"Studies on genetic variability and DNA marker diversity in selected lines for Sterility Mosaic Disease resistance in pigeonpea [*Cajanus cajan* (L.) Millsp.]"

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"Studies on genetic variability and DNA marker diversity in selected lines for Sterility Mosaic Disease resistance in pigeonpea [*Cajanus cajan* (L.) Millsp.]"

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AFFECTIONATELY DEDICATED TO GOD ALMIGHTY

DEPARTMENT OF GENETICS AND PLANT BREEDING
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CERTIFICATE

This is to certify that thesis entitled "**Studies on genetic variability and DNA marker diversity in selected lines for Sterility Mosaic Disease resistance in pigeonpea [*Cajanus cajan* (L.) Millsp.]**" submitted by **Gangadhara, A. V., in partial fulfillment of the requirement for the award of degree of Master of Science (Agriculture) in Genetics and Plant Breeding,** to the University of Agricultural Sciences, Bangalore is a record of bonafide research work done by him during the period of his study in University, under my guidance and supervision and the Thesis has not previously been formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Introduction

I. INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is the most important pulse crop in India next to chickpea, producing about 2.21 m. tonnes from an area of 3.38 m. ha with the productivity of 653 kg/ha. Major states in terms of area and production are Maharashtra, Uttar Pradesh, Madhya Pradesh, Karnataka, Gujarat and Andhra Pradesh together contributes for about 90% of area and 90% of production of Pigeonpea. In Karnataka, it occupies an area of 5.8 lakh hectares with annual production of 2.6 lakh tonnes with an average productivity of 476 kg / ha (Anonymous, 2004). Pigeonpea is cultivated in multitude of production systems for diverse uses *viz*., grain, vegetable, forage, green manure and fuel.

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 lack of improved production technology and due to various abiotic and biotic stresses. So far any crop improvement programmes aiming to increase the productivity, a sound breeding programme utilizing indigenous and exotic germplasm is very much essential.

Pigeonpea being a long duration crop is prone to both biotic and abiotic stresses. Diseases of economic importance in the Indian sub continent are *Fusarium* wilt, sterility mosaic disease and *Phytopthora* blight (Reddy *et al*., 1990). Among the diseases, sterility mosaic disease (SMD) transmitted by the eriophid mite (*Aceria cajani)* is a major threat to pigeonpea production in the Indian subcontinent causing significant yield losses up to 90 per cent (Kannaiyan *et al*., 1984).

Yield being a complex trait is collectively influenced by various component characters, which are polygenically inherited and highly influenced by environmental variations. So, the observed variability for these characters is the sum total of hereditary effects and the influence of environment. Hence, it becomes necessary to partition the observed variability in to heritable and non-heritable components measured as genotypic and phenotypic co-efficient of variations, heritability and genetic advance. The knowledge of the degree of association existing between yield and its attributes, among

yield components is possible through the precise estimation of genotypic and phenotypic correlation coefficients. Path coefficient analysis splits the correlation coefficients into components of direct and indirect effects so that relative influence of each component character on the yield could be assessed which helps a great deal to formulate selection strategies, to develop suitable genotypes.

Genetic diversity at genomic level is a pre-requisite for finding variations in the selection of genotypes. Though a range of plant morphological traits are accurately used for distinguishing the genotypes, environment plays an important role in influencing their expression. Thus, a reliable tool for variety characterization and identification is to know DNA polymorphism. Advances in DNA markers allow such detection and it is becoming increasingly possible to detect variation between individuals of similar phenotype at DNA level.

There are varieties of molecular markers like RFLPs, RAPDs, AFLPs and STS which are commonly being used for molecular tagging of disease resistant genes. RAPDs and SSRs are the best to start with for identifying markers and convert them later into more reliable, user-friendly marker types for tagging genes and for marker assisted selection.

Breeding for disease resistance is very important, but in breeding programme it takes time. Though, conventional breeding has done much, but has not substantially improved productivity of this crop, mainly due to its out crossing nature. Because of its long life cycle, it is a problem to screen the varieties and breeding population for SMD resistance. Accurate phenotyping or genotyping is a must for resistance breeding. The use of molecular markers provides an excellent solution to these problems.

For development of markers for SMD, it is a pre-requisite to develop F_2 segregating population from the resistant and susceptible parents. This could be developed based on the diversity of the parents for resistance and susceptibility, which could serve as a database and ready reckoner for identification of parents unambiguously and subsequently can be used in marker assisted plant breeding.

Realizing the importance of such an investigation, the present study was carried out with the following objectives

1. To study the variability, heritability and genetic advance in Pigeonpea.
2. To study the association among yield and yield attributing characters
3. To study the direct and indirect effects of quantitative characters on yield.
4. To study the DNA marker diversity in the selected genotypes differing in resistance levels to sterility mosaic disease with RAPD and SSR markers.

Review of Literature

II. REVIEW OF LITERATURE

Pigeonpea is the second most important grain legume crop in India. It belongs to the family *Leguminoceae,* Sub family *Papilionoceae* and group *Phaseoleae.*

Numerous types of pigeonpea are known to differing in plant height, maturity, color, size and shape of pods and seeds. Shaw *et al.* (1933) distinguished 86 different types of collections from all over India. Mehtha and Deve (1931) recognized 36 types and broadly grouped them under two varieties (*Cajanus cajan* var. *bicolor* and *C. cajan* var. *flavus).* Cajanus *cajan* var. *flavus* is described as a relatively small plant having 2 to 3 seeds in a pod which are more spotted, while *Cajanus cajan* var. *bicolor* is a large plant with 4 to 5 seeds in the pods which are marked with dark streaks.

In the present investigation the literature pertaining to the objectives has been reviewed and presented under the following headings.

1 Genetic variability, heritability and genetic advance studies.

2 Correlation and path coefficient analysis.

3 Molecular diversity studies

2. 1 Studies on genetic variability, heritability and genetic advance

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, which indicates the relative magnitude of genetic diversity present in the material, will help to compare the genetic variability present for different characters.

Rathnaswamy *et al.* (1973) noted high genotypic coefficient of variation for seeds per pod, pods per plant, pod weight and days to flowering. High heritability values were reported for plant height, branches per plant, pods per plant and days to 50 % flowering.

Chandra *et al.* (1975) reported high genetic variation for yield, primary and secondary branches and days to flowering, of which days to flowering and number of primary and secondary branches showed high heritability values.

Ram *et al.* (1976a) reported high genotypic coefficient of variability and heritability for clusters per plant followed by grain yield where as, highest genetic advance observed for clusters per plant followed by harvesting index. The grain yield and primary branches exhibited low values of genetic advance.

High genetic variation was reported by Malhothra and Sodhi (1977) for branches per plant, grain yield and pod number. They also observed high heritability for grain yield, pod number and branches per plant accompanied by high genetic advance.

High heritability and genetic gain values were observed for days to flowering, days to maturity, harvest index and seed yield per plant by Singh *et al.* (1978). Bashiruddin and Sreeramulu (1981) observed high genotypic coefficient of variation for 100 seed weight, cluster per plant, pods per plant and branches per plant.

Jagashoram (1983) studied one hundred genotypes of pigeonpea in two years and reported that magnitude of phenotypic variability was high for all the characters studied except for seeds per pod. High estimates of genotypic co-efficient of variation and heritability accompanied by moderate to high genetic advance for pods per plant, days to maturity, plant height and days to flowering were observed across the environments.

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 of 50% 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 12 pigeonpea genotypes, Balyan and Sudhakar (1985a) reported high estimates of phenotypic and genotypic coefficient of variation, heritability and expected genotypic advance for primary and secondary branches, pods per plant, 100 seed weight and seed yield.

Sindhu *et al.* (1985) found that variability was highest for pods per plant. Heritability estimates were high for all the traits studied except for the 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 50 % flowering, branches per plant, plant height, pods per plant, pod length, pod weight and 100 seed weight.

High genotypic coefficient of variation was observed for pod number followed by cluster number and seed yield while, it was lowest for seeds per pod. High heritability and genetic advance were observed for pod number, cluster number and seed yield (Natarajan *et al.* 1990).

Holkar *et al.* (1991) evaluated 27 Pigeonpea genotypes, high GCV and PCV estimates were observed 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 genetic advance for grain yield per plant, whereas heritability was high for all the traits except primary branches per plant.

Ghodke *et al.* (1994) reported high estimates of heritability for number of pods, days to 50%flowering and days to maturity.

Gamber *et al.* (1996) reported moderate PCV and GCV estimates for days to 50% flowering, pod length and 100 seed weight at genotypic and phenotypic variations.

Aher (1998) observed wide genetic variability for plant height, number of secondary branches per plant and days to 50% flowering. High heritability accompanied by high genetic advance was observed for number of secondary and primary branches per plant, followed by grain yield per plant, days to 50% flowering and plant height.

Pansuriya (1998) reported information on variability, heritability and correlation coefficients from 20 early maturing pigeonpea genotypes. Plant height and pods per plant showed a wide range of phenotypic variation. The genotypic and phenotypic coefficients of variation were highest for dry matter per plant, harvest index, pods per plant and grain 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 (1998) reported 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 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.

Basavarajaiah *et al.* (2000) reported high phenotypic and genotypic coefficient of variation values for days to 50% 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 50% flowering, yield per plant, length of pod bearing branches and 100-seed weight.

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 number of seeds/pod. Heritability estimates were high for all the traits, except seeds/pod.

Deshmukh *et al.* (2000) reported that number of secondary branches/plant showed the highest genetic variation. Heritability estimates were high for days to 50% 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, grain 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 genotypic and phenotypic coefficient of variation values for branches for plant, pods per plant and low values for days to 50% 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 pods per plant exhibited high genotypic and phenotypic coefficient of variation.

Chattopadyaya and Dhiman (2005) reported high genotypic and phenotypic variation for 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.

2.2 Correlation and Path Coefficient Analysis

2.2.1 Association of characters

Correlation coefficient analysis helps to determine the nature and degree of relationship between the two measurable characters. It resolves the complex relation between the events into simple forms of association. Knowledge of phenotypic and genotypic correlation between important characters is of immense help in the selection of suitable plant type.

Strong positive correlation was found between grain yield and length of pod bearing branch, number of pods and seeds per plant as reported by Ravi Prakash (1979).

Awatade *et al.* (1980) recorded positive and highly significant correlation of grain yield with pods per plant, clusters per plant, plant height, secondary branches per plant and days to maturity both at phenotypic and genotypic levels.

The grain yield per plant showed significant and positive correlation with number of primary branches, 100 grain weight, number of pods per plant and pod length (Godawat 1980).

Asawa *et al.* (1981) revealed that grain yield was positively correlated with secondary branches, pods per plant, seeds per pod and days to maturity.

Pahuja *et al.* (1981) observed highly significant correlation of grain yield with plant height, non- productive as well as productive branches, seed size, pods per plant and days to maturity.

Singh *et al.* (1981) found highly significant positive correlation of grain yield with pods per plant, plant height, days to 50% flowering, seeds per pod and days to maturity.

Balyan and Sudhakar (1985a) noted a significant positive correlation between grain yield and seven yield related characters. *viz.*, days to maturity, plant height, number of primary and secondary branches, pods per plant, seeds per pod and 100 seed weight. Significant positive association of grain yield with plant height and pods per plant was also reported by Sindhu *et al.* (1985).

Singh *et al.* (1985) reported that seed yield had highest positive association with number of pods per plant followed by 100 seed weight. Days to 50% flowering however indicated a negative association with the yield.

Bhongale and Raut (1987) noted significant positive correlation of grain yield with plant height, branches per plant, pods per plant, pod weight and seeds per pod.

Merker and Nerker (1987 a and b) observed a significant positive correlation of seed yield with plant height, secondary branches per plant, clusters per plant, pods per plant, biomass and harvest index both at the genotypic and phenotypic levels .

Angadi *et al.* (1988) studied eleven hybrids and nine varieties of pigeonpea. Association studies indicated that pod yield, plant height, branches per plant, days to flower and pods per plant were strongly associated with grain yield.

Choudhary *et al.* (1988) reported positive correlation of grain yield with plant density, pods per plant, branches per plant and seeds per pod. They also revealed that, earliness had negative correlation with plant height, number of branches and clusters per plant.

Patel *et al.* (1988) reported that, seed yield was strongly correlated with plant height, branches per plant and pods per plant.

Patil *et al.* (1989) observed a positive correlation of seed yield with branches per plant, pods per plant and hundred seed weight.

Balakrishna and Natarajarathnam (1989) reported that seed yield per plant was highly correlated with pods per plant. The pods per plant and 100 seed weight were negatively correlated with dry matter efficiency and harvest index.

Ganesh Murthy and Stephen Dorairaj (1990) observed significant positive correlation of seed yield with a dry matter production, pods per plant, clusters per plant, branches per plant, plant height, seeds per pod, days to flowering, days to maturity, 100 seed weight, harvest index and pod length.

Studies conducted by Henry and Krishna (1990) on character association revealed that, seed yield had highly significant and positive correlation coefficient with the plant height, number of branches, number of clusters and number of seeds per pod.

Natarajan *et al.* (1990) reported significant positive correlation of seed yield with pod number, cluster number and plant height.

The Correlation coefficients worked out by Holkar *et al.* (1991) indicated that, hundred seed weight and pods per plant had highly positive genotypic correlation with seed yield. On the other hand, days to maturity and days 50% flowering showed negative correlation with seed yield at both phenotypic and genotypic levels.

Jahargirdhar *et al.* (1991) indicated that number of pods, 100 seed weight and plant height were strongly associated with grain yield. High correlation between grain yield and pods per plant also reported by Paul and Upadhaya (1991).

Khapre and Nerkar (1992) reported that, days to maturity, plant height, pods per plant and number of total biomass per plant had highly significant positive association with grain yield under different cropping system.

Patel and Patel (1992) indicated that grain yield was positively and significantly associated with plant height and pods per plant at both genotypic and phenotypic levels.

Virangama and Goyal (1994) observed significant positive association of grain yield with pods per plant, number of primary branches, plant height, length of secondary branches, 100 seed weight, days to flowering and pod length. Number of pods per plant was significantly and positively correlated with all the traits except pod length and number of seeds per pod.

Significant positive association for grain yield with number of pods per plant, plant height, seeds per pod and hundred seed weight was reported by Byre Gowda *et al.* (1996).

Significant positive associations of grain yield with days to 50% flowering, days to maturity and pod set was observed Gamber *et al.* (1996).

In study of 28 pigeonpea experimental hybrids by Paul *et al.* (1996) indicated that, yield was positively and significantly correlated with number of pods per plant and number of secondary branches at phenotypic level while at genotypic level the association was highest for dry matter at maturity.

Dhedi *et al.* (1997) observed that seed yield was significantly and positively correlated at phenotypic level with plant height, branches per plant and pods per plant

Kingshlin and Subbaraman (1997 a and b) indicated that branches per plant, clusters per plant, pods per plant, pod length, seeds per pod and 100-seed weight were strongly associated with seed yield.

Singh (1997) reported positive correlation for number of pods per plant, number of seeds per pod and 100 seed weight.

Pansuriya *et al.* (1998) reported that grain yield was significantly and positively correlated with pods per plant and dry matter per plant, indicating that selection on the basis of these traits would be effective for yield improvement.

Basavarajaiah *et al.* (1999) reported that significant positive correlation of grain yield with pod weight, pods per plant, straw weight, branches per plant and shelling percentage.

Jagdish Singh (1999) reported that seed yield per plant showed significant positive association with plant height, branches per plant, pods per plant, seeds per pod and biological yield per plant. Maximum correlated response was recorded for biological yield per plant followed by pods per plant, branches per plant, harvest index and seeds per pod.

Srinivas (1999) reported that seed yield showed significant positive relationship with plant height, number of primary branches, secondary branches and pods per plant.

Kingshlin and Subbaraman (1999) indicated that branches per plant, clusters per plant, pods per plant, pod length, seeds per pod and 100-seed weight were strongly associated with seed yield.

Deshmukh *et al.* (2000) reported that characters exhibited significant positive correlations with grain yield exhibited positive correlations with pods per plant, days to flowering, days to maturity, primary and secondary branches.

Pandey and Singh (2001 a and b) reported that seed yield per plant had positively significant genotypic correlation with days to initial flowering, number of primary branches per plant and number of pods per plant.

Firoz Mahamad (2003) reported that days to 50% flowering, number of branches, pods per plant, pod yield per plant and 100 seed weight had high positive and significant correlation coefficient values with seed yield.

Ram Dhari *et al.* (2004) reported that pods per plant and fruiting branches per plant had positive and high correlation values with seed yield.

Chattopadhyay and Dhiman (2005) reported that seed yield had high and positive correlation values with plant height and number of seeds per pod, whereas 100 seed weight was negatively associated with seed yield.

2.2.2 Path Coefficient Analysis

Measure of correlation does not consider dependence of one variable over the other. Direct contribution of each component to the yield and the indirect effect through its association with other components cannot be differentiated from mere correlation studies. A Statistical tool called path coefficient analysis suggested by Wright (1921) offers a solution to this problem. It is a tool in genetic analysis, which partitioned the association of components on yield and indirect effects of the characters through other components.

Pankaj Reddy *et al.* (1975) indicated that, the maturity period and pod number had direct effect on seed yield.

The path coefficient analysis by Veeraswamy *et al.* (1975) revealed that, the number of branches had maximum influence both directly and indirectly on seed yield. The number of days to flowering had a direct negative influence on the yield.

Wakankar and Yadav (1975) found that number of pods had high positive direct effect on seed yield followed by number of secondary branches.

Ram *et al.* (1976b) reported that, the primary branches, cluster per plant and pods per cluster contributed directly as well as indirectly to grain yield. Plant height and number of pods exerted maximum influence on seed yield as reported by Gunaseelan and Hanumantha Rao (1976).

The path coefficient analysis in 25 genotypes of pigeonpea by Raviparkash (1979) indicated that, the direct contributions from number of inflorescence and number of pods per plant, where as contribution of seeds per plant was high.

Awatade *et al.* (1980) noticed that, the number of clusters per plant and 100 seed weight had direct effects on seed yield whereas days to maturity and seeds per pod had low indirect effects on seed yield. High direct effect of 100 seed weight on grain yield per plant was also reported by Godawat (1980).

Asawa *et al.* (1981) reported that, most of the yield contributing traits expressed strong indirect effects on seed yield via secondary branches.

Pahuja *et al.* (1981) found that pods per plant had high direct positive effect on seed yield while the productive branches had high positive indirect effect through pods per plant.

Singh *et al.* (1982) observed that maximum direct positive effect on grain yield was shown by the number of pods per plant followed by 100 seed weight. Days to 50% flowering however indicated a negative direct effect on the yield.

Balyan and Sudhakar (1985b) found that days to maturity, plant height, number of primary and secondary branches, pods per plant, seeds per pod and hundred seed weight had positive direct effect on seed yield

Sindhu *et al.* (1985) observed that pods per plant, plant height and seed size were major contributing traits to the seed yield.

Singh *et al.* (1999) reported that pods per plant had highest positive direct effect on grain yield followed by 100- seed weight, seeds per pod and days to 50% flowering whereas, pod length had low direct effect on seed yield.

Deshmukh *et al.* (2000) reported maximum positive indirect effects on seed yield through pods per plant followed by branches per plant, pod length and shelling percentage. The plant height exhibited high and negative indirect effect on seed yield.

Firoz Mahamad (2003) reported that pod yield, branches per plant and 100 seed volume had high direct effect on seed yield.

Ram Dhari *et al.* (2004) reported that pods per plant and branches per plant had high positive and direct influence on seed yield.

Chattopadhyay and Dhiman (2005) reported that plant height and number of seeds per pod had high and direct positive influence on seed yield, whereas the contribution of 100 seed weight was negative to seed yield.

2.3 Molecular diversity studies

The use of molecular markers in pigeonpea has been limited to the studies on cytoplasmic variation, polygenic relations and finger printing of accessions of the cultivated species.

Hence literature partitioning to diversity studies is collected from related crops also.

Restriction fragment length polymorphism (RFLPs) have 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. cajanifolia* (Nadimpalli *et al.,* 1993).

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 α, atp δ, cox -I and cox- II (Sivaramakrishnan *et al.*,1996).

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

RAPD markers associated with resistance to *Fusarium* wilt race 1 were identified in RIL population of chickpea and allele specific associated primers CS-27F/CS-27 developed from RAPD markers amplified a fragment linked to the allele for susceptibility to race 1 of *Fusarium* wilt (Mayer *et al.*, 1997).

Tyagi *et al.* (1997) reported that, RAPD markers were used for investigating quantitative trait loci (QTLs) in two strains of pigeonpea and in the F_1 and F_2 progeny. DNA was extracted from young leaves and subjected to polymerase chain reaction (PCR) for amplification. The use of single primers of arbitrary nucleotide sequence resulted in the selective amplification of DNA fragments that were unique to parents, F_1 and F_2 progeny. However, the level of polymorphism among parents was very low. F_1 was intermediate between two parents, but F_2 showed little variation, indicating that both parents were different morphologically, but with little genetic variation at DNA level.

Banerjee *et al.* (1998) isolated DNA from four cultivated chickpea accessions. RAPD analysis with 12 primers revealed 424 amplified DNA bands. The wild species produced a maximum of 34 polymorphic bands, while all cultivars except ICCV 92504 produced 2 or 3 polymorphic bands. Analysis of accessions using microsatellite probes revealed amplified bands not visible on ethydium bromide-stained gel. Combination of RAPD analysis and probing with microsatellite repeats (TG) 10, (GATA) 4 and (CAC) 5 revealed polymorphic and genotype-specific unique DNA banding patterns.

Tiwari *et al.* (1998) identified both in coupling and repulsion phase RAPD markers for powdery mildew resistant gene *er*-1 in pea, bulks were constituted from F_3

individuals and analyzed. Markers OPO-18 was found to be linked in coupling phase, while the other OPE-16 and OPL-6 were in repulsion phase to *er* -1.

Pigeonpea line ICPL 87 was evaluated for improvements in qualitative and quantitative traits. The somaclonal variants could be 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 and these 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 that in pigeonpea, a set of 10 simple sequence repeat (SSR) markers were developed. Screening for polymorphic SSRs was conducted on a set of 12 diverse pigeonpea accessions using 20 primer pairs. Of the 20 primer pairs, 10 loci exhibited polymorphism when applied to the set of 12 diverse pigeonpea accessions.

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

Souframanien *et al.* (2003) used randomly amplified polymorphic DNA (RAPD) markers for the identification of two pigeonpea cytoplasmic male sterile (CMS) lines derived from crosses between the wild (*Cajanus scarabaeoides* and *C. sericeus*) and the cultivated species of *Cajanus cajan*. The male sterile (A) line and its maintainer (B) line could be easily differentiated with certain random primers. The two male sterile (288 A and 67 A) systems are based on *C. scarabaeoides* and the other is based on *C. sericeus* could also be differentiated. 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 line and wild species studied.

Fernandes *et al.* (2003) reported in pigeonpea that DNA amplification with the SSR primer resulted in high level of genetic diversity with all the isolates of *rhizobium* showing unique profiles of DNA.

Rajesh *et al.* (2005) studied the usefulness of cleaved amplified polymorphic sequence (CAPS) and derived cleaved amplified polymorphic sequence (dCAPS) markers to identify polymorphism in a region of chickpea genome lacking visible polymorphism. CAPS analysis did not detect polymorphism in the product amplified with the primer (MF) designed from forward end of 4m10 BAC clone. dCAPS technique was used by designing primers with a single nucleotide mismatch adjacent to SNP position creating restriction site in the amplified PCR product of one parent but not the other. The size of the product amplified by MF is 553 bp and Taq1 restriction site was created by replacing an adenosine with a thymidine at the third position 5' to the SNP. The amplified products were digested using Taq1 enzyme and separated on 2% agarose gel to detect polymorphism between the parental lines and the segregating population. Metaphor agarose gel or 6% acrylamide gel is recommended for improved resolution of the digested bands. SNP detection between parental allelic sequences was verified by comparing replicate sequences.

Bonn (2006) isolated SSR loci of pigeonpea by screening non-enriched and enriched partial genomic libraries with SSR probes. 220 Soybean markers were tested in pigeonpea, 39 of which amplified interpretable bands. Nine of the markers developed were polymorphic to the parental lines of F_6 *Fusarium* wilt RIL mapping population. Analysis of segregation in the RIL segregation revealed that all the 9 SSR segregated in the expected 1:1 ratio and were further tested for any possible linkage with QTL for resistance to *Fusarium* wilt.

Kotresh *et al.* (2006) identified RAPD markers associated with wilt pathogen by using F_2 populations derived from the contrasting parents. DNA samples were extracted from the seedlings and used for marker identification. PCR reactions using 340 decamer primers with genomic DNA of the parents resulted in detection of 45 polymorphic amplicons from 39 ramdom decamer primers. PCR testing revealed that presence of two amplicons at 704 bp and 500 bp with susceptibilty.

Material and Methods

III. MATERIAL AND METHODS

The present study was conducted during kharif 2005 at the All India Co-ordinated Research Project on Pigeonpea, GKVK farm, University of Agricultural Sciences, Bangalore which is situated at an altitude of 930 M above mean sea level (MSL), $12^{0}\ 58^{1}$ north latitude and $77^{0}\ 35^{1}$ east longitude. The details of the materials, techniques adopted and method of analysis of the data are furnished in this chapter.

3.1 MATERIAL

The material for the study comprised of 100 genotypes of pigeon pea of diverse origin procured from the All India Coordinated Research Project on pigeonpea, University of Agricultural Sciences, Bangalore. List of the genotypes used in the variability study is presented in the Appendix-I.

3.2 METHODS

3.2.1 Experimental layout

The field experiment was laid out in Randomized Complete Block Design with three replications. Each genotype was grown in two rows of four meters length. A spacing of 60 cm between rows and 20 cm between plants was adopted.

3.2.2 Crop management

The crop was raised providing all the necessary agronomic inputs and plant protection measures as per the package of practice recommended for the crop.

3.2.3 Method of sampling and recording observations

Five plants were selected at random from each genotype in each replication for recording the observations. Each character was observed for eliciting the information and described here under.

3.2.3.1 Plant height (cm)

The plant height was measured at maturity from the base of the plant to the tip of main branch and expressed in centimeters.

3.2.3.2 Number of branches per plant

The number of branches per plant was counted at the time harvest.

3.2.3.3 Days to 50% flowering

Total number of days taken from the day of sowing to the day on which 50 % of the plants showed anthesis was recorded.

3.2.3.4 Length of pod bearing branch (cm)

The length of pod bearing branch from the five randomly selected branches was measured from the base of the pod bearing branch to the tip and mean was calculated and expressed in centimeters.

3.2.3.5 Days to maturity

The number of days taken from the day of sowing to physiological maturity of all the pods was recorded.

3.2.3.6 Pods per plant

The total number of filled pods per plant were counted from the five plants selected at random at the time of harvest and mean was calculated.

3.2.3.7 Pod length (cm)

Five pods were selected from each genotype and length of the pod was measured and the mean was calculated.

3.2.3.8 Seeds per pod

Number of seeds per pod were counted from randomly selected five pods in each of the randomly selected five plants and expressed as the mean number of seeds per pod.

3.2.3.9 Seed yield per plant (g)

Total weight of the seeds per plant was recorded in grams in each of the randomly selected five plants after threshing, cleaning and drying and the mean was calculated.

3.2.3.10 Shelling percentage (%)

The ratio of seed yield per plant to pod yield per plant was worked out and expressed in percentage.

$$\text{Shelling percentage} = \frac{\text{Seed weight (g)}}{\text{Pod weight (g)}} \times 100$$

3.2.3.11 100 seed weight (g)

The weight of hundred randomly selected seeds in each of five randomly selected plants was recorded in grams and mean was computed.

3.2.3.12 Scoring for sterility mosaic disease resistance in selected genotypes

An effective technique called "Leaf stapling technique" for screening pigeonpea germplasm and breeding material for resistance to sterility mosaic virus. This technique involves stapling of a portion of SMD infected pigeonpea leaves on the healthy seedlings. Mites from the stapled leaf migrate and transmit the virus to the test plants. The severity of sterility mosaic disease incidence was computed by counting the number of infected plants and total number of plants in each genotype and expressed as per cent incidence.

3.3 Statistical analysis

The mean values of 11 characters were analyzed for their variances following randomized complete block design (Sundararaj *et al.*1972).

ANOVA

Source of variation	Degrees of freedom	Mean sum of squares
Treatment	K-1	MSSt
Replication	V-1	MSSr
Error	(K-1)(V-1)	MSSe
Total	(KV-1)	

Where,

V = Replication

K= Treatment

MSSt, MSSr and MSSe =Mean sum of squares for treatments, replication and error respectively.

The F value ratio is compared with table F ratio at t-1 degrees of freedom both at 1% and 5% level of significance.

3.3.1 Estimation of genetic parameters

3.3.1.1 Genotypic and Phenotypic co-efficient of variability (GCV and PCV)

The co-efficient of variability both at phenotypic and genotypic level of all the characters were computed according to formulae suggested by Burton and De Vane (1953).

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

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

PCV and GCV were classified as suggested by Siva Subramanian and Menon (1973), and are given below.

1-10%	:	low
10-20%	:	moderate
20% and above	:	high

3.3.1.2 Heritability (Broad sense)

Heritability in Broad sense (h^2) estimates were computed by the formula as suggested by Hansen, *et al.* (1956).

$$h^2 = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

Where,

$\sigma^2 g$ = genotypic varience
$\sigma^2 p$ = phenotypic varience

The heritability per cent was categorized as suggested by Robinson *et al.* (1949) and is mentioned below.

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

3.3.1.3 Genetic advance (GA)

The extent of genetic advance expected through selection for each character is calculated as per the formula suggested by Johnson *et al.* (1955).

$$GA = H \times \sigma p \times k$$

Where,

GA = genetic advance
H = heritability (broad sense)
σp = phenotypic standard deviation
k = selection differential which is equal to 2.06 at 5% intensity of selection (Lush, 1949).

$$\text{GA as per cent of mean} = \frac{\text{GA}}{\overline{X}} \times 100$$

Where,

GA = Genetic advance

$\overline{X}$ = General mean

The genetic advance as per cent of mean was categorized as suggested by Johnson *et al.* (1955) and the same is given below.

1 - 10%	:	Low
10 - 20%	:	Moderate
20% and above	:	High

3.3.2 Estimation of correlation coefficients

The phenotypic and genotypic correlation coefficients were estimated among all possible combination, in each population as suggested by Al.-Ji bouri *et al.* (1958).

$$r_{12} = \frac{Cov_{12}}{\sqrt{V_1 \times V_2}} \times 100$$

where,

r_{12}= correlation coefficients between character 1 and character 2

Cov_{12} = covariance between character 1 and character 2

V_1 = variance of character 1

V_2 = variance of character 2

The significance of correlation coefficients is tested using "t" value of Fisher and Yates (1963).

3.4 Path coefficient analysis

The estimates of direct and indirect contribution of various yield components to the yield, the path coefficient analysis was followed as suggested by Wright (1921) and illustrated by Dewey and Lu (1959). The direct and indirect effects of different traits are

calculated by solving the sets of simultaneous equations through the use of "Doo-Little Technique" as described by Goulden (1959).

3.5 DNA marker diversity studies using RAPD and SSR markers

Plant material

The material for the study comprised of six genotypes of pigeonpea differing in their resistance levels to SMD. List of genotypes used in the DNA markers diversity study is presented in Table 1. The six genotypes were sown under green house under controlled condition and the healthy seedlings were used for DNA extraction. The RAPD and SSR primers used are mentioned in the Table 2 and Table 3 along with their sequences.

Table 1. List of pigeonpea genotypes selected for marker diversity studies

SI. No.	Genotype	Reaction to SMD
1	BRG 1	Moderately susceptible
2	BRG 3	Resistant
3	HY 3C	Moderately resistant
4	ICP 7035	Resistant
5	ICP 8863	Susceptible
6	TTB 7	Susceptible

(Saifulla *et al.*, 2005)

DNA extraction

Sixteen days old seedlings were used for DNA extraction. DNA was extracted as per the modified CTAB (Cetyl Trimethyl Alluminium Bromide) method (Cao and Oard, 1997). The protocol for DNA extraction is illustrated in Fig.1 However, the procedure adopted is outlined briefly as follows:-

1. Fresh and healthy leaves (1.0g) of about sixteen days old seedlings were collected.
2. The leaves were cut into pieces and homogenized completely by adding 400 µl of extraction buffer.
3. Again added 400 µl of extraction buffer and mixed well and from this around 400 µl homogenized solution was taken into an eppendof.
4. 400 µl of chloroform was added and centrifuge this mixture at 14, 000 rpm for one minute.
5. The supernatant was transferred through mira cloth into another centrifuge tube.
6. To the supernatant obtained, 800 µl of absolute alcohol was added and centrifuged for another 3 minutes at 14,000 rpm.
7. The supernatant was discarded and the DNA pellet was washed with 70 per cent alcohol and dried to remove the residues of alcohol.
8. Finally, DNA pellet was dissolved in 50 µl of TE buffer and stored at -20^0C for further use.

RAPD and SSR primers

The RAPD (14) and cowpea specific SSR primers (15) used in the study along with their sequences are presented in Table 2 and 3.

RAPD reaction mixture

The RAPD reaction mixture consisted of 2.0 µl of template DNA, 1.5 µl primer, 1.5 µl dNTPs, 0.3 µl Taq, 2.0 µl of 1 X PCR buffer (20 M Tris HCL pH 8.8, 10 mM KCl,

Table 2. RAPD primers used for DNA marker diversity studies in pigeonpea

Sl. No.	Primer	Sequence
1	OPM-9	5'-GTCTTGCGGA-3'
2	OPM-12	5'-GGGACGTTGG-3'
3	OPL-7	5'-AGGGTCGTTC-3'
4	OPL-18	5'-GTAACCAGCC-3'
5	OPF-8	5'-AGGTCTTGGG-3'
6	OPO-5	5'-CAGCCCAGAG-3'
7	OPN-7	5'-ACCTCAGCTC-3'
8	OPN-8	5'-AGCGTCACTC-3'
9	OPN-13	5'-AGGCGGGAAC-3'
10	OPM-14	5'-ACCACCCACC-3'
11	OPM-16	5'-GGGATATCGG-3'
12	OPM-20	5'-CCCAGTCACT-3'
13	OPO20	5'-ACACACGCTG-3'
14	OPO-19	5'-GGTGCACGTT-3'

Source: MAS lab Department of Genetics and Plant Breeding UAS, GKVK, Bangalore.

2.0 mM Mg SO_4 0.1 % Triton X-100 and 10 mM $(NH_4)_2$ SO_4) and 12.7 µl of sterile water in an volume of 20 µl and to this one drop of mineral oil (sigma) was added.

SSR reaction mixture

The SSR reaction mixture consists of 1.0 µl of template DNA, 2.0 µl forward primer, 2.0 µl reverse primer, 3.0 µl dNTPs, 1.5 µl Taq, 2.0 µl of 1 X PCR buffer (20 M Tris HCL pH 8.8, 10 mM KCl, 2.0 mM Mg SO_4 0.1 % Triton X-100 and 10 mM $(NH_4)_2$ SO_4) and 5.2 µl of sterile water in an volume of 20 µl.

Amplification conditions

Amplification was carried out on a MJ Research PTC 200 Thermal Cycle. The amplification profile was as follows:

For RAPD

Initial denaturation temperature	:	94^0 C – 4 minutes
Denaturation	:	94^0 C 1 minute
Primer annealing	:	36^0 C - 1 minute
Primer extension	:	72^0 C – 2 minutes

Later three stages were repeated 35 times

Complete primer extension	:	72^0 C 5 minutes
Soak temperature	:	4^0C until removed

For SSR

The amplification condition for SSR is same as that of RAPD amplification condition except primer annealing temperature at 56^0C for one minute for all the primers.

Gel electrophoresis

Agarose gel of 0.8% each for RAPD and SSR was prepared using electrophoresis grade Agarose (Sigma) in a volume of electrophoresis buffer (1 X TAE) sufficient for

Table 3. Cowpea specific SSR primers used for DNA marker diversity in pigeonpea

Sl. No.	Primer	Sequence
1.	AY_189137-3A	5¹GACACCGGGCGTATCCTT3¹
	AY_189137-3B	5¹CTTGCTTATTATATGTTGCCTTAG3¹
2.	AY_189137-1A	5¹TTCGTGTCTGGGGGAGGAT3¹
	AY_189137-1B	5¹TACGCCCGGTGTCATAGTGTT3¹
3.	AY_189138-3A	5¹GGCTGCAAGGGTTCTCAATG3¹
	AY_189138-3B	5¹CAACAATATGCCTCCTTCTGC3¹
4.	AY_193835-3A	5¹AAATGGTTGCTTTCTCTGACA3¹
	AY_193835-3B	5¹GCAACATTTGTATGGGGAACCT3¹
5.	AY_193836-3A	5¹GGGCAACCAAACCGTGTG3¹
	AY_193836-3B	5¹ATGGAAGCAGAAATTTGAGTAAC3¹
6.	AY_193837-3A	5¹ATATCGGCGCCTCTTCCCTACAGT3¹
	AY_193837-3B	5¹GACATAAAACTCCCACGAAATCAG3¹
7.	AY_193837-1A	5¹CCATTTGTACCACCCAGGAG3¹
	AY_193837-1B	5¹ATCGGCAATGACAGGAACA3¹
8.	AY_257179-1A	5¹AGCCAAGCCTCTGCCATTC3¹
	AY_257179-1B	5¹GACAAAGCGATCTGCCTGAGTT3¹
9.	D_83970-3A	5¹TCAACCAGTATAATCGCAAGACAT3¹
	D_83970-3B	5¹CCAGCGACATCATCACAACAATAA3¹
10.	D_83971-3A	5¹GCTTGGGGCTTGAATTTACTCCT3¹
	D_83971-3B	5¹AATGCAAACTCTTACAAACCACAC3¹
11.	D_83971-1A	5¹CCAGCTTTGAAGGGGACTCT3¹
	D_83971-1B	5¹TGGGCAATTGCAACATCTCT3¹
12.	D_83972-3A	5¹AGAAAGGGATAGTGGACAAGATTA3¹
	D_83972-3B	5¹TTACATATCCATTGGCAGAACATC3¹
13.	D_88121-3A	5¹CCTAACGATGTGGCAGAAGC3¹
	D_88121-3B	5¹ATGGCTAGATTTGAGTGAGGATTG3¹
14.	D_88122-3A	5¹CGCCGGACGAGGAGTAT3¹
	D_88122-3B	5¹AAAAGAAATTGGGTTAAAAAGTAT3¹
15.	U_30875-5A	5¹TTGGGATTAAGCTTCTGATTTTGA3¹
	U_30875-5B	5¹ATTGATGGCGCTAGTGATGATT3¹

Note

A= Forward primer, B= Reverse primer

Source: Department of Plant Biotechnology, UAS, GKVK, Bangalore.

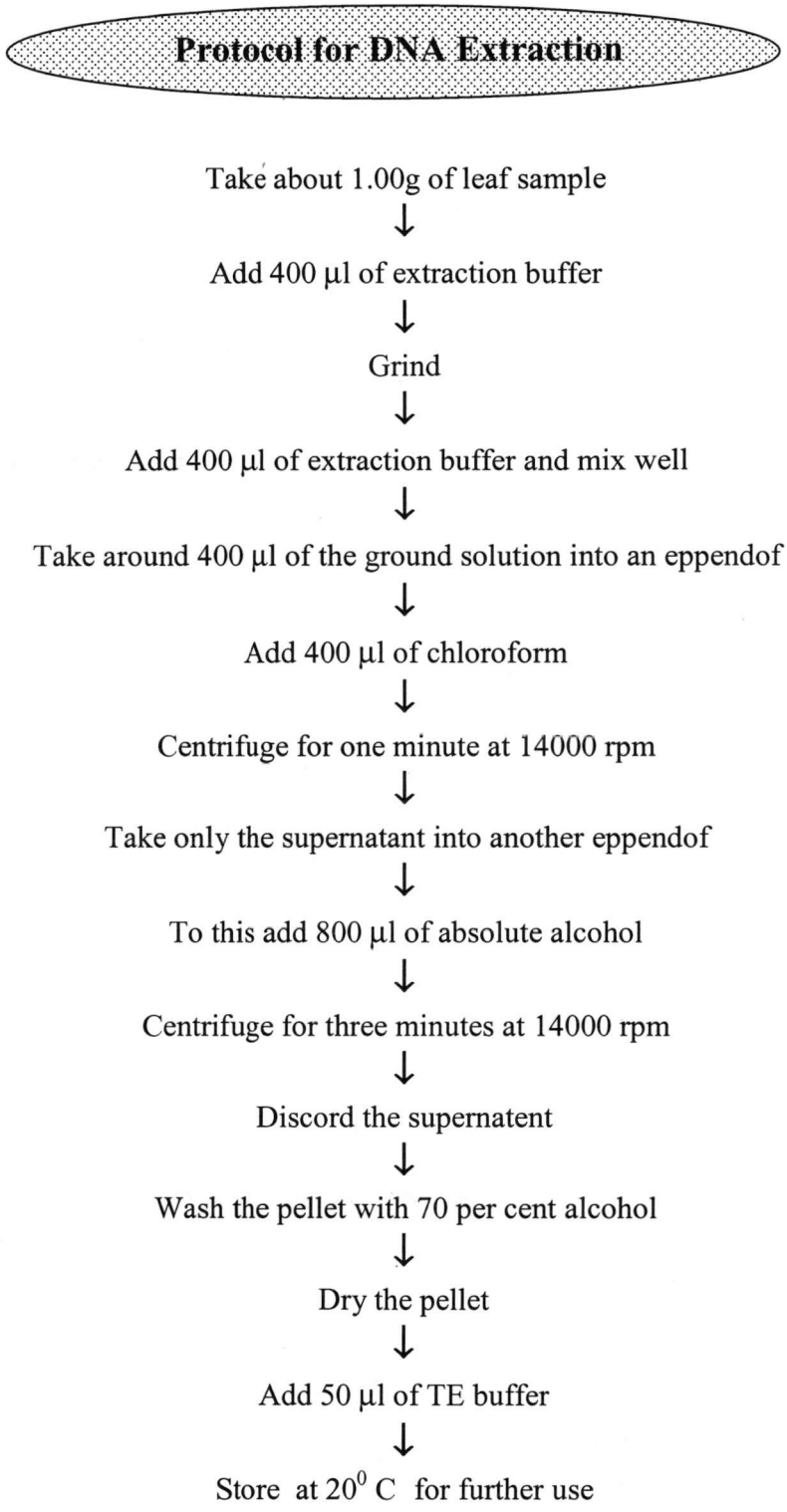

Fig. 1 Schematic representation of procedure for DNA extraction.

constructing a gel (300 ml for 18X 30 cm gel). Ethydium Bromide was added at the concentration of 0.5 µg/ml of gel. The gel was allowed to set fully before removing the comb and loading the sample. Five µl of loading dye was added to 20 µl of PCR products and mixed well before loading into the wells. Care was taken to prevent mixing of samples between hours for separation of PCR fragments. After the run, the gel was viewed under UV light and the DNA banding pattern was recorded directly.

Scoring of RAPD and SSR generated bands

The bands generated by random primers and SSR primers were scored by binary coding treating '0' for the absence of band, '1' for the presence of band in each primer.

DNA analysis

Analysis of banding pattern was done using a "STATISTICA" package. The programme used was cluster analysis joining (tree clustering) with raw input data of each genotype separately. The main parameters which guided the joining (tree clustering) process linkage rule is unweighted pair grouping average (UPGMA) and the distance was computed from raw data using euclidean distance. Both RAPD and SSR data was separately analyzed and dendrogram was drawn. Combined dendrogram was constructed by pooled raw data (both RAPD and SSR).

Experimental results

IV. EXPERIMENTAL RESULTS

The results obtained from the present investigation are furnished under the following headings

1. Variability and genetic parameters
2. Character Association
3. Path coefficient analysis
4. DNA marker diversity studies using RAPD and SSR markers

Analysis of variance on 100 genotypes for the eleven traits studied for variability is presented in the Table 4. The results indicated highly significant differences among the genotypes for all the characters studied.

4.1 Variability and genetic parameters

To understand the extent to which the observed variation is due to genetic factors, the mean, range, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2) in broad sense and genetic advance (GA) as per cent of mean for yield and yield attributing characters were computed for eleven characters and are furnished in the Table 5. The graphical reprcsentation of PCV and GCV and heritability along with genetic advance are given in Fig 2 and Fig 3 respectively.

In general, the PCV values were higher than the GCV values for all the eleven traits studied. The difference between PCV and GCV was wide for the characters viz., seed yield per plant, shelling percentage, branches per plant, seeds per pod and pods per plant and moderate for length of pod bearing branches, pod length and plant height. The differences were low for 100 seed weight, days to 50 % flowering and days to maturity indicating the low sensitivity to environment on these traits.

4.1.1 Plant height (cm)

A moderate variation was noticed for the plant height with the over all mean value of 150.79 cm. Plant height was least in the genotype PH 502 (38.6 cm) and the tallest genotype was BDN 2001-6 (258 cm).

Table 4. ANOVA for 11 quantitative characters in Pigeonpea

Source of variation	DF	MSS values for 11 quantitative characters — Characters										
		X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
Replication	2	1937.24	5.33	5.84	212.14	9.38	1338.99	0.05	0.04	0.63	179.56	326.98
Treatment	99	6802.61**	20.21**	728.21**	1711.95**	859.04**	11885.65**	1.74**	1.65**	10.95**	107.90**	1430.73**
Error	198	365.85	3.53	7.73	121.39	4.08	901.44	0.33	0.26	0.68	70.47	108.80

** = **Significant at 1 % ; * = Significant at 5 %**

X_1 = Plant height (cm)

X_2 = Branches per plant

X_3 = Days to -50%flowering

X_4 = Length of pod bearing branch (cm)

X_5 = Days to maturity

X_6 = Pods per plant

X_7 = Pod length (cm)

X_8 = Seeds per pod

X_9 =100 Seed weight (g)

X_{10} = Shelling percentage

X_{11} = Seed yield (g)

The estimates of phenotypic and genotypic coefficients of variability were high with the values of 33.23 per cent and 30.72 per cent respectively. High heritability (85.4 %) and high genetic advance as percent of mean (58.48 %) values were observed for this character.

4.1.2 Branches per plant

A wide variation was observed for this trait and it ranges from 3.8 branches per plant in PH 504 to the maximum of 17.17 branches per plant in the genotype GRG 205 and the over all mean was 8.54 branches per plant.

Branches per plant exhibited high estimates of PCV (35.31 %) and GCV (27.62 %) and also high estimates of heritability (61.2 %) and of GA as per cent of mean (44.49 %).

4.1.3 Days to 50 % flowering

Maximum days taken to 50% flowering was in the genotype GRG -205 (124 days) while minimum days was taken in the genotype AL 1492 (52 days) with over all mean of 88.99 days.

Phenotypic and genotypic coefficient of variabilities for this trait were moderate with the values of 17.69 and 17.41 per cent respectively. The estimate of heritability was very high (96.9 %) and also the genetic advance as per cent mean (35.30 %).

4.1.4 Length of pod bearing branch (cm)

This character exhibited a high variability ranging from 19.2 cm in ICPL 87 to 129.2 cm in BDN 2 with an over all mean of 76.1 cm.

Phenotypic and genotypic coefficients of variability estimate values were high for this trait with 33.54 % and 30.26 % respectively. This trait also recorded high values of heritability (81.4 %) and genetic advance as per cent of mean (56.22 %).

Table 5. Estimates of range, mean, phenotypic and genotypic co-efficient of variability, heritability and genetic advance as per cent of mean for 11 traits in pigeonpea

Sl. No.	Character	Range		Mean	Coefficient of Variability		Heritability in broad sense (%)	Genetic advance as per cent of mean
		Max	Min		PCV	GCV		
1.	Plant height (cm)	258.00	38.60	150.79	33.23	30.72	85.40	58.48
2.	Branches per plant	17.70	3.80	8.54	35.31	27.62	61.20	44.49
3.	Days to 50% flowering	124.00	52.00	88.99	17.69	17.41	96.90	35.30
4.	Length of pod bearing branch (cm)	129.20	19.20	76.10	33.54	30.26	81.40	56.22
5.	Days to maturity	214.00	135.00	180.41	9.42	9.36	98.60	19.13
6.	Pods per plant	308.20	9.40	111.79	60.42	54.13	80.20	99.88
7.	Pod length (cm)	9.00	3.60	5.50	16.31	12.50	58.70	19.71
8.	Seeds per pod	6.20	2.00	4.20	14.99	8.66	33.40	10.31
9.	100 seed weight (g)	18.10	7.20	11.20	18.10	16.52	83.30	31.06
10.	Shelling percentage	83.30	42.86	69.60	13.09	5.07	15.00	4.05
11.	Seed yield (g)	110.00	6.40	33.93	70.47	62.02	77.40	114.65

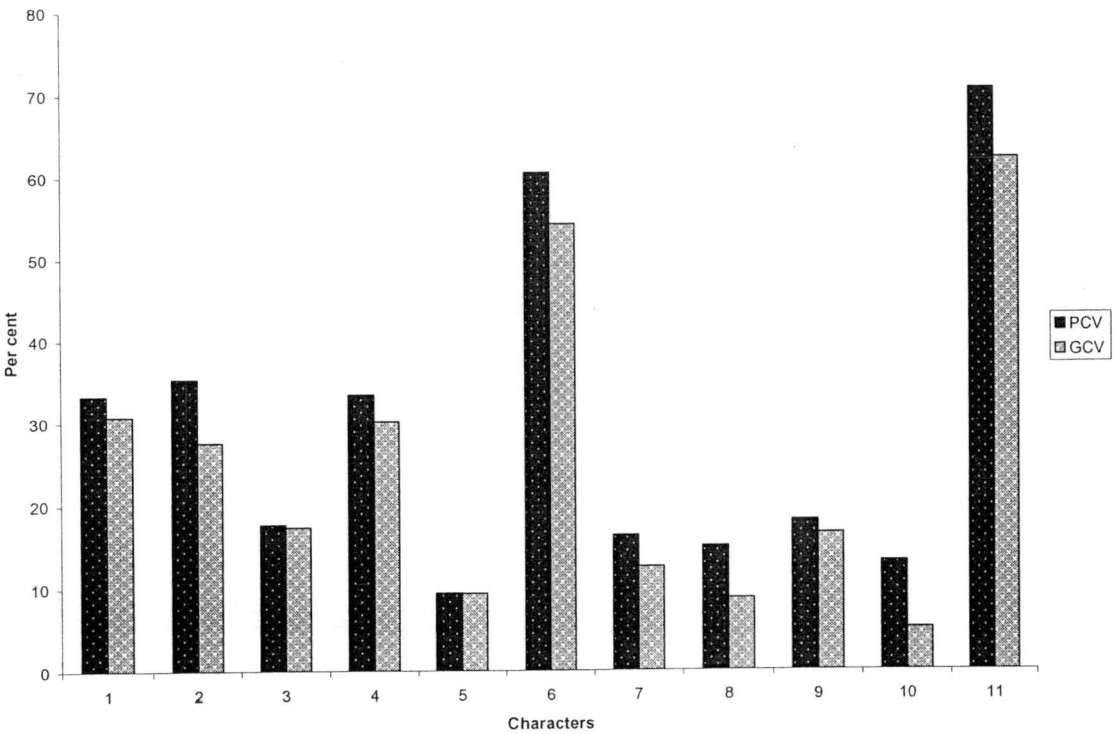

Fig. 2. Phenotypic co-efficient of variation (PCV) and Genotypic co-efficient of variation (GCV)

X_1 = Plant height (cm)

X_2 = Branches per plant

X_3 = Days to 50% flowering

X_4 = Length of pod bearing branch (cm)

X_5 = Days to maturity

X_6 = Pods per plant

X_7 = Pod length (cm)

X_8 = Seeds per pod

X_9 = 100 Seed weight (g)

X_{10} = Shelling percentage

X_{11} = Seed yield (g)

4.1.5 Day to maturity

The range observed for this trait was from 135 days in the genotype H 2000-3 7 to 214 days in GRG 205 with an over all mean of 180.41 days.

Co efficient of variability values were low for this character with the values of 9.42 per cent and 9.36 per cent at genotypic and phenotypic levels respectively. Very high heritability estimates of 98.60 per cent were recorded for this character; while the genetic advance as per cent mean was moderate (19.13 %).

4.1.6 Pods per plant

The genotypes studied exhibited wide range of variability for this character. The genotype GRG -205 produced highest number of pods per plant (308.2 pods per plant) and lowest number of pods per plant was produced in the genotype PH 502 (9.4).

Phenotypic and genotypic coefficients of variability values were high with 60.42 and 54.13 per cent respectively. This trait exhibited very high estimates of heritability (80.2 %) and genetic advance as percent of mean (99.88 %).

4.1.7 Pod length (cm)

This character exhibited a moderate range of variation with an overall mean of 5.5 cms. The maximum pod length was noticed in the genotype BRG 3 (9.00 cm) and the minimum pod length was in PH 504 (3.6 cm).

The phenotypic and genotypic coefficient variability for this character was moderate with the values of 16.31 % and 12.5 % respectively. This character had moderate estimates of heritability (58.7 %) and genetic advance as per cent of mean (19.71 %).

4.1.8 Seeds per pod

This character exhibited maximum seeds per pod in LCV10 (6.2) while, the genotype PH 506 (2.0) of showed minimum number of seeds per pod with an over all mean value of 4.2 seeds per pod.

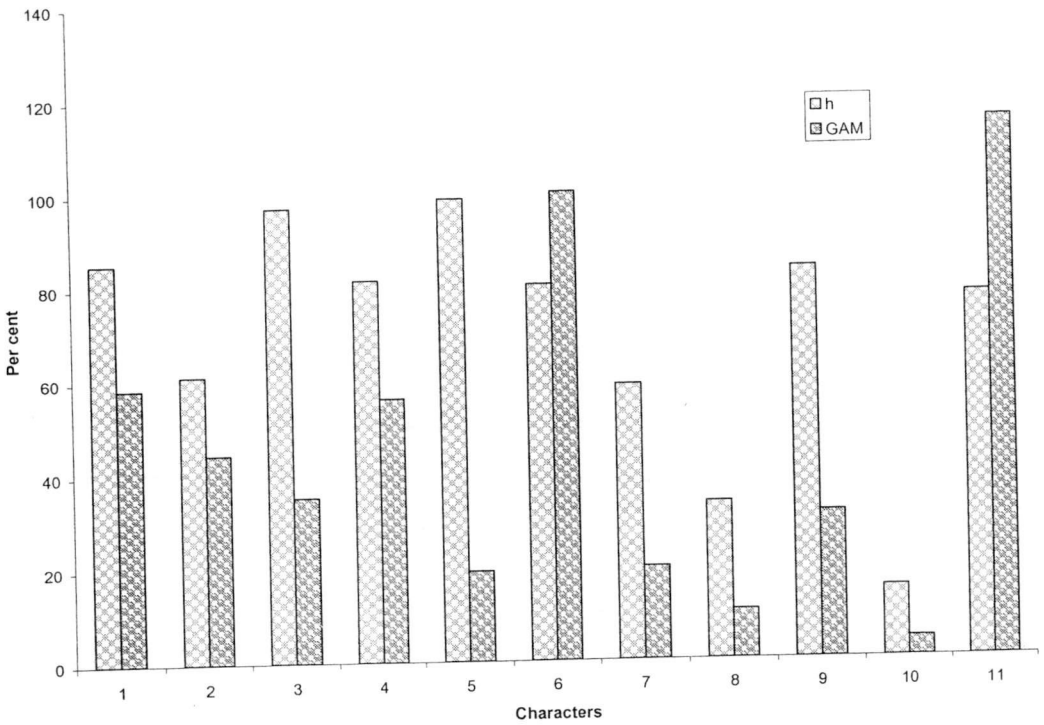

Fig. 3. Heritability (h^2) and Genetic advance as per cent of mean (GAM)

$\mathbf{X_1}$ = Plant height (cm)

$\mathbf{X_2}$ = Branches per plant

$\mathbf{X_3}$ = Days to 50% flowering

$\mathbf{X_4}$ = Length of pod bearing branch (cm)

$\mathbf{X_5}$ = Days to maturity

$\mathbf{X_6}$ = Pods per plant

$\mathbf{X_7}$ = Pod length (cm)

$\mathbf{X_8}$ = Seeds per pod

$\mathbf{X_9}$ = 100 Seed weight (g)

$\mathbf{X_{10}}$ = Shelling percentage

$\mathbf{X_{11}}$ = Seed yield (g)

Seeds per pod exhibited moderate estimate of PCV (14.99 %) and low estimate of GCV (8.66 %). Estimates of heritability (33.4 %) and genetic advance as per cent of mean (10.31 %) were moderate for this trait.

4.1.9 100 Seed weight (g)

A wide range of variation was observed for this trait. Minimum 100 seed weight of 7.2 g was observed in BDN 2001 - 6, while the genotype Hy 3C recorded maximum 100 seed weight of 18.1 g .The overall mean value was 11.2 g.

Moderate estimates of phenotypic and genotypic coefficient of variability of 18.1 % and 16.52 % respectively were recorded for this trait, while the estimate of heritability (83.3 %) and genetic advance as per cent of mean (31.06 %) were high for this character.

4.1.10 Shelling percentage

Shelling per cent among genotypes studied was ranged from 42.86 % in SKNP 0202 to 83.3 % in BRG 2-5 with an over all mean value of 69.6 %.

Moderate estimate of phenotypic (13.09 %) and low estimate of genotypic coefficient of variability (5.07 %) values were observed for this trait. This character showed very low heritability (15 %) and genetic advance as percent mean (4.05 %).

4.1.11 Seed yield per plant (g)

This character recorded high range of variability with an over all mean value of 33.93 g. The maximum seed yield per plant was observed in the genotype BRG 1 (W) with 110 g while minimum seed yield was noticed in SKNP 0203 with 6.4g seed yield per plant.

High estimates of phenotypic and genotypic coefficient of variability values (70.47 % and 62.02 %) were observed. Estimates of heritability (77.4 %) and a genetic advance as per cent of mean (114.65 %) were also high for this trait.

4.2 Character Association

The genotypic and phenotypic correlation coefficients among all the character combinations are presented in the Table 6 and 7 respectively. In general, the genotypic correlation coefficient values were found to be higher than their respective phenotypic correlation coefficients.

4.2.1 Association of seed yield with the component characters

Grain yield exhibited highly significant and positive association at both genotypic and phenotypic levels with plant height (0.657, 0.547) number of branches (0.797, 0.583), days to 50% flowering (0.676, 0.589), days to maturity (0.454, 0.393), pods per plant (0.801, 0.740), pod length (0.561,0.332) and seeds per pod (0.666, 0.337) while, with the Length of pod bearing branch, the association was significant (0.181, 0.147) at both the levels. Shelling percentage exhibited highly significant and positive association with seed yield at genotypic level (0.411) while at phenotypic level the association was non significant. Hundred seed weight exhibited positive and significant association at genotypic level (0.150) while, at phenotypic level the association was non significant.

4.2.2 Association among yield components

4.2.2.1 Plant height (cm)

The association of plant height was highly significant and positive with number of branches (0.761, 0.597), days to 50 % flowering (0.753, 0.694), length of inflorescence (0.717, 0.637), days to maturity (0.704, 0.645), pods per plant (0.776, 0.685), pod length (0.341, 0.255), seeds per pod (0.283, 0.502) and Shelling percentage (0.68, 0.281) at both phenotypic and genotypic level. With 100 seed weight, its association was significantly positive (0.181) at genotypic level and negatively significant at phenotypic level (-0.160).

4.2.2.2 Number of branches per plant

This trait showed highly significant and positive association with plant height (0.761, 0.597), days to 50 % flowering (0.726, 0.567), Length of pod bearing branch (0.37, 0.277), days to maturity (0.626, 0.491), number of pods (0.918, 0.727) seeds per

Table 6. Estimate of genotypic correlation co-efficients for 11 characters in Pigeonpea

Characters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
X_1	1.000	0.761**	0.753**	0.717**	0.704**	0.776**	0.341**	0.502**	0.181*	0.680**	0.657**
X_2		1.000	0.726**	0.370**	0.626**	0.918**	0.278**	0.467**	-0.060	0.685**	0.797**
X_3			1.000	0.473**	0.740**	0.700**	0.371**	0.483**	0.010	0.454**	0.676**
X_4				1.000	0.696**	0.470**	-0.198**	-0.201**	-0.295**	0.311**	0.181*
X_5					1.000	0.593**	0.000	0.014	-0.225**	0.529**	0.454**
X_6						1.000	0.321**	0.428**	-0.008	0.568**	0.801**
X_7							1.000	0.989**	0.387**	-0.150*	0.561**
X_8								1.000	0.316**	0.144*	0.666**
X_9									1.000	-0.333**	0.150*
X_{10}										1.000	0.411**
X_{11}											1.000

** = Significant at 1 % ; * = Significant at 5 %

X_1 = Plant height (cm)

X_2 = Branches per plant

X_3 = Days to -50%flowering

X_4 = Length of pod bearing branch (cm)

X_5 = Days to maturity

X_6 = Pods per plant

X_7 = Pod length (cm)

X_8 = Seeds per pod

X_9 =100 Seed weight (g)

X_{10} = Shelling percentage

X_{11} = Seed yield (g)

pod (0.467, 0.189) and shelling percentage (0.685, 0.232) both at genotypic and phenotypic level. The association was positive and highly significant with pod length at genotypic level (0.278) and significant and positive at phenotypic level (0.140).

4.2.2.3 Days to 50 % flowering

Days to 50 % flowering showed highly significant and positive association with plant height (0.753, 0.694), branches per plant (0.726, 0.567), length of pod bearing branch (0.473, 0.425), days to maturity (0.74, 0.722), pods per plant (0.7, 0.625) pod length (0.371, 0.261) seeds per pod (0.483, 0.269), shelling percentage (0.454, 0.185) both at genotypic and phenotypic levels.

4.2.2.4 Length of pod bearing branch (cm)

At both genotypic and phenotypic levels, length of pod bearing branch exhibited positive and highly significant association with plant height (0.717, 0.637), number of branches (0.370, 0.277), days to 50 % flowering(0.473, 0.425), days to maturity (0.696, 0.623), pods per plant (0.47, 0.403) and shelling percentage (0.311, 0.134). Its association with 100 seed weight was significant and negative (-0.295, -0.256) at both the levels while with pod length (-0.198) and seeds per pod (-0.201), its associations were negative and highly significant at genotypic level.

4.2.2.5 Days to maturity

At both genotypic and phenotypic levels, days of maturity exhibited highly significant and positive association with plant height (0.704, 0.645), number of branches (0.626, 0.491), days to 50% flowering (0.74, 0.722), length of pod bearing branch (0.696, 0.623), pods per plant (0.593, 0.526) and shelling percentage (0.529, 0.212). Its association was highly significant and negative with 100 seed weight (-0.225, -0.197) at both the levels.

4.2.2.6 Number of pods per plant

This character exhibited highly significant and positive association with plant height (0.777, 0.685), number of branches (0.918, 0.727), days to 50% flowering (0.700,

Table 7. Estimates of phenotypic correlation co-efficients for 11 quantitative characters in Pigeonpea

Characters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
X_1	1.000	0.597**	0.694**	0.637**	0.645**	0.685**	0.255**	0.283**	-0.160*	0.281**	0.547**
X_2		1.000	0.567**	0.277**	0.491**	0.727**	0.140*	0.189**	-0.068	0.232**	0.583**
X_3			1.000	0.425**	0.722**	0.625**	0.261**	0.269**	0.007	0.185**	0.589**
X_4				1.000	0.623**	0.403**	-0.097	-0.104	-0.256**	0.134*	0.147*
X_5					1.000	0.526**	0.011	0.017	-0.197**	0.212**	0.393**
X_6						1.000	0.200**	0.239**	-0.004	0.209**	0.740**
X_7							1.000	0.644**	0.277**	-0.022	0.332**
X_8								1.000	0.159*	0.059	0.337**
X_9									1.000	-0.123	0.108
X_{10}										1.000	0.134
X_{11}											1.000

** = Significant at 1 % ; * = Significant at 5 %

X_1 = Plant height (cm)

X_2 = Branches per plant

X_3 = Days to -50%flowering

X_4 = Length of pod bearing branch (cm)

X_5 = Days to maturity

X_6 = Pods per plant

X_7 = Pod length (cm)

X_8 = Seeds per pod

X_9 =100 Seed weight (g)

X_{10} = Shelling percentage

X_{11} = Seed yield (g)

The association was positive and significant with plant height at genotypic level (0.181) and negative and significant at phenotypic level (-0.160).

4.2.2.10 Shelling percentage

This attribute exhibited positive and highly significant association with plant height (0.680, 0.281), branches per plant (0.685, 0.232), days to 50 % flowering (0.454, 0.185), days to maturity (0.529, 0.212), pods per plant (0.568, 0.209) at both the levels. At genotypic level, its association was highly significant and positive with length of pod bearing branch (0.311), significant and positive with seeds per pod (0.144), significant and negative with pod length (-0.150) and highly significant and negative with 100 seed weight (-0.333).

4.3 Path coefficient Analysis

Path coefficient analysis indicating the nature and magnitude of direct and indirect effects of 10 different characters on the seed yield are estimated at genotypic level and presented in Table 8.

Among all the direct effects, pod length exhibited highest positive direct effect (0.461) followed by pods per plant (0.367), branches per plant (0.319), shelling percentage (0.222), length of inflorescence (0.154) and days to 50 % flowering (0.111), while plant height exhibited high and considerable amount of negative direct effect (-0.400) on seed yield. Seeds per pod (0.041), 100 seed weight and days to maturity (0.018) showed low and positive direct effects on seed yield

Though plant height exhibited high and considerable amount of negative direct effect (-0.400) on seed yield, it has contributed considerable amount of positive indirect effect to the seed yield via, pods per plant (0.285), branches per plant (0.243), pod length (0.157), shelling percentage (0.151) and length of inflorescence (0.11) which resulted its significant association with seed yield.

Number of branches per plant registered high and positive direct effect (0.319) on seed yield and this was further magnified by its high indirect effects via pods per plant

Table 8. Genotypic path co-efficient analysis showing direct (diagonal) and indirect effects (above and below diagonal) of different characters on seed yield in Pigeonpea

Characters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	Correlation Coefficients (r)
X_1	**-0.400**	0.243	0.084	0.110	0.012	0.285	0.157	0.020	-0.006	0.151	0.657**
X_2	-0.305	**0.319**	0.081	0.057	0.011	0.337	0.128	0.019	-0.002	0.152	0.797**
X_3	-0.301	0.231	**0.111**	0.073	0.013	0.257	0.171	0.020	0.000	0.101	0.676**
X_4	-0.287	0.118	0.053	**0.154**	0.012	0.173	-0.091	-0.008	-0.009	0.069	0.181*
X_5	-0.282	0.199	0.082	0.107	**0.018**	0.218	0.000	0.001	-0.007	0.117	0.454**
X_6	-0.311	0.293	0.078	0.072	0.010	**0.367**	0.148	0.017	0.000	0.126	0.801**
X_7	-0.137	0.089	0.041	-0.030	0.000	0.118	**0.461**	0.040	0.012	-0.033	0.561**
X_8	-0.201	0.149	0.054	-0.031	0.000	0.157	0.456	**0.041**	0.01	0.032	0.666**
X_9	0.072	-0.019	0.001	-0.045	-0.004	-0.003	0.178	0.013	**0.031**	-0.074	0.150
X_{10}	-0.272	0.218	0.051	0.048	0.009	0.209	-0.069	0.006	-0.01	**0.222**	0.411**

Residual effect: 0.2218

X_1 = Plant height (cm)

X_2 = Branches per plant

X_3 = Days to -50%flowering

X_4 = Length of pod bearing branch (cm)

X_5 = Days to maturity

X_6 = Pods per plant

X_7 = Pod length (cm)

X_8 = Seeds per pod

X_9 = 100 Seed weight (g)

X_{10} = Shelling percentage

(0.337), shelling percentage (0.152) and pod length (0.128). It had high and negative indirect effect through plant height (-0.305).

Days to 50% flowering had moderate direct effect on seed yield and this was further magnified by its high indirect effects via pods per plant (0.257), branches per plant (0.231), pod length (0.171) and shelling percentage (0.101), while considerable amount of negative indirect effect (-0.301) was recorded via plant height.

Length of pod bearing branch had moderate direct effect on seed yield and it also exhibited moderate indirect effects via pods per plant (0.173) and branches per plant (0.118). Its indirect effect through plant height was negative and high (-0.287) on seed yield.

Days to maturity exhibited negligible amount of positive direct effect on seed yield but its high indirect effects via pods per plant (0.107), branches per plant (0.199), shelling percentage (0.117) and length of inflorescence (0.107) resulted in its significant association with seed yield. However its indirect influence via plant height (-0.282) was high and negative on seed yield.

Pods per plant registered high and positive direct effect (0.367) on seed yield and this was magnified by its positive and high influence through branches per plant (0.293) followed by pod length (0.148) and shelling per cent (0.126). Its indirect effect was high and negative on seed yield through plant height (-0.311).

Pod length has a very high direct effect (0.461) on seed yield and its indirect effects through pods per plant (0.118) and branches per plant (0.089) were moderate. It also exhibited moderate negative indirect influence through plant height (-0.137).

Though seeds per pod exhibited low amount of positive direct effect (0.041) on seed yield, its association with seed yield was magnified mainly by their indirect effects via pod length (0.456), pods per plant (0.157) and branches per plant (0.149). Its high negative indirect influence via plant height (-0.201) did not effect on its association with seed yield.

Hundred seed weight had very low amount of positive and direct effect influence (0.031) on seed yield, and its indirect influence via all other characters were low except through pod length (0.178).

Shelling percentage exhibited high and positive direct effect (0.222) on seed yield and its indirect influences were also high through branches per plant (0.218)and pods per plant (0.209) while through plant height, it exerted negative and indirect influence (-0.271) on seed yield.

4.3 DNA marker diversity studies using RAPD and SSR markers

4.3.1 Screening for SMD incidence

Six genotypes of pigeonpea were scored for incidence of SMD (Table 9). Amongst the genotypes tested for SMD incidence, BRG 3, ICP 7035 and Hy 3C recorded lower levels of incidence of 8.33, 10.79 and 15.83 per cent respectively. Highest incidence of 100 per cent was recorded in ICP 8863 and TTB 7. The genotype BRG 1 recorded SMD incidence of 37.56 per cent.

4.3.2 RAPD markers

The results of all RAPD marker data and amplified RAPD markers based on the banding pattern are presented in the Table 10 and gel picture in Plate 1 and 2. All the 14 RAPD primers generated PCR markers. The RAPD markers used for amplification generated totally 99 marker levels for six pigeonpea genotypes. This consists of 39 polymorphic bands (39.39 %) and 66 (60.60 %) monomorphic bands. Primer OPM-9 generated highest number of polymorphic bands (6) followed by OPO-20 and OPF-8 (5 each), OPM-20 and OPN-7 (4 each), OPL-18 and OPL-7 (3 each), OPM-12, OPN-8, OPM-14 and OPM-16 (2 each), whereas OPO-19 generated single polymorphic band. The highest number of monomorphic bands was noticed in OPN-13 and OPO-5 (8 each) both of them which did not produce polymorphic bands.

Table 9. List of pigeonpea genotypes selected for marker diversity studies and their per cent incidence to Sterility Mosaic Disease

SI. No.	Genotype	Per cent incidence to SMD
1	BRG 1	37.56
2	BRG 3	8.33
3	HY 3C	15.83
4	ICP 7035	10.79
5	ICP 8863	100.00
6	TTB 7	100.00

Primer OPM-9

A total of six polymorphic bands (54.5%) were produced out of 11 bands and five bands were monomorphic. This primer was amplified in all the genotypes but unique banding pattern was noticed in ICP 8863 and TTB 7 these bands were absent in rest of the genotypes.

OPM-12

This primer generated totally six bands; out of which two bands (33.33%) were polymorphic and the remaining four are monomorphic. This primer was amplified in all the genotypes.

OPL-7

A total of three polymorphic bands (50%) were produced out of six bands. The genotype BRG 1 exhibited specific banding pattern for this primer and this band is absent in other genotypes.

OPL-18

This primer amplified in all the genotypes and generated 50% (three bands) polymorphism out of total six bands.

OPF-8

This primer exhibited totally eight bands, out of which five bands were polymorphic (63 %) and rest are monomorphic.

OPN-7

About 67 per cent of polymorphism was seen with four bands out of six. OPN-7 was amplified in ICP 7035, which is unique for this genotype and absent in rest of the genotypes. This primer did not amplify the genotype BRG 1.

Table 10. Number of polymorphic bands generated by RAPD primers and their percentage

Sl. No.	Primers	Total number of bands	Polymorphic bands	Monomorphic bands	Per cent of polymorphism
1	OPM-9	11	6	5	81.81
2	OPM-12	6	2	4	33.33
3	OPL-7	6	3	3	50.00
4	OPL-18	6	3	3	50.00
5	OPF-8	8	5	3	62.50
6	OPO-5	8	---	8	0.00
7	OPN-7	6	4	2	66.66
8	OPN-8	8	2	6	25.00
9	OPN-13	8	----	8	0.00
10	OPM-14	5	2	3	40.00
11	OPM-16	8	2	6	25.00
12	OPM-20	8	4	4	50.00
13	OPO20	7	5	2	71.42
14	OPO-19	4	1	3	25.00
	Total	99	39	60	39.39

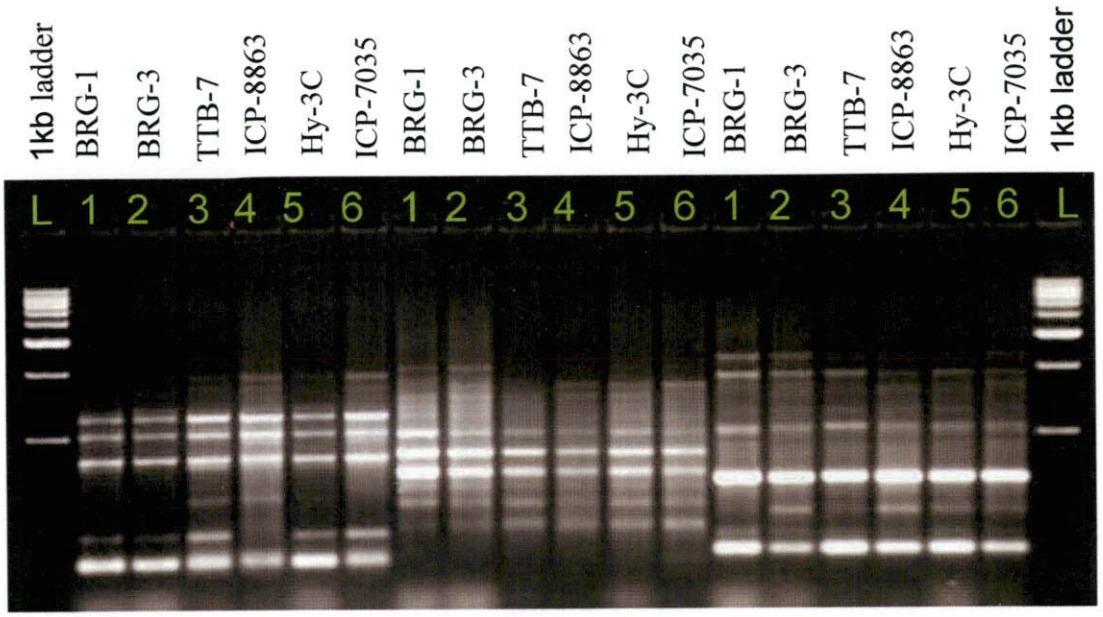

Plate 1. Gel picture showing the banding pattern of 6 genotypes for RAPD markers OPM-9 (1st set 6 wells), OPM-12 (2nd set 6 wells) and OPL-7 (3rd set 6 wells)

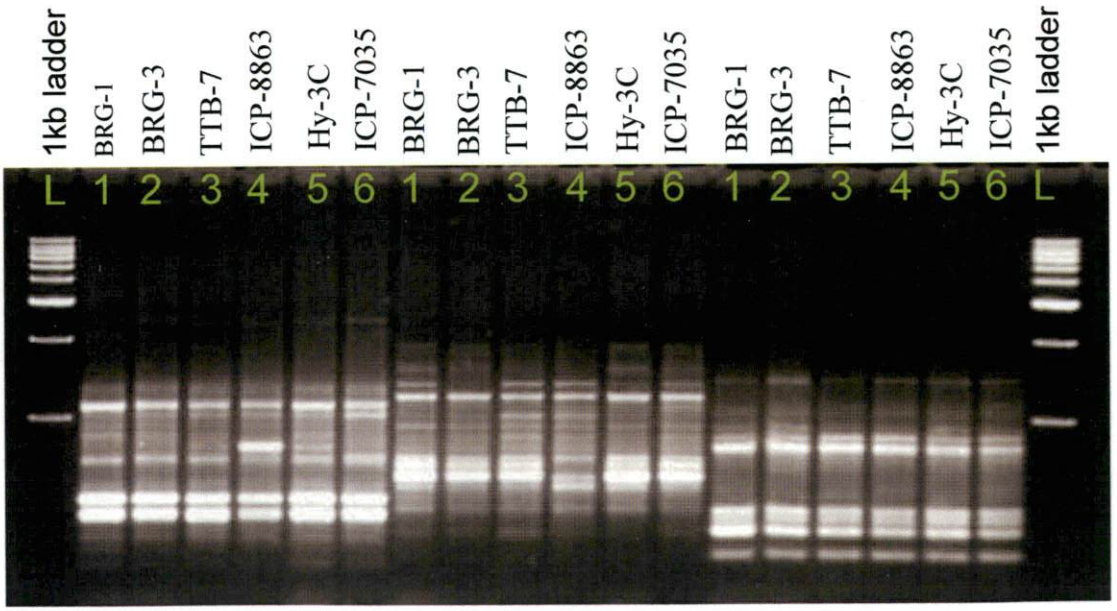

Plate 2. Gel picture showing the banding pattern of 6 genotypes for RAPD markers OPL-18 (1st set 6 wells), OPF-8 (2nd set 6 wells) and OPO-5 (3rd set 6 wells)

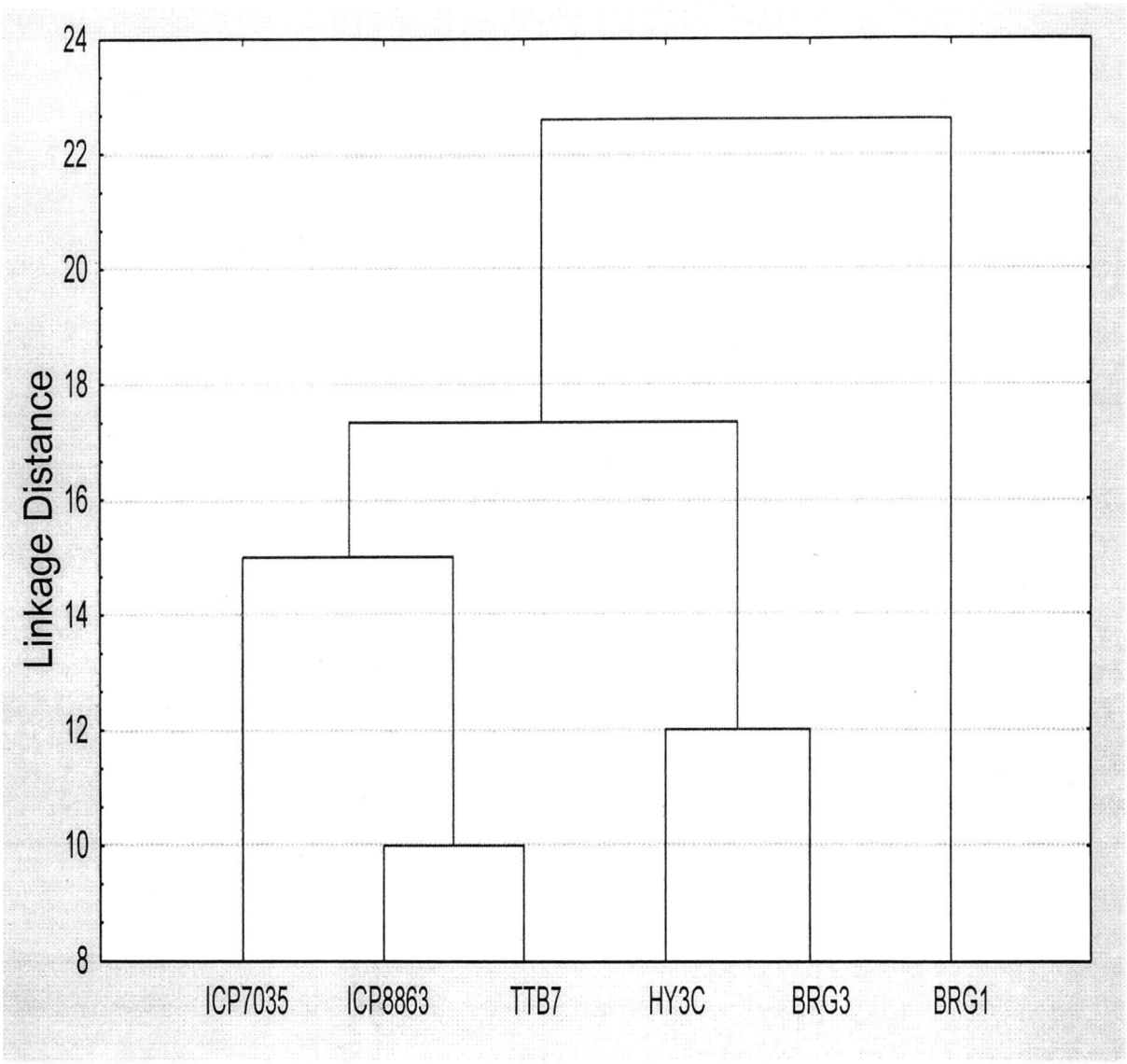

Fig. 4. RAPD tree diagram for six pigeonpea genotypes

OPN-8

Out of eight bands produced two were polymorphic which accounts for 25 per cent of polymorphism. This primer did not amplify the genotype BRG 1.

OPM-16

About 25 per cent of polymorphism was seen with two bands out of eight bands. All the genotypes exhibited polymorphism except BRG 1 in which the primer was not amplified.

OPM-14

About 40% of polymorphism was seen with two out of five bands and all the genotypes were amplified except ICP 7035.

OPO-20

About 50 per cent polymorphism was exhibited with four bands out of eight bands. This primer was amplified in all the genotypes.

OPO-19

Out of four bands produced, only one (25%) was polymorphic which is specific to BRG 1.

OPM-20

This primer produced 50% polymorphism but there is no specific banding pattern for any genotype.

The dendrogram constructed by unweighted paired group method is shown in the Fig. 4. The genotype ICP 8863 and TTB 7 clustered at 10% linkage distance indicating that they were closely related. Hy 3C and BRG 3 are clustered at the linkage distance of 12% and sub clustered at the 17% linkage distance with ICP 8863, TTB 7 and ICP 7035. BRG 1 genotype formed a distinct group of cluster with all other genotypes.

4.4.2 SSR markers

The results of all the SSR marker data and amplified SSR markers based on the banding pattern are presented in Table 11 and gel picture in Plate 3. A total of 38 bands were generated, out of which 35 are polymorphic. The highest number of polymorphic bands were generated by D_83971-1(12) followed by D_88122-3(6), D_88121-3 and AY_189137-1(4 each), AY_189137-3(3), AY_193836-3(2) and the primers AY_193837-1, AY_257179-1, D_83970-3 and U_30875-5 generated single polymorphic band each. AY_189138-3 has generated one monomorphic band while the remaining primers did not produce any bands.

Primer AY_189137-1

This primer exhibited 67 per cent of polymorphism with four bands out of six. This primer was not amplified in ICP 7035, while other genotypes were amplified.

AY_189138-3

This primer was not found useful in differentiating the genotypes, as all the bands were monomorphic.

AY _ 193836-3

Out of two bands produced, all were polymorphic and generated 100 per cent polymorphism and it was able to clearly differentiate the genotype ICP 8863 from other genotypes.

AY _ 193837-1, AY _ 257179-1 and D_83970 – 3

These primers produced only one level of polymorphism, which accounts for 100 per cent. These primers amplified all the genotypes except TTB 7.

AY _ 189137- 3

This primer generated three polymorphic bands and two bands were specific for BRG 3 and ICP 7035 at different levels.

Table 11. Number of polymorphic bands generated by SSR primers and their percentage

Sl. No.	Primer	Total no. of bands	Polymorphic bands	Monomorphic bands	Per cent of polymorphism
1	AY_189137-3	3	3	---	100.00
2	AY_189137-1	6	4	2	66.66
3	AY_189138-3	1	---	1	0.00
4	**AY_193835-3**	---	---	---	0.00
5	AY_193836-3	2	2	---	100.00
6	**AY_193837-3**	---	---	---	0.00
7	AY_193837-1	1	1	---	100.0
8	AY_257179-1	1	1	---	100.00
9	D_83970-3	1	1	---	0.00
10	**D_83971-3**	---	---	---	00.00
11	D_83971-1	12	12	---	100.00
12	**D_83972-3**	---	---	---	00.00
13	D_88121-3	4	4	---	100.00
14	D_88122-3	6	6	---	100.00
15	U_30875-5	1	1	---	100.00
	Total	38	35	3	92.10

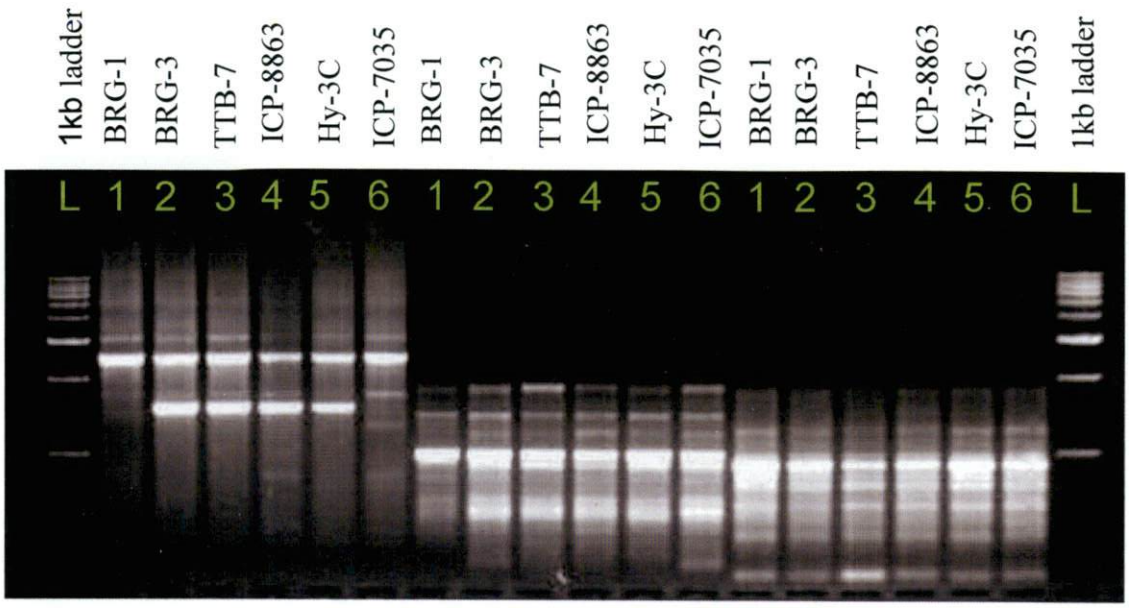

Plate 3. Gel picture showing the banding pattern of 6 genotypes for RAPD markers OPN-7(1st set 6 wells), OPN-8(2nd set 6 wells) and OPN-13(3rd set 6 wells)

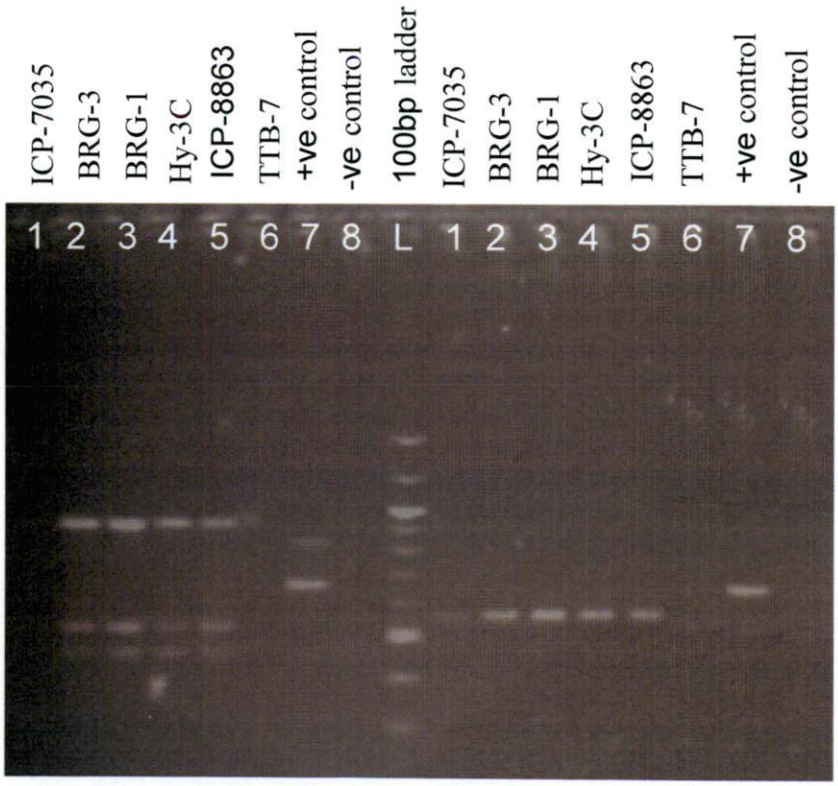

Plate 4. Gel picture showing the banding pattern of 6 pigeonpea genotypes for cowpea specific SSR primers AY_193836-3 (1st set 6 wells), AY_189138-3(2nd set 6 wells).

D_ 83971 – 1

Though this primer generated maximum number of bands (12), there was no specific band for any of genotype selected.

D_88121 –3

This primer has generated four polymorphic bands (100%) and was able to amplify ICP 7035, BRG 3 and Hy 3C while; BRG 1, ICP 8863 and TTB 7 were not amplified.

D_88122 –3

BRG 3 was able to clearly differentiated by this primer form other genotypes by the polymorphic bands (six) present at different levels and absent in rest of the genotypes.

U_ 30875 –5

This primer produced a single polymorphic band in BRG 3 and Hy 3C, while in rest of the genotypes this band was absent.

The dendrogram for six pigeonpea cultivars was constructed based on the SSR data Fig. 5. It indicated that at linkage distance of 6%, TTB 7 and ICP 8863 shared the common cluster and were more closely related than other genotypes. Hy 3C and BRG 3 farmed clusters at nine per cent indicating that these genotypes are 91 per cent similarity. ICP 7035 formed sub cluster to HY 3C and BRG 3 at 13% linkage distance indicating 87% similarity with HY 3C and BRG 3.The genotype BRG 1 formed extreme sub cluster with all other clusters at 19 % linkage distance indicating that this genotype is highly distinct from the rest.

The combined dendrogram constructed based on both RAPD and SSR data Fig. 6 indicated two major clusters formed a separate clusters. BRG-1 formed separate and distinct cluster from other genotypes at 42 % linkage distance. TTB-7 and ICP-8863 showed closely related about 84 %, similarly HY-3C and BRG-3 formed other cluster at about 22 % linkage distance indicating 78 % similarity between them. ICP 7035 formed

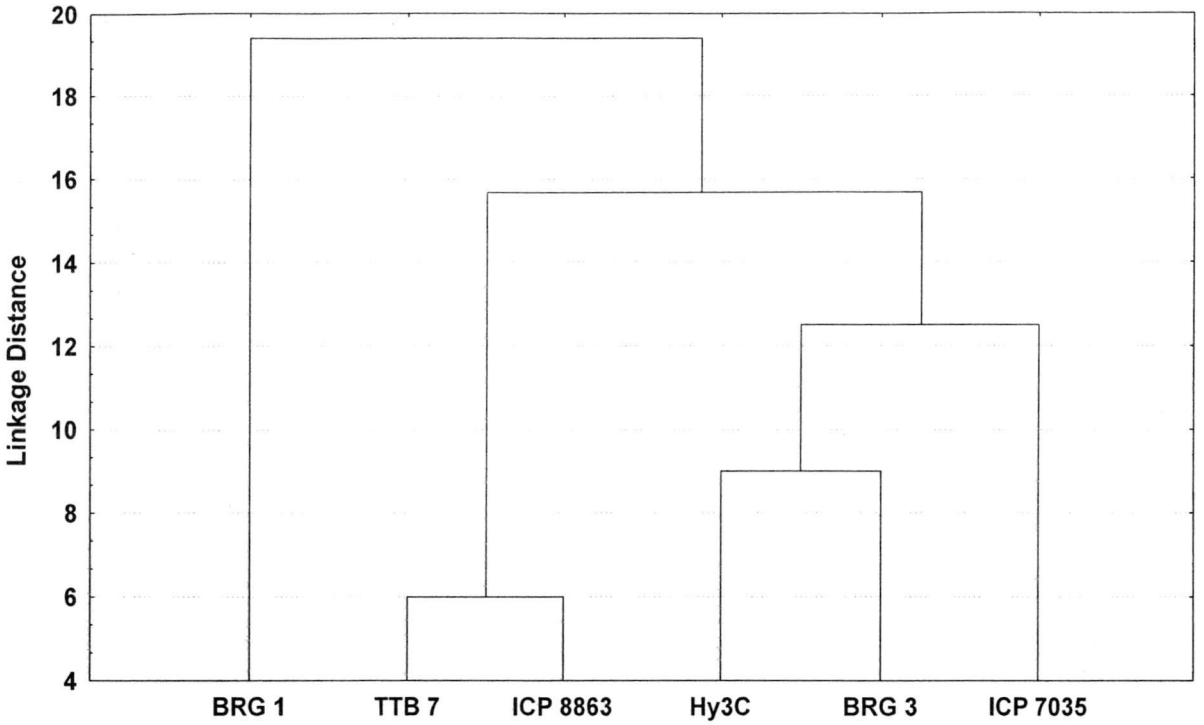

Fig. 5. SSR tree diagram for 6 pigeonpea genotypes

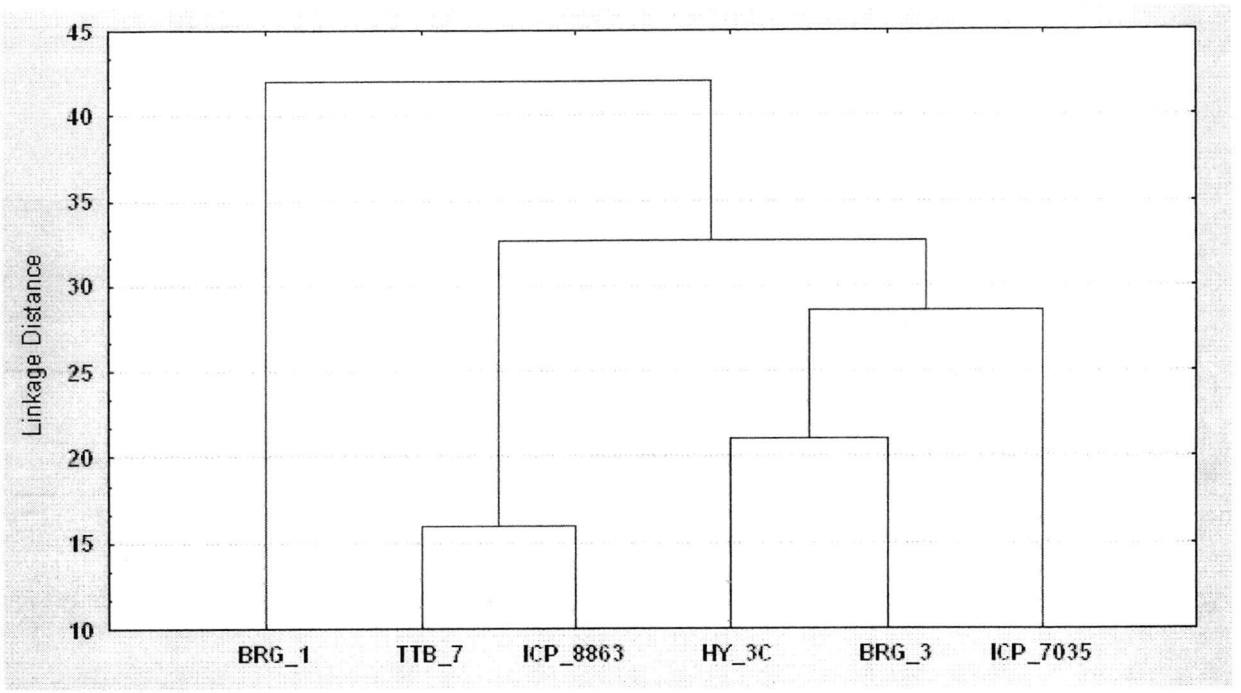

Fig. 6. Combined dendrogram of RAPD and SSR data

sub cluster to HY 3C and BRG 3 at 27 % linkage distance indicating 73 % similarity with HY 3C and BRG 3. TTB 7 and ICP 8863 formed a separate cluster and are closely related. They formed a cluster at 16 % linkage distance indicating that they are 84 % similarity.

Discussion

V. DISCUSSION

Plant breeding is known as art and science of improving genetic pattern in relation to their economic use. In the process of improvement, desirable plants are continuously being selected from genetically variable population. Improvement through breeding programme in any crop depends on the presence of genetic variability in the germplasm material available. The utility of such material could be judged based on the knowledge of extent of variability present in the crop species with respect to the nature and degree of relationship between any two measurable characters.

In the present study, a set of hundred genotypes collected from different sources was used to estimate its variability parameters, its correlation and path coefficient analysis. A set of six genotypes differing in their levels of resistance to SMD were used to study the DNA marker diversity using RAPD and SSR markers. Standard methods were adopted and the results obtained are discussed under the following headings to arrive at valid conclusions.

1 Genetic variability studies

2 Correlation coefficient studies

3 Path coefficient analysis and

4 DNA marker diversity using RAPD and SSR markers.

5.1 Genetic Variability Studies.

The results of analysis of variances for 11 quantitative characters studies revealed highly significant differences among the genotypes both at 5% and 1% level of significance.

Genetic variability is a basic need for breeders to improve the crops by adopting selection criteria based on the type of variability existing in the material. The genotypes included in the present study exhibited considerable amount of variability for all the 11 characters The wide range of variation indicates the scope for the selection of suitable basic material in breeding for further improvement and it values reflects the extent of phenotypic variability present in the material.

The range in the mean values does not reflect the total variance in the material being studied. Hence actual variance has to be estimated for all the characters to know the extent of variability. The phenotypic variances indicate the amount of variance which is due to differences in the phenotypic values whereas the genotypic variances indicates the magnitude of variances arising due to differences in genotypic values.

The absolute values for phenotypic and genotypic variances cannot be used for comparing the degree of variability for different characters, because the means for the characters to be measured could also be different. Hence coefficient of variation, which is calculated by considering the respective means, has to be used for further comparison. High estimates of these characters indicate wide variability. In the same way narrow differences between the phenotypic and genotypic coefficient of variation imply less response or influence of environmental effects.

The PCV and GCV values were relatively high for plant height branches per plant, length of pod bearing branch, pods per plant and seed yield per plant indicating high degree of variability exhibited by these traits. These results are in conformity with the findings of Rathnaswamy *et al.* (1973), Malhothra and Sodhi (1977) Jagashoram (1983) and Balyan and Sudhakar (1985), Aher *et al.* (1998), Vikas and Singh (1998) and Venkateshwaralu (2001).

Moderate PCV and GCV estimates were observed for days to 50% flowering, pod length and 100 seed weight indicating substantial influence of environment. On the contrary high estimates of PCV and GCV for these characters were reported by Premsagar and Jatasra (1984), Natarajan *et al.* (1990) Aher *et al.* (1998) Basavarajaiah (2000) and Deshmukh *et al.* (2000).

Seeds per pod and shelling percentage showed moderate estimates of PCV and low estimates of GCV, while days to maturity showed low GCV and PCV. On the contrary high PCV and GCV values for these traits were reported by Singh *et al.* (2003), Chattopadhyaya and Dhiman (2005).

The coefficient of variation reveals the extent of variability present for different characters and it does not indicate the heritable portion. To obtain the magnitude of heritable portion of variability, it is essential to know the heritability estimates of the different characters. The heritability estimates separates the variances to environmental effects from total variability and indicates the accuracy with which a genotype can be identified by its phenotypic performance, thus making the selection more effective. As such heritability in broad sense include both additive and non-additive gene effects (Hansen *et al.*1956).

High heritability estimates were obtained for plant height, branches per plant, days to 50% flowering, length of inflorescence, days to maturity, pods per plant, 100 seed weight and seed yield indicating these traits are least influenced by the environment. These results are in accordance with Jagashoram (1983), Balyan and Sudhakar (1985a), Natarajan *et al* (1990), Sunil Holkar *et al.* (1991) and Basararajaiah *et al.* (2000).

Pod length and seeds per pod exhibited moderate heritability indicating substantial influence of environment of these characters. These results are in conformity with findings of Khapre and Nerker (1992), Ghodke *et al.* (1994) and Aher *et al.* (1998). On the contrary, Chattopadhyaya and Dhiman (2005) reported high heritability for seeds per pod.

Heritability values alone provides no information of the amount of genetic progress that would result from selecting the best individuals since their scope is restricted by their interaction with environment. Johnson *et al.* (1955) reported that heritability estimates along with genetic gain would be more useful than the former alone, in predicting the effectiveness of selecting the best individuals. Therefore, it is essential to consider the predicted genetic advance along with heritability estimates as a tool in the selection programme for better efficiency in the selection.

A relative comparison of heritability values and expected genetic gain expressed as per cent of mean gives an idea about the nature of gene action governing a particular character. High heritability coupled with high genetic advance reveals the presence of

lesser environmental influence and prevalence of additive gene action in their expression, but lower values of genetic advance indicates the prevalence of non-additive gene action for moderate values of genetic advance both additive and non-additive gene action might be involved in their expression.

High heritability coupled with high genetic advance as per cent mean was noticed for the character plant height, branches per plant, days to 50% flowering, length of pod bearing branch, pods per plant, 100 seed weight and seed yield indicating selection is effective and controlled by additive gene action. Similar results were reported by Jagashoram (1983), Balyan and Sudhakar (1985) Natarajan *et al.* (1990) and Sunil Holkar *et al.* (1991), Singh *et al.* (2003), Chattopadyaya and Dhiman (2005).

Moderate heritability coupled with moderate genetic advance as per cent mean was noticed for the characters, pod length and seeds per pod indicating substantial influence of environment on these characters and controlled by both additive and non-additive gene action. These results are in accordance with Khapre and Nerker (1992). On the contrary, Srinivas (1999) reported high heritability for pod length and moderate heritability for seeds per pod.

5.2 Correlation Coefficient Analysis.

The Phenotype of the plant is the result of interaction of large number of factors. Therefore, the final yield is the sum total of all the effects of several components characters and thus it is a polygenically controlled character. These characters may be either related positively or negatively with each other and yield. Genetic correlation between various plant characters may arise because of linkage, pleiotropy or developmentally induced functional relationship. It is advisable to understand the correlation between economically important character like seed yield and pod yield with other trait so that selection objectives can be achieved using indirect selection for these associated traits. In the present investigation genotypic and phenotypic correlation of seed yield with some of its yield components and also among themselves, were studied. In general genotypic correlation coefficient was higher magnitude than phenotypic correlation coefficients.

5.2.1 Association of Seed Yield with Yield Components

In the present investigation, plant height, branches per plant, days to 50% flowering, days to maturity, pods per plant, pod length and seeds per pod exhibited highly significant positive association with seed yield per plant at both genotypic and phenotypic levels. These results are in conformity with Pahuja *et al.* (1981) Balyan and Sudhakar (1985 a and b), Angadi *et al.* (1988), Patel *et al.* (1988), Patil *et al.* (1989), Henry and Krishna (1990), Natarajan *et al.* (1990), Jahargiridhar *et al.* (1991), Virangama and Goyal (1994), Pandey and Singh (2001 a and b) and Ram Dhari *et al.* (2004). On the contrary Holkar *et al.* (1991) reported negative association of grain yield with days to 50% flowering and days to maturity.

At genotypic level, shelling percentage and 100 seed weight showed highly significant and positive association with seed yield. These results are in accordance with the findings of Jahargiridhar *et al.* (1991), Patel (1992) and Byre Gowda *et al.* (1996).

Length of pod bearing branch showed positive and significant association with grain yield at both phenotypic and genotypic level. Basavarajaiah *et al.* (1999) also reported positive association between Length of pod bearing branch and grain yield.

5.2.1.1 Association among Yield Components

Plant height showed positive and significant association with branches per plant, days to 50% flowering, length of inflorescence, days to maturity, pods per plant, pod length, seeds per pod and shelling percentage at both the levels. Similar results were reported by Henry and Krishna (1990), Aher *et al.* (1998) and Srinivas (1999). It had positive association with 100 seed weight at genotypic level while negative association at phenotypic level.

Branches per plant exhibited positively significant association with days to 50% flowering length of pod bearing branch, days to maturity, pod per plant, pod length, seeds per pod and shelling percentage at both phenotypic and genotypic levels. These results are in conformity with Ganesh Murthy and Stephen Dorairaj (1990), Henry and Krishna (1990) and Aher *et al.* (1998). It had negative association with 100 seed weight at both

the levels. Similar results are similar to that of Deshmukh *et al.* (2000) and Pandey and Singh (2001), Chattopadhyaya and Dhiman (2005).

Days to 50% flowering and positive association with length of inflorescence, days to maturity, pods per plant, pod length, seeds per pod and shelling percentage both at genotypic and phenotypic levels. These results are in accordance with Aher *et al.* (1998), Deshmukh *et al.* (2000), Pandey and Singh (2001 a and b). It had negative association with 100 seed weight at both the levels. On the contrary Firoz Mahamad (2003) reported high positive correlation.

Length of pod bearing branch exhibited positive association with days to maturity, pods per plant and shelling percentage at both the levels. These results are in accordance with Basavarajaiah *et al.* (1999).

Days to maturity had positive association with pods per plant and shelling percentage both at phenotypic and genotypic levels. This is in accordance with Ganesh Murthy and Stephen Dorairaj (1990), Khapre and Nerker (1992), It also had negative association with 100 seed weight at both the levels and similar results were reported by and Chattopadyaya and Dhiman (2005).

Pods per plant exhibited positive association with pod length, seeds per pod and shelling percentage at both the levels. Similar results are reported by Brar (1993) and Tikka (1986).

Pod length had positive association with seeds per pod and 100 seed weight. This is in accordance with Virangama and Goyal (1994). Its association with shelling percentage was negative and significant.

Seeds per pod showed positive association with 100 seed weight. Similar results were reported by Byre Gowda *et al.* (1996). On the contrary Chattopadhyaya and Dhiman (2005) reported negative association of seeds per pod with 100 seed weight. Shelling percentage had negative association with 100 seed weight at genotypic levels.

The correlation studies of the present study, indicated that seed yield had highly significant positive association with plant height, branches per plant, days to 50% flowering, days to maturity, pods per plant, pod length, seeds per pod, 100 seed weight and shelling percentage and significant association with length of inflorescence. Hence improvement in any of these characters would also improve seed yield and indirect selection to seed yield using these traits will be effective.

5.3 Path Coefficient Analysis.

The estimation of correlation coefficient does not consider the dependence of one variable on the other independent variable. Path coefficient analysis, a biological technique developed Wright (1921) and later elaborated by Deway and Lu (1959) has been widely used by different workers to separate the effect of particular cause among others. It is simply a standardized partial regression coefficient analysis that separates the correlation coefficient into components of direct and indirect effects and measures the relative importance of each further involved in contributing to the final product. If the correlation between dependent and independent variables arise due to direct effect of the character, it reflects a true relationship between them and selection can be done on such characters to improve the dependent character. In order to obtain cause and affect relationship between yield and yield attributing components, path coefficient analysis was done in pigeonpea and results are discussed below.

In the present study, ten yield attributing characters *viz*., plant height, branches per plant, days to 50% flowering, length of inflorescence, days to maturity, pods per plant, pod length, seeds per pod, 100 seed weight and shelling percentage which have significant correlation coefficient values with yield were considered.

Pod length exerted maximum direct effect on seed yield per plant followed by pods per plant, branches per plant, shelling percentage, length of inflorescence and days to 50% flowering. These results are in accordance with Paul and Upadhyaya (1991) and Paul *et al.* (1996), Singh *et al.* (1999). Plant height showed highest negative direct effect on seed yield per plant. On the contrary, Balyan and Sudhakar (1985a), Natarajan *et al.* (1990) and Basavarajaiah *et al.* (1999) reported highest positive effect.

Among the indirect effects, the maximum positive indirect effects of component characters on seed yield was shown through pods per plant followed by branches per plant, pod length and shelling percentage. These results are in conformity with the reported results of Veraswamy *et al.* (1975), Ram *et al.* (1976b), Gunaseelan and Hanumantha Rao (1976), Pahuja (1981), Henry and Krishna (1990). The indirect effects of component characters on seed yield through plant height were high and negative. These results are in accordance with Deshmukh *et al.* (2000).

5.4 DNA marker diversity using RAPD and SSR markers

Six pigeonpea genotypes having contrasting levels of SMD resistance were selected for DNA marker diversity analysis. They were screened for resistance against SMD and for DNA marker diversity using RAPD and SSR primers.

5.4.1 Screening for SMD

Amongst six Pigeonpea genotypes studied for resistant to SMD, BRG 3 and ICP 7035 recorded lower levels of incidence where as ICP 8863 and TTB 7 showed 100 % incidence and hence they are susceptible. These results are in conformity of the findings of Rangaswamy *et al.* (1997) and Saifulla *et al.* (2005).

5.4.1 RAPD Analysis

In the present study, a total of 99 distinct DNA bands were generated from fourteen primers. Out of which, 39 (39.39 %) were polymorphic and 60 (60.60%) bands were monomorphic. The DNA profile based on RAPD data clearly suggested the presence of polymorphism in the selected pigeonpea genotypes. Lohithashwa *et al.* (2003) reported 52 DNA bands from eleven entries using eight random RAPD primers of which 33 (63.46 %) were polymorphic in pigeonpea.

Amongst the six selected pigeonpea genotypes, some of them generated genotypic specific bands. ICP 8863 and TTB 7 exhibited genotype specific band for the primer OPM 9. This is clearly differentiated that, these two genotypes are distinct from other genotypes with respect to their susceptibility reaction to SMD compared to other

genotypes by showing specific bands. Both these genotypes recorded very high incidence of SMD compared to others. Similarly, the primers OPO 19 and OPL 7 generated distinct bands for BRG 1, which is differed from other genotypes. Similar results indicated unique banding pattern specific to different varieties using RAPD markers as reported by Lohitashwa *et al.* (2003) and Rathnaparke *et al.* (1995) in pigeonpea and Banerjee *et al.* (1998) in chickpea.

Form the dendrogram constructed, it is clearly depicts that both the SMD susceptible genotypes TTB 7 and ICP 8863 clustered together. The resistant genotype ICP 7035 formed a separate sub cluster which is very close to other resistant genotypes Hy-3C and BRG-3, both were grouped in a separate cluster. BRG-1 formed a separate and independent cluster. Lohithashwa *et al.* (2003) reported the grouping of wilt susceptible genotype ICPL 87 and the resistant genotype WRP 1 in different clusters based on the dendrogram.

5.4.2 SSR Analysis

In the present study, 38 distinct bands were generated from 15 primers. Out of which 35 were polymorphic bands. The highest number of bands were generated by D_ 83971-1 (12) followed by D_88122-3(6), AY_189137 -1 and D_88121-3(4 each), AY_189137-3(3) and AY_ 193836-3(2) while single bands was generated by the primers AY_193837-1, AY_257179-1, D_83970-3 and U_ 30875-5.

Primer AY_189137-3 generated specific bands in BRG 3 and ICP- 7035, both have recorded resistant reaction to SMD, which gives the information about the extent of similarity between them. The genotype BRG -3 was clearly differentiated by the primer D_88122-3 from the rest. AY_193836-3 generated specific band in ICP -8863, which recorded susceptible reaction to SMD.

Dendrogram depicted that the susceptible genotypes TTB 7 and ICP 8863 clustered together at 6% while the moderately resistant genotypes Hy 3C and resistant genotype BRG 3 clustered together at 9% which was again sub clustered with resistant genotype ICP 7035. This indicated that susceptible and resistant genotypes were formed a

separate clusters. BRG 1, a moderately resistant variety formed a separate cluster. Similar Studies by Bonn (2006) in RIL (F_6) populations of pigeonpea, using 220 soybean specific primers indicated 39 interpretable bands and nine of these markers were polymorphic to the parental lines of *Fusarium* wilt.

Dendrogram constructed by combined RAPD and SSR data depicted that the susceptible genotypes TTB 7 and ICP 8863 clustered together at 16 % linkage distance while the resistant genotypes Hy 3C and BRG 3 clustered together at 22 % which was again sub clustered with another resistant genotype ICP 7035 at 28 %. These results indicated that susceptible and resistant genotypes were formed a separate clusters. BRG 1, a moderately resistant variety formed a separate cluster. Combined dendrogram of RAPD and SSR data by Nethra (2003) also revealed clustering of rice genotypes based on morphological, biochemical, biological and molecular markers.

Summary

VI. SUMMARY

In the present study hundred genotypes of pigeonpea were evaluated using randomized complete block design for eleven quantitative characters to elucidate the information on nature and magnitude of genetic variability, pattern of correlation among characters, direct and indirect effects of components characters on seed yield. Six diverse genotypes with contrasting levels of resistance to Sterility mosaic disease were selected to study DNA marker diversity with reference to expression of disease.

The analysis of variance revealed that significant differences among the genotypes for the characters studied. The estimates of PCV and GCV are high for plant height, branches per plant, length of inflorescence, pods per plant and seed yield. Days to 50% flowering, pod length and 100 seed weight exhibited moderate GCV and PCV while days to maturity exhibited low GCV and PCV.

High heritability coupled with high genetic advance as per cent of mean was noticed for the character plant height, branches per plant, days to 50% flowering, pod bearing branches, pods per plant, 100 seed weight and seed yield indicating selection is effective for these characters and were controlled by additive gene action.

Moderate heritability coupled with moderate genetic advance as per cent mean was noticed for the characters, pod length and seeds per pod indicating substantial influence of environment on these characters and controlled by both additive and non-additive gene actions.

Grain yield exhibited highly significant and positive association at both genotypic and phenotypic levels with plant height, branches per plant, days to 50% flowering, days to maturity, pods per plant, pod length and seeds per pod. At genotypic level, shelling percentage exhibited highly significant and positive association with seed yield, while hundred seed weight exhibited positive and significant association.

The path coefficient analysis revealed that highest positive direct effect on seed yield was exerted by pod length, which was followed by pods per plant, branches per

plant, shelling per cent, length of pod bearing branch and days to 50 % flowering. Plant height exhibited high and considerable amount of negative direct effect on seed yield. Seeds per pod and 100 seed weight showed low and positive direct effect on seed yield. Indirect contributions of branches per plant, pods per plant and pod length was high and positive, while the indirect contribution through plant height was negative.

Amongst the six pigeonpea genotypes studied for SMD incidence, BRG 3 and ICP 7035 recorded lower levels of incidence whereas ICP 8863 and TTB 7 showed 100 % incidence.

RAPD Analysis generated totally 99 distinct DNA bands from fourteen primers. Out of which 39 (39.39%) were polymorphic and 60 (60.60%) bands were monomorphic indicating the presence of polymorphism in the selected pigeonpea genotypes. The primer OPM-9 exhibited genotype specific band in ICP 8863 and TTB 7, which clearly differentiated these two susceptible genotypes from other genotypes with respect to their susceptibility reaction to SMD. Similarly, primers OPO-19 and OPL-7 generated distinct bands for BRG 1.

Dendrogram constructed based on RAPDs, indicates that SMD susceptible genotypes TTB 7 and ICP 8863 clustered together. The resistant genotype ICP 7035 formed a separate sub cluster, which is very close to other resistant genotype Hy 3c and BRG 3. BRG 1 formed a separate cluster.

In the SSR analysis, 38 distinct bands were generated from 15 primers. Out of which 35(92%) were polymorphic bands indicating the presence of polymorphism in the selected pigeonpea genotypes.

The primer D_ 83971-1 generated highest number of bands. Primer AY_189137-3 generated specific bands in BRG-3 and ICP 7035, both have recorded resistant reaction to SMD. The genotype BRG 3 was clearly differentiated by the primer D_88122-3 from rest. AY_193836-3 generated specific band in ICP 8863, which recorded susceptible reaction to SMD.

Dendrogram based on SSR Analysis, showed that susceptible genotypes TTB 7 and ICP 8863 are clustered together, while the genotypes Hy 3C and BRG 3 together formed separate cluster which was sub clustered with highly resistant genotype ICP 7035 indicating that susceptible and resistant genotypes formed separate clusters.

Dendrogram constructed by combined RAPD and SSR data depicted that the susceptible genotypes TTB 7 and ICP 8863 clustered together, while the genotypes Hy 3C and BRG 3 were sub clustered with ICP 7035 from the susceptible genotypes. Whereas BRG 1 formed separate, unique and distinct cluster from rest of the genotypes, indicating ICP 7035, BRG 3, Hy 3C and BRG 1 are differing at genotypic level from susceptible genotypes TTB 7 and ICP 8863.

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Appendix

Appendix 1. Treatment means of 11 characters for 100 genotypes

Sl. No.	GENOTYPE	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
1	JKM 197	207.07	12.40	100.33	99.00	194.67	172.33	5.23	4.07	9.13	76.35	38.93
2	JSA 34	201.40	14.67	97.33	98.67	194.67	261.33	5.20	4.07	9.33	75.63	71.60
3	CORG 99013	216.20	11.47	98.67	94.27	196.00	181.07	5.80	4.47	11.23	74.15	63.20
4	ICP8863	197.53	9.53	94.67	99.40	195.33	130.07	4.77	4.13	10.67	81.30	40.80
5	BDN 2003-2	168.13	10.33	96.33	74.33	191.00	159.33	5.30	4.60	8.80	77.95	39.67
6	AK 222492	180.87	8.13	105.00	114.60	199.67	117.13	5.10	3.60	11.43	63.59	29.67
7	GRG-205	168.07	17.17	124.00	85.93	214.00	308.2	5.33	3.80	11.70	67.92	90.40
8	BDN 2	191.60	6.47	94.67	129.2	196.33	198.87	5.40	4.07	10.90	66.03	48.13
9	JSA 75	197.07	9.87	113.00	119.47	195.33	125.20	5.17	4.17	9.03	69.96	30.20
10	AK 222498	184.53	9.47	101.67	105.40	212.00	111.07	5.50	4.03	11.33	71.72	27.67
11	PT 01-27	161.80	10.07	88.00	81.35	180.00	240.73	5.23	4.27	10.40	64.18	65.33
12	ICPL 87119	218.73	11.87	96.00	101.07	196.67	167.63	4.84	4.30	11.40	77.96	50.50
13	WRG 136	228.67	13.13	98.67	101.60	195.00	214.60	5.53	4.40	8.73	79.08	51.80
14	BRG 2-5	208.80	13.47	112.67	80.47	204.67	204.17	6.13	5.00	8.03	83.30	72.00
15	NDA 05- 33	218.67	13.13	118.33	90.53	208.33	160.80	6.20	4.47	12.43	70.52	37.67
16	BDN 2001-9	200.00	12.60	98.33	105.27	195.33	213.53	5.53	4.13	12.77	80.61	59.07
17	JSA 73	203.13	12.40	101.67	98.27	194.67	214.93	5.43	3.97	13.27	77.70	57.67
18	TJT 502	186.27	9.47	85.67	95.47	180.33	176.37	5.57	4.00	10.23	72.31	35.67
19	WRG 123	172.53	8.87	95.00	96.40	195.33	148.93	5.79	4.67	12.40	77.40	51.80
20	NDA-05-01	207.47	11.73	99.67	77.73	196.00	257.87	6.30	4.60	8.87	74.79	58.40
21	PT 02-25	180.33	11.27	90.67	90.47	182.67	168.47	5.33	4.00	8.73	84.93	38.13
22	JSA 41	168.47	13.73	97.00	93.07	195.00	236.40	5.27	3.87	11.47	66.10	57.93

Continued appendix 1

23	SKNP 0224	177.93	8.60	100.00	120.73	195.33	122.27	5.33	3.93	11.43	76.84	33.33
24	JKM 204	202.87	12.13	111.67	94.33	195.33	162.07	5.43	4.47	12.67	69.76	38.60
25	TTB -7	209.53	13.80	113.33	55.00	183.33	176.00	6.20	5.33	8.53	73.89	69.07
26	BDN 2001-6	258.00	6.93	96.67	103.07	192.00	76.33	4.60	4.00	7.2	73.51	73.33
27	ICPL 8863 Sel	185.27	9.40	94.00	78.67	191.00	105.47	4.53	3.73	11.70	82.75	27.87
28	BDN 2 sel	186.00	6.93	93.00	96.73	193.00	165.60	4.67	3.53	9.93	74.23	24.27
29	ICPL 87119 Sel	205.20	10.93	102.33	101.20	192.67	162.13	4.87	4.07	8.37	74.60	41.13
30	TTB -7 Sel	200.67	11.60	114.33	43.73	186.00	163.20	6.67	5.33	11.00	74.84	48.93
31	BDN 702	188.00	8.87	96.00	79.87	176.00	153.20	5.37	4.33	16.83	70.43	41.80
32	WRG 65	193.80	9.27	97.33	66.40	190.67	161.07	5.40	4.13	8.70	77.25	51.73
33	PT 02 -5	187.47	9.00	89.00	78.90	191.33	98.73	5.93	4.67	10.03	72.04	40.53
34	Phule T 8208 -1	195.20	8.20	94.00	85.27	191.67	115.87	5.07	4.07	8.33	74.87	32.93
35	WRG 79	222.27	6.87	96.00	108.33	192.67	70.73	4.83	3.80	9.37	77.18	12.20
36	PA 303	93.33	5.80	67.00	49.93	141.00	41.90	5.20	4.20	12.40	65.76	9.67
37	H 2000-37	86.07	6.27	55.00	40.07	135.00	27.07	5.00	4.00	10.67	70.56	24.00
38	AL 1507	78.67	6.13	57.33	40.40	136.33	23.80	5.33	4.13	15.37	71.11	9.67
39	ASJ 105	118.53	7.40	72.67	66.93	175.33	65.67	5.13	4.07	10.70	66.03	13.93
40	SKNP 0203	136.80	5.53	72.67	75.40	175.33	60.40	5.13	4.00	11.73	58.43	6.4
41	PUSA 2005-1	78.47	5.07	56.67	41.40	147.00	29.07	5.00	3.87	10.70	70.00	8.20
42	AL 1492	99.93	8.13	52.00	59.40	144.00	47.67	5.10	4.13	11.00	64.55	8.73
43	H 2000-47	66.87	5.73	57.00	42.73	147.00	20.13	5.00	3.67	10.37	61.52	9.13
44	WRGE 42	101.73	7.20	96.00	60.07	174.67	45.73	5.83	3.73	12.37	63.59	8.47
45	UPAS 120	84.80	5.67	70.67	42.20	175.00	34.20	5.33	4.07	11.03	67.69	8.40
46	CORG 200402	88.13	4.93	72.67	52.53	160.67	35.40	5.20	3.53	11.70	69.55	10.60

Continued appendix 1

47	PUSA 2005-2	81.47	5.73	57.67	41.20	145.00	25.73	5.00	4.07	9.00	64.45	9.00
48	TJT 501	116.73	7.20	73.67	63.87	163.00	77.53	6.00	4.20	12.37	69.54	14.00
49	JSA 28	114.33	6.80	72.67	64.20	162.67	65.33	6.17	4.53	12.00	66.67	14.07
50	PT 02 -9	105.13	7.53	70.67	51.67	172.33	78.40	5.60	3.47	10.97	65.88	16.20
51	PA 322	109.40	6.40	71.33	63.40	158.33	62.67	5.33	4.07	12.67	65.99	14.00
52	SKNP 0202	126.67	7.67	82.67	103.20	163.33	104.40	4.40	3.87	11.00	42.86	15.13
53	WRGE 39	133.20	8.63	106.00	59.13	162.67	115.53	6.00	4.73	11.30	67.22	21.87
54	AJS 104	71.67	6.20	62.00	36.67	161.67	22.67	5.07	4.07	14.33	67.02	10.47
55	ICPL 87	54.73	6.60	64.67	19.20	176.67	24.87	4.60	4.07	12.00	68.75	15.27
56	PH 501	142.00	7.73	96.67	98.53	195.33	124.93	4.40	3.87	12.03	70.70	31.33
57	PH 502	38.60	6.20	95.33	26.93	178.00	9.40	4.41	3.80	8.90	73.33	12.27
58	PH 503	134.13	6.07	95.33	92.53	201.67	62.93	4.87	3.80	11.67	67.52	26.60
59	PH 504	139.67	3.80	93.33	96.00	182.67	55.93	3.60	3.87	9.70	63.44	19.27
60	PH 505	137.20	6.53	92.67	83.80	198.67	113.93	5.07	3.93	12.40	65.66	27.33
61	PH 506	123.60	10.27	92.33	84.53	197.67	67.80	4.40	2.00	11.00	69.60	22.60
62	PH 507	161.33	11.13	104.00	97.33	200.00	107.60	4.90	3.87	11.03	66.34	16.67
63	PH 508	110.27	5.47	93.00	76.00	180.00	58.80	4.90	3.40	11.70	69.44	24.87
64	JKPH 3103	138.13	7.87	80.67	76.73	186.00	100.07	5.77	4.27	11.03	69.54	26.80
65	JKPH 2103	141.67	8.13	88.33	75.80	188.67	156.00	5.53	4.40	11.03	68.44	27.20
66	JKPH 3101	106.13	6.07	89.00	83.87	185.00	71.67	4.70	4.53	10.33	63.41	17.13
67	JKPH 6103	154.73	7.47	89.33	84.20	183.33	97.13	5.07	4.13	11.70	66.03	20.80
68	JKPH 2101	127.47	7.53	91.33	94.30	184.67	93.00	5.27	3.73	10.70	66.27	59.33
69	JKPH 6101	120.53	9.00	91.67	75.73	183.67	74.13	5.53	4.27	11.00	68.44	23.07
70	JKPH 1101	143.00	8.20	82.33	74.00	199.67	109.13	5.70	3.80	10.00	70.28	28.33

Continued appendix 1

71	JKPH 1103	162.73	9.60	91.33	94.73	199.00	123.20	5.57	3.93	11.03	68.02	32.00
72	BRG-1	188.33	9.13	112.33	53.80	174.00	155.60	7.13	5.47	16.37	72.32	70.80
73	BRG-2	166.27	8.47	103.00	46.73	173.33	155.20	7.53	5.00	13.00	58.15	53.80
74	BRG-3	151.80	7.27	102.33	49.93	175.00	121.27	9.00	5.53	15.67	71.17	61.33
75	BRG 2-2	155.57	8.73	94.67	60.80	175.00	110.47	7.33	5.33	13.67	63.95	56.40
76	BRG 2-6	165.93	10.87	91.00	79.87	174.67	145.33	4.80	4.00	13.10	50.95	36.93
77	BRG-1(W)	185.93	9.40	107.67	65.00	174.33	200.27	7.47	5.13	17.03	70.63	110.00
78	BRG-2-7	205.40	13.00	106.00	52.80	181.33	124.67	6.27	4.87	9.50	78.15	42.53
79	LCV-8	169.93	9.60	94.33	65.13	172.33	183.40	7.00	4.53	11.03	60.48	75.33
80	LCV-10	168.47	7.47	102.33	47.33	175.33	83.67	7.80	6.20	11.13	58.18	68.07
81	TT-2000	199.27	9.20	89.67	94.40	175.33	124.33	5.60	4.40	10.07	64.88	33.07
82	PT-92-30	182.93	11.47	100.67	64.20	177.00	174.00	5.33	4.47	12.00	67.33	64.00
83	HY-3C	159.13	11.67	96.33	49.07	175.67	171.93	6.74	4.60	18.1	71.10	86.00
84	GT-1	235.67	8.60	94.67	121.80	200.67	85.07	7.13	4.93	11.03	67.78	26.87
85	SKNP-9711	168.40	6.20	87.00	111.60	175.67	99.80	5.87	4.13	8.67	65.74	25.53
86	SKNP-110	67.27	7.40	75.00	37.60	174.00	49.27	4.93	3.40	10.93	62.70	66.67
87	SKNP-111	219.87	8.47	86.00	121.07	207.33	65.27	6.07	4.07	8.60	65.48	26.67
88	TT 401	116.67	6.67	74.33	78.73	159.67	52.40	5.90	4.33	11.33	75.08	18.07
89	UPAS 120 Sel	110.67	5.80	71.33	60.13	159.00	87.67	5.00	4.20	9.70	72.58	18.20
90	TT 402	119.30	6.93	72.33	75.47	174.33	70.27	5.57	4.13	10.37	65.38	16.00
91	ICPL 87 Sel	45.53	5.40	67.33	20.40	176.67	22.27	4.73	3.80	13.33	69.80	9.13
92	CORG 2004- 01	126.47	5.93	77.00	63.53	162.67	63.47	5.63	4.00	12.00	73.08	13.67
93	WRGE 38	130.27	7.60	60.67	79.87	175.00	82.27	5.60	4.33	10.70	64.96	11.93
94	PUSA -2004 -2	88.27	4.33	65.00	62.13	159.00	37.07	4.93	3.87	9.80	64.65	12.27

Continued appendix 1

95	BRG- 2-1	116.93	7.60	85.67	76.40	167.67	101.07	5.40	4.47	13.37	71.33	22.47
96	JKE 110	115.07	5.47	93.00	69.67	171.00	55.00	4.70	3.47	11.33	72.11	8.47
97	PA 296	109.00	6.20	66.67	54.67	159.00	59.53	5.20	4.13	9.67	72.11	15.80
98	SKNP - 0207	107.13	5.53	83.67	69.80	175.00	50.27	5.33	4.00	12.00	61.54	10.67
99	WRGE 37	95.20	6.13	103.33	64.80	178.00	38.60	5.83	3.80	11.00	64.10	9.20
100	CORG 9701	133.53	7.00	75.00	77.40	158.33	81.33	5.47	4.00	10.17	73.81	13.00
	Mean	150.79	8.54	88.99	76.10	180.41	111.79	5.50	4.20	11.20	69.60	33.39
	SEm±	15.62	1.53	2.27	8.99	1.65	24.51	0.47	0.41	0.67	6.85	9.27
	CD	30.79	3.02	4.47	17.74	3.25	48.34	0.92	0.82	1.33	13.51	16.79
	CV	12.68	22.00	3.12	14.47	1.12	26.85	10.48	12.22	7.39	12.06	33.47

$\mathbf{X_1}$ = Plant height (cm)

$\mathbf{X_2}$ = Branches per plant

$\mathbf{X_3}$ = Days to -50%flowering

$\mathbf{X_4}$ = Length pod bearing branch (cm)

$\mathbf{X_5}$ = Days to maturity

$\mathbf{X_6}$ = Pods per plant

$\mathbf{X_7}$ = Pod length (cm)

$\mathbf{X_8}$ = Seeds per pod

$\mathbf{X_9}$ = 100 Seed weight (g)

$\mathbf{X_{10}}$ = Shelling percentage

$\mathbf{X_{11}}$ = Seed yield (g)