (14.60g), ICP8921 (14.40g), ICP9045 (14g), ICP10397 (14.60g), ICP11015 (14.60g), ICP13270 (14.60g), ICP13579 (14.80g), ICP14229 (15.60g), ICP14444 (17.20g), ICP14638 (15g), ICP14071 (14.60g), ICP14722 (14g), ICP14832 (14.76g), ICP15161 (14.40g), ICP15382 (15.80g), ICP4575 (15.80g), ICP4019 (17g), BRG-9-2 (14.40g), BRG-207 (15.77g), ICP1224 (15.60g), BRG-7-5 (15g), ICP1550 (14.80g), ICP12674 (15.40g), ICP12266 (16.20g), UPAS-120 (15.60g), GC-11-39 (15.75g), ICP4275 (14.40g), ICP12282 (15g), BRG-7-6 (14.60g), ICP12692 (14.60g), Jamadhar local (14.80g), PG-12 (15.40g), Chaple (16.60g), and Bennur local (16.20g),
3	Seed yield per plant (g)	ICP655 (47.80g), ICP3046 (45g), ICP3049 (84.40g), ICP3451 (49.60g), ICP3576 (47.60g), ICP4167 (48.40g), ICP4392 (58.40g), ICP6128 (56.20g), ICP6971 (55.91g), ICP7223 (45.68g), ICP14638 (51.23g), ICP14071 (55g), ICP14722 (45.55g), ICP14801 (55.55g), ICP14976 (46.27g), ICP10804 (45.20g), ICP4878 (45.41g), ICP4275 (52.61g), ICP12282 (57.88g), ICP13270 (91.33g), BRG-7-6 (58.33g), DM-158 (49g), ICPL-87 (52.83g), Jamadhar local (56.22g), Bidabr local (51.26g), jke-144 (57.04g), PUSA-92 (62.28g), PG-12 (95.03g), MA-29 (62.62g), Karithogari (58.27g), ICPB2091 (53.80g), CORG-295 (59.16g), Chaple (55g), Gullyal white (46.80g), CORG-970 (53.60g), CORG-2004-01 (47.55), PJT-501 (48.30g) and Bennur local (45.80)
4	Primary branches	ICP-3451 (6.90g), ICP7071 (7.20g), ICP9750 (8.0g), ICP11910 (6.56g), ICP12654 (7.0g) and HY-3 (6.43g)
5	Days to maturity	ICP-655, (163.40), ICP-995 (157.00), ICP-1071 (160.00), ICP-2577 (165.00), ICP-6128 (160.20), ICP-6815 (165.00), ICP-6992 (156.00), ICP7148 (164.50), ICP12515 (165.60), ICP13167 (100.00), ICP14819 (115.70), ICP 14832 (143.65), BRG-7-3 (165.00), ICP1730 (157.00), ICP12333 (158.00), ICP12674 (160.00), ICP4878 (146.00), ICP6528 (155.00), ICP12266 (156.00), ICP4395 (135.00), BRG-7-2 (135.00), Jamadhar local (160.00), Bidar local (135.00), JKE-144 (154.00), Chaple (159.00), WRP-1 (165), JKM-197 (155) and GRG-801 (155.00) Bennur local (164.00)

The days from flowering to maturity recorded significant and positive association with days to fifty per cent flowering, plant height and days to maturity at phenotypic level. Days from flowering to maturity exhibited negative and non significant association with seed yield per plant in the present study.

The days to maturity had positive and significant association with days from flowering to maturity, plant height and days to fifty per cent flowering at phenotypic level. Days to maturity registered significant negative association with seed yield per hectare. Salunke *et al.*, 1995 and Aher *et al.* (1998) reported positive and significant association of days to maturity with days from flowering to maturity and plant height.

Straw yield recorded positive and significant association with harvest index at phenotypic level. A study from Chauhan *et al.* (2002) reported similar findings. Similarly, harvest index showed positive and significant association with test weight, straw yield at phenotypic level. The harvest index exhibited positive association with seed yield per hectare at phenotypic level. A study from Mittal *et al.* (2010) reported similar findings, wherein, seed yield was positively associated with harvest index.

Seed yield per plant recorded significant and positive association with harvest index. Similarly it had negative and significant association with plant height, days to fifty per cent flowering, days to maturity and straw yield at phenotypic level. The results of the present study are in conformity with the reports of Aher *et al.* (1998); Musaana and Nahdy (1998) and Chandrikala *et al.* (2010). Specific gravity showed a positive and significant association with test weight.

## 5.5 Path coefficient analysis

The correlation coefficient provides basic information about the association prevailing between pairs of characters. However, a dependent character is an interaction production of many jointly associated component characters and modification in any one factor will disturb whole network of cause and effect system. The path coefficient analysis, a statistical device developed by Wright (1921), which takes into account the cause and effect relation between the variables is unique in partitioning the association into direct and indirect effect through other independent variables. The path coefficient analysis also measures the comparative significance of causal factors involved. This is simply a standardized partial regression analysis, wherein total correlation value is sub divided into causal scheme. Li (1956) emphasized the importance of path diagram, which facilitates the understanding of the nature of cause and effect system. In the present study, the path coefficient analysis carried at phenotypic level and the results are discussed below.

### 5.5.1 Path analysis for seed yield

In the current study, path analysis was used to work out the direct and indirect effect of the 5 characters on seed yield per plant (Table 9).

The characters *viz.*, harvest index and days to 50 per cent flowering exhibited highest direct effects as analyzed through phenotypic path coefficients. Similar results of exertion of positive direct effects of harvest index were reported by Sodavadiya *et al.* (2009); Salunke *et al.* (1995); Thanki and Sawargaonkar (2010).

In the present study harvest index had direct positive effect on seed yield per ha. The results obtained in the present study are in conformity with the studies of Sawant *et al.* (2009); Thanki and Sawargaonkar (2010). Among the traits studied in the present study, days to 50 per cent flowering recorded high positive direct effect on the seed yield. It exhibited positive indirect effect via, plant height and days to maturity. The results are similar to the reports of Sawant *et al.* (2009).

Harvest index had highest positive direct effect on seed yield per hectare. So that selecting the plants with high grain yield per plant leads to increase in the total seed yield.

Days to maturity exhibited negative direct effect on seed yield per plant. It also exhibited positive indirect effect on seed yield per plant via harvest index. The results are in conformity with the earlier reports made by Sodavidya *et al.* (2009) and Chandrikala *et al.* (2010).

## 5.6 Genetic diversity

An estimation of genetic diversity within and between groups of germplasm is essential and useful for proper selection of parents to exploit heterosis and also to get potential segregants. Numerous approaches have been advocated by various workers to assess the genetic divergence in

crop plants. Of the several methods available, Mahalanobis' generalized distance estimated by  $D^2$  statistic (Rao, 1952) is a unique tool for discriminating populations, considering a set of traits together rather than concluding from indices based upon morphological resemblances, eco-geographical diversity and phylogenetic associations. In the present study 192 accessions, were assessed for the diversity, using concept of Mahalanobis' generalized distance ( $D^2$ ) (1936).

### 5.6.1 Genetic diversity in different groups

Based on  $D^2$  values, 192 accessions in the present study were grouped into nine distinct clusters (Fig.1), representing frequency of diversity (Table 10). Cluster I was the largest with 108 genotypes followed by cluster II with 50 genotypes, cluster III and cluster V with 12 genotypes each, cluster VII (4 genotypes), cluster VI with 3 genotypes, cluster IV, cluster VIII and cluster IX were solitary clusters containing one genotype each. The genotypes, which have fell within the same cluster exhibited narrow range of genetic variability among them, while between clusters indicated wide range of variability depending on the intra and inter cluster distance, respectively.

Occurrence of nine clusters in the present study indicated the existence of large amount of genetic diversity among them. The results obtained are in conformity with studies of Katiyar *et al.* (2004). They also observed fourteen clusters for the 221 accessions studied. The formation of solitary clusters (VII, X, XI, XII, XIII and XIV) in the present study may be due to total isolation preventing the gene flow or intensive/ human selection for diverse adaptive complexes.

The intra cluster distance was maximum in cluster VII (146.77) followed by cluster V (100.1), III (89.53), II (85.59), I (77.46) and VI (68.34). Thus considerable variability existed among the genotypes within these clusters. It provides an indication to select the genotypes based on the genetic distance for realizing heterosis.

Based on inter cluster  $D^2$  values, the genotypes belonging to cluster I and VI appeared to be more diverse (385.01) followed by cluster VI and VII (383.46), cluster V and VII (374.15), III and VI (352.06).cluster VII and VIII (312.17) which suggested that selection of genotypes from these divergent groups would result in higher magnitude of heterosis for the characters concerned.

### 5.6.2 Contribution of characters towards divergence

Among the 13 quantitative characters studied, the most important character contributing to the divergence was grain yield per hectare followed by number of pods per plant, plant height and days to maturity (Table 10) while, number of primary branches per plant, number of seeds per pod, number of days to 50 per cent flowering and harvest index contributed little to the genetic diversity. These observations are in accordance with Muralidharan (1980) for pod length, days to 50 per cent flowering and days to maturity and Pandey *et al.* (1983) for pod length. The results of the present study are in agreement with the reports of Singh *et al.* (2010), Shivani *et al.*, (2010) and Bhadru (2011).

### 5.6.3 Cluster mean analysis

Analysis of cluster means indicated substantial variation among clusters grouped according to  $D^2$  analysis. Based on the range of means, it is possible to know the characters which influence divergence. The cluster mean values for the 13 characters are presented in Table 13.

The genotypes having highest plant height, highest seeds per pod, lowest number of days from flowering to maturity and highest test weight were grouped in cluster VII. Cluster IX contained the genotypes with highest pod length, days from flowering to maturity, number of days to 50 per cent flowering, days to maturity and highest harvest index. Whereas, the clusters VIII contained the genotypes with lowest amount of plant height, lowest number of primary branches, lowest number of days to maturity and highest number of pods per plant.

The cluster VI contained the genotypes with lowest number of seeds per pod, highest seed yield per plant and highest seed yield per hectare.

The genotypes in the cluster V recorded highest number of primary branches and lowest pod length. Whereas, the test weight recorded lowest mean value identified with the cluster IV. Cluster I was associated with genotypes having lowest amount of seed yield per plant, lowest harvest index and lowest amount of seed yield per hectare.

Lowest number of days to 50 per cent flowering was observed in the genotypes belonging to the cluster III.

## 5.7 Evaluation of genotypes for *Fusarium* wilt and *Helicoverpa armigera*

An effort was made to evaluate the 192 genotypes for *Fusarium* wilt and *Helicoverpa armigera* incidence under natural conditions without any control measures. The present findings of resistance of genotypes to disease might be due to apparent resistance mechanisms such as non existence of pathogen, tolerance or disease escape mechanism of host plant. Hence, one has to evaluate these genotypes under heavy incidence of insects/diseases as certain the genotypes resistance.

## 5.8 Screening of genotypes for sterility mosaic disease

Sterility mosaic disease was first described in 1931 from Pusa, Bihar State, India (Mitra, 1931). The sterility mosaic disease is also called as 'green plague' is caused by pigeonpea sterility mosaic virus (PPSMV) and transmitted by the vector, *Aceria cajani* (Kannaiyan *et al.*, 1984). It has been found to cause more than 95 per cent of the loss in India (Reddy *et al.*, 1981) and the losses of sterility mosaic disease is estimated to be 205,000 tons (Kannaiyan *et al.*, 1984). The sterility mosaic disease is characterized by bushy and pale green appearance of plants, with excess vegetative growth, stunting, mosaic and mottling of leaves and partial or complete cessation of reproductive structures (Reddy *et al.*, 1990). Evaluation of 16 genotypes for resistance to sterility mosaic disease at ICRISAT, Patancheru and 9 different locations in India, revealed large variation in disease incidence and symptom expression (Reddy *et al.*, 1993). In the study conducted by Barhate *et al.* (2000), out of 22 genotypes studied, 19 entries showed resistance to sterility mosaic disease.

The leaf stapling method described by Nene and Reddy (1976) was used to inoculate plants by mites in both field and green house. The technique had shown to facilitate inoculation at primary leaf stage and rapidly express disease symptoms. Screening of accessions in susceptible plot for sterility mosaic disease is considered as most efficient means to identify sterility mosaic disease resistant accessions. The susceptible plot was created in Main Agriculture Research Station UAS, Dharwad by sowing susceptible variety, Maruti (ICP 8863) as an infected row and sowing same susceptible variety intermittently to increase disease load in experimental plot. The leaf stapling technique was adopted to transfer pigeonpea sterility mosaic virus (PPSMV), where mites (*Aceria cajani*) help in transmission of virus

In the present investigation, under natural conditions the per cent disease incidence in 192 accessions ranged from 0 to 100 per cent. Based on the results, accession 121,14, 4, 37 and 16 genotypes were grouped as resistant, moderately resistant, susceptible, moderately susceptible and highly susceptible respectively (Table 15).

## 5.9 Molecular characterization

Even though morphological descriptors are useful for identification and discrimination between varieties or genotypes, they are limited in number and may be affected by environmental factors. Molecular markers score over morphological traits as they are plenty in number, independent of environmental effects and allow cultivar identification at early stages of plant growth.

A variety of DNA marker system *viz.*, RAPD, RFLP, SSR, SNP and DArT's have been identified and developed from time to time for use as tools for application in crop improvement. Among these, SSRs markers are of choice, because of their abundance, high polymorphism and co-dominance inheritance. They are well suited for the assessment of genetic variability within crop species and of the genetic relationships among species. The 'touchdown' Polymerase Chain Reaction conditions were employed along with annealing temperature of 58°C to improve the amplification clarity which helps in scoring. The touchdown programmes also helps to optimize enzymes and primer concentrations resulting in great cost reductions and thus has implications for similar projects in the future (Odeny *et al.*, 2007)

Based on phenotypic observations 48 accessions, comprising of 24 resistant and 24 susceptible genotypes for fusarium wilt and 50 accessions comprising of 25 resistant and 25 susceptible genotypes for sterility mosaic disease were subjected for molecular characterization (Jaggal *et al.*, 2012). In the present study molecular investigation was undertaken to characterize the diversity between the selected accessions of pigeonpea for protein content (Thimmaraju.2012). The study comprised of 46 accessions comprising of 23 high protein and 23 low protein content accessions employing a set of one hundred and fifty simple sequence repeat (SSR) primers.

### 5.9.1 Molecular characterization of sterility mosaic disease.

Out of 150 markers used, 62 markers generated amplifications in the accessions selected for molecular characterization of sterility mosaic disease resistance and susceptibility. Among 62 SSR markers, 58 primers generated polymorphism (98.72%) with an average of 5.06 polymorphic alleles per locus. Earlier studies reported an average of 3.10 for 10 primers (Burns, 2001).

The cluster analyses of sterility mosaic disease, grouped the selected accessions into three major clusters A, B and C. Among the three major clusters, sub cluster I-A with 11 accessions, Cluster II-A with 5 accessions and cluster III-A with 9 accessions were found resistant. Similarly the sub cluster I-B which contained 10 accessions, cluster II-B which contained 3 accessions and cluster III-B which contained 7 accessions were found susceptible. The major cluster C which diverged to form a separate cluster consisted of 5 accessions which were also found susceptible (Table 19).

Gangadhara (2006) who studied the parental polymorphism in pigeonpea using RAPD and SSR markers also reported grouping of susceptible and resistant genotypes into separate clusters

### 5.9.2 Molecular characterization of fusarium wilt

Out of 150 SSR markers used, 41 markers generated amplifications in the accessions selected for fusarium wilt resistance and susceptibility. Out of 41 markers, only 37 markers generated polymorphism on 3 per cent agarose used for the study. However, 37 markers yielded 163 alleles (97.5 per cent polymorphism), with an average of 3.9 polymorphic allele per locus. Similar results were reported by Saxena *et al.* (2010a) and Saxena *et al.* (2010b) who noticed 2.6 alleles per locus.

The cluster analysis based on the marker data for fusarium wilt clustered the genotypes into three major clusters A, B and C as provided in the Table.18. Among the three major clusters, sub cluster I-A with 15 accessions, Cluster II-A with 9 accessions and cluster III-A with 1 accessions were found resistant. Similarly the sub cluster I-B which contained 9 accessions, cluster II-B which contained 11 accessions and cluster III-B which contained 2 accessions were also found susceptible. The major cluster C which diverged to form a separate cluster consisted of a lone accession which was found susceptible.

### 5.9.3 Molecular characterization of protein content

Out of 150 markers used, 46 markers generated amplifications in the accessions selected for molecular characterization of high and low protein content genotypes. Among 46 SSR markers, 45 primers generated polymorphism (99.53%) with an average of 4.63 alleles per locus.

The cluster analysis which took into consideration the alleles generated for protein content clustered the genotypes into three major clusters A, B and C as provided in the Table.19. The first cluster A, subdivided into three sub clusters *viz.*, sub cluster I-A consisted of 8 accessions, cluster II-A which consisted of 3 accessions along with 10 accessions of cluster III A which had low protein content. The clusters I-B (13 accessions), II-B (2 accessions), I-C (5 accessions) and II-C (3 accessions) recorded high protein content. The molecular characterization for protein content could differentiate the accessions with high protein content and low protein content into divergent groups.

The mean Polymorphic Information Content found in both fusarium wilt (0.43) and sterility mosaic disease (0.53) characterization was found maximum. The mean Polymorphic Information Content recorded for protein content was 0.52. As compared to earlier reports of Saxena *et al.* (2010b) (0.43), SSR-based diversity studies in pigeonpea by Burns *et al.* (2001) and Odeny *et al.* (2007) (0.39), a higher level of polymorphism was noticed in the present study.

The most abundant motif's in the present study were AT based (AT, AAT, TTAT) followed by TC class of repeats. The AT based motif' was reported the most abundant in plants (Morgante and Oliveri, 1993, Cardle *et al.*, 2000, Murghante *et al.*, 2002) even though previous studies have excluded them due to the problems with self-complimentarity and difficulties in amplification (Su *et al.*, 1996). Odeny *et al.* (2007) reported the existence of abundant AT based motif's followed by TC class of repeats. AT based (AT, AAT, TTAT) followed by TC class of repeats was reported (Jaggal 2012). The amplification of these motifs in the present study was greatly enhanced by optimization making them equally good source of microsatellites. The polymorphism of SSR markers is generally directly correlated to the number of repeats that are present.

The cluster analysis based on UPGMA- radial dendrogram, revealed three major clusters were which could be further divided into sub clusters. The sub clusters contained both resistant and

susceptible genotypes of fusarium wilt, sterility mosaic diseases and also contained high and low protein content genotypes. Therefore, it will be interesting to understand the inheritance/genetics of resistance to fusarium wilt and sterility mosaic disease (Saxena *et al.*, 2010b). Higher resolution in such clusters may be possible if sufficiently large number of markers are made use of, leading to distinction between the resistant and susceptible genotypes.

The results illustrate, the potential of marker systems to distinguish the content of seed protein in pulses crop at genus level. The information will facilitate selection of accession to serve as parents for effective breeding programs for increasing seed protein and in crop improvement programs. Dendrogram also explains the existence of diversity among the selected accessions which will help in hybridization work for the improvement of seed protein in pigeonpea.

The developed markers exhibited polymorphism across a range of pigeonpea accessions. Use of these markers also offers an efficient system for the assessment of genetic diversity within minicore set of pigeonpea. The markers *viz.*, Cc15 exhibited twelve alleles per marker, Cc13, Cc12, Cc40, Cc21, Cc26, Cc27 and CcM185 exhibited eight alleles per marker followed by Cc29, Cc31, Cc34, Cc35, Cc45 and CcM181 markers which amplified seven alleles per marker. These markers can be successfully used for molecular characterization of genotypes.

The allele size ranged from 100 to 220 bp for fusarium wilt, 100 to 300 bp for sterility mosaic disease and 100 to 200 for protein content. While, comparing the allele numbers and polymorphic information content values with repeat units, no consistent relationship was noticed. Based on marker polymorphism data *i.e.* allele numbers, Polymorphic Information Content values and cluster analysis, it can be generalized that genetic diversity in pigeonpea gene pool is very high. In total, 98.72 per cent, 97.5 and 99.53 per cent of the alleles were found polymorphic for sterility mosaic disease, fusarium wilt and protein content respectively.

In the present investigation the allele number ranged from 2 to 12 alleles per locus with the mean of 5 alleles per locus. The results were in concomitant with the reports of Saxena *et al.* (2010 a & b) and Datta *et al.* (2010) who noticed 2.6 and Odeny *et al.* (2009) study of 3.14 alleles per locus. Previous diversity analysis of cultivated pigeonpea by Burns *et al.* (2001) species reported an average of 3.10 for 10 polymorphic loci and 3.4 for 9 polymorphic loci was reported by Odeny *et al.* (2001). In the same study of Odeny *et al.* (2001) a low level of diversity was found in cultivars than 4.8 when wild relatives were included in similar analysis. While, Yang *et al.* (2006) reported less diversity within cultivated pigeonpea by using DArT markers. The present investigations revealed higher mean major allele frequency (MAF), PIC value and allele number compared to the reported results of Jaggal (2012) where the recorded MAF, PIC value and allele number of 0.84, 0.18 and 3 respectively.

## 5.10 Molecular identification of alleles

In the present study resistant genotypes exhibited amplicon size of 160 bp which are generated by the marker Cc12 for SMD. In a similar study, Jaggal *et al.* (2012) reported as amplicon size of 126 bp to 300 bp generated for sterility mosaic disease. Similarly the marker, CcM185 generated amplicon size of 180 bp for resistant genotypes of FW Jaggal *et al.* (2012) and also reported the allele size ranges from 130 bp to 300 bp for fusarium wilt and the resistant genotypes exhibited amplicon size of 160 bp generated by the marker Cc13 for protein content. The marker RGA-1TG, which produced an amplicon size of 450 bp in the resistant genotypes of mungbean. Similarly the marker, MtB99 produced amplicons of size 420bp, which were specific to MYMV resistance was reported by Ashwini *et al.* (2011), obtained through the characterization study of NBS-LRR degenerate markers.

## Future line of work

Based on the results obtained in the present study following future line of work is suggested.

1. The mini core is genetically diverse and possesses potential variation for economic traits and hence it could be subjected for further exploitation in the breeding programmes.
2. Seed yield per plant exhibited high GCV, PCV, heritability and genetic advance as per cent of mean. In addition there was close correspondence between GCV and PCV implying the selection of these traits to make significant improvement.
3. Through correlation and path analysis, it was revealed that harvest index had positive and significant association with the seed yield and also positive direct effect on seed yield. Hence these traits could be considered for selection.

4. The accessions PG-12 (95.03g) and ICP 13270(91.33g) recorded high yield along with resistance to both fusarium wilt and sterility mosaic diseases. The future hybridization programmes can be based on such genotype, which has been reported in the study.
5. Future breeding strategies in pigeonpea must focus on broadening genetic base by introgressing favorable alleles from other landraces and wild populations in order to maximize gains from selection.
6. Resistant genotypes exhibited amplicon size of 160 bp generated by the marker Cc12 for SMD. Similarly, the markers CcM185 and Cc13 generated amplicon size of 180 bp and 160bp for resistant genotypes of FW and high protein content of genotypes respectively. Similarly considered for further validating and use in molecular characterization and thus build up set of markers for MAS.

## SUMMARY AND CONCLUSIONS

One hundred and ninety-two genotypes of pigeonpea (*Cajanus cajan* (L.) Millspaugh (2n=22), that includes mini core collection set (124 pigeonpea accessions) from International Crop Research Institute for Semi-arid Tropics (ICRISAT) Patancheru, Andhra Pradesh and 68 local germplasm from ARS Gulbarga were evaluated for yield and yield contributing traits, associations, direct and indirect effects of yield contributing components on seed yield. The present study was also taken up to assess the magnitude of variance prevalent in the pigeonpea genotypes. In addition, an effort has been made for molecular characterization of resistant and susceptible genotypes of pigeonpea with respect to SMD and FW along with molecular characterization of genotypes contrastingly different for protein content.

The genotypes were evaluated in an augmented design during 2012-13 *kharif* at Botanical garden, Main Agricultural Research Station, University of Agricultural Sciences Dharwad. The salient findings of the present study are summarized as below.

1. Analysis of variance revealed highly significant difference among the genotypes for the 10 characters among the 14 characters studied.
2. Genotypic and phenotypic coefficients of variation were high for number of primary branches per plant, number of pods per plant, seed yield per plant, straw yield and seed yield per hectare.
3. The characters viz., plant height, seed yield per plant, number of days to 50 per cent flowering, number of days to maturity, harvest index and specific gravity exhibited high heritability coupled with a high genetic advance as per cent of mean which indicated that simple selection scheme would be sufficient for these traits to bring about genetic improvement in desired directions.
4. At the phenotypic level, seed yield showed positive and significant association with harvest index. Hence, the selection based on these traits would improve the yield. It is also suggested that hybridization of genotypes possessing different combinations of such characters will be the most useful approach for obtaining desirable high yielding segregants.
5. Path coefficient analysis revealed that, harvest index and days to 50 per cent flowering exhibited highest direct effects on seed yield per plant.
6. Using Mahalanobis'  $D^2$  statistics method, 192 genotypes were grouped into 9 divergent clusters. Cluster I was the largest with 108 genotypes followed by cluster II with 50 genotypes, cluster III and cluster V with 12 genotypes each, cluster VII 4 genotypes, cluster VI with 3 genotypes, cluster IV, cluster VIII and cluster IX were solitary clusters containing one genotype each. The intra cluster distance was maximum in cluster VII (146.77) followed by cluster V (100.1), III (89.53), II (85.59), I (77.46) and VI (68.34). Thus considerable variability existed among the genotypes within these clusters. It provides an indication to select the genotypes based on the genetic distance for realizing appreciable heterosis.
7. Based on inter cluster  $D^2$  values, the genotypes belonging to cluster I and VI appeared to be more diverse (385.01) followed by cluster VI and VII (383.46), cluster V and VII (374.15), III and VI (352.06) and cluster VII and VIII (312.17). Hence it is suggested that selection of genotypes from these divergent groups would result in higher magnitude of heterosis for the characters concerned. It is desirable to select accessions from clusters having high inter cluster distance and high seed yield as parents in the recombination breeding programmes.
8. Evaluation of genotypes for the *Fusarium* wilt and *Helicoverpa* incidence under natural conditions revealed that 130 genotypes and 19 genotypes showed less than ten per cent of infection respectively. However, the resistance of these genotypes needs to be evaluated under artificial epiphytotic conditions to confirm the results obtained in the present investigation.
9. The disease screening of 192 pigeonpea accessions in the field revealed that 121 accessions were found resistant for sterility mosaic disease. These genotypes will have to be tested further for valve of disease resistance.
10. The 150 simple sequence repeat oligos with high PIC value based on the previous studies were utilized to screen the selected accessions for sterility mosaic disease, fusarium wilt and

protein content genotypes. Among 150 SSR markers, 41 for fusarium wilt, 62 for sterility mosaic disease and 46 for protein content generated amplifications.

11. The mean PIC value generated by the oligo's was 0.43 for FW, while it was 0.53 for SMD. The mean PIC value recorded for protein content was 0.52.
12. The markers Cc15 and CcM181 generated allele's size of 180 bp (resistant genotypes) and 200 bp (susceptible genotypes) for sterility mosaic disease. Similarly, the marker Cc9 generated allele's size of 150 bp (resistant genotypes) and 180 bp (susceptible genotypes) for fusarium wilt. Two markers viz., Cc9 and Cc15 generated polymorphic allele sizes of 180 bp (high protein genotypes) and 200 bp (low protein genotypes) in the present investigation.

## REFERENCES

- Ae, N., Arihara, J., Okada, K., Yoshihara, T. and Johansen, C., 1990, Phosphorus uptake by pigeonpea and its role in cropping systems of the Indian sub-continent. *Sci.*, 248 : 477-480.
- Aggarwal, R. K., Brar, D. S., Nandi, S., Hang, N. and Kush, G. S., 1999, Phylogenetic relationships among *Oryza* species revealed by AFLP markers. *Theor. Appl. Genet.*, 98 : 1320 – 1328.
- Aggarwal, R. K., Shenoy, V. V., Ramadevi, J., Rajkumar, R. and Singh, L., 2002, Molecular characterization of some Indian Basmati and other elite rice genotypes using fluorescent AFLP. *Theor. Appl. Genet.*, 105 : 680–690.
- Aher, R. P., Thombre, B. B. and Dahal, D. V., 1998, Genetic variability and character association in pigeonpea (*Cajanus cajan* (L) Millsp). *Legume Res.*, 21(1) : 41-44.
- Ahmad Neyaz and Bajpai, G. C., 2002, Physical and cooking qualities of pigeonpea and their correlation. *Legume Res.*, 25(3) : 192-195.
- Akkaya, M. S., Bagwhatt, A. A. and Cregan, P. B., 1992, Length polymorphisms of simple sequence repeat DNA in soybean. *Genetics.*, 132 : 1131–1139.
- Amala Balu, P. and Rathnasamy, R., 2003, Inheritance to sterility mosaic disease in pigeonpea (*Cajanus cajan* (L.) Millsp). *Crop Res.*, 25 (2) : 301- 304.
- Anantharaju, P. and Muthiah, A. R., 2008, Studies on genetic variability in F<sub>2</sub> population of pigeonpea. *Madras Agric. J.*, 5 (7-12) : 431-436.
- Arora, S. K., 1977, Legume Carbohydrates : in Chemistry and Biochemistry of legumes. Edited by oxford and IBH, New Delhi.
- Ashwini, N., 2011, Genome wide identification of “R” genes and exploitation of candidate RGA markers in mungbean (*Vigna radiata*) and (*Vigna mungo*). *M. Sc. (Agri.) Thesis*, Univ., Agric., Sci., Dharwad, Karnataka (India).
- Atachi, P. and Dihou, Z. C., 1994, Record of host plants *Maruca testularis* (Geyer) (Lepidoptera : Pyralidae) in the Republic of Benin. *Ann. Soc. Entomol.*, 30 : 169-174.
- Balyan, H. S. and Sudhakar, M. V., 1985, Variability, character association and path coefficient studies and genotype of early maturity group in pigeonpea. *Madras Agric. J.*, 72 : 165-172.
- Barhate, B. G., Bendre, N. J., Kute, N. S. and Gaikwad, R. T., 2000, Source of resistance to fusarium wilt and sterility mosaic disease of pigeonpea. *Legume Res.*, 23(1) : 136-138.
- Basavarajaiah, D., Byre Gowda, M., Lohithaswa, H. C., Kulkarni, R. S., 2000, Assessment of pigeonpea germplasm and isolation of elite genotypes for Karnataka. *Crop Res.*, 20(3) : 444-448.
- Bashiruddin, M. D. and Sreeramulu, C., 1981, Genetic variability in pigeonpea. *Andhra Agric. J.*, 28 : 207-209.
- Baskaran, K. and Muthiah, A. R. 2006, Variability studies in pigeonpea (*Cajanus cajan* (L.) Millsp.) *Res on Crops.*, 7(1) : 249-252.
- Baskaran, K. and Muthiah, A. R., 2007, Associations between yield and yield attributes in Pigeonpea (*Cajanus cajan* (L.) Millsp.) *Legum. Res. - An Internat. J. Year* 30.
- Becker, J., Vos, P., Kuiper, M., Salamini, F. and Heun, M., 1995, Combined mapping of AFLP and RFLP marker in barley. *Mol. Gen. Genet.*, 249 : 65–73.
- Bensnard, G., Tagmount, A., Baradat, P., Vigouroux, A. and Berville, A., 2002, Molecular approach of genetic affinities between wild and ornamental platanus. *Euphytica.*, 126 : 401–412.
- Bhadru, D. 2010, Correlation and path analysis for yield and yield attributing traits in white coated pigeonpea (*Cajanus cajan* (L) Millsp). *Legume Res.*, 33(3) : 196-200.
- Bhadru, D. 2011, Variability and genetic divergence studies in white seed coated pigeonpea (*Cajanus cajan* (L.) Millsp.) *Crop Res.*, 42 (1/2/3) : 263-268.

- Bhadru, D., 2010a, Studies on genetic parameters and interrelationships among yield and yield contributing traits in pigeonpea (*Cajanus cajan* (L.) Millsp). *Legume Res.*, 33(1) : 23-27.
- Bhadru, D., 2010b, Character association and path analysis in Pigeonpea. *J. Food Legume.*, 24(1) : 69-71.
- Blair, M. W., Rodriguez, L. M., Pedraza, F., Morales, F. and Beebe, S., 2007, Genetic mapping of the bean golden yellow mosaic geminivirus resistance gene *bgm-1* and linkage with potyvirus resistance in common bean (*Phaseolus vulgaris* L.). *Theor. Appl. Genet.*, 114 (2) : 261-271.
- Borojevic, S., 1990, Principles and method of plant breeding, Elsevier Science Publishers, Amsterdam, Holland.
- Bressani, R., Elias, S. G., 1974, Nutritive value of legume crops for humans and animals. *Advances in Legume Science*. Eds. Summerfield, RJ and Bunting A. H., Royal Botanical
- Burns, M. J., Edwards, K. J., Newbury, H. J., Ford-Lloyd, B. V. and Baggott, C. D., 2001, Development of simple sequence repeat (SSR) markers for the assessment of gene flow and genetic diversity in pigeonpea (*Cajanus cajan*). *Mol Ecol Notes.*, 1 : 283-285.
- Burton, G. W. and Davane, E. M., 1953, Estimating heritability in tall Fuscus (*Festuca arundinaceae*) from replicated clonal material. *Agron. J.*, 45 : 478-481.
- Cardle, L., Ramsay, L., Melbourne, D., Macaulay, M., Marshall, D. and Waugh, R., 2000, Computational and experimental characterization of physically clustered simple sequence repeats in plants. *Genet.*, 156 (2) : 847-854.
- Chandirakala, R. Subbaraman, N., 2010, Character association and path analysis for yield attributes in full sib progenies in pigeonpea (*Cajanus cajan* (L.) Mill sp.). *Electronic J. Plant Breed.*, 1 : 4 : 824-827.
- Chattopadhyay, K. and Dhiman, K. R., 2005, Characterization, variability, diversity and path coefficient analysis of pigeonpea germplasm from North-east India under rainfed upland condition in Tripura. *Legume Res.*, 28(2) : 140-142.
- Chauhan, Y. S, Johansen, C, Jung-Kyung Moon, Yeong-Ho Lee, and Suk-Ha Lee, 2002, Photoperiod Responses of Extra-Short-Duration Pigeonpea Lines Developed at Different Latitudes. *Crop Sci.*, 42 : 1139–1146.
- Chitra U., Singh U., Venkateswara Rao P., 1996. Phytic acid, in vitro protein digestibility, dietary fiber and minerals of pulses as influenced by processing methods. *Plant Foods for Human Nutrition*, 49 : 307–316.
- Choudhary, R. P., Singh, I. P., Verma, A. K. and Datta, S., 2007, Identification of genotypic specific markers and assessment of genetic relatedness among pigeonpea cultivars using RAPD. *J. Food Legumes*, 20 : 12- 15.
- Choudhury, R. P., Singh, I. P., Shulabhi, V., Singh, N. P. and Kumar, S., 2008, RAPD markers for identification of cytoplasmic genic male sterile, maintainer and restorer lines of pigeonpea. *J. Food Legumes*, 21 : 218- 221.
- Datta, J., Nand, L., Mayank, K., Prasoon, P. and Gupta, V. S., 2010, Efficiency of Three PCR based Marker Systems for Detecting DNA Polymorphism in *Cicer arietinum* (L.) and *Cajanus cajan* (L.) Millspaugh. *Genet. Engg. Biotechnol. J.*, 25 : 58-62.
- Datta, S., Kumavat, G., Bikram, P. S., Deepak, K. G., Singh, S., Dogra, V., Gaikwad, K., Tilak, R. S., Ranjeet, S. R., Tapas, K. B., Subhojit, D., Mahendra, N. S., Fhakrudin, B., Pawan, K., Wanjari, K. B., Rajeev, K. V., Douglas, R. C. and Nagendra, K. R., 2011, Development of genic-SSR markers by deep transcriptome sequencing in pigeonpea [*Cajanus cajan* (L.) Millsp]. *BMC Plant Bio.*, 11(17) : 1471-2229.
- Datta, S., Rai, R., Tiwari, K., Kashyap, M., Hena and Kumar, S., 2007, Phylogenetic analysis of pulse crops using RAPD markers. *J. Food Legumes*, 20(1) : 9-11.
- De, D. N., 1974, Pigeonpea in evolutionary studies in world crops : diversity and change in the Indian subcontinent. (Hutchinson J, ed.). London : Cambridge press. pp. 79-87.
- Deshmukh, R. B., Rodge, R. G., Patil, J. V., Sahane, D. V., 2000, Genetic variability character

- association and path analysis in pigeonpea under different cropping systems. *J. Maharashtra Agric. Univ.*, 25(2) : 176-178.
- Dewey, D. R. and Lu, K. N., 1959, A correlation and path coefficient analysis of components of crested wheat grass seed production. *Agron. J.*, 51 : 515-518.
- Dhahat, D. V., Deshmukh, R. B. and Patil, J. V., 1995, Path analysis in pigeonpea in sole crop and inter-cropping with pearl millet. *Legume Res.*, 18 (3) : 167-169.
- Dhamelia, H. R. and Pathak, A. R., 1994, Correlation studies in segregating generations of pigeonpea. *ICPN*, 1 : 30-32.
- Donald, C. M., 1962, In Search of yield. *J. Australian Agric. Sci.*, 28 : 171-178.
- Edwards, K. J., Barker, J. H. A., Daly, A., Jones, C. and Karp, A., 1996, Microsatellite libraries enriched for several microsatellite sequences in plants. *Biotechniq.*, 20 : 758-759.
- Falconer, D. S., 1981, Introduction to quantitative genetics, 2<sup>nd</sup> Ed. Oliver and Boyd, Edinburg, London, pp. 164-176.
- Farindra Singh, Singh, I. P., Mazumder., 2012, Identification of *Fusarium* wilt-resistant sources of long-duration pigeonpea (*Cajanus cajan*). *AJCS.*, 6(7) : 1154-1158.
- Fatokun, C. A., Danesh, D., Menancio-Hautea, D. I. and Young, N. D., 1993, A linkage map for cowpea (*Vigna unguiculata* (L.) Walp) based on DNA markers ( $2n = 22$ ). In *Genetic maps 1992 : A Compilation of Linkage and Restriction Maps of Genetically Studied Organisms*. Edited by J. S. O'Brien. Cold Spring Harbour Laboratory Press, New York, pp. 6256-6258.
- Firoz Mahamad, 2003, Variability studies in vegetable pigeonpea (*Cajanus cajan* (L.) Millsp) accessions. *M. Sc. (Agri.) Thesis*, submitted to Univ., Agric., Sci., Bangalore, Karnataka (India).
- Fisher, R. A. and Yates, F., 1963, *Statistical Tables for Biological, Agricultural and Metrical Research*, Oliver and Boyd, Edinburgh.
- Fisher, R. A., 1918, The correlation between relatives on the supposition of Mendalian Inheritance *Trans Rou. Soc. Edinb.*, 52 : 399-433.
- Galton, F., 1889, *Natural Inheritance*, McMillan and Co., London.
- Gamber, R. K., Sandeet Singh and Sandhu, T. S., 1996, Variability, associations of seed yield with different phases of the reproductive period in pigeonpea. *Indian J. Genet.*, 56 : 318-322.
- Ganapathy, K. N., 2009, Genetic analysis and identification of DNA markers linked to sterility mosaic disease resistance in pigeonpea (*Cajanus cajan* (L.) Millsp.). *Ph. D Thesis* submitted to Univ., Agric., Sci., Bangalore, Karnataka (India).
- Ganapathy, K. N., Byre Gowda, M., Ajay, B. C., Venkatesha, S. C., Gnanesh, B. N., Sunil, S., Gomashe, Prasanth Babu, Girish, G., Prasad, P. S., Veerakumar, G. N., Patil, J. V., 2012, Inheritance studies of sterility mosaic disease (SMD) resistance in vegetable type pigeonpea (*Cajanus cajan* (L.) Millsp.). *Indian J. Agric. Sci.*, 81 (11) : 1046-51.
- Ganapathy, K. N., Byre Gowda, M., Venkatesh, S. C., Ramachandra, R., Gnanesh, B. N. and Girish, G., 2010, Identification of AFLP markers linked to sterility mosaic disease in pigeonpea *Cajanus cajan* (L.) Millsp. *Intl. J. Integrative Biotechnol.*, 7 (13) : 145-149.
- Ganapathy, K. N., Byre Gowda, M., Venkatesha, S. C., Ramachandra, R., Gnanesh, B. N. and Girish, G., 2009, Identification of AFLP markers linked to sterility mosaic disease in pigeonpea (*Cajanus cajan* (L.) Millsp.) *IJIB.*, 7 : 145-149.
- Gangadhara, A. V., 2006, Studies on genetic variability and DNA marker diversity in selected lines for sterility mosaic disease resistance in pigeonpea (*Cajanus cajan* (L.) Millsp). *M. Sc. (Agri.) Thesis*, Univ., Agric., Sci., Bangalore, Karnataka (India).
- Ghadge, P. N., Shewalkar, S. V. and Wankhede, D. B., 2008, Effect of processing methods on qualities of instant whole legume : pigeon pea (*Cajanus cajan* L.). *Agricultural engineering international : the CIGR Ejournal*. Manuscript FP 08 004. Vol. X.
- Ghodke, M. K., Makne, V. G., Jahargirdhar, J. E. and Khapra, P. R., 1994, Heritability and genetic

- advance in pigeonpea under three cropping systems. *J. Maharashtra Agric. Univ.*, 19 : 278-279.
- Gnanesh, B. N., Bohraa, A., Sharmaa, M., Byregowda, M., Pande, S., Wesleya, V., Saxenaa, R. K., Saxenaa, K. B., KaviKishorc, P. B., Varshney R. K., 2011, Genetic mapping and Quantitative Trait Locus analysis of resistance to sterility mosaic disease in pigeonpea (*Cajanus cajan* (L.) Millsp.) *Field Crops Res.*, 5470 : 1-9.
- Gnanesh, B. N., Ganapathy, K. N., Ajay, B. C., Byre Gowda, M., 2011, Inheritance of sterility mosaic disease resistance to Bangalore and Patancheru isolates in pigeonpea (*Cajanus cajan* (L.) Millsp. *Electron. J. Pl. Breed.*, 2 (2) : 218-223.
- Gohil, R. H., 2006, Genetic divergence in pigeonpea (*Cajanus cajan* (L.) Millsp.). *Res. on Crops*, 7(3) : 748-750.
- Gopalan, C., Shastri, B. V. R., Balsubramanian, S. C., 1985, *Nutritive Value of Indian Foods*, National Institute of Nutrition (NIN), Indian Council of Medical Research, Hyderabad, India.
- Gopali, J. B., Teggelli, R., Mannur, D. M. and Yelshetty, S., 2010, Web-forming Lepidopteran, *Maruca vitrata* (Geyer) : An emerging and destructive pest in pigeonpea. *Karnataka J. Agric. Sci.*, 23 (1) : 35-38.
- Grafius, J. E., 1964, A geometry of plant breeding. *Crop Sci.*, 4 : 241-246.
- Graham P. H. and Vance C. P., 2003 Legumes- importance and constraints to greater use. *Plant Physiology*, 131 : 872–877.
- Greilhuber, J. and Obermayer, R., 1998, Genome size variation in *Cajanus cajan* (Fabaceae) : a reconsideration. *Plant Syst. Evol.*, 212 : 135-141.
- Gupta, P. K. and Varshney, R. K., 2000, The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica*, 113 : 163-185.
- Hamid, A. Husna, A. Haque, M. M. Islam, M. R., 2011, Genetic variability in pigeonpea (*Cajanus cajan* L. Millspaugh). *Electronic J. Plant Breed.*, 2 (1) : 117-123.
- Hammond-Kosack, K. E. and Jones, J. D. G., 1997, Plant disease resistance genes. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 48 : 575–607.
- Hanson, C. H., Robinson, H. F. and Comstock, R. K., 1956, Biometrical studies on yield in segregating population of Korean Lasphadezia. *Agron. J.*, 48 : 314- 318.
- Hazel, L. N., 1943, Genetic basis for constructing selection indices. *Genet.*, 28 : 476-490.
- Hill, M., Witsenboer, H., Zabeau, M., Vos, P., Kesseli, R. and Michelmore, R., 1996, PCR based fingerprinting using AFLP as a tool for studying genetic relationships in *Lactuca*. *Theor. Appl. Genet.*, 93 : 1202–1210.
- Holkar, S., Nigam, P. K. and Mishra, V. K., 1991, Note on path analysis in pigeonpea. *Intl. Pigeonpea Newslett.*, 14 : 4-5.
- Jagashoram, 1983, Studies on genetic diversity for some quantitative characters in pigeonpea. *Madras Agric. J.*, 70 : 146-148.
- Jagdish Singh, 1999, Studies on variability and heterosis in pigeonpea (*Cajanus cajan* (L.) MillSp). *Adv. in Plant. Sci.*, 12(1) : 215-221.
- Jaggal, L. G., 2012, Molecular characterization of pigeonpea (*Cajanus cajan* (L.) Millsp) accessions to sterility mosaic virus and fusarium wilt. *M. Sc. (Agri.) Thesis*, Univ., Agric., Sci., Dharwad, Karnataka (India).
- Jain, K. C. and Reddy, M. V., 1995, Inheritance of resistance to fusarium wilt in pigeonpea (*Cajanus cajan* L.). *Indian J. Genet. Plant Breed.*, 55 : 434-437.
- Johnson, H. W., Robinson, H. F. and Comstock, R. E., 1955, Estimation of genetics and environmental variability in soybean. *Agron. J.*, 47 : 477-483.
- Joshi, A. B., 1957, Genetics of resistance to diseases and pests. *Indian J. Genet.*, 17 : 305–317.

- Kannaiyan, J., Nene, Y. L., Reddy, M. V., Ryan, J. G. and Raju, T. N., 1984, Prevalence of pigeonpea diseases and associated crop losses in Asia, Africa and the America. *Trop. Pest Manag.*, 30 : 62-71.
- Kanwan, B. K. and Hazarika, M. H., 1988, Genetic variability in pigeonpea. *Crop Improv.*, 15 : 98-100.
- Karimi, R., James, O., Owuoché and Silim, N., 2010, Inheritance of fusarium wilt resistance in pigeonpea (*Cajanus cajan* (L.) Millspaugh). *Indian J. Genet.*, 70 (3) : 271-276.
- Katiyar, P. K. Dua, R. P. Singh, I. P. Singh, B. B. Singh, F. 2004, Multivariate analysis for genetic diversity in early pigeonpea accessions. *Legume Res.*, 27 (3) : 164-170.
- Khan, T. N. and Rachie, K. O., 1972, Preliminary evaluation and utilization of pigeonpea germplasm in Uganda. *E. African Agric. J.*, 38 : 78-82.
- Khapre, P. R. and Nerkar, M. Y. S., 1992, Genetic variability and character association in pigeonpea under different cropping system. *J. Maharashtra Agric. Univ.*, 17 : 445-448.
- Kimani, P. M., Nyende, A. B. and Silim, S., 1994, Development of early maturing fusarium wilt resistant pigeonpea cultivars. *African Crop Sci. J.*, 2 : 35-41.
- Knauff, D. A. and Garbet, D. W., 1989, Genetic diversity among peanut cultivars. *Crop Sci.*, 29 : 1417-1422.
- Kotresh, H., Fakrudin, B., Punnuri, S. M., Rajkumar, B. K., Thudi, M., Paramesh, H., Lohithswa, H. and Kuruvinashetti, M. S., 2006, Identification of two RAPD markers genetically linked to a recessive allele of a fusarium wilt resistance gene in pigeonpea (*Cajanus cajan* L. Millsp.). *Euphytica.*, 149 : 113 – 120.
- Kumar, A. S., Reddy, T. P. and Reddy, G. M., 1983, Plant regeneration from different callus of pigeonpea (*Cajanus cajan* L.). *Plant Sci. Lett.*, 32 : 271-278.
- Kumar, P. L., Jones, A. T. and Reddy, D. V. R., 2003, A novel mite transmitted virus with a divided RNA genome closely associated with Pigeonpea sterility mosaic disease. *Phytopathol.*, 93 : 81-91.
- Kumar, P. L., Latha, T. K. S., Kulkarni, N. K., Raghavendra, N., Saxena, K. B., Waliyar, F., Rangaswamy, K. T., Muniyappa, V., Sabitha Doraiswamy and Teifion, A., 2005, Broad based resistance to pigeonpea sterility mosaic disease in wild relatives of pigeonpea (*Cajanus* : Phaseoleae), *Ann. Appl. Biol.*, 146 : 371 – 379.
- Lateef, S. S. and Reed, W., 1990, Insect pests of pigeonpea. In S. R Sing (ed) *Insect Pests Trop. Food Legum.*, (Chichester, UK : Wiley), pp. 193-242.
- Lavakumar, P., Jones, A. T. and Reddy, D. V. R., 2002, Methods manual pigeonpea sterility Mosaic Virus, Detection and Screening for Resistance, ICRISAT, Patancheru, AP, India.
- Lee, M., 1995, DNA markers and plant breeding programs. *Adv. Agron.*, 55 : 265-344.
- Li, Y. C., Korol, A. B., Fahima, T., Beiles, A. and Nevo, E., 2002, Microsatellites : genomic distribution, putative functions and mutational mechanisms : a review. *Mol. Ecol.*, 11 : 2453-2465.
- Li, Y. C., Roder, M. S., Fahima, T., Kirzher, V. M., Beiles, A., Korol, A. B. and Nevo, E., 2000, Natural selection causing microsatellite divergence in wild emmer wheat at the ecologically variable microsite at Ammiad, Israel. *Theor. Appl. Genet.*, 100 : 985-999.
- Linnaeus, C., 1753, *Species plantarum*. 739 (cited by van der Maesen 1986).
- Litt, M. and Luty, J. A., 1989, A hypervariable microsatellite revealed *in vitro* amplification of dinucleotide repeat within the cardiac muscle actin gene. *Am. J. Hum. Genet.*, 44 : 397- 401.
- Liu, K. and Muse, S. V., 2005, Powermarker : Integrated analysis environment for genetic marker data. *Bioinform.*, 21 : 2128-2129.
- Lohithaswa, H. C., Shailaja Hittalmani, Shashidhar, H. E. and Dharmaraj, P. S., 2003, Assessment of genetic variability in some pigeonpea (*Cajanus cajan* (L.) Millsp.) genotypes using RAPD markers. *Indian J. Genet.*, 63 : 329-330.

- Lush, J. L., 1940, Inter-size correlation regression of offspring on dams as a method of estimating heritability of character. *Proc. American Soc. Animal Prod.*, 33 : 293-301.
- Lush, J. L., 1949, Heritability of quantitative characters in farm animals. *Proc. 8<sup>th</sup> Congress of Genetics and Hereditary*, 35 : 356-375.
- Mahate, D. N. and Dave, B. B., 1931, Studies in *Cajanus indicus*. *Dept. Agric. India Bot.*, 19 : 1-25.
- Maheswaran, M., Subudhi, P. K., Nandi, S., Xu, J. C., Parco, A., Yang, D. C. and Huang, N., 1997, Polymorphism, distribution, and segregation of AFLP markers in a doubled haploid rice population. *Theor. Appl. Genet.*, 94 : 39-45.
- Maksem, K., Leister, D., Peleman, J., Zadeau, M., Salamini, F. and Gebhardt, C., 1995, A high resolution map of the vicinity of two RI locus on chromosome V of potato based RFLP and AFLP markers. *Mol. Gen. Genet.*, 249 : 74-81.
- Mallikarjuna, N. and Saxena, K. B., 2002, Production of hybrid between *Cajanus acutifolius* and *C. cajan*. *Euphytica*, 124 : 107-110.
- Manyasa, E. O. Silim, S. N. Githiri, S. M. Christiansen, J. L. 2008, Diversity in Tanzanian pigeonpea (*Cajanus cajan* (L.) Millsp.) landraces and their response to environments. *Genetic Resou. and Crop Evolut.*, 55 (3) : 379-387.
- Manyasa, E. O., Silim, S. N., Githri, S. M. and Christiansen, J. L., 2007, Diversity in Tanzanian pigeonpea (*Cajanus cajan* (L.) Millspaugh) landrace and their response to environments (In Press); Genetic resource and crop Evolution.
- Metkar, A. P., Gawande, V. L. and Gahukar, S. J., 2010, Molecular characterization of CMS lines in pigeonpea (*Cajanus cajan* (L.) Millsp.). *J. Food Legume.*, 23(3&4) : 234-237.
- Meyers, B. C., Dickerman, A. W., Michelmore, R. W., Sivaramakrishnam, S., Sobral, B. W. and Young, N. D., 1999, Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide- binding superfamily. *Plant J.*, 20 : 317-332.
- Michelmore, R. W. and Meyers, B. C., 1998, Clusters of resistance genes evolve by divergent selection and a birth and death process. *Genome Res.*, 8 : 1113-1130.
- Mir, R. R., Saxena, R. K., Saxena, K. B., Upadhyaya, H. D., Kilian, A., Cook, D. R. and Varshney, R. K., 2012, Whole-genome determinacy in pigeonpea (*Cajanus spp.*) *Plant Breed.*, p. 7.
- Mitra, M., 1931, Report of the Imperial Mycologist. *Scientific Rep. Agric. Res. Inst., Pusa*, 19 : 58-71.
- Mittal, V. P., Paramjit Singh and Brar, K. S. 2010, Character association and path coefficient analysis for yield components in pigeonpea, *Cajanus cajan* L. *Madras Agric. J.*, 97 : 319-320.
- Muralidharan, K., 1980, Studies on genetic divergence and breeding behavior of few inter varietal crosses in field bean. *M. Sc. (Agri.) Thesis*, Uni. Agric. Sci., Bangalore, Karnataka (India)
- Murghante, M. and Oliveri, A., 1993, PCR amplified microsatellites as markers in plant Genetics. *Plant.*, 3 : 175-182.
- Murghante, M., Hanafey, M. and Powell, W., 2002, Microsatellites are preferentially associated with non-repeatative DNA in plant genomes. *Nat. Genet.*, 30(2) : 194-200.
- Murray, M. G. and Thompson, W. F., 1980, Rapid isolation of high molecular weight DNA. *Nucleic Acids Res.*, 8 : 4321-4325.
- Murugesan, S., Murugan, E. and Nadarajan, N., 1997, Inheritance of duration, leaf colour, sterility mosaic disease resistance and growth habit in pigeonpea. *Madras Agric. J.*, 84 (1) : 10-12.
- Musaana, M. S. and Nahdy, M. S., 1998, Path coefficient analysis of yield and its components in pigeonpea. *African Crop Sci. J.*, 6 : 143-148.
- Nadimpalli, B. G., Jarret, R. L., Pathak, S. C. and Kochert, G., 1992, Phylogenetic relationships of the pigeonpea (*Cajanus cajan*) based on nuclear restriction fragment length polymorphism. *Genome*, 36 : 216-223.

- Nagaraj, K. M., Chikkadevaiah, and Kulkarni, R. S., 2004, Inheritance of resistance to sterility mosaic virus in pigeonpea (*Cajanus cajan* (L.) Millsp.). *Indian J. Genet.*, 64 (2) : 118 – 120.
- Narendra, P., Rajesh Kumar Singh, S. N., Ranjeet Singh Verma, H. R., 2011, Genetic divergence in pigeonpea collections. *Agril. Biol. Res.*, 27(1) : 14-19.
- Natarajan, G. C., Thiyyaranjan, K. and Ayyamperumal, 1990, Genetic variability and path analysis in pigeonpea. *Madras Agric. J.*, 77 : 378-381
- Nene, Y. L. and Reddy, M. V., 1976, A technique to screen pigeonpea for resistance to sterility mosaic. *Trop. Grain Leg. Bull.*, 5 : 23.
- Nene, Y. L. and Reddy, M. V., 1977, Leaf stapling technique to screen pigeonpea for resistance to sterility mosaic. *Indian Phytopathol.*, 30 (1) : 153.
- Odeny, A., Jayashree, B., Ferguson, M., Hoisington, D. Crouch, J. and Gebhardt, C., 2007, Development, characterization and utilization of microsatellite markers in pigeonpea. *Plant Breed.*, 126 : 130–136.
- Odeny, D. A., Jayashree, B., Gebhardt, C. and Crouch, J., 2009, New microsatellite markers for pigeonpea (*Cajanus cajan* (L.) millsp.). *BMC Res. Notes.*, 2 : 35.
- Okori, P., Fahleson, J., Rubaihayo, P. R., Adipala, E. and Dixelius, C., 2003, Assessment of genetic variation among East African *Cercosporazeae- maydis* populations using AFLP and RFLP. *J. Afri. Crop Sci.*, 11 : 75-86.
- Pal, B. P., 1934, Recent progress in plant breeding at Pusa, Bihar. *Agric. Livest. India*, 4 : 511–512.
- Pandey, R. N., Pawar, S. E. and Bhatia, C. R., 1996, Inheritance of wilt resistance in pigeonpea. *Indian J. Genet. Plant Breed.*, 56 : 305-308.
- Pandey, R. P., Assawa, B. M. and Tiwari, J. P., 1983, Genetic divergence in *Dolichos lablab*. *JNKVV Res. J.*, 17 (1 & 2) : 66-70.
- Panguluri, S. K., Janaiah, J., Govil, J. N., Kumar, P. A. and Sharma, P. C., 2005, AFLP fingerprinting in pigeonpea (*Cajanus cajan* (L.) Millsp.) and its wild relatives. *Genet. Res. Crop. Evol.*, 53 : 523- 531.
- Pansuriya, A. G., Pandya, H. M., Kathiria, K. B., 1998, Genetic variability and correlation in early maturing genotypes of pigeonpea. *Gujarat Agric. Univ. Res. J.*, 23(2) : 23-27.
- Paterson, A. H., Stalker, H. T., Gallo-Meagher, M., Burrow, M. D., Dwivedi, S. L., Crouch, J. H. and Mace, E. S., 2004, Genomics and Genetic Enhancement of Peanut. In : *Legume Crop Genomics*. (Eds.) Wilson, R. F., Stalker, H. T. and Brummer, E. C., Champaign, Ill : AOCS Press. pp. 97-109.
- Pathak, G. N., 1970, Redgram, In : Pulse crops of India. Indian Council of Agricultural Research, New Dehli, India, pp. 14-53.
- Paul, S., Wachira, F. N., Powell, W. and Waugh, R., 1997, Diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia inensis* (L.) Kuntze) revealed by AFLP markers. *Theor. Appl. Genet.*, 94 : 255–263.
- Pawar, N. B. and Mayee, C. D., 1986, Reaction of pigeonpea genotypes and their crosses to Fusarium wilt. *Indian Phytopath.*, 39 : 70-74.
- Perrier, X., Flori, A. and Bonnot, F., 2003, *Data Analysis Methods*, Science Publishers Montpellier, pp. 43-76.
- Pillen, K., Binder, A., Ramsay, K. L., Waugh, R., Forster, J. and Leon, J., 2000, Mapping new EMBL derived barley microsatellites and their use in differentiating German barley cultivars. *Theor. Appl. Genet.*, 101 : 652-660.
- Polanco, C. and Ruiz, M. L., 2002, AFLP analysis of somaclonal variation in *Arabidopsis thaliana* regenerated plants. *Plant Sci.*, 162 : 817–824.
- Prasannalatha, C. H., Parampreet Kaur and Bhalla, J. K., 1999, Molecular characterization of somaclonal variants in pigeonpea. *Curr. Sci.*, 76(5) : 693-695.
- Premsagar and Jatasra, 1984, Variability, heritability and genetic advance in segregating lines of

- pigeonpea. *Pulse Crop Newsl.*, 4 : 9-11.
- Quedraogo, J. T., Maheshwari, V., Berner, D. K., St-Pierre, C. A., Belzile, F. and Timko, M. P., 2002, Identification of AFLP markers linked to resistance of cowpea (*Vigna unguiculata* L.) to *Striga gesnerioides*. *Theor. Appl. Genet.*, 102 : 1029–1036.
- Quedraogo, T. J., Tignegre, J. B., Timko, M. P. and Belzile, F. J., 2002, AFLP markers linked to resistance against *Striga gesnerioides* race 1 in cowpea (*Vigna unguiculata*). *Genome*, 45 : 787–793.
- Raju, N. L., Belaghihalli, N., Gnanesh, Pazhamala Lekha, Balaji Jayashree, Suresh Pande, Pavana, J., Hiremath, Munishamappa, Byregowda, Nagendra, K., Singh, Rajeev K Varshney, 2010, The first set of EST resource for gene discovery and marker development in pigeonpea (*Cajanus cajan* L.). *BMC Plant Bio.*, 10(45) : 1471-2229.
- Ram Dhari, Waldia, R. S. and Malik, B. P. S., 2004, Association of seed yield with its component traits in pigeonpea. Department of Plant Breeding, C. C. S. Haryana Agricultural University, Hisar (Haryana), India. 6(2) : 119-120.
- Ramanujam, S. and Thirumalachar, D. K., 1967, Genetic variability of certain characters in red pepper (*Capsicum annum* L.). *Mysore J. Agril. Sci.*, 1 : 32-36.
- Rangaswamy, K. T., Muniyappa, V., Lava Kumar, P., Saxena, K. B., Byregowda, M., Raghavendra, N., Pandurangaiah, K., Vijay Kumar, R., Waliyar, F., and Jones, AT., 2005, ICP 7035 – A sterility mosaic resistant vegetable and grain purpose pigeonpea variety, SAT eJournal., 1 : 1-3.
- Rao, C. R., 1952, Advanced statistical methods in biometrical research. John Wiley and Sons, New York.
- Rao, C. R., 1960, Multivariate analysis : An indispensable tool in statistical and in applied research : *Sankya*, 22 : 317-338.
- Rao, S. C., Coleman, S. W. and Mayeux, H. S., 2002, Forage production and nutritive value of selected pigeonpea ecotypes in the southern great plains. *Crop Sci.*, 42 : 1259-1263.
- Rathnaparke, M. B., Gupta, V. S., Murthy, M. R. V. and Ranjekar, P. K., 1995, Genetic fingerprinting of pigeonpea and its wild relatives using RAPD markers. *Theor. Appl. Genet.*, 91 : 893-898.
- Reddy, L. J., Upadhyaya, H. D., Gowda, C. L. L. and Sube Singh, 2005, Development of core collection in Pigeonpea [*Cajanus cajan* (L.) Millspaugh] using geographic and qualitative morphological descriptors. *Genet. Reso. Crop Evolut.*, 52 : 1049–1056.
- Reddy, M. V. and Nene, Y. L., 1981, Ring spot symptom : a genotypic expression of pigeonpea sterility mosaic. *Trop. Grain Leg. Bull.*, 15 : 27-29.
- Reddy, M. V., Raju, T. N., Sharma, S. B., Nene, Y. L. and McDonald, D., 1993, Handbook of pigeonpea disease. Information Bulletin No. 42. Patancheru 502 324, Andhra Pradesh, India : International Crops Research Institute for the Semi-arid Tropics, p. 61.
- Reddy, M. V., Sharma, S. B. and Nene, Y. L., 1990, Pigeonpea disease management. The Pigeonpea CAB International Wallingford, UK. pp. 303-347.
- Reddy, M. V., Sheila, V. K., Murthy, A. K. and Padma, N., 1995, Mechanism of resistance to *Aceria cajani* in pigeonpea. *Int. J. Tropical Plant Diseases.*, 13 : 51 – 57.
- Rekha, R., Prasanthi, L., Sekhar, M. R., Latha, P., Sudhakar, S., 2011, Genetic diversity in pigeonpea [*Cajanus cajan* (L.) millsp]. *Legume Res.*, 34 (2) : 139-142.
- Robinson, H. F., Compstock, R. E. and Harey, P. H., 1949, Estimates of heritability and degrees of dominance in corn. *Agron. J.*, 43 : 353-359.
- Rockland, L. B., Radke, T. M., 1981, Legume protein quality. *Food Technology*, 35(3) : 79.
- Saifulla, M., Mahesh, M. and Byre Gowda, M., 2006, Reaction of pigeonpea genotypes for sterility mosaic disease. In : *National Seminar on New Frontiers in Plant Pathology*, September 28 – 30, Shimoga, India, p. 43.

- Saifulla, M., Mahesh, M., Nagesha, G. K. and Byre Gowda, M., 2005, Management of sterility mosaic disease through host resistance in pigeonpea. In : *Indian Society of Plant Pathologists and Centenary Symposium on Plant Pathology*, Shimla. India, p. 36.
- Salunke, J. S., Aher, R. P., Shinde, G. C. and Kute, N. S., 1995, Correlation and path coefficient analysis in early Pigeonpea. *Legume Res.*, 18(3) : 162-166.
- Salunke, J. S., Aher, R. P., Shinde, G. C. and Kute, N. S., 1995, Correlation and path coefficient analysis in early Pigeonpea. *Legume Res.*, 18(3) : 162-166.
- Satish Kumar, D., Koteswara Rao, Y., Rama Kumar, P. V. and Srinivasa Rao, V., 2006, Genetic variability and character association in pigeonpea [*Cajanus cajan* (L.) Millsp.]. *The Andhra Agric J.*, 53 (1&2) : 116.
- Sawant, M. N. ; Sonone, A. H. ; Anarase, S. A. 2009 Character association, path coefficient analysis and genetic diversity in pigeonpea. *J. Maharashtra Agric., Univ.*, 34 : 134-137
- Saxana, R. K., Saxena, K. B., Kumar, R. V., Hosington, D. A. and Varshney, R. K., 2010b, Simple sequence repeat-based diversity in elite Pigeonpea genotypes for developing mapping populations to map resistance to *Fusarium* wilt and sterility mosaic disease, *Plant Breed.*, 129, 135-141.
- Saxena K. B., Faris D. G., Kumar R. V., 1987 Relationship between seed size and protein content in newly developed high protein lines of pigeonpea. *Plant Foods Hum Nutrition* 36 : 335–340
- Saxena, K. B. and Sharma, D., 1995, Source of dwarfism in pigeonpea. *Indian J. Pulse Res.*, 8 : 1-6.
- Saxena, K. B., 2008, Genetic Improvement of Pigeonpea – a Review. *Trop. Plant. Biol.*, 1 : 159-178.
- Saxena, K. B., Kumar, R. V. and Rao, P. V., 2002, Pigeonpea nutrition and its improvement. In : Quality improvement in crops. The Food Products Press, *Crop Sci.*, pp. 227–260.
- Saxena, K. B., Kumar, R. V. and Sultana, R., 2010, Quality nutrition through pigeonpea - a review, pp. 1335-1344.
- Saxena, K. B., Kumar, R. V., Madhavilatha, K. and Dalvi, V. A., 2006, Commercial pigeonpea hybrids are just a few steps away. *Indian J. Pulses Res.*, 19 : 7–16.
- Saxena, K. B., Kumar, R. V., Saxena, R. K., Sharma, M., Srivastava, R. K., Sultana, R., Varshney, R. K., • M. I. Vales, M. I., Pande, S., 2012, Identification of dominant and recessive genes for resistance to *Fusarium wilt* in pigeonpea and their implication in breeding hybrids. *Euphytica.*, 188 : 221–227.
- Saxena, R. K., Saxena, K. and Varshney, R. K., 2010a, Application of SSR markers for molecular characterization of hybrid parents and purity assessment of ICPH 2438 hybrid of pigeonpea (*Cajanus cajan* (L.) Millspaugh) *Mol. Breed.*, 26 : 371–380.
- Saxena, R. K., Prathima, C., Saxena, K., Hoisington, D. A., Singh, N. K. and Varshney, R. K., 2009a, Novel SSR markers for polymorphism detection in pigeonpea (*Cajanus* spp.). *Plant Breed.*, doi : 10. 1111 / j. 1439-0523. 2009. 01680. x.
- Saxena, R. K., Saxena, K., Kumar, R. V., Hoisington, D. A. and Varshney, R. K., 2009b, Simple sequence repeat-based diversity in elite pigeonpea genotypes for developing mapping populations to map resistance to *Fusarium* wilt and sterility mosaic disease. *Plant Breed.*, 1-7.
- Selvi, R., Muthaih, A. R. and Manivannan, N., 2006, Tagging of RAPD marker for MYMV resistance in mungbean [*Vigna radiata* (L.) wilczek]. *Asian J. Pl. Sci.*, 5 : 277- 280.
- Sharma, D., Gupta, S. C., Rai, G. S. and Reddy, M. V., 1984, Inheritance of resistance to sterility mosaic disease in pigeonpea. *Indian J. Genet.*, 44 (1) : 84 -90.
- Sharma, S. K., Knox, M. R. and Ellis, T. H. N., 1996, AFLP analysis of the diversity and phylogeny of Lens and its comparison with RAPD analysis. *Theor. Appl. Genet.*, 93 : 751–758.

- Shaw, F. J. F., 1933, Studies in Indian Pulses : 3. The type of *Cajanus indicus* Spreng. *Indian J. Agric. Sci.*, 3 : 1-36.
- Shaw, F. J. F., 1936, Studies in Indian pulses : The inheritance of morphological characters and wilt resistance in arhar (*Cajanus indicus* Spreng.). *Indian J. Agric. Sci.*, 6 : 139–187.
- Shivani D., Sreelakshmi Ch., Kumar C. V. Sameer 2010 Genetic divergence and stability analysis in pigeonpea (*Cajanus cajan* L.) *Electronic J. Plant Breed.* : 1 (4) : 530-535.
- Shreelakshmi, Ch., Shivani, D., Sameer Kumar, C. V., 2011, Inheritance of *Fusarium* Wilt resistance in pigeonpea. *Madras Agric. J.*, 98 (10-12) : 331-333.
- Sidramappa, B., 2008, Relationship of phenological traits with productivity in chickpea (*Cicer arietinum* L.). *M. Sc. (Agri.) Thesis*, Univ., Agril., Sci., Dharwad.
- Sindhu, P. S., Varma, H. and Seema, H., 1985, Genetic relationship among yield components in pigeonpea. *Indian J. Agric. Sci.*, 55 : 232-235.
- Singh, A. P., Singh, U. P., Singh, R. M. and Rajni Raina., 2003, Relative efficiencies of four population improvement schemes in pigeonpea [*Cajanus cajan* (L.) Millsp.]. *Crop Res.*, 25(2) : 305-311.
- Singh, B. V., Pandya, B. P., Gautam, P. L., Beniwal, S. P. S. and Pandey, M. P., 1983, Inheritance of resistance to sterility mosaic virus in pigeonpea. *Ind. J. Genet.*, 43 : 487- 493.
- Singh, F., Singh, I. P., Mazumder, N. D., 2011, Identification of *Fusarium* wilt-resistant sources of long-duration pigeonpea (*Cajanus cajan*) *Indian J. Agric., Sci.*, 81 (11) : 1046–51.
- Singh, I. P., Vishwa, D., Chaudhary, R. C. and Panday, D. K., 1998, Genetics of *Fusarium* wilt resistance in pigeonpea, In : *National Symposium on Management of Biotic and Abiotic Stress in Pulse Crops*, 26-28<sup>th</sup> June, IIPR Kanpur, India, PK.
- Singh, J., Bhadana, V. P. and Datt, S., 2010a, Correlation and Path Coefficient Analysis among yield and its contributing traits in pigeonpea (*Cajanus cajan* (L.) Millsp.). *Environ and Ecol.*, 26(3A) : 1396-1399
- Singh, M. N., Awasthi, S. K. and Singh, R. S., 2010b, Genetic divergence in Pigeonpea, *J. Food Legumes*, 23(1) : 82-83.
- Singh, S. R. and van Emden, H. F., 1979, Insect pests of grain legumes. *Ann. Rev. Entomol.*, 24 : 255 - 278.
- Singh, S., Singh, K. N., Kant, R., Mehfooz, S. and Dutta, S., 2008, Assessment of genetic diversity among pigeonpea genotypes using SSR markers. *Indian J. Genet.*, 68 : 255- 260.
- Singh, U. and Eggum, B. O., 1984, Factors affecting the protein quality of Pigeonpea (*Cajanus cajan* L.). *Qual Plant Plant Foods Hum Nutr*, 34 : 2 73-283.
- Singh, U., Jain, R. C., Jambunathan, R. J., Faris, P. G., 1984, Nutritive value of vegetable
- Sivaramakrishnan, S., Seetha, K., Nageshwar Rao, A. and Singh, L., 1996, RFLP analysis of cytoplasmic male sterile lines of pigeonpea developed by interspecific crosses. *Euphytica*, 93 : 304-312.
- Sivusubramanian, S. and Menon, M., 1973, Heterosis and in breeding in rice. *Madras Agric. J.*, 60 : 1139.
- Sodavadiya, P. R., Pithia, M. S., Savaliya, J. J., Pansuriya, A. G. and Korat, V. P., 2009, Studies on characters association and path analysis for seed yield and its components in pigeonpea (*Cajanus cajan* (L.) Millsp.). *Legume Res.*, 32 (3) : 203-205.
- Souframanien, J., Manjaya, J. G., Krishna, T. G. and Pawar, S. E., 2003, Random amplified polymorphic DNA analyses of cytoplasmic male sterile and male fertile pigeonpea (*Cajanus cajan* (L.) Millsp.). *Euphytica*, 129 (3) : 293-299.
- Sreelakshmi, C., Sameer, Kumar, C. V. and Shivani, D., 2010, Genetic analysis of yield and its component traits in drought tolerant genotypes of pigeonpea (*Cajanus cajan* L. Millspaugh). *Electronic J. Plant Breed.*, 1(6) : 1488-1491
- Srinivas, T., Jain, K. C., Reddy, M. V., Reddy, M. S. S., 1999, Genetic relationships among yield

components in pigeonpea. *Indian J. Pulses Res.*, 12(2) : 180-186.

- Srinivas, T., Reddy, M. V., Jain, K. C. and Reddy, M. S. S., 1997, Studies on inheritance of resistance and allelic relationships for strain 2 of pigeonpea sterility mosaic pathogen, *Ann. Appl. Biol.*, 130 : 105 – 110.
- Su, X. Z., Wu, Y., Sifri, C. D. and Wellems, T. E., 1996, Reduced extension, temperatures required for PCR amplification of extremely A + T rich DNA. *Nucleic Acids Res.*, 24 : 1574-1575.
- Sunitha, V., Vijaya Lakshmi and Ranga Rao, G. V., 2008, Screening of pigeonpea genotypes against *Maruca vitrata* (Geyer). *J. Food Legume.*, 21(3) : 193-195.
- Takalkar, N. G., Rewale, A. P., Birari, S. P., 1998, Studies on genetic variability, heritability and genetic advance in early group of pigeonpea. *Annals of Agric. Res.*, 19(2) : 117-119.
- Tanksley, S. D., Young, N. D., Paterson, A. H. and Bonierbal, M. W., 1989, RFLP mapping in plant breeding- new tools for an old science. *Bio-Technology.*, 7 : 257-264
- Tautz, D. and Rentz, P., 1984, Simple sequences are ubiquitous repetitive components of eukaryotic genome. *Nucleic Acids Res.*, 12 : 4127-4138.
- Thanki, H. P. and Sawargaonkar, S. L., 2010, Path coefficient analysis in pigeonpea (*Cajanus cajan* L. Millsp.). *Electronic J. Plant Breed.*, 1(4) : 936-939
- Thimmaraju, 2012, Evaluation of pigeonpea (*Cajanus cajan* (L.) Millsp) minicore collection set for yield and yield related traits. *M. Sc. (Agri.) Thesis*, Univ., Agri., Sci., Dharwad, Karnataka (India).
- Tikka, S. B. S., 1986, Pigeonpea genetics and improvement, A review in : *Breeding for vegetable type pigeonpea* (Eds. Tikka, S. B. S., Shah, R. M. and Sakhela, R. R.), shared publishing academy, Ahuja
- Tiwari, K. R., Penner, G. A. and Warkentin, T. D., 1998, Identification of coupling and repulsion phase RAPD markers for powdery mildew resistance gene *er-1* in pea. *Genome*, 41 : 440-444.
- Tyagi, A. P., 1997, Investigation of quantitative trait loci (QTLs) in two diverse strains of pigeonpea (*Cajanus cajan*) using RAPD markers. *J. South Pacific Agric.*, 4 : 92-98.
- Udensi, O., Edu, E. A., Umana, E. J. and Ikema, E. V., 2011, Estimation of genetic variability in locally grown pulses (*Cajanus cajan* (L.) Millsp and *Vigna unguiculata* (L.) Walp) : Apanacea for sourcing superior genotypes. *Pakistan J. Biol. Sci.*, 14(6) : 404-407.
- Upadhyaya, D., Gowda, C. L. L. Buhariwalla, H. K. and Crouch, J. H., 2006, Efficient Use of Crop Germplasm Resources : Identifying Useful Germplasm for Crop Improvement through Core and Mini-core Collections and Molecular Marker Approaches, *Plant Genetic Resources : Characterization and Utilization*, 4 : 25-35.
- Upadhyaya, H. D., Reddy, K. N., Singh, Sube and Gowda, C. L. L., 2013, Phenotypic diversity in *cajanus species* and identification of promising sources for agronomic traits and seed protein content. *Genet. Reso. Crop Evolut.*, 60(2) : 639-659.
- Upadhyaya, H. D., Yadav, D., Dronavalli, N., Gowda, C. L. L. and Singh, S., 2010, Mini core germplasm collections for infusing genetic diversity in plant breeding programs. *Electronic J. Plant Breed.*, 1(4) : 1294-1309.
- Van Der and Maesen, L. J. G., 1980, India is the native home of the pigeonpea. In : Arends, J. C, Boelma, G, de Grant, C. T, Leeuwenberg, A. J. M. (Eds) Libergratularious in Honrem, H. C. D. de Wit. *Agril. Univ., Miscellan. Paper*, 19 : 257-262.
- Van Der and Maesen, L. J. G., Nene, Y. L., Hall, S. D. and Sheila, V. K., 1990, Pigeonpea : origin, history, evolution and taxonomy in the pigeonpea. CAB International, Wallingford, Oxon, UK, pp. 15–46
- Van der Maesen L. J. G., 1986, *Cajanus* DC. and *Atylosia* W&A. (Leguminosae). Agricultural University Wageningen Papers 85-4 Wageningen, the Netherlands : Agricultural University, p. 225.
- Vange T. and Egbe M. O., 2009, Studies on genetic characteristics of pigeonpea germplasm. *World J.*

*Agric. Sci.*, 5(6) : 714-719.

- Varshney, R. K., Graner, A. and Sarrells, M. E., 2005, Genic microsatellite markers in plants : features and application. *Trends Biotechnol.*, 23 : 48-55.
- Varshney, R. K., Hoisington, A. and Tyagi, A. K., 2006, Advances in cereal genomics and applications in crop breeding. *Trends Biotechnol.*, 24 : 490-499.
- Vasconcelos, M. J. V., De Barros, E. G., Moreira, M. A. and Vieira, C., 1996, Genetic diversity of commonbean *Phaseolus vulgaris* L. determined by DNA based molecular markers. *Braz. J. Genet.*, 19 : 447 – 451.
- Vavilov, N. I., 1939, The new systematics of cultivated plants. In the new systematics. (Huxley J, ed.). London : Oxford University Press. pp. 549-566.
- Venkateshwarlu, O., 2001, Genetic variability in pigeonpea (*Cajanus cajan* (L.) Millsp). *Legume Res.*, 24(2) : 205-206.
- Vikas and Singh, S. P., 1998, Variability and character association in early and extra early genotypes of Pigeonpea (*Cajanus cajan* (L) Millsp). *Legume Res.*, 21(3/4) : 229-232.
- Vos, P., Hogers, R., Bleekers, M., Rejians, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M., 1995, AFLP : A new technique for DNA fingerprinting. *Nucleic Acids Res.*, 23 : 4407-4414.
- Wasike, S., Okori, P. and Rubaihayo, P. R., 2005, Genetic variability and relatedness of the Asian and African pigeonpea as revealed by AFLP. *Afr. J. Biotech.*, 4 : 1228- 1233.
- Waugh, R., Bonar, N., Baird, E., Thomas, B., Graner, A., Hayes, P. and Powell, W., 1997, Homology of AFLP products in three mapping populations of barley. *Mol. Gen. Genet.*, 255 : 311–321.
- Weber and Morthy, B. R., 1952, Heritable and non-heritable relationship and variability of oil content and agronomic characteristics in the F<sub>2</sub> generation of soybean crosses. *Agron. J.*, 44 : 202-209.
- Weising, K., Kaemmer, D., Weigand, F., Epplen, J. T. and Kahl, G., 1992, Olionucleotide fingerprinting reveals various probe dependent levels of informativeness in chickpea (*Cicer arietinum*). *Genome*, 35 : 436- 442.
- Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V., 1990, DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, 18 : 6531–6535.
- Wright, S., 1921, Correlation and causation. *J. Agril. Res.*, 20 : 557-585.
- Yang, S. Y., Saxena, R. K., Kulwal, P. L., Gavinjash, Dube A., Harper, J. D. I., Upadhyaya, H. D., Gothalwal, R., Kilian A. and Varshney, R. K., 2011, The first genetic map of pigeonpea based on diversity arrays technology (DArT) markers, *J. Genet.*, 90.
- Yang, S., Pang, W., Ash, G., Harper, J., Carling, J., Wenzl, P., Huttner, E., Zong, X. and Kilian, A., 2006, low level of genetic diversity in cultivated pigeonpea compared to its wild relatives is revealed by diversity arrays technology. *Theor. Appl. Genet.*, 113 : 585-595.
- Young, N. D., 1999, A cautiously optimistic vision for marker-assisted breeding. *Mol. Breed.*, 5 : 505–51.
- Zavodra, M., Kraic, J., Paglia, G., Gregova, E. and Morgante, M., 2000, Differentiation between closely related lentil (*Lens culinaris* Medik.) cultivars using DNA markers. *Seed Sci. Tech.*, 28 : 217-219.
- Zhou, J. J., Singh, R. J. and Hymowitz, T., 2004, SSR marker and ITS cleared amplified polymorphic sequence analysis of soybean x *Glycine tomentella* subgeneric derived lines. *Theor. App. Genet.*, 109 : 769-774.
- Zhu, J., Gale, M. D., Quarrie, S., Jackson, M. T. and Bryan, G. J., 1998, AFLP markers for the study of rice biodiversity. *Theor. Appl. Genet.*, 96 : 602–611.

**Appendix I: Monthly mean meteorological data during crop growth period (2012) and the average of past 62 years (1950-2011) at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad**

Month	Rainfall (mm)		Temperature (°C)				Relative humidity (%)	
			Maximum		Minimum			
	2012	1950-2011	2012	1950-2011	2012	1950-2011	2012	1950-2011
January	0.0	0.8	29.8	28.7	14.0	14.1	55.6	64.8
February	0.0	11.5	32.3	31.6	15.9	16.6	53.5	54.4
March	0.0	1.5	35.8	34.9	15.9	19.7	40.7	64.2
April	56.6	48.6	35.7	36.6	18.4	20.0	57.1	78.1
may	3.8	20.0	35.6	35.2	21.2	20.9	50.8	75.8
June	43.4	106.4	30.6	30.2	21.5	21.7	73.7	86.3
July	112.2	153.8	27.2	27.3	20.9	20.9	84.6	89.2
August	90.0	101.0	32.0	27.2	19.3	20.2	80.4	88.6
September	89.6	107.5	28.0	27.9	19.8	19.9	75.8	86.7
October	89.2	125.9	28.4	29.5	17.8	18.7	64.7	79.4
November	35.7	32.0	27.7	28.9	15.5	15.9	57.8	73.6
December	19.6	4.9	29.5	27.8	14.8	13.2	55.9	69.1
<b>Total</b>	<b>540.1</b>	<b>713.8</b>	-	-	-	-	-	-

**Appendix II: Mean values of 14 quantitative characters in 192 pigeonpea accessions**

ICP No.	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	X <sub>6</sub>	X <sub>7</sub>	X <sub>8</sub>	X <sub>9</sub>	X <sub>10</sub>	X <sub>11</sub>	X <sub>12</sub>	X <sub>13</sub>	X <sub>14</sub>
348	219.00	5.50	159.80	4.60	3.60	110.00	63.80	168.80	180.00	11.60	15.78	0.77	28.40	157.76
655	221.80	5.60	172.60	4.60	3.60	108.00	54.00	163.40	113.07	10.20	42.28	0.69	47.80	265.53
772	242.00	6.20	223.60	4.60	4.00	117.00	57.00	174.00	140.00	11.60	23.43	0.91	32.80	182.20
939	237.00	5.40	128.60	5.50	3.80	130.00	72.10	202.10	191.00	10.20	21.47	0.75	41.00	227.76
995	231.80	5.60	150.00	4.30	3.80	101.00	57.00	157.00	160.00	10.40	8.25	0.85	13.20	73.33
1071	245.20	4.85	130.00	5.40	4.00	105.00	56.95	160.00	107.59	11.20	39.41	0.89	42.40	235.53
1156	231.00	4.80	110.00	4.80	3.80	115.00	55.30	170.30	195.00	12.40	26.36	0.89	51.40	285.53
2577	226.80	4.98	75.80	4.90	4.00	108.00	58.45	165.00	79.21	13.50	27.52	0.96	21.80	121.10
2698	228.00	5.60	76.00	4.90	3.80	125.00	72.95	200.00	125.58	12.80	31.06	1.14	39.00	216.65
2746	240.20	4.80	78.40	6.00	4.00	115.00	15.30	174.00	200.00	13.60	21.00	1.42	42.00	233.31
3046	227.80	5.40	105.00	5.00	3.80	121.00	58.50	178.90	235.60	12.20	19.10	0.84	45.00	249.98
3049	231.20	4.87	77.40	5.10	3.80	119.00	57.80	176.80	130.00	12.60	64.92	1.13	84.40	468.84
3451	261.40	6.90	172.00	5.30	3.00	133.00	57.75	190.76	122.80	13.90	40.39	1.34	49.60	275.53
3576	225.00	4.94	122.00	6.00	3.00	100.00	72.95	172.80	170.12	13.80	27.98	1.25	47.60	264.42
4029	252.80	5.20	131.00	4.50	4.00	118.90	55.70	173.40	82.93	13.00	51.85	0.78	43.00	238.87
4167	256.00	4.38	164.60	4.90	3.80	120.30	50.80	175.00	140.00	9.56	34.57	0.64	48.40	268.86
4317	214.40	4.92	153.00	4.80	4.00	102.00	56.33	168.80	60.92	10.20	36.11	0.73	22.00	122.21
4392	221.00	4.94	109.00	5.00	4.00	123.40	56.75	179.81	61.79	10.60	94.52	1.00	58.40	324.41
5142	241.00	4.82	110.00	6.25	4.00	129.00	59.00	180.00	104.62	13.50	28.10	1.18	29.40	163.32
5863	259.00	4.87	131.80	4.10	3.60	155.50	50.86	206.85	67.20	12.20	41.37	1.07	27.80	154.43
6049	220.80	4.67	173.60	5.00	3.80	113.00	60.95	173.50	73.00	10.40	24.11	1.00	17.60	97.77

*Contd....*

ICP No.	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	X <sub>6</sub>	X <sub>7</sub>	X <sub>8</sub>	X <sub>9</sub>	X <sub>10</sub>	X <sub>11</sub>	X <sub>12</sub>	X <sub>13</sub>	X <sub>14</sub>
6123	252.00	4.90	130.00	5.30	3.80	119.00	75.60	194.60	127.00	11.00	25.67	0.98	32.60	181.09
6128	238.00	5.80	139.20	4.80	3.80	102.30	60.20	160.20	121.00	15.80	46.45	1.44	56.20	312.19
6668	256.00	4.89	88.80	4.70	3.80	131.50	60.35	190.00	138.00	15.00	12.61	1.44	17.40	96.66
6739	243.00	4.89	181.00	4.20	4.00	130.00	73.50	200.00	178.00	10.60	13.37	0.82	23.80	132.21
6815	236.60	4.87	84.00	4.90	4.00	116.00	45.00	165.00	131.00	11.40	18.93	0.76	24.80	137.76
6859	266.00	4.99	215.50	4.30	4.00	133.00	60.02	195.05	102.03	11.40	21.86	0.73	22.30	123.88
6929	253.60	4.76	123.40	5.00	3.60	120.00	49.50	170.40	180.00	10.40	12.56	0.73	22.60	125.54
6971	249.80	5.80	136.28	4.80	3.00	126.00	56.00	184.00	141.00	11.20	39.66	0.86	55.91	310.60
6992	220.20	4.85	128.90	5.80	3.00	103.00	55.55	156.00	150.00	9.66	20.99	0.78	31.49	174.94
7057	287.00	5.87	164.60	4.90	4.00	130.00	55.70	187.55	200.00	15.92	12.33	1.09	24.67	137.03
7076	238.80	7.20	195.40	5.30	4.00	143.50	65.70	200.05	72.29	13.39	51.01	1.42	36.87	204.82
7148	248.00	5.20	171.60	4.80	3.60	100.50	56.75	164.50	112.00	11.40	23.78	1.33	26.63	147.95
7221	292.00	4.80	146.20	4.40	3.80	109.50	60.75	170.75	84.31	13.00	32.98	1.12	27.81	154.48
7223	217.00	5.60	138.80	3.90	3.80	127.00	58.45	185.45	80.58	15.00	56.69	0.94	45.68	253.77
7260	233.40	4.85	151.20	4.50	3.80	114.00	67.75	181.75	85.80	15.60	19.54	1.16	16.77	93.14
7314	254.20	4.69	107.00	5.30	3.40	127.00	61.75	188.75	156.00	14.20	21.81	1.22	34.02	188.97
7366	213.60	4.20	135.60	4.90	4.20	99.00	71.10	170.10	130.00	13.50	15.30	0.95	19.89	110.50
7426	232.80	5.80	153.00	5.40	4.20	126.00	58.85	185.00	150.00	12.40	19.67	0.89	29.51	163.91
7507	238.00	5.60	210.60	6.00	4.20	145.00	54.00	200.00	178.05	14.60	16.38	1.12	29.17	162.03
7803	247.00	6.00	90.60	5.00	4.00	125.00	64.30	198.00	179.00	12.60	13.21	0.97	23.65	131.39
7869	245.00	4.50	128.20	6.00	4.00	125.00	66.25	191.25	141.00	14.60	13.84	1.20	19.51	108.38
8012	247.60	4.87	119.20	4.50	3.00	130.00	53.90	175.00	183.69	11.60	12.72	1.04	23.37	129.83
8255	250.00	7.20	152.20	5.10	4.20	120.00	50.00	176.00	121.54	11.60	15.06	0.87	18.30	101.67
8266	254.00	4.80	158.80	5.30	4.20	130.00	55.95	185.95	260.88	13.32	14.48	1.21	37.77	209.82
8384	218.78	4.40	144.00	4.40	4.00	114.00	62.05	176.05	76.00	13.60	27.08	0.93	20.58	114.31

Contd....

ICP No.	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	X <sub>6</sub>	X <sub>7</sub>	X <sub>8</sub>	X <sub>9</sub>	X <sub>10</sub>	X <sub>11</sub>	X <sub>12</sub>	X <sub>13</sub>	X <sub>14</sub>
8602	280.00	6.20	169.40	4.90	3.80	120.00	63.25	180.00	160.92	11.60	16.03	1.18	25.80	143.32
8757	255.60	4.77	139.80	4.00	4.00	131.00	53.20	184.20	160.00	14.20	16.02	1.42	25.63	142.36
8840	283.60	4.68	150.60	4.50	3.60	127.00	62.15	189.15	100.94	11.60	14.14	1.00	14.28	79.30
8860	224.60	4.78	170.00	4.10	3.60	125.00	60.75	185.00	191.13	12.60	14.30	1.03	27.32	151.77
8921	247.00	5.60	180.00	4.90	4.00	120.00	50.70	180.00	138.60	14.40	16.60	1.18	23.01	127.84
8949	222.00	6.20	188.80	5.00	3.60	141.00	70.00	200.00	114.82	13.60	27.09	0.93	31.10	172.76
9045	220.00	4.90	165.60	4.40	4.00	120.00	63.60	191.60	130.00	14.00	16.37	1.08	21.28	118.19
9414	241.00	5.40	86.80	4.80	3.80	130.00	60.00	190.00	160.00	11.00	11.18	0.98	17.89	99.36
9655	236.00	4.87	90.60	4.90	3.80	131.00	73.80	200.00	142.00	12.20	13.58	1.03	19.29	107.13
9691	221.00	5.88	80.60	4.50	4.00	135.00	56.85	191.85	180.57	11.20	12.65	0.81	22.85	126.93
9750	259.00	8.00	73.00	4.00	4.00	149.00	62.65	211.65	138.80	13.40	17.94	0.96	24.90	138.32
10094	235.00	6.32	68.80	5.00	5.00	110.00	66.25	176.25	138.00	11.44	16.22	0.81	22.38	124.33
10228	237.80	4.50	86.00	5.20	4.00	118.00	75.90	193.90	81.94	11.00	32.18	0.82	26.36	146.45
10397	239.00	5.60	108.80	4.70	4.00	135.00	75.35	210.35	46.29	14.60	53.55	0.86	24.79	137.70
10447	210.60	4.40	124.20	4.50	4.00	98.00	65.29	167.25	169.00	9.80	13.76	0.64	23.25	129.16
10503	248.00	4.98	124.80	4.20	4.00	130.00	60.00	190.00	160.00	13.00	22.88	0.93	36.60	203.32
10559	250.00	4.65	73.00	4.80	3.40	135.00	73.75	208.75	200.00	12.20	8.22	0.97	16.44	91.35
10654	225.40	4.60	108.60	5.00	3.60	129.00	57.20	186.20	180.00	12.20	8.31	0.90	14.95	83.06
11015	243.00	4.86	95.40	4.70	4.00	108.00	69.55	177.55	155.00	14.60	8.32	1.04	12.89	71.60
11059	263.00	4.76	118.00	4.72	3.80	150.00	58.00	208.00	140.00	13.00	16.36	0.93	22.90	127.21
11230	243.00	5.60	148.00	4.80	4.00	148.00	81.20	219.50	125.00	11.20	26.47	0.90	33.09	183.81
11281	265.00	4.87	90.00	4.70	3.40	150.00	56.95	206.95	109.20	11.80	20.95	0.83	22.88	127.10
11320	250.00	4.89	173.00	3.80	3.40	149.00	58.90	200.70	115.39	13.80	30.50	1.06	35.19	195.49
11477	218.00	4.80	31.60	4.75	3.80	118.00	51.00	170.00	145.00	11.00	19.15	0.86	27.77	154.27

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ICP No.	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	X <sub>6</sub>	X <sub>7</sub>	X <sub>8</sub>	X <sub>9</sub>	X <sub>10</sub>	X <sub>11</sub>	X <sub>12</sub>	X <sub>13</sub>	X <sub>14</sub>
11627	227.00	4.80	96.00	4.70	3.80	120.00	65.95	195.95	115.00	13.22	17.18	1.05	19.76	109.74
11690	259.60	4.48	128.40	5.00	3.80	148.00	72.55	220.55	74.32	12.96	33.27	0.95	24.73	137.36
11823	263.60	5.50	135.00	4.80	5.00	140.00	57.30	197.30	180.00	14.20	9.69	0.97	17.43	96.85
11833	257.00	4.50	128.00	5.25	3.00	143.00	70.85	213.85	81.72	13.40	26.36	1.00	21.54	119.68
11910	222.00	6.56	133.40	4.50	4.00	127.00	59.25	186.25	130.00	17.00	15.39	1.21	20.01	111.13
11946	255.80	5.20	115.40	4.40	4.00	140.00	59.00	199.00	136.00	15.20	14.55	0.92	19.78	109.89
12105	266.00	4.80	129.00	4.46	4.60	135.00	71.15	206.15	234.67	14.00	10.37	1.43	24.32	135.12
12123	254.00	5.80	165.80	4.70	3.60	136.00	57.75	193.75	208.00	12.60	12.45	1.03	25.89	143.82
12142	238.20	5.80	159.00	5.84	3.60	119.00	50.50	171.90	200.00	13.60	11.50	1.00	23.00	127.77
12298	221.00	4.80	135.60	5.20	4.60	108.00	49.50	157.50	246.34	14.00	13.35	1.19	32.89	182.70
12410	245.00	5.60	120.00	5.50	4.40	135.00	43.35	180.00	173.91	14.00	12.22	1.46	21.25	118.02
12515	223.00	5.80	145.80	5.50	4.00	100.00	60.60	165.60	89.36	12.40	17.19	0.94	15.36	85.31
12596	226.00	4.80	129.00	6.00	4.60	110.00	63.00	168.00	190.00	14.20	13.14	1.13	24.96	138.64
12654	241.00	7.00	129.00	5.00	5.20	130.00	60.00	190.00	145.00	10.30	10.75	0.60	15.58	86.56
12680	266.00	5.35	142.40	5.00	3.80	150.00	51.95	200.00	133.34	11.16	15.60	0.90	20.80	115.54
13139	266.00	5.42	159.40	4.00	4.30	161.00	57.10	215.00	217.20	12.52	9.18	0.83	19.93	110.72
13167	302.00	4.68	84.00	5.60	4.60	141.00	66.00	100.00	148.00	13.24	11.00	0.83	16.28	90.45
13191	304.00	5.60	128.60	5.40	4.60	169.00	54.75	200.00	128.00	12.60	16.94	0.84	21.68	120.43
13244	292.00	5.20	88.80	5.10	4.00	132.00	59.90	190.00	190.00	13.60	13.07	0.93	24.84	137.99
13270	295.00	6.20	121.60	4.80	3.80	132.00	85.50	200.00	190.00	14.60	13.06	1.04	24.82	137.86
13304	263.00	5.60	56.60	4.70	3.80	155.00	72.00	200.00	150.00	13.40	26.39	1.00	39.58	219.89
13359	243.00	4.65	63.20	5.04	4.20	130.00	60.00	190.00	158.00	11.66	20.16	1.08	31.86	176.97
13431	323.00	6.00	139.00	4.90	4.00	145.00	54.45	204.45	164.20	12.20	15.42	1.02	25.32	140.63
13571	243.20	4.60	145.20	4.10	4.20	138.00	39.00	178.00	174.26	11.20	17.98	1.30	31.33	174.04

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ICP No.	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	X <sub>6</sub>	X <sub>7</sub>	X <sub>8</sub>	X <sub>9</sub>	X <sub>10</sub>	X <sub>11</sub>	X <sub>12</sub>	X <sub>13</sub>	X <sub>14</sub>
13575	279.00	5.00	100.40	6.20	3.80	139.00	72.20	200.00	145.00	13.00	17.45	0.90	25.30	140.54
13577	292.60	5.60	156.00	4.58	4.60	138.00	53.00	192.00	236.00	13.20	15.58	1.02	36.76	204.20
13579	246.00	4.60	96.89	5.60	3.40	139.00	45.60	185.00	200.00	14.80	6.65	0.94	13.29	73.85
13633	317.00	6.40	12.60	5.60	4.40	135.00	60.35	195.35	186.00	12.20	20.01	0.83	37.21	206.70
13662	284.00	4.80	43.78	5.40	4.60	139.00	56.50	195.50	158.00	13.60	14.47	1.08	22.86	127.01
13884	229.00	6.20	153.00	5.00	4.60	118.00	55.00	173.00	120.00	10.00	23.29	0.63	27.95	155.26
14116	304.00	4.50	149.80	4.80	3.00	136.00	46.95	182.95	190.00	12.33	13.16	0.99	25.01	138.92
14147	312.00	4.89	145.80	6.00	3.00	139.00	59.50	198.50	220.72	11.80	6.95	1.00	15.35	85.26
14229	242.00	5.98	141.20	7.90	4.00	147.00	74.75	221.75	217.40	15.60	10.52	1.22	22.88	127.10
14294	236.60	5.60	66.80	7.06	5.80	135.00	60.40	195.40	133.89	13.20	17.14	0.78	22.94	127.45
14368	200.87	5.80	96.50	4.70	5.20	77.00	67.50	144.50	115.58	12.60	15.05	0.88	17.39	96.60
14444	216.00	5.60	77.00	4.80	4.00	83.00	54.30	137.30	172.26	17.20	15.43	1.56	26.57	147.61
14471	247.00	6.10	71.60	4.80	3.40	125.00	61.50	186.50	171.38	12.40	10.55	0.79	18.08	100.41
14569	239.00	6.20	89.60	5.16	4.20	131.00	50.00	181.00	160.00	9.68	13.37	0.65	21.38	118.79
14638	235.00	5.60	86.00	5.00	4.60	118.00	63.50	181.50	87.45	15.00	58.58	1.14	51.23	284.57
14071	241.00	6.00	201.80	5.60	4.20	125.00	53.00	178.00	200.00	14.60	27.50	1.07	55.00	305.53
14722	272.00	4.94	87.30	5.66	4.00	145.00	54.90	199.90	108.84	14.00	41.85	1.15	45.55	253.03
14801	271.00	5.40	89.00	6.20	4.40	167.00	68.05	235.05	42.95	13.40	129.34	0.91	55.55	308.58
14819	247.00	4.56	79.00	5.76	4.80	80.00	35.70	115.70	114.56	10.80	22.96	0.73	26.31	146.14
14832	232.20	4.86	185.80	5.56	5.00	90.00	53.65	143.65	131.25	14.76	14.08	1.12	18.48	102.67
14900	205.00	4.87	70.40	6.66	4.00	127.00	50.25	177.25	145.00	13.00	19.95	0.87	28.93	160.71
14976	214.00	5.00	81.20	7.10	4.60	123.00	48.20	171.20	155.00	13.44	29.85	0.87	46.27	257.02
15049	263.00	5.20	76.60	4.90	5.20	87.00	61.80	148.80	117.07	12.00	17.68	0.85	20.70	114.98
15068	243.00	5.20	89.80	4.90	3.80	137.00	60.25	197.25	180.00	8.60	16.92	0.53	30.46	169.23

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ICP No.	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	X <sub>6</sub>	X <sub>7</sub>	X <sub>8</sub>	X <sub>9</sub>	X <sub>10</sub>	X <sub>11</sub>	X <sub>12</sub>	X <sub>13</sub>	X <sub>14</sub>
15161	251.00	5.60	96.50	4.54	4.00	154.00	48.10	202.10	82.64	14.40	22.01	1.03	18.19	101.05
15185	249.00	4.80	123.00	5.70	3.80	140.00	47.70	187.70	250.00	13.00	13.11	1.05	32.77	182.03
15382	255.00	4.80	228.80	4.84	4.20	134.00	60.70	194.70	183.05	15.80	13.18	1.16	24.13	134.03
16264	243.00	5.34	63.20	4.90	3.60	130.00	60.00	190.00	155.00	11.66	15.11	0.75	23.42	130.09
4575	240.00	4.78	279.20	5.30	4.00	114.00	59.00	173.00	86.40	15.80	32.54	1.18	28.11	156.15
BRG-7-3	239.00	4.56	127.00	4.52	4.00	104.00	61.00	165.00	95.06	12.40	26.30	0.90	25.00	138.88
BRG-6-12	260.00	5.21	149.80	5.06	4.20	140.00	55.00	195.00	82.50	11.80	18.62	0.83	15.36	85.32
1730	253.60	5.24	104.20	6.40	3.80	102.00	55.00	157.00	103.29	12.80	25.04	0.91	25.86	143.65
4019	255.60	5.00	100.00	4.50	4.80	114.00	72.00	186.00	20.93	17.00	134.76	1.20	28.21	156.71
BRG-9-2	248.00	4.80	98.50	5.00	4.00	112.00	63.00	175.00	240.00	14.40	9.60	0.96	23.05	128.04
BRG-207	268.00	5.80	49.00	5.00	4.00	114.00	55.00	169.00	21.24	15.77	104.01	1.19	22.09	122.73
TTB-7	228.00	5.76	73.20	6.00	4.00	119.00	50.00	169.00	189.00	13.10	11.08	1.07	20.94	116.33
TM224	236.00	4.50	69.80	6.30	3.00	138.00	59.00	197.00	158.21	11.00	15.57	0.76	24.63	136.81
1224	240.00	6.26	116.00	6.20	4.80	136.00	56.00	192.00	91.29	15.60	25.74	1.04	23.50	130.54
BRG-7-5	217.00	6.20	73.60	5.70	5.00	99.00	54.00	153.00	200.00	15.00	17.85	1.09	35.70	198.30
TM-26	255.00	4.76	95.00	4.90	4.60	144.00	68.00	212.00	195.00	13.20	11.04	0.90	21.53	119.59
5641	265.00	4.60	156.80	5.30	4.20	129.00	55.00	184.00	135.00	13.60	22.27	0.96	30.06	166.99
12460	255.00	5.00	156.80	4.60	3.80	129.00	68.00	197.00	126.61	12.20	23.84	0.89	30.18	167.65
1550	234.00	4.89	78.00	5.80	4.00	114.00	71.00	185.00	115.00	14.80	22.07	1.12	25.38	141.00
DPD-2-38	240.00	5.20	65.00	5.40	4.80	131.00	49.00	180.00	145.00	12.00	24.69	0.82	35.80	198.87
12333	242.00	4.97	109.20	4.50	4.00	100.00	58.00	158.00	180.00	13.60	22.22	0.99	40.00	222.20
10804	238.00	5.20	96.50	5.30	3.60	108.00	63.00	171.00	198.00	13.40	22.83	1.06	45.20	251.09
12674	226.00	5.60	54.75	6.70	4.00	103.00	57.00	160.00	200.00	15.40	19.45	1.20	38.90	216.09
Gullyal local	253.00	4.87	49.75	5.90	5.40	143.00	55.00	198.00	185.22	14.20	14.89	1.01	27.58	153.23

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ICP No.	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	X <sub>6</sub>	X <sub>7</sub>	X <sub>8</sub>	X <sub>9</sub>	X <sub>10</sub>	X <sub>11</sub>	X <sub>12</sub>	X <sub>13</sub>	X <sub>14</sub>
4878	231.00	4.60	115.00	5.90	4.60	81.00	65.00	146.00	100.39	12.72	45.24	0.95	45.41	252.26
PUSA992	214.00	5.87	123.00	4.50	4.80	114.00	60.00	174.00	127.25	13.36	16.12	0.88	20.51	113.93
6528	210.00	6.00	153.80	4.50	4.00	112.00	43.00	155.00	210.00	13.60	13.14	1.05	27.60	153.30
Gullyal red	241.00	5.20	153.60	6.50	4.00	130.00	60.00	190.00	190.00	11.00	20.78	0.83	39.49	219.34
HY-3-C	245.00	6.43	112.00	4.80	4.00	114.00	58.00	172.00	180.29	14.20	15.30	0.96	27.58	153.23
12266	244.00	5.55	133.00	4.88	4.00	103.00	53.00	156.00	182.00	16.20	22.78	1.12	41.45	230.27
9903	233.00	4.98	156.00	7.30	4.00	128.00	60.00	188.00	121.37	14.00	27.17	1.47	32.97	183.17
1366	245.00	4.99	137.20	4.86	5.20	134.00	55.00	189.00	180.00	12.40	14.19	1.01	25.54	141.86
4395	216.00	5.60	104.20	4.96	4.20	91.00	44.00	135.00	130.00	13.60	26.18	1.11	34.04	189.09
UPAS-120	243.00	4.89	118.20	4.64	4.20	130.00	63.00	193.00	175.11	15.60	13.38	0.99	23.43	130.16
BRG-9-2	214.00	5.80	81.20	5.20	4.00	114.00	72.00	186.00	120.82	13.40	16.28	0.97	19.67	109.29
TS-3@	263.00	4.80	100.80	5.10	4.20	117.00	68.00	185.00	190.00	13.80	13.98	1.11	26.57	147.59
DM-155	201.00	4.65	41.25	4.56	4.20	128.00	55.00	183.00	196.00	14.20	14.10	1.04	27.64	153.52
TM-389	216.00	4.89	52.25	5.00	3.60	136.00	63.00	199.00	120.77	14.00	24.06	0.90	29.06	161.43
BRG-7-2	233.00	6.00	74.00	5.00	5.00	90.00	45.00	135.00	150.00	14.20	14.94	1.06	22.41	124.49
GC-11-39	276.00	5.50	77.80	6.50	4.00	114.00	60.00	174.00	160.00	15.75	14.86	1.14	23.77	132.03
12301	210.00	4.80	51.80	5.10	3.00	127.00	63.00	190.00	168.00	12.75	11.35	0.90	19.06	105.88
BRG-7-4	200.00	4.87	73.00	5.10	4.20	118.00	50.00	168.00	178.00	10.50	19.42	0.75	34.56	191.98
GRG-2-95	222.00	5.45	121.20	5.00	4.00	136.00	61.00	197.00	139.04	13.20	31.16	0.93	43.33	240.70
12674	229.00	5.80	83.80	6.70	4.00	149.00	58.00	207.00	192.00	12.20	8.52	0.81	16.36	90.88
4275	221.00	4.80	92.80	4.86	4.80	135.00	55.00	190.00	160.00	14.40	32.51	1.09	52.01	288.92
12282	268.00	4.80	82.00	5.80	3.80	114.00	69.00	183.00	159.50	15.00	36.29	1.23	57.88	321.52
13270	224.00	5.30	105.00	5.60	4.20	128.00	71.00	199.00	122.96	13.80	74.28	0.96	91.33	507.36
BRG-7-6	227.00	4.68	103.88	5.20	3.80	132.00	60.00	192.00	145.00	14.60	40.23	0.97	58.33	324.02

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ICP No.	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	X <sub>6</sub>	X <sub>7</sub>	X <sub>8</sub>	X <sub>9</sub>	X <sub>10</sub>	X <sub>11</sub>	X <sub>12</sub>	X <sub>13</sub>	X <sub>14</sub>
DM-158	238.00	5.80	151.96	5.90	4.20	77.00	55.00	132.00	220.00	14.20	22.27	1.03	49.00	272.20
ICPL-87	239.00	4.76	208.80	5.80	4.40	131.00	50.00	181.00	200.00	11.10	26.42	0.88	52.83	293.47
12692	243.00	5.10	156.40	5.40	4.00	110.00	60.00	170.00	131.67	14.60	32.07	1.14	42.22	234.53
Jamadhar local	265.00	5.60	158.30	5.40	4.00	115.00	45.00	160.00	114.05	14.80	49.30	1.06	56.22	312.30
WRP-1	233.00	4.50	92.98	5.16	4.00	130.00	63.00	193.00	117.60	12.60	35.31	1.00	41.52	230.64
Bidabr local	244.00	4.40	98.00	5.40	4.00	91.00	44.00	135.00	180.61	11.88	28.38	0.87	51.26	284.75
UPAS-120	255.00	6.32	123.25	5.60	4.00	107.00	59.00	166.00	130.81	13.20	16.85	0.94	22.04	122.43
JKE-144	243.00	4.50	108.21	4.50	3.00	94.00	60.00	154.00	158.00	12.60	36.10	0.90	57.04	316.86
PUSA-92	231.00	4.54	113.01	5.10	5.00	128.00	53.00	181.00	110.93	13.44	56.14	1.08	62.28	345.97
8863	232.00	5.60	67.60	5.50	4.60	129.00	62.00	189.00	160.00	14.00	21.71	0.99	34.74	192.98
PG-12	238.00	5.40	138.50	5.36	3.80	122.00	61.00	183.00	210.00	15.40	45.25	1.10	95.03	527.91
MA-29	235.00	4.80	93.40	5.80	4.00	123.00	53.00	176.00	114.53	12.80	54.67	1.00	62.62	347.85
Karithogari	223.00	4.20	274.95	5.80	4.20	163.00	50.00	213.00	330.00	14.20	17.66	1.13	58.27	323.68
ICPB 2091	226.00	5.40	179.42	5.20	4.00	133.00	55.00	185.00	211.15	14.60	25.48	1.07	53.80	298.85
CORG-295	216.00	5.20	190.24	5.10	3.80	128.00	60.00	188.00	127.51	14.40	46.40	0.99	59.16	328.63
Chaple	229.00	5.40	148.38	5.10	4.00	109.00	50.00	159.00	124.52	16.60	44.17	1.24	55.00	305.53
Gulyal white	239.00	4.80	98.19	5.80	4.20	104.00	55.00	159.00	120.32	12.00	38.90	0.77	46.80	259.97
CORG-9701	228.00	5.20	140.00	6.36	4.60	130.00	60.00	190.00	200.00	12.00	26.80	0.72	53.60	297.75
WRP-1 (W)	212.00	5.87	78.30	5.24	4.80	102.00	70.00	165.00	190.00	13.20	22.18	1.08	42.14	234.09
Rudrawadi local	232.00	5.43	84.20	5.00	4.20	128.00	60.00	180.00	180.00	12.30	23.43	0.77	42.18	234.31
JKM-197	225.00	5.20	95.08	5.80	3.60	113.00	45.00	155.00	195.00	10.90	16.49	0.85	32.16	178.65

Contd....

ICP No.	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	X <sub>6</sub>	X <sub>7</sub>	X <sub>8</sub>	X <sub>9</sub>	X <sub>10</sub>	X <sub>11</sub>	X <sub>12</sub>	X <sub>13</sub>	X <sub>14</sub>
CORG-2004-01	234.00	4.79	82.38	5.00	3.80	114.00	65.00	175.00	293.00	12.11	16.23	0.87	47.55	264.13
PJT-501	214.00	5.00	124.83	5.60	4.00	115.00	58.00	173.00	180.00	11.60	26.83	0.73	48.30	268.31
GRG-801	212.00	5.34	151.18	5.50	3.00	100.00	55.00	155.00	180.00	12.80	20.12	0.85	36.22	201.20
Bennur local	211.00	5.60	110.36	5.12	5.00	116.00	50.00	164.00	190.00	16.20	24.11	1.19	45.80	254.42
GRG-206	239.00	5.62	89.23	5.40	3.60	107.00	60.00	167.00	165.00	14.00	23.63	0.88	38.99	216.59
<b>Checks</b>														
MARUTI	217.96	5.48	108.8	5.12	3.8	117	57.1	168.6	142.86	12.08	37.1	1.02	44.82	293.42
BSMR	242.92	5.32	170.28	5.26	3.76	130.4	48.94	178.8	107.69	12.3	78.85	1.07	34.34	190.75
ASHA	229	5.05	133.76	5.11	3.64	132.4	61.7	187.4	149.77	14.34	0.93	0.93	37.56	208.62
GS-1	241.96	5.24	114.63	5.17	4	130.2	62.5	188.4	227.07	13.44	19.28	1.01	43.97	244.27
<b>CD @ 5%</b>	7.72	0.962	20.67	0.45	0.73	6.602	0.57	0.61	3.25	708.56	1.743	0.29	0.225	0.58

\*CD @5 % (between means of a check and test genotypes)

X<sub>1</sub> - Plant height (cm)

X<sub>2</sub> - Primary branches per plant

X<sub>3</sub> - Pods per plant

X<sub>4</sub> - Pod length (cm)

X<sub>5</sub> - Seeds per pod

X<sub>6</sub> - Number of days to 50% flowering

X<sub>7</sub> - Number of days taken from flowering to maturity

X<sub>8</sub> - Number of days to maturity

X<sub>9</sub> - Straw yield per plant (g)

X<sub>10</sub> - Test weight (g)

X<sub>11</sub> - Harvest index (%)

X<sub>12</sub> - Specific gravity

X<sub>13</sub> -Yield per plant (g)

X<sub>14</sub> - Grain yield (kg/ha)

**GENETIC VARIABILITY STUDIES IN MINICORE  
COLLECTION SET OF PIGEONPEA (*Cajanus cajan* (L.)  
Millsp.) AND MOLECULAR CHARACTERIZATION IN  
SPECIAL RELATION TO STERILITY MOSAIC DISEASE,  
FUSARIUM WILT AND PROTEIN CONTENT**

**NETHRAVATHI K H**

**2013**

**Dr. B. R. PATIL  
Major Advisor**

**ABSTRACT**

A minicore collection set comprising 192 pigeonpea accessions were evaluated in an augmented design during 2012-13 *kharif* at Botanical garden, Main Agricultural Research Station, University of Agricultural Sciences Dharwad. The genotypes were evaluated for the genetic parameters, correlation, and path coefficient analysis and for reaction to fusarium wilt (FW), sterility mosaic disease (SMD) and protein content. High genotypic and phenotypic co-efficient of variation, was recorded for the characters *viz.*, Number of primary branches per plant, number of pods per plant and seed yield. However High heritability coupled with high genetic advance as per cent of mean was exhibited by the characters *viz.*, plant height, days to fifty per cent flowering, days to maturity, seed yield per plant, harvest index and specific gravity. Aforesaid characters may be stressed during selection for genetic gain. The accessions, ICP13270, ICP3049, PUSA-92 and MA-29 recorded significantly high seed yield per plant compared to the standard check, (Maruti). These genotypes can be considered for hybridization programme.

Divergences studies classified the accessions into 9 clusters. The intra cluster distance was maximum in cluster VII (146.77). Selection of genotypes from these divergent groups would result in higher magnitude of heterosis for the characters concerned. Based on phenotypic observations 48, 50 and 46 accessions were selected for molecular characterization of FW, SMD and protein content respectively employing a set of 150 simple sequence repeat (SSR) primers. The allele size's ranged from 100 to 220 bp for fusarium wilt, 100 to 300 bp for sterility mosaic disease and 100 to 200 for protein content. These markers can be successfully used for molecular characterization of genotypes. Most abundant motif's in the present study were AT based (AT, AAT, TTAT) followed by TC class of repeats. Such repeats may be emphasized during marker assisted selection.