

**MORPHOLOGICAL AND MOLECULAR DIVERSITY ANALYSIS, AND  
GENETIC ENHANCEMENT OF PIGEONPEA (*CAJANUS CAJAN* L.)  
FOR PODBORER RESISTANCE THROUGH WIDE HYBRIDIZATION**

**THESIS SUBMITTED TO  
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DOCTOR OF PHILOSOPHY  
IN GENETICS**

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### CERTIFICATE

This is to certify that the Thesis entitled **“Morphological and Molecular Diversity Analysis, and Genetic Enhancement of Pigeonpea (*Cajanus cajan* L.) for Podborer Resistance through Wide Hybridisation”**, submitted for award of the degree of Doctor of Philosophy in Genetics, Osmania University, is a record of the *bona fide* research carried out by Ms. R. Aruna, under my supervision, and no part of the Thesis has been submitted for any other degree or diploma.

The assistance and help taken during the course of this investigation and the sources of literature referenced have been fully acknowledged.

Date: 10.10.2003

  
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## DECLARATION

I hereby declare that the research work presented in this Thesis entitled **“Morphological and Molecular Diversity Analysis, and Genetic Enhancement of Pigeonpea (*Cajanus Cajan* L.) for Podborer Resistance through Wide Hybridisation”**, has been carried out by me at the Department of Genetics, Osmania University, Hyderabad and at ICRISAT, Patancheru, under the supervision of Prof. D. Manohar Rao, Department of Genetics, Osmania University.

This work is original and no part of the Thesis has been submitted earlier for the award of any other degree or diploma of any University.

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# INTRODUCTION

## INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millspaugh), also known as red gram, is the sixth most important grain legume crops grown in the tropics and sub-tropics between 30°N and 30°S latitudes. Pigeonpea is cultivated in about 50 countries of Asia, Africa and Americas. This crop is adaptable in a number of cropping systems and is grown on marginal to rich lands. Pigeonpea is cultivated in almost 4 million hectares worldwide. In India, this crop is grown over 2.9 million hectares for multiple uses such as food, fodder and fuel (Nene and Sheila, 1990). Grain legumes or pulses are the major source of dietary protein in developing countries. The high protein content and relatively low cost of these food legumes has earned them the title, "Poor man's Meat" which effectively underlines their vital importance in developing nations. Legumes have also played a major role in patterning the agricultural systems in the tropics.)

Insects are the most important among biotic constraints to pigeonpea production worldwide, causing losses of more than \$ 1000 million every year. More than 200 species of insects feed on pigeonpea, of which *Helicoverpa armigera*, *Maruca vitrata*, *Melanagromyza obtusa*, *Clavigralla* spp., *Nezara viridula* and *Callosobruchus* spp. are the most important (Lateef and Reed, 1992). Of these, legume podborer, *Helicoverpa armigera*, is the most destructive and notorious pest of the field crops (Lateef and Reed, 1992). It is widely distributed from the Cape Verde Islands in Atlantic Ocean, through Africa, Asia and Australia to the South Pacific Islands and from Southern Europe to New Zealand (Reed and Pawar, 1982). It is the most difficult insect due to its wide host range, high fecundity and strong migratory ability of adult moths, and the ability to undergo a facultative diapause (Fitt, 1989). It is a polyphagous pest and has been recorded feeding on 181 cultivated and uncultivated plant species belonging to 45 families; 40 of dicots and 5 monocots (Manjunath *et al.*, 1989). However, most serious losses have been recorded on crops such as pigeonpea, chickpea, tomato, cotton, sorghum, pearl millet, peas and groundnut. The productivity of chickpea (Reed *et al.*, 1987) and pigeonpea (Singh *et*

*al.*, 1990) crops is drastically affected by *H. armigera*. *Helicoverpa* causes an estimated loss of US\$ 927 million in chickpea and pigeonpea, and possibly, over US\$ 2 billion on other crops worldwide. A conservative estimate is that over US\$ 1 billion is spent on insecticides to control this pest. Therefore, in addition to huge economic losses caused by the pest, there are several indirect costs from the deleterious effects of pesticides on the environment and human health (Sharma *et al.*, 2001). Continuous use of insecticides and chemicals has led to the insecticide resistance in this insect, which resulted in several crop failures. Therefore, host plant resistance plays an important role in the management of this pest. It offers a viable economic solution in this situation.)

The identification and utilization of cultivars resistant or tolerant to *H. armigera* would have a number of advantages, particularly for a few relatively value crops such as pigeonpea. Notwithstanding the availability of vast germplasm with wide degree of variability for various economic characters within the cultivated types, little progress has been made in evolving varieties/hybrids with durable resistance to biotic stresses. Resistant and less susceptible cultivars would provide an equitable, environmentally sound, and sustainable pest management tool. More than 14,000 pigeonpea accessions have been screened at ICRISAT center and in the national agricultural research programs. Though, several genotypes have been identified, which suffer no or less pod damage, these genotypes have not been widely used. The level of tolerance in the cultivated genotypes is low, and some of the genotypes are susceptible to major pigeonpea fungal and viral pathogens. Wild relatives of *Cajanus* species, especially *C. scarabaeoides*, have been identified as a potential source of resistance (Pundir and Singh, 1987; Saxena *et al.*, 1990; Shanower *et al.*, 1997). There is also some evidence that the wild species have different mechanisms of resistance against podborer than in the cultivated types. The genes from the wild relatives can be tapped through wide hybridization for use in the crop improvement to diversify the basis of resistance to these pests. However, despite the availability of a wide array of wild sources of resistance, their utility in pigeonpea improvement has not been fully explored. A few isolated reports of utilization of these wild species in pigeonpea breeding include their exploitation as

sources of resistance to podborer, pod wasp and *Phytophthora* blight (Reddy *et al.*, 1982; Saxena *et al.*, 1996; Sharma *et al.*, 2000).

Recent studies at ICRISAT have focused on identifying and characterizing physical and biochemical parameters/components which may contribute to the insect resistance in wild species of *C. scarabaeoides* and *C. platycarpus*. Among the physical components, trichomes and the nature of pod wall surface are being investigated. The distribution of trichomes in different accessions and pigeonpea varieties and their correlation with mechanisms of resistance and inheritance of the various types of trichomes are yet to be investigated.

Compounds in trichome exudates and / or on the pod surface may stimulate or deter *H. armigera* larvae from feeding. Acetone extracts from the pod surface of *C. scarabaeoides* have shown some antifeedant activity, which was absent in *C. cajan*. It is possible that the feeding stimulant in the susceptible species is associated with the presence of phagostimulants or absence of antifeedant compounds that are released from the glandular trichomes on the surface of the pods.

Pest management strategies of *Helicoverpa* include cultural management of the crop and its environment; biological control using predators, parasites and microbial pesticides; sex pheromones for population monitoring and mating disruptions; host plant resistance, and chemical control. Environmentally safe technologies are not yet ready, in a form, to be delivered to farmers, although all agree that use of integrated pest management (IPM) strategies can reduce the negative environmental effects of chemical pesticides (Sharma *et al.*, 2001).

Continuing efforts to conserve the germplasm of diverse array of species of *Cajanus* and to transfer the desirable genes from these wild plants into cultivated *C. cajan* have emphasized the need to understand the genetic diversity and phylogenetic relationships among the crop species in more detail (Pundir, 1981; Pundir and Singh, 1985; Saxena *et al.*, 1990). Wild relatives of pigeonpea serve as a rich source of disease and insect resistance that can be introgressed into the cultivated genotypes (Shanower, 1999). The use of wild relatives in pigeonpea

improvement for various qualitative traits has been reported long back but to a very limited extent. The attention paid towards pigeonpea improvement, with the use of the wild relatives, though started in early 1970s, yet, remarkable improvements in this area have been very few. Plant breeding history shows that diverse gene pools are the foundations for effective crop improvement programmes. Exotic germplasm from weedy species has been used nearly exclusively as a source of genes for improving qualitatively inherited characters, such as disease resistance. The assessment of diversity has focused mainly on cultivated types in the primary gene pool and little is known of the extent of variation or the nature of traits available in wild species belonging to other gene pools. Further, the taxonomic confusions and lack of evaluation information on traits of interest, particularly with reference to resistance to serious pests and diseases seem to have precluded their intensive study and utilization.

The primary objective in plant breeding is to widen the genetic base of a cultivated species. If the needed variation is limited, as in the case of pigeonpea, the options for the breeders are incorporation of alien variation, induction of mutations or exploitation of Somaclonal variation. The other and possibly the most viable resource of introducing variation into a species is through transfer of genetic material from one species to another by hybridization. The genetic potential of wild relatives is widely demonstrated in plant breeding and in the evolutionary studies. Wild relatives have helped to fill the voids in traditional breeding programmes. Pigeonpea is the only cultivated species under the genus *Cajanus* and the rest 31 species belonging to this genus are wild. Of these, 13 are endemic to Australia, 8 to Indian subcontinent and Myanmar, and the rest of them occur in more than one country. Apart from *C. cajan*, only the other species, *Cajanus scarabaeoides*, is common and widespread throughout South and Southeast Asia, the Pacific Islands, and northern Australia (Van der Maesen, 1986). In addition to India, the greatest diversity of wild species of *Cajanus* is found in Myanmar, Yunnan-China, and northern Australia. Several species, such as *C. villosus*, *C. elongatus*, *C. granadiflorus* and *C. niveus* that were earlier collected or known to occur in northeastern India are either rare or extinct. Wild relatives of pigeonpea

possess several valuable traits, including cytoplasmic, genetic male sterility systems (Reddy and Faris, 1981; Ariyanayagam *et al.*, 1995), partially cleistogamous trait, which ensures very high purity of genotypes (Saxena *et al.*, 1998), podborer resistance (Lateef *et al.*, 1981; Dodia *et al.*, 1996; Shanower *et al.*, 1997), nematode resistance (Sharma *et al.*, 1993), and salinity tolerance (Subba Rao, 1988). With the possibility of large-scale adoption of transgenic techniques to combat intractable problems in pigeonpea, there is a possibility of gene escape into the non-target organisms, including wild and weedy relatives through gene flow. This could result in the loss of valuable traits that are required for future breeding programs. To develop precautionary measures to arrest this problem, knowledge on the possibility of gene flow between pigeonpea and various wild relatives needs to be gained. This will help in preserving the valuable species by following necessary regulatory measures.

The objective of the backcross method is to improve one or two specific defects of a high yielding variety, which is well adapted to the area and has other desirable characteristics. The character(s) lacking in this variety are transferred to it from a donor parent without changing the genotype of this variety, except for the genes being transferred. Thus, the end results of a backcross programme will be a well-adapted variety with one or two improved characters. Backcross method has been used to transfer simply inherited characters, mostly insect and disease resistance, from wild or related species into a cultivated species. For example, transfer of resistance into wild fire and black fire from *Nicotiana longiflora* to *N. tabaccum* leaf and stem rust resistance from *Triticum timopheevii*, *T. monococcum*, *Aegilops speltoides* and *Secale cereale* (rye) to *T. aestivum*; and black-arm resistance from several *Gossypium* species to *G. hirsutum*

Scientific approaches for conservation and utilization of plant genetic resources require an accurate assessment of the amount and distribution of genetic variation within a gene pool. An important pre-requisite for using wild species is the identification of closely related species as potential gene donors and finding useful genes for pigeonpea improvement. Traditionally, phenological and morphological



characters have been used for the identification of pigeonpea cultivars and their wild relatives. Among the 271 accessions of 47 wild species, related to *Cajanus* available in the gene bank at ICRISAT (Remanandan *et al.*, 1988), *Cajanus scarabaeoides* is the most widely distributed. Since, *C. scarabaeoides* can be easily crossed with pigeonpea, its useful genes can be utilized in the improvement of the latter. Except the morpho-taxonomical description of the species (Van der Maeson, 1986) no information is available on the variation within the species for economical traits, and only a limited molecular work has been carried out (Sivaramakrishnan *et al.*, 2001).

A large number of methodologies exist for the assessment of genetic diversity in plant species. A combination of morphological traits and protein profiling methods, such as isozymes (Nevo *et al.*, 1986), allozymes (May, 1992) and seed storage proteins (Doll and Brown, 1977) have conventionally been applied. However, such traits are influenced by environmental factors and so the results elucidated based on such studies do not provide a true measure of genetic diversity. The advent of environmentally neutral molecular markers will allow better quantification of genetic diversity (Clegg, 1984; Gepts, 1995). New technological developments have expanded the range of DNA polymorphism assays for genetic fingerprinting and for investigating genetic diversity and genetic relatedness. Assignment of levels and distribution of polymorphism (usually conceptualized as 'allelic richness' and 'allelic evenness') in a crop, permit the sampling and utilization of genetic resources in a more systematic and efficient manner, and also allow an enhanced understanding of evolutionary relationships both for breeding and conservation. These technologies include restriction fragment length polymorphism (RFLP) (Botstein and White, 1980; Rafalski and Vogel, 1996), random amplified polymorphic DNA markers (RAPD) (Bowcock *et al.*, 1994), amplified fragment length polymorphism (AFLP) (Zabeau and Vos, 1993), and simple sequence repeats or micro-satellites (SSR) (Tantz, 1989, Weber and Powell, 1992). These methods detect polymorphism by assaying subsets of the total amount of DNA sequence variation in a genome. Polymorphisms detected with AFLP and RFLP assays reflect restriction size variations. RFLPs have been used to characterize the genetic diversity in cultivated pigeonpea and its wild relatives (Beckmann and Soller, 1983; Tansley

and Miller 1990; Wang *et al.*, 1992; Sivaramakrishnan *et al.*, 2001). There are many advantages of RFLPs in the estimation of genetic relationships (Melchinger *et al.*, 1991; Smith and Smith, 1991; Stuber, 1992). AFLP methodology has been used to assess genetic diversity in rice (Zhu *et al.*, 1998), lactuca (Hill *et al.*, 1996), Soybean (Sharma *et al.* 1996, Maughan *et al.*, 1997;), sunflower (Hongtrakul *et al.*, 1997). SSRs are highly polymorphic and are becoming the marker of choice in both animal and plant species (Condit and Hubell, 1991; Akkaya *et al.*, 1992; Morgante and Oliveri, 1993; Wang *et al.*, 1994).

There are no reports, in pigeonpea, for diversity analysis using AFLP markers. The cultivated pigeonpea lines were studied for variation at genomic level, using SSR primers but there are no reports of diversity analysis in wild pigeonpea accessions (Malcolm, 2001). However, the cluster analysis of southern blot hybridization, data with 15 restriction enzyme – probe combinations placed various species of pigeonpea into ten major groups and revealed a phylogenetic relationship among these groups. This study suggests that RFLP of mt DNA can be used for the diversity analysis of pigeonpea. The variations in mitochondrial DNA hybridization patterns also suggest the extensive rearrangement of the organelle genome among the *Cajanus* species (Sivaramakrishnan *et al.*, 1999).

Assessment of genome relationships is a first step in the exploitation of wild species in the improvement of any cultivated species. The next step is utilization of such hybrids in the breeding programme before which it would be essential to study the inheritance pattern and also assess the quantum of variability generated. Studies on inheritance provide information on the possible number of genes governing a character and their interaction. Evaluation of variation in F<sub>2</sub> generation helps in understanding the extent of recombination and variability. Genetic studies provide a clear direction for handling the segregating generations. Few studies have been made on the genetics of qualitative and quantitative traits in pigeonpea (Deshpande and Jeswani, 1956; D'Cruz and Deokar, 1970; Munoz and Abrams, 1971; Pandey, 1972; Sharma *et al.*, 1972, Joshi, 1973; Choudhary and Thombre, 1977; Dahiya and Brar,

1977; Dahiya *et al.*, 1977; Kapur, 1977; Malhotra and Sodhi, 1977; Reddy *et al.*, 1979; Singh *et al.*, 1997).

Most of the interspecific hybridization work done at ICRISAT, in pigeonpea, was mostly confined to breeding for high protein lines (Reddy *et al.*, 1979) and to a limited extent for breeding for insect resistance, dwarfs and isolation of cytoplasmic male sterile involving a few wild accessions of *Cajanus* (Saxena *et al.*, 1990). Genome relationships between wild and cultivated *Cajanus* species are still obscure. *C. cajanifolius*, which is morphologically very similar to *Cajanus cajan*, except for the seed strophiole, was identified as early as 1920 (Van der Maesen, 1980) but an attempt to cross these two species was not reported until 1981 (Pundir, 1981). The studies on the hybrid progenies of *C. cajan* x *C. scarabaeoides* cross suggested that the antibiosis mechanism of resistance was governed by a single dominant gene (Verulkar *et al.*, 1997).

Thus, with an aim of further understanding of wild *Cajanus* species and their potential significance in pigeonpea improvement for pest resistance, to understand the genetic basis of different qualitative and quantitative traits, including the resistance against podborer, the present investigation was undertaken with the following objectives:

- Morphological, molecular and biochemical characterization of wild *Cajanus scarabaeoides* accessions.
- To screen the *C. scarabaeoides* accessions for resistance against podborer and to identify the highly resistant ones to involve them in the hybridization programme.
- To incorporate the podborer resistant gene(s) from the wild *C. scarabaeoides* accessions to the cultivated *C. cajan* genotypes through backcross programme and wide hybridization.
- To study the genetic basis of qualitative and quantitative traits
- To study the genetic basis and different mechanisms of resistance against podborer.

# REVIEW OF LITERATURE

## REVIEW OF LITERATURE

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) is one of the major grain legumes of the tropics and subtropics. Small and marginal farmers in India, Myanmar, Kenya, Malawi, Uganda and a few countries of Central America produce it commercially. It belongs to the family Leguminosae, subfamily Papilionidae, tribe Phaseolae and subtribe Cajaninae. After the merger of genus *Atylosia* with *Cajanus*, the latter has 32 species (Van der Maesen, 1986) of which *C. cajan* is the only cultivated species.

### Diversity analysis

Pigeonpea was originated in India, as evidenced by the presence of several wild relatives (including the nearest one), larger diversity of crop gene pool, ample linguistic evidence, a few archeological remains and wider usage in daily cuisine. De (1974) and Vernon Royes (1976) prepared reviews that including discussions on the pigeonpea's origin. The later considered it in favor of Indian origin. Further considerations also clarified this (Van der Maesen, 1980). Several authors considered eastern Africa as the "Center of origin", since pigeonpea seems to occur wild in Africa. The scarce but often cited archeological evidence of one seed in an ancient Egyptian tomb and the wild occurrence in Africa made many authors (Purseglove, 1968; Rachie and Roberts, 1974) favor an African origin. However, the range of diversity of the crop in India is much larger, and this made Vavilov (1951) list pigeonpea as of Indian origin.

Pigeonpea is the only cultivated food crop of the Cajaninae subtribe of the economically most important leguminous tribe Phaseolae, which contains many bean species consumed by man (e.g., *Phaseolus*, *Vigna*, *Cajanus*, *Lablab*, *Macrotyloma*). The cultivated pigeonpea stands alone as a crop species in the sub-tribe, of which most of the species belong outside the pigeonpea gene-pool, or at the most in its tertiary genepool, while several *Cajanus* species can be placed in the secondary gene pool. The different species of *C. cajan* and other related genera are grouped into different gene pools as follows

## **Gene pool**

Primary gene pool

Secondary gene pool

Tertiary gene pool

## **Genus / species**

Cultivar collections

*Cajanus acutifolius*, *C. albicans*, *C. cajanifolius*,  
*C. lanceolatus*, *C. latiseptus*, *C. lineatus*,  
*C. reticulatus*, *C. scarabaeoides* var.  
*scarabaeoides*, *C. sericeus*, *C. trinervius*

*C. goensis*, *C. heynei*, *C. kerstingii*, *C. mollis*,  
*C. platycarpus*, *C. rugosus*, *C. volubilis*, and  
other *Cajanus* spp. Other *Cajaninae* (e.g.,  
*Rhynchosia*, *Dunbaria*, *Eroisema*)

## **Use of wild accessions**

The collection and study of wild species assume crucial significance as the discovery and incorporation of alien genes provide an active means to sustain crop improvement, particularly when levels of resistance in the cultigens are low and virulent strains of pests and pathogens overcome host plant resistance. Further, an assessment of the levels and patterns of genetic diversity within and among wild relatives would substantially help in understanding the static and dynamic properties of genetic variation in natural populations and evolutionary processes of domestication and utility of wild gene pools in further plant breeding programmes. Additionally, critical reviews of the state of diversity within various gene pools of a crop would help to provide a more objective basis for determining the most appropriate way to overcome a suspected bottleneck, and in choosing the most suitable approach.

Very little information about morphological and molecular diversity analysis, interspecific hybridization and backcross breeding program is available in pigeonpea.

## **Morphological diversity**

Data on ecogeographic patterns of variability in crop species, including their wild and weedy relatives, are useful for basic studies on crop evolution (Jain and Singh 1972; Harlan, 1975; Simmonds, 1976; Jain, 1977), for planning efficient

germplasm collection expeditions (Moseman and Craddock, 1976; Cristopher *et al.*, 1984) and for selecting parents to use them in plant breeding programmes (Ward, 1962; Munck *et al.*, 1970; Bartual *et al.*, 1985; Dale *et al.*, 1985).

For several crop species, evaluation of entries in germplasm collections has provided large data bases on the plant traits that can be used to study patterns of genetic diversity (Harlan, 1975; Frankel and Hawkes, 1975; Kumar *et al.*, 1984; Polignano and SpagnolettiZeuli, 1985).

Saxena *et al.* (1990) evaluated 33 accessions of *C. scarabaeoides* for variation in some of the useful traits to identify parents for inter-generic hybridization. A large variation was observed for leaf components, seed size, pod length, seeds/pod, days to flowering, seed protein, sulphur containing amino acids, resistance to cyst nematode, *phytophthora* blight, sterility mosaic, *fusarium* wilt, pod borer, pod fly and pod wasp. Only four accessions were found to have more than 28.5% protein content. Methionine and cysteine contents were marginally higher than in cultivated pigeonpea but the variation was not large enough to utilize them in the breeding program. In *C. scarabaeoides* accessions resistant to *Fusarium* wilt, *Phytophthora* blight, sterility mosaic, and cyst nematode were detected. Compared to pigeonpea, the *C. scarabaeoides* accessions were less susceptible to the Lepidopteran borer and were immune to pod fly damage. The accessions ICPW 89, ICPW 111 in short duration – (100-120 days), and ICPW 94 and ICPW 118 in the medium duration (140-180days) were identified as potential parents for use in interspecific hybridization.

One hundred and ninety six genotypes from the local germplasm collections of *Cajanus cajan* were grown in a randomized block design with three replications to study the variation in harvest index (Singh and Srivatsava, 1977). Results indicated that these genotypes did manifest marked variation in harvest index and growth characteristics under different planting systems.

Plant population effects on interrelationship of seed yield and its components on the pigeonpea genotypes were studied (Satpute, 1994). Correlation and path

analysis in the populations based on the genotype-plant density combinations revealed that the dry matter production, number of pods per plant, seeds per plant and harvest index were closely associated with the seed yield. The direct effect of these characters on seed yield was not similar. Over the seasons and plant densities, the number of seeds per plant and dry matter yields were identified to be the important yield characters.

## **Molecular diversity**

Classical methods of estimating genetic diversity and / or relatedness among groups of plants relied upon phenotypic traits. However, these have two disadvantages: firstly the traits were subject to environmental influences and secondly the levels of polymorphism (allelic variation) are limited. These limitations were significantly overcome by development of environment- neutral biochemical makers (Isozymes) and protein electrophoresis (Hunter and Markert, 1957) and molecular markers that focus directly on the variation controlled by genes. The higher resolution of molecular markers makes them a valuable tool for a variety of purposes, such as fingerprinting, facilitates appropriate choice of parents for breeding programs, analysis of quantitative traits, location and detection of quantitative trait loci (QTLs), gene mapping, marker assisted selection and gene transfer, understanding evolutionary pathways, and for the assessment of genetic diversity of plant germplasm. Hillis (1987) recommended that morphological work on large samples combined with molecular analysis on smaller samples maximize both information and usefulness. Kresovich and Mc Pherson (1992) believed that molecular markers could resolve biological, operational and logistical questions dealing with four broad areas of germplasm characterization: the determination of correct identity of an individual (whether it was true to type, duplicate etc.) the estimation of degree of similarity among individuals understanding of hierarchical structure and partitioning of variations among individuals, accessions, populations and species, and identification and detection of the presence of particular alleles in individuals, accessions, populations, chromosomes or cloned DNA segments.



The range of molecular markers (Table 1) that can be relatively easily used on most plant germplasm is quite extensive (Mohan *et al.*, 1997; Gupta and Varshney, 2000). Techniques vary from identifying polymorphism in actual DNA sequence to the use of DNA hybridization methods to identify RFLPs (Restriction Fragments Length Polymorphism), or the use of PCR – based (Polymerase Chain Reaction) technology to find polymorphisms using RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequence Repeat) and AFLP (Amplified Fragment Length Polymorphism). The different methods differ in their cost, ease of application, type of data generated (dominant or Co- dominant markers), the degree of polymorphism they reveal, the way they resolve genetic differences, and in the taxonomic levels at which they can be most appropriately used (Karp *et al.*, 1997).

The application of different techniques to genetic diversity analysis have been well reviewed (Malyshev and Kartel, 1997; Newbury and Ford – Lloyd, 1997; Westman and Kresovich, 1997; Karp *et al.*, 1998). Assignment of levels and distribution of polymorphism (usually conceptualized as ‘ allelic richness’ and ‘ allelic evenness’) in a crop permit the sampling and utilization of genetic resources in a more systematic and efficient manner, and also allow an enhanced understanding of evolutionary relationships both for breeding and conservation. Some applications of diversity analysis using molecular marker tools including: identification of genetic diversity (Hamrick and Godt, 1990), determining collection priorities and sampling strategies (Schoen and Brown, 1991), guiding the designation of *in situ* or on farm conservation strategies (Bonierbale *et al.*, 1997), monitoring genetic erosion (Robert *et al.*, 1991) or vulnerability (Adams, 1977), guiding the management of *ex situ* collections (Kresovich *et al.*, 1997), maximizing the genetic diversity in core collections (Gepts, 1995), comparing agronomically useful regions of genomes of different crops (Paterson *et al.*, 1995), defining the identity of improved varieties or other plant genetic resources (Lee *et al.*, 1995), monitoring the movement of plant genetic resources ( Hardon *et al.*, 1994) and assisting in taxonomic evolution and enhancing understanding of relationships between crop gene pools (Gepts, 1995), achieving precise, unambiguous and accurate identification of germplasm at species / subspecies levels (Wang and Tanksley, 1989; Virk *et al.*, 1995; Martin *et al.*, 1997;

**Table 1: Molecular marker techniques**

S.No	Acronym	Technique / reference
1	AFLP	Amplified Fragment Length Polymorphism Vos <i>et al</i> , 1995
2	ALP	Amplicon Length Polymorphism Ghareyazie <i>et al</i> , 1995
3	AP-PCR	Arbitrarily Primed PCR Welsh and Mac Clelland, 1990
4	AS-PCR	Allele Specific PCR Sarkar <i>et al</i> , 1990
5	CAPS	Cleaved Amplified Polymorphic Sequence Lyamichev <i>et al</i> , 1993
6	DAF	DNA Amplified Fingerprints Caetano-Anolles <i>et al</i> , 1991
7	IMP	Inter-MITE (Miniature Inverted-Repeat Transposable Elements) Polymorphism, Change <i>et al</i> , 2001
8	ISA = ISSR	Inter – SSR Amplification = Inter Simple Sequence Repeat Zietkiewicz <i>et al</i> , 1994
9	MP – PCR	Microsatellite – Primed PCR Meyer <i>et al</i> , 1993
10	RAMS	Randomly Amplified Microsatellite Ender <i>et al</i> , 1996
11	RAPD	Random – Amplified Polymorphic DNA Williams <i>et al</i> , 1990
12	REMAP	Retrotransposon – Microsatellite Amplified Polymorphism Kalender <i>et al</i> , 1999
13	RFLP	Restricted Fragment Length Polymorphism Botstein <i>et al</i> , 1980
14	SAP	Specific – Amplicon Polymorphism Williams <i>et al</i> , 1991
15	SCAR	Sequence Characterized Amplified Region Williams <i>et al</i> , 1991
16	SNP	Single Nucleotide Polymorphism Nikiforov <i>et al</i> , 1994
17	SSCP	Single Strand Confirmation Polymorphism Orita <i>et al</i> , 1989
18	SSLP	Microsatellite Simple Sequence Length Polymorphism Rongwen <i>et al</i> , 1995
19	SSLP	Minisatellite Simple Sequence Length Polymorphism Jarwan and Wells, 1989
20	SSR	Simple Sequence Repeat Hearne <i>et al</i> , 1992
21	STMS	Sequence Tagged Micro Satellite sites Bakemann and Soller, 1990
22	STS	Sequence Tagged Sites Fukuoka <i>et al</i> , 1994

Zhu *et al.*, 1998), identifying duplicates within collections particularly in gene banks (Virk *et al.*, 1995). The genetic variability of 38 grape fruits (*Citrus paradisi*) and three pummelos (*C. maxima*) accessions was evaluated using RAPDs and SSRs analyses. Approximately 49% of the 198 RAPD were polymorphic and 4.6 alleles per SSR loci were identified (Corazza-Nunes, 2002).

The AFLP system (Vos *et al.*, 1995) has been shown to be effective and reproducible for analysis of genetic linkage and gene mapping (MacKill *et al.*, 1996; Voorrips *et al.*, 1997), map based cloning (Cnops *et al.*, 1996), plant evolution (Huem *et al.*, 1997). Biodiversity technology has been applied to wheat in localized situations (Parker *et al.*, 1998; Barrett and Kidwell, 1998; Burkhamer *et al.*, 1998; Law *et al.*, 1998; Ma and Lapitan, 1998; Hartl *et al.*, 1999; Bai *et al.*, 1999; Bhon *et al.*, 1999; Singh *et al.*, 1999). No studies have been reported in pigeonpea for diversity analysis using AFLP markers. RFLP analysis, using genomic single copy probes, has been generally used to characterize the variation among wild and cultivated species (Miller and Tanskley 1990, Jena and Kochert 1991; Gawel *et al.*, 1992; Jarret *et al.*, 1992). Variation in chloroplast DNA (Close *et al.*, 1989) and mitochondrial DNA (Deu *et al.*, 1995; Moeykens *et al.*, 1995; Tozuka *et al.*, 1998) has been used to study the diversity of cytoplasm in crop species like soyabean, an out-crossing species like pigeonpea. Compared to chloroplast genome, the mitochondrial genome has many variations within or between closely related species (Close *et al.*, 1989; Grabau *et al.*, 1992, Deu *et al.*, 1995.). Diversity in 28 accessions of pigeonpea and its wild relatives representing 11 species belonging to six sections of genus *Cajanus*, and four species of genus *Rynchosia* was assessed by analyzing the mt DNA hybridization patterns (Sivaramakrishnan *et al.*, 1999). Highly polymorphic hybridization banding patterns with maize mt DNA probes in RFLP were observed both in the wild and cultivated accessions. Cluster analysis of southern blot hybridization data with 15 restriction enzyme – probe combinations placed various species of pigeonpea into ten major groups and revealed phylogenetic relationship among these groups. This study suggests that RFLP of mt DNA can be used for the diversity analysis of pigeonpea. The variations in mitochondrial DNA hybridization patterns also suggest the extensive rearrangement of the organelle

genome among the *Cajanus* species (Sivaramakrishnan *et al.*, 1999). Twenty four accessions representing 12 species of four genera (*Cajanus*, *Dunbaria*, *Eriosema* and *Rhynchosia*) were examined to determine phylogenetic relationships in the genus *Cajanus* sufficient RFLP polymorphisms were detected among species to resolve in – group taxa into distinct clusters. Topologies of trees from parsimony and similarity matrix analyses were similar but not identical, and clustering patterns agreed broadly with published phylogenies based on seed protein data and to a lesser extent, data from cytology and breeding experiments (Nadimpalli and Jarret, 1993). The cultivated pigeonpea lines were studied for variation at genomic level, using SSR primers but there are no reports of diversity analysis in wild pigeonpea accessions (Malcolm, 2001).

## **Biochemical diversity**

### **Proteinase inhibitors**

Plants use proteins as a part of their defense strategies. An interesting class of defense protein is the inhibitors of digestive enzymes that occur in many plants. The two main classes of inhibitors discovered so far are the protease inhibitors and the amylase inhibitors. Among them, protease inhibitors play an important role in defense of plants against herbivorous insects. They act as competitive inhibitors of enzymes by binding tightly to the active site of the enzyme. The antimetabolic activity of the protease inhibitors is due to direct inhibition of the larval proteolysis and utilization of proteins leading to the death of larvae by slow starvation.

Proteinase inhibitors are widely distributed in the plant kingdom, particularly in seeds and tubers, where they often represent several percent of total protein (Liener and Kakade, 1969; Ryan, 1973; Richardson, 1977). They have been most extensively studied in leguminosae, gramineae and solanaceae, presumably because of the large number of species in these families, (Richardson, 1977). According to specificity, proteinase inhibitors can be divided into four classes, inhibiting serine, cysteine, metallo- or aspartyl proteases. Several non-homologous families of protease inhibitors are recognized among the animal, microorganism, and plant

kingdoms ( Laskowski *et al.*, 1980). In plants about ten protease inhibitor families have been recognized (Garcia *et al.*, 1987). Members of the serine and cysteine proteinase inhibitors have been more relevant to the area of plant defense than metallo- and aspartyl proteinase inhibitors, since only a few of these latter two families of inhibitors have been found in plants.

## **Trypsin inhibitors**

Soyabean trypsin inhibitor (Kunitz inhibitor) was the first plant inhibitor to be well characterized. Its isolation and crystallization from soyabean and that of its complex with trypsin is one of the classic achievements of the inhibitor chemistry (Kunitz, 1947). It has a molecular weight of 20,000 to 25,000 with relatively a few disulphide bonds and posses a specificity, which is directed primarily towards trypsin. Trypsin (Mw 23,300) is the main intestinal digestive enzyme responsible for the hydrolysis of food proteins. Due to the ability of this inhibitor to inhibit trypsin from the insect gut, it has received much attention as a target for the control of insect pests. Steffens *et al.*,(1978) reported that when trypsin inhibitor and the weak inhibitor of trypsin from corn were fed to larvae of European corn borer larvae at 2-5% of diets, SBTI inhibited growth of larvae and delayed the pupation, whereas the corn inhibitors have no effect on the growth or metamorphosis of the larvae. Soyabean trypsin inhibitor retarded larval growth of *Maruca sexta*, when added to the artificial diet at 5% level (Shukle *et al.*, 1983). Broadway and Duffey (1986) tested the effect of purified SBTI and potato inhibitor II ( an inhibitor of both trypsin and chymotrypsin) on the growth and digestive physiology of larvae of *Helicoverpa zea*. Soyabean trypsin inhibitor significantly affected the growth and digestive physiology of *H. armigera*, when 0.84% (dry weight) incorporated into the artificial diet, SBTI significantly reduced the high alkaline trypsin-like enzyme activity by 18% (Wang *et al.*, 1996). Potato proteinase inhibitor I was most effective in reducing growth rate, followed by Soyabean trypsin inhibitor (Marwick *et al.*, 1995).

## Lectins

Many plant species contain carbohydrate binding proteins, which are commonly called as lectins or agglutinins. Lectins bind reversibly to the specific mono- or oligo saccharides. The first description of a lectin, the Ricin, a toxic ferment contained a miniature of toxic ricin molecules and non- toxic agglutinins, in seeds of *Ricinus communis* and in some other euphorbiaceae species. For the first time the term 'Blutkorperchenagglutinin' (hemagglutinin) was introduced as a common name for all the plant proteins that cause clumping of cells (Elfstrand, 1898). The idea that toxicity is an intrinsic property of lectins was abandoned in the beginning of the century and reported the presence of nontoxic lectins in the legumes, *Phaseolus vulgaris*, *Pisum sativum*, *Lens culinaris* and *Vicia sativa* (Lanmdstener and Raubitschek, 1907). Later more non-toxic plant haemagglutinins were discovered. The lectins are now defined as all plant proteins possessing atleast one non-catalytic domain, which binds reversibly to a specific mono- or oligosaccharide (Peumans and Van Damme, 1995). Lectins are found abundantly in many plant tissues in storage organs and protective structures of some of the plants. They have been isolated from various plant tissues, the seeds being the richest source (Etzler, 1986). This is especially true among the members of leguminosae family (Strosberg *et al.*, 1986).

Effect of protein on the normal development of insects was first reported that phytohemagglutinin (PHA), in the black bean, *Phaseolus vulgaris*, produced deleterious effects on the larvae of bruchid beetle, *Callosobruchus maculatus* (Janzen *et al.*, 1976).

Murdock *et al.* (1990) screened seventeen plant lectins and found that at dietary levels of 0.2 and 0.1% (w/w) of orange (*Maclura pomifera*), peanut (*Arachis hypogea*), potato (*Solanum tuberosum*), jimson weed (*Datura stramonium*) and wheat germ (*Triticum aestivum*) delayed the developmental time of *C. maculatus*.

Each of the three germ isolectins showed similar effect against cowpea weevil. N-acetylglucosamine binding lectins from *Oryza sativa* and *Urtica dioica*

also showed increased mortality and increased development time when fed to the cowpea weevil (Huesing *et al.*, 1991b). Gatehouse *et al.* (1992) showed that lectins from *Allium sativum* and *Galanthus nivalis* (snowdrop) affected the survival of cowpea weevil larvae. Larvae reared on artificial seeds containing 2% lectin suffered 90% mortality compared to the control larvae. Larval mortality was more than 50% at 1% level of lectin. The bioassays with soyabean lectin inhibited larval growth of *M. sexta* incorporated into an artificial diet at 1% level (Shukle and Mudrock, 1983).

## **Biology, nature of damage and management of *H. armigera***

*H. armigera* is a pest of world wide agricultural importance. It feeds on a wide range of wild and cultivated host plants. The larvae, particularly the later instars, feed on the reproductive parts of the plant. In India, it is a dominant pest on cotton, pigeonpea, and chickpea. On pigeonpea and chickpea, it commonly destroys more than half of the grain yield. The biological characteristics such as high fecundity, extensive polyphagy, strong flying ability and facultative diapause contribute to the devastating pest status of *H. armigera* (Fitt, 1989). The ability to feed on various plants enables *H. armigera* populations to develop continuously during the entire cropping season (Bhatnagar *et al.*, 1982).

The biology of *H. armigera* is typical of noctuidae. Morphology of various life stages has been described by Pearson (1958), Jayraj (1982) and Zalucki *et al.*, (1986). Distinguishing features have been described by Dominguez Garcia- Tejero (1957), Hardwick (1965), King (1994) and Mathews (1999). The legume pod borer females lay eggs singly, on the upper surface of the leaves along the midrib, flowers, pods and stems. The number of eggs per female ranges from 387 to 1364 on different host plants (Dhandapani and Subramaniam, 1980). The eggs are white and nearly spherical when freshly laid, and darken with age. Eggs hatch in 2-5 days. Larval duration varies from 8 to 28 days (Singh and Singh, 1975), and there are 5 to 7 larval instars, which vary with temperature and the host plant. Pupation takes place in soil, and the adults emerge in 7 to 10 days. One generation can be completed in just over 4 weeks under favorable conditions. The number of generations vary according to

agroclimatic conditions. It passes through four generations in Punjab (Singh and Singh, 1975), seven to eight generations in Andhra Pradesh (Bhatnagar, 1980), and five generations in Uttar Pradesh (Tripathi, 1985).

The lepidopteran borers viz., *Helicoverpa armigera*, *Exelastis atomosa*, *Maruca testualis* and dipteran pod fly, *Melanagromyza obtusa* have been reported as the most damaging at the reproductive phase of the plants (Singh and Singh, 1978). Insecticides have been an important component of the management strategy for pigeonpea pests, especially borers.

The young larvae of *H. armigera* feed by scraping green tissues and wander about nibbling various parts of the plant until they find a flower bud or flower, when a bud is hollowed out, leaving an empty shell. In pigeonpea and chickpea, the older larvae chew voraciously into the buds, flowers, and pods, leaving characteristic round holes. In cotton, older larvae feed on the buds and young bolls and habitually feed with only the front portion of its body inside the hole thus commonly showing an accumulation of larval faces between the surface and the enclosing bracteoles.

The estimated crop losses due to *H. armigera* vary in different countries viz., US \$ 600 million in chickpea and pigeonpea per annum in semi-Arid tropics (ICRISAT, 1992); A\$16 million in 1979 (Alcock and Twine, 1981), A\$23.5 million (Wilson, 1982), and A\$25 million annually in Australia (Twine, 1989). In India, crop losses in pulses, chickpea and pigeonpea were estimated at over \$300 million per annum (Reed and Pawar, 1982).

*H. armigera* is a pest of major importance in most areas, damaging a wide variety of food, fiber, oilseed, fodder and horticultural crops. Its major pest status is rooted in its mobility, polyphagy, high reproductive rate, and diapause, all of which make it well adapted to exploit transient habitats such as man-made agro – ecosystems. The natural control means, chemical or the integrated control methods need to be adopted to minimize the losses due to this pest. The key issue of moth immigration, and movement in general, is of general relevance to the long-term effectiveness of any control strategy aimed to suppress more than one generation. It



may have little value if crop infestation is mainly by the immigrant moths of distant origin.

Integrated pest management strategies for *Helicoverpa* require integration of different control tactics to implement a threshold based on the relationship between population density and economic loss. It is often difficult to obtain precise data on relationship because many extraneous factors, both environmental and socio-economic, influence it. Tactics that have been evaluated against *Helicoverpa* include cultural manipulation of crop and its environment, biological control including the use of microbial pesticides, sex pheromones for population monitoring or mating disruption, sterile backcross techniques, chemical control and host plant resistance.

## **Evaluation for podborer resistance**

Several workers have reported serious lepidopteran borers damage on determinate clustering and early and medium maturing pigeonpea cultivars (Lateef and Reed, 1980; Reddy *et al.*, 1983; Yadava *et al.*, 1988). Mali and Patil (1993) in field screening of some pigeonpea varieties against Pod borers, reported minimum percentage of damage due to *H. armigera* on variety T-21 (8.98), Sehore – 68 (12.07%) and maximum damage on variety, ICPL-87 (32.77%).

## **Mechanisms of resistance**

The mechanism of resistance needs to be understood for any genetic enhancement programme. An empirical approach was proposed by Painter (1936,1941 and 1951). Painter's proposed mechanism of resistance was grouped into three main categories 1) Non-preference is avoidance of insect by plants and is often projected as a property of the plant. For this reason Kogan and Ontman (1978) proposed to substitute antixenosis for the term 'non-preference'. It is a parallel term to 'antibiosis' and conveys the idea that the plant is avoided as a bad host. 2) Antibiosis includes all adverse effects exerted by the plant on the insect's biology including development, reproduction and survival. 3) Tolerance includes all plant

responses resulting in the ability to withstand infection and to support insect populations that would severely damage susceptible plants. Plant physical characters (Southwood, 1986) are prime factors to be considered for host plant resistance. Biochemical (Isoprenoids, acetogenins, aromatic derived from shikmic acid and acetate, alkaloids, protease inhibitors and nonprotein aminoacids and glycosides) and morphological basis of resistance (thickening of cell walls, rapid proliferation of plant tissues, toughness of stem, trichomes effect on feeding and digestion, on oviposition, as a mechanical barrier to locomotion, attachment, association with allelochemical factors, incrustation of minerals in cuticles, surface waxes and anatomical adaptations of organs) were reported .

## **Antibiosis**

Antibiosis, one of the three types of mechanisms of resistance proposed by Painter (1951) is described as those adverse effects on the insect life history when a resistant host plant variety is used as food. The adverse effect on the insect can be in the form of reduced fecundity, decreased size, abnormal shortened life and/or increased mortality. Antibiotic effect (Dodia *et al.*, 1996) of *C. scarabaeoides*, *C. cajanifolius*, *C. reticulatus*, *C. sericeus* and F<sub>1</sub> hybrids of cross, (*C. scarabaeoides* x *C. cajan*), and the cultivated pigeonpea lines were observed on *H. armigera*. The results of the study clearly showed that the larval and pupal mass fed on wild pigeonpea flowers and F<sub>1</sub> hybrid was significantly lower than those larvae fed on the cultivated pigeonpeas. The developmental period of larvae fed on wild pigeonpea flowers was longer than those fed on the cultivated pigeonpea flowers. Similarly, pupal size of larvae fed on the F<sub>1</sub>s and the wild species was significantly reduced compared to the cultivated pigeonpeas. Growth index and larval fecundity were adversely affected for larvae reared on the wild species and F<sub>1</sub>s. The studies on the hybrid progenies of a *C. cajan* x *C. scarabaeoides* cross by Verulkar *et al.* (1997) suggested that the antibiosis mechanism of resistance was governed by single dominant gene.

## Trichomes

Trichomes are unicellular or pluricellular outgrowths from the epidermis of leaves, shoots and roots (Uphof, 1962). The collective trichome cover of a plant surface is called pubescence. Several authors have attempted to classify the variety of plant trichomes (Uphof, 1962 and Hummel and Staesche, 1962). Structure, color, growth habit pubescence of plants and chemical composition of plants confer resistance against insects. Levin (1973) discussed the ecological functions of trichomes as defense against herbivores.

Plant hairs were reported to be associated with resistance against insects in at least 19 plant genera (Webster, 1975). Poos and Smith (1931) reported that potato leafhopper (*Empoasca fabae*) heavily infested and seriously injured the glabrous soybeans, whereas the rough hairy varieties were relatively free from leafhoppers. Genung and Green (1962) reported that growing soyabean varieties, with dense hairiness of foliage manifested non-preference to female leafhoppers for oviposition.

Afzal and Abbas (1943) and Parnell *et al.* (1949) reported a very close relationship between hairiness of cotton and resistance to jassids (*Empoasca* spp.). Without exception, all distinctly hairy types have been found highly resistant and all the non-hairy types fully susceptible. Intermediate degrees of hairiness were associated with intermediate degrees of resistance (Parnell *et al.*, 1949). They found such a relationship between varieties of *G. hirsutum* and *G. barbadense* species and also in the segregating progenies of the hybrids between the two species, the lack of hairiness in the early stages of the growth was associated with the lack of resistance. Hairiness and resistance to jassids develop concurrently. Length of hairs was shown to be of prime importance and densities without adequate length were ineffective.

Two pairs of genes,  $H_1$  and  $H_2$ , appear to play a role in the genetic control of pubescence of leaves in cotton, *G. hirsutum*,  $H_1$  seems to induce lengthy hair and density, and is incompletely dominant to  $h_1$ . The  $H_2$  allele seems to induce hairiness but to a small degree. It acts additively to  $H_1$ , giving profusely hairy plants. Effects of pubescence on pink bollworm (*P. gossypiella*) damage were studied in strains of

cotton (*G. hirsutum*). Damage was lower in TM 1, a densely pubescent strain carrying the pilose allele  $H_2$ , than in most other strains tested, but the levels of pubescence below that of TM 1 did not ensure resistance (Wilson and Wilson, 1977).

Pods of mustard (*Brassica hirta*) with stiff hairs showed no significant flea beetle (*Phyllotreta crucifera*) damage, while adjacent plots of rapeseed showed heavy pod damage. Removal of hairs from mustard pods caused an increase in feeding damage by the flea beetle (Lamb, 1980).

The damage caused by bollworm (*H. zea*) to the glanded and glandless version of 12 diverse lines in cotton was compared (Oliver *et al.*, 1970). The data on oviposition and damage to squares and bolls by larvae showed significant differences between two versions. Glabrous cottons were reported to suppress the population of cotton bollworm and tobacco bud worm (*H. virescens*) by reducing the total number of eggs deposited by the adults (Lukefahr *et al.*, 1971, Shaver and Lukefahr, 1971 and Robinson *et al.*, 1980). However, the glabrous condition causes greater susceptibility to thrips (Lee, 1971).

In tomatoes, a correlation between resistance to spider mites and concentration of glandular hairs on the leaves was observed (Stoner *et al.*, 1968). Glandular hairs on leaves and stems stuck onto naturally occurring aphids in field plants of *Solanum polyadnium* and had fewest free aphids (Gibson, 1976 a). Glandular hairs of this species of potato also provide a form of resistance to larvae of Colorado beetle, *Leptinotarsa decemlineata* (Gibson 1976b).

Wheat leaf pubescence was reported to confer resistance to cereal leaf beetle, *Oulema melanopus* (Gallun *et al.*, 1966) and to Hessian fly, *M. destructor*, (Robberts *et al.*, 1979). Significant correlation between larval weight of cereal leaf beetle and pubescence density in common wheat, *Triticum aestivum* was observed (Ringlund and Everson, 1968). The resistance to oviposition was due to greater hair density and hair length in wheat (Webster *et al.*, 1975). Three dominant genes governed pubescence density in *Triticum turgidum*. The genes might be operating in additive manner to determine the length of pubescence (Liesle, 1974). Starks and Merkle (1977) noted

that the dense and long hairs did not necessarily result in resistance to green bugs (*Schizaphis graminum*) in wheat.

In cultivated tobacco, three major types of trichomes were recognized; glandular, simple and hydathodes. Differences for trichome traits among *Nicotiana* species and genotypes of *N. tabaccum* have long been known (Barrera *et al.*, 1966; Delon, 1979). Johnson (1988) reported that analyses of the F<sub>2</sub> data for presence and absence of the glandular trichomes indicated that some of the genotypes in tobacco had alleles at two loci and some others alleles at three loci. Genes at these loci may affect glandular trichome density and presence or absence of Glandular trichomes.

The discovery of erect glandular trichomes, in various *Medicago* species, condition a high level of resistance to several key alfalfa insect pests (Shade, 1979; Thompson, 1975; Johnson, 1980a,) has created an interest in the utilization of this character to produce alfalfa cultivars with multiple insect resistance. The genetic mechanism governing the expression of glandular hair character appears complex and polygenic. Observations on interspecific crosses have indicated that expressivity of this character is much greater in some crosses than in others. A study was conducted to determine the magnitudes of genetic and non genetic variance components, an interspecific cross between erect glandular haired, tetraploid population of alfalfa, involving *Medicago sativa* and *M. prostrata* with adequate density of hairs for resistance to several insect pests (Kitch, 1985). Parent offspring regression analysis gave an estimate of 0.55 for the narrow sense heritability. The phenotypic variance was partitioned into additive (29%), non-additive (16%), general environmental (29%), and specific environmental (26%) variances were calculated.

The combining ability and heritability of pubescence and its relationship with resistance to potato leaf hopper, *Empoasca faba* , was investigated in the progeny of fixed set of clones in two alfalfa (*Medicago sativa*) populations (Elden, 1986). General and specific combining ability effects were highly significant in all crosses. Heritability estimates were non-significant for stem and very low (0.28,  $P < 0.05$ ) for

leaf Pubescence. Significant negative correlation for all crosses and clones were found between stem pubescence, and potato leaf hoppers feeding damage and nymphal populations.

In a study, two groups of near-isogenic strains of soybean, *Glycine max*, with different types of pubescence,) it is reported that glabrous strains were damaged more by the Potato leaf hopper than pubescent strains (Broersma *et al.*, 1972). The orientation of hairs was more important for resistance to leaf hopper than the density of hairs. Campell *et al.* (1976) working with a large number of peanuts, demonstrated that potato leaf hopper resistance was associated with high percentage of long and straight trichomes extending outward at a 45 angle. Susceptible lines had appressed trichomes. The major plant factor affecting potato leaf hopper nymphal population, on two cultivars of snap beans, *Phaseolus vulgaris* , was density of hooked trichomes (Pillemer and Tingey, 1978). A negative correlation between trichome density and insect pest abundance and / or damage has been observed in a number of crops including cowpea for *Maruca testulalis* (Oghiakhe *et al.*, 1992) and strawberry for *Othiorhynchus sulcatus* by (Doss *et al.*, 1987). Chickpea trichomes and exudates have a negative impact on growth, development time and survival and of *Helicoverpa armigera* larvae (Shrivastava and Shrivastava, 1990).

Presence of trichomes play an important role in resistance against insects. Van der Maesen (1986) reported glandular and non-glandular trichomes on vegetative and reproductive plant parts of *Cajanus* spp. Bisen and Sheldrake (1981) reported three types of trichomes in *C.cajan* viz., simple nonglandular, yellow bag like and tubular glandular trichomes. Shanower *et al.*, (1996) reported the presence of five types of trichomes on pods of *C.cajan* and they play a major role in protection against *H.armigera*.

## **Interspecific hybridization**

Inter-varietal or intra specific crosses are preferred because the hybrids possess maximum fitness value. That is, such hybrids are viable and fertile, hence favored under both nature and domestication. In contrast, wide crosses or more precisely inter-specific and inter-generic hybrids suffer either from non-viability or

sterility or from both. As a consequence, the forces of natural selection promptly eliminate such distant hybrids of wide crosses. In crop improvement programmes the parents used in the hybridization are generally different varieties of the same species. But, in many cases, it may be desirable or even necessary, to crosses individuals belonging to two different species or genera. In certain crops, plant breeders in the 20<sup>th</sup> century have increasingly used interspecific hybridization for transfer of genes from a non-cultivated plant species to a crop variety in a related species.

The first recorded interspecific hybrid was in 1717 between carnation and sweet William by Thomas Fairchild (Allard, 1960). The first man-made cereal, "Triticale" was an outcome of intergeneric hybridization. Extensive studies on distant hybridization have been made in crops like wheat (Sears, 1972; Sears, 1975) barley (Bothmer and Hagberg, 1983), maize (Mangelsdorf, 1974; Harlan and De wet, 1977), *Solanum* (Swaminathan and Magoon, 1961; Motskaitis and Vinitkus, 1975), cotton (Blank *et al.*, 1972; Meyer, 1973; Meyer, 1974), *Nicotiana* (Mann *et al.*, 1963; Smith, 1968; Berbec, 1974), tomato (Rick, 1982), and rice (Nayar, 1973).

Distant hybridization studies have also been carried out in several important legumes such as, *Phaseolus*, *Vigna*, *Vicia*, *Pisum*, *Arachis*. Species of genus *Phaseolus* have been a subject of wide interest. The possibility of gene exchange between species had led to several studies on interspecific hybridization, especially between *P. vulgaris* and *P. coccineus* (Mendel, 1866; Tschermak-Seysenegg, 1942; Lamprecht, 1948; Rudrof, 1953; Kedar and Bemis, 1960; Thomas, 1964; Al-yasiri and Coyne, 1966; Rutger and Beckman, 1970; Smartt, 1970; Marechal, 1971; Haq *et al.*, 1980; Savova, 1981; Shii *et al.*, 1982; Conti, 1983). Hybridization with other *Phaseolus* species including the wild forms has received wide attention (Honma, 1956; Coyne, 1964; Smartt, 1970; Braak and Koistra, 1975; Le Merchand *et al.*, 1976; Tan Boun Suy, 1979; Hwang, 1979). Interspecific hybridization in *phaseolus* in recent years has been widely used in the improvement of *phaseolus* species with respect to disease resistance, insect resistance, nitrogen fixation and several agronomic characters (Lapinskas, 1980; Alvarez, 1981; Bannerot *et al.*, 1981; Zapata *et al.*, 1982; Hunter *et al.*, 1982). Wide hybridization has also received a fair degree

of attention in the genus *Glycine*. Studies on hybridization between *G.max* and *G.soja* have been extensive (Karasawa, 1936; Ting, 1946; Williams, 1948; Tang and Chen, 1959; Tang and Tai, 1962; Ahmed *et al.*, 1977 and 1979; Kiazuma *et al.*, 1980).

Interspecific hybridisation in *cajanus* dates back to 1956 when Deodikar and Thakur (1956) made the first cross between *C. cajan* with *C. lineatus*. The hybrid was fairly fertile. Kumar *et al.* (1958) extended the earlier work on to hybrid cytology and found regular bivalent formation in the hybrid. A hybrid between *C. cajan* and *C. scarabeoides* was obtained by Roy and De (1967) and expressed the doubts about the generic status of *Cajanus*. Reddy (1973) analysed pachytene chromosome pairing in *Cajanus cajan*, *C. lineata*, *C. scarabaeoides* and *C. sericeus* and their hybrids. These pachytene studies in general revealed a high degree of chromosome homology between *C. cajan* and the three species of Wild *Cajanus*. Ariyanayagam and Spence (1978) reported hybrids between *C. cajan* and *C. platycarpus* while further attempts (Reddy *et al.*, 1980; Pundir, 1981) to cross *Cajanus cajan* with *C. platycarpa* failed. Further attempts in *C. cajan* and wild relatives hybridization by Pundir (1981) involved karyotype comparisons between cultivated and wild species and meiotic pairing in the F<sub>1</sub> hybrids. These studies revealed a great degree of karyotypic similarities between species. All the studies on *Cajanus* - wild hybridization revealed a close relationship between the species and regular pairing in their hybrids which nevertheless exhibited a fair degree of sterility (Table 2).

Most of the interspecific hybridization work was done at ICRISAT and was mostly confined to breeding high protein lines (Reddy *et al.*, 1979) and to a limited extent for breeding for insect resistance, dwarfs and isolation of cytoplasmic male steriles involving a few wild accessions of *Cajanus*. Genome relationships between wild and cultivated *Cajanus* species are still obscure. *C. cajanifolius*, which is morphologically very similar to *Cajanus* except for the seed strophiole, was identified as early as 1920 (Van der Maesen, 1980) but an attempt to cross these two species was made by Pundir (1981).



**Table 2 : Interspecific hybridization in *Cajanus***

<i>Cajanus</i> species	Author(s)
<i>C. lineatus</i>	Deodikar and Thakur (1956)
<i>C. lineatus</i>	Kumar <i>et al.</i> (1958)
<i>C. scarabaeoides</i>	Roy and De (1967)
<i>C. lineatus</i> , <i>C. scarabaeoides</i> and <i>C. sericeus</i>	Reddy <i>et al.</i> (1973)
<i>C. platycarpus</i>	Ariyanayagam and Spence (1978)
<i>C. albicans</i> , <i>C. sericeus</i> , <i>C. lineatus</i> , <i>C. scarabaeoides</i> , <i>C. trivervea</i> and <i>C. cajanifolius</i>	Pundir (1981)
<i>C. reticulatus</i> (sub-sp. <i>Reticulata</i> ), <i>C. pluriflorus</i> and <i>C. acutifolius</i>	Dundas (1984)
<i>C. sericeus</i>	Singh <i>et al.</i> (2000)

Distant hybridization is mostly aimed at introducing new genetic variability or to achieve a new genomic constitution in such a way that the characters of the parental species are recombined effectively. These possibilities are directly related to the degree of genetic relatedness between the parents. It has been found that, the closer the genome relationship between the cultivated and the wild species the greater the amount of genetic recombination, and consequently variability.

Assessment of genome relationships is a first step in the exploitation of wild species in the improvement of any cultivated species. The next step is utilization of such hybrids in the breeding programme before which it would be essential to study the inheritance pattern and also assess the quantum of variability generated. Studies on inheritance provide information on the possible number of genes governing a character and their interaction. Evaluation of variation in the F<sub>2</sub> generation helps in

understanding the extent of recombination and variability. Genetic studies provide a clear direction to handling of segregating generations. A few studies have been made on the genetics of qualitative and quantitative traits in Pigeonpea (Deshpande and Jeswani, 1956; D'Cruz and Deokar, 1970; Munoz and Abrams, 1971; Pandey, 1972; Sharma *et al.*, 1972; Joshi, 1973; Choudhary and Thombre, 1977; Dahiya and Brar, 1977; Dahiya *et al.*, 1977; Kapur, 1977; Malhotra and Sodhi, 1977; Reddy *et al.*, 1979).

Though, today pigeonpea is an unsophisticated tall and is beset with number of problems it serves the farmer well, and is not unsuited to the modern agriculture. Since 1925, the scientific attention has been paid to improvement of this crop mainly in India, where emphasis on improvement is now centered at Indian Council of Agricultural Research, the International Crops Research Institute for Semi-arid Tropics, and in various agricultural Universities.

## **Inheritance of qualitative traits in pigeonpea**

Deshpande and Jeswani (1952) and Deokar and D'Cruz (1971) reported that the prostrate growth habit was recessive to normal erect type, and controlled by a single gene. However, Patil and D'Cruz (1965) and Shinde *et al.* (1971) observed the  $F_2$  ratio of 13 normal: 3 creeping types. Deokar *et al.* (1971) observed that the growth habit was controlled by three genes  $Cgr_a$ ,  $Cgr_b$  and  $Cgr_c$  giving a ratio of 45 erect: 9 creeping: 10 prostrate in the  $F_2$  generation.

A number of genetic studies have been reported on plant height and branching habit (erect, compact, spreading). Sen *et al.* (1966) identified bushy dwarf pigeonpea phenotypes where the dwarfness was controlled by a recessive gene, *d*. Waldia and Singh (1987b) reported that dwarf phenotype in the Do dwarf line was governed by two non – allelic recessive genes  $t_1$  and  $t_2$ . Saxena *et al.* (1989a) studied inheritance of three dwarfs D6, PD1, and PBNA and reported that the dwarfing trait in each line was controlled by a single recessive gene. Shaw (1931) observed dominance of erect growth habit over spreading type. D' Cruz and Deokar (1970), reported that a single dominant gene, *Sbr* controlled spreading habit, and the erect

types were homozygous recessive. According to De Menezes (1956) branching angle is quantitatively inherited. D'Cruz *et al.*, (1971) observed that the branching habit was governed by three duplicate complementary factors  $Sbr_{a2}$ ,  $Sbr_{b2}$  and  $Sbr_{c2}$ , giving an  $F_2$  ratio of 54 spreading: 10 erect types.

In general, the trifoliate leaflet of pigeonpea is lanceolate, but some morphological variations in the leaflet shape have been reported. The first report of inheritance of leaflet shape in pigeonpea was published by Pandey *et al.* (1954). They referred to both obovate and round shaped leaflets and reported a  $F_2$  ratio of 3 lanceolate: 1 round leaflets. The monogenic inheritance of lanceolate leaflet shape was also confirmed by Patil and D'Cruz (1967), Deokar *et al.*, (1971), D'Cruz and Deokar (1970), and D'Cruz *et al.*, (1971). Deshpande and Jeswani (1956) observed segregation ratio of 3:1 for lanceolate and 15:1 for obcordate leaflets in the  $F_2$  generation of two different crosses. D'Cruz *et al.* (1971) reported a ratio of 117 oblong or oval (round) leaflets with obtuse apices: 75 lanceolate leaflets with acute apices: 64 obcordate leaflets with retuse apices in the  $F_2$  population of a cross, involving obcordate and round leaflet types. D'Cruz *et al.* (1973) reported monogenic inheritance in round x obcordate leaflet types, and assigned the gene symbols *lltr* and *llt*. They concluded that three allelic genes *Llt*, *lltr*, and *llt* are involved in the inheritance of lanceolate, round, and obcordate leaflet shapes.

The predominate stem color in pigeonpea germplasm of Indian origin is green; while in African germplasm predominant color is purple. In certain cases, unstable purple stem pigmentation due to the exposure of stems to direct sunlight is observed. Purple stem color was found dominant to green and was found to be controlled by a single factor, *Pst* (D'Cruz and Deokar, 1970; D'Cruz *et al.* 1971; D'Cruz *et al.* 1974). However, in a cross between cultivars, N. Black x Purple grained, Deokar and D'Cruz (1972) reported an  $F_2$  ratio of 45 purple: 19 green, and suggested that three genes *Psta*, *Pstb*, and *Pstc*. These simply inherited contrasting stem colors i.e., have been used as markers to detect the extent of natural out-crossing in pigeonpea (Bhatia *et al.*, 1983).

Some *Cajanus* species are characterized by the presence of a prominent strophiole on the seed surface. Reddy (1973) reported 9:7 F<sub>2</sub> ratio, from a cross between *C. cajan* and *Atylosia* species, suggesting that the involvement of two genes with complementary gene action. Reddy *et al.* (1981a), Kumar *et al.*, (1985) reported that in *C. scarabaeoides*, *C. sericeus* and *C. albicans* the presence of strophiole was controlled by two genes (NS and SDI) with inhibitory action. But Pundir and Singh (1985) reported that seeds with strophioles in *Cajanus* spp. are due to the presence of two genes (*s*<sub>1</sub> and *s*<sub>2</sub>) with duplicate gene action. Singh *et al.* (2000) reported that the strophioled character is dominant over the non- strophioled character and is governed by a single gene.

Pundir and Singh (1985) studied inheritance of seed colour in *C. scarabaeoides* and *C. cajanifolius* in crosses with orange seeded pigeonpea lines. They reported that a single partially dominant gene, *Osc*, governed the dark seed color of *Cajanus* spp. Reddy *et al.* (1981a) and Kumar *et al.*, (1985) found that seed mottling, was controlled by two complementary genes, *Msd<sub>a</sub>* and *Msd<sub>b</sub>*.

Pods of *C. scarabaeoides* have dense hairs on their surface. Reddy (1973) and Pundir and Singh (1985) reported that a single dominant gene, designated as *Hp*, governed this trait. Singh *et al.* (2000) reported F<sub>2</sub> segregation of 3 hairy: 1 non-hairy in crosses of *C. cajan* with four accessions of *C. sericeus*, suggesting that the character is expressed by a single gene *Ph* with complete dominance of hairiness over non-hairiness of pods. However, Reddy *et al.* (1980) observed two phenotypic ratios, 3:1 and 13: 3 in crosses between *C. scarabaeoides* and two different accessions of pigeonpea.

The obovate leaflet shape in *C. scarabaeoides* and *C. albicans* was found to be controlled by a single partially dominant gene (Reddy, 1973, Kumar *et al.* 1985; Pundir and Singh, 1985). Pundir and Singh (1985) designed the gene symbols *L*<sub>1</sub> and *L*<sub>2</sub> However, in a cross between *C. cajan* and *C. lineatus*, Reddy (1973) reported dominance of lanceolate over ovate leaflet shape. Singh *et al.* (2000) observed that in the F<sub>1</sub> generation in all the plants of all four crosses of *C. cajan* with *C. sericeus*, the

leaflet shape was observed to be intermediate to both the parents suggesting the incomplete dominance. In the  $F_2$  generation the individuals segregated in a ratio of 1 (oblong ovate): 2 (intermediate): 1 (lanceolate) in all the crosses.

Kumar *et al.* (1985) and Pundir and Singh (1985) reported the twining growth habit of *C. scarabaeoides* and *C. albicans*, controlled by two genes with epistatic gene action resulting in a ratio of 13 non- twining : 3 twining The erect growth habit of pigeonpea was dominant to the spreading growth habit of *C. scarabaeoides*. The plants were intermediate between erect and spreading habit and in  $F_2$  generation they observed a ratio of 1 erect: 1 spreading: 14 intermediate, suggesting that two genes ( $Eg_1$  and  $Eg_2$ ) with partial dominance were responsible for the growth habit. Pundir and Singh (1986) studied inheritance for pod length and ovule number in six interspecific  $F_2$  populations. The interspecific crosses of *C. lineatus* and *C. scarabaeoides* showed transgressive segregation for pod length, However, in the interspecific crosses involving Pigeonpea a restricted segregation was observed which was attributed to a negative gene interaction in the two species.

Singh *et al.* (2000) reported that in crosses of *C. cajan* with *C. sericeus* the  $F_1$  generation of all the crosses the individuals had the seeds of intermediate shape with respect to the parents. In  $F_2$  generation of these crosses the plants segregated in a simple Mendelian ratio of 1 (flat): 2 (intermediate): 1 (round) suggesting that the seed shape was controlled by a single gene *Ss* with incomplete dominance.

## Gene action in quantitative traits

Besides estimates of genetic parameters, inbreeding depression in pigeonpea, beyond the  $F_2$  generation, indicates that dominance is not an important genetic variance component for yield in this crop. Knowledge of plant characteristics is essential for planning an effective breeding programme. This is useful in selection of individuals with adaptation to different agro- ecological zones. Measurement of genetic variability and understanding of inheritance of characters is of prime importance in pigeonpea to formulate a sound crop improvement program.

Sharma *et al.* (1972) reported predominance of additive gene action for the seed size from a 10 – parent diallel study. However, the genes controlling smaller seed size were found to be dominant over the genes controlling the larger seeds. Gupta *et al.* (1981) confirmed additive gene action and reported that only two or three genes governed the seed size.

For days to flower Gupta *et al.*, (1981) reported the predominance of additive gene effects, while Pandey (1972), Sharma *et al.*, (1973b), and Dahiya and Satija (1978) observed additive gene action with partial dominance for earliness.

Plant height was studied in a nine-parent diallel by Sharma (1981). He reported the importance of both additive and dominance gene effects. Genes controlling tall stature were dominant over genes controlling short stature. Only a very few studies have been conducted so far on the genetics of wild X cultivated crosses in Pigeonpea (Reddy *et al.* 1980 ; Pundir, 1981).

Time of flowering plays an important role when growing season is restricted by climatic factors like drought and high temperature. Duration of flowering period is a major yield determinate in the indeterminate growth habit of certain pigeonpea genotypes. Sharma *et al.*, (1973a), Dahiya and Brar (1977), Dahiya and Satija (1978), Gupta *et al.* (1981), Reddy *et al.* (1981b) reported additive gene action for days to flower, and non – additive gene action was reported by Reddy *et al.*, (1981b). Both additive and non-additive gene action for days to flower was reported to be by Sidhu and Sandhu (1981), Saxena *et al.* (1981b) and Chaudhari *et al.* (1980).

Pandey (1972) and Sharma *et al.* (1972) reported additive gene action for days to maturity While Kapur (1977) and Sidhu and Sandhu (1981) reported both additive and non-additive gene action.

Sharma *et al.* (1973a) and Sharma (1981) reported additive gene action for plant height, whereas Pandey (1972) and Reddy *et al.* (1979) reported non-additive gene action for days to maturity. Kapur (1977), Sidhu and Sandhu (1981), Saxena *et al.* (1981b) and Reddy *et al.* (1981b) reported additive and non-additive gene action.

Chaudhari *et al.* (1980) reported additive gene action for number of primary and secondary branches. Pandey (1972), Saxena *et al.* (1981b) and Mohamed *et al.* (1985) reported the additive gene action for number of seeds per pod while Venkateshwarulu and Singh (1982) and Kapur (1977) reported both additive and non-additive gene action.

Pandey (1972), Sharma *et al.* (1973a), Chaudhari *et al.*, (1980) and Saxena *et al.* (1981b) reported additive gene action for grain yield. Dahiya and Brar (1977), Sidhu and Sandhu (1981) and Laxman Singh and Pandey (1974) reported the non-additive gene action for yield and yield components. Reddy *et al.* (1981b), Venkateshwarulu and Singh (1982) and Sidhu and Sandhu (1981) reported of both additive and non-additive gene action.

Pandey (1972) reported additive gene action for protein content. Sharma *et al.* (1973b) and Sharma *et al.* (1974) reported the non-additive gene action for the seed protein content. Sharma *et al.* (1974) reported the predominance of both additive and non-additive gene action.

## **Heritability**

Heritability is the ratio of genetic variance to the phenotypic variance (Singh, 1977) expressed in percent. It is a good index of transmission of characters from parents to offspring (Falconer, 1989). The knowledge of heritability helps in predicting the behavior of succeeding generations and making desirable selections. It depends on the variability present in the material and the environmental effects it. Heritability estimates provide guidelines on the efficiency of selection as they refer to the proportion of phenotypic variance that is due to genetic variance. A high heritability estimate suggests that the character can be easily selected in the test environment.

The estimate can also be used to calculate the genetic advance under a given selection intensity, and hence helps in determining the population size necessary to exercise selections. However, the heritability estimate is valid for a given population, and the environment in which it was obtained. Therefore, it is difficult to generalize heritability estimates from one population to another (Dudley and Moll, 1966). In pigeonpea, a number of reports on heritability estimate for various quantitative traits have been published. For the sake of convenience, the estimate has been grouped as high (>75%), medium (50-75%) and low (<50%).

Munoz and Abrams (1971), Khan and Rachie (1972), Rubaihayo and Onim (1975), Dahiya and Brar (1977) and Sidhu and Sandhu (1981) reported medium heritability for days to flower and high heritability values were reported by Munoz and Abrams (1971), Khan and Rachie (1972), Pandey (1972), Sharma *et al.* (1973b) and Sidhu and Sandhu (1981) reported low heritability values for days to maturity and medium heritability was reported by Dahiya and Satija (1978). Kumar and Reddy (1982) Sidhu *et al.* (1985) reported high heritability.

Munoz and Abrams (1971), Khan and Rachie (1972), Sidhu and Sandhu (1981) and Sharma (1981) reported low heritability for plant height. Medium heritability was reported by Pandey (1972), Kumar and Reddy (1982) and Sidhu *et al.* (1985). High heritability values were reported by Munoz and Abrams (1971), Khan and Rachie (1972), Sharma *et al.* (1973a), Rubaihayo and Onim (1975) and Sheriff and veeraswamy (1977).

Kumar and Reddy (1982) reported medium heritability for pod bearing length and number of secondary branches. Kumar and Reddy (1982) reported low heritability values for number of primary branches.

Sharma *et al.* (1973a) and Sidhu and Sandhu (1981) reported low heritability values for number of seeds per pod. Kumar and Reddy (1982) reported medium heritability. Munoz and Abrams (1971), Khan and Rachie (1972), Sharma *et al.* (1973a), Sharma *et al.* (1973b) and Rubaihayo and Onim (1975) reported low heritability values for grain yield. Medium heritability values were reported by



Munoz and Abrams (1971), Pandey (1972), Malhotra and Sodhi (1977) and Sidhu *et al.*, (1985). Khan and Rachie (1972) and Sharif and Veeraswamy (1977) reported high heritability values.

Sharma *et al.* (1973b), Sharma *et al.* (1974), Rubaihayo and Onim (1975) and Dahiya *et al.* (1977) reported the low heritability values for protein content. Pandey (1972) and Dahiya *et al.* (1977) reported medium heritability.

## **Heterosis**

Heterosis is the superiority in the performance of hybrid over both the parents. Commercial exploitation of heterosis in crop plants is regarded as a major breakthrough in the realm of plant breeding. It has lead to considerable yield improvement of several cereals and other crops (Rai, 1979).

Solomon *et al.* (1957) were the first to report the hybrid vigour in pigeonpea in ten inter-varietal crosses. In some crosses they observed hybrid vigour over the better parent upto a maximum of 24.5 % for grain yield together with; plant height, plant spread, stem girth, number of fruiting branches and leaf length and width. Singh, *et al.* (1983) reported upto 22.1% mid parent heterosis in the cross Mukta (medium – duration) x UPAS – 120 (short –duration). Evaluation of medium and short duration pigeonpea hybrids in multi- locational trials has shown 20 to 49% heterosis over the well adapted, control cultivar (Saxena *et al.* 1986b).

Generally a high level of hybrid vigour is observed among crosses involving parents with diverse phonologies. Hybrids involving different species of *Cajanus* manifest very high vigour for vegetative growth

## **Backcross breeding**

In the Backcross, the hybrid and the progenies in the subsequent generations are repeatedly backcrossed to one of their parents. As a result, the genotype of backcross progeny becomes increasingly similar to that of the parent to which the backcrosses are made. At the end of 6-8 backcrosses, the progeny would be almost

identical with the parent used for backcrossing. The objective of the backcross method is to improve one or two specific defects of a high yielding variety, which is well adapted to the area and has other desirable characteristics. The characters lacking in this variety are transferred to it from a donor parent without changing the genotype of this variety, except for the genes being transferred. Thus the end result of a backcross programme will well-adapted variety with one or two improved characters.

Back cross method has been used to transfer simply inherited characters, mostly insect and disease resistance, from related species into a cultivated species. For example, transfer of resistance into wild fire and black fire from *Nicotiana longiflora* to *N. tobaccum* a leaf and stem rust resistance from *Triticum timopheevii*, *T. monococcum*, *Aegilops speltoides* and rye (*S. cereale*) to *T. aestivum*, of black-arm resistance from several *Gossypium* species to *G. hirsutum* etc. Interspecific transfer of genes is easy when the chromosomes of the two species pair regularly. But often chromosomes of the concerned species are differentiated by structural changes that reduce pairing between also transferred along with the desirable gene. Another difficulty in interspecific gene transfers is that the transferred gene may not be able to function in the same way in the genetic environment of the new species.

Wild relatives of wheat are a rich source of new genes for resistance to various wheat pathogens (Sharma and Gill, 1983; Gale and Miller, 1987; Jauhar, 1993; Jaing *et al.*, 1994; Friebe *et al.*, 1996; Harjit-Singh *et al.*, 1998). A number of new genes for resistance to various wheat diseases included the three rust viz., leaf rust (*Puccinia econdita* f. sp. Tritici), stripe rust (*P. striiformis*) and stem rust (*P. graminis* tritici), have been transferred from closely and distantly related wild species (McIntosh, 1998). A number of genes for disease resistance transferred from wild relatives of wheat Viz. Lr9, Yr9, Pm8, Lr26 etc. have been overcome by the emergence of virulent pathotypes of pathogens when deployed in wheat cultivars.

# MATERIALS AND METHODS

## MATERIALS AND METHODS

The present investigation was undertaken mainly to study the following:

1. Diversity analysis among the accessions of *Cajanus scarabaeoides*
2. The genetic basis podborer resistance and other qualitative and quantitative characters; and incorporation of podborer resistance gene(s) through wide hybridisation.

### Diversity analysis

In the present investigation, 30 accessions of *C. scarabaeoides*, a wild relative of pigeonpea, and six cultivated varieties of *C. cajan* (Table 3) were used. Wild accessions were selected from the world germplasm collection available at Rajendra S. Paroda GeneBank, at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT). Morphological, molecular and biochemical diversity analysis, among the accessions, for various characters, including podborer resistance, was done. Experiments were conducted in 2000 and 2001 Kharif seasons at ICRISAT, situated at an altitude of 545m above the mean sea level, 17°N latitude, and 78° E longitudes.

### Morphological diversity analysis

Thirty accessions, belonging to *C. scarabaeoides* and six cultivars of *C. cajan* were morphologically characterised (Table 3). Plants were grown in deep black vertisols. The experiment was laid in Randomized Complete Block Design (RCBD), with three replications. Seeds of each accession were sown on a 4 m long ridge with an inter- plant spacing of 10 cm and an inter-row spacing of 60 cm. Normal agronomic practices were followed to raise the crop. The crop was occasionally irrigated and minimal quantities of insecticides were sprayed to reduce the crop damage to capture the maximum diversity. Observations on different qualitative and quantitative characters (Table 4) were recorded on five randomly selected plants from each accession following the morphological and taxonomical descriptors (ICRISAT, 1993) during the 2000 and 2001 Kharif seasons. Mean of five observations on five plants was used for statistical data analysis.

**Table 3: Diversity analysis of wild and cultivated pigeons**

S.No.	ICP Number	ICPW Number	Species name	Origin of accessions/country
1	ICP 15683	ICPW 82	<i>C scarabaeoides</i>	Maharashtra, India
2	ICP 15684	ICPW 83	<i>C scarabaeoides</i>	Maharashtra, India
3	ICP 15687	ICPW 86	<i>C scarabaeoides</i>	Karnataka, India
4	ICP 15691	ICPW 90**	<i>C scarabaeoides</i>	Himachal Pradesh, India,
5	ICP 15695	ICPW 94	<i>C scarabaeoides</i>	Sri Lanka, India
6	ICP 15696	ICPW 95	<i>C scarabaeoides</i>	Burma
7	ICP 15697	ICPW 96	<i>C scarabaeoides</i>	Uttar Pradesh, India
8	ICP 15699	ICPW 98	<i>C scarabaeoides</i>	Uttar Pradesh, India
9	ICP 15702	ICPW 101	<i>C scarabaeoides</i>	West Bengal India,
10	ICP 15712	ICPW 111	<i>C scarabaeoides</i>	Maharashtra, India,
11	ICP 15716	ICPW 115	<i>C scarabaeoides</i>	Assam India,
12	ICP 15717	ICPW 116	<i>C scarabaeoides</i>	Sikkim India,
13	ICP 15720	ICPW 119	<i>C scarabaeoides</i>	Nueva Vizcaya, Philippines
14	ICP 15723	ICPW 122	<i>C scarabaeoides</i>	Tamilnadu India
15	ICP 15726	ICPW 125	<i>C scarabaeoides</i>	Tamilnadu India
16	ICP 15731	ICPW 130	<i>C scarabaeoides</i>	Andhra Pradesh, India
17	ICP 15733	ICPW 132	<i>C scarabaeoides</i>	Orissa, India
18	ICP 15738	ICPW 137	<i>C scarabaeoides</i>	Orissa, India
19	ICP 15739	ICPW 138	<i>C scarabaeoides</i>	Orissa, India
20	ICP 15742	ICPW 141	<i>C scarabaeoides</i>	Australia
21	ICP 15748	ICPW 147	<i>C scarabaeoides</i>	Madhya Pradesh, India
22	ICP 15753	ICPW 152**	<i>C scarabaeoides</i>	Betutu-Rote Island, Indonesia
23	ICP 15879	ICPW 278	<i>C scarabaeoides</i>	Flores Island, Indonesia
24	ICP 15881	ICPW 280	<i>C scarabaeoides</i>	Flores Island, Indonesia
25	ICP 15882	ICPW 281	<i>C scarabaeoides</i>	West Tripura, India
26	ICP 15903	ICPW 302	<i>C scarabaeoides</i>	Anuradhapura, Sri Lanka
27	ICP 15906	ICPW 305	<i>C scarabaeoides</i>	, Polonnaruwa, Sri Lanka
28	ICP 15909	ICPW 308	<i>C scarabaeoides</i>	Pelennaruwa, Sri Lanka
29	ICP 15911	ICPW 310	<i>C scarabaeoides</i>	Anuradhapura, Sri Lanka
30	ICP 15916	ICPW 315	<i>C scarabaeoides</i>	Candy, Sri Lanka,
31	ICP 15743	ICPW 142 *	<i>C scarabaeoides</i>	Australia
32	ICP 15744	ICPW 143 *	<i>C scarabaeoides</i>	Australia
33	ICP 15745	ICPW 144 *	<i>C scarabaeoides</i>	Australia
34	ICP 15760	ICPW 159 *	<i>C sericeus</i>	India
35	ICP 15761	ICPW 160 *	<i>C sericeus</i>	India
36	ICP 15762	ICPW 161 *	<i>C sericeus</i>	India
37	ICP 15763	ICPW 162 *	<i>C sericeus</i>	Australia
38	ICP 15675	ICPW 74 *	<i>C reticulatus</i>	Australia
39	ICP 26		<i>C cajan</i>	India
40	ICP 28		<i>C cajan</i>	India
41	ICP 8518		<i>C cajan</i>	India
42	ICP 8863		<i>C cajan</i>	India
43	ICP 14722		<i>C cajan</i>	India
44	ICP 14770		<i>C cajan</i>	India

\*\* = Not included in molecular characterization

\* = included only in molecular characterization

**Table 4 : Description of qualitative and quantitative characters in pigeonpea**

Character	Description
<b>Qualitative characters</b>	
Plant habit	Erect, semi-spreading or spreading
Stem color	Green, purple or mixed
Leaflet shape	Lanceolate or obovate
Pod color	Purple or green
Seed strophiole	Present or absent
Seed mottles	Present or absent
Pod hairiness	Pubescent or glabrous
<b>Quantitative characters</b>	
No. of days to flower	No. of days from sowing to 50% flowering.
No. of days to maturity	No. of days from sowing to maturity.
Leaflet length (cm)	Length from leaf tip to the petiole.
Leaf width (cm)	Width of leaf in middle region.
Pod length (cm)	Length of fully grown pod.
Pod width (cm)	Width of fully-grown pod.
Pod bearing length (cm)	Distance between the bottom most and top most pods on plant.
No. of locules / pod	No. of locules / pod.
No. of seeds / pod	No. of seeds / pod.
No. of primary branches	No. of branches borne directly on main stem at the time of maturity.
No. of secondary branches	No. of branches arising from primary branches at the time of maturity.
100 – seed weight (g)	(Seed yield / plant (g) / Total No. of seeds / plant) X 100
Harvest index	Ratio of seed yield (g) to total dry weight of the plant (g)
Seed protein (%)	Total seed protein as estimated by Technicon Auto Analyzer (TAA)
Trichome density (No./mm <sup>2</sup> )	Measured using Ocular microgrid

### **Estimation of seed protein - (Technicon Auto Analyzer method)**

The protein content of seeds was estimated in the Crop Quality Service Laboratory at ICRISAT by adopting the following procedure. The seeds of each plant were ground in Udy cyclone grinding mill and passed through 0.4 mm mesh to obtain flour. Sixty mg of flour was transferred to a Technicon digestion tube (75 ml) and 3 ml of acid mixture, consisting of 5 parts (v/v) of orthophosphoric acid in 100 parts of sulphuric acid and one kjel tab containing 1.5g K<sub>2</sub>SO<sub>4</sub> and 7.5 mg selenium, were added to each tube. Each set of 40 tubes, consisting of 36 unknown samples under study, 2 standard checks and 2 blanks were included to estimate the protein<sup>4</sup>

content. The set was heated to 36°C for 1 h 15 min for digestion in a block digester. The digest was cooled and dissolved in 75 ml of water and was mixed thoroughly. Aliquots of sample was transferred into a Technicon sample cup for analysis using TAA. Protein content was estimated by multiplying the total nitrogen content, obtained by TAA, with a factor 6.25.

## Statistical analysis

Data on quantitative traits was subjected to preliminary statistical analysis. Data was analyzed for RCBD using REML (Residual Maximum Likelihood) analysis using random effect model on GENSTAT 6.0, considering season as fixed and genotype as random. The components of variance, due to wild and cultivated individually and together, and their interactions with environment, were estimated for all traits to know whether the genotypes differed or interacted with environment as a group or not. Genotype x environment analysis, considering all the genotypes as one group, was done and the components of phenotypic variance ( $\delta^2 p$ ), due to genotypic variance ( $\delta^2 g$ ), genotype x environment variance ( $\delta^2 ge$ ), and residual ( $\delta^2 e$ ) and their standard errors were calculated. Heritability (broad sense) was estimated from the phenotypic and genotypic components of variance using the formula:

$$\text{Heritability (h}^2\text{)} = \text{genotypic variance } (\delta^2 g) / \text{phenotypic variance } (\delta^2 p)$$

where  $\delta^2 p = \delta^2 g + \delta^2 ge / n_e + \delta^2 e / (n_r \times n_e)$  ; where  $n_e$  is the number of environments / seasons and  $n_r$  is the number of replications. Assuming asymptotic normality, the ratio of the variance component estimate to its standard error was compared to standard normal deviate, at 5 and 1 percent levels of significance to test the significance of variance component estimates. Data on qualitative characters was summarized for the wild and cultivated genotypes. Data on quantitative characters was standardized.

The mean observations for each trait were standardized by subtracting the mean value of the character and subsequently dividing by its respective standard deviation for each observation. This resulted in the standardised values for each trait with an average of 0 and standard deviation of 1. The standardised values were used to perform the cluster analysis. Phenotypic relationships, among accessions were

assessed using Euclidean distance (Sneath and Sokal, 1973). Statistical analysis was done using NTSYS - Pc version 2.11 (Rohlf, 1992). The resulting phenotypic distance matrix was subjected to non-metric Multi-Dimensional Scaling (MDS) to graphically visualize any evidence of clustering among the accessions in the two-dimensional Euclidean space. The inter-relationships among accessions in MDS plot was confirmed by subjecting the distance matrix to sequential agglomerative hierarchical non-overlapping (SHAN) cluster analyses using the average linkage unweighed pair group arithmetic mean (UPGMA) clustering algorithm. Co-phenetic correlation coefficients were estimated to assess the degree of agreement between the observed similarity matrix and their resultant dendrogram and MDS plots.

### **Molecular diversity analysis**

For molecular characterization, four accessions of *C.sericeus* and one accessions of *C. reticulatus* were also included in the study along with thirty one accessions of *C. scarabaeoides* and six varieties of pigeonpea and thus in all, 42 genotypes were included (Table 3) Molecular marker diversity was assessed among the wild and cultivated pigeonpeas using AFLP (Amplified Fragment Length Polymorphism) with 5 primer combinations, RFLP (Restriction Fragment Length Polymorphism) with 9 maize mitochondrial probe – enzyme combinations and 10 SSR (Simple Sequence Repeats) primer pairs.

### **Isolation and purification of genomic DNA**

In the present study, CTAB procedure (Murray and Thompson, 1980) was adopted for isolation of genomic DNA with a few modifications Seeds were grown in the green house in 20cm diameter pots with sterilized potting mixture (Alfisol: sand; 2:1).

About 3 - 5 g of tender leaves were collected and ground to a fine powder with liquid nitrogen using a pre-cooled mortar and pestle. About 70 mg of PVP (Polyvinyl pyrrolidine) was added during the process to avoid phenol formation. The powder, without being allowed to thaw, was transferred to 50 ml polypropylene tubes containing 15 ml of CTAB extraction buffer (100 mM Tris-HCl pH 8.0, 50 mM EDTA pH 8.0, 100 mM NaCl and 2% SDS), mixed gently by inversion, and



incubated for 90 min at 65°C in a water bath. An equal volume (15 ml) of chloroform-isoamyl alcohol (24:1) was added to the tubes containing sample and buffer. They were mixed by gentle inversion for 5 min and centrifuged at 8000 rpm for 10 min at 20°C in RC-5 Sorval centrifuge. The top aqueous phase was transferred to fresh 50 ml polypropylene tubes. Chloroform- isoamyl (24:1 v/v) alcohol extraction was repeated, and later, an equal volume of chilled isopropanol was added to the clear supernatant. The solution was mixed gently by inversion and kept at room temperature for 1h. The DNA was then spooled out with a bent pasteur pipette and suspended into 15 ml falcon tubes containing 70 % ethanol, washed twice with 5 ml of 70% ethanol and air dried. Four ml of T<sub>50</sub>E<sub>10</sub> (50 mM Tris-HCl pH 8.0 and 10 mM EDTA pH 8.0) buffer was added and DNA was allowed to dissolve. Subsequently, 80µl of RNase (10 mg/ ml) was added and incubated overnight at 37°C.

For purification of extracted DNA, an equal volume (4 ml) of chloroform-phenol (1:1) was added, mixed gently by inversion, and centrifuged at 5000 rpm for 10 min. The clear supernatant was transferred to a fresh tube and the previous step was repeated. An equal volume (4 ml) of chilled isopropanol and 200 µl of sodium acetate was added to the supernatant, mixed gently by inversion and DNA was allowed to precipitate. The DNA was hooked into 1.5 ml eppendorfs containing 1 ml of 70 % ethanol. The eppendorfs were centrifuged at 10,000 rpm for 5min at 4°C, ethanol was decanted and the DNA was air-dried for 30min. Depending upon the pellet, 80-300 µl of T<sub>10</sub>E<sub>1</sub> (10 mM Tris-HCl and 1 mM EDTA pH 8.0), was added and the tubes were stored at 4°C for further use.

### **Qualitative and quantitative estimation of DNA**

To test the quality of DNA, the OD values were recorded at 260 and 280nm and the ratio of OD<sub>260</sub> to OD<sub>280</sub> was calculated to check the purity of each DNA sample. Pure DNA preparations show the values of ratio OD<sub>260</sub> to OD<sub>280</sub> between 1.7 and 1.8 (Maniatis *et al.*, 1982). Further, to test the quality of DNA, samples were also subjected to gel electrophoresis, using 0.8% TAE-agarose gel as described by Maniatis *et al.* (1982). Gels were stained with ethidium bromide and viewed on a UV-transilluminator, photographed with a camera fitted with UV filter and checked

for RNA contamination. DNA quality was assessed by comparing with different concentrations of undigested lambda DNA sample. DNA was quantified based on the spectrophotometer measurements of UV absorption at 260 nm, assuming 1 OD at 260 nm is equal to 50 ng of DNA (Maniatis *et al.*, 1982).

### **Molecular diversity analysis using AFLP**

AFLP analysis of the wild and cultivated pigeonpeas (Table 3) was carried out using the commercial kit (Life Technologies, USA) following the manufacturer's protocols with slight modifications. Five AFLP primer combinations viz., E - ACG M-CTT, E- ACG M-CAT, E- ACG M CTA, E-ACT M-CTC and E- AGG M-CAC, were used for the analysis. DNA samples were diluted to 80 ng/ $\mu$ l.

The AFLP technique involved five major steps: (a) restriction digestion of DNA by restriction endonucleases, (b) ligation of fragments by adapters, (c) PCR pre-amplification of the restriction fragments, (d) selective amplification of pre-amplified products, and e) gel analysis of the amplified fragments.

#### ***Restriction digestion of genomic DNA***

About 250 ng of genomic DNA was digested with 1.5 units each of EcoRI and Mse I, 2.5 $\mu$ l of 10X reaction buffer was added and made upto a final volume of 12.5 $\mu$ l with distilled water in 1.5 ml micro-centrifuge tubes. The contents were mixed gently by centrifugation and incubated at 37°C for 2 h. The mixture was further incubated at 70°C for 15 min to inactivate the restriction endonucleases. The tube was then placed on ice and the contents were collected after brief centrifugation.

#### ***Ligation of digested DNA***

To 5 ml of digested DNA, 4  $\mu$ l of adapter ligation solution and 1 $\mu$ l of T<sub>4</sub> DNA ligase were added, mixed gently by brief centrifugation, and incubated at 20°C for 2 h.

### ***Pre-amplification of restricted DNA fragments***

Ten folds diluted the ligated sample. To 2µl of ligated diluted DNA sample, which was used as the template in a PCR reaction, 16µl of pre-amplification primer mix, 2µl of 10 x PCR buffer, 1 unit (0.2µl) of Taq DNA polymerase (Amersham, Pharmacia, U.K), were added along with distilled water to make up the volume to 20µl. The contents were mixed gently and samples were pre-amplified in a Perkin-Elmer 9600 Thermocycler with the following conditions: 30 cycles were performed at 94 °C for 30 sec followed by 30 cycles at 56 °C for 60 sec and finally for 30 cycles at 72 °C for 60 sec. The pre-amplified samples were diluted by 50 times with TE buffer.

### ***Selective amplification of pre-amplified products***

In a 50µl reaction, 5 µl of 10 X buffer, 16µl of [ $\gamma^{32}$ P] dATP (3,000 Ci/mmol) and 2µl of T<sub>4</sub> PNK were added to 18µl of the selected EcoRI primer (E-ACG or E-ACT or E-AGG), mixed gently by brief centrifugation and incubated at 37 °C for 1 h. The enzyme was heat inactivated at 70 °C for 10 min. AFLP ladder was labelled by mixing 2µl of 30-300bp unlabelled AFLP ladder, 1µl of 5 x exchange buffer, 1µl of [ $\gamma^{32}$ P] dATP (3,000 Ci/mmol) with 1µl of T<sub>4</sub> PNK in a total reaction mixture of 5µl. The reaction mixture was incubated at 37 °C for 30 min. Reaction was terminated by incubating the mix for 15 min at 70 °C. To this, 5 µl of T<sub>10</sub>E<sub>1</sub> and 25 µl of loading buffer (19% formamide, 10 mM EDTA, 0.1% bromophenol blue and 0.1 % xylene cyanolene cyanol) were added and stored at -20°C for further use.

For each primer, selective amplification was performed by adding 2.5µl of the diluted pre-amplified DNA, 0.25µl of the labelled EcoRI primer, 2.25µl of Mse I primer containing dNTPs, 4µl of sterile distilled water, 1µl of 10X PCR buffer and 1 unit of Taq DNA polymerase. The PCR conditions for selective amplification of DNA were as follows: one cycle was performed at 94 °C for 30sec, 65 °C for 30 sec, and 72 °C for 60 sec; during the next 12 cycles the annealing temperature was progressively lowered by 0.7 °C; and 23 cycles were performed at 94 °C for 30 sec, 56 °C for 30 sec and 72 °C for 60 sec.

### ***Gel electrophoresis***

After PCR, an equal volume (10 $\mu$ l) of formamide dye (19% formamide, 10 mM EDTA, 0.1% bromophenol blue and 0.1 % xylene cyanol) was added to each reaction. The samples were heated for 3 min at 95 °C and placed on ice immediately. The fragments were separated using model S2 sequencing unit (GIBCO BRL). Six percent polyacrylamide was poured (20:1: acrylamide : bis; 7.5 M urea; 1 X TBE buffer) into gel plates with 0.4 mm spacers and shark-tooth combs. The gel was pre-electrophoresed at 1500 V for 20 min. A sample of 3 $\mu$ l was loaded on the gel and electrophoresed at 1700 V until xylene cyanol reached two-thirds down the length of the gel. The gel was dried using a Bio-Rad gel drier. The gel was transferred to Whatman 3 filter paper, covered with Saran wrap and dried under vacuum for 1 h at 80 °C. Autoradiograms were obtained by exposing the gel for varying periods in a cassette with intensifying screen using Kodak-X-OMAT film. Fragment sizes were determined using end labelled AFLP marker (30-300 bp; Life technologies, USA). The autoradiogram was manually scored for the presence and absence of bands for each locus for all the accessions. The dried gel was exposed to X-ray film at room temperature overnight and developed.

### **RFLP analysis using maize mitochondrial DNA probes**

RFLP analysis of wild and cultivated pigeonpeas (Table - 3) was carried out to study the diversity among them. The RFLP technique involved five major steps, a) restriction endonuclease digestion of DNA, b) separation of DNA fragments by gel electrophoresis, c) transfer of DNA fragments to a nylon membrane, d) hybridisation of DNA fragments using radioactively labelled probe, and e) autoradiography analysis of results.

### ***Restriction enzyme digestion***

Genomic DNA (15 $\mu$ g) of each accession was separately digested with EcoRI, EcoRV and Hind III restriction endonucleases following the supplier's instructions (Amersham Pharmacia Biotech, Ltd.). The digestion was carried out in a total volume of 30  $\mu$ l and incubated overnight at 37°C. The reaction was terminated by addition of 3  $\mu$ l of loading buffer (25% sucrose, 0.1% bromophenol blue and 20 mM

EDTA) to each 30  $\mu$ l sample. Digestion was confirmed by running the samples on 0.8% agarose gel in TAE buffer and viewing on a UV trans - illuminator after staining with ethidium bromide.

### ***Gel electrophoresis***

Fragments of digested DNA were separated by electrophoresis in 0.8% TAE-agarose in a horizontal slab gel (Bio-Rad DNA Sub Cell<sup>TM</sup>) electrophoretic unit (Owl Separation Systems Model No.A-1) for 16 h at 38 V  $\text{cm}^{-1}$  in TAE buffer (0.04 M Tris-acetate, 0.001 M EDTA, pH 7.8). Gels were prepared in the same buffer that was used for electrophoresis. Hind III digested lambda DNA was used as molecular size markers with fragment sizes of 23.1 kb, 9.4 kb, 6.6 kb, 4.4 kb, 2.3 kb and 2.0 kb. Gels were stained in 0.5  $\mu\text{g ml}^{-1}$  of ethidium bromide for 15 min, destained for 30 min in distilled water, viewed on a UV trans-illuminator and photographed to assess the digestion quality.

### ***Preparation of southern blots***

The electrophoretically separated DNA fragments were transferred from agarose gel to a Hybond - N+ nylon membrane (Amersham Pharmacia Biotech, Ltd.) following the procedure of Southern (1975). The gel and membrane were placed on a sponge which was partially dipped in an alkali solution of 0.4 M NaOH to serve as a denaturing agent and vehicle for a capillary transfer of DNA fragments. As the alkali solution passes through the gel, DNA fragments are carried out of the gel and bound to the nylon membrane. The membranes with DNA fragments were soaked in 2x SSC for 2 min to neutralize the alkali, air dried and cross linked using Strtagene UV cross linker (Stratagene, Germany), wrapped with cling film and stored at -20°C for future use.

### ***Hybridisation of DNA fragments using labelled probes***

Maize clones, containing known mitochondrial (mt) DNA genes, were obtained for use as probes in Southern blot hybridizations. The atp 6 clone (F1F0 ATPase subunit 6, Dewey *et al.*, 1985), as purified plasmid DNA with corresponding inserts, was supplied by C.S Leveings III, Department of Genetics, North Carolina

State University, Raleigh, NC, USA. Clones, *cox I* (Cytochrome Oxidase Subunit 1; Issac *et al.*, 1985), and *atp  $\alpha$*  (Issac *et al.*, 1985) were provided by C.J.Leaver, Department of Plant Science, University of Oxford, Oxford, U.K.

The random-primed method of Feinberg and Vogelstein (1983) was used for labelling DNA with  $\alpha$ - $^{32}\text{P}$ . A purified insert DNA sample of 4  $\mu\text{l}$  was denatured by heating at 95°C for 10 min, then quenched on ice for 5 min and labelled using  $\alpha$ - $^{32}\text{P}$  – deoxyadenosine 5 triphosphate (dATP), supplied by the New England Labs, labelling kit. The probe was labelled in 50 $\mu\text{l}$  reaction mixture containing about 25–50ng of denatured probe DNA, 1 x labelling buffer, 2 ml equimolar concentrations of dCTP, dGTP and dTTP and 1.5 units of klenow enzyme. The reaction mixture was incubated at 37 °C for 1 h. The reaction was again terminated by adding 400 $\mu\text{l}$  of 200mM EDTA. The labelled probe was again denatured by heating at 95°C for 10 min. Lambda *Hind III* marker was also labelled similarly and added to the reaction mixture prior to hybridization.

Southern blots were pre-hybridized, overnight at 65°C, with 30 ml of pre-hybridization solution (7% SDS, 1% BSA, 0.5 M  $\text{Na}_2\text{HPO}_4$  and 20 mg/ ml sheared and denatured salmon sperm DNA) per two blots (20 x 15 cm) in standard hybridization bottles (30 x 3.5 cm) for 6 h in case of new blots and 1 h for stripped blots in a Techne Hybridizer (HB-1D). While placing the blots in the bottle, care was taken to remove all air bubbles trapped between the blots and the sides of the bottle.

Hybridization was carried out by adding labelled probe to the pre-hybridisation solution and incubating for 16 h at 65°C in hybridization oven (Hybaid, U.K). Care was taken to remove air bubbles present between the blot and the hybridization bottle. Following hybridization, the blots were washed four times with of 50 ml each of  $^{32}\text{P}$ -wash solution for 15 min at 65 °C in hybridization bottles in hybridization oven. The first two washes were done using wash 1 solution (100 ml 20 x SSC, 25 ml 20 % SDS and diluted to a volume of 1 liter with distilled water) followed by two washes with wash 2 solution (10 ml of 20x SSC, 25 ml of 20 % SDS and diluted to a volume of 1 L with distilled water). Blots were dried between sheets of tissue, enclosed in saran wrap in cling films.

## ***Autoradiography***

Autoradiography was conducted at -70 °C by exposing the membrane to photographic film (Kodak, X-OMAT™ and XK-5) using Kodak intensifying screens in a cassette for various exposure times depending on radioactivity counts. The X-ray films were developed with Kodak developer for 2 min followed by treatment for 1 min in 1% acetic acid, fixed with Kodak fixer for 5 min, washed in running tap water and then air dried. The autoradiograms were photographed using Kodak 100 ASA color print films. The fragment sizes were determined using lambda Hind III standard marker.

## **Simple Sequence Repeat (SSR) analysis**

Ten sets of SSR primers (Table - 5), supplied by University of Birmingham, were used for genotyping the wild and cultivated pigeonpeas (Table - 3). The analysis involved two steps: (a) PCR amplification and site specific annealing of genomic segments flanked by repeats and (b) gel electrophoresis.

**Table 5: SSR primer sets in genotyping of wild and cultivated pigeonpeas**

S No	Locus	Composition of repeats (SSRs)	Denaturing temp	Fragment size in ICPL 86012	No. of alleles in diverse set in <i>C. cajan</i>	Size range of alleles in diverse set
1	CCB1	(CA) <sub>10</sub>	55°C	198bp	3	196-204 bp
2	CCB2	(CA) <sub>21</sub>	50°C	163bp	3	160-166 bp
3	CCB3	(CA) <sub>11</sub>	55°C	222bp	4	220-231 bp
4	CCB4	(CA) <sub>31</sub> Imperfect	50°C	226bp	3	220-245 bp
5	CCB5	(CT) <sub>22</sub>	55°C	201bp	5	190-215 bp
6	CCB6	(CA) <sub>6</sub>	55°C	205bp	2	202-208 bp
7	CCB7	(CT) <sub>16</sub>	55°C	155bp	3	150-158 bp
8	CCB8	(CT) <sub>30</sub>	50°C	139bp	3	138-148 bp
9	CCB9	(CT) <sub>22</sub>	50°C	178bp	4	155-180 bp
10	CCB10	(CA) <sub>15</sub> Imperfect	55°C	242bp	3	244-250 bp

### ***PCR amplification***

PCR reactions were carried out in a PTC-100 Thermocycler (MJ Research Inc, USA.). Each 25µl reaction contained 25 ng of genomic DNA, 1 X PCR buffer (50mM KCl, 20mM Tris-HCl pH 8.4), 10 pmol of each primer, 2mM MgCl<sub>2</sub>, 200 µM each of dCTP, dGTP, dTTP, dATP and 1µCi of [ $\alpha$ -<sup>32</sup>P]-dATP and 1 unit of Taq DNA polymerase (Amersham Pharmacia, UK) using the following PCR programme at 94 °C for 5 min, denaturation at 94°C for 1 min and the annealing temperature at 50 °C or 55 °C depending on the Primer set used (Table - 5) for 50 sec, extension was carried out at 72 °C for 50 sec and final extension at 72 °C for 5 min, in all 30 cycles were carried out.

### ***Electrophoresis***

PCR products were electrophoresed on a denaturing polyacrylamide gel (6% acrylamide, 7.5 M urea, 1 X TBE) at 1500 V for 2 h. The gel was transferred to Whatman 3 filter paper, covered with saran wrap and dried under vacuum for 1 h at 80°C. Autoradiograms were obtained by exposing the gel for varying periods in a cassette with intensifying screen using Kodak-X-OMAT film. Fragment sizes were determined using end labelled AFLP marker (30-300bp; Life Technologies, USA). The autoradiogram was manually scored for the presence or absence of the band for each locus for all the accessions.

### ***Statistical analysis***

For each accession, polymorphism was scored as 1 for the presence and 0 for absence of a band and data were analyzed using NTSYS-Pc version 1.70 (Rohlf, 1992). Allele sharing or the proportion of alleles, Ps (Bowcock, 1994) shared between two of the accessions screened, averaged over the loci, was used as a measure of similarity for all marker types. This corresponds to the simple matching coefficient (Sokal, 1958) for the dominant marker (AFLP) and the Dice indices or Nei and Li coefficient (Nei and Li, 1979) for co-dominant markers (RFLP and SSR).

The genetic distance between individuals (u,v) was calculated as  $d_{uv} = (1-P_{uv})$  resulting in N x N matrix  $D=\{d_{uv}\}$ . The distance matrix, D, was subjected to



sequential agglomerative hierarchical cluster analysis (SHAN), based on the similarity matrices, using unweighted pair group method arithmetic average (UPGMA) and relationship between accessions were visualized as dendrograms (Rohlf, 1992). Differences between dendrograms were tested by generating cophenetic values for each dendrogram and the assembly of the cophenetic matrix for each marker type. The mantel correspondence test was used to compare the similarity matrices, to define the degree of congruence in the estimation of genetic relationships for each marker type.

#### ***Arithmetic mean heterozygosity, Effective Multiplex ratio, and Marker Index***

The efficiency for polymorphism detection was analyzed for each marker type using different indices. Expected heterozygosity for each of the genetic marker was calculated from the square of sum of the allele frequencies (Nei, 1973). The Arithmetic mean heterozygosity was calculated for each marker class (AFLP, RFLP and SSR). Gene diversity ( $H_j$  or  $H_{av}$ ), also termed as the polymorphism information content, and expected heterozygosity were calculated as

$$H_{av} = \Sigma(1 - \Sigma P_i^2)/n,$$

where  $(1 - \Sigma P_i^2)$  is the expected heterozygosity, was estimated for each individual locus as follows:  $j = 1 \dots n$  (Nei, 1973 and 1987),  $H_j = [N/(N-1)]$  to account for the effect of sampling from a finite population. Nei (Nei and Li, 1973) derived  $H_j$ , is the estimated probability that the two members of a population, chosen at random without replacement, differ in their allelic composition. As evident from the equation, gene diversity  $H_j$  at a locus  $j$  depends on the number of detected alleles  $a_j$ , their frequencies  $P_{ij}$  and the sample size  $N$ , the average gene diversity ( $H_{ij}$ ) was estimated as,

$$H_{av} = 1 - \sum_{i=1}^n P_i^2$$

Where,  $P$  is the frequency of the  $i^{th}$  allele of each marker (Nei and Li, 1973) and  $n$  is the number of alleles. Anderson *et al.*, (1993) suggested that the gene diversity is the same as the polymorphism information content (PIC). The Marker Index can be calculated according to Powell (1996), as  $MI = EMR/H_{av}$  ( $P$ ). The Effective Multiplex

ratio ( $EMR=n_p\beta$ ) is the number of polymorphic loci in the germplasm and  $\beta (n_p/(n_p + n_{np}))$  is the polymorphic fraction.

A Principal Co-ordinate Analysis (PCoA) was carried out on the distance matrix and the (D) to visualize the genetic interrelationships among the accessions in two-dimensional PcoA plots, with resultant scores for samples on the first two components plotted pairwise in each case. Multidimensional scaling (MDS) plots were constructed on the Distance Matrix, D, and the stress values have been calculated.

## Biochemical estimation

In the present study, total content of protein, trypsin inhibitors, and the lectins of pods, of the wild and cultivated pigeonpeas (Table 3), at different developmental stages viz, juvenile or the milk stage, immature and the mature stages, were estimated.

About 1g of pod, along with seeds at three developmental stages was ground separately in 8 ml of 0.0025 M HCl. The ground paste was transferred to 25 ml centrifuge tubes for centrifugation at 10,000 rpm for 10 min. The supernatant was collected in 1.5 ml eppendorff tubes and was used for the estimation of total protein, trypsin inhibitors and lectins.

### Protein Estimation

Protein content in the pods of the wild and cultivated pigeonpeas was estimated using Lowry's (1977) method. The following solutions were required for the protein estimation

1. Solution A – 2 % Sodium carbonate, was dissolved in 200 ml of 0.1M NaOH.
2. Solution B – 5% Copper sulphate.
3. Solution C – 1 % Potassium sodium tartarate.
4. Solution D – 192 ml of solution A + 4 ml of solution B + 4 ml of solution C.

5. **Solution E – Follin Ciocalten Reagent with a dilution of 1:1 (15 ml of distilled water + 15 ml Follins reagent).**

A total of 300 $\mu$ l of sample was prepared. Two dilutions, one with 275 $\mu$ l of distilled water and 25 $\mu$ l of the supernatant and the other with 250 $\mu$ l of distilled water and 50 $\mu$ l of the supernatant, were prepared. To both the dilutions, 2.5 ml of solution D and 250 $\mu$ l of solution E were added. The ingredients were incubated at room temperature for 30 min and protein was estimated at 600nm. Bovine Serum Albumin (BSA) was used as the standard at a concentration of 2 mg/ ml. Protein content in each sample was calculated using the following formula.

For dilution 1 -

O.D value of the sample  $\times$  8.192 = protein in mg

For dilution 2 –

O.D value of the sample  $\times$  4.816 = protein in mg

**Estimation of trypsin inhibitor (s)**

The supernatant used for protein estimation was also used for estimating the content of trypsin inhibitor. Trypsin inhibitor content was estimated using BAPNA ((N- $\alpha$ -Benzoyl-DL-Arginine P-Nitroanilidine Hydrochloride - C<sub>19</sub>H<sub>22</sub>N<sub>6</sub>O<sub>4</sub>. HCl) (Erlanger *et al.*, 1961). Following method was used in estimating the trypsin inhibitor. The solutions to be used in the estimation should be prepared afresh before the experiment the following . Reagents are used in the estimation of trypsin inhibitors.

**BAPNA (Sigma Ltd.):**- This solution is a chromogenic trypsin substrate and dissolves only in Dimethyl Sulphoxide (DMSO) at room temperature. In 2 ml of the DMSO, 60 mg of BAPNA was dissolved. The resulting solution was mixed in 20 ml of Tris HCl buffer pH 8.0 and 4 ml of 1 M CaCl<sub>2</sub> . The total volume was made upto 200 ml with sterile distilled water.

**Trypsin Solution :** -Five grams of trypsin (Amersham Life Sciences; source – Bovine pancreas crystalline powder ; 2,739 units/ mg powder to be stored at -20°C)

powder was dissolved in 100 ml of 0.0025M HCl (500µl of HCl in 100 ml of distilled water) and stored at 4°C.

**Acetic Acid** : Acetic acid, 15 ml, was mixed in 35 ml of distilled water,

For the trypsin inhibitor activity, 1:1 dilution of the supernatant (100µl of supernatant with 100µl of sterile distilled water) was used. Diluted sample, 10µl, was dissolved in 990µl of sterile distilled water. Two sets of the sample, with and without trypsin were prepared. In each accession, a set of three samples each from juvenile, immature and mature pods were included. In the first set with trypsin, 400µl of trypsin solution was added and the second without trypsin, only 400µl of distilled water was added. The samples were incubated at room temperature for 15 min. Later, 1.5 ml of BAPNA solution was added to above samples and incubated at 37 °C for 30 min. After incubation, 300µl of 30 % acetic acid were added to the tubes and optical density was measured at 410 nm. One standard each for the samples with and without trypsin were maintained. To 1 ml of distilled water without any sample, 1.5 ml of the BAPNA and 300 µl of the 30 % acetic acid were added. To the standard with trypsin, 400 µl of the trypsin solution and to the other standard without trypsin 400 µl of distilled water were added.

Trypsin inhibitor activity was calculated with the following formula and the activity was expressed in the units of inhibition per mg protein.

% trypsin inhibited =

$$\frac{\text{O.D value of sample with trypsin} - \text{O.D value of the sample without trypsin}}{1.038} \times 100$$

No. of trypsin units inhibited = % trypsin inhibited x 0.0166

#### **Estimation of lectin by haemagglutination**

Lectin activity was calculated in the wild and cultivated pigeonpeas (Table - 3). For measurement of haemagglutinating activity, rabbit red blood cells were used. Rabbit blood was collected in an equal volume of Alsever solution (20.5 g glucose + 0.80g Na-citrate + 0.42g NaCl in 100 ml distilled water and adjusted to pH 7.2 with

10% citric acid), containing 2 drops of heparin/5 ml Alsever. Cells were collected by centrifugation (1500 rpm for 15 min), washed 5 times with saline buffer pH 6.5 (100 mg of azide and 4.5 g of NaCl were dissolved in 500 ml of sterile distilled water) to give 4% (v/v) suspension. The lectin samples were serially diluted in a microtiter plate with equal volumes of saline, pH 6.5, to give a final volume of 0.025 ml. To each dilution 0.05 ml of the rabbit erythrocytes suspension was added. After 2 hh, the end point of the titration was estimated visually as the lowest dilution, which showed the agglutination (titer). The lectin content is expressed in the form of specific haemagglutination units (HAU) and is calculated as,

$$\text{Specific Haemagglutination units (HAU)} = \frac{\text{(agglutination titer / protein content)} \times \text{dilution factor}}{1}$$

### Trichome density

Trichomes generally play an important role in plant-insect interactions (Jeffree, 1986; David and Easwaramoorthy, 1988; Smith, 1989; Peter *et al.*, 1995). Therefore, the study was conducted to identify different types of trichomes and their distribution in cultivated pigeonpeas and *C. scarabaeoides* accessions. A minimum of 10 pods was collected from each accession, in all the three replications in both the seasons. The pods were preserved in a fixative (Acetic acid: absolute alcohol:: 1:3) and examined under the light microscope at a magnification of 100x with an ocular measuring grid to identify different types of trichomes and also their distribution. Because of obvious differences in density among trichome types, they were counted in an area of 4.84 mm<sup>2</sup> (A, B and D) and in 1.21 mm<sup>2</sup> (Type C). Calculations for density of trichomes were based on the mean values of 10 pods in each accession, in 3 replications and two seasons. The pods were scanned under the Scanning Electron Microscope (SEM) using the methodology described by Reddy *et al.*, (1995). Electron Micrographs were taken with a JEOL JSM 35 CF.

### Screening for podborer resistance

Pigeonpea cultivars and *C. scarabaeoides* accessions (Table 3), were screened for podborer resistance in field under multi-choice conditions during the 2000 and 2001 kharif seasons. Eleven plants of each accession were grown at a space

of 30 cm, in the plots of 10 rows, spaced 75 cm apart on black vertisols. The material was classified into three groups based on the days to flowering (early = 50 – 75, medium = 76 - 100 and late = 101-125 days). The plants were raised in three replications, in each experiment, in a RCBD. Appropriate susceptible (ICP 8863) and resistant (ICPL 332) genotypes were included as controls / checks in the screening. Plant protection measures, such as spraying of insecticides, were not taken so as to capture the maximum damage ratings.

The data was recorded for number of eggs and larvae per inflorescence and the percentage of buds, flowers and pods damaged on five random plants in each row. Two inflorescences, 30 - 40 cm long, per plant at flowering stage, were tagged, and observations were recorded on the marked areas. Data were recorded on 5<sup>th</sup>, 7<sup>th</sup>, 11<sup>th</sup>, 21<sup>st</sup> and 31st day after tagging the inflorescences.

## Interspecific hybridization

Five accessions of *C. scarabaeoides* (ICPW 94, ICPW 116, ICPW 125, ICPW 130 and ICPW 141) and two cultivated varieties of *C. cajan* (ICP 26 and ICP 28) were used in the study. The wild accessions were selected based on their resistance levels against *Helicoverpa armigera* (Sharma *et al.*, 2001). The two cultivated varieties, are the popular high-yielding varieties grown all over India .

The study was carried out at ICRISAT, Patancheru during 2000, 2001 and 2002 Kharif seasons. Seeds were sown in the field (Alfisol) manually at a spacing of 60 cm and at an interrow distance of 75 cm. Mechanical support was provided to the wild creeping types. As the seeds of wild *C. scarabaeoides* accessions possess a hard seed coat, the seeds were scarified with a scalpel to remove the seed coat opposite to the coleoptiles to enable it to germinate. Irrigation was provided at regular intervals and endosulphan was sprayed to check insect damage. Crop was covered with nylon nets to avoid any cross pollination and pest damage.

For interspecific hybridization, manual emasculation and pollination was done. In *C. cajan*, flower opening begins in the morning at about 7 AM with the anthesis continuing until late in the afternoon. The flowers remain open for 20 – 24 h and the anthers dehisce before flower opening. The accessions of *C. scarabaeoides* 57

have similar floral biology except that they have delayed flower opening. For hybridization, buds of the appropriate size were opened with the help of forceps and the anthers were removed without injuring the stigma. The forceps was dipped in spirit after each emasculation and immediate pollination. Pollinated flowers were tagged to differentiate from the unpollinated ones. In the interspecific hybridization studies, ten crosses each in the direct (*C.cajan* as female and *C.scarabaeoides* as male) and reciprocal (*C.scarabaeoides* as female and *C.cajan* as male) crosses were made.

At maturity, pods were harvested from individual plants separately and their identity maintained. The  $F_1$  seed from each cross were scarified and sown in Alfisol field in a single row covered by nylon cages, with an interplant spacing of 75cm to obtain the progeny.  $F_1$  seeds of their reciprocals were avoided, as very less seed was available from each cross. During the time of flowering, selfed plants could be visually identified and were removed from the field.

Hybrid seed of individual  $F_1$  plants from each of the seven crosses (ICP 28 x ICPW 94, ICP 28 x ICPW 125, ICP 28 x ICPW 130, ICP 28 x ICPW 141, ICP 26 x ICPW 94, ICP 26 x ICPW 125 and ICP 26 x ICPW 130) were sown, in fifteen rows, after scarification. Seeds of other three crosses and reciprocals were not sown because of shortage of hybrid seed. One row of parents were also sown along with each  $F_2$  population under the cover of nylon cages. Appropriate plant protection measures were taken by providing irrigation and weeding at regular intervals. Seed was harvested from each individual  $F_2$  plant and stored separately.

The  $F_3$  seeds of three crosses (ICP 28 X ICPW 94, ICP 28 X ICPW 130 and ICP 26 X ICPW 125) were sown in the field to raise  $F_3$  generation. Twenty seeds from each  $F_2$  family were sown and the crop was covered with nylon nets. Seeds of individual plant were harvested separately in each cross.

## **Pollen fertility**

Pollen fertility was tested with 1% acetocarmine stain. All shriveled, unstained, and poorly stained pollen were counted as sterile. Three microscopic fields per flower of five flowers per plant were observed for pollen fertility. 58

Observations for pollen fertility were made on all the plants in both the parents (*C. scarabaeoides* and *C. cajan*), F<sub>1</sub> and F<sub>2</sub> generations.

### **Screening for podborer resistance**

Plants were screened for podborer resistance under multi-choice conditions in the field, following the method of Sharma *et al.* (2001). The crop was maintained without any insecticidal spray during the screening period and the plants were exposed to natural infestation. Two inflorescences per plant were tagged and observations were recorded on 5<sup>th</sup>, 7<sup>th</sup>, 11<sup>th</sup>, 21<sup>st</sup> and 31<sup>st</sup> day of tagging for the number of buds, flowers and pods damaged; and the number of eggs and larvae on each inflorescence. This screening was done on plants of each of the F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub> and backcross progenies before carrying it to the next generation.

### **Production of backcross progenies**

After screening, the F<sub>1</sub> plants showing resistance against podborer was selected for further crossing work. Only three crosses (ICP 28 x ICPW 94, ICP 28 x ICPW 130, and ICP 26 x ICPW 125) were selected for the production of backcross generations. The plot was maintained in insecticide free conditions during the first few days of screening. After screening the plants were covered with nylon nets and 2% endosulfan was sprayed to protect the plants from insect damage. Screening was done during the first week of September and continued for over a month's time. Resistant F<sub>1</sub> plants were used as male in backcrossing and *C. cajan* as the female parent. At the time of harvest, the seed from each BC<sub>1</sub> plant was harvested separately and stored for further use.

BC<sub>1</sub> seeds of three crosses were sown at an interplant distance of 60cm and at an inter row spacing of 75 cm, as the plants were spreading in habit. BC<sub>1</sub> plants in all three crosses were screened for podborer resistance and 15 resistant plants were chosen for further backcrossing. The BC<sub>2</sub> seed from each individual plant was harvested and stored separately for further use.

BC<sub>2</sub> seeds were sown in three rows with an interplant distance of 60 cm and the inter-row distance of 75 cm. The BC<sub>2</sub> plants were screened for podborer



resistance and the resistant plants were used as male and the *C.cajan* were used as the female parents in the backcrossing program. BC<sub>3</sub> seed was collected from each individual plant separately and stored for further use.

## **Genetic basis of qualitative and quantitative characters**

### **Qualitative characters**

Data was recorded on parents, F<sub>1</sub> and F<sub>2</sub> plants in all seven crosses for the following qualitative characters: Plant habit (erect, semi-spreading and spreading), leaflet shape (lanceolate, intermediate and obovate), stem color (purple, mixed and green), seed strophiole (presence or absence), seed mottles (presence or absence) and pod hair (glabrous or pubescent) (Table 4). Plants were classified into above distinct categories and  $\chi^2$  test was used to test the goodness of fit of the observed ratio.

### **Quantitative traits**

Inheritance of quantitative traits was studied in two different experiments; (a) in 2001 kharif, the F<sub>2</sub> plants along with parents and F<sub>1</sub>s were evaluated for all the characters (Table - 4) in all the seven crosses (ICP 28 x ICPW 94, ICP 28 x ICPW 125, ICP 28 x ICPW 130, ICP 28 x ICPW 141, ICP 26 x ICPW 94, ICP 26 x ICPW 125 and ICP 26 x ICPW 130). Heterosis, broad sense heritability and inbreeding depression were calculated for all the characters in all the seven crosses. and (b) in 2002 kharif season, the parents, F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub> and BC<sub>1</sub> (*C.cajan* x F<sub>1</sub>) plants were evaluated for all the characters (Table 4) and the data was subjected to Cavalli's scaling test. The five- parameter generation mean analysis was used to study the gene effects. This analysis was done for three crosses (ICP 28 x ICPW 94, ICP 28 x ICPW 130 and ICP 26 x ICPW 125). For the other four crosses data was recorded till F<sub>2</sub> generation and means and variances were reported for different characters.

### **Inheritance of trichome type and density**

Data on type and density of trichomes were recorded for two crosses (ICP 28 x ICPW 94 and ICP 26 x ICPW 125). Five pods from ten plants each of the four parents (ICP 28 and ICP 26; ICPW 94 and ICWP 125), ten plants each of their F<sub>1</sub>s and 250 plants of F<sub>2</sub> and 75 plants of BC<sub>1</sub> progeny were evaluated for the type and

density of trichomes. The pods were observed under a light microscope with a magnification of 100 x and also scanned under a JEOL JSM 35 CF Scanning electron microscope (SEM) and electron micrographs were also taken. Trichome density was estimated using light microscope with an ocular measuring grid. Because of the differences in the density among trichome types, trichomes were counted on an area of 4.84mm<sup>2</sup> (Types A, B and D) or 1.21 mm<sup>2</sup> (Type C). For data analysis, means of five pods per plant at three microscopic fields were used. The  $\chi^2$  test was used to test the goodness of fit of the observed ratio (high density of trichomes : low density of trichomes ) of segregation in the F<sub>2</sub> and BC<sub>1</sub>F<sub>1</sub> generations.

### **Genetic basis of mechanisms of resistance against podborer**

Mechanisms of resistance against podborer was recorded as antixenosis and antibiosis. The antixenosis mechanism was studied in the field under multi- choice conditions while the antibiosis was studied in the laboratory under no- choice condition.

#### ***Antixenosis or Non-preference mechanism of resistance***

The parents (P<sub>1</sub> and P<sub>2</sub>), F<sub>1</sub>, F<sub>2</sub> and the BC<sub>1</sub> ( *C. cajan* x F<sub>1</sub>) hybrids of three crosses (ICP 28 x ICPW 94, ICP 28 x ICPW 130 and ICP 26 x ICPW 125) were screened in the field for podborer resistance under multi-choice conditions in 2001 Kharif season. Plants were exposed to multi-choice conditions and were allowed natural infestation by pests. The plot was divided into 4 m long rows with an inter plant distance of 30 – 40 cm and an inter - row spacing of 75cm in view of the semi-spreading nature of the plants. One row each of the parents (*C. scarabaeoides* ICPW 94, ICPW 130, ICPW 125 and *C. cajan*; ICP 26 and ICP 28), their F<sub>1</sub>s, 20 rows of the F<sub>2</sub> population of each cross and 5 rows of BC<sub>1</sub> (*C. cajan* x F<sub>1</sub>) were grown. During the Kharif 2002, 125 F<sub>3</sub> families in cross ICP 28 x ICPW 94, 116 families in cross ICP 28 x ICPW 130 and 109 families in cross ICP 26 X ICPW 125 were screened.

### ***Data collection***

For screening of plants against podborer, two inflorescences, of 25 – 30 cm in length per plant were tagged with plastic ribbons. Observations were recorded on 5<sup>th</sup>, 7<sup>th</sup>, 11<sup>th</sup>, 21<sup>st</sup> and the 31<sup>st</sup> day of tagging. All plants in a single cross (Parents, F<sub>1</sub> and F<sub>2</sub>) were tagged on the same day and thus the plants in three crosses were tagged on three consecutive days. Observations on total number of buds, flowers, and pods present and the number of buds, flowers, and pods infected by podborer per inflorescence were recorded. Data on number of eggs and larvae present in each inflorescence were also recorded.

### ***Data analysis***

Average damage caused by insets to buds, flowers and pods on the 5<sup>th</sup>, 7<sup>th</sup>, 11<sup>th</sup>, 21<sup>st</sup> and the 31<sup>st</sup> day, from the day of tagging, was used for analysis. The average was expressed as the percentage of buds, flowers, and pods damaged and average number of eggs and larvae present per inflorescence per plant. Based on the percent damage of buds, flowers and pods, the damage rating was given. Rating was given a scale of 1 – 5 with 1 as no damage to the pods, 2 as  $\leq 10\%$  damage, 3 as  $\leq 20\%$  damage with minimum of two egg masses and one larvae, 4 as  $\leq 30\%$  damage with more than three egg masses and two larvae and 5 as  $> 40\%$  damage with more than five egg masses and five larvae.

The plants in each generation was classified into resistant and susceptible categories according to the percentage damage. Plants with damage rating between 1 and 2 were grouped into resistant types and those with damage rating from 3 to 5 were grouped into the susceptible types.  $\chi^2$  test was used to test the goodness of fit of the observed ratio of segregation for the antixenosis mechanism in the F<sub>2</sub> and BC<sub>1</sub>F<sub>1</sub> population in all the three crosses. The results were further confirmed by comparing with the data on F<sub>3</sub> families following the same approach.

### ***Antibiosis mechanism of resistance***

Pods obtained from the progeny of only one cross (ICP 28 X ICPW 94) were screened in the laboratory against podborer to study the antibiosis mechanism of resistance. Pods from 20 plants each of the two parents (*C. scarabaeoides* and 62

*C. cajan*), 10 plants of F<sub>1</sub>, 250 plants of F<sub>2</sub> and 70 plants of BC<sub>1</sub> population were collected in polythene bags in the morning and screened for podborer resistance in the insect rearing laboratory of ICRISAT.

### **Bioassay**

To culture the *H. armigera* in the laboratory, 75 g of chickpea flour, 12 g yeast, 1.175 g L-ascorbic acid, 1.25 g of methyl -4-hydroxybenzoate, 0.75 g of sorbic acid and 2.875 g of aureomycin and were mixed in 1 ml of formaldehyde, 2.5 ml of commercially available vitamin stock solution and 112.5 ml of water were added to it and mixed thoroughly. To this, 4.375 g of agar in 200 ml of water was added and mixed thoroughly to obtain media with even consistency. This diet was then poured into small plastic cups and allowed to cool in a laminar flow cabinet. Neonate larvae were reared individually at 27°C under photoperiod of 12:12 (L:D) h.

The larvae and adults of *H. armigera* used in the feeding tests and oviposition experiments, were obtained from the laboratory culture maintained at ICRISAT, Patancheru, India. The culture was established and maintained by providing with field-collected larvae at regular intervals. Larvae were reared on chickpea diet at 27°C (Armes *et al.*, 1992). Adults were kept at 25°C in a cage and mappylinous were provided as a substrate for oviposition. The moths were provided 10% honey solution on absorbent cotton for oviposition.

In the present study the pods were screened under no – choice conditions, where in each the third instar larvae was given no other option but to feed on the pod provided to it. Moistened filter paper was kept in alcohol-cleaned petridishes. The third instar larvae were released into the petridish containing only five pods from single plant. These petridishes were covered with lids containing moistened filter paper. The petriplate was tightened with a rubber band, as a precautionary measure, to prevent the larvae from escaping out of the petriplate. The filter papers were moistened at regular intervals to provide enough moisture to the larvae for their survival. Three replications, each with five pods per plant were screened.

### ***Data collection and analysis***

Weights of larvae were noted down before their release into the petridishes. Gain or loss in the larval weights was recorded. Observation of the pods after 72 h under a magnifier gave the damage rating of the pods. Damage rating of pods was scored on a scale of 1-9. Ratings were given after observation under magnifier so that the minute details of the damage could be captured without fail. The damage rating was given based on the mortality and loss or gain of weights and also pod damage by the insects. Pods without any damage and caused the death of insects were given a rating of 1 but on the other hand the insects gained weight by eating pods and did not cause the death of insects were given the damage rating of 9; and the others were given rating from 2 to 8 depending on the proportionate damage caused to the pods. The plants with pods in the damage rating of 1-4 were clustered as the resistant types, while those falling under 5 – 9 were categorized as the susceptible types. The  $\chi^2$  analysis was used to test the goodness of fit of the observed ratio of resistant : susceptible to the expected ratio of segregation for the damage rating in the F<sub>2</sub> population and backcross generations.

### **Statistical methods**

Data on the various aspects of interspecific hybrids was analysed using different statistical methods. The hybrid vigour among F<sub>1</sub>s was calculated in the form of mid-parent and better- parent heterosis. The inbreeding depression was calculated from data on F<sub>1</sub> and F<sub>2</sub> generation. Heritability and genetic advance were calculated from the variances of the parents, F<sub>1</sub> hybrids and F<sub>2</sub> generation in each cross. The components of variance (d, h, l, j and i) were calculated.

### **Generation mean analysis**

Generation mean analysis was used to estimate the components of genetic variation. Testing of epistasis was necessary before estimating the components of genetic variation to decide the method of analysis for components of variation. Components of genetic variation were estimated for days to flower and maturity, leaf length and width, pod length and width, pod bearing length, number of locules and

seeds per pod, 100-seed weight, number of primary and secondary branches and harvest index.

### **Scaling test**

The test of adequacy of scales is important because in most of the cases the estimation of additive and dominance components of variance is by assuming the absence of gene interaction. Cavalli (1952) and proposed the following tests for estimating the scale effects:

$$A = 2BC_1 - P_1 - F_1;$$

$$B = 2 BC_2 - P_2 - F_1;$$

$$C = 4 F_2 - 2 F_1 - P_1 - P_2;$$

$$D = 4 F_3 - 2 F_2 - P_1 - P_2$$

The variances were calculated using the following formula:

$$VA = 4 V (BC_1) + V (P_1) + V (F_1);$$

$$VB = 4V (BC_2) + V (P_2) + V (F_1)$$

$$VC = 16 V (F_2) + 4 V (F_1) + V (P_1) + V (P_2);$$

$$VD = 16 V (F_3) + 4V(F_2) + V (P_1) + V (P_2)$$

When the scale is adequate, the values of A, B, C and D should be zero within the limits of their respective standard errors. However, in the present study the B scales could not be estimated because of the absence of the backcross progeny with the other parent.

### ***Five parameter model:***

This model was proposed by Hayman (1958) and is used in the absence of backcross progenies ( $BC_1$  and  $BC_2$ ) and instead when  $F_3$  is available. Analysis is based on five populations viz.,  $P_1$ ,  $P_2$ ,  $F_1$ ,  $F_2$  and  $F_3$  generations of a single cross. Five parameters,  $m$ ,  $d$ ,  $h$ ,  $i$  and  $l$  were estimated. This model does not provide information about additive x dominance type of epistasis and requires three crop seasons for generation of material and the fourth season for evaluation.

The gene effects are estimated as follows:

$$m = F_2;$$

$$d = \frac{1}{2} P_1 - \frac{1}{2} P_2;$$

$$h = \frac{1}{6} (4F_1 + 12F_2 - 16F_3);$$

$$i = P_1 - F_2 + (\frac{1}{2}) (P_1 - P_2 + h);$$

$$l = \frac{1}{3} (16 F_3 - 24F_2 + 8F_1) ;$$

The variances of these estimates were calculated as below:

$$V_m = VF_2,$$

$$V_d = (\frac{1}{4}) (VP_1 + VP_2) ;$$

$$V_h = (1/36) (16VF_1 + 144VF_2 + 256VF_3)$$

$$V_i = VP_1 + VF_2 + (\frac{1}{4}) (VP_1 + VP_2 + V_h) + (1/16) V_l$$

$$V_l = (1/9) (256 VF_3 + 576 VF_2 + 64VF_1);$$

Their standard errors are estimated as follows:

$$S.E.m = (V_m)^{1/2}; S.E. d = (V_d)^{1/2}; S.E. h = (V_h)^{1/2}; S.E. i = (V_i)^{1/2}; S.E. l = (V_l)^{1/2}$$

In this model the parameters were estimated with increased precision and it also provides  $\chi^2$  test for the model.

### *Test of significance*

The significance of the above parameters is tested with the help of t value. The t value was calculated for each component by dividing the value of gene effect of respective components by their S.E. The calculated value of t was compared with 1.96, which is the table value of t at 5% level of significance. If the calculated value is greater than 1.96, it is considered as significant.

### **Heritability**

Heritability ( $h^2$ ) for 13 quantitative traits along with trichome density was estimated using the formula of Falconer (1989).

$$h^2_{bs} = \frac{\text{Genotypic variance}}{\text{Phenotypic variance}} \times 100 = \frac{VF_2 - VE ((VP_1 + VP_2 + VF_1))/3}{VF_2} \times 100$$

VE, VP<sub>1</sub>, VP<sub>2</sub>, VF<sub>1</sub>, and VF<sub>2</sub> are the variance of environment, variance of parent one, variance of parent two, variance of F<sub>1</sub> and variance of F<sub>2</sub> respectively. VE was provided by the variate in the non-segregating generation, P<sub>1</sub>, P<sub>2</sub> and F<sub>1</sub>. The variation in F<sub>2</sub> consists of both environment and genetic variance.

### Heterosis

The performance of hybrid in relation to its parents can be expressed in two ways; Mid parent heterosis (average heterosis) is the performance of a hybrid compared to the average performance of both of its two parents. Better parent heterosis (heterobeltiosis) is the comparison of the performance of the hybrid with that of better parent. Heterosis is usually expressed as percentage and computed by using the formula of Fehr (1987).

$$\text{Mid parent heterosis (\%)} = \frac{\text{Mean of } F_1 - \text{Mean of parents}}{\text{Mean of parents}} \times 100$$

$$\text{Better parent heterosis (\%)} = \frac{\text{Mean of } F_1 - \text{mean of better parent}}{\text{Mean of better parent}} \times 100$$

### Inbreeding depression

The inbreeding depression refers to decrease in fitness and vigour due to inbreeding was calculated using the following formula given by Phundan Singh and Narayanan (1997).

$$\text{Inbreeding depression (\%)} = \frac{\text{Mean of } F_1 - \text{Mean of } F_2}{\text{Mean of } F_1} \times 100$$

The significance of heterosis and inbreeding depression was tested with the help of critical difference (CD). The general formula for estimation of CD = SE difference  $\times$  t value at 5% or 1% level. Mean error variance from combined analysis of variance of parents, F<sub>1</sub>s and F<sub>2</sub>s is used for calculating the SE of difference.

### Chi - square ( $\chi^2$ ) analysis

Chi - square test ( $\chi^2$ ) to find the goodness of fit was calculated as per the formula given by Panse and Sukhatme (1967).



$$\chi^2 = \sum \frac{(\text{Observed frequencies} - \text{Expected frequencies})^2}{\text{Expected frequencies}}$$

Calculated Chi- square values were compared with the table values given by Fischer and Yates (1963).

### Test of significance of means

Test of significance of means is a procedure for distinguishing whether the observed difference corresponds to any real difference among the genotypes or can be ascribed to mere sampling fluctuations. In the present study, t test was used to test the significance of differences for the quantitative characters studied between the means of both the parents used in the crossing program using the formula of Kapur and Saxena (1969).

$$t = \frac{|\text{Mean of } P_1 - \text{mean of } P_2|}{\text{Standard deviation of the population } \sqrt{1/n_1 + n_2}}$$

Where  $n_1$  and  $n_2$  are the sizes of the samples for parent 1 and 2 respectively.

$$S = \frac{1}{n_1 + n_2 - 2} [(n_1 - 1) S_1^2 + (n_2 - 1) S_2^2]$$

$S_1$  and  $S_2$  are the standard deviations for parent 1 and 2 respectively.

### Correlation coefficient

Changes in one variable may be accompanied by changes in the other, indicating the relationship between the two variables. Correlation coefficient (r) is the measure of direction and degree of closeness of the linear relationship between two variables. Simple correlation coefficients among different characters were calculated using the formula suggested by Panse and Sukhatme (1967).

$$\text{Correlation coefficient } (r) = \frac{\sigma_{XY}}{\sigma_X \cdot \sigma_Y}$$

$$\sigma_{XY} = \frac{\sum f \cdot dx \cdot dy}{N}$$

$\sigma_{XY}$  = The co variance between X and Y

$\sigma_X$  = standard deviation of X

$\sigma_Y$  = standard deviation of Y

$dx$  and  $dy$  = deviations.

***Significance of correlation coefficient***

$$t = \frac{r \sqrt{n-2}}{\sqrt{1-r^2}}$$

$r$  is the estimate obtained from  $n$  pairs and compared to standard 't' value at 5% and 1% level of significance (Snedecor and Cochran, 1968).

# RESULTS

## RESULTS

In the present investigation; morphological, molecular and biochemical diversity analysis, among 30 accessions of *C. scarabaeoides* and six varieties of *C. cajan* (Table 3), for various characters (Table 4), including podborer resistance has been done (Fig 1). Further, the study includes the incorporation of podborer resistance gene(s) from the wild accessions of *C. scarabaeoides* to cultivated *C. cajan* through backcrossing programme, and in order to investigate the genetic basis of various characters by raising  $F_1$ ,  $F_2$ ,  $F_3$  and backcross generations.

### Diversity analysis

#### Morphological diversity analysis

Morphological diversity analysis, among 30 different accessions of *C. scarabaeoides* and six varieties of pigeonpea (Table 3) for 14 traits (Table 4) including density of trichomes on pods, was done in 2000 and 2001 Kharif seasons. Season was found to be significant for days to flower, pod width and number of locules per pod. Significant differences were found for days to flower and maturity, leaf area, leaf dry weight, specific leaf area, pod length and width, number of locules per pod, number of seeds per pod, 100 seed weight and number of secondary branches for habit (the wild and cultivated pigeonpea). Interaction between season and the accessions/varieties was significant for days to flower and leaf area. Interaction between season and wild accessions was significant for days to flower, leaf dry weight and 100 - seed weight. The interaction of season and cultivated pigeonpeas was significant for days to maturity and leaf area (Table 6). Genotype was found significant for days to flower, leaf dry weight, specific leaf area, pod length, number of locules per pod, number of seeds per pod, 100- seed weight, number of secondary branches and total seed protein (Table 7). Genotype x season interaction was significant for leaf area, leaf dry weight, pod length and width. Habit

Table - 6: Significance of differences between seasons, habits and their interaction in *Cajanus*

Character	Days to flower (No.)			Days to maturity (No.)			Leaf area (mm <sup>2</sup> )			Leaf dry weight (g)		
	Walds statistics	Wald/df $\chi^2$ value	$\chi^2$ Probability	Walds statistics	Wald/df $\chi^2$ value	$\chi^2$ Probability	Walds statistics	Wald/df $\chi^2$ value	$\chi^2$ Probability	Walds statistics	Wald/df $\chi^2$ value	$\chi^2$ Probability
Season	29.12	29.12	<0.001**	0.1	0.1	0.748	1.38	1.38	0.24	4	4	0.046
Habit	999.32	999.32	<0.001**	1980.75	1980.75	<0.001**	6326.63	6326.63	<0.001**	1190.61	1190.61	<0.001**
Season x Habit	5.13	5.13	0.023*	0	0	0.948	3.67	3.67	0.055	0.07	0.07	0.795
Season x Habit (Wild)	11.59	2.32	0.041*	6.55	1.31	0.257	2.38	0.48	0.794	7.01	7.01	0.008**
Season x Habit (Cult.)	31.59	1.09	0.338	46.66	1.61	0.02 *	52.58	1.81	0.005**	23.88	0.82	0.735
	Leaf specific area (mm <sup>2</sup> /g)			Pod length (cm)			Pod width (cm)			No. of locules / pod		
Season	3.81	3.81	0.051	0.04	0.04	0.849	34.64	34.64	<0.001**	24.88	24.88	<0.001**
Habit	31.94	31.94	<0.001**	9959.44	9959.44	<0.001**	95.74	95.74	<0.001**	104.2	104.2	<0.001**
Season x Habit	0.49	0.49	0.486	0.04	0.04	0.851	0.01	0.01	0.922	1.26	1.26	0.261
Season x Habit (Wild)	2.53	0.51	0.772	2.69	0.67	0.611	5.47	1.09	0.361	1.2	0.3	0.878
Season x Habit (Cult.)	26.23	0.9	0.613	17.86	0.62	0.927	29.69	1.03	0.416	11.56	0.41	0.997
	No. of seeds/ pod			100-seed weight (g)			No. of primary branches			No. of secondary branches		
Season	5.61	5.61	0.081	3.41	3.41	0.065	0.86	0.86	0.355	2.69	2.69	0.101
Habit	51.9	51.9	<0.001**	31813.80	31813.80	.001**	8.21	8.21	0.004**	11096.75	11.96.75	<0.001**
Season x Habit	0.48	0.48	0.49	2.75	2.75	0.098	0.30	0.30	0.582	0.00	0.00	0.969
Season x Habit (Wild)	0.08	0.04	0.962	10.24	2.05	0.069	0.01	0.00	1.000	1.89	0.38	0.865
Season x Habit (Cult.)	19.46	0.69	0.883	8.98	0.31	1.000	33.58	1.20	0.215	31.85	1.10	0.326

\* = significant at 5% level; \*\* = significant at 1% level;

Cond...

Fig. 1: *C. cajan* and *C. scarabaeoides* accessions in the field used to study morphological diversity



Fig 1

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Character	Density of Trichome A (No./mm <sup>2</sup> )			Density of Trichome B (No./mm <sup>2</sup> )			Density of Trichome C (No./mm <sup>2</sup> )		
Interaction	Walds statistics	Wald/df (χ <sup>2</sup> value)	χ <sup>2</sup> Probability	Walds statistics	Wald/df (χ <sup>2</sup> value)	χ <sup>2</sup> Probability	Walds statistics	Wald/df (χ <sup>2</sup> value)	χ <sup>2</sup> Probability
Season	10.25	10.25	0.001**	0.01	0.01	0.931	0.00	0.00	0.975
Habit	3401.08	3401.08	<0.001**	5364.90	5364.90	>0.001**	39637.70	39637.7	<0.001**
Season x Habit	3.67	3.67	0.056	0.00	0.00	0.969	0.00	0.00	0.989
Season x Habit (Wild)	0.00	0.00	-	0.00	-	-	0.00	0.00	-
Season x Habit (Cult.)	58.80	2.03	<0.01**	0.27	0.02	1.000	0.03	0.00	1
	Density of Trichome D (No./mm <sup>2</sup> )			Bud damage (%)			Flower damage (%)		
Season	0	0	-	0.01	0.01**	0.927	0.17	0.17	0.68
Habit	1170.53	1170.53	<0.001**	455.85	455.85	<0.001	438.46	438.46	<0.001
Season x Habit	2.11	2.11	0.147	0.36	0.36	0.547	1.65	1.65	0.20
Season x Habit (Wild)	3.93	0.98	0.415	7.02	1	0.426	9.07	1.30	0.248
Season x Habit (Cult.)	11.29	0.49	0.98	35.88	1.12	0.291	50.35	1.57	0.021*
	Pod damage (%)			No. of eggs/ inflorescence			No. of larvae/inflorescence		
Season	0.80	0.80	0.37	0.64	0.64	0.423	0.54	0.54	0.461
Habit	1109.9	1109.9	<0.001**	1038.08	1038.08	<0.001**	774.36	774.36	<0.001**
Season x Habit	3.75	3.75	0.053	10.67	10.67	0.001**	0.66	0.66	0.416
Season x Habit (Wild)	35.54	5.08	<0.001**	33.75	4.82	<0.001**	37.18	5.31	<0.001**
Season x Habit (Cult.)	19.42	0.61	0.961	117.93	3.69	<0.001**	88.37	2.76	<0.001**



**Table - 7 : Estimates of components of variance and heritability for different characters**

Trait	Days to flowering (No.)		Days to maturity (No.)		Leaf area (mm <sup>2</sup> )		Leaf dry weight (g)		Leaf specific area (mm <sup>2</sup> /g)		Pod length (cm)		Pod width (cm)	
	Variance	Standard Error	Variance	Standard Error	Variance	Standard Error	Variance	Standard Error	Variance	Standard Error	Variance	Standard Error	Variance	Standard Error
Interaction														
Genotype	176.93	42.373	290.45	696.533	67.592	16.184	0.03	0.006	299.12	73.692	1.21	0.290	0.01	0.009
Genotype x Season	0.09	0.080	0.64	0.468	0.825	0.051	0.00	0.000	0.01	4.824	0.00	0.003	0.00	0.000
Habit x Wild	8.74	5.6033	26.79	17.368	61.15	38.716	0.01	0.00	413.92	267.35	0.19	0.126	0.01	0.00
Habit x cult	208.14	54.69	304.48	80.137	57.74	15.180	0.00	0.00	278.49	75.451	0.04	0.012	0.00	0.00
Residual	0.69	0.082	3.67	0.439	0.367	0.044	0.00	0.000	54.05	6.466	0.02	0.003	0.00	0.000
Genotypic variance component	176.90		290.40		67.58		0.003		299.12		1.210		0.004	
Phenotypic variance component	172.25		265.40		65.28		0.046		308.20		1.448		0.059	
Heritability (%)	90.2		92.5		95.6		92.32		92.53		92.3		82.1	

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Trait	Locules per pod (No.)		Seed per pod (No.)		Seed weight (g)		Number of primary branches		Number of secondary branches		Total seed protein (%)	
	Variance	Standard error	Variance	Standard error	Variance	Standard error	Variance	Standard error	Variance	Standard error	Variance	Standard error
Genotype	0.33	0.080	0.31	0.077	15.14	3.622	7.64	3.144	109.21	26.312	6.67	1.562
Genotype x Season	0.00	0.005	0.00	0.005	0.00	0.009	0.00	2.482	0.00	0.067	0.00	0.000
Habit. x Wild	0.33	0.214	0.151	0.102	3.08	1.957	4.46	5.76	5.21	3.377	3.10	1.791
Habit x cult.	0.31	0.082	0.327	0.088	0.20	0.057	7.15	3.13	82.49	21.696	3.96	1.022
Residual	0.05	0.005	0.06	0.007	0.09	0.011	27.83	3.333	0.76	0.091	0.00	0.001
Genotypic variance component	0.328		0.319		15.46		7.638		109.20		6.668	
Phenotypic variance component	0.459		0.452		16.54		12.280		112.25		6.07	
Heritability (%)	96.5		95.4		90.4		62.2		91.2		98.5	

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Trait	Density of Trichome A (No./mm <sup>2</sup> )		Density of Trichome B (No./mm <sup>2</sup> )		Density of Trichome C (No./mm <sup>2</sup> )		Density of Trichome (No./mm <sup>2</sup> )	
	Variance	Standard error	Variance	Standard error	Variance	Standard error	Variance	Standard error
Genotype	2.82	0.678*	10.64	2.552**	2613.27	624.876**	11.09	2.663**
Genotype x Season	0.01	0.008	0.00	0.009	0.00	0.423	0.00	0.022
Habit x wild	5.56	3.518	14.59	9.235	4090.46	2587.426	24.54	15.549
Habit x cult.	1.39	0.369	7.32	1.926**	1375.42	361.671**	7.44	1.965**
Residual	0.05	0.007	0.09	0.016	4.71	0.563	0.25	0.000
Genotypic variance component	4.56		5.89		4.25		5.29	
Phenotypic variance component	5.98		6.52		4.98		5.84	
Heritability (%)	85.6		86.25		91.25		92.3	

x wild interaction was significant for leaf dry weight and pod width (Table 7). Habit x cultivated interaction was significant for days to flower and maturity, leaf dry weight, leaf specific area, pod width, number of locules and seeds per pod and 100-seed weight. Heritability was high for all the traits studied, ranging from 70.00 to 97.75 % except for number of primary branches (Table 7).

### **Days to flower**

Among the *C. scarabaeoides* accessions, ICPW 98 was the earliest to flower in 50.5 days and ICPW 308 was the last to flower in 104.6 days (Table 11). Among the *C. cajan* genotypes, ICP 26 was the earliest to flower in 52.3 days and the genotype ICP 14722 took maximum of 71.8 days to flower. As a group, on an average *C. scarabaeoides* flowered in 75.7 days while *C. cajan* in 63.7 days, with an overall mean of 73.15 days (Table 8).

### **Days to maturity**

Among the *C. scarabaeoides* accessions, ICPW 86 was the earliest to mature in 85.83 days. However, this accession flowered later (54.01 days) than the earliest flowering ICPW 98. The accession, ICPW 147 took maximum of 156.7 days to mature. The mean number of days for maturity of wild accession was 118 (Table 11). Among the cultivated, ICP 14770 matured earliest in 93 days compared to 106.1 days of ICP 8863, with a group mean of 99.5 days and with an overall mean of 113.7 days (Table 8).

### **Leaf area**

Leaves of different *C. scarabaeoides* genotypes were comparatively smaller than those of *C. cajan*. Among the *C. scarabaeoides* accessions, the leaf area ranged from 17.36 mm<sup>2</sup> in ICPW 302 to 42.11 mm<sup>2</sup> in ICPW 101, with a group mean of 32.55 mm<sup>2</sup>, whereas, among the *C. cajan* genotypes the leaves were smallest in ICP 28 with an area of 32.95 mm<sup>2</sup> and that of ICP 14722 were the largest with an area of

**Table - 8: Mean of different quantitative traits in *C.scarabaeoides* and *C.cajan***

Accessions	Days to flower (No.)	Days to maturity (No.)	Leaf area (mm <sup>2</sup> )	Leaf dry Wt. (g)	Leaf specific area (mm <sup>2</sup> /g)	Pod length (cm)	Pod width (cm)	No. of locules per pod	No. of seed per pod	Seed weight (g)	No. of primary branches	No. of secondary branches	Seed protein (%)
<b><i>C.scarabaeoides</i> accessions</b>													
ICPW 82	51.48	98.56	29.62	0.22	148.51	2.32	0.57	5.45	5.43	2.33	12.57	19.52	27.63
ICPW 83	54.26	101.13	18.15	0.1	180.82	1.92	0.67	4.54	4.15	1.84	12.98	15.29	26.81
ICPW 86	54.01	85.83	38.89	0.23	168.91	2.58	0.66	3.85	3.89	2.99	9.93	17.53	27.34
ICPW 90	55.74	97.73	41.11	0.28	147.32	2.57	0.66	5.6	5.38	1.84	8.16	17.87	25.44
ICPW 94	67.16	104.43	18.52	0.14	133.11	2.11	0.64	4.64	4.72	1.87	13.05	17.61	26.18
ICPW 95	69.14	98.73	37.04	0.22	168.22	2.25	0.65	5.71	5.69	2.64	11.23	28.94	26.7
ICPW 96	75.84	112.93	41.69	0.23	180.81	2.43	0.56	4.6	4.55	1.88	9.28	27.14	29.58
ICPW 98	50.48	93.73	36.28	0.21	172.52	2.49	0.56	5.53	5.51	2.78	10.91	13.67	25.46
ICPW 101	69.23	96.32	42.11	0.24	175.11	2.63	0.66	5.35	5.25	1.9	8.92	25.98	28.58
ICPW 111	81.48	124.83	34.69	0.24	145.12	2.34	0.69	4.51	4.36	2.26	8.77	8.43	27.72
ICPW 115	75.35	126.72	32.18	0.22	146.81	2.49	0.67	4.69	4.73	1.81	9.35	15.12	28.38
ICPW 116	71.89	126.23	38.22	0.23	166.12	2.56	0.68	5.71	5.54	2.54	13.67	41.02	26.44
ICPW 119	75.21	112.22	32.41	0.21	154.61	2.61	0.67	5.5	5.22	2.61	8.79	12.69	21.71
ICPW 122	70.62	125.43	41.31	0.25	165.22	2.26	0.66	5.34	5.22	2.47	10.82	16.57	24.62
ICPW 125	69.02	113.24	38.54	0.21	182.93	2.44	0.64	5.34	5.35	2.39	8.75	12.52	25.24
ICPW 130	66.8	93.93	37.01	0.22	168.13	2.64	0.69	5.55	5.65	2.61	12.89	16.82	26.46
ICPW 132	78.1	112.61	31.51	0.23	137.73	2.59	0.67	5.86	5.39	2.17	12.57	31.49	27.64
ICPW 137	66.7	105.13	34.25	0.24	143.33	2.3	0.69	5.42	5.54	2.15	13.72	34.63	24.68
ICPW 138	78.75	120.32	21.35	0.11	193.13	2.42	0.75	4.73	4.47	3.45	10.88	22.05	25.74
ICPW 141	53.05	101.73	37.82	0.24	159.63	2.62	0.68	5.27	5.43	2.85	9.78	12.39	24.77
ICPW 147	98.92	156.73	24.13	0.13	181.32	2.23	0.65	4.69	4.51	2.04	17.56	11.64	22.54
ICPW 152	88.07	136.32	31.85	0.19	164.82	2.57	0.73	4.54	4.15	2.71	8.73	16.42	21.54
ICPW 278	85.18	117.41	40.84	0.22	185.23	2.60	0.60	5.58	5.12	2.05	13.19	21.76	23.43
ICPW 280	85.43	133.93	21.75	0.12	180.63	2.53	0.66	5.76	5.35	1.76	9.17	28.79	23.44
ICPW 281	95.64	148.72	36.52	0.26	141.11	2.57	0.65	4.31	4.23	1.88	9.37	26.17	23.82
ICPW 302	84.23	106.85	17.36	0.11	164.13	2.45	0.57	4.57	4.46	1.72	11.44	38.24	24.44
ICPW 305	94.84	144.22	32.81	0.23	143.31	1.98	0.74	4.33	4.39	1.52	11.65	30.69	24.55
ICPW 308	104.57	133.33	22.17	0.15	151.72	2.61	0.64	4.61	4.55	1.71	9.37	37.53	25.44
ICPW 310	101.2	113.25	33.41	0.23	145.81	2.51	0.53	3.85	3.92	2.59	9.41	19.03	27.28
ICPW 315	98.56	103.31	32.82	0.22	149.53	1.91	0.69	4.38	4.31	2.76	11.50	24.44	27.62
<b>Mean</b>	<b>75.73</b>	<b>118</b>	<b>32.55</b>	<b>0.204</b>	<b>161.52</b>	<b>2.42</b>	<b>0.65</b>	<b>4.99</b>	<b>4.88</b>	<b>2.27</b>	<b>10.95</b>	<b>21.73</b>	<b>25.71</b>
<b>SEM</b>	<b>2.893</b>	<b>3.687</b>	<b>1.387</b>	<b>0.009</b>	<b>3.007</b>	<b>0.039</b>	<b>0.011</b>	<b>0.104</b>	<b>0.108</b>	<b>0.085</b>	<b>0.389</b>	<b>1.657</b>	<b>0.368</b>
<b><i>C.cajan</i> varieties</b>													
ICP 26	52.25	103.91	49.11	0.32	153.81	5.36	0.73	4.7	4.67	11.64	6.13	4.53	23.45
ICP 28	56.49	97.93	32.95	0.21	159.32	5.5	0.79	4.59	3.91	11.36	9.79	2.87	23.84
ICP 8518	66.94	98.22	35.98	0.19	188.61	4.68	0.66	4.8	4.88	10.45	8.97	4.57	19.71
ICP 8863	66.64	106.1	44.53	0.31	144.21	5.62	0.68	5.58	4.94	14.49	9.66	8.91	21.62
ICP 14722	71.75	105.5	50.83	0.26	194.62	5.61	0.75	4.52	4.72	14.6	8.89	2.81	19.72
ICP 14770	68.39	93.02	34.57	0.23	172.51	4.67	0.58	4.7	4.7	11.57	11.93	6.32	19.73
<b>Mean</b>	<b>63.74</b>	<b>99.5</b>	<b>41.38</b>	<b>0.253</b>	<b>168.85</b>	<b>5.24</b>	<b>0.69</b>	<b>4.82</b>	<b>4.63</b>	<b>12.352</b>	<b>9.23</b>	<b>5.02</b>	<b>21.34</b>
<b>SEM±</b>	<b>3.1</b>	<b>2.133</b>	<b>3.915</b>	<b>0.012</b>	<b>8.148</b>	<b>0.182</b>	<b>0.031</b>	<b>0.158</b>	<b>0.152</b>	<b>0.751</b>	<b>0.765</b>	<b>0.944</b>	<b>0.789</b>
<b>Trial mean</b>	<b>73.15</b>	<b>113.7</b>	<b>34.01</b>	<b>0.21</b>	<b>162.7</b>	<b>2.89</b>	<b>0.66</b>	<b>4.98</b>	<b>4.84</b>	<b>3.95</b>	<b>10.66</b>	<b>18.94</b>	<b>24.97</b>
<b>SEM</b>	<b>0.336</b>	<b>0.776</b>	<b>0.246</b>	<b>0.003</b>	<b>2.973</b>	<b>0.064</b>	<b>0.011</b>	<b>0.086</b>	<b>0.098</b>	<b>0.128</b>	<b>1.699</b>	<b>0.357</b>	<b>0.014</b>
<b>CD (5%)</b>	<b>0.937</b>	<b>2.164</b>	<b>0.684</b>	<b>0.008</b>	<b>8.289</b>	<b>0.178</b>	<b>0.031</b>	<b>0.24</b>	<b>0.275</b>	<b>0.357</b>	<b>4.738</b>	<b>0.994</b>	<b>0.038</b>
<b>CV (%)</b>	<b>1.126</b>	<b>1.675</b>	<b>1.769</b>	<b>3.342</b>	<b>4.544</b>	<b>5.422</b>	<b>4.253</b>	<b>4.295</b>	<b>5.059</b>	<b>7.933</b>	<b>49.51</b>	<b>4.614</b>	<b>0.135</b>

50.83 mm<sup>2</sup>. The mean leaf area of *C. cajan* group was 41.38 mm<sup>2</sup> and the overall mean was 34.01 mm<sup>2</sup> (Table 8).

### **Leaf dry weight**

Among the *C. scarabaeoides* accessions, ICPW 83 had a leaf dry weight of 0.10 g and ICPW 90, with highest dry weight of 0.28 g, with a group mean of 0.20 g whereas, in *C. cajan* genotypes it ranged from 0.19 g (ICP 8518) to 0.32 g (ICP 26) with a group mean of 0.25 g and with an overall mean leaf dry weight of 0.21 g (Table 8)

### **Specific leaf area**

Specific leaf area was greater in the wild accessions compared to *C. cajan* genotypes. Among the *C. scarabaeoides* accessions it ranged from 133.11 mm<sup>2</sup> / g in ICPW 94 to 193.13 mm<sup>2</sup> / g in ICPW 138 with a group mean of 161.52 mm<sup>2</sup> / g. Among the *C. cajan* genotypes it ranged between 144.21 mm<sup>2</sup> / g in ICP 8863 to 194.62 in ICP 14722 with a group mean of 168.9 mm<sup>2</sup> / g and with an overall mean of 162.70 mm<sup>2</sup> / g (Table 8).

### **Pod length**

Pods of *C. cajan* genotypes were longer than the wild types. Among the *C. scarabaeoides* accessions, pods of ICPW 315 were the shortest with a mean length of 1.91 cm whereas, those of ICPW 130 were the longest with a length of 2.64 cm and a group mean of 2.42 cm . Among the *C. cajan* genotypes, ICP 14770 with a pod length of 4.67 cm had the shortest pods and ICP 8863 with a mean length of 5.62 cm had the longest pods. The *C. cajan* group mean pod length was 5.24 cm, with an overall mean length of 2.89 cm (Table 8).

## **Pod width**

*C. scarabaeoides* accessions and *C. cajan* varieties had comparable pod width. ICPW 310 had the least pod width of 0.53 cm, whereas it was the maximum of 0.75 cm in ICPW 138, with a group mean of 0.65 cm . Among the *C. cajan* genotypes it ranged from 0.58 cm in ICP 14770 to 0.79 cm in ICP 28 with a group mean of 0.69 cm and with an overall mean of 0.66 cm (Table 8).

## **Number of locules per pod**

Among the *C. scarabaeoides* accessions, the number of locules was least in ICPW 86 and ICPW 310 (3.9 locules), whereas the highest number of 5.9 locules was in ICPW 132, while among *C. cajan* genotypes the number of locules ranged from 4.5 in ICP 14722 to 5.6 in ICP 8863. The *C. scarabaeoides* group mean (4.99 locules) was slightly higher than the *C. cajan* group mean (4.82 locules). Overall mean for this trait was 4.98 locules / pod (Table 8).

## **Number of seeds per pod**

ICPW 86 had the least number of seeds per pod (3.9) compared to the highest number (5.7) in ICPW 95 among the wild types, with a group mean of 4.9 seeds per pod. Among the cultivated, ICP 28 had the least number of 3.9 seeds per pod and ICP 8863 had a maximum of 4.9 seeds , with a group mean of 4.6 seeds per pod. The overall mean was 4.8 seeds per pod (Table 8).

## **100 - seed weight**

*C. cajan* genotypes had larger and heavier seeds (12.35 g) compared to *C. scarabaeoides* seeds (2.27 g) . Among the *C. scarabaeoides* accessions the 100 – seed weight ranged from 1.52g in ICPW 305 to 3.45g in ICPW 138 and among the *C. cajan* genotypes it was from 10.45g in ICP 8518 to 14.60g in ICP 14722. The overall mean of 100 – seed weight was 3.95g (Table 8)

## **Number of primary branches**

Among the *C. scarabaeoides* accessions, ICPW 90 had the least number of primary branches (8.2) compared to the highest number 17.6 in ICPW 147 with a group mean of 11 branches. Among the *C. cajan* genotypes the mean number of primary branches ranged from 6.1 in ICP 26 to 11.9 in ICP 14770 with a group mean of 9.2 and with an overall mean of 10.7 branches (Table 8).

## **Number of secondary branches**

Secondary branches were more in *C. scarabaeoides* accessions compared to the cultivated. Among the wild types, ICPW 111 had the least number of 8.4 branches compared to the maximum number of 41 branches in ICPW 116. The *C. scarabaeoides* group mean was 21.7 branches. Among the *C. cajan* genotypes the mean number of secondary branches ranged from 2.8 in ICP 14722 to 8.9 in ICP 8863. The *C. cajan* group mean was 5.0, with an overall mean of 18.9 branches (Table 8).

## **Seed protein**

Compared to the cultivated genotypes, the wild accessions had higher content of seed protein. Among the wild, ICPW 152 had the least protein content of 21.54 % compared to the highest content of 29.58 % in ICPW 96, with a group mean of 25.71 %. Among the *C. cajan* genotypes the protein content ranged from 19.71 % in ICP 8518 to 23.84 % in ICP 28 with a group mean of 21.34 % and with an overall mean protein content of 24.98 % (Table 8).

## **Interrelationships among accessions for morphological traits**

The MDS clustering (Fig 3) of 42 accessions based on combined data of all the 13 traits revealed that the wild and cultivated accessions belong to different



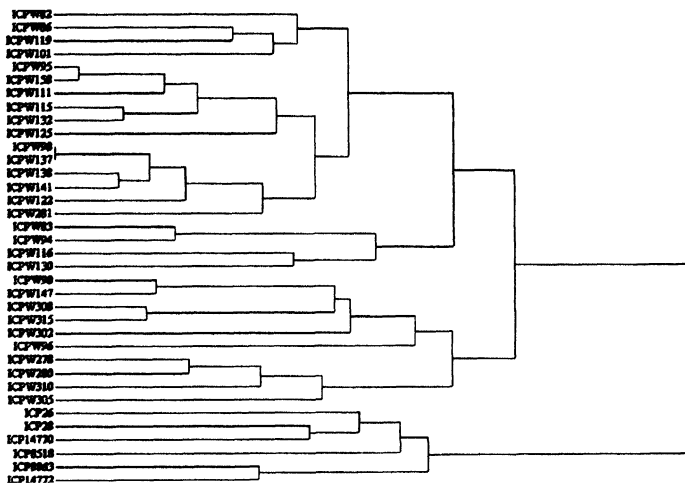


Fig. 2 UPGMA dendrogram of 36 genotypes for morphological and agronomic characters. List of accessions is given in Table 3.

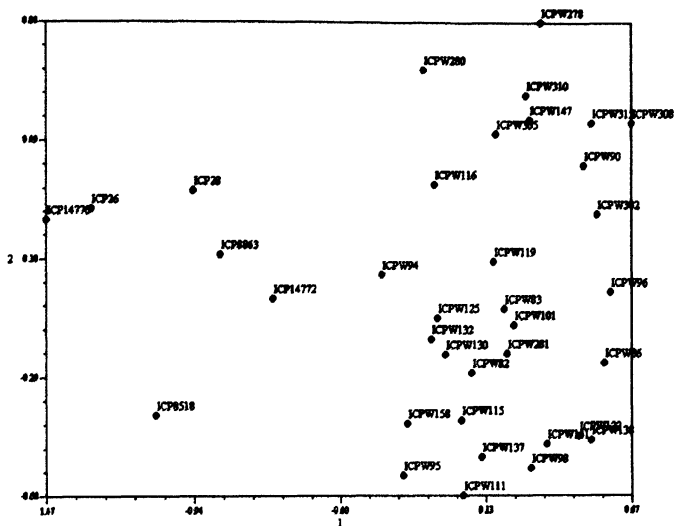


Fig. 3 MDS plot for of genotypes for various morphological and agronomic characters ( $r = 0.98$ ). List of accessions is given in Table 3

clusters. Under the major cluster of wild the accessions of Indian, Sri Lankan and Australian origin formed different sub-clusters. The accessions belonging to Philippines and Myanmar were grouped into another single sub cluster under the major wild cluster. Whereas, the cultivated genotypes formed only single cluster different from the wild accessions. The UPGMA grouping (Fig 2) of the combined data revealed hierarchical nature of the accessions for different characters. The grouping pattern observed in MDS (Fig 3) and UPGMA exhibited similar clustering and sub- clustering of the accessions.

## **Molecular diversity analysis**

Molecular diversity among the wild and cultivated pigeonpeas was studied using RFLP, AFLP and SSR markers.

### **RFLP analysis with maize mitochondrial DNA probes**

The three maize mitochondrial DNA probes (*atp 6*, *atp α* and *cox I*) in three restriction enzyme (*EcoR I*, *EcoRV* and *Hind III*) combinations hybridized to 42 accessions, representing three wild species (*C. scarabaeoides*, *C. sericeus* and *C. reticulatus*) and one cultivated pigeonpea, *C. cajan*. A high level of polymorphism was detected among various accessions by 9 enzymes – probe combinations (Table 9). Representative hybridization patterns are shown in (Fig 4 a, b and c).

The *EcoR I*- *atp 6* combination generated a maximum number of 14 hybridization bands ranging from 2.1 to 23.1 Kb. The *ECOR V* – *cox 1* combination was least polymorphic yielding only 5 bands ranging in size from 2 to 11.2 kb respectively (Table 9). With high levels of polymorphisms detected, the different enzyme – probe combinations were able to uniquely fingerprint (distinguish) all the 42 accessions, except *EcoR V* – *atp α* and *EcoR V* – *cox 1* which could not distinguish the accessions of *C. reticulatus* from *C. sericeus*. Number of unique banding patterns / haplotypes ranged from 8 in *EcoR V* – *atp α* to 10 in *Hind III* –

**Fig. 4: RFLP Southern blot, of wild *C. scarabaeoides* accessions and cultivated pigeonpea varieties, obtained using maize mt DNA probes**

a) *Eco* RI - atp  $\alpha$

b) *Eco* RI - atp  $\alpha$

M is the marker *Hind* III  $\lambda$  DNA

The accessions in the gel from L to R are listed in Table – 3.

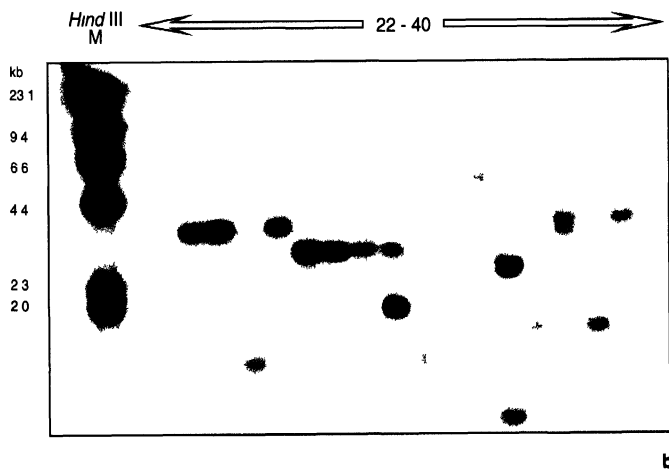
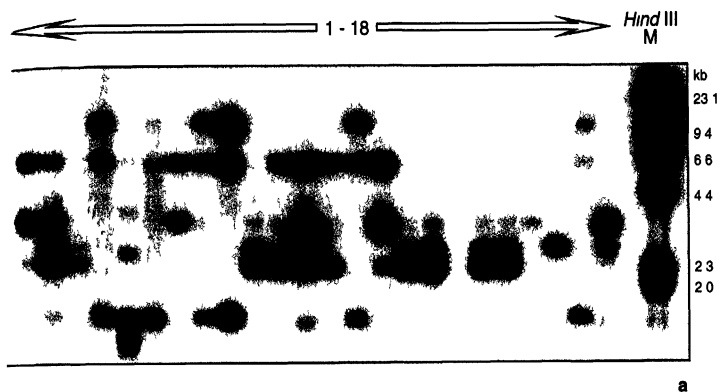


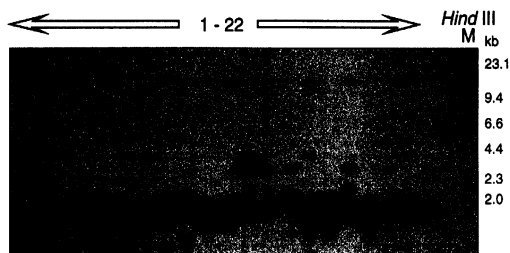
Fig: 4

**Fig. 4. RFLP Southern blot, of wild *C. scarabaeoides* accessions and cultivated pigeonpea varieties, obtained using maize mt DNA probes**

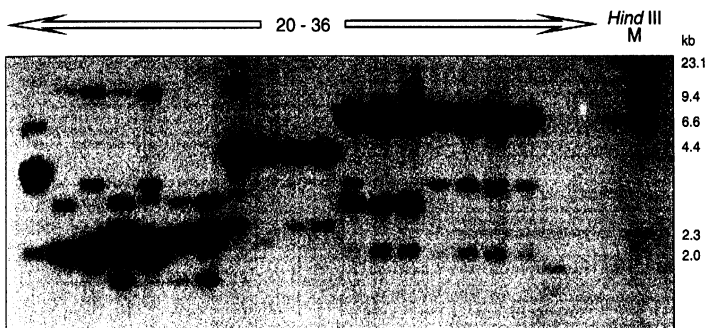
**c) *Eco* RI - atp 6      d) *Eco* RI - atp 6      e) *Hind* III - atp 6**

**M is the marker *Hind* III  $\lambda$  DNA**

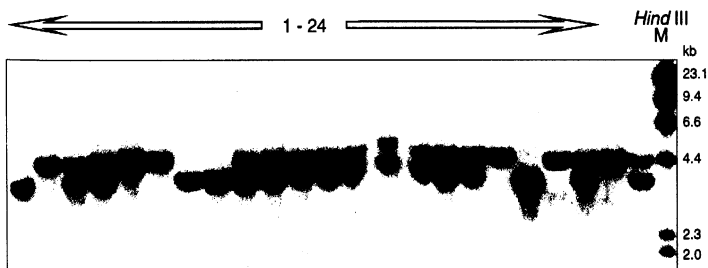
**The accessions in the gel from L to R are listed in Table – 3.**



c



d



e

Fig: 4

**Table - 9: Polymorphism and range of band sizes in *Cajanus* spp. with maize mt probes**

Enzyme – Probe combination	No. of bands	No. of polymorphic bands	Diversity index	Effective Multiplex Ratio	Marker Index	Size (Kb) of bands
ECoR1- <i>atp6</i>	14	14	0.88	14.00	12.32	2.1 to 23.1
Hind 111 – <i>atp 6</i>	10	9	0.89	8.10	7.21	2.0 to 9.2
ECoRV – <i>atp6</i>	6	5	0.84	4.17	3.50	3.0 to 14.8
ECoR1- <i>atp α</i>	13	13	0.82	13.00	10.66	2.0 to 15.8
Hind 111 – <i>atp α</i>	10	10	0.87	10.00	8.70	7.7 to 14.4
ECoRV – <i>atp α</i>	8	7	0.82	6.13	5.03	2.1 to 11.2
ECoR1- <i>cox 1</i>	11	11	0.94	11.00	10.34	4.1 to 14.2
Hind 111 – <i>cox 1</i>	9	8	0.81	7.11	5.76	3.4 to 11.6
ECoRV – <i>cox 1</i>	5	5	0.82	5.00	4.10	2.0 to 11.2
Mean	9.55 ± 0.987	9.11 ± 1.070	0.85 ± 0.050	8.73 ± 1.16	7.51 ± 1.050	
Polymorphism (%)	95.34%					
Bootstrap value (0.85 – 0.96) at 95% confidence level						

**Table - 10: RFLP Banding pattern in species of *Cajanus***

Enzyme – Probe combination	<i>C.sc</i>	<i>C.se</i>	<i>C.re</i>	<i>C.ca</i>	No. of unique patterns across species
ECoR1- <i>atp6</i>	7	2	1	3	13 (2 shared between <i>C.scarabaeoides</i> and <i>C.cajan</i> )
Hind 111 – <i>atp 6</i>	6	1	1	2	10 (none shared between the species )
ECoRV – <i>atp6</i>	3	1	0	2	6 (none shared between the species)
ECoR1- <i>atp α</i>	8	1	1	3	13 (1 shared between <i>C.scarabaeoides</i> and <i>C.sericeus</i> and 1 shared between <i>C.scarabaeoides</i> and <i>C.cajan</i> )
Hind 111 – <i>atp α</i>	5	2	1	2	10 (2 shared between <i>C.scarabaeoides</i> and <i>C.cajan</i> )
ECoRV – <i>atp α</i>	4	1	1	2	8 (1 shared between <i>C.scarabaeoides</i> and <i>C.cajan</i> )
ECoR1- <i>cox 1</i>	7	1	1	2	11 (1 shared between <i>C.scarabaeoides</i> and <i>C.sericeus</i> ; 2 shared between <i>C.scarabaeoides</i> and <i>C.cajan</i> )
Hind 111 – <i>cox 1</i>	5	2	0	2	9 ) (none shared between species)
ECoRV – <i>cox 1</i>	2	1	0	2	5 (none shared between species)
<i>C.sc</i> = <i>C.scarabaeoides</i> ; <i>C.se</i> = <i>C.sericeus</i> ; <i>C.re</i> = <i>C.reticulatus</i> ; <i>C.ca</i> = <i>C. cajan</i>					



*atp 6* combination (Table 10). More hybridizing restriction fragments were revealed in *C. scarabaeoides* accessions than *C. cajan*.

*EcoR V – atp 6* and *EcoR V – atp  $\alpha$*  were the only two combinations in which none of the 6 and 8 patterns generated respectively were shared between any of the genotypes (Table 10).

The effective multiplex ratio ranged from 5.00 in *EcoR V- cox 1* to 14.00 in *EcoR 1 – atp 6* combination with an average value of  $12.33 \pm 5.56$ . Marker Index ranged between 4.10 in *ECOR V- cox 1* to 12.32 in *ECOR 1 – atp 6* with an average value of  $10.65 \pm 4.99$  (Table 9).

### Inter-relationships among the accessions

The diversity index (Hav) ranged from 0.81 in *Hind 111 – cox 1* combination to 0.94 in *EcoR 1 – cox 1* combination. Pair wise similarities (Sij) among the *C. scarabaeoides* accessions ranged from 0.52 to 1.00 with an average of  $0.71 \pm 0.21$ . Accessions belonging to the same geographical region grouped together with similarity values for accessions of Indian origin ( $S_{ij} = 0.61 \pm 0.12$ ), Australian origin ( $S_{ij} = 0.70 \pm 0.11$ ), Sri Lankan origin ( $S_{ij} = 0.64 \pm 0.04$ ) and accessions from Indonesia and Myanmar ( $S_{ij} = 0.62 \pm 0.11$ ) (Fig. 5). Further, the *C. scarabaeoides* accessions from Australia were the most distinct from that of Indian and Sri Lankan origin accessions. The similarity values for the accessions of *C. sericeus* were  $S_{ij} = 0.75 \pm 0.14$ , *C. reticulatus*  $S_{ij} = 0.24$  and *C. cajan*  $S_{ij} = 0.71 \pm 0.15$  (Fig. 5).

The MDS plot (Fig. 6) revealed four distinct groups. First group of *C. scarabaeoides* comprises four distinct sub – groups. The accessions from India, Sri Lanka and Australia belong to three different subgroups while the accessions from Phillipines and Myanmar formed the fourth sub-group lying in between the Indian and Sri Lankan sub-groups. The accessions of *C. sericeus*, *C. reticulatus* and the genotypes of *C. cajan* formed the other three major groups. The stress value was  $r =$

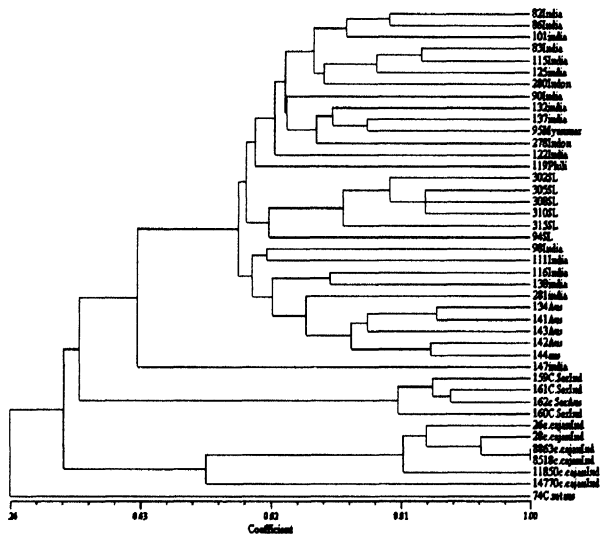


Fig. 5 UPGMA Dendrogram of 42 genotypes of wild and cultivated *Cajanus* spp. using RFLP markers ( $r = 0.98$ ). Accession identities are listed in Table 3.



0.95. The UPGMA dendrogram (Fig 5) revealed the hierarchical structure of 42 accessions. UPGMA dendrogram distinguished the four groups of *C. scarabaeoides*, *C. sericeus*, *C. reticulatus* and *C. cajan*. The four sub-groups of *C. scarabaeoides* accessions from Indian, SriLankan, Australian and Phillipines and Myanmar were placed in group I. Group 2 comprised of four accessions of *C. sericeus*, Group 3 comprises only the single accession of *C. reticulatus* and group 4 comprises the genotypes of *C. cajan*.

## AFLP analysis

AFLP analysis, of forty-two accessions belonging to four species, was done using five primer pair combinations; E- ACT M-CTC, E – AGG M – CAC, E-ACG M- CAT, E-ACG M- CTA and E-ACG M-CTT. Representative AFLP profiles were given in Fig 7 a, b and c. A total of 438 scorable bands were detected across the 42 accessions. All bands that could be reliably read on the autoradiogram were treated as individual dominant loci and scored as 1. The alternative form of an allele was scored as zero. The number of scorable bands ranged from 69 for E- ACG M- CAT to 129 for E – ACT M- CTC. Fraction of polymorphic bands ( $\beta$ ) ranged from 0.94 in E-AGG M-CAC to 0.97 in E-ACG M-CTT, with an average value of  $0.97 \pm 0.02$  (Table 11). Polymorphism, gene diversity, Effective Multiplex ratio and Marker Index values are given in Tables 11 and 12. The gene diversity ( $H_{av}$ ) ranged from 0.66 for E-AGG M- CAC to 0.83 for E-ACG M-CAT. The Effective Multiplex ratio ranged from 55.61 in E- ACG M- CAT to 113.74 in E-ACT M-CTC (Table 11).

## Inter relationships among accessions

Jaccards pair wise similarity coefficient ( $S_{ij}$ ) for all the 42 accessions ranged from 0.24 to 1.00 with an average of  $0.51 \pm 0.26$ . In *C. scarabaeoides*, the similarity coefficient for accessions of Indian origin was  $0.64 \pm 0.14$ , Australian origin was  $0.75 \pm 0.05$  and Sri Lankan origin was  $0.72 \pm 0.12$ . Two separate groupings for accessions

**Fig. 7: AFLP profiles of wild and cultivated pigeonpeas**

**The primer combination:**

**a) EACT MCTC**

**The accessions in the gel from L to R are listed in Table – 3.**

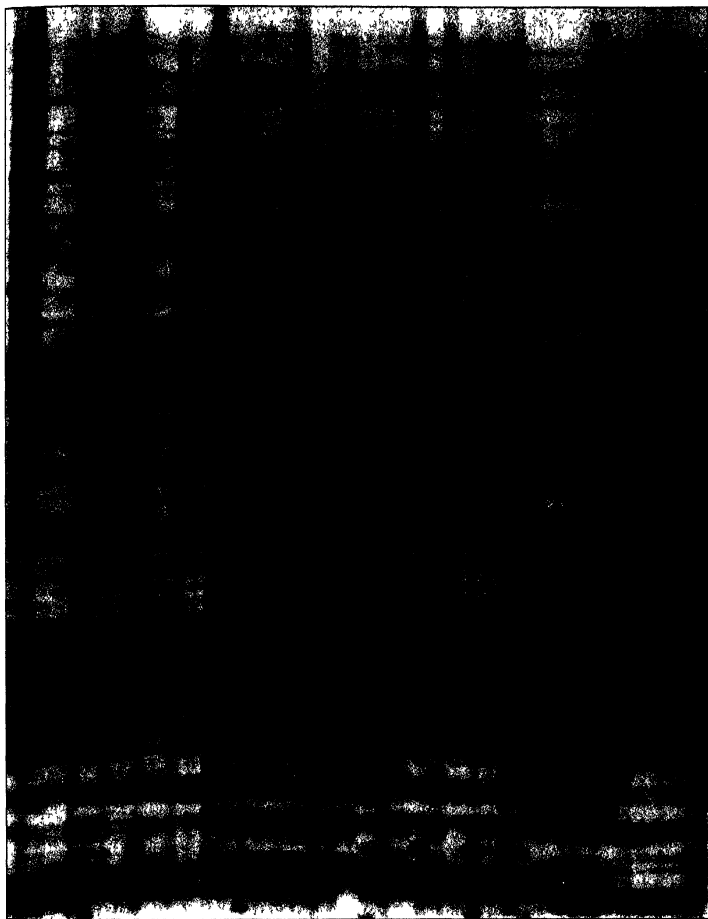


Fig: 7

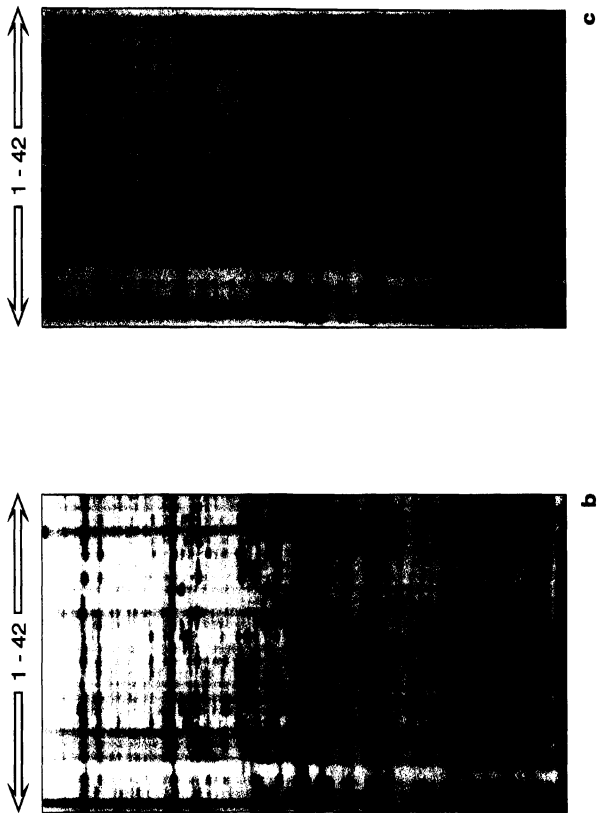
**Fig. 7: AFLP profiles of wild and cultivated pigeonpeas**

**The primer combination:**

**b) EACG MCAC; c) EAGG MCTT**

**The accessions in the gel from L to R are listed in Table – 3.**

**Fig: 7**





**Table - 11: Diversity index, effective multiplex ratio and Marker Index for AFLP markers.**

Primer combination	Total No. of bands	No. of polymorphic bands	Fraction of polymorphic bands ( $\beta$ )	Diversity Index (Hav)	Effective multiplex ratio (EMR)	Marker Index (MI)
E-ACT M-CTC	129	121	0.94	0.77	113.74	87.58
E-AGG M- CAC	94	89	0.94	0.66	83.66	55.25
E-ACG M-CAT	69	67	0.97	0.83	55.61	46.17
E-ACG M-CTA	78	72	0.92	0.74	66.24	49.02
E-ACG M -CTT	77	75	0.97	0.74	72.75	53.84
Total	447	426			404.70	303.53
Mean	89.45	85.2	0.95	0.75	80.94	60.71
Polymorphism (%)	95.31%					
Bootstrap value (0.85 – 0.91) at 95% confidence interval						

**Table - 12: Polymorphism and gene diversity in pigeonpeas using AFLPs**

Primer combination	P (%) G (Hav)	<i>C.scarabaeoides</i>	<i>C.sericeus</i>	<i>C.reticulatus</i>	<i>C.cajan</i>
E-ACT M-CTC	P (%) G (Hav)	91.27 0.77	76.74 0.70	a 0.79	84.49 0.79
E-AGG M- CAC	P (%) G (Hav)	91.49 0.79	77.66 0.67	a 0.79	79.78 0.72
E-ACG M-CAT	P (%) G (Hav)	98.56 0.82	82.61 0.77	a 0.74	82.61 0.80
E-ACG M-CTA	P (%) G (Hav)	94.87 0.81	79.49 0.71	a 0.81	79.14 0.75
E-ACG M -CTT	P (%) G (Hav)	96.10 0.82	75.32 0.76	a 0.82	75.32 0.75
Combined primer combinations	P (%) G (Hav)	92.6 0.80±0.07	78.07 0.72± 0.13	a 0.79±0.0 3	80.76 0.76±0 .87
P = Polymorphism (%); G = Gene diversity(Hav); Boot strap at 95% confidence level (0.85 – 0.91)					
<sup>a</sup> = only one accessions in this species was included					

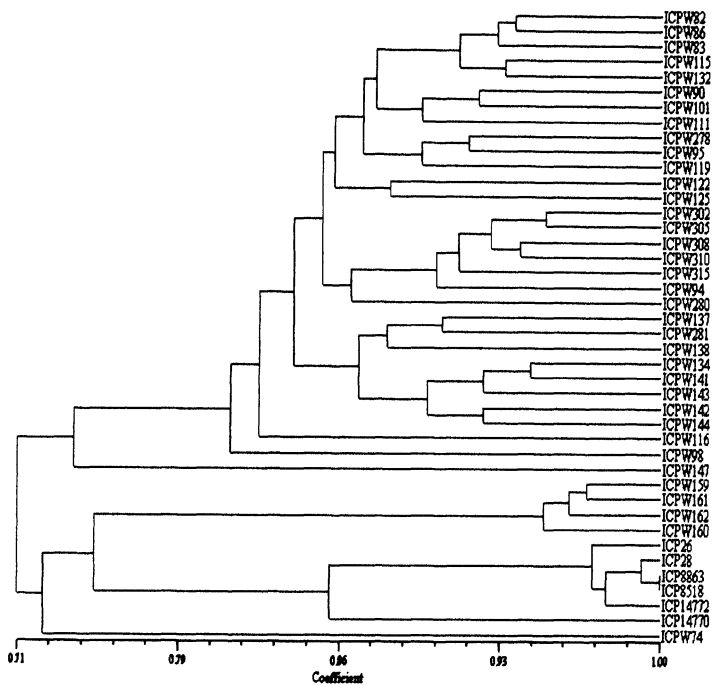


Fig. 8 UPGMA Dendrogram of 42 genotypes of wild and cultivated *Cajanus* spp. using AFLP markers ( $r = 0.98$ ). Accession identities are listed in Table 3.

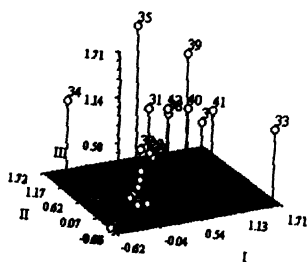


Fig. 9. MDS plot of 42 genotypes of wild and cultivated pigeonpea using AFLP markers ( $r = 0.95$ ).

of *C. scarabaeoides* from India were seen, the early flowering group ( $S_{ij} = 0.79 \pm 0.12$ ) and the medium flowering group ( $0.62 \pm 0.14$ ) separated by late flowering Sri Lankan accessions ( $S_{ij} = 0.75 \pm 0.04$ ) and medium to late flowering Indonesian and Myanmar accessions ( $S_{ij} = 0.71 \pm 0.05$ ). The Australian origin medium flowering accessions formed a completely different group ( $S_{ij} = 0.84 \pm 0.07$ ). Similarity coefficient values were  $0.81 \pm 0.10$  for *C. sericeus* and  $0.56 \pm 0.21$  for *C. cajan*. The least similarity to all other species was found in *C. reticulatus* ( $S_{ij} = 0.24$ ) (Fig 8).

The MDS plot (Fig 9) grouped the 42 accessions into four distinct major clusters. The hierarchical structures of these clusters were revealed in UPGMA based dendrogram (Fig 8). All *C. scarabaeoides* accessions clustered together, were subclustered based on the geographical regions viz ;the Indian, Sri Lankan Australian and Myanmar and Indonesian origin. *C. sericeus*, *C. reticulatus* and *C. cajan* formed the other three different groups with no specific sub- clusters (Fig 8).

## SSR analysis

Ten SSR primer pairs were used to study the diversity among 42 accessions of 4 *Cajanus* species, of which only eight primer pairs amplified the alleles in all the accessions. High polymorphism was observed among the *C. cajan* accessions where all the eight pairs amplified the alleles, while among the wild species *C. scarabaeoides*, *C. sericeus*, and *C. reticulatus* only seven out of eight amplified the alleles (Fig 10 a, b and c).

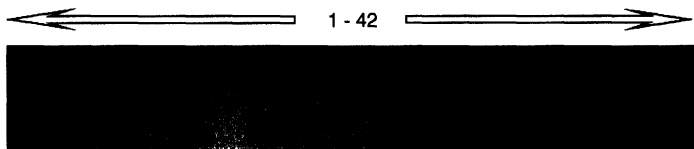
A total of 71 alleles were detected with an average allelic richness of 7.63 alleles per locus (Table 13). The number of alleles ranged from 3 for CCB4 to 14 for CCB1. Gene diversity was generally high, ranging from 0.62 to 0.92, with an average of 0.85 over all the loci (Table 13). When classified at the species level, the gene diversity was highest for *C. cajan* (0.80) followed by *C. scarabaeoides* (0.71), *C. sericeus* (0.68) and *C. reticulatus* (0.41) (Table 14). Primer pairs CCB4 amplified only in *C. cajan* (3 alleles) and failed to amplify in all other species. The number of

**Fig. 10. SSR profiles of wild and cultivated pigeonpeas**

**The primer combination:**

**a) CCB 3**

**The accessions in the gel from L to R are listed in Table – 3.**



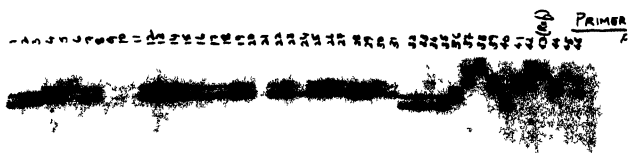
**Fig: 10**

**Fig. 10: SSR profiles of wild and cultivated pigeonpeas**

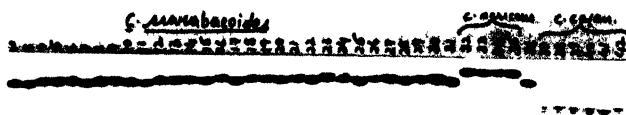
**The primer combination:**

**b) CCB 5;    c) CCB 7**

**The accessions in the gel from L to R are listed in Table – 3.**



a



b

Fig: 10



**Table - 13: Polymorphism and gene diversity in wild and cultivated Pigeonpeas with SSRs**

[illegible]

**Table - 14: Gene diversity and polymorphism in four species of *Cajanus* with SSRs**

S.No	Locus name	Total No. of alleles	H*	No. of alleles	H*	No. of alleles	H*	No. of alleles	H*	No. of alleles	H*
				<i>C.scaberrimoides</i>	<i>C.sericeus</i>	<i>C.reticulatus</i>		<i>C.cajan</i>			
1	CCB1	14	0.89	8	0.85	2	0.62	1	0.00	5	0.79
2	CCB2	*	*	*							
3	CCB3	*	*	*							
4	CCB4	3	0.62	0	0.00	0	0.00	0	0.00	3	0.75
5	CCB5	8	0.91	5	0.89	2	0.56	2	0.82	3	0.75
6	CCB6	9	0.86	4	0.81	3	0.84	2	0.71	2	0.68
7	CCB7	5	0.92	2	0.71	2	0.79	1	0.00	3	0.84
8	CCB8	5	0.89	3	0.81	2	0.66	2	0.68	3	0.82
9	CCB9	5	0.82	2	0.65	2	0.52	1	0.00	3	0.88
10	CCB10	12	0.89	7	0.89	3	0.84	2	0.72	5	0.89
	Total	61		31		16		11		27	
	Avg. H		0.89		0.71		0.68		0.41		0.80
	Polymorphism (%)		100%		82%		100%		90%		100%
Bootstrap –based on 95% Confidence level (0.89 – 0.94)											
*H : Gene diversity											

observed alleles was highest in *C. scarabaeoides* (31) followed by *C. cajan* (27), *C. sericeus* (16) and *C. reticulatus* (11). The Effective Multiplex ratio ranged from 3.00 for locus CCB4 to 14.00 for locus CCB1. The Marker Index ranged from 1.47 in CCB4 to 12.46 in locus CCB1. Bootstrapping value, based on 95 % confidence interval, ranged from 0.89 to 0.94 (Table 13).

### **Interrelationships among accessions**

The accessions belonging to different regions (India, Sri Lanka, Australia, Phillipines and Indonesia) were significantly differentiated based on the MDS clustering (Fig. 12) pattern at a stress value of  $r = 0.94$ . All the four different species *C. scarabaeoides*, *C. sericeus*, *C. reticulatus* and *C. cajan* formed four different clusters. The hierarchical structure of these clusters was revealed in UPGMA based dendrogram (Fig. 11). The Indian origin *C. scarabaeoides* accessions (both early and medium duration flowering) formed only one group, separate from the Sri Lankan, Australian and Indonesian and Phillipines clusters. *C. sericeus* accessions clustered into two different groups, one sub group of Indian origin and the other of Australian origin. *C. reticulatus* was placed between *C. cajan* and *C. sericeus* (Fig. 11).

Among the three markers, 100% polymorphism was observed for SSR markers, followed by the AFLPs and RFLPs, but the highest effective multiplex ratio of 80.94 and marker index value of 60.71 were observed for AFLPs. The diversity index was maximum for SSRs (0.89) (Table 15).

### **Screening for podborer resistance**

Season was not found to be significant for any of the parameters, of the podborer resistance, studied. Habit was found to be significant for percentage bud, flower and pod damage, number of eggs/inflorescence and number of larvae / inflorescence (Table 16). Season x habit interaction was non- significant for all the parameters recorded except for number of eggs per inflorescence. However, the season x wild habit was found significant for number of eggs and larvae per

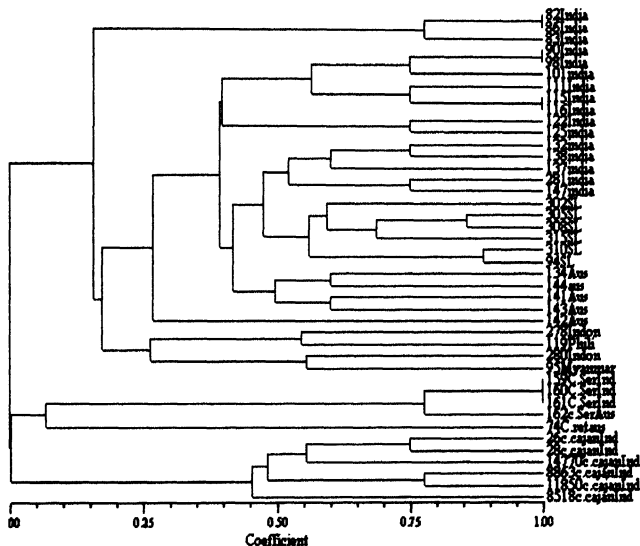


Fig. 11 UPGMA Dendrogram of 42 genotypes of wild and cultivated *Cajanus* spp. using SSR markers ( $r = 0.95$ ). Accession identities are listed in Table 3.

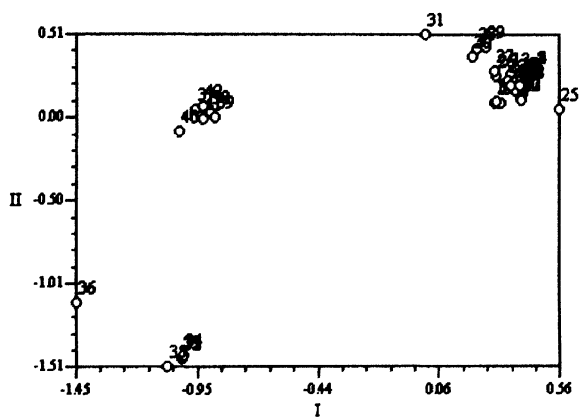


Fig. 12. MDS plot of 42 genotypes of wild and cultivated pigeonpea using SSR markers ( $r = 0.95$ ). Accessions are listed in Table 3.

**Table - 15: Relative effectiveness of Molecular Markers**

Marker	No. of Assay units	Total No. of Bands	No. of Polymorphic Bands	Percentage polymorphism (%)	No. of Bands / Assay unit	Effective Multiplex ratio	Marker Index	Diversity Index
AFLP	5 (Primer Combinations)	447	426	95.4%	89.82	80.94	60.71	0.75
RFLP	9 (Probe-Enzyme Combinations)	86	82	95.34%	14.00	8.72	7.51	0.85
SSR	8 (Primer Pairs)	61	61	100%	7.65	7.39	6.39	0.89

**Table - 16: Percentage damage among *C. scarabaeoides* and *C. cajan* accessions**

Accessions	Bud damage (%)	Flower Damage (%)	Pod damage (%)	No. of eggs	No. of larvae
<b><i>C. scarabaeoides</i> accessions</b>					
ICPW 82	0.42 (2.91) *	0.00 (0.00)	1.51(5.81)	0	0
ICPW 83	2.51 (7.32)	0.52 (2.31)	0.92(5.32)	0.24	0.11
ICPW 86	0.52 (3.32)	0.53 (2.42)	0.72(3.83)	0.12	0
ICPW 90	1.51 (5.63)	0.64 (3.73)	1.81(6.11)	0.14	0.12
ICPW 94	0.00 (0.00)	0.00 (0.00)	0.00(0.00)	0	0
ICPW 95	1.42 (5.35)	0.00 (0.00)	1.71(6.11)	0.16	0.11
ICPW 96	0.71 (3.93)	0.00 (0.00)	2.72(7.82)	0	0.21
ICPW 98	0.00 (0.00)	0.00 (0.00)	2.62(9.23)	0	0
ICPW 101	3.00 (8.26)	2.12 (6.92)	2.63(8.71)	0	0
ICPW 111	1.81 (6.22)	0.00 (0.00)	0.84(5.25)	0	0
ICPW 115	0.12 (1.22)	0.00 (0.00)	3.01(9.44)	0.12	0
ICPW 116	0.00 (0.00)	0.00 (0.00)	0.00(0.00)	0	0
ICPW 119	1.81 (6.21)	0.20 (1.43)	1.91(6.44)	0.16	0.11
ICPW 122	0.72 (2.82)	0.00 (0.00)	0.62(4.45)	0.22	0
ICPW 125	2.11 (7.83)	0.00 (0.00)	0.00(0.00)	0	0
ICPW 130	0.00 (2.32)	0.32 (1.73)	0.93(5.21)	0	0
ICPW 132	0.72 (2.82)	0.00 (0.00)	1.12(5.61)	0	0
ICPW 137	2.84 (5.63)	0.00 (0.00)	1.21(3.62)	0	0.11
ICPW 138	1.12 (4.91)	1.71 (6.15)	2.52(8.94)	0	0
ICPW 141	0.00 (0.00)	0.00 (0.00)	0.00(0.00)	0	0
ICPW 142	0.92 (3.23)	1.51 (5.31)	3.21(9.86)	0	0.11
ICPW 144	0.93 (3.21)	1.53 (5.32)	3.22(9.84)	0	0.11
ICPW 147	0.00 (0.00)	0.00 (0.00)	0.21(1.68)	0	0
ICPW 152	1.95 (4.63)	0.00 (0.00)	2.21(7.59)	0.25	0
ICPW 278	0.00 (0.00)	0.71 (2.84)	4.25(11.32)	0.26	0
ICPW 280	1.62 (5.33)	3.92 (11.33)	4.12(11.13)	0	0.12
ICPW 281	0.00 (0.00)	0.00 (0.00)	0.00(0.00)	0	0
ICPW 302	0.86 (4.24)	0.52 (3.41)	1.61(5.91)	0.11	0.1
ICPW 308	3.32 (10.14)	4.32 (11.74)	2.62(8.82)	0.11	0.11
ICPW 305	0.00 (0.00)	0.32 (2.45)	0.33(2.43)	0	0
ICPW 310	0.23 (1.52)	0.55 (2.33)	0.62(3.54)	0.11	0
ICPW 315	0.33 (1.74)	0.55 (2.43)	0.33(1.73)	0.12	0
Total	0.99	0.77	1.77	0.07	0.04
SEM±	0.174	0.209	0.228	0.016	0.011
<b><i>C. cajan</i> genotypes</b>					
ICP 26	20.12 (33.36)	27.91 (31.64)	27.12(31.44)	4.81	4.59
ICP 28	23.83 (27.94)	4.22 (9.76)	29.33(32.75)	5.62	5.98
ICP 8863	13.32 (17.55)	14.74 (22.13)	36.11(55.83)	3.92	5.29
ICP 14770	15.33 (22.86)	22.83 (28.55)	26.72(37.38)	2.43	4.59
ICP 14772	15.63 (23.22)	5.24 (12.84)	12.41(20.66)	3.35	6.98
ICPL 332 (Resistant)	4.33 (11.91)	8.40 (16.16)	6.23(14.35)	1.25	0.96
ICP 8518 (Susceptible)	27.84 (31.33)	14.23 (22.23)	32.33(56.23)	7.13	8.97
Total	17.65	14.99	26.34	4.03	5.49
SEM±	1.904	4.688	3.868	0.55	0.454
Overall mean	4.33	8.41	6.23	1.25	0.96
SEM±	27.84	14.23	32.33	7.13	8.97
CD (5%)	8.709	4.482	3.391	0.565	0.157
CV (%)	29	88.24	30.43	65.19	56.6

\* Figures in parenthesis are the angular transformation of the original data.

inflorescence (Table 6). Season x cultivated habit was significant for percentage pod damage, number of eggs and larvae / inflorescence (Table 6).

Damage on *C. scarabaeoides* accessions was less compared to the pigeonpea genotypes. The percentage bud, flower and pod damage; and the number of eggs and larvae/ inflorescence on *C. scarabaeoides* accessions were less compared to pigeonpea genotypes.

### **Bud damage**

Nine (ICPW 94, ICPW 98, ICPW 116, ICPW 130, ICPW 141, ICPW 147, ICPW 278, ICPW 281 and ICPW 305) of the thirty *C. scarabaeoides* accessions screened showed 0.00 % bud damage, 11 accessions (ICPW 82, ICPW 86, ICPW 96, ICPW 115, ICPW 122, ICPW 132, ICPW 142, ICPW 144, ICPW 302, ICPW 310 and ICPW 315) showed less than 1.00 % (Table 16). Among the *C. scarabaeoides* accessions, ICPW 308 showed the maximum bud damage of 3.32 % which was however lower than the resistant pigeonpea check ICPL 332 (4.33 %), which was included as a control. Among six *C. cajan* genotypes (excluding ICPL 332), ICP 8863 showed minimum bud damage of 13.32 % while ICP 8518, the susceptible check, exhibited the maximum damage of 27.84 % (Table 16). The mean damage in this group was 17.65 % and an overall damage mean was 4.33 % (Table 16).

### **Flower damage**

Sixteen accessions (ICPW 82, ICPW 94, ICPW95, ICPW 96, ICPW 98, ICPW 111, ICPW 115, ICPW 116, ICPW 122, ICPW125, ICPW 132, ICPW 137, ICPW141, ICPW147, ICPW 152 and ICPW 281) showed 0.00 % flower damage (Table 16). Ten accessions showed less than 1.00 % flower damage. ICPW 308 showed the maximum damage of in this group of 4.32 %. The mean flower damage was 0.77 %. Among the six *C. cajan* genotypes the flower damage ranged from 4.22 % in ICP 28 to 27.91 % in ICP 26 with a group mean of 14.99 % (Table 16). Two genotypes, ICP 28 and ICP 14722 had significantly lower flower damage than the

resistant check ICPL 332 (8.40 %) while ICP 26 and ICP 14770 exhibited significantly higher flower damage than the susceptible check ICP 8518 (14.23 %) (Table 16).

### **Pod damage**

Six (ICPW 94, ICPW 141, ICPW 116, ICPW 130, ICPW 125 and ICPW 281) of the thirty *C. scarabaeoides* accessions showed a pod damage of 0.00 %. Nine accessions exhibited less than 1.00 percentage damage. The maximum damage of 4.25 percentages was observed in ICPW 278, in this group, with a group mean of 1.77 percentages. Among the six *C. cajan* genotypes, the pod damage ranged from 12.41 percentage in ICP 14722 to 36.11 percentage in ICP 8863 with a group mean of 26.34 percentage (Table 16). All the *C. cajan* accessions showed significantly higher damage compared to the resistant check ICPL 332 (6.23 percentage) while four accessions (ICP 26, ICP 28, ICP 14770 and ICP 14722) exhibited significantly lower damage than the susceptible check ICP 8518 (32.33 percentage). The overall mean percentage of pod damage was 6.23 percentage (Table 16).

### **Number of eggs / inflorescence**

Nineteen accessions of *C. scarabaeoides* (ICPW 82, ICPW 86, ICPW 94, ICPW 98, ICPW 101, ICPW 111, ICPW 115, ICPW 116, ICPW 122, ICPW 125, ICPW 130, ICPW 132, ICPW 138, ICPW 141, ICPW 147, ICPW 152, ICPW 278, ICPW 281 and ICPW 305) did not have eggs on their inflorescences (Table 16). The remaining accessions had on an average less than 1.00 egg per inflorescence. In this group, the mean number of eggs per inflorescence was 0.07. Among the *C. cajan* genotypes ICP 14770 had the lowest (2.43) number of eggs/ inflorescence and ICP 28 had the highest (5.62) number of eggs / inflorescence, with a group mean of 4.03 eggs / inflorescence. All the *C. cajan* genotypes had higher number of eggs / inflorescence compared to the resistant check ICPL 332 (1.25 eggs / inflorescence) and hence, all of them had less number of eggs/ inflorescence than the susceptible check, ICP 8518 (7.13 eggs / inflorescence) (Table 16).



## Number of larvae / inflorescence

Twenty-one (ICPW 82, ICPW 86, ICPW 94, ICPW 98, ICPW 101, ICPW 111, ICPW 115, ICPW 116, ICPW 122, ICPW 125, ICPW 130, ICPW 132, ICPW 138, ICPW 141, ICPW 147, ICPW 152, ICPW 278, ICPW 281 and ICPW 305, ICPW 310 and ICPW 315) of the thirty *C. scarabaeoides* accessions had 0.00 larvae on the inflorescence and a maximum of 0.21 was noticed on ICPW 96 with a group mean of 0.04 larvae per inflorescence. Among the *C. cajan* genotypes ICP 26 had lowest number of larvae (4.59 larvae/ inflorescence) and ICP 14772 had the highest number (6.98 larvae / inflorescence) with a group mean of 5.49 larvae / inflorescence. All *C. cajan* genotypes had more number of larvae than the resistant pigeonpea check ICPL 332 (0.96 larvae / inflorescence), but less number of larvae compared to the susceptible check ICP 8518 (8.97 larvae / inflorescence). The overall mean number of larvae / inflorescence was 0.96 for all the 36 genotypes (Table 16).

## Biochemical diversity analysis

Trypsin inhibitors and lectins play an important role in conferring resistance against podborer. The quantification was carried at three pod stages, the juvenile, immature and mature. Juvenile, immature and mature stages were found significant for protein and lectin contents, but wild and cultivated habit was significant for protein and trypsin inhibitor content. Stage x habit interaction was significant for only trypsin inhibitor content. Genotype and genotype x stage interaction was significant for all the three traits studied. Genotype x wild habit was significant for protein and lectin content, while it was non-significant in the cultivated habit (Table 17).

## Protein content

In *C. scarabaeoides* accessions, mature pods had maximum protein content (3.19g) followed by immature (3.08g) and juvenile pods (2.76g) (Fig. 13). In the

**Table - 17: Significance of differences between three maturity stages, habit and their interaction in wild and cultivated *Cajanus***

Character	Protein (mg)			Trypsin inhibitor (units/mg)			Lectin content (HAU/mg)		
Interaction	Walds statistics	Wald / df ( $\chi^2$ value)	$\chi^2$ probability	Walds statistics	Wald / df ( $\chi^2$ value)	$\chi^2$ probability	Walds statistics	Wald / df ( $\chi^2$ value)	$\chi^2$ probability
Stage	28.83	14.41	<0.001**	3.73	1.86	0.155	108.91	54.45	<0.001**
Habit	15.94	15.94	<0.001**	30.77	30.77	<0.001**	1.24	1.24	0.265
Stage x Habit	6.74	3.37	0.034*	20.25	10.13	<0.001**	0.78	0.39	0.678
Variance components									
Interaction	Variance component	Standard error	Variance component	Standard error	Variance component	Standard error			
Genotype	0.40	0.107**	0.01	0.004**	3075.89	717.27**			
Genotype x stage	0.11	0.021**	0.02	0.005*	51406.49	8819.45**			
Genotype x habit (wild)	0.12	0.02**	0.05	0.005	55362.00	10174.10**			
Genotype x Habit (cult.)	0.03	0.017	0.03	0.014	18339.70	8039.60			
* = significant at 5% level; ** = significant at 1% level;									

**Fig: 13 Protein content in the pods of *C.scarabaeoides***

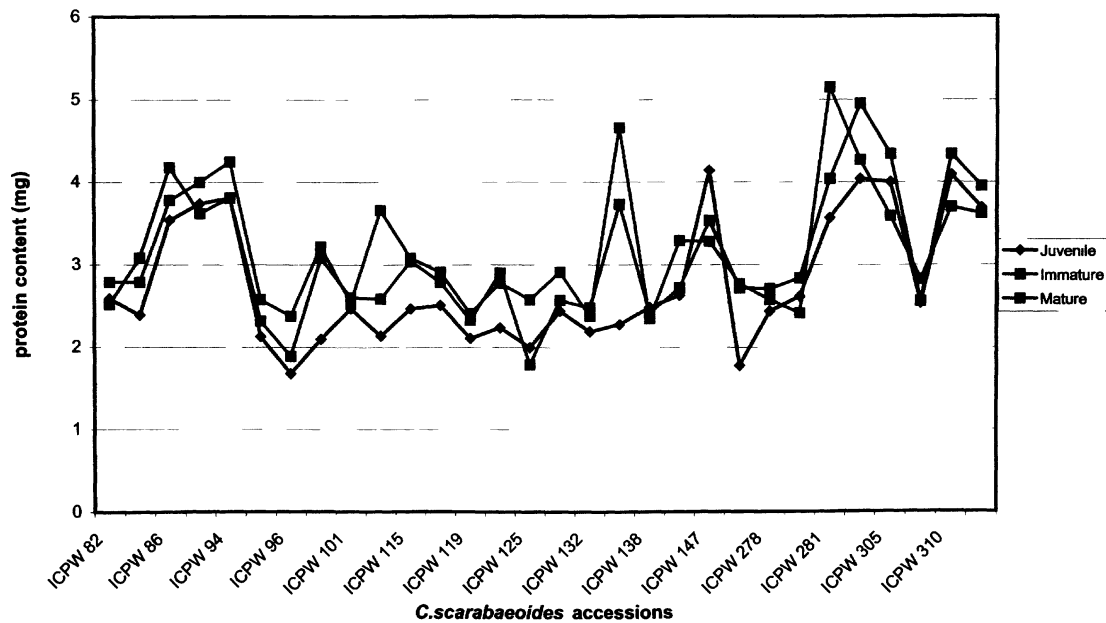
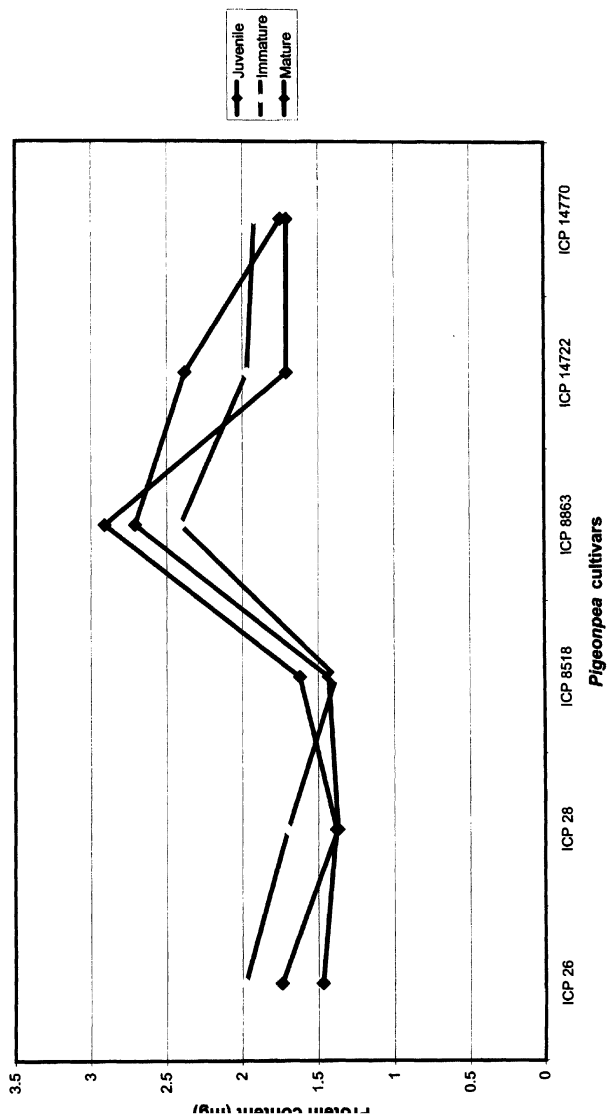


Fig: 14 Protein content in pods of cultivated Pigeonpea



juvenile pods, the highest protein content was observed in ICPW 147 (4.14g) and the least in ICPW 96(1.68g). In the immature pods the highest protein content was observed in ICPW 302 (4.95g) and the least in the ICPW 125 (1.79g). The mature pods of ICPW 281 showed the highest protein content (5.15g) and ICPW 138 the least with 2.35g proteins (Fig. 13). However, in *C. cajan* the protein content was equal in the juvenile and immature pods stages with a mean of 1.90 g followed by 1.80 g in the mature pods (Fig 14). In the juvenile pods, it was highest in ICP 8863 with 2.71g and the least content was in ICP 28 with 1.37g. The immature pods of ICP 8863 contained the highest protein content (2.41g) while ICP 8518 had the lowest (1.38g). The mature pods of ICP 8863 contained the highest protein content (2.91g) while ICP 28, the lowest (1.38g). The overall mean of both the wild and cultivated genotypes, the protein content was highest in mature pods (2.95g) followed by immature pods (2.88g) and the juvenile pods (2.62g) (Table 18).

### Trypsin inhibitor

The three pod stages of all the 30 wild accessions did not differ in the mean trypsin inhibitor content. The content of trypsin inhibitor was 1.55 units/mg in mature, 1.54 units/mg in immature and 1.55 units/mg in juvenile pods (Fig 15). Similarly, among the *C. scarabaeoides* accessions, significant differences were not observed in the trypsin inhibitor content in the juvenile (1.60 units/mg), immature (1.62 units/mg) and the mature pods (1.64 units/mg). However, individually the 30 *C. scarabaeoides* accessions differed significantly for trypsin inhibitor contents at the juvenile, immature and mature pod stages. In the juvenile pods, maximum trypsin inhibitor content was observed in ICPW 302 and ICPW 122 (1.79 units/mg) and the least in ICPW 111 (0.39 units/ mg). At the immature stage, maximum content was observed in ICPW 308 and ICPW 137 (1.85 units/mg) and minimum content in ICPW 111 (1.03 units/ mg). In the mature pods, maximum trypsin inhibitor content (1.86 units/mg) was found in ICPW 152 and the least content (1.44 units/mg) in ICPW 111. In the cultivated genotypes, the three pod stages differed significantly in their trypsin inhibitor content (Fig. 16). The juvenile pods had highest mean trypsin

**Table - 18: Content of protein, trypsin inhibitor and lectins at three maturity stages (juvenile, immature and mature) of *C.scarabaeoides* and *C.cajan***

Accession	Protein content (g)			Trypsin inhibitor (units/ g protein)			Specific Haemagglutination activity (HAU / g protein)		
	Juvenile	Immature	Mature	Juvenile	Immature	Mature	Juvenile	Immature	Mature
<i>C.scarabaeoides</i> accessions									
ICPW 82	2.59	2.52	2.79	1.46	1.46	1.51	988	127	0
ICPW 83	2.39	3.08	2.79	1.63	1.68	1.71	536	13	0
ICPW 86	3.54	4.18	3.78	1.72	1.62	1.54	362	77	0
ICPW 90	3.74	3.62	4	1.71	1.66	1.65	343	23	0
ICPW 94	3.81	3.81	4.25	1.68	1.61	1.57	672	168	0
ICPW 95	2.13	2.32	2.58	1.5	1.44	1.48	601	138	0
ICPW 96	1.68	1.89	2.38	1.34	1.46	1.56	762	170	0
ICPW 98	2.1	3.08	3.22	1.49	1.54	1.48	1220	0	0
ICPW 101	2.47	2.6	2.54	1.54	1.59	1.54	260	16	0
ICPW 111	2.14	2.59	3.66	0.39	1.03	1.44	300	31	0
ICPW 115	2.47	3.04	3.08	1.53	1.56	1.47	519	0	0
ICPW 116	2.51	2.79	2.91	1.59	1.72	1.7	1020	115	0
ICPW 119	2.11	2.33	2.41	1.71	1.71	1.77	304	0	0
ICPW 122	2.24	2.9	2.78	1.79	1.7	1.67	143	0	0
ICPW 125	2	1.79	2.58	1.76	1.76	1.8	320	0	0
ICPW 130	2.44	2.57	2.91	1.74	1.74	1.7	525	250	0
ICPW 132	2.19	2.48	2.38	1.7	1.74	1.69	293	0	0
ICPW 137	2.28	3.73	4.66	1.71	1.85	1.79	561	43	0
ICPW 138	2.49	2.42	2.35	1.57	1.61	1.64	2057	67	0
ICPW 141	2.63	2.72	3.29	1.43	1.51	1.51	487	59	0
ICPW 147	4.14	3.53	3.28	1.61	1.65	1.75	309	91	0
ICPW 152	1.78	2.72	2.77	1.73	1.77	1.86	719	0	0
ICPW 278	2.44	2.71	2.58	1.76	1.57	1.66	525	31	0
ICPW 280	2.62	2.84	2.42	1.51	1.41	1.58	31	0	0
ICPW 281	3.57	4.04	5.15	1.69	1.59	1.66	12	0	0
ICPW 302	4.04	4.95	4.27	1.79	1.79	1.72	159	33	0
ICPW 305	4	4.34	3.59	1.66	1.75	1.73	640	148	0
ICPW 308	2.54	2.56	2.83	1.77	1.85	1.78	32	0	0
ICPW 310	4.09	4.34	3.7	1.71	1.66	1.67	157	37	0
ICPW 315	3.69	3.95	3.62	1.75	1.61	1.58	174	21	0
Mean	2.76	3.08	3.19	1.6	1.62	1.64	501.14	53.98	0
SE±	0.765	0.786	0.74	0.258	0.162	0.113	419.44	66.944	0
<i>C.cajan</i> genotypes									
ICP 26	1.74	1.98	1.47	1.57	1.07	1.09	736	324	0
ICP 28	1.37	1.7	1.38	1.54	1.16	1.04	934	188	0
ICP 8518	1.43	1.38	1.62	1.12	1.11	1.06	224	58	0
ICP 8863	2.71	2.41	2.91	1.4	1.24	1.04	473	266	0
ICP 14722	2.38	1.97	1.71	1.11	1.17	1.25	538	163	0
ICP 14770	1.75	1.92	1.71	1.13	1.11	0.97	732	84	0
Mean	1.89	1.89	1.8	1.31	1.14	1.08	605.88	180.13	0
SE±	0.536	0.341	0.56	0.218	0.06	0.094	248.57	102.419	0
Mean	2.62	2.88	2.95	1.55	1.54	1.55	518.6	75	0
SEM±	0.13	0.14	0.15	0.05	0.04	0.04	65.86	14.42	0
SED	0.8	0.85	0.88	0.27	0.23	0.24	395.18	86.52	0
Range	1.37 - 4.14	1.38 - 4.95	1.38 - 5.15	0.39 - 1.75	1.03 - 1.85	0.97 - 1.86	11.00-2056.00	0.00-323.00	0.00-0.00

Fig. 15 Trypsin inhibitor content in wild accession of *Pigeonpea*

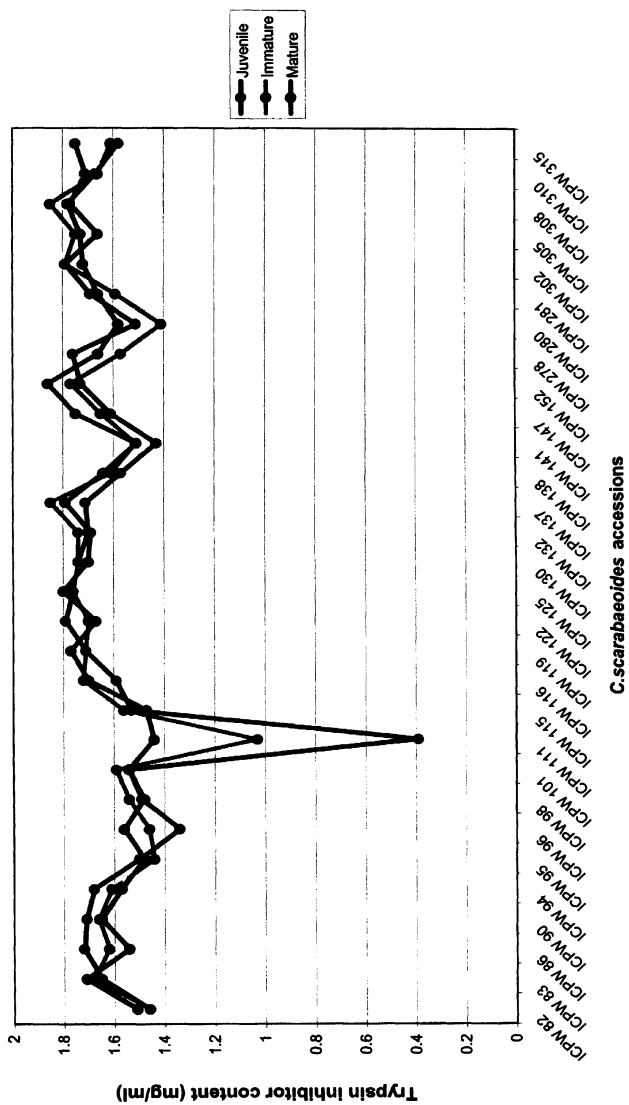
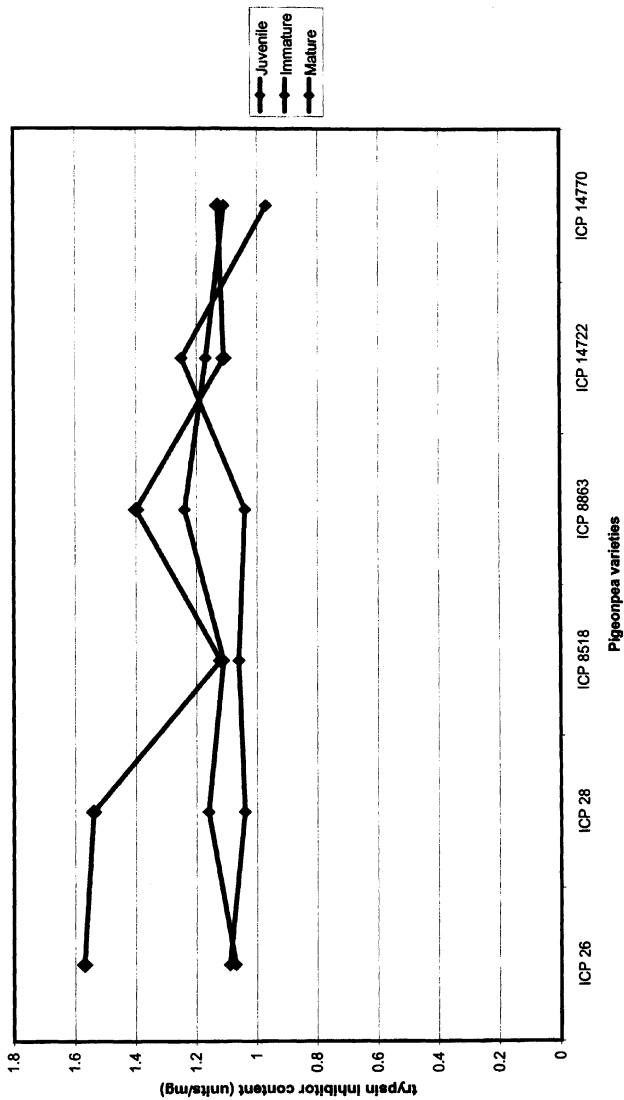


Fig: 16 Trypsin inhibitor content in the cultivated pigeonpea





content (1.31 units/mg) followed by immature (1.14 units /mg) and mature pods (1.10 units/mg). The juvenile pods of ICP 26 contained maximum trypsin inhibitor content (1.57 units/mg) followed by ICP 28 (1.54 units/mg) and ICP 8863 (1.40 units/mg). ICP 14722 contained lowest trypsin inhibitor (1.11 units/mg). The immature pods of ICP 8863 had the highest trypsin inhibitor (1.24 units/mg) followed by ICP 14722 (1.17 units/mg) and ICP 28 (1.16 units / mg). ICP 26 contained the least trypsin inhibitor (1.07 units/mg). The mature pods of ICP 14722 had the highest trypsin inhibitor (1.25 units/mg) followed by ICP 26 (1.09). ICP 14770 had the lowest trypsin inhibitor in its mature pods (0.97 units/mg) (Table 18).

## Lectin

The three pod stages differed significantly for lectin content (Fig.18). Dilution plates showing the agglutination pattern are shown in figure 17. The juvenile pods contained maximum mean lectin content of 518.6 HAU/mg, followed by immature pods with a mean value of 75.0 HAU/mg. The mature pods did not contain any lectin in any of the 36 genotypes (Fig. 18). In 30 *C. scarabaeoides* accessions, juvenile pods had mean lectin content of 501.14 HAU/mg and immature pods with 53.9 HAU/mg. The juvenile pods of ICPW 138 showed maximum lectin (2057 HAU/mg) followed by ICPW 98 (1220 units/mg). ICPW 281 had the lowest lectin content in its juvenile pods (12 HAU/mg). The immature pods of ICPW 130 had a maximum lectin of (250 HAU/mg) followed by ICPW 96 (170 HAU/mg). No lectin content was detected in the immature pods of 11 accessions; ICPW 98, ICPW 115, ICPW 119, ICPW 122, ICPW 125, ICPW 132, ICPW 152, ICPW 278, ICPW 280, ICPW 281 and ICPW 308. In the *C. cajan* group also mean lectin content was maximum in the juvenile pods (605 HAU/mg) followed by immature pods (180 HAU/mg) (Fig. 19). In the juvenile stage, maximum content was observed in ICP 28 (934 HAU/mg) followed by ICP 26 (736 HAU/mg) and ICP 14770 (732 HAU/mg) (Fig. 19). ICP 8518 had the lowest lectin content (224 HAU/mg). In the immature stage, the highest lectin content (323 HAU/mg) was observed in ICP 26 followed by

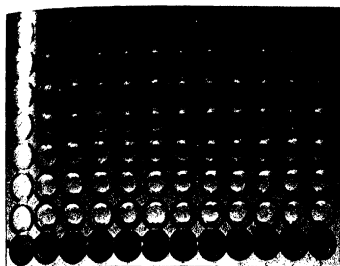
**Fig. 17: Haemagglutination profiles of lectins in wild and cultivated pigeonpeas**

- a) Con A – Standard used in the lectin assay
- b) Wild accessions ICPW 94 and ICPW 130
- c) Wild accessions ICPW 116 and ICPW 125
- d) *C. cajan*, ICP 26 and ICP 28

J = Juvenile stage of pod

IM = Immature stage of pod

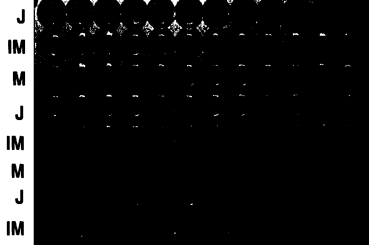
M = Mature stage of pod



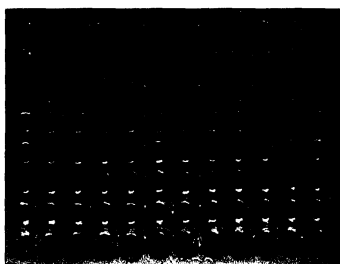
Con A

a

Con A

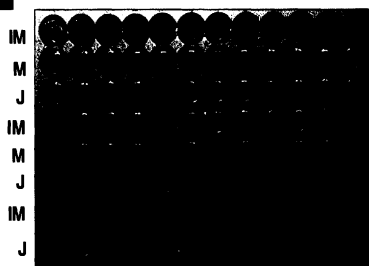


b



c

J  
IM  
M  
J  
IM  
M  
J  
IM



d

Fig: 17

Fig: 18 Lectin content in Wild accessions of pigeonpea

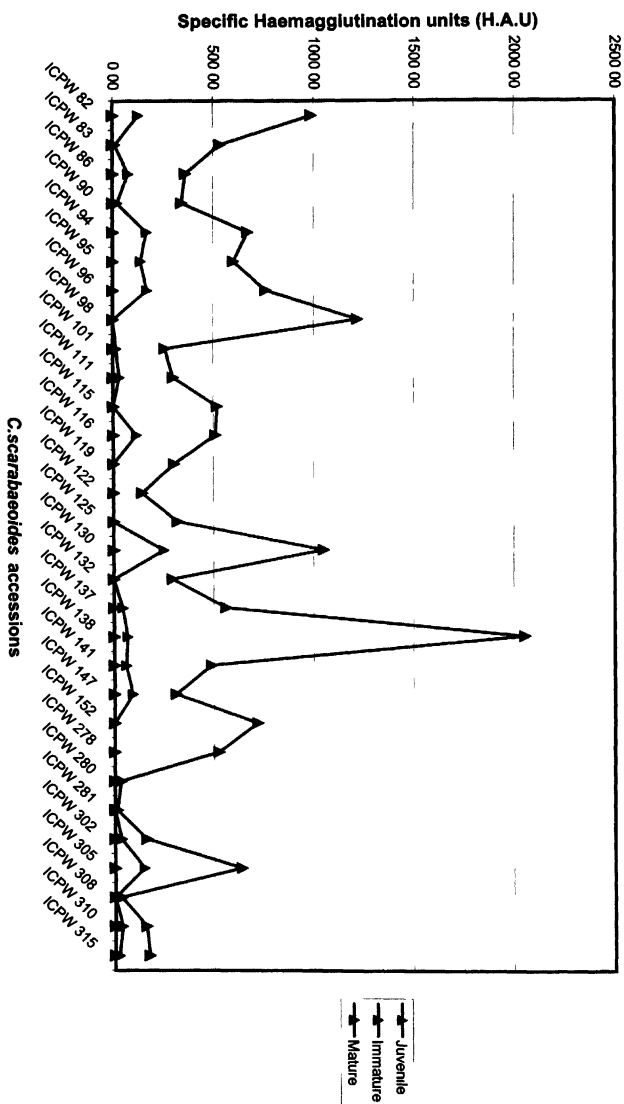
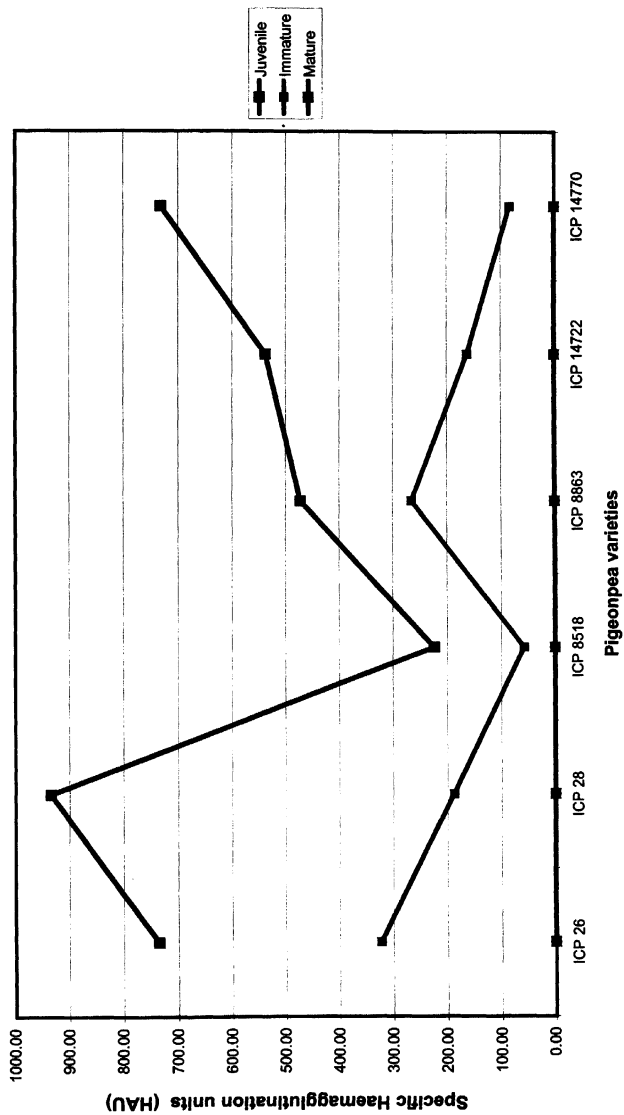


Fig: 19 Lectin content in Pigeonpea varieties



ICP 8863 (266HAU/mg). ICP 8518 had the least lectin content of 58 HAU/mg (Table 18).

## **Density and type of trichomes**

Five morphologically distinct types of trichomes (A, B, C, D and E) were identified on pods of 30 *C. scarabaeoides* accessions and 6 pigeonpea genotypes by light and scanning electron microscopy (Fig 20, 21 a and b ). Type A had a long tubular neck containing a clear viscous fluid. It is longer than other trichomes except the type D. The base of type A trichomes is enlarged and consists of 6 to 10 cells and the neck comprises of 4 to 8 cells (Fig 21 b). Type B is yellowish, unsegmented globular sac. Its contents are released only after the cell wall is ruptured. Unsegmented, nonglandular trichomes were separated into short (Trichome C) and long (Trichome D) trichomes (Fig 20). Type D was 4 to 11 times longer than Type C in all the accessions. In addition, electron micrographs showed a small, multi-lobed fifth glandular trichome (Type E), attached to the pod surface by a short stalk (Fig 21 a). Type E was too small to measure the density using light microscope hence its density was not recorded.

Season was significant for densities of trichomes A and B. Habit (Wild and cultivated) was significant for densities of trichomes A, B and C (Table 6). Interaction between season and habit was significant for density of trichome A. Interaction between season and wild habit was significant for density of trichome A, B and C. Interaction of season and cultivated habit was significant for density of trichome A (Table 6). Genotype was significant for the densities of four types of trichomes. Genotype x season and habit x wild interaction was non – significant for all the trichome types. Habit x cultivated was significant for densities of B, C and D trichomes (Table 7). Heritability was high for all the trichome types (Table 7).

Fig. 20: Types of trichomes on pods of *C. scarabaeoides* accession,  
ICPW 94

B = Type B trichome

C = Type C trichome

D = Type D trichome

**Fig: 20**

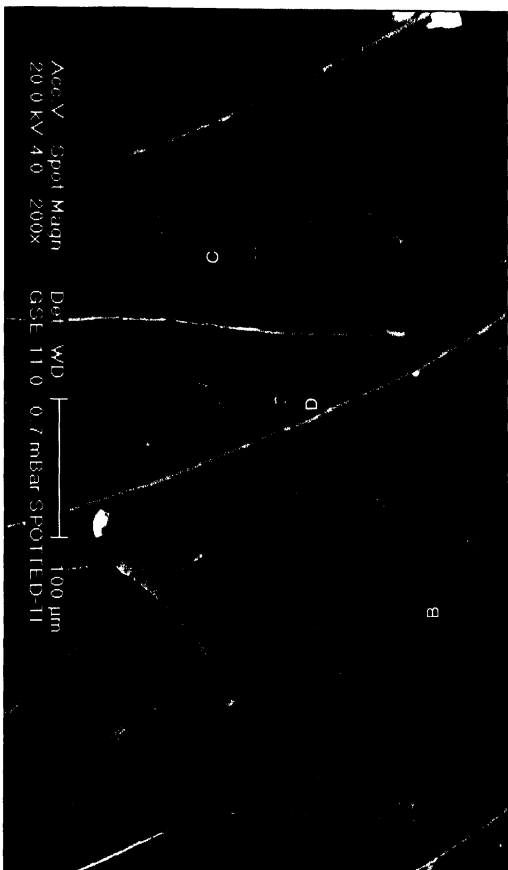
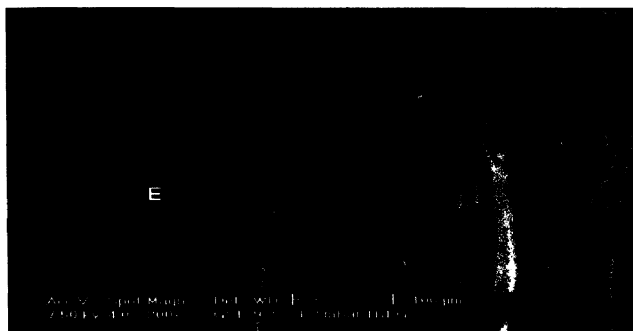




Fig. 21. Types of trichomes on pods of wild and cultivated pigeonpeas

- a) E = Type E trichome on pods of *C. scarabaeoides* accession  
ICPW 116
- b) A, B, C & D = Type A, B, C & D trichomes on pods of *C. cajan*  
genotype ICP 26



b

**Fig: 21**

## Variation in trichome density

Types B, C and D trichomes were present on pods of all the *C. scarabaeoides* accessions and *C. cajan*. Type A was absent in most of the *C. scarabaeoides* accessions and present only in a few accessions with less density. Density of each trichome type differed significantly among the pods of wild and cultivated accessions. Pods of *C. scarabaeoides* were more pubescent than pods of *C. cajan* because of the higher densities of types B, C and D trichomes. Pods of *C. scarabaeoides* had more of non-glandular trichomes types C and D and glandular type B, compared to only a few number of glandular trichome type A. However, the density of glandular trichome type A was more on the pods of *C. cajan*, compared to the *C. scarabaeoides* accessions. Trichome types A, B, C and D were found on pods of all pigeonpea genotypes studied (Table 19). The densities of types A, C and D trichomes varied significantly among the wild genotypes. Significant seasonal variation for type and density of trichomes was not found in wild and cultivated genotypes.

### Density of trichome A

Among the *C. scarabaeoides* accessions, trichome A was absent and even if present in some of the accessions, the frequency was very low. For example, accessions ICPW 82, ICPW 94, ICPW 119, ICPW 130, ICPW 141, ICPW 147, ICPW 152, ICPW 308 and ICPW 315 did not have type A trichomes on their pods. ICPW 95 and ICPW 278 had only 0.13 trichomes/mm<sup>2</sup> and remaining accessions had 0.03 – 0.11 trichomes/mm<sup>2</sup> (Table 19). The mean density of trichomes on *C. scarabaeoides* was only 0.04 trichomes / mm<sup>2</sup>. Among the *C. cajan* genotypes the density ranged from 3.67 trichomes / mm<sup>2</sup> in ICP 26 to 5.53 trichomes/mm<sup>2</sup> in ICP 8863 with a group mean of 4.81 trichome/mm<sup>2</sup>. The overall mean density of trichome A was 0.84 trichomes /mm<sup>2</sup> (Table 19).

**Table - 19: Means for trichome density (No./mm<sup>2</sup>)\* among *C.scarabaeoides* and *C.cajan***

Accession	Trichome A (No./mm <sup>2</sup> )	Trichome B (No./mm <sup>2</sup> )	Trichome C (No./mm <sup>2</sup> )	Trichome D (No./mm <sup>2</sup> )
<i>C.scarabaeoides</i> accessions				
ICPW 82	0	1.13	141.07	10.8
ICPW 83	0.08	1.67	141.8	13.2
ICPW 86	0.07	3.87	116.07	8
ICPW 90	0.03	4.07	142.13	10.17
ICPW 94	0	3.8	190.4	8.733
ICPW 95	0.13	2.27	123.87	6.67
ICPW 96	0.03	3.8	131.93	5.67
ICPW 98	0.03	3.6	122.07	11.6
ICPW 101	0.09	1.07	126	10.87
ICPW 111	0.11	1.67	122.6	5.07
ICPW 115	0.08	3.73	169	5.13
ICPW 116	0.04	1.4	173.73	9.67
ICPW 119	0	4.8	160.07	7.2
ICPW 122	0.05	1.8	165.47	7.2
ICPW 125	0.07	3.27	160.67	6.2
ICPW 130	0	2.27	161.33	6.53
ICPW 132	0.07	2.13	143.73	6.53
ICPW 137	0.05	3.73	169	9.86
ICPW 138	0.03	3.6	146.67	6.6
ICPW 141	0	3.55	179.33	7.73
ICPW 147	0	2.4	188.23	11.87
ICPW 152	0	3.8	170.7	6.31
ICPW 278	0.13	3.13	155.87	7.8
ICPW 280	0.05	1.6	157.4	4.73
ICPW 281	0.07	3.33	159.07	5.4
ICPW 302	0.04	2	140.87	10.6
ICPW 305	0.04	3.07	153.73	11.33
ICPW 308	0	2.87	145.33	6.06
ICPW 310	0.04	3.07	153.73	11.33
ICPW 315	0	2	140.87	10.6
Mean	0.044	2.82	151.76	8.32
SEM±	0.007	0.183	3.567	0.448
<i>C.cajan</i> genotypes				
ICP 26	3.67	1.13	25.73	2.33
ICP 28	5.23	1.73	29.33	3.07
ICP 8518	4.93	0.8	25.26	2.06
ICP 8863	5.53	1.47	23.06	1.33
ICP 14722	4.73	1.53	26.06	1.13
ICP 14770	4.8	0.93	22.41	1.072
Mean	4.82	1.27	25.31	1.83
SEM±	0.259	0.15	1.006	0.324
Trial mean	0.84	2.55	130.68	7.23
SEM ±	0.303	0.182	8.5	0.556
SED	1.687	1.014	51.002	3.334
Range	0.27-5.53	0.00-3.80	22.41-190.40	1.07-13.20
CD (5.0%)	0.571	0.343	17.257	1.128

\* Density based on observation on 5 pods per plant and three microscopic fields per pod

## Density of trichome B

Though trichome B was present both in the wild and cultivated genotypes, a slightly higher density was seen in the wild than in the cultivated accessions. Density of trichome B ranged from 1.07 /mm<sup>2</sup> in ICPW 101 to 4.80 /mm<sup>2</sup> in ICPW 119 with a group mean of 2.82 /mm<sup>2</sup> (Table 22). Among the *C. cajan* genotypes, the density of trichomes ranged from 0.80 /mm<sup>2</sup> in ICP 8518 to 1.73 /mm<sup>2</sup> in ICP 28 with a group mean of 1.27 /mm<sup>2</sup>. The overall mean density of trichome B was 2.56 /mm<sup>2</sup> (Table 19).

## Density of trichome C

Trichome C was more densely found on pods of both *C. scarabaeoides* and *C. cajan* genotypes and more than any other trichome types on pods of both wild and cultivated. The density of type C is four to five times more on *C. scarabaeoides* accessions compared to on *C. cajan* genotypes. The density of this trichome ranged from 116.07 / mm<sup>2</sup> in ICPW 86 to 190.40 / mm<sup>2</sup> in ICPW 94 with a group mean of 151.70 / mm<sup>2</sup> (Table 19) Among *C. cajan* genotypes it ranged from 22.41 / mm<sup>2</sup> in ICP 14770 to 29.33 /mm<sup>2</sup> in ICP 28 with a group mean of 25.31 / mm<sup>2</sup>, with an overall mean of 130.68 trichomes / mm<sup>2</sup> (Table 19).

## Density of trichome D

Trichome D was present on pods of both *C. cajan* and *C. scarabaeoides* accessions, with significant differences in the density of this trichome in the wild and cultivated genotypes. Among the *C. scarabaeoides* accessions the density ranged from 4.73 trichomes / mm<sup>2</sup> in ICPW 280 to 13.20 trichomes / mm<sup>2</sup> in ICPW 83 with a group mean of 8.32 trichomes / mm<sup>2</sup> (Table 19), while among the *C. cajan* genotypes the density ranged from 1.07 trichomes / mm<sup>2</sup> ICP 14770 to 3.07 trichomes / mm<sup>2</sup> in ICP 28 with a group mean of 1.85 trichomes / mm<sup>2</sup> and with an overall mean density of 7.20 trichomes / mm<sup>2</sup> (Table 19).

## **Correlation between different traits**

The phenotypic, genotypic and environmental correlation coefficient values obtained for various characters are presented in tables 20, 21 and 22 respectively.

### **Phenotypic correlation**

#### **Density of trichome A**

Density of trichome A was significantly correlated positively with pod length (0.50), seed weight (0.63), percentage flower damage (0.65), bud damage (0.69), pod damage (0.82), number of eggs (0.77) and number of larvae (0.74) but significantly correlated negatively with density of trichome B (-0.68), trichome C (-0.89), trichome D (-0.73), number of secondary branches (-0.44) and seed protein content (-0.35).

#### **Density of trichome B**

Density of trichome B was significantly correlated positively with density of trichome C (0.77), trichome D (0.66) and seed protein content (0.42) but correlated negatively with pod length (-0.43), seed weight (-0.52), percentage bud damage (-0.65) and flower damage (-0.63), pod damage (-0.71), number of eggs (-0.71) and number of larvae (-0.70).

#### **Density of trichome C**

Density of trichome C was significantly correlated positively with density of trichome D (0.68), days to flower (0.37), days to maturity (0.41) and number of secondary branches (0.38) but was significantly correlated negatively with pod length (-0.51), seed weight (-0.63), percentage bud damage (-0.77), flower damage (-0.73), pod damage (-0.81), number of eggs (-0.82) and number of larvae (-0.82).

Table - 20: Genotypic correlation coefficient for various morphological characters

Character	DA	DB	DC	DD	DF	DM	LF	Lfd	Lfa	PL	PW	LP	SP	SW	NP	NS	TP	BD	FD	PD
DB	-0.69**																			
DC	-0.90**	0.72**																		
DD	-0.74**	0.67**																		
DF	-0.25	0.26	0.37*	0.07																
DM	-0.28	0.34*	0.42	0.10	0.81**															
LF	0.24	-0.12	-0.30	-0.24	-0.32	-0.34*														
Lfd	0.20	-0.09	-0.23	-0.19	-0.29	-0.25	0.90**													
Lfa	0.10	-0.04	-0.16	-0.11	-0.05	-0.13	0.16	-0.26												
PL	0.51**	-0.44**	-0.52**	-0.36*	-0.12	-0.34	0.49**	0.42*	0.17											
PW	-0.04	0.13	0.18	0.13	0.13	0.08	0.13	0.13	0.09	0.37*										
LP	-0.11	0.08	0.13	0.00	-0.15	-0.09	0.09	0.05	0.12	-0.17	-0.17									
SP	-0.20	0.08	0.19	0.04	-0.17	-0.13	0.23	0.16	0.13	-0.14	-0.18	0.93**								
SW	0.63**	-0.53**	-0.63**	-0.43**	-0.37*	0.46**	0.37*	0.21	0.97**	0.35*	-0.19	-0.18								
NP	-0.32	0.16	0.51	0.62	0.48	0.64	-0.72	-0.77	0.13	-0.59	-0.28	0.28	0.31	-0.49						
NS	-0.45**	0.33*	0.38*	0.26	0.36*	0.28	-0.35*	-0.26	-0.23	-0.58**	-0.22	0.25	0.21	-0.61**	0.35					
TP	-0.36*	0.42**	0.28	0.30	-0.18	-0.03	-0.15	-0.03	-0.30	-0.61**	-0.32	0.04	0.06	-0.63**	-0.01	0.40*				
BD	0.91**	-0.82**	-0.90**	-0.73**	-0.38	-0.28	0.18	0.14	0.09	0.38	-0.20	-0.13	-0.20	0.49**	-0.33	-0.38	-0.31			
FD	0.72**	-0.68**	-0.80**	-0.63**	-0.29	-0.21	0.12	0.11	0.04	0.40*	-0.30	-0.05	-0.07	0.47**	-0.26	-0.37*	-0.34	0.98**		
PD	0.84**	-0.71**	-0.82**	-0.67**	-0.27	-0.26	0.23	0.25	-0.01	0.52**	-0.14	0.06	-0.07	0.63**	-0.23	-0.33*	-0.38*	0.83**	0.81**	
EG	0.81**	-0.74**	-0.86**	-0.69**	-0.29	-0.24	0.26	0.22	0.11	0.57**	-0.13	-0.05	-0.05	0.64**	-0.35	-0.47**	-0.44**	0.98**	0.91**	0.82**
LR	0.78**	-0.74**	-0.84**	-0.67**	-0.30	-0.24	0.27	0.21	0.11	0.54**	-0.13	-0.06	-0.05	0.62**	-0.37	-0.45**	-0.43*	0.94**	0.91**	0.78**

DA = Density of trichome A, DB = Density of trichome B, DC = Density of trichome C, DD = Density of trichome D, DF = Days to maturity, LF = leaf area, Lfd = leaf dry weight, Lfa = specific leaf area, PL = Pod length, PW = Pod width, LP = Locules per pod, SP = Seeds per pod, SW = 100 - Seed weight, NP = number of primary branches, NS = number of secondary branches, TP = Total protein, BD = percent bud damage, FD = percent flower damage, PD = percent pod damage, EG = number of eggs and LR = number of larvae.

## **Density of trichome D**

Density of trichome D was significantly correlated negatively with pod length (-0.35), seed weight (-0.42), percentage bud damage (-0.55), flower damage (-0.57), pod damage (-0.64), number of eggs (-0.65) and number of larvae (-0.63).

## **Days to flower**

Days to flower was significantly correlated positively with days to maturity (0.80) but was significantly correlated negatively with percentage bud damage (-0.31).

## **Days to maturity**

Days to maturity was significantly correlated negatively with leaf area (-0.33) and specific leaf area (-0.34).

## **Leaf area**

Leaf area was significantly correlated positively with leaf dry weight (0.89), pod length (0.49) and seed weight (0.46) but was significantly correlated negatively with number of primary branches (-0.24) and number of secondary branches (-0.35).

## **Leaf dry weight**

Leaf dry weight was significantly correlated positively with pod length (0.41) and seed weight (0.36) but was correlated negatively with specific leaf area (-0.31) and number of primary branches (-0.26).

## **Leaf specific area**

Leaf specific area was significantly correlated positively with pod width (0.37), 100- seed weight (0.97), percentage flower damage (0.40), pod damage (0.52), number of eggs (0.57) and number of larvae (0.54) but correlated negatively with number of secondary branches (-0.58), total protein (-0.61).



### **Pod length**

Pod length was significantly correlated positively with pod width (0.35), seed weight (0.96), percentage bud damage (0.30), flower damage (0.37), pod damage (0.51), number of eggs (0.54) and number of larvae (0.52) but was significantly correlated negatively with number of secondary branches (-0.57) and seed protein (-0.61).

### **Pod width**

Pod width was not significantly correlated with any of the characters studied.

### **Number of locules per pod**

Number of locules per pod was significantly correlated positively with seed protein (0.88) .

### **Number of seeds per pod**

This character was not correlated with any of the characters studied.

### **Seed weight**

Seed weight was significantly correlated positively with percentage bud damage (0.39), flower damage (0.44), pod damage (0.62), number of eggs (0.62) and number of larvae (0.59) but was significantly correlated negatively with number of secondary branches (-0.61) and seed protein (-0.63) .

### **Number of primary branches**

This trait was not correlated with any of the characters studied.

## **Number of secondary branches**

This trait was significantly correlated positively with seed protein (0.40) but was significantly correlated negatively with percentage bud damage (-0.31), flower damage (-0.35), number of eggs (-0.45) and number of larvae (-0.43).

## **Total seed protein**

Seed protein was significantly correlated negatively with percentage flower damage (-0.31), pod damage (-0.38), number of eggs (-0.43) and number of larvae (-0.41).

## **Bud damage**

Percentage bud damage was significantly correlated positively with the percentage flower damage (0.74), pod damage (0.66), number of eggs (0.74) and number of larvae (0.75).

## **Flower damage**

Percentage flower damage was significantly correlated positively with the percentage pod damage (0.74), number of eggs (0.82) and number of larvae (0.82).

## **Pod damage**

Percentage pod damage was significantly correlated positively with the number of eggs (0.79) and number of larvae (0.74).

## **Number of eggs**

Numbers of eggs were significantly correlated positively with number of larvae (0.99).

## **Genotypic correlation**

### **Density of trichome A**

Density of trichome A was significantly correlated positively with pod length (0.51), seed weight (0.63), percentage flower damage (0.72), bud damage (0.91), percentage pod damage (0.84), number of eggs (0.81) and number of larvae (0.78) but significantly correlated negatively with density of trichome B (-0.69), trichome C (-0.90), trichome D (-0.74), number of secondary branches (-0.45) and seed protein content (0.36).

### **Density of trichome B**

Density of trichome B was significantly correlated positively with density of trichome C (0.72), density of trichome D (0.67), days to maturity (0.34), number of secondary branches (0.33) and seed protein content (0.42) but was significantly correlated negatively with pod length (-0.44), seed weight (-0.53), bud damage (-0.82) and flower damage (-0.68), pod damage (-0.71), number of eggs (-0.74) and number of larvae (-0.74).

### **Density of trichome C**

Density of trichome C was significantly correlated positively with density of trichome D (0.69), days to flower (0.37), days to maturity (0.42) and number of secondary branches (0.33) but was significantly correlated negatively with pod length (-0.52), seed weight (-0.63), bud damage (-0.96), flower damage (-0.80), pod damage (-0.82), number of eggs (-0.86) and number of larvae (-0.84).

### **Density of trichome D**

Density of trichome D was significantly correlated negatively with pod length (-0.36), seed weight (-0.43), number of primary branches (0.62), bud damage

Table - 21: Phenotypic correlation coefficient for morphological characters

Phenotypic	DA	DB	DC	DD	DF	DM	Lf	Ltdw	Ltisa	PL	PW	LP	SP	SW	NP	NS	TP	BD	FD	PD	EGG
DB	0.25																				
DC	-0.88**	0.72**																			
DD	-0.73**	0.66**	0.68**																		
DF	-0.25	0.26	0.37*	0.07																	
DM	-0.27	0.34	0.41*	0.10	0.80**																
Lf	0.24	-0.11	-0.30	-0.24	-0.32	-0.33*															
Ltd	0.20	-0.09	-0.23	-0.18	-0.28	-0.24	0.89**														
Ltisa	0.09	-0.03	-0.13	-0.09	-0.04	-0.12	0.16	-0.31*													
PL	0.50**	-0.43**	-0.51**	-0.35*	-0.12	-0.34*	0.48**	0.41*	0.15												
PW	-0.04	0.12	0.17	0.12	0.13	0.08	0.13	0.12	0.07	0.35*											
LP	-0.10	0.08	0.13	-0.01	-0.15	-0.09	0.09	0.06	0.08	-0.16	-0.16										
SP	-0.19	0.08	0.18	0.05	-0.16	-0.12	0.22	0.15	0.09	-0.13	-0.17	0.88**									
SW	0.63**	-0.52**	-0.63**	-0.42**	-0.19	-0.36	0.48**	0.36*	0.19	0.96**	0.33	-0.19	-0.17								
NP	-0.10	0.06	0.16	0.22	0.15	0.21	-0.24*	-0.26*	0.09	-0.21	-0.07	0.07	0.04	-0.16							
NS	-0.44**	0.33	0.38*	0.26	0.36	0.28	-0.35*	-0.25	-0.19	-0.57**	-0.21	0.24	0.20	-0.61**	0.11						
TP	-0.35*	0.42*	0.28	0.29	-0.18	-0.03	-0.15	-0.03	-0.26	-0.61**	-0.31	0.04	0.06	-0.63**	0.00	0.40*					
BD	0.69**	-0.65**	-0.77**	-0.55**	-0.31*	-0.23	0.14	0.10	0.07	0.30*	-0.17	-0.13	-0.14	0.39**	-0.08	-0.31*	-0.25				
FD	0.65**	-0.63**	-0.73**	-0.57**	-0.27	-0.19	0.10	0.03	0.37*	-0.27	-0.05	-0.06	0.44**	-0.08	-0.35*	-0.31*	0.74**				
PD	0.82**	-0.70**	-0.81**	-0.64**	-0.27	-0.25	0.23	0.24	0.00	0.51**	-0.14	0.05	-0.08	0.62**	-0.07	-0.33	-0.38*	0.66**	0.74**		
EG	0.77**	-0.71**	-0.82**	-0.65**	-0.28	-0.23	0.25	0.20	0.10	0.54**	-0.13	-0.06	-0.05	0.62**	-0.11	-0.45**	-0.43**	0.74**	0.82**	0.79**	
LR	0.74**	-0.70**	-0.82**	-0.63**	-0.28	-0.23	0.26	0.20	0.11	0.52**	-0.13	-0.06	-0.05	0.59**	-0.11	-0.43**	-0.41*	0.75**	0.82**	0.74**	0.99**

DA = Density of trichome A, DB = Density of trichome B, DC = Density of trichome C, DD = Density of trichome D, DF = Days to maturity, Lf = leaf area, Ltd = leaf dry weight, Ltisa = specific leaf area, PL = Pod length, PW = Pod width, LP = Locules per pod, SP = Seeds per pod, SW = 100 - Seed weight, NP = number of primary branches, NS = number of secondary branches, TP = Total protein, BD = percent bud damage, FD = percent flower damage, PD = percent pod damage, EG = number of eggs and LR = number of larvae.

(-0.73), flower damage (-0.63), pod damage (-0.67), number of eggs (-0.69) and number of larvae (-0.67).

### **Days to flower**

Days to flower was significantly correlated positively with days to maturity (0.81) and number of secondary branches (0.36).

### **Days to maturity**

Days to maturity was significantly correlated negatively with leaf area (-0.34) and seed weight (-0.37), while it significantly correlated positively with number of primary branches (0.64).

### **Leaf area**

Leaf dry weight was significantly correlated positively with leaf dry area (0.90), pod length (0.49) and seed weight (0.46) but was significantly correlated negatively with number of primary branches (-0.72) and number of secondary branches (-0.35).

### **Leaf dry weight**

Leaf area was significantly correlated positively with pod length (0.42) and seed weight (0.37) but was significantly correlated negatively with number of primary branches (-0.77).

### **Specific leaf area**

Specific leaf area was not significantly correlated with any of the characters studied.

## **Pod length**

Pod length was significantly correlated positively with pod width (0.37), seed weight (0.97), flower damage (0.40), pod damage (0.52), number of eggs (0.57) and number of larvae (0.54) but was significantly correlated negatively with number of primary branches (-0.59), number of secondary branches (-0.58) and seed protein (-0.61).

## **Pod width**

Pod width was significantly correlated positively with seed weight (0.35).

## **Number of locules per pod**

Number of locules per pod was significantly correlated positively with seeds per pod (0.93).

## **Number of seeds per pod**

Number of seeds per pod was not significantly correlated with any of the characters studied.

## **Seed weight**

Seed weight was significantly correlated positively with percentage bud damage (0.49), flower damage (0.47), pod damage (0.63), number of eggs (0.64) and number of larvae (0.62) but was significantly correlated negatively with number of primary branches (-0.49), number of secondary branches (-0.61) and seed protein (-0.63).

## **Number of primary branches**

Number of primary branches was not significantly correlated with any of the characters studied.

## **Number of secondary branches**

Number of secondary branches was significantly correlated positively with seed protein (0.40) but was significantly correlated negatively with percentage flower damage (-0.37), pod damage (-0.33), number of eggs (-0.47) and number of larvae (-0.45) .

## **Total seed protein**

Seed protein was significantly correlated negatively with pod damage (-0.38), number of eggs (-0.44) and number of larvae (-0.43) .

## **Bud damage**

Percentage bud damage was significantly correlated positively with the percentage flower damage (0.89), pod damage (0.83), number of eggs (0.96) and number of larvae (0.94).

## **Flower damage**

Percentage flower damage was significantly correlated positively with the percentage pod damage (0.81), number of eggs (0.91) and number of larvae (0.91) .

## **Pod damage**

Percentage pod damage was significantly correlated positively with the number of eggs (0.82) and number of larvae (0.76).

## **Number of eggs**

Numbers of eggs were significantly correlated positively with number of larvae (0.99).

## **Environmental correlation**

### **Density of trichome A**

Density of trichome A was significantly correlated positively to number of secondary branches (0.24) but significantly correlated negatively with percentage bud damage (-0.34) and number of larvae (-0.22).

### **Density of trichome B**

Density of trichome B was significantly correlated positively with the leaf area (0.26).

### **Density of trichome C**

Density of trichome C was significantly correlated positively with leaf area (0.23).

### **Density of trichome D**

Density of trichome D was significantly correlated negatively with pod length (-0.36), seed weight (-0.43), number of primary branches (0.62), bud damage (-0.73), flower damage (-0.63), pod damage (-0.67), number of eggs (-0.69) and number of larvae (-0.67).

### **Days to flower**

Days to flower were significantly correlated negatively to flower damage (-0.24).

### **Days to maturity**

Days to maturity did not show any significant correlations with any of the characters studied.



Table - 22: Environmental correlation coefficients for morphological characters

Environment	DA	DB	DC	DD	DF	DM	LF	LfW	LfA	PL	PW	LP	SP	SW	NPfb	Nseb	TP	BD	FD	PD	EGG
DB	-0.10																				
DC	0.17	0.14																			
DD	-0.11	0.16	0.08																		
DF	0.01	0.10	-0.18	-0.14																	
DM	0.06	0.10	-0.03	0.04	0.02																
LF	0.17	0.28**	0.23*	0.02	0.11	0.01															
LfW	-0.05	-0.15	-0.11	-0.05	-0.09	-0.01	-0.05														
LfA	0.08	0.17	0.21	0.04	0.08	0.01	0.39**	-0.90**													
PL	0.10	-0.15	0.05	-0.10	-0.09	0.04	0.01	-0.01	0.02												
PW	0.08	0.01	0.03	-0.09	0.19	-0.22	-0.06	-0.03	0.01	0.13											
LP	0.23	0.11	-0.12	-0.20	0.05	0.21	-0.06	0.32	-0.30	0.07	-0.04										
SP	-0.02	0.20	-0.08	0.19	0.02	0.15	0.04	0.07	-0.12	-0.02	-0.10	0.09									
SW	0.10	0.05	0.00	0.02	0.23	0.20	0.31**	-0.14	0.18	0.18	-0.11	0.14	0.14								
NPfb	0.01	0.08	-0.04	0.15	-0.03	0.10	-0.07	-0.12	0.11	-0.15	0.08	-0.12	-0.23**	0.01							
Nseb	0.24**	-0.01	0.04	-0.05	-0.13	-0.05	0.05	-0.28**	0.24	0.06	0.11	0.04	-0.09	-0.11	-0.01						
TP	-0.01	-0.05	0.00	-0.03	-0.04	0.01	0.06	0.47**	0.28**	0.02	0.07	0.28**	-0.19	0.00	-0.02	-0.22					
BD	-0.34**	0.05	-0.01	0.19	-0.14	-0.08	-0.08	-0.05	0.04	-0.04	-0.05	-0.25**	0.07	-0.06	0.01	-0.21	0.01				
FD	-0.14	-0.02	-0.02	-0.04	-0.24*	0.00	-0.09	-0.01	0.00	-0.10	-0.15	-0.05	-0.05	-0.05	0.00	-0.28**	0.02	0.36**			
PD	-0.02	0.05	0.12	0.23*	-0.04	0.17	0.15	-0.09	0.14	0.03	-0.11	-0.17	-0.20	0.22	0.03	0.01	-0.01	0.09	0.07		
EGG	-0.20	0.12	-0.02	0.10	0.00	0.03	-0.01	-0.04	0.06	-0.05	-0.12	-0.16	-0.03	-0.13	-0.02	-0.03	0.02	0.03	0.13	0.23*	
LAR	-0.22*	0.18	-0.02	0.14	0.02	-0.01	-0.02	-0.07	0.08	-0.01	-0.14	-0.10	-0.02	-0.11	0.01	0.05	0.02	0.11	0.14	0.23**	0.92**

DA = Density of trichome A, DB = Density of Trichome B, DC = Density of trichome D, DF = Days to flower, DM = Days to maturity, LF = leaf area, LfW = leaf dry weight, LfA = specific leaf area, PL = Pod length, PW = Pod width, LP = Locules per pod, SW = 100 - Seed weight, NPfb = number of primary branches, NSeb = number of secondary branches, TP = Total protein, BD = percent bud damage, FD = percent flower damage, PD = percent pod damage, EG = number of eggs and LR = number of larvae.

### **Leaf area**

Leaf area was significantly correlated positively with leaf dry weight (0.39) and seed weight (0.31).

### **Leaf dry weight**

Leaf area was significantly correlated positively with total protein (0.47) but was significantly correlated negatively with specific leaf area (-0.90), number of secondary branches (-0.26).

### **Specific leaf area**

Specific leaf area was significantly correlated positively with total protein (0.28).

### **Pod length**

Pod length was not significantly correlated with any of the characters studied.

### **Pod width**

Pod width was not significantly correlated with any of the characters studied.

### **Number of locules per pod**

Number of locules per pod was significantly correlated positively with total protein (0.26) but negatively significantly correlated with percentage bud damage.

### **Number of seeds per pod**

Number of seeds per pod was significantly correlated negatively with number of primary branches (-0.23).

## **Seed weight**

Seed weight was not significantly correlated with any of the characters studied.

## **Number of primary branches**

Number of primary branches was not significantly correlated with any of the characters studied.

## **Number of secondary branches**

Number of secondary branches was significantly correlated negatively with percentage flower damage (-0.28).

## **Total seed protein**

Seed protein was not significantly correlated with any of the characters studied.

## **Bud damage**

Bud damage was significantly correlated positively with the percentage flower damage (0.36).

## **Flower damage**

Flower damage was not significantly correlated with any of the characters studied.

## **Pod damage**

Percentage pod damage was significantly correlated positively with the number of eggs (0.23) and number of larvae (0.23).

## Number of eggs

Numbers of eggs were significantly correlated positively with number of larvae (0.39).

## Interspecific hybridization

The second part of the present study includes the incorporation of podborer resistance gene (s) from the wild accessions of *C. scarabaeoides* to cultivated *C. cajan* through back cross programme and also to study the genetic basis of various characters by raising  $F_1$ ,  $F_2$ ,  $F_3$  and  $BC_1F_1$  generations.

Interspecific hybrids were produced by crossing the wild accessions of *C. scarabaeoides* (ICPW 94, ICPW 116, ICPW 125, ICPW 130 and ICPW 141) (Fig. 22) with cultivated varieties of *C. cajan* (ICP 28 and ICP 26) (Fig. 23). Though, *C. scarabaeoides* falls under the secondary gene pool it has 90 – 95 % is crosses compatibly with cultivated pigeonpeas giving fertile hybrids.

## Production of $F_1$ , $F_2$ , $F_3$ and backcross progenies

In the present wide hybridization programme the following crosses were made to raise different generations.

### $F_1$ generation

ICP 28 x ICPW 94 **	ICP 26 x ICPW 94*
ICP 28 x ICPW 116	ICP 26 x ICPW 116
ICP 28 x ICPW 125*	ICP 26 x ICPW 125**
ICP 28 x ICPW 130**	ICP 26 x ICPW 130*
ICP 28 x ICPW 141*	ICP 26 x ICPW 141

\* crosses involved in raising  $F_2$  generation

\*\* crosses involved in raising  $F_2$ ,  $F_3$ ,  $BC_1F_1$ ,  $BC_1F_2$ ,  $BC_2F_1$ ,  $BC_2F_2$  and  $BC_3$

**Fig. 22. *C. scarabaeoides* parents used in the crossing program**

**a) ICPW 94**

**b) ICPW 125**

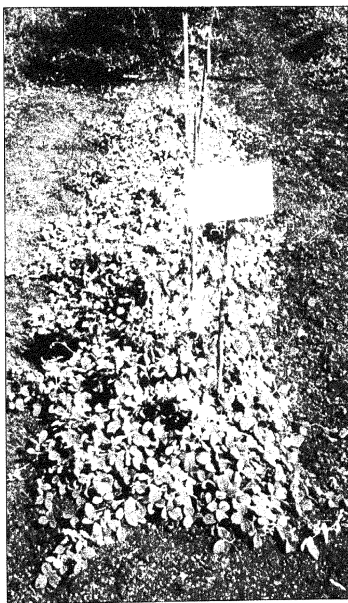
**c) ICPW 130**



a



b



c

Fig: 22

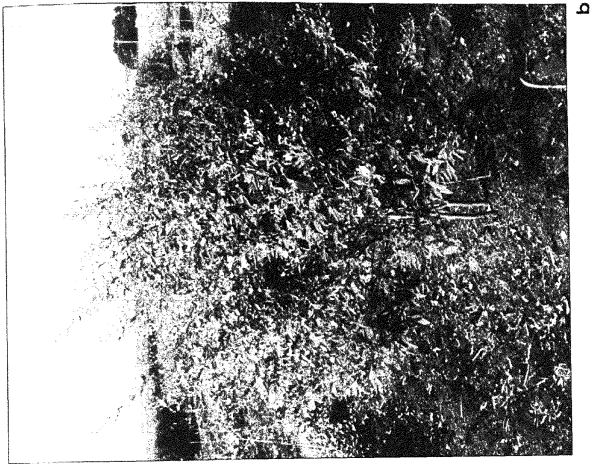
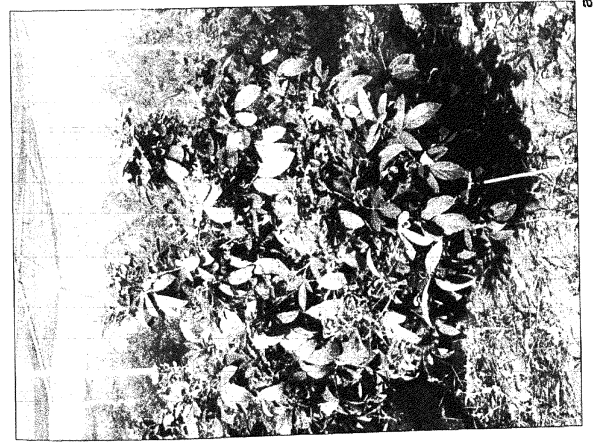


Fig: 23

**Fig. 23. *C. cajan* parents used in the crossing program**

**a) ICP 28**

**b) ICP 26**



The data regarding the number of pollination made in  $F_1$ ,  $BC_1F_1$  and  $BC_2F_1$  and  $BC_3F_1$  is presented in Tables 23, 24 and 25 respectively. While the percentage of seed germination in  $F_1$ ,  $F_2$ ,  $F_3$ ,  $BC_1$  and  $BC_2$  is presented in Tables 26, 27, 28 and 29 respectively.

In every generation, the plants were screened for podborer resistance and only the resistant lines were used in the subsequent generations.

## Evaluation of parents for different characters

Differences among means of parents, involved in the crosses, were evaluated using t- test. The t – values and the p – values for differences among the cultivated (ICP 28 and ICP 26) and wild (ICPW 94, ICPW 116, ICPW 125, ICPW 130 and ICPW 141) parents are presented (Tables 30 and 31). Highly significant differences were recorded among the parents for all the characters viz., days to flower and maturity, leaf length and width, pod length and width, pod bearing length, number of locules per pod, number of seeds per pod, 100 – seed weight, number of primary and secondary branches, seed protein, density of trichome A, B, C and D.

## Pollen fertility

Percentage pollen fertility in the hybridisation  $F_1$ ,  $F_2$  and  $BC_1F_1$ , in all the interspecific crosses is given in Table 32. The pollen fertility in pigeonpea cultivars of ICP 28 is 96.27 % and ICP 26 is 94.21 %; while in the *C. scarabaeoides* accessions is ICPW 94 it is 96.27 %, ICPW 116 it is 90.58 %, ICPW 125 is 90.58 %, ICPW 130 is 89.27 % and ICPW 141 is 81.45 %. Among the  $F_1$  hybrids; ICP 28 x ICPW 94 the most fertile hybrid with a mean of 87.02 % and ICP 28 x ICPW 116 was the least fertile hybrid with mean percentage hybrid fertility of 66.25 %. Among the  $F_2$  population, the most fertile plants were from ICP 28 x ICPW 94 segregants with a mean pollen fertility of 69.68 % and the least fertile hybrids belonged to ICP 28 x ICPW 125 with a mean pollen fertility of 40.44 %. Among the  $F_1$  hybrids, ICP 26 x ICPW 125 was the most fertile hybrid with a mean of 80.57 % and ICP 26 x

**Table 23: Number of pollinations to produce  $F_1$  seed**

S.No.	Cross	No. of pollinations	No. of pods formed	Pod setting (%)	No. of seeds
1	ICP 28 X ICPW 94	125	21	16.8	31
2	ICP 28 X ICPW 116	160	15	9.4	37
3	ICP 28 X ICPW 125	125	39	31.2	59
4	ICP 28 X ICPW 130	130	27	20.8	32
5	ICP 28 X ICPW 141	60	18	30	41
6	ICP 26 X ICPW 94	220	13	5.9	27
7	ICP 26 X ICPW 116	225	11	4.9	25
8	ICP 26 X ICPW 125	160	10	6.3	24
9	ICP 26 X ICPW 130	210	11	5.2	24
10	ICP 26 X ICPW 141	325	9	2.8	18

**Table 24 : Number of pollinations to produce  $BC_1F_1$  seed**

S.No	Cross	Plant No.	No. of Pollinations	No. of pods	No. of seeds obtained
1	ICP 28 X (ICP 28XICPW 94)	1	180	65	103
		2	280	85	171
		3	25	89	175
		4	150	37	72
		5	150	34	52
		6	150	50	86
2	ICP 28 X (ICP 28 X ICPW 130)	1	280	81	189
		2	280	62	158
		3	280	75	183
		4	130	50	106
		5	130	34	69
3	ICP 26 X (ICP 26 X ICPW 125)	1	200	33	61
		2	200	36	56
		3	200	23	39
		4	200	16	40
		5	200	19	37
4	ICPW 94 X (ICP 28 X ICPW 94)		200	36	101
5	ICPW 130 X (ICP 28 X ICPW 130)		250	47	192
6	ICPW 125 X (ICP 26 X ICPW 125)		210	17	60

**Table 25: Number of pollinations to produce BC<sub>2</sub>F<sub>1</sub> and BC<sub>3</sub>F<sub>1</sub> seed**

BC <sub>1</sub>	ICP 28 X( ICP 28 X( ICP 28X ICPW130))			ICP 28 X( ICP 28 X( ICP 28X ICPW130))			ICP 26 X( ICP 26 X( ICP 26X ICPW125))		
Plant No.	No. of pollinations	No. of pods	No. of seeds	No. of pollination	No. of pods	No. of seeds	No. of pollination	No. of pods	No. of seeds
1	151	30	47	141	52	80	141	21	33
2	125	44	83	121	63	145	121	25	51
3	156	58	102	125	33	50	125	32	10
4	100	60	184	142	89	125	142	59	148
5	100	42	64	112	49	83	112	28	55
6	125	62	142	95	17	20	95	46	108
7	142	68	119	41	5	85	112	59	74
8	125	67	149	114	90	220	114	57	134
9	121	75	140	115	64	100	115	34	79
10	124	65	229	121	75	160	121	102	203
11	125	60	111	124	104	207	124	38	66
12	126	24	32	95	15	29	95	63	126
13	128	85	170	96	35	45	96	24	53
14	125	69	98	98	35	38	98	60	59
15	111	36	54	42	39	77	42	41	76
BC <sub>3</sub>	ICP 28 x BC <sub>2</sub>			ICP 28 x BC <sub>2</sub>			ICP 26 x BC <sub>2</sub>		
1	51	30	29	45	21	80	36	21	45
2	65	14	21	56	63	125	11	25	51
3	56	35	49	36	33	50	25	32	69
4	100	20	39	42	56	125	42	59	69
5	92	42	64	12	49	83	12	28	55
6	59	26	42	95	17	85	95	46	58
7	65	45	36	41	15	59	12	59	74
8	74	52	100	59	59	78	14	57	134
9	29	11	29	69	64	78	15	34	79
10	89	65	229	21	75	91	21	18	59
11	59	39	46	24	39	59	24	14	66
12	69	29	32	52	48	68	30	21	51
13	53	29	85	59	44	71	42	24	53
14	59	29	68	55	35	58	59	36	59
15	65	36	54	42	39	100	42	26	76

**Table 26: Percentage of seed germination in F<sub>1</sub> generation**

S.No	Cross	No. of seeds sown	No. of hybrids	%
1	ICP 28 X ICPW 94	20	13	65
2	ICP 28 X ICPW 116	17	1	5.88
3	ICP 28 X ICPW 125	21	9	42.86
4	ICP 28 X ICPW 130	21	16	76.19
5	ICP 28 X ICPW 141	19	1	5.25
6	ICP 26 X ICPW 94	19	2	10.53
7	ICP 28 X ICPW 116	16	1	6.25
8	ICP 26 X ICPW 125	18	3	16.66
9	ICP 26 X ICPW 130	19	1	5.26
10	ICP 26 X ICPW 141	16	1	6.25

**Table 27: Percentage of seed germination in F<sub>2</sub> generation of seven crosses**

Cross	No. of seeds		Germination%
	sown	Germinated	
ICP 28 X ICPW 94	500	472	94.4
ICP 28 X ICPW 125	275	245	89.1
ICP 28 X ICPW 130	263	251	95.4
ICP 28 X ICPW 141	249	232	93.2
ICP 26 X ICPW 94	262	252	96.2
ICP 26 X ICPW 125	272	247	90.8
ICP 26 X ICPW 130	281	250	88.9

**Table 28: Percentage of seed germination in the F<sub>3</sub> generation of three crosses**

Cross	No. of F <sub>3</sub> families sown	No. of seeds		Germination %
		Sown	Germinated	
ICP 28 x ICPW 94	125	2256	2013	89.29
ICP 28 x ICPW 130	116	2141	1963	91.69
ICP 26 x ICPW 125	109	2000	1800	90.00

**Table 29: Percentage of seed germination in BC<sub>1</sub> and BC<sub>2</sub> generations**

Cross	No. of seeds		Germination (%)
	Sown	Germinated	
BC <sub>1</sub>			
[ICP 28 x (ICP 28 x ICPW 94)]	75	70	93.3
[ICP 28 x (ICP 28 x ICPW 130)]	81	71	87.7
[ICP 26 x (ICP 26 x ICPW 125)]	60	50	83.3
BC <sub>2</sub>			
[ ICP 28 x (ICP 28 x (ICP 28 x ICPW 94)]	110	95	86.4
[ ICP 28 x (ICP 28 x (ICP 28 x ICPW 130)]	125	112	89.6
[ ICP 26 x (ICP 26 x (ICP 26 x ICPW 125)]	95	80	84.2

Table- 30: Test of significance of means for morphological characters of two parents

Character	CP 26 x ICPW 94		CP 26 x ICPW 116		ICP 26 x ICPW 125		ICP 26 x ICPW 130		ICP 26 x ICPW 141	
	t-value	P value	t-value	P value	t-value	P value	t-value	P value	t-value	P value
Days to Flowering (50%) (No.)	4.09	0.004	55.15	<0.001	42.43	<0.001	22.64	<0.001	15.18	<0.001
Days to Maturity (75%) (No.)	10.11	<0.001	22.36	<0.001	11.63	<0.001	16.33	<0.001	1.63	0.141
Leaf Length (cm)	37.12	<0.001	33.00	<0.001	20.14	<0.001	17.78	<0.001	24.82	<0.001
Leaf Width (cm)	26.19	<0.001	14.98	<0.001	29.94	<0.001	4.73	0.006	28.34	<0.001
Pod Length (cm)	17.52	<0.001	19.25	<0.001	17.41	<0.001	18.23	<0.001	19.45	<0.001
Pod Width (cm)	2.53	0.035	2.53	0.035	2.12	0.067	3.54	0.008	1.41	0.195
Pod Bearing Length (cm)	21.43	<0.001	16.43	<0.001	17.46	<0.001	21.562	<0.001	24.621	<0.001
No. of Locules / Pod (No.)	1.23	0.864	1.00	0.347	2.14	0.065	3.21	0.012	3.21	0.012
No. of Seeds/ Pod (No.)	0.63	0.545	2.31	0.050	5.20	<0.001	5.06	<0.001	5.20	<0.001
100 – Seed Weight (g)	1069.33	<0.001	687.91	<0.001	2154.00	<0.001	2199.00	<0.001	1070.32	<0.001
No. of Primary Branches (No.)	19.61	<0.001	13.28	<0.001	13.91	<0.001	24.75	<0.001	38.58	<0.001
No. of Sec Branches (No.)	37.53	<0.001	11.50	<0.001	23.67	<0.001	34.64	<0.001	86.60	<0.001
Total Protein (mg)	124.12	<0.001	61.96	<0.001	106.57	<0.001	82.67	<0.001	212.29	<0.001
Trichome A (no./mm <sup>2</sup> )	41.28	<0.001	40.41	<0.001	35.37	<0.001	27.88	<0.001	29.95	<0.001
Trichome B (no./mm <sup>2</sup> )	38.54	<0.001	58.51	<0.001	48.86	<0.001	43.05	<0.001	29.85	<0.001
Trichome C (no./mm <sup>2</sup> )	441.15	<0.001	75.47	<0.001	127.22	<0.001	176.00	<0.001	173.43	<0.001
Trichome D (no./mm <sup>2</sup> )	90.93	<0.001	14.18	<0.001	81.44	<0.001	74.00	<0.001	63.06	<0.001

Table - 31: Test of significance of means for morphological characters of two parents

Character	ICP 28 x ICPW 94		ICP 28 x ICPW 116		CP 28 x ICPW 125		ICP 28 x ICPW 130		ICP 28 x ICPW 141	
	t-value	P value	t-value	P value	t-value	P value	t-value	P value	t-value	P value
Days to Flowering (50%) (No.)	15.78	<0.001	43.64	<0.001	26.56	<0.001	7.06	<0.001	4.33	0.003
Days to Maturity (75%) (No.)	6.72	<0.001	23.60	<0.001	14.65	<0.001	18.57	<0.001	5.69	<0.001
Leaf Length (cm)	12.11	<0.001	23.15	<0.001	10.96	<0.001	9.06	<0.001	16.40	<0.001
Leaf Width (cm)	16.25	<0.001	1.34	0.245	3.29	0.011	14.44	<0.001	6.33	0.002
Pod Length (cm)	44.95	<0.001	50.94	<0.001	46.49	<0.001	38.73	<0.001	47.63	<0.001
Pod Width (cm)	0.74	1.000	4.00	0.004	4.43	0.002	5.69	<0.001	3.13	<0.001
Pod Bearing Length (cm)	13.423	<0.001	21.16	<0.001	17.23	<0.001	18.731	<0.001	19.24	<0.001
No. of Locules / Pod (No.)	0.53	0.608	1.90	0.094	3.54	0.008	4.93	<0.001	4.95	<0.001
No. of Seeds / Pod (No.)	0.89	1.000	1.90	0.094	5.06	<0.001	4.95	<0.001	5.06	<0.001
100 - Seed Weight (g)	356.76	<0.001	349.67	<0.001	378.49	<0.001	386.5	<0.001	378.8	<0.001
No. of Primary branches (No.)	0.63	0.545	5.69	<0.001	5.06	<0.001	3.54	0.008	19.61	<0.001
No. of Sec branches (No.)	31.98	<0.001	12.94	<0.001	21.75	<0.001	29.85	<0.001	68.22	<0.001
Total Protein (mg)	37.95	<0.001	34.54	<0.001	32.21	<0.001	16.5	<0.001	20.02	<0.001
Trichome A (no./mm <sup>2</sup> )	50.54	<0.001	37.12	<0.001	47.71	<0.001	38.33	<0.001	42.19	<0.001
Trichome B (no./mm <sup>2</sup> )	27.75	<0.001	51.81	<0.001	39.18	<0.001	34.7	<0.001	18.54	<0.001
Trichome C (no./mm <sup>2</sup> )	417.28	<0.001	73.38	<0.001	118.38	<0.001	160.1	<0.001	160.51	<0.001
Trichome D (no./mm <sup>2</sup> )	75.92	<0.001	10.81	<0.001	37.84	<0.001	43.38	<0.001	42.34	<0.001

**Table - 32: Pollen fertility in parents, F<sub>1</sub>, F<sub>2</sub> & BC<sub>1</sub>F<sub>1</sub> hybrids**

Cross	Pollen fertility Mean $\pm$ SE	Range	Cross	Pollen fertility Mean $\pm$ SE	Range
<b>ICP 28 X ICPW 94</b>			<b>ICP 26 X ICPW 94</b>		
ICP 28	96.27 $\pm$ 1.802	94.42 - 98.21	ICP 26	94.21 $\pm$ 0.721	97.9 - 99.21
ICPW 94	93.20 $\pm$ 1.498	92.23 - 94.96	ICPW 94	93.20 $\pm$ 1.498	92.23 - 94.96
F <sub>1</sub>	87.02 $\pm$ 4.956	77.42 - 91.12	F <sub>1</sub>	69.21 $\pm$ 4.698	56.98 - 72.56
F <sub>2</sub>	69.68 $\pm$ 24.568	9.82 - 97.56	F <sub>2</sub>	62.58 $\pm$ 2.569	60.25 - 68.25
BC <sub>1</sub> F <sub>1</sub>	78.66 $\pm$ 20.82	25.00 - 99.65	BC <sub>1</sub> F <sub>1</sub> *	-	-
<b>CP 28 X ICPW 116</b>			<b>ICP 26 X ICPW 116</b>		
ICP 28	96.27 $\pm$ 1.802	94.42 - 98.21	ICP 26	94.21 $\pm$ 0.721	97.9-99.21
ICPW 116	90.58 $\pm$ 1.078	89.13-91.32	ICPW 116	90.58 $\pm$ 1.078	89.13-91.32
F <sub>1</sub>	66.25 $\pm$ 2.445	58.26-77.05	F <sub>1</sub>	57.69 $\pm$ 9.845	53.28-70.15
F <sub>2</sub>	52.36 $\pm$ 5.899	46.35-59.36	F <sub>2</sub>	45.69 $\pm$ 18.26	36.59-58.89
BC <sub>1</sub> P <sub>1</sub> *	-	-	BC <sub>1</sub> F <sub>1</sub> *	-	-
<b>CP 28 X ICPW 125</b>			<b>ICP 26 X ICPW 125</b>		
ICP 28	96.27 $\pm$ 1.802	94.42 - 98.21	ICP 26	94.21 $\pm$ 0.721	97.9-99.21
ICPW 125	90.58 $\pm$ 1.078	89.34-91.23	ICPW 125	90.58 $\pm$ 1.078	89.34-91.23
F <sub>1</sub>	74.59 $\pm$ 7.598	76.52-80.46	F <sub>1</sub>	80.57 $\pm$ 5.78	73.25-87.54
F <sub>2</sub>	40.44 $\pm$ 8.970	50.49-69.68	F <sub>2</sub>	54.11 $\pm$ 22.74	14.29-93.65
BC <sub>1</sub> F <sub>1</sub> *	-	-	BC <sub>1</sub> F <sub>1</sub>	84.66 $\pm$ 13.96	39.64-88.56
<b>CP 28 X ICPW 130</b>			<b>ICP 26 X ICPW 130</b>		
ICP 28	96.27 $\pm$ 1.802	94.42 - 98.21	ICP 26	94.21 $\pm$ 0.721	97.9-99.21
ICPW 130	89.27 $\pm$ 3.866	85.21-92.51	ICPW 130	89.27 $\pm$ 3.866	85.21-92.51
F <sub>1</sub>	75.56 $\pm$ 2.569	82.17-89.56	F <sub>1</sub>	70.59 $\pm$ 16.96	60.79-90.52
F <sub>2</sub>	59.68 $\pm$ 22.57	4.83-91.48	F <sub>2</sub>	59.26 $\pm$ 21.26	32.26-69.58
BC <sub>1</sub> F <sub>1</sub>	85.90 $\pm$ 13.99	7.41-98.52	BC <sub>1</sub> F <sub>1</sub> *	-	-
<b>CP 28 X ICPW 141</b>			<b>ICP 26 X ICPW 141</b>		
ICP 28	96.27 $\pm$ 1.802	94.42 - 98.21	ICP 26	94.21 $\pm$ 0.721	97.9-99.21
ICPW 141	81.45 $\pm$ 2.235	78.95-82.15	ICPW 141	81.45 $\pm$ 2.235	78.95-82.15
F <sub>1</sub>	71.69 $\pm$ 10.598	68.95-88.31	F <sub>1</sub>	67.82 $\pm$ 2.256	64.59-73.60
F <sub>2</sub>	54.11 $\pm$ 22.74	14.79-93.57	F <sub>2</sub>	56.29 $\pm$ 12.256	46.25-59.68
BC <sub>1</sub> F <sub>1</sub> *	-	-	BC <sub>1</sub> F <sub>1</sub> *	-	-
<b>* BC<sub>1</sub>F<sub>1</sub> not produced</b>					



ICPW 116 was the least fertile hybrid with mean percentage hybrid fertility of 57.69 %. Among the F<sub>2</sub> hybrids the most fertile plants were of ICP 26 x ICPW 94 segregants with a mean pollen fertility of 62.58 % and the least fertile belonged to ICP 26 x ICPW 116 cross with a mean pollen fertility of 45.69 %. Among the three backcross populations, [ICP 26 x (ICP 26 x ICPW 125)] population was the most fertile with a mean of 84.66 % and ICP 28x (ICP 28 x ICPW 94) was the least fertile with a mean of 78.66 %.

### **Mid parent and better parent heterosis and inbreeding depression**

Mid parent and Better parent heterosis were calculated for the morphological (days to flowering and maturity, leaf length and width, pod length and width, number of primary and secondary branches), agronomic (pod bearing length, number of locules and seeds per pod, 100- seed weight and harvest Index) and resistance related characters (density of trichomes A, B, C, and D). In the wide crosses of *C. scarabaeoides* with ICP 28 the mid parent and better parent heterosis and inbreeding depression values are presented in tables from 33 to 37; while in the crosses with ICP 26 they were presented in tables from 38 to 42. The F<sub>1</sub> interspecific hybrids produced are shown in figures 24, 25 and 26.

### **Days to flower**

In crosses of *C. scarabaeoides* with ICP 28 the mid parent heterosis for days to flower ranged from -17.71 in ICP 28 x ICPW 130 to 1.68 in ICP 28 x ICPW 116 and in crosses with ICP 26 the values ranged from - 4.80 in ICP 26 x ICPW 141 to 4.65 in ICP 26 x ICPW 125. Better parent heterosis in crosses with ICP 28 ranged from -9.49 (ICP 28 x ICPW 141) to 15.48 (ICP 28 x ICPW 130) and in crosses with ICP 26 from 0.39 in (ICP 26 x ICPW 94) to 21.22 in (ICP 26 x ICPW 125). The inbreeding depression values for days to flower in crosses of *C. scarabaeoides*

**Table - 33 Heterosis and Inbreeding depression for different traits in ICP 28 x ICPW 94**

Character	Mid Parental value	Better Parental value	F <sub>1</sub> Mean	F <sub>2</sub> Mean	Mid- parent Heterosis (%)	Better parent Heterosis (%)	Inbreeding depression (%)
Days to flower (No.)	59.8	53.2	52.9	50.2	-11.47**	-0.47	5.21
Days to maturity(No.)	91.7	85.6	93.5	91.2	1.91	9.16	2.41
Leaf length (cm)	5.6	6.4	5.6	3.8	0.90	-11.48	32.64
Leaf width (cm)	2.6	3.0	3.2	1.7	25.10*	7.05	47.96*
Pod length (cm)	4.0	5.5	4.1	3.0	2.01	-25.73	26.40**
Pod width (cm)	0.6	0.7	0.9	0.8	28.57*	50.00**	11.11
Pod bearing length (cm)	12.8	18.3	11.2	9.5	-12.16*	-38.80**	15.45
No. of locules /pod	3.9	4.1	4.3	3.9	9.11	4.61	9.23
No. of seeds /pod	3.8	3.8	4.3	3.9	12.83*	13.42	9.23
No. of primary branches	9.5	9.4	7.2	6.7	-22.26**	23.09**	7.33
No. of secondary branches	9.9	17.4	20.6	13.9	107.86**	18.51**	32.47**
Seed weight (g)	7.0	11.0	5.53	5.0	-20.77*	-49.82**	10.38
Harvest Index	12.2	18.2	15.4	15.9	26.00*	-15.47**	9.09
Seed protein (%)	24.8	27.2	25.9	#	4.43	-4.85	#
Density of Trichome A (No. / mm <sup>2</sup> )	1.8	5.0	0.9	0.8	-48.88*	80.00**	12.09
Density of Trichome B (No. / mm <sup>2</sup> )	3.8	5.3	5.3	3.6	41.33*	-0.39	32.83**
Density of Trichome C (No. / mm <sup>2</sup> )	110.6	190.3	154.0	129.7	39.23**	-19.06**	15.77
Density of Trichome D (No. / mm <sup>2</sup> )	6.2	8.8	6.7	5.7	8.49	-23.69*	14.48

# = not estimated; \* = Significant at 5% level; \*\* = Significant at 1% level

**Table - 34: Heterosis and Inbreeding depression for different traits in ICP 28 x ICPW 116**

Character	Mid-Parental value	Better Parental value	F <sub>1</sub> mean	Mid- parent Heterosis(%)	Better parent Heterosis(%)
Days to flower (No.)	73.3	66.4	74.6	1.68	12.29*
Days to maturity (No.)	110.4	97.8	105.2	-4.79	7.60*
Leaf length (cm)	5.3	6.4	4.7	-9.91	-25.63
Leaf width (cm)	2.2	2.2	3.0	35.32*	36.36*
Pod length (cm)	3.9	5.5	3.3	-16.02	-40.69**
Pod width (cm)	0.6	0.7	0.9	28.57*	50.00**
Pod bearing length (cm)	13.2	19.3	11.3	-14.97**	-41.56**
No. of locules /pod	4.1	4.4	4.2	2.44	-4.55
No. of seeds /pod	4.1	4.4	4.4	7.32	-0.23
Seed weight (g)	6.5	11.0	6.9	5.29	-43.92**
No. of primary branches	8.3	9.3	6.6	-20.48**	-28.26*
No. of secondary branches	5.9	9.4	10.9	84.75**	48.72**
Harvest Index	11.25	18.23	18.52	64.62**	1.59
Seed protein (%)	25.4	28.3	24.8	-2.21	-12.24**
Density of Trichome A (No. / mm <sup>2</sup> )	2.7	4.8	0.6	-76.87**	-87.03
Density of Trichome B (No. / mm <sup>2</sup> )	6.8	11.8	11.3	68.00**	-4.06
Density of Trichome C (No. / mm <sup>2</sup> )	99.5	169.6	162.4	63.13**	-4.35
Density of Trichome D (No. / mm <sup>2</sup> )	6.0	8.4	8.4	40.10*	0.24
* Significant at 5% level;                      ** Significant at 1% level					

Table - 35: Heterosis and Inbreeding depression for different traits in ICP 28 x ICPW 125

Character	Mid-Parental value	Better Parental value	F <sub>1</sub> mean	F <sub>2</sub> mean	Mid-parent Heterosis (%)	Better parent Heterosis (%)	Inbreeding Depression %
Days to flower (No.)	70.6	66.4	71.6	70.9	1.39	7.82	0.92
Days to maturity (No.)	105.1	97.8	103.3	99.0	-1.64	5.65	4.02
Leaf length (cm)	5.9	6.4	5.3	1.4	-10.22	-17.14	73.79**
Leaf width (cm)	2.0	2.1	2.2	2.1	9.36	4.72	6.31
Pod length (cm)	4.0	5.5	3.8	2.4	-5.49-	-30.84	37.24
Pod width (cm)	0.6	0.7	1.1	0.8	83.61**	64.71**	25.56
Pod bearing length (cm)	11.2	15.2	12.3	11.7	9.27	-19.55*	4.40
No. of locules / pod	4.3	4.8	4.4	2.4	2.33	-8.51-	44.54**
No. of seeds / pod	4.6	5.4	4.4	2.5	-4.35	-18.52	44.37**
Seed weight (g)	6.9	11.0	5.7	5.1	-16.52*	-48.64*	9.19
No. of primary branches	8.5	9.3	7.3	7.0	-13.10	-20.65*	2.87
No. of secondary branches	7.6	12.6	13.9	6.7	83.86**	10.32	51.51**
Harvest Index	11.63	18.25	18.86	14.12	57.01**	1.34*	25.13
Seed protein (%)	24.5	26.6	23.6	#	-3.63	-11.07	#
Density of Trichome A (No. / mm <sup>2</sup> )	2.6	4.6	0.7	#	-75.38**	-85.99	#
Density of Trichome B (No. / mm <sup>2</sup> )	4.9	7.6	6.5	#	32.65*	-14.47	#
Density of Trichome C (No. / mm <sup>2</sup> )	95.1	159.3	160.2	#	68.88**	0.56	#
Density of Trichome D (No. / mm <sup>2</sup> )	4.7	6.2	5.6	#	20.00	-10.63	#

# = Not estimated;

\* = Significant at 5% level;

\*\* = Significant at 1% level

Table - 36: Heterosis and Inbreeding depression for different traits in ICP 28 x ICPW 130

Character	Mid - Parental value	Better Parental value	F <sub>1</sub> Mean	F <sub>2</sub> Mean	Mid- parent Heterosis (%)	Better parent Heterosis (%)	In breeding Depression (%)
Days to flower (No.)	68.2	66.4	56.1	53.1	-17.71**	15.48**	5.33
Days to maturity (No.)	110.6	97.8	105.8	102.1	-4.32	-8.20	3.48
Leaf length (cm)	6.0	6.4	5.5	3.0	-7.23	-13.21	45.53**
Leaf width (cm)	2.7	3.2	2.7	1.3	0.00	-15.22*	51.22**
Pod length (cm)	3.9	5.5	3.4	2.7	-11.66	-37.77**	22.29
Pod width (cm)	0.6	0.7	0.8	0.7	33.33*	6.94*	6.15
Pod bearing length (cm)	13.3	19.4	10.4	9.6	-22.03**	-46.51**	7.44
No. of locules /pod	4.5	5.3	3.8	2.8	-15.11*	-27.38*	25.63
No. of seeds / pod	4.6	5.2	3.6	3.2	-20.17	-30.00*	12.09
Seed weight (g)	6.7	11.0	6.0	5.5	-11.06	-46.01	7.23
No. of primary branches	9.7	10.2	8.3	7.3	-14.74*	-19.16*	12.21
No. of secondary branches	9.5	16.4	17.5	3.9	84.66**	6.40**	20.23
Harvest Index	12.82	18.23	18.46	17.92	43.99**	1.24	2.92
Seed protein (%)	23.5	24.6	20.9	#	-11.06	-14.86**	#
Density of Trichome A (No. / mm <sup>2</sup> )	2.6	4.6	0.7	#	-74.42**	-85.56	#
Density of Trichome B (No. / mm <sup>2</sup> )	5.0	7.9	7.7	#	50.55*	3.66	#
Density of Trichome C (No. / mm <sup>2</sup> )	95.4	160.1	157.1	#	64.59**	-1.82	#
Density of Trichome D (No. / mm <sup>2</sup> )	5.2	7.2	6.7	#	29.08	-5.82	#

# = Not estimated;

\* = Significant at 5% level;

\*\* = Significant at 1% level

**Table - 37: Heterosis and Inbreeding depression for different traits in ICP 28 x ICPW 141**

Character	Mid Parental value	Better Parental value	F <sub>1</sub> Mean	F <sub>2</sub> Mean	Mid- parent Heterosis (%)	Better parent Heterosis (%)	Inbreeding Depression (%)
Days to flower (No.)	68.0	66.4	60.1	59.1	-11.55*	-9.49**	1.66
Days to maturity(No.)	101.2	97.8	96.5	95.1	-4.63	-1.84	1.43
Leaf length (cm)	5.5	6.4	5.2	3.7	-5.78	-17.93*	28.88
Leaf width (cm)	1.9	2.1	3.1	1.3	59.38*	44.34*	56.64
Pod length (cm)	3.8	5.5	3.9	2.9	2.61	-28.29	26.08
Pod width (cm)	0.6	0.7	0.8	0.7	26.23	13.24	6.15
Pod bearing length (cm)	14.4	21.6	14.3	13.1	-0.90	-33.91*	7.86
No. of locules /pod	4.5	5.3	4.4	9.3	-2.22	-16.16	25.00
No. of seeds /pod	4.7	5.4	4.4	6.8	-6.52	-19.74	9.20
Seed weight (g)	6.5	11.0	4.5	2.8	-30.37**	-58.80**	37.67
No. of primary branches	12.3	15.4	6.3	3.0	-49.59**	-59.74**	4.64
No. of secondary branches	18.4	34.4	20.3	3.9	9.99	-41.86**	38.00
Harvest Index	11.85	18.21	15.63	14.14	31.89*	-16.50	9.53
Seed protein (%)	25.0	27.6	22.0	#	-11.31*	-20.17	#
Density of Trichome A (No. / mm <sup>2</sup> )	2.9	4.6	0.7	#	-75.79*	-85.13**	#
Density of Trichome B (No. / mm <sup>2</sup> )	3.3	4.4	4.4	#	32.83*	0.00	#
Density of Trichome C (No. / mm <sup>2</sup> )	104.6	178.6	168.8	#	61.37**	-5.34	#
Density of Trichome D (No. / mm <sup>2</sup> )	5.6	7.7	6.5	#	17.20*	-14.84**	#

# = Not estimated; \* = Significant at 5% level; \*\* = Significant at 1% level



Table - 39: Heterosis and Inbreeding depression for different traits in ICP 26 x ICPW 116

Character	Mid Parental value	Better Parental value	F <sub>1</sub> Mean	Mid- parent Heterosis (%)	Better parent Heterosis (%)
Days to flower (No.)	68.4	56.8	67.4	-1.45	18.68*
Days to maturity(No.)	114.6	103.3	105.6	-7.81	2.27
Leaf length (cm)	5.6	6.9	5.7	1.62	-18.44**
Leaf width (cm)	2.9	3.6	3.4	15.46	-6.15
Pod length (cm)	4.0	5.8	3.6	-11.17	-38.49*
Pod width (cm)	0.6	0.7	0.9	53.45**	30.88**
Pod bearing length (cm)	14.4	21.6	18.5	28.37*	-14.38
No. of locules /pod	4.3	4.4	4.3	0.00	-3.85
No. of seeds /pod	4.3	4.5	4.4	-2.22	2.33
Seed weight (g)	6.8	11.6	4.5	-33.43*	-60.92**
No. of primary branches	5.4	7.5	4.6	-14.18	-38.26*
No. of secondary branches	6.9	9.3	11.3	64.23**	21.62**
Harvest Index	10.25	16.25	15.98	35.88*	-1.69
Seed protein (%)	24.3	28.3	23.2	-4.41	-18.06
Density of Trichome A(No. / mm <sup>2</sup> )	2.0	3.4	0.5	-72.73**	-84.02**
Density of Trichome B(No. / mm <sup>2</sup> )	6.3	11.8	11.6	85.03**	-1.69
Density of Trichome C(No. / mm <sup>2</sup> )	97.2	169.3	165.2	69.94**	-2.42
Density of Trichome D(No. / mm <sup>2</sup> )	5.4	8.4	6.5	19.85	-22.57*

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



**Table - 40: Heterosis and Inbreeding depression for different traits in ICP 26 x ICPW 125**

[illegible]

**Table 41: Heterosis and Inbreeding depression for different traits in ICP 26 x ICPW 130**

Character	Mid Parental value	Better Parental value	F <sub>1</sub> Mean	F <sub>2</sub> Mean	Mid- parent Heterosis (%)	Better parent Heterosis (%)	Inbreeding Depression (%)
Days to flower (No.)	63.4	56.8	61.6	59.7	-2.92	8.34**	3.04
Days to maturity (No.)	113.3	103.3	108.7	106.7	-4.00	5.31	1.87
Leaf length (cm)	6.2	6.9	5.5	4.6	-11.54	-20.46*	16.30
Leaf width (cm)	3.4	3.6	2.7	1.5	-20.18**	-23.74*	45.06*
Pod length (cm)	4.0	5.8	3.4	2.5	-15.17	-41.41**	25.81**
Pod width (cm)	0.7	0.7	0.8	0.8	14.29	14.29	0.00
Pod bearing length (cm)	13.3	19.4	10.2	9.3	-23.06*	-47.24**	89.13**
No. of locules /pod	4.4	5.3	3.8	2.7	-13.58	-27.38**	30.37*
No. of seeds /pod	4.6	5.2	3.6	2.5	-21.21*	-30.53**	31.87
Seed weight (g)	7.0	11.6	5.6	5.1	-19.68*	-51.64**	8.41
No. of primary branches	6.7	10.3	8.3	8.0	23.06*	-19.32*	3.14
No. of secondary branches	10.4	16.4	17.3	9.4	65.55**	5.05**	77.74**
Harvest Index	11.45	17.25	18.13	17.00	58.34**	5.10	6.23
Seed protein (%)	22.2	24.6	20.9	#	-5.60	-14.87	#
Density of Trichome A (No. / mm <sup>2</sup> )	2.1	3.4	2.9	#	37.91*	13.91	#
Density of Trichome B (No. / mm <sup>2</sup> )	4.3	7.9	4.6	#	7.19	-41.23**	#
Density of Trichome C (No. / mm <sup>2</sup> )	92.4	160.2	146.1	#	58.09**	-8.79	#
Density of Trichome D (No. / mm <sup>2</sup> )	4.8	7.1	4.4	#	-9.55	-38.85*	#

# = not estimated; \* - Significant at 5% level; \*\* = Significant at 1% level

Table - 42: Heterosis and Inbreeding depression for different traits in ICP 26 x ICPW 141

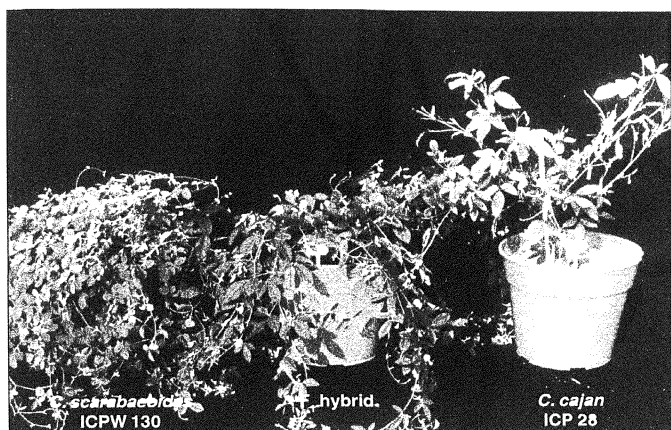
Character	Mid Parental value	Better Parental value	F <sub>1</sub> Mean	Mid- parent Heterosis (%)	Better parent Heterosis (%)
Days to flower (No.)	63.2	56.8	60.1	-4.80	5.81**
Days to maturity (No.)	103.8	103.3	97.0	-6.62	-6.11
Leaf length (cm)	5.8	6.9	5.2	-10.46	-24.79**
Leaf width (cm)	2.7	3.7	3.1	15.47	-14.53
Pod length (cm)	4.0	5.8	3.9	-1.50	-32.47**
Pod width (cm)	0.7	0.7	0.8	14.29	14.29
Pod bearing length (cm)	14.4	21.6	11.3	-21.70*	-47.87**
No. of locules /pod	4.4	5.2	4.4	0.23	-15.30
No. of seeds /pod	4.7	5.4	4.3	-8.69	-20.48
Seed weight (g)	6.8	11.6	4.5	-33.14	-60.73**
No. of primary branches	9.3	15.4	6.2	-33.15**	-59.60**
No. of secondary branches	19.4	34.4	20.1	3.60	-41.53*
Harvest Index	11.25	16.25	15.69	39.47	-3.45
Seed protein (%)	23.7	27.7	22.2	-6.08*	-19.60
Density of Trichome A (No. / mm <sup>2</sup> )	2.2	3.4	2.6	16.22	-23.67
Density of Trichome B (No. / mm <sup>2</sup> )	2.6	4.4	4.3	65.91**	-2.48
Density of Trichome C (No. / mm <sup>2</sup> )	101.2	178.2	149.2	47.42**	-16.27
Density of Trichome D (No. / mm <sup>2</sup> )	5.1	7.7	4.6	-10.04	-40.50**

\* = Significant at 5% level;

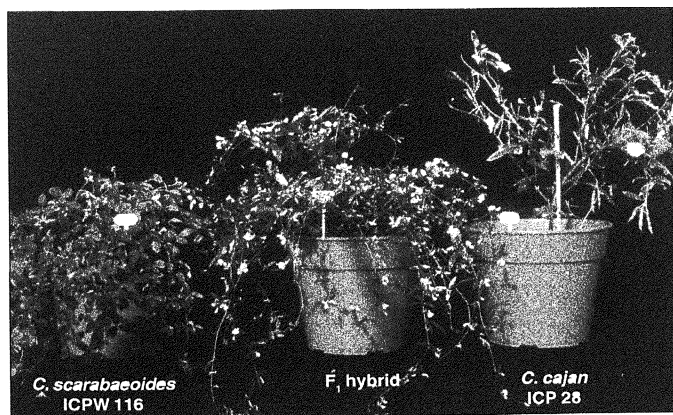
\*\* = Significant at 1% leve

**Fig. 24: F<sub>1</sub> hybrids and its parents used in wide hybridization**

- a)      ICP 28 x ICPW 130**
- b)      ICP 28 x ICPW 116**



a

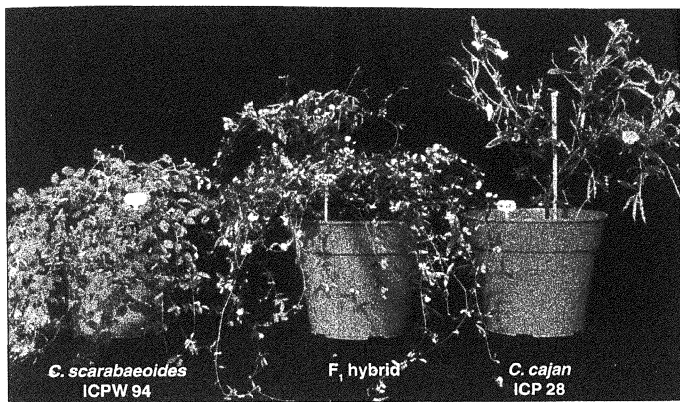


b

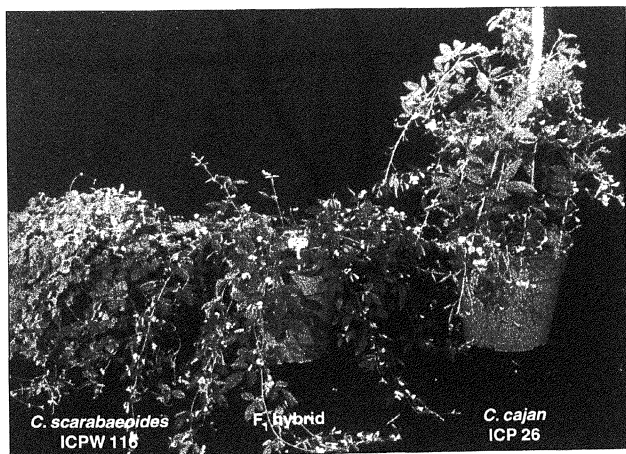
Fig: 24

**Fig. 24: F<sub>1</sub> hybrids and its parents used in wide hybridization**

- c)      ICP 28 x ICPW 94
- d)      ICP 26 x ICPW 116



c



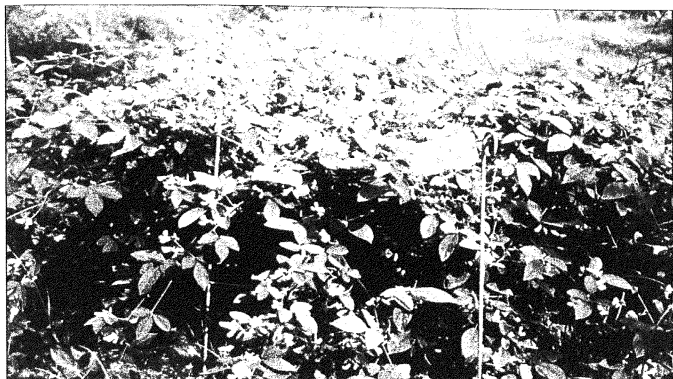
d

Fig: 24

**Fig. 25: F<sub>1</sub> hybrids produced in wide hybridization**

- a)      ICP 28 x ICPW 125**
- b)      ICP 28 x ICPW 130**





a



Fig: 25

**Fig. 25: F<sub>1</sub> hybrids produced in wide hybridization**

- c)        ICP 28 x ICPW 94**
- d)        ICP 28 x ICPW 116**



c



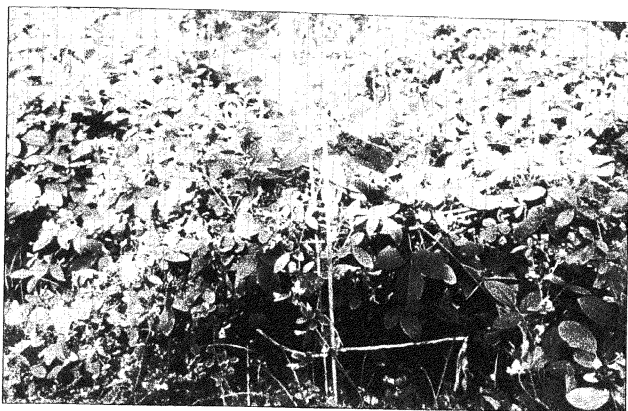
d

Fig: 25

**Fig. 25: F<sub>1</sub> hybrids produced in wide hybridization**

**e)        ICP 26 x ICPW 125**

**f)        ICP 28 x ICPW 141**



e



f

Fig: 25

Fig. 25: F<sub>1</sub> hybrids produced in wide hybridization

g) ICP 26 x ICPW 94

h) ICP 26 x ICPW 130



g



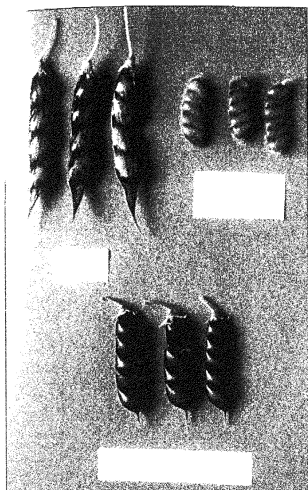
h

**Fig: 25**

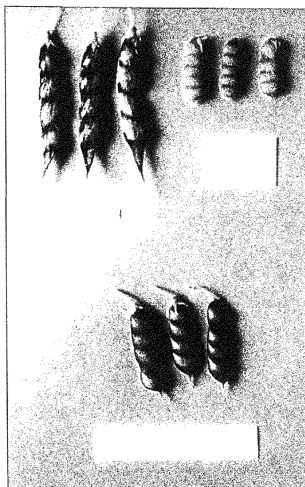
Fig. 26: Pods of  $F_1$  along with their parents

- a) ICP 28 x ICPW 94
- b) ICP 28 x ICPW 130
- c) ICP 28 x ICPW 125
- d) ICP 26 x ICPW 125

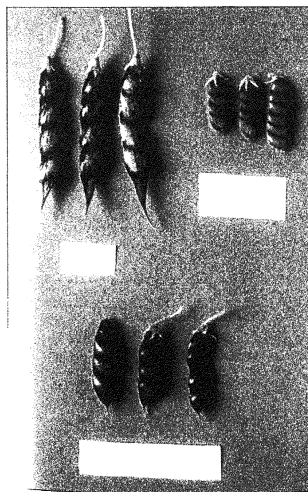




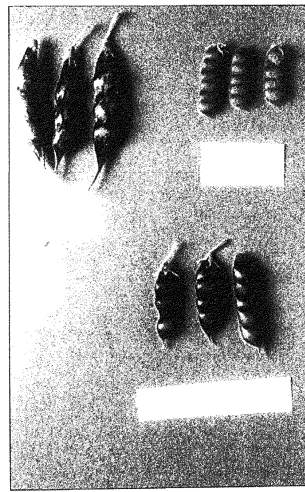
a



b



c



d

Fig: 26

accessions (ICPW 94, ICPW 125, ICPW 130, ICPW 141) with *C. cajan* ICP 28, ranged from 0.92 (ICP 28 x ICPW 125) to 5.33 (ICP 28 x ICPW 130) and in crosses with ICP 26 the values ranged from 2.42 (ICP 26 x ICPW 94) to 3.04 (ICP 26 x ICPW 130) (Tables 33 – 42).

### **Days to maturity**

Days to maturity, in crosses with ICP 28 the mid- parent heterosis ranged from – 4.799 (ICP 28 x ICPW 116) to 1.91 (ICP 28 x ICPW 94) and with ICP 26 it ranged from –7.81 (ICP 26 x ICPW 116) to 2.65 (ICP 26 x ICPW 94). Better parent heterosis in crosses with ICP 28 ranged from – 8.20 (ICP 28 x ICPW 130) to 9.16 (ICP 28 x ICPW 94) and in crosses with ICP 26 from –13.08 (ICP 26 x ICPW 94) to 5.31 (ICP 26 x ICPW 130). Inbreeding depression for days to maturity the values ranged from 1.43 (ICP 28 x ICPW 141) to 4.20 (ICP 28 x ICPW 125) and in crosses with ICP 26 it ranged from 1.87 (ICP 26 x ICPW 130) to 2.69 (ICP 26 x ICPW 94) (Tables 33 – 42).

### **Leaflet length**

Mid parent heterosis for leaflet length in crosses with ICP 28 ranged from – 10.22 (ICP 28 x ICPW 125) to 0.90 (ICP 28 x ICPW 94) and in crosses with ICP 26 from –12.05 (ICP 26 x ICPW 94) to 1.62 (ICP 26 x ICPW 116). Better parent heterosis for leaflet length, in crosses with ICP 28, ranged from –25.63 (ICP 28 x ICPW 116) to 17.14 (ICP 28 x ICPW 125) and in crosses with ICP 26 from –25.36 (ICP 26 x ICPW 94) to –18.44 (ICP 26 x ICPW 125). Inbreeding depression in crosses with ICP 28 ranged from 28.88 (ICP 28 x ICPW 141) to 73.79 (ICP 28 x ICPW 125) and in crosses with ICP 26 from 16.30 (ICP 26 x ICPW 130) to 70.42 (ICP 26 x ICPW 94) (Tables 33 – 42).

## **Leaflet width**

Mid parent heterosis for leaflet width, in crosses with ICP 28, the values ranged from 2.25 (ICP 28 x ICPW 130) to 59.38 (ICP 28 x ICPW 141) and in crosses with ICP 26 from -20.18 (ICP 26 x ICPW 130) to 15.47 (ICP 26 x ICPW 141). Better parent heterosis in crosses with ICP 28 ranged from -15.22 (ICP 28 x ICPW 130) to 44.34 (ICP 28 x ICPW 141) and in crosses with ICP 26 from -3.26 (ICP 26 x ICPW 125) to 15.46 (ICP 26 x ICPW 116). Inbreeding depression values ranged from 6.31 (ICP 28 x ICPW 125) to 56.64 (ICP 28 x ICPW 141) and in crosses with ICP 26 from 9.16 (ICP 26 x ICPW 94) to 48.31 (ICP 26 x ICPW 125) (Tables 33 – 42).

## **Pod length**

Mid parent heterosis for pod length, in crosses with ICP 28, ranged from -16.02 (ICP 28 x ICPW 116) to 2.61 (ICP 28 x ICPW 141) and in crosses with ICP 26 from -15.17 (ICP 26 x ICPW 130) to 1.50 (ICP 26 x ICPW 141). Better parent heterosis for pod length, in crosses with ICP 28, ranged from -40.69 (ICP 28 x ICPW 116) to -25.7 (ICP 28 x ICPW 94) and in crosses with ICP 26 from -41.41 (ICP 26 x ICPW 130) to -32.47 (ICP 26 x ICPW 141). Inbreeding depression for pod length, in crosses with ICP 28, ranged from 22.29 (ICP 28 x ICPW 130) to 37.24 (ICP 28 x ICPW 125) and in crosses with ICP 26 from 25.81 (ICP 26 x ICPW 130) to 41.31 (ICP 26 x ICPW 125) (Tables 33 – 42).

## **Pod width**

For pod width, in crosses with ICP 28, the mid parent heterosis ranged from 33.33 (ICP 28 x ICPW 130) to 83.61 (ICP 28 x ICPW 125) and in crosses with ICP 26 the mid parent heterosis ranged from 14.29 (ICP 26 x ICPW 130) to 53.45 (ICP 26 x ICPW 116). Better parent heterosis ranged from 6.94 (ICP 28 x ICPW 130) to 64.71 (ICP 28 x ICPW 125) and in crosses with ICP 26 from 14.29 (ICP 26 x ICPW 130) to 35.90 (ICP 26 x ICPW 94). Inbreeding depression for pod width the values

ranged from 11.11 (ICP 28 x ICPW 94) to 25.56 (ICP 28 x ICPW 125) and in crosses with ICP 26 from 1.30 (ICP 26 x ICPW 130) to 9.52 (ICP 26 x ICPW 94) ) (Tables 33 – 42).

### **Pod bearing length**

Mid parent heterosis for pod bearing length, in crosses with ICP 28, the values ranged from –22.03 (ICP 28 x ICPW 130) to 9.27 (ICP 28 x ICPW 125) and in crosses with ICP 26 from –25.80 (ICP 26 x ICPW 94) to 28.37 (ICP 26 x ICPW 116). Better parent heterosis, in crosses with ICP 28, ranged from – 46.51 (ICP 28 x ICPW 130) to -19.55 (ICP 28 x ICPW 125) and in crosses with ICP 26 from – 48.31 (ICP 26 x ICPW 94) to –14.38 (ICP 26 x ICPW 116). Inbreeding depression for pod bearing length ranged from 4.40 (ICP 28 x ICPW 125) to 15.45 (ICP 28 x ICPW 94) and in crosses with ICP 26 from 3.59 in (ICP 26 x ICPW 94) to 89.13 (ICP 26 x ICPW 130) (Tables 33 – 42).

### **Number of locules per pod**

Mid parent heterosis for number of locules per pod, in crosses with ICP 28, ranged from –15.11 (ICP 28 x ICPW 130) to 9.11 (ICP 28 x ICPW 94) and in crosses with ICP 26 from –13.58 (ICP 26 x ICPW 130) to 10.53 (ICP 26 x ICPW 94). Better parent heterosis for number of locules per pod, in crosses with ICP 28, ranged from – 27.38 (ICP 28 x ICPW 130) to 4.61 (ICP 28 x ICPW 94) and in crosses with ICP 26 from – 27.38 (ICP 26 x ICPW 130) to 5.25 (ICP 26 x ICPW 94). Inbreeding depression in crosses with ICP 28 ranged from 9.23 (ICP 28 x ICPW 94) to 44.54 (ICP 28 x ICPW 125) and in crosses with ICP 26 from 23.01 (ICP 26 x ICPW 125) to 34.92 (ICP 26 x ICPW 94) (Tables 33 – 42).

### **Number of seeds per pod**

Mid parent heterosis for number of seeds per pod, in crosses with ICP 28, ranged from –20.17 (ICP 28 x ICPW 130) to 12.83 (ICP 28 x ICPW 94) and in crosses with ICP 26 ranged from –21.21 (ICP 26 x ICPW 130) to 10.56 (ICP 26 x

ICPW 125). Better parent heterosis for number of seeds per pod, in crosses with ICP 28 ranged from – 30.00 (ICP 28 x ICPW 130) to 13.42 (ICP 28 x ICPW 94) and in crosses with ICP 26 from –30.53 (ICP 26 x ICPW 130) to 5.72 in (ICP 26 x ICPW 94). The inbreeding depression for number of seeds per pod the values ranged from 9.20 (ICP 28 x ICPW 141) to 44.37 (ICP 28 x ICPW 125) and in crosses with ICP 26 from 20.33 (ICP 26 x ICPW 125) to 43.53 (ICP 26 x ICPW 94) (Tables 33 – 42).

### **Number of primary branches**

Mid parent heterosis for number of primary branches, in crosses with ICP 28, ranged from –49.59 (ICP 28 x ICPW 141) to -13.10 (ICP 28 x ICPW 125) and in crosses with ICP 26 ranged from -33.15 (ICP 26 x ICPW 141) to 23.06 (ICP 26 x ICPW 130). Better parent heterosis in crosses with ICP 28 ranged from –59.74 (ICP 28 x ICPW 141) to 23.09 (ICP 28 x ICPW 94) and in crosses with ICP 26 from –59.60 (ICP 26 x ICPW 141) to –19.32 (ICP 26 x ICPW 130). The inbreeding depression for number of primary branches, in crosses with ICP 28, ranged from 2.87 (ICP 28 x ICPW 125) to 12.21 (ICP 28 x ICPW 130) and in crosses with ICP 26 from 3.14 (ICP 26 x ICPW 130) to 14.01 (ICP 26 x ICPW 125) (Tables 33 – 42).

### **Number of secondary branches**

Mid parent heterosis for number of secondary branches in crosses with ICP 28 ranged from 9.99 (ICP 28 x ICPW 141) to 84.75 (ICP 28 x ICPW 116) and in crosses with ICP 26 ranged from 3.60 (ICP 26 x ICPW 141) to 68.81 (ICP 26 x ICPW 94). Better parent heterosis, in crosses with ICP 28 ranged from –41.86 (ICP 28 x ICPW 141) to 48.72 (ICP 28 x ICPW 116) and in crosses with ICP 26 from –41.53 (ICP 26 x ICPW 141) to 21.62 (ICP 26 x ICPW 116). Inbreeding depression in crosses with ICP 28 from 20.23 (ICP 28 x ICPW 130) to 51.51 (ICP 28 x ICPW 125) and in crosses with ICP 26 from 24.86 (ICP 26 x ICPW 94) to 77.74 (ICP 26 x ICPW 130) (Tables 33 – 42).

## **100 – seed weight**

Mid parent heterosis for 100 – seed weight, in crosses with ICP 28, ranged from –30.37 (ICP 28 x ICPW 141) to 5.29 (ICP 28 x ICPW 116) and in crosses with ICP 26 from –39.90 (ICP 26 x ICPW 94) to –19.68 (ICP 26 x ICPW 130). Better parent heterosis for 100 seed- weight, in crosses with ICP 28, ranged from –58.80 (ICP 28 x ICPW 141) to – 43.92 (ICP 28 x ICPW 116) and in crosses with ICP 26 from – 62.28 (ICP 26 x ICPW 94) to –51.64 (ICP 26 x ICPW 130). The inbreeding depression, in crosses with ICP 28, ranged from 7.23 (ICP 28 x ICPW 130) to 37.67 (ICP 28 x ICPW 141) and in crosses with ICP 26 from 8.41 (ICP 26 x ICPW 130) to 16.37 (ICP 26 x ICPW 125) (Tables 33 – 42).

## **Seed protein**

Mid parent heterosis for seed protein, in crosses with ICP 28, ranged from – 11.06 (ICP 28 x ICPW 130) to 4.43 (ICP 28 x ICPW 94) and in crosses with ICP 26 from –11.40 (ICP 26 x ICPW 125) to –1.75 (ICP 26 x ICPW 94). Better parent heterosis in crosses with ICP 28 ranged from –20.17 (ICP 28 x ICPW 141) to –4.85 (ICP 28 x ICPW 94) and in crosses with ICP 26 from –22.78 (ICP 26 x ICPW 125) to –14.87 (ICP 26 x ICPW 130) (Tables 33 – 42).

## **Harvest Index**

Mid- parent heterosis for harvest index, in crosses with ICP 28, ranged from 26.00 (ICP 28 x ICPW 116) to 64.62 (ICP 28 x ICPW 116) and in crosses with ICP 26 from 30.87 (ICP 26 x ICPW 125) to 58.34 (ICP 26 x ICPW 130). Better parent heterosis, in crosses with ICP 28, ranged from –19.50 (ICP 28 x ICPW 141) to 3.34 (ICP 28 x ICPW 125) and in crosses with ICP 26 from –6.49 (ICP 26 x ICPW 94) to 5.10 (ICP 26 x ICPW 130). The inbreeding depression in crosses with ICP 28, ranged from 2.92 (ICP 28 x ICPW 130) to 25.13 (ICP 28 x ICPW 125) and in crosses with ICP 26 from 1.70 (ICP 26 x ICPW 94) to 6.23 (ICP 26 x ICPW 130) (Tables 33 – 42).

### **Density of trichome A**

Mid parent heterosis for density of trichome A, in crosses with ICP 28, ranged from -76.87 (ICP 28 x ICPW 116) to 48.88 (ICP 28 x ICPW 94) and in crosses with ICP 26 ranged from -72.73 (ICP 26 x ICPW 116) to 37.91 (ICP 26 x ICPW 130). Better parent heterosis, in crosses with ICP 28, ranged from -90.08 (ICP 28 x ICPW 94) to -85.13 (ICP 28 x ICPW 141) and in crosses with ICP 26 from -84.02 (ICP 26 x ICPW 116) to 13.91 (ICP 26 x ICPW 130) (Tables 33 - 42).

### **Density of trichome B**

Mid parent heterosis for density of trichome B, in crosses with ICP 28, ranged from 32.65 (ICP 28 x ICPW 125) to 68.00 (ICP 28 x ICPW 116) and in crosses with ICP 26 from -19.62 (ICP 26 x ICPW 125) to 85.53 (ICP 26 x ICPW 94). Better parent heterosis, in crosses with ICP 28, ranged from -14.47 (ICP 28 x ICPW 125) to 3.65 (ICP 28 x ICPW 130) and in crosses with ICP 26 from -55.91 (ICP 26 x ICPW 125) to 6.02 (ICP 26 x ICPW 94) (Tables 33 - 4).

### **Density of trichome C**

Mid parent heterosis for density of trichome C, in crosses with ICP 28, the values ranged from 39.23 (ICP 28 x ICPW 94) to 68.88 (ICP 28 x ICPW 125) and in crosses with ICP 26 from 47.42 (ICP 26 x ICPW 141) to 72.54 (ICP 26 x ICPW 94). Better parent heterosis, in crosses with ICP 28, ranged from -19.06 (ICP 28 x ICPW 94) to 0.81 (ICP 28 x ICPW 125) and in crosses with ICP 26 from -16.27 (ICP 26 x ICPW 141) to -2.41 (ICP 26 x ICPW 116) (Tables 33 - 42).

### **Density of trichome D**

Mid parent heterosis for density of trichome D, in crosses with ICP 28, the values ranged from 8.49 (ICP 28 x ICPW 94) to 40.10 (ICP 28 x ICPW 116) and in crosses with ICP 26 from -21.56 (ICP 26 x ICPW 125) to 35.70 (ICP 26 x ICPW 94). Better parent heterosis in crosses with ICP 28 ranged from -23.69 (ICP 28 x ICPW 94)

to 0.24 (ICP 28 x ICPW 116) and in crosses with ICP 26 from -45.23 (ICP 26 x ICPW 125) to -12.98 (ICP 26 x ICPW 94) (Tables 33 - 4).

## Genetic basis of quantitative characters

In the present investigation the genetic basis of 13 quantitative characters viz., days to flower, days to maturity, leaf length, leaf width, pod length, pod width, pod bearing length, number of locules per pod, number of seeds per pod, number of primary and secondary branches, 100 - seed weight and harvest index has been studied. To determine the genetic basis of the above traits the means and variances for various characters have been calculated in ICP 28 x ICPW 94, ICP 28 x ICPW 130 and ICP 26 x ICPW 125 crosses.  $F_2$  populations grown in nets are shown in Figure 27. Different  $F_2$  and  $F_3$  segregants are shown in figures 28 and 29 respectively. The  $BC_1F_1$  plants are shown in figure 30.

### Days to flower

#### *ICP 28 x ICPW 94*

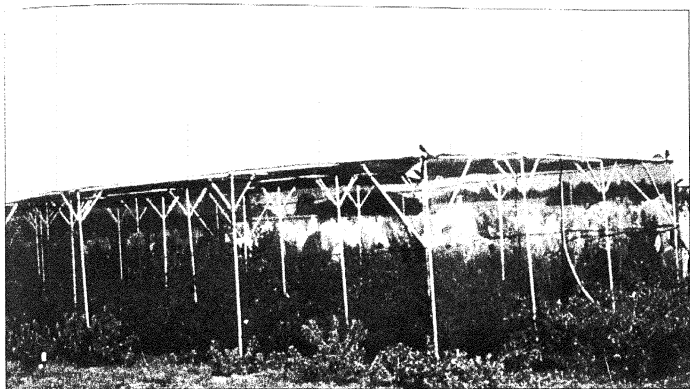
The mean number of days to flower ( $52.9 \pm 0.31$ ) in  $F_1$  generation was less than the mean of ICP 28 ( $66.40 \pm 0.163$ ) and ICPW 94 ( $53.20 \pm 0.533$ ). The  $F_2$  mean ( $50.2 \pm 0.057$ ) was less than the means of  $F_1$ , ICP 28, and ICPW 94. Mean of  $BC_1F_1$  (ICP 28 x  $F_1$ ) ( $54.79 \pm 0.141$ ) was less than the mean of ICP 28 but greater than the means of ICPW 94,  $F_1$ , and  $F_2$ . The  $F_3$  mean ( $54.78 \pm 0.179$ ) was equal to the mean of  $BC_1F_1$  but greater than the means of ICPW 94,  $F_1$ , and  $F_2$  but less than ICP 28 (Table 43).

The variance in  $F_1$  (2.976) was greater than the variance in ICP 28 (0.27) and ICPW 94 (2.84). Variance in  $F_2$  (9.808) was greater than the variances in ICP 28, ICPW 94, and  $F_1$ . Variance in  $BC_1F_1$  (ICP 28 x  $F_1$ ) (6.21) was greater than the variances in ICP 28, ICPW 94, and  $F_1$  but less than the variance in  $F_2$ . Variance in  $F_3$

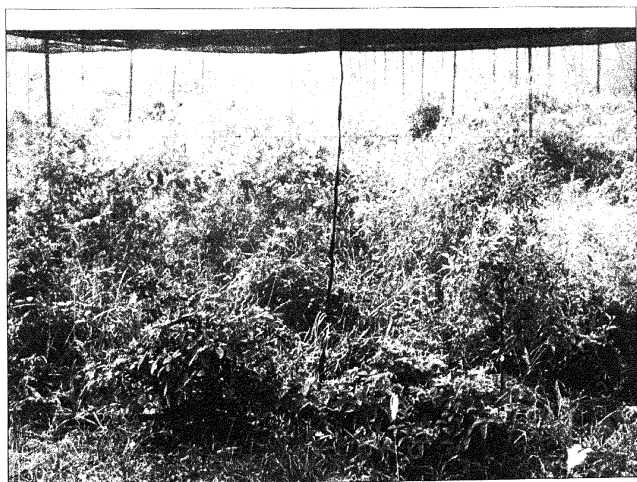


Fig. 27: Segregating  $F_2$  progenies in field (Covered with nylon nets)

- a) ICP 28 x ICPW 94
- b) ICP 28 x ICPW 130



a



b

Fig: 27

Fig. 28: Segregants in  $F_2$  generation

- a) Plant with green pods
- b) Plant with purple, non hairy pods
- c) Plant with mixed pod colour & hairy pods
- d) Plant with mixed pod colour
- e) Plant with semi-spreading plant habit



a



b



c



d



e

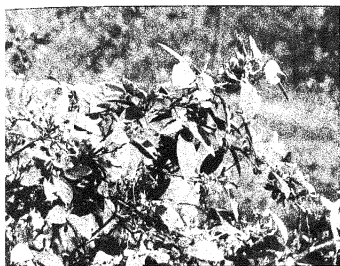
Fig: 28

Fig. 28: Segregants in F<sub>2</sub> generation

- f) Plant with spreading plant habit
- g) Plant with intermediate leaves and spreading habit
- h) Plant with pigeonpea like leaf, flower and pod
- i) Plant with compact inflorescence, pigeonpea like flower but *C. scarabaeoides* like pod
- j) Plant with *C. scarabaeoides* like leaves and spreading plant habit
- k) Plant with pigeonpea like pods and intermediate leaflet shape



f



h



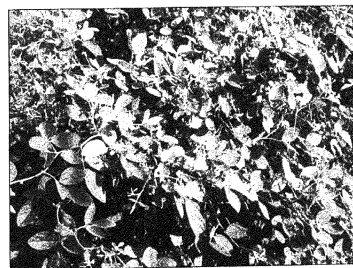
j



g



i



k

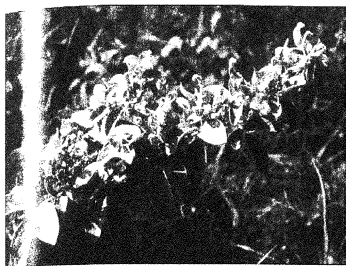
Fig: 28

Fig. 28. Segregants in  $F_2$  generation

- l) Compact plant with small pod bearing length
- m) Spreading plant with large pod bearing length
- n) Compact plant with small pod bearing length
- o) Spreading plant with large pod bearing length
- p) Semi-spreading plant habit with large pod bearing length
- q) Plant with small pod bearing length with pigeonpea like pods

Fig. 28. Segregants in  $F_2$  generation

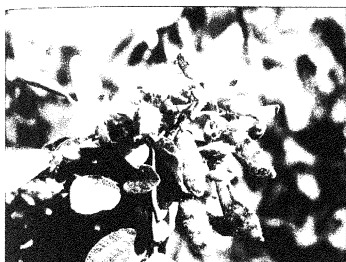




r



s



t



u



v



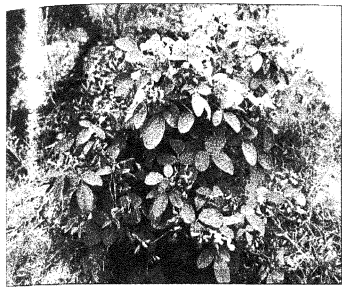
w



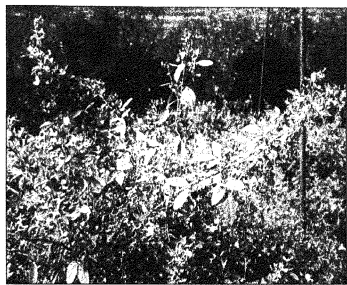
x

Fig. 28

Fig. 29. Segregants in  $F_3$  generation



a



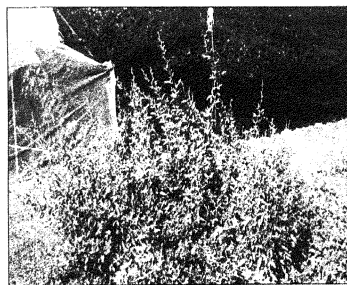
b



c



d



e

Fig: 29

Fig. 29. Segregants in  $F_3$  generation



**f**



**g**



**h**

**Fig: 29**

Table - 43: Mean values for various characters of ICP 28 x ICPW 94

Character	Mean in ICP 28 (P <sub>1</sub> )	Mean in ICPW 94 (P <sub>2</sub> )	F <sub>1</sub> mean	F <sub>2</sub> mean	F <sub>3</sub> mean	BC <sub>1</sub> F <sub>1</sub> mean
Days to flower (No.)	66.40 ± 0.163	53.20 ± 0.533	52.9 ± 0.31	50.2 ± 0.057	54.78 ± 0.179	54.79 ± 0.141
Days to maturity (No.)	97.80 ± 0.442	85.60 ± 1.127	93.4 ± 0.351	91.2 ± 0.107	103.79 ± 0.126	98.43 ± 0.143
Leaf length (cm)	6.36 ± 0.044	4.80 ± 0.073	5.63 ± 0.054	3.79 ± 0.042	3.67 ± 0.039	3.18 ± 0.051
Leaf width (cm)	2.12 ± 0.014	2.98 ± 0.033	3.2 ± 0.032	1.65 ± 0.024	1.66 ± 0.028	1.37 ± 0.023
Pod length (cm)	5.48 ± 0.039	2.50 ± 0.020	4.06 ± 0.051	2.99 ± 0.037	2.90 ± 0.040	3.25 ± 0.039
Pod width (cm)	0.74 ± 0.014	0.54 ± 0.017	0.85 ± 0.014	0.81 ± 0.022	0.78 ± 0.006	0.70 ± 0.008
Pod Bearing length (cm)	5.90 ± 0.348	23.50 ± 0.601	11.2 ± 0.592	9.5 ± 0.201	13.77 ± 0.44	10.48 ± 0.289
No. of Locules per pod	3.80 ± 0.133	4.00 ± 0.211	4.30 ± 0.133	3.91 ± 0.029	4.06 ± 0.025	2.99 ± 0.034
No. of seed per pod	3.25 ± 0.133	3.95 ± 0.214	4.30 ± 0.133	3.91 ± 0.029	4.89 ± 0.045	2.89 ± 0.045
No. of Primary branches	9.20 ± 0.133	9.40 ± 0.163	7.23 ± 0.257	6.68 ± 0.036	6.09 ± 0.089	9.66 ± 0.163
No. of Secondary branches	2.40 ± 0.267	17.40 ± 0.163	20.61 ± 0.180	13.92 ± 0.059	12.91 ± 0.095	6.16 ± 0.150
100 seed weight (g)	11.05 ± 0.015	2.95 ± 0.002	5.53 ± 0.112	4.95 ± 0.016	4.32 ± 0.042	7.49 ± 0.067
Harvest Index	18.23 ± 1.255	6.23 ± 0.259	5.41 ± 0.259	5.27 ± 0.259	5.12 ± 1.256	10.24 ± 2.563

Fig. 30. Back cross progeny ( $BC_1F_1$ )

a) ICP 26 x (ICP 26 x ICPW 125)

b) ICP 28 x (ICP 28 x ICPW 94)



a



b

**Fig: 30**



population (9.524) was greater than the variances in ICP 28, ICPW 94,  $F_1$ , and  $BC_1F_1$  but less than the variance in  $F_2$  population (Table 44).

The estimates of  $d$  (additive),  $h$  (dominance),  $l$  (dominance x dominance) were significant but  $i$  (additive x additive) was non- significant (Table 45). The maximum contribution was from  $h$  (81.86%) followed by  $d$  (3.7%), and  $l$  (2.97%) in explaining variance of this trait (Table 46).

#### ***ICP 28 x ICPW 130***

Mean of  $F_1$  ( $56.1 \pm 0.597$ ) was less than the means of ICPW 130 ( $70.0 \pm 0.298$ ) and ICP 28 ( $66.4 \pm 0.167$ ). Mean of  $F_2$  ( $53.1 \pm 0.399$ ) was less than the means of  $F_1$ , ICP 28, and ICPW 130. The  $BC_1F_1$  (ICP 28 x  $F_1$ ) mean ( $64.27 \pm 0.243$ ) was greater than the means of  $F_1$  and  $F_2$  but less than that of ICP 28 and ICPW 130. The  $F_3$  mean ( $60.64 \pm 0.290$ ) was greater than  $F_1$  and  $F_2$  but less than ICP 28, ICPW 130, and  $BC_1F_1$  (Table 47).

Variance in  $F_1$  (3.567) was greater than the variances in ICP 28 (0.277) and ICPW 130 (0.889). Variance in  $F_2$  population (40.002) was greater than the variances in ICP 28, ICPW 130, and  $F_1$ . Variance in  $BC_1F_1$  (ICP 28 x  $F_1$ ) (3.314) was greater than ICP 28 and ICPW 130 but less than that in  $F_1$  and  $F_2$ . Variance in  $F_3$  (16.954) was greater than that of ICP 28, ICPW 130,  $F_1$  and  $BC_1F_1$  but less than the  $F_2$  variance (Table 48).

The estimates of  $d$ ,  $h$ ,  $l$  and  $i$  were significant (Table 49), and the contribution of  $h$  (57.49%) was maximum, followed by  $l$  (22.63%),  $d$  (14.68%), and  $i$  (5.19%) in explaining the variation in this character (Table 46).

#### ***ICP 26 x ICPW 125***

The  $F_1$  mean ( $68.9 \pm 0.407$ ) was greater than the mean of ICP 26 ( $56.80 \pm 0.249$ ) but less than ICPW 125 ( $74.80 \pm 0.133$ ). Mean of  $F_2$  ( $67.23 \pm 0.353$ ) was greater than

**Table - 44: Variance and heretability for various characters of ICP 28 x ICPW 94**

Character	Variance in ICP 28	Variance in ICPW 94	Variance in $F_1$	Variance in $F_2$	Variance in $F_3$	Variance in $BC_1 F_1$	Environmental variance	Genotypic variance	Broad -sense heritability (%)
Days to flower (No.)	0.27	2.84	2.976	9.808	9.524	6.206	2.03	7.78	79.32
Days to maturity (No.)	1.96	12.71	1.233	12.884	13.256	5.256	5.30	7.58	58.86
Leaf length (cm)	0.02	0.05	0.029	0.440	0.324	0.159	0.03	0.41	92.50
Leaf width (cm)	0.02	0.01	0.010	0.150	0.162	0.133	0.01	0.14	91.11
Pod length (cm)	0.02	0.04	0.025	0.304	0.335	0.097	0.01	0.29	90.68
Pod width (cm)	0.01	0.03	0.07	0.118	0.058	0.054	0.04	0.08	68.93
Pod Bearing length (cm)	1.21	3.61	3.48	10.093	39.909	5.111	2.77	7.33	72.59
No. of Locules per pod	0.18	0.44	0.18	0.606	0.529	0.073	0.27	0.34	56.00
No. of seed per pod	0.18	0.36	0.18	0.206	0.408	0.122	0.24	0.35	50.92
No. of Primary branches	0.18	0.27	0.66	1.329	1.629	1.626	0.37	0.96	72.16
No. of Secondary branches	0.71	0.27	1.32	3.869	3.828	2.573	0.77	3.10	80.18
100 seed weight (g)	0.02	0.01	0.12	0.162	0.355	0.276	0.04	0.12	69.14
Harvest Index	0.42	0.11	0.28	2.56	2.56	1.45	0.27	2.29	89.45

Table - 45: Estimates of different parameters with five parameter model (ICP 28 X ICPW 94)

	Mean	t-value	Additive (d)	t-value	Dominance (h)	t-value	I = h x h	t-value	i = d x d	t-value
Days to flower (50%)	66.41±0.057	1168.00*	6.60±0.256	23.67**	-21.57±1.421	14.64**	-16.36±3.06	5.35*	-0.11±0.121	NS
Days to maturity (50%)	102.6±0.107	955.80**	6.10±0.625	10.07**	-89.25±2.532	35.36**	141.70±5.17	27.44*	-78.77±1.950	40.24**
Leaf length (cm)	3.79±0.042	90.42**	0.78±0.049	18.16**	1.56±0.141	10.97**	4.28±0.422	10.14*	3.04±0.147	20.68**
Leaf width (cm)	1.65±0.024	67.75**	-0.43±0.071	24.38**	0.995±0.092	10.81**	4.145±0.261	15.92*	-0.507±0.085	5.88**
Pod length (cm)	2.99±0.035	85.88**	1.49±0.022	67.39**	-1.71±0.132	12.85**	7.712±0.377	20.45*	1.19±0.128	9.82**
Pod width (cm)	0.91±0.022	41.84**	0.19±0.115	3.46**	0.31±0.045	6.46**	-0.83±0.181	4.61*	-0.01±0.059	NS
Pod bearing length (cm)	9.48±0.021	47.12**	-8.81±0.125	25.34**	-37.27±1.305	28.52**	111.71±3.26	34.28*	-58.94±1.172	50.3**
No. of locules/pod	3.91±0.029	136.21**	-0.11±0.125	NS	-0.14±0.125	NS	1.85±0.445	4.21*	-0.75±0.225	3.32**
No. of seed / pod	3.91±0.284	136.65**	-0.35±0.125	2.74**	-2.348±0.156	14.73**	6.28±0.486	12.95*	3.76±0.238	15.92**
No. of primary branches	13.92±0.056	236.10**	-7.5±0.156	47.94**	7.145±0.303	23.54**	12.49±0.848	14.82*	-18.57±0.425	45.53**
No. of secondary branches	9.68±0.036	267.21**	-0.10±0.105	NS	7.95±0.302	26.21**	-25.65±0.886	29.02*	9.79±0.319	30.68**
100 seed weight	4.95±0.158	314.25**	4.05±0.007	53.10**	-0.59±0.137	4.25**	3.47±0.393	8.89*	8.98±0.122	73.62**
Harvest Index	5.12±1.122	158.29**	5.69±0.009	55.52**	-0.52±1.254	5.26**	4.15±0.421	7.52**	7.15±1.251	52.32**

NS - Non significant; \*significance at 5% level, \*\* significant at 1% level

Table - 46: Variability of different parameters for various traits in three crosses

	Days to flower (No.)	Days to maturity (No.)	Leaf length (cm)	Leaf width (cm)	Pod length (cm)	Pod width (cm)	Pod Bearing length (cm)	No. of seed per pod	No. of locules per pod	No. of Primary branches	No. of secondary branches	Seed weight (g)	Harvest index
<b>ICP 28 x ICPW 94</b>													
d	3.7	NS	35.67	10.34	87.50	NS	34.79	6.81	NS	43.70	NS	95.10	94.56
h	81.86	15.30	NS	2.68	1.53	48.86	3.88	9.08	NS	50.49	13.22	4.67	3.56
i	NS	47.79	60.36	76.78	2.74	NS	60.89	8.26	49.87	NS	28.25	NS	NS
l	2.97	36.30	3.70	10.27	8.17	46.58	NS	75.85	42.86	5.79	58.49	NS	NS
<b>ICP 28 x ICPW 130</b>													
d	14.68	69.35	26.16	11.56	47.79	17.59	6.34	NS	NS	1.19	67.29	98.59	98.00
h	57.48	14.33	NS	26.89	13.75	NS	64.29	65.06	37.28	27.03	NS	NS	NS
i	5.19	15.67	71.89	58.79	NS	70.59	29.38	NS	2.48	52.59	2.09	NS	NS
l	22.63	NS	1.92	2.82	13.79	11.39	NS	28.47	59.68	19.38	30.38	NS	NS
<b>ICP 26 x ICPW 125</b>													
d	86.56	35.37	14.84	70.46	1.88	NS	61.84	3.46	13.17	44.69	80.48	98.56	97.28
h	9.08	8.87	3.15	NS	NS	13.87	6.34	42.77	45.56	NS	NS	2.56	2.54
i	3.48	55.78	65.78	23.76	20.18	3.84	9.21	NS	10.96	26.84	2.37	NS	NS
l	NS	NS	16.24	4.15	77.71	81.57	22.58	53.37	30.49	21.49	14.98	NS	NS

**Table – 47: Mean values for various characters in different generations of ICP 28 x ICPW 130**

Character	Mean in ICP 28	Mean in ICPW 130	F <sub>1</sub> mean	F <sub>2</sub> mean	F <sub>3</sub> mean	BC <sub>1</sub> F <sub>1</sub> mean
Days to flowering (No.)	66.44±0.167	70.00±0.298	56.1±0.597	53.1±0.399	60.64±0.290	64.27±0.243
Days to maturity (No.)	98.00±0.418	123.40±0.805	105.80±0.919	102.1±0.238	102.19±0.257	126.54±0.369
Leaf length (cm)	6.36±0.048	5.54±0.040	5.53±0.054	3.01±0.035	3.68±0.040	3.09±0.074
Leaf width (cm)	2.12±0.014	3.22±0.049	2.72±0.021	1.33±0.017	1.67±0.028	1.32±0.034
Pod length (cm)	5.49±0.040	2.24±0.041	3.41±0.063	2.7±0.028	3.90±0.041	3.13±0.053
Pod width (cm)	0.54±0.07	0.72±0.014	0.77±0.022	0.72±0.006	0.78±0.006	0.72±0.010
Pod Bearing length (cm)	4.78±0.307	14.80±1.289	10.4±0.67	9.6±0.480	11.77±0.444	4.04±0.201
No. of Locules per pod	3.78±0.139	5.20±0.133	3.81±0.133	2.83±0.039	4.06±0.025	2.08±0.072
No. of seed per pod	3.84±0.139	5.60±0.133	3.60±0.133	3.20±0.039	4.23±0.025	2.83±0.072
No. of Primary branches	9.22±0.139	10.20±0.133	8.20±0.249	7.3±0.106	4.33±0.043	8.15±0.046
No. of Secondary branches	4.61±0.139	16.50±0.133	17.5±0.249	3.9±0.601	8.69±0.043	8.69±0.046
100 seed weight (g)	11.06±0.014	2.33±0.001	5.97±0.119	5.5±0.052	7.70±0.060	3.73±0.075
Harvest Index	18.23±1.259	7.41±2.598	8.46±1.259	7.92±0.865	7.14±1.256	10.42±1.256

**Table - 48: Variance and heretability for various characters of ICP 28 x ICPW 130**

Character	Variance in ICP 28	Variance in ICPW 130	Variance in F <sub>1</sub>	Variance in F <sub>2</sub>	Variance in F <sub>3</sub>	Variance in BC <sub>1</sub> F <sub>1</sub>	Environ-mental variance	Genotypic variance	Broad -sense heritability (%)
Days to flower (No.)	0.277	0.889	3.567	40.002	16.954	3.314	1.58	38.42	96.05
Days to maturity (No.)	1.750	6.489	8.444	14.253	13.256	7.650	5.56	8.69	60.97
Leaf length (cm)	0.322	0.416	0.429	0.308	0.522	0.306	0.39	0.08	25.97
Leaf width (cm)	0.032	0.024	0.054	0.072	0.162	0.067	0.04	0.04	55.56
Pod length (cm)	0.216	0.016	0.040	0.200	0.334	0.156	0.09	0.11	55.00
Pod width (cm)	0.012	0.012	0.015	0.056	0.018	0.016	0.01	0.11	82.14
Pod Bearing length (cm)	0.944	4.622	4.489	12.956	19.713	2.257	3.35	9.60	74.10
No. of Locules per pod	0.194	0.178	0.178	0.376	0.128	0.288	0.18	0.19	50.53
No. of seed per pod	0.194	0.178	0.178	0.376	0.128	0.288	0.18	0.19	55.53
No. of Primary branches	0.658	0.178	0.622	2.808	1.365	1.121	0.49	2.32	82.62
No. of Secondary branches	1.194	1.178	1.622	6.808	6.523	5.121	1.33	5.48	80.49
100 seed weight (g)	0.042	0.014	0.412	0.675	0.729	0.316	0.57	0.52	77.04
Harvest Index	0.486	0.442	0.682	1.589	1.256	1.316	0.54	1.05	66.08

**Table - 49: Estimates of different parameters with five parameter model (ICP28 X ICPW 130)**

	Mean	t-value	Additive (d)	t-value	Dominance (h)	t-value	l = h x h	t-value	i = d x d	t-value
Days to flower (50%)	63.63±0.400	159.12**	-1.778±0.173	10.27**	-20.97±1.182	17.73**	12.52±3.893	3.22**	-12.55±1.25	10.19**
Days to maturity (50%)	126.21±0.23	528.4**	-12.70±0.452	27.66**	-24.16±1.456	16.59**	-32.35±3.996	8.158**	-44.86±1.405	31.92**
Leaf length (cm)	3.012±0.035	85.75**	0.41±0.0321	12.69**	-0.09±0.133	NS	10.26±0.381	26.96**	1.14±0.135	8.445**
Leaf width (cm)	1.36±0.017	77.98**	-0.549±0.025	21.46**	0.02±0.083	NS	5.56±0.209	26.42**	-1.132±0.075	15.04**
Pod length (cm)	2.62±0.135	92.54**	1.62±0.029	55.81**	-2.91±0.129	22.49**	9.00±0.356	25.30**	0.79±0.127	6.39**
Pod width (cm)	0.73±0.001	104.12**	-0.08±0.011	7.96**	-0.13±0.026	4.857**	0.44±0.086	5.11**	-0.44±0.033	13.18**
Pod bearing length (cm)	18.58±0.485	38.59**	-5.011±0.668	7.58**	-23.16±1.58	14.59**	22.39±4.861	4.61**	-35.99±1.692	21.20**
No. of locules /pod	2.85±0.038	72.89**	-0.711±0.099	7.167**	-2.65±0.135	19.49**	9.19±0.491	18.73**	-3.38±0.226	14.73**
No. of seed per pod	2.58±0.106	25.40**	-0.39±0.492	NS	-2.85±0.604	4.725**	11.79±2.035	5.792**	-3.109±1.036	3.01**
No. of primary branches	9.26±0.106	87.41**	-0.49±0.099	4.93**	12.46±0.292	42.64**	-29.18±1.123	26.50**	12.99±0.374	34.74**
No. of secondary branches	14.20±0.106	134.21**	-6.18±0.308	20.06**	2.43±0.299	8.38**	15.41±1.151	14.00**	12.06±0.751	16.05**
100 seed weight	3.92±0.052	75.54**	4.35±0.001	567.5**	-8.72±0.207	42.21**	25.62±0.613	41.78**	0.72±0.193	3.749**
Harvest Index	4.56±0.025	55.26**	4.53±0.002	541.95**	-7.59±0.256	52.12**	36.25±0.715	41.25**	0.56±0.251	3.256**

\* \*\* Significant at 5% and 1 % level, NS - non significant

the mean of ICP 26 but less than the means of ICPW 125 and  $F_1$ . The  $BC_1F_1$  (ICP 26  $\times$   $F_1$ ) mean ( $62.99 \pm 0.175$ ) was greater than the mean of ICP 26 but less than the means of ICPW 125,  $F_1$ , and  $F_2$ . Mean of  $F_3$  ( $66.72 \pm 0.308$ ) was greater than the means of ICP 26 and  $BC_1F_1$  but less than ICPW 125,  $F_1$ , and  $F_2$  means (Table 50)

Variance in  $F_1$  (1.656) was greater than variances in ICP 26 (0.622) and ICPW 125 (0.178). Variance (7.002) in  $F_2$  was greater than that in ICP 26, ICPW 125, and  $F_1$ . Variance in  $BC_1F_1$  (ICP 26  $\times$   $F_1$ ) (5.582) was greater than the variances in ICP 26, ICPW 125, and  $F_1$  but less than  $F_2$ . Variance in  $F_3$  (23.667) was greater than ICP 26, ICPW 125,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  (Table 51).

The estimates of  $d$ ,  $h$  and  $i$  were significant (Table 52) and the maximum contribution was from  $d$  (86.564%) followed by  $h$  (9.08%) and  $i$  (3.48%), in explaining the variation in this character (Table 46).

## **Days to maturity**

### ***ICP 28 $\times$ ICPW 94***

The mean number of days ( $93.47 \pm 0.351$ ) for  $F_1$  plants was between the means of ICPW 94 ( $85.60 \pm 1.127$ ) and ICP 28 ( $97.80 \pm 0.442$ ). The  $F_2$  mean ( $91.2 \pm 0.11$ ) was greater than the mean of ICPW 94 but less than the means of ICP 28 and  $F_1$ . Mean of the  $BC_1F_1$  (ICP 28  $\times$   $F_1$ ) ( $98.43 \pm 0.143$ ) was greater than the means of ICP 28, ICPW 94,  $F_1$  and  $F_2$ . The  $F_3$  mean ( $103.79 \pm 0.126$ ) was greater than  $F_1$ ,  $F_2$ , ICP 28, ICPW 94, and  $BC_1F_1$  means (Table 43).

Variance in  $F_1$  (1.233) was less than ICP 28 (1.96) and ICPW 94 (12.71). Variance in  $F_2$  (12.884) was greater than the variance in ICPW 94, ICP 28, and  $F_1$ . Variance in the backcross population  $BC_1F_1$  (ICP 28  $\times$   $F_1$ ) (5.256) was greater than the variance in ICP 28 and  $F_1$  but less than that in ICPW 94 and  $F_2$ . Variance in the  $F_3$  population (13.256) was greater than the variances in ICPW 94, ICP 28,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  (Table 44).



**Table1 - 50: Mean values for various characters in different generations of ICP 26 x ICPW 125**

Character	ICP 26 mean $\pm$ SE	ICPW 125 mean $\pm$ SE	F <sub>1</sub> mean $\pm$ SE	F <sub>2</sub> mean $\pm$ SE	F <sub>3</sub> mean $\pm$ SE	BC <sub>1</sub> F <sub>1</sub> mean $\pm$ SE
Days to flower (No.)	56.80 $\pm$ 0.249	74.80 $\pm$ 0.133	68.9 $\pm$ 0.407	67.23 $\pm$ 0.353	66.72 $\pm$ 0.308	62.99 $\pm$ 0.165
Days to maturity (No.)	103.00 $\pm$ 0.211	112.00 $\pm$ 0.596	103.2 $\pm$ 0.326	101.2 $\pm$ 0.572	101.23 $\pm$ 0.263	118.95 $\pm$ 0.382
Leaf length (cm)	6.94 $\pm$ 0.034	5.38 $\pm$ 0.039	5.66 $\pm$ 0.030	2.58 $\pm$ 0.074	3.99 $\pm$ 0.050	2.81 $\pm$ 0.039
Leaf width (cm)	3.58 $\pm$ 0.013	1.94 $\pm$ 0.034	2.67 $\pm$ 0.030	1.38 $\pm$ 0.049	1.67 $\pm$ 0.024	1.29 $\pm$ 0.019
Pod length (cm)	5.18 $\pm$ 0.099	2.54 $\pm$ 0.016	3.8 $\pm$ 0.029	2.23 $\pm$ 0.066	3.97 $\pm$ 0.041	3.13 $\pm$ 0.033
Pod width (cm)	0.62 $\pm$ 0.013	0.68 $\pm$ 0.013	0.76 $\pm$ 0.027	0.7 $\pm$ 0.091	0.78 $\pm$ 0.005	0.74 $\pm$ 0.003
Pod Bearing length (cm)	6.00 $\pm$ 0.516	27.00 $\pm$ 1.506	11.2 $\pm$ 0.731	10.72 $\pm$ 0.894	15.77 $\pm$ 0.402	4.14 $\pm$ 0.095
No. of Locules per pod	4.00 $\pm$ 0.211	4.80 $\pm$ 0.122	4.30 $\pm$ 0.153	2.44 $\pm$ 0.091	2.06 $\pm$ 0.023	2.69 $\pm$ 0.039
No. of seed per pod	3.60 $\pm$ 0.163	5.40 $\pm$ 0.163	3.50 $\pm$ 0.163	2.45 $\pm$ 0.091	3.27 $\pm$ 0.029	2.69 $\pm$ 0.039
No. of Primary branches	3.20 $\pm$ 0.133	7.60 $\pm$ 0.163	5.7 $\pm$ 0.213	4.9 $\pm$ 0.036	4.33 $\pm$ 0.038	3.70 $\pm$ 0.013
No. of Secondary branches	4.40 $\pm$ 0.163	12.60 $\pm$ 0.163	12.60 $\pm$ 0.452	6.7 $\pm$ 0.122	9.27 $\pm$ 0.072	8.69 $\pm$ 0.169
100 seed weight (g)	11.15 $\pm$ 1.414	2.54 $\pm$ 0.002	4.52 $\pm$ 0.172	3.9 $\pm$ 0.271	7.70 $\pm$ 0.053	7.63 $\pm$ 0.259
Harvest Index	17.42 $\pm$ 1.256	5.24 $\pm$ 1.255	6.39 $\pm$ 0.956	5.41 $\pm$ 0.256	5.59 $\pm$ 0.563	8.46 $\pm$ 0.549

**Table - 51: Variance and heretability for various characters of ICP 26 x ICPW 125**

Character	Variance in ICP 26	Variance in ICPW 125	Variance in F <sub>1</sub>	Variance in F <sub>2</sub>	Variance in F <sub>3</sub>	Variance in BC <sub>1</sub> F <sub>1</sub>	Environmental variance	Genotypic variance	Broad-sense heritability (%)
Days to flower (No.)	0.622	0.178	1.656	7.002	23.666	5.582	0.82	6.18	88.26
Days to maturity (No.)	0.444	3.555	1.067	18.356	17.221	30.642	1.69	16.67	90.81
Leaf length (cm)	0.061	0.015	0.089	0.310	0.628	0.315	0.05	0.25	80.65
Leaf width (cm)	0.012	0.012	0.019	0.136	0.164	0.072	0.01	0.12	88.24
Pod length (cm)	0.099	0.073	0.028	0.242	0.429	0.228	0.07	0.17	70.25
Pod width (cm)	0.017	0.022	0.017	0.167	0.098	0.065	0.02	0.15	89.82
Pod Bearing length (cm)	2.667	3.667	5.344	14.749	14.300	1.851	3.89	10.86	73.63
No. of Locules per pod	0.444	0.178	0.233	0.463	0.130	0.328	0.28	0.18	38.81
No. of seed per pod	0.267	0.267	0.266	0.465	0.209	0.328	0.27	0.19	40.86
No. of Primary branches	0.178	0.267	0.455	1.174	1.307	0.137	0.30	0.87	74.11
No. of Secondary branches	0.820	1.267	2.044	4.570	4.302	5.865	1.37	3.21	70.02
100 seed weight (g)	0.026	0.044	0.030	0.073	0.074	0.067	0.03	0.04	54.79
Harvest Index	0.089	0.124	1.158	4.456	3.112	4.512	0.46	3.99	89.54

The estimates of *d*, *h*, *l* and *i* were significant (Table 45), the contribution was maximum for *i* (47.78%) followed by *l* (36.30%), and *h* (15.37%) in explaining the variation in this character (Table 46).

### ***ICP 28 x ICPW 130***

Mean of  $F_1$  ( $105.80 \pm 0.919$ ) was in between the means of ICP 28 ( $98.00 \pm 0.418$ ) and ICPW 130 ( $123.40 \pm 0.805$ ). The  $F_2$  mean ( $102.1 \pm 0.238$ ) was greater than ICP 28 but less than ICPW 130, and  $F_1$ . Mean of the backcross progeny  $BC_1F_1$  (ICP 28 x  $F_1$ ) ( $126.54 \pm 0.369$ ) was greater than ICP 28, ICPW 130,  $F_1$ , and  $F_2$ . The  $F_3$  mean ( $102.19 \pm 0.257$ ) was similar to  $F_2$  but greater than ICP 28 and less than ICPW 130,  $F_1$ , and  $BC_1F_1$  means (Table 47).

The variance in  $F_1$  population (8.444) was greater than ICP 28 (1.750) and ICPW 130 (6.489). The variance in  $F_2$  (14.253) was greater than ICP 28, ICPW 130, and  $F_1$ . Variance in  $BC_1F_1$  (ICP 28 x  $F_1$ ) (7.650) was greater than ICP 28 and ICPW 130 but less than  $F_1$  and  $F_2$  generations. The variance in  $F_3$  (13.256) was greater than ICP 28, ICPW 130,  $F_1$ , and  $BC_1F_1$ , but less than  $F_2$  generation (Table 48).

The estimates of *d*, *h*, *l* and *i* were significant (Table 49) and the maximum contribution was from *d* (69.35%) followed by *i* (15.67%) and *h* (14.33%) in explaining the variation in this character (Table 46).

### ***ICP 26 x ICPW 125***

The Mean of  $F_1$  ( $103.2 \pm 0.326$ ) was equal to ICP 26 ( $103.00 \pm 0.211$ ) but less than ICPW 125 ( $112.00 \pm 0.596$ ).  $F_2$  mean ( $101.2 \pm 0.572$ ) was less than ICP 26, ICPW 125, and  $F_1$  means. The  $BC_1F_1$  (ICP 26 x  $F_1$ ) mean ( $118.95 \pm 0.382$ ) was greater than  $F_1$ ,  $F_2$ , ICP 26, and ICPW 125.  $F_3$  mean ( $10.23 \pm 0.263$ ) was equal to  $F_2$  but less than ICP 26, ICPW 125,  $F_1$ , and  $BC_1F_1$  means (Table 50).

Variance in  $F_1$  (1.067) was greater than that in ICP 26 (0.444) but less than the variance in ICPW 125 (3.555). Variance in  $F_2$  (18.356) was greater than the

variances in ICP 26, ICPW 125, and  $F_1$ .  $BC_1F_1$  variance ( $ICP\ 26 \times F_1$ ) (30.642) was greater than ICP 26, ICPW 125,  $F_1$ , and  $F_2$ . Variance in the  $F_3$  population (17.221) was greater than ICP 26, ICPW 125, and  $F_1$  but less than  $F_2$ , and  $BC_1F_1$  (Table 51).

The estimates of  $d$  (additive),  $h$  (dominance), and  $i$  (additive  $\times$  additive) were significant but  $l$  (dominance  $\times$  dominance) was non-significant (Table 52) in explaining the variation in this character. The maximum contribution was from  $i$  (55.78%) followed by  $d$  (35.37%),  $h$  (8.87%) in explaining the variation in this character (Table 46).

## Leaflet Length

### *ICP 28 $\times$ ICPW 94*

Mean length ( $5.63 \pm 0.054$ ) of leaflet was between the means of ICP 28 ( $6.36 \pm 0.044$ ) and ICPW 94 ( $4.80 \pm 0.073$ ). The  $F_2$  ( $3.79 \pm 0.042$ ) was less than ICP 28, ICPW 94 and  $F_1$ . The  $BC_1F_1$  mean ( $ICP\ 28 \times F_1$ ) ( $3.2 \pm 0.05$ ) was less than the means of ICP 28, ICPW 94,  $F_1$ , and  $F_2$ . The  $F_3$  mean ( $3.67 \pm 0.039$ ) was greater than the mean of  $BC_1F_1$  but less than ICP 28, ICPW 94,  $F_1$  and  $F_2$  means (Table 43).

Variance in  $F_1$  (0.029) was greater than the variance in ICP 28 (0.020) but less than that in ICPW 94 (0.053). Variance in  $F_2$  (0.440) was greater than the variances in ICP 28, ICPW 94, and  $F_1$ . Variance in  $BC_1F_1$  ( $ICP\ 28 \times F_1$ ) (0.159) was less than that in  $F_2$  but greater than ICP 28, ICPW 94 and  $F_1$ . Variance in  $F_3$  (0.324) was greater than ICP 28, ICPW 94,  $F_1$  and  $BC_1F_1$  but less than  $F_2$  variances (Table 44).

The estimates of  $d$ ,  $h$ ,  $i$  and  $l$  were significant (Table 45), however, the maximum contribution was from  $i$  (60.36%), followed by  $d$  (35.67%) and  $l$  (3.70%) in explaining the variation in this character (Table 46).

### ***ICP 28 x ICPW 130***

The  $F_1$  mean ( $5.53 \pm 0.054$ ) was equal to ICPW 130 ( $5.54 \pm 0.04$ ) but less than ICP 28 ( $6.36 \pm 0.048$ ). The  $F_2$  mean ( $3.01 \pm 0.035$ ) was less than ICP 28, ICPW 130, and  $F_1$ .  $BC_1F_1$  (ICP 28 x  $F_1$ ) mean ( $3.09 \pm 0.074$ ) was less than ICP 28, ICPW 130 and  $F_1$  mean but greater than  $F_2$  mean. The  $F_3$  mean ( $3.68 \pm 0.04$ ) was less than ICP 28, ICPW 130 and  $F_1$  but greater than the means of  $F_2$  and  $BC_1F_1$  (Table 47).

Variance in  $F_1$  (0.429) was greater than the variance in ICP 28 (0.322) and ICPW 130 (0.416). Variance in  $F_2$  (0.308) was less than that in ICP 28, ICPW 130, and  $F_1$ .  $BC_1F_1$  variance (ICP 28 x  $F_1$ ) (0.306) was less than the variance in ICP 28, ICPW 130,  $F_1$ , and  $F_2$ . Variance in  $F_3$  (0.522) was greater than the variance in ICP 28, ICPW 130,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  (Table 48).

The estimates of  $d$ ,  $i$  and  $l$  were significant but  $h$  is non-significant (Table 49). The contribution of  $i$  (71.89 %) was maximum followed by  $d$  (26.16 %),  $l$  (1.92 %) in explaining the variation in this character (Table 46).

### ***ICP 26 x ICPW 125***

The  $F_1$  mean ( $5.66 \pm 0.03$ ) was less than the mean of ICP 26 ( $6.94 \pm 0.034$ ), but greater than that of ICPW 125 ( $5.38 \pm 0.039$ ). The  $F_2$  mean ( $2.58 \pm 0.074$ ) was less than ICP 26, ICPW 125, and  $F_1$  means.  $BC_1F_1$  (ICP 26 x  $F_1$ ) ( $2.81 \pm 0.039$ ) was greater than the  $F_2$  but less than ICP 26, ICPW 125, and  $F_1$  means. The  $F_3$  mean ( $3.99 \pm 0.05$ ) was greater than  $F_2$  and  $BC_1F_1$  but less than ICP 26, ICPW 125, and  $F_1$  means (Table 50).

The  $F_1$  variance (0.089) was greater than the variance in ICP 26 (0.061) and ICPW 125 (0.05). Variance in  $F_2$  (0.310) was greater than ICP 26, ICPW 125, and  $F_1$ . The  $BC_1F_1$  (ICP 26 x  $F_1$ ) (0.315) was equal to  $F_2$  mean but greater than the means of ICP 26, ICPW 125, and  $F_1$ . The variance in  $F_3$  (0.628) was greater than ICP 26, ICPW 125,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  variance (Table 51).

The estimates of d, h, i and l were significant in explaining the variation in this character) (Table 52). The maximum contribution was from i (65.78%) followed by l (16.24%), d (14.84%) and h (3.2%) in explaining the variation (Table 46).

### **Leaflet width**

#### ***ICP 28 x ICPW 94***

The  $F_1$  mean ( $3.2 \pm 0.032$ ) was greater than ICP 28 ( $2.12 \pm 0.014$ ) and ICPW 94 ( $2.98 \pm 0.033$ ). The  $F_2$  mean ( $1.65 \pm 0.024$ ) was less than ICP 28, ICPW 94, and  $F_1$  means. The  $BC_1F_1$  (ICP 28 x  $F_1$ ) ( $1.37 \pm 0.023$ ) was less than ICP 28, ICPW 94,  $F_1$ , and  $F_2$  means. The  $F_3$  mean ( $1.66 \pm 0.028$ ) was equal to the  $F_2$  mean but less than ICP 28, ICPW 94 and  $F_1$  and greater than  $BC_1F_1$  means (Table 43).

Variance in  $F_1$  (0.010) was equal to the variance in ICPW 94 (0.011) but less than that in ICP 28 (0.02). The  $F_2$  (0.15) was greater than ICP 28, ICPW 94, and  $F_1$  variances. Variance in  $BC_1F_1$  (ICP 28 x  $F_1$ ) (0.133) was less than  $F_2$  but greater than ICP 28, ICPW 94 and  $F_1$ . Variance in  $F_3$  (0.162) was greater than the variance in ICP 28, ICPW 94,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  (Table 44).

The estimates of d, h, i and l were significant (Table 45), with the maximum contribution from i (76.78%) followed by d (10.34%), l (10.27%) and h (2.68%) in explaining the variation in this character (Table 46).

#### ***ICP 28 x ICPW 130***

The  $F_1$  mean ( $2.72 \pm 0.021$ ) was greater than ICP 28 ( $2.12 \pm 0.014$ ) but less than ICPW 130 ( $3.22 \pm 0.049$ ).  $F_2$  mean ( $1.33 \pm 0.017$ ) was less than ICP 28, ICPW 130, and  $F_1$  means. The  $BC_1F_1$  (ICP 28 x  $F_1$ ) mean ( $1.32 \pm 0.034$ ) was approximately equal to  $F_2$  mean but less than ICP 28, ICPW 130, and  $F_1$  mean.  $F_3$  mean ( $1.67 \pm 0.028$ ) was less than ICP 28, ICPW 130 and  $F_1$  but greater than the means of  $F_2$  and  $BC_1F_1$  (Table 47).

Variance in  $F_1$  (0.054) was greater than the variance in ICP 28 (0.032) and ICPW 130 (0.024). Variance in  $F_2$  (0.072) was greater than the variances in ICP 28, ICPW 130 and  $F_1$ . Variance in  $BC_1F_1$  (ICP 28  $\times$   $F_1$ ) (0.067) was greater than variance in ICP 28, ICPW 130, and  $F_1$  but less than  $F_2$ .  $F_3$  variance (0.162) was greater than ICP 28, ICPW 130,  $F_1$ ,  $F_2$  and  $BC_1F_1$  (Table 48).

The estimates of  $d$ ,  $l$  and  $i$  were significant but  $h$  (dominance) was non-significant (Table 49), with the maximum contribution from  $i$  (58.79 %) followed by  $l$  (26.89 %),  $d$  (11.56 %) in explaining the variation in this character (Table 46).

### *ICP 26 $\times$ ICPW 125*

Mean of  $F_1$  ( $2.67 \pm 0.030$ ) was less than the mean of ICP 26 ( $3.58 \pm 0.013$ ) but greater than ICPW 125 ( $1.94 \pm 0.034$ ).  $F_2$  mean ( $1.38 \pm 0.049$ ) was less than ICP 26, ICPW 125 and  $F_1$  means. The  $BC_1F_1$  mean (ICP 26  $\times$   $F_1$ ) ( $1.29 \pm 0.019$ ) was less than ICP 26, ICPW 125,  $F_1$ , and  $F_2$  mean.  $F_3$  mean ( $1.67 \pm 0.025$ ) was less than ICP 26, ICPW 130 and  $F_1$  but greater than the means of  $F_2$  and  $BC_1F_1$  (Table 50).

Variance in  $F_1$  (0.019) was greater than the variance in ICP 26 (0.012) and ICPW 125 (0.012). Variance in  $F_2$  (0.136) was greater than ICP 26, ICPW 125, and  $F_1$  variances. Variance in  $BC_1F_1$  (ICP 26  $\times$   $F_1$ ) (0.072) was greater than ICP 26, ICPW 125, and  $F_1$  variance but less than the variance in  $F_2$ .  $F_3$  (0.164) was greater than ICP 26, ICPW 125,  $F_1$ ,  $F_2$  and  $BC_1F_1$  variances (Table 51).

The estimates of  $d$ ,  $l$  and  $i$  were significant but was non-significant (Table 52), with the maximum contribution from  $d$  (70.46 %) followed by  $i$  (23.76 %) and  $l$  (4.15 %) in explaining the variation in this character (Table 46).

## Pod length

### *ICP 28 x ICPW 94*

The  $F_1$  mean ( $4.06 \pm 0.051$ ) was greater than ICPW 94 ( $2.50 \pm 0.026$ ) mean, but less than mean of ICP 28 ( $5.48 \pm 0.039$ ).  $F_2$  mean ( $2.99 \pm 0.037$ ) was greater than the means of ICPW 94 but less than ICP 28 and  $F_1$ . The  $BC_1F_1$  (ICP 28 x  $F_1$ ) mean ( $3.25 \pm 0.039$ ) was greater than the means of ICPW 94 and  $F_2$  but less than ICP 28 and  $F_1$ . The  $F_3$  mean ( $2.90 \pm 0.04$ ) was greater than ICPW 94 but less than ICP 26,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  means (Table 43).

Variance in  $F_1$  (0.025) was greater than the variance in ICP 28 (0.02) but less than the variance in ICPW 94 (0.04). Variance in  $F_2$  (0.304) was greater than ICP 28, ICPW 94, and  $F_1$  variances.  $BC_1F_1$  (ICP 28 x  $F_1$ ) (0.097) was greater than variances in ICP 28, ICPW 94, and  $F_1$  but less than  $F_2$ . Variance (0.335) in  $F_3$  was greater than ICP 28, ICPW 94,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  (Table 44).

The estimates of  $d$ ,  $h$ ,  $l$  and  $i$  were significant (Table 45) with the maximum contribution from  $d$  (87.50 %) followed by  $l$  (8.17 %),  $i$  (2.79 %), and  $h$  (1.53 %) in explaining the variation in this character (Table 46).

### *ICP 28 x ICPW 130*

$F_1$  mean ( $3.41 \pm 0.063$ ) was less than ICP 28 ( $5.49 \pm 0.040$ ) but greater than ICPW 130 ( $2.24 \pm 0.041$ ).  $F_2$  mean ( $2.7 \pm 0.023$ ) was less than means of ICP 28 and  $F_1$  but greater than ICPW 130. The  $BC_1F_1$  (ICP 28 x  $F_1$ ) ( $3.13 \pm 0.053$ ) was less than ICP 28 and  $F_1$  but greater than ICPW 130 and  $F_2$ . The  $F_3$  mean ( $3.90 \pm 0.041$ ) was less than ICP 28 but greater than ICPW 130,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  means (Table 47).

The variance in  $F_1$  (0.040) was less than the variance in ICP 28 (0.216) but greater than ICPW 130 (0.016). Variance in  $F_2$  (0.200) was greater than that in, ICPW 130, and  $F_1$  but less than in ICP 28. The variance in  $BC_1F_1$  (ICP 28 x  $F_1$ ) (0.156) was greater than ICPW 130 and  $F_1$  but less than ICP 28 and  $F_2$ . Variance in



F<sub>3</sub> (0.334) was greater than the variances in ICP 28, ICPW 130, F<sub>1</sub>, F<sub>2</sub>, and BC<sub>1</sub>F<sub>1</sub> (Table 48).

The estimates of d, h, i and l were significant (Table 49) with the maximum contribution from d (47.79%) followed by l (13.79%), h (13.75%) in explaining the variation in this character (Table 46).

#### ***ICP 26 x ICPW 125***

F<sub>1</sub> mean ( $3.8 \pm 0.09$ ) was less than the mean of ICP 26 ( $5.18 \pm 0.09$ ) but greater than ICPW 125 ( $2.54 \pm 0.06$ ). F<sub>2</sub> ( $2.23 \pm 0.07$ ) was less than the means of ICP 26, ICPW 125, and F<sub>1</sub>. The BC<sub>1</sub>F<sub>1</sub> mean (ICP 26 x F<sub>1</sub>) ( $3.13 \pm 0.033$ ) was greater than means of ICPW 125 and F<sub>2</sub> but less than ICP 26 and F<sub>1</sub>. The F<sub>3</sub> mean ( $3.97 \pm 0.041$ ) was less than ICP 26 but greater than ICPW 125, F<sub>1</sub>, F<sub>2</sub>, and BC<sub>1</sub>F<sub>1</sub> means (Table 50).

The F<sub>1</sub> variance (0.028) was less than the variances in ICP 26 (0.099) and ICPW 125 (0.073). Variance in F<sub>2</sub> (0.242) was greater than the variances in ICP 26, ICPW 125, and F<sub>1</sub>. Variance in BC<sub>1</sub>F<sub>1</sub> (ICP 26 x F<sub>1</sub>) (0.228) was greater than ICP 26, ICPW 125, and F<sub>1</sub> but less than F<sub>2</sub>. The F<sub>3</sub> variance (0.429) was greater than ICP 26, ICPW 125, F<sub>1</sub>, F<sub>2</sub>, and BC<sub>1</sub>F<sub>1</sub> (Table 51).

The estimates of d, h, i and l were significant (Table 52) with maximum contribution was from l (77.71 %), followed by i (20.18 %) and d (1.88 %) in explaining the variation in this character (Table 46).

#### **Pod width**

#### ***ICP 28 x ICPW 94***

F<sub>1</sub> mean ( $0.85 \pm 0.014$ ) was greater than the means of ICP 28 ( $0.740 \pm 0.014$ ) and ICPW 94 ( $0.540 \pm 0.017$ ). F<sub>2</sub> mean ( $0.81 \pm 0.022$ ) was less than the mean of F<sub>1</sub> but greater than that of ICPW 94 and ICP 28 means. Mean of BC<sub>1</sub>F<sub>1</sub> (ICP 28 x F<sub>1</sub>)

( $0.70 \pm 0.008$ ) was less than the means of ICP 28,  $F_1$  and  $F_2$  but greater than ICPW 94. The  $F_3$  mean ( $0.78 \pm 0.006$ ) was less than  $F_1$  and  $F_2$  means but greater than the ICP 28, ICPW 94, and  $BC_1F_1$  means (Table 43).

Variance in  $F_1$  (0.07) was greater than the variances in ICP 28 (0.01) and ICPW 94 (0.03). Variance in  $F_2$  (0.118) was greater than the variances in ICP 28, ICPW 94, and  $F_1$ . Variance in  $BC_1F_1$  (ICP 28  $\times$   $F_1$ ) (0.054) was less than the variance in  $F_2$  and  $F_1$  but greater than in ICP 28 and ICPW 94. Variance in  $F_3$  (0.058) was greater than that in ICP 28, ICPW 94, and  $BC_1F_1$  but less than  $F_1$  and  $F_2$  (Table 44).

The estimates of  $d$ ,  $h$  and  $l$  were significant but  $i$  was non-significant (Table 45) and the maximum contribution was from  $h$  (48.86 %) followed by  $l$  (46.58 %) and the contribution of  $i$  in explaining the variation in this character (Table 46).

#### *ICP 28 $\times$ ICPW 130*

$F_1$  mean ( $0.77 \pm 0.022$ ) was greater than ICPW 130 ( $0.72 \pm 0.014$ ) and ICP 28 ( $0.54 \pm 0.017$ ).  $F_2$  mean ( $0.72 \pm 0.006$ ) was equal to ICPW 130 less than  $F_1$  but greater than ICP 28. The  $BC_1F_1$  (ICP 28  $\times$   $F_1$ ) ( $0.72 \pm 0.010$ ) was equal to ICPW 130 and  $F_2$ , less than  $F_1$  but greater than ICP 28. The  $F_3$  mean ( $0.78 \pm 0.006$ ) was greater than ICP 28, ICPW 130,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  (Table 47).

Variance in  $F_1$  (0.015) was greater than the variances in ICP 28 (0.012) and ICPW 130 (0.012). The variance in  $F_2$  (0.056) was greater than the variances ICP 28, ICPW 130, and  $F_1$ . Variance in  $BC_1F_1$  (ICP 28  $\times$   $F_1$ ) (0.016) was equal to variance in  $F_1$  but greater than in ICP 28 and ICPW 130 but less than  $F_2$ . Variance in  $F_3$  (0.018) was equal to  $BC_1F_1$  and  $F_1$  but greater than ICP 28 and ICPW 130, but less than  $F_2$  (Table 48).

The estimates of d, h, i and l were significant (Table 49) with maximum contribution from i (70.59 %) followed by d (17.59 %), l (11.39 %), in explaining the variation in this character (Table 46).

### ***ICP 26 x ICPW 125***

$F_1$  mean ( $0.76 \pm 0.027$ ) was greater than ICP 26 ( $0.62 \pm 0.013$ ) and ICPW 125 ( $0.68 \pm 0.013$ ). Mean in  $F_2$  ( $0.7 \pm 0.019$ ) was greater than ICP 26, ICPW 125 but less than the  $F_1$  mean. The  $BC_1F_1$  mean (ICP 26 x  $F_1$ ) ( $0.74 \pm 0.003$ ) was greater than ICP 26, ICPW 125, and  $F_2$  but less than  $F_1$  mean. The  $F_3$  mean ( $0.78 \pm 0.005$ ) was greater than the means of ICP 26, ICPW 125,  $F_1$ ,  $F_2$  and  $BC_1F_1$  (Table 50).

Variance in  $F_1$  (0.017) was equal to variance in ICP 26 (0.017) but less than that in ICPW 125 (0.022). Variance in  $F_2$  (0.167) was greater than the variances in ICP 26, ICPW 125, and  $F_1$ . Variance in  $BC_1F_1$  (ICP 26 x  $F_1$ ) (0.065) was greater than variance in ICP 26, ICPW 125, and  $F_1$  but less than  $F_2$ . The  $F_3$  variance (0.098) was greater than ICP 26, ICPW 125,  $F_1$ , and  $BC_1F_1$  variances but less than that in  $F_2$  (Table 51)

The estimates of d, h, i and l were significant (Table 52) with the maximum contribution from l (81.57 %) followed by h (13.87 %), i (3.84 %), in explaining the variation in this character (Table 46).

### **Pod bearing length**

#### ***ICP 28 x ICPW 94***

$F_1$  mean ( $11.2 \pm 0.592$ ) pod bearing length was greater than the mean of ICP 28 ( $5.90 \pm 0.384$ ) but less than that of ICPW 94 ( $23.50 \pm 0.601$ ). Mean of  $F_2$  ( $9.50 \pm 0.201$ ) was less than the means of ICPW 94, and  $F_1$  but greater than ICP 28 means. The  $BC_1F_1$  mean (ICP 28 x  $F_1$ ) ( $10.48 \pm 0.289$ ) was greater than the means of ICP 28 and  $F_2$  but less than ICPW 94 and  $F_1$ . The  $F_3$  mean ( $13.77 \pm 0.44$ ) was less than ICPW 94, but greater than the means of ICP 28,  $F_1$ ,  $F_2$  and  $BC_1F_1$  (Table 43).

Variance in  $F_1$  (3.48) was less than the variance in ICPW 94 (3.61) but greater than that in ICP 28 (1.21). Variance in  $F_2$  (10.093) was greater than ICP 28, ICPW 94, and  $F_1$ . Variance in  $BC_1F_1$  (ICP 28 x  $F_1$ ) (5.11) was less than  $F_2$  but greater than ICP 28, ICPW 94 and  $F_1$ . Variance in  $F_3$  (39.909) was greater than the variances in ICP 28, ICPW 94,  $F_1$ ,  $F_2$  and  $BC_1F_1$  (Table 44).

The estimates of d, h, i and l were significant (Table 45), and the maximum contribution was from i (60.89 %) followed by d (34.79 %) and h (3.88 %) in explaining the variation in this character (Table 46).

### *ICP 28 x ICPW 130*

$F_1$  mean ( $10.04 \pm 0.67$ ) was greater than the means ICP 28 ( $4.78 \pm 0.307$ ) but less than ICPW 130 ( $14.80 \pm 1.289$ ).  $F_2$  mean ( $9.6 \pm 0.480$ ) was greater than ICP 28 but less than ICPW 130 and  $F_1$  means.  $BC_1F_1$  mean (ICP 28 x  $F_1$ ) ( $4.04 \pm 0.201$ ) was less than ICP 28, ICPW 130,  $F_1$ , and  $F_2$ .  $F_3$  mean ( $11.77 \pm 0.444$ ) was greater than ICP 28,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  but less than ICPW 130 mean (Table 47).

Variance in  $F_1$  (4.489) was greater than that in ICP 28 (0.944) but less than in ICPW 130 (4.622). Variance in  $F_2$  (12.956) was greater than that in ICP 28, ICPW 130, and  $F_1$ . The  $BC_1F_1$  variance (ICP 28 x  $F_1$ ) (2.257) was greater than that in ICP 28 but less than ICPW 130,  $F_1$ , and  $F_2$ .  $F_3$  (19.713) variance was greater than that in ICP 28, ICPW 130,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  (Table 48).

The estimates of d, h, i and l were significant (Table 49), with the maximum contribution from h (64.35%) followed by i (29.39%) and d (6.35 %) in explaining the variation for this character (Table 46).

### *ICP 26 x ICPW 125*

$F_1$  mean ( $11.2 \pm 0.731$ ) was greater than ICP 26 ( $6.00 \pm 0.516$ ) mean but less than the mean of ICPW 125 ( $27.00 \pm 0.506$ ).  $F_2$  ( $10.72 \pm 0.894$ ) mean was greater than ICP 26 but less than ICPW 125, and  $F_1$ . The  $BC_1F_1$  mean (ICP 26 x  $F_1$ ) ( $4.14 \pm 0.095$ )

was less than means of ICP 26, ICPW 125,  $F_1$ , and  $F_2$ . The  $F_3$  ( $15.77 \pm 0.402$ ) mean was less than the means of ICPW 125 but greater than the means of ICP 26,  $F_1$ ,  $F_2$  and  $BC_1F_1$  (Table 50).

Variance in  $F_1$  (5.344) was greater than the variances that in ICP 26 (2.667) and ICPW 125 (3.67). Variance in  $F_2$  (14.749) was greater than that in ICP 26, ICPW 125, and  $F_1$ . Variance in  $BC_1F_1$  (ICP 26  $\times$   $F_1$ ) (1.851) was less than that in ICP 26, ICPW 125,  $F_1$ , and  $F_2$ . The  $F_3$  variance (14.300) was greater than ICP 26, ICPW 125,  $BC_1F_1$  and  $F_1$  but less than  $F_2$  (Table 51).

The estimates of  $d$ ,  $h$ ,  $i$  and  $l$  were significant (Table 52), however, the maximum contribution was from  $d$  (61.84 %) followed by  $l$  (22.58 %),  $i$  (9.24 %), in explaining the variation in this character (Table 46).

## Number of locules per pod

### *ICP 28 $\times$ ICPW 94*

$F_1$  mean ( $4.30 \pm 0.133$ ) for number of locules per pod was greater than the means of ICP 28 ( $3.80 \pm 0.133$ ) and ICPW 94 ( $4.0 \pm 0.21$ ). The  $F_2$  mean ( $3.91 \pm 0.029$ ) was less than ICPW 94, and  $F_1$  but greater than ICP 28. The  $BC_1F_1$  (ICP 28  $\times$   $F_1$ ) mean ( $2.99 \pm 0.034$ ) was less than ICP 28, ICPW 94,  $F_1$ , and  $F_2$  means. The  $F_3$  mean ( $4.06 \pm 0.025$ ) was greater than ICP 28, ICPW 94,  $F_2$ , and  $BC_1F_1$  but less than  $F_1$  (Table 43).

Variance in  $F_1$  (0.18) was equal to the variance in ICP 28 (0.18) but less than ICPW 94 (0.44). Variance in  $F_2$  (0.606) was greater than the variances in ICP 28, ICPW 94 and  $F_1$ . Variance in  $BC_1F_1$  (ICP 28  $\times$   $F_1$ ) (0.073) was less than the variance in ICP 28, ICPW 94,  $F_1$  and  $F_2$ . Variance in  $F_3$  (0.529) was less than  $F_2$  but greater than the variances in ICP 28, ICPW 94,  $F_1$ , and  $BC_1F_1$  (Table 44).

The estimates of *i* and *l* were significant but the estimates of *d* and *h* were non-significant (Table 45) with the maximum contribution was from *i* (47.9%) followed by *l* (42.9%) in explaining the variation in this character (Table 46).

#### ***ICP 28 x ICPW 130***

$F_1$  mean ( $3.81 \pm 0.133$ ) was less than ICPW 130 ( $5.22 \pm 0.133$ ) but was equal to ICP 28 ( $3.78 \pm 0.139$ ).  $F_2$  mean ( $2.83 \pm 0.039$ ) was less than the means of ICP 28, ICPW 130, and  $F_1$ . The  $BC_1F_1$  (ICP 28 x  $F_1$ ) ( $2.08 \pm 0.072$ ) was less than the means of ICP 28, ICPW 130,  $F_1$ , and  $F_2$  means.  $F_3$  mean ( $4.06 \pm 0.025$ ) was greater than the means of ICP 28,  $F_1$ ,  $F_2$  and  $BC_1F_1$  but less than ICPW 130 (Table 47).

Variance in  $F_1$  (0.178) was similar to the variance in ICPW 130 (0.18) but less than ICP 28 (0.194). Variance in  $F_2$  (0.376) was greater than the variances in ICP 28, ICPW 130, and  $F_1$ . Variance in  $BC_1F_1$  (ICP 28 x  $F_1$ ) (0.288) was greater than ICP 28, ICPW 130, and  $F_1$  but less than  $F_2$ . The  $F_3$  (0.128) was less than ICP 28, ICPW 130,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  (Table 48).

The estimates of *d*, *h*, *i* and *l* were significant (Table 49), however, the maximum contribution was from *l* (59.68 %) followed by *h* (37.28 %), *i* (2.48 %) in explaining the variation for this character (Table 46).

#### ***ICP 26 x ICPW 125***

$F_1$  mean ( $4.30 \pm 0.153$ ) was greater than the mean of ICP 26 ( $4.0 \pm 0.211$ ) but less than that of ICPW 125 ( $4.8 \pm 0.122$ ).  $F_2$  mean ( $2.4 \pm 0.091$ ) was less than the means of ICP 26, ICPW 125 and  $F_1$ . The  $BC_1F_1$  mean (ICP 26 x  $F_1$ ) ( $2.7 \pm 0.039$ ) was less than the means of ICP 26, ICPW 125, and  $F_1$  but greater than  $F_2$ . The  $F_3$  ( $4.1 \pm 0.023$ ) was less than ICPW 125, and  $F_1$  but greater than ICP 26,  $F_2$  and  $BC_1F_1$  (Table 50).

Variance in  $F_1$  (0.233) was less than that of ICP 26 (0.444) but greater than the variance in ICPW 125 (0.181). Variance in  $F_2$  (0.468) was greater than ICP 26,

ICPW 125, and  $F_1$ . Variance in  $BC_1F_1$  ( $ICP\ 26 \times F_1$ ) (0.330) was less than the variances in ICP 26 and  $F_2$  but greater than that in ICPW 125 and  $F_1$ . The  $F_3$  variance (0.130) was less than ICP 26, ICPW 125,  $F_1$ ,  $F_2$  and  $BC_1F_1$  (Table 51).

The estimates of  $d$ ,  $h$ ,  $i$  and  $l$  were significant (Table 52) with maximum contribution was from  $h$  (45.56%) followed by  $l$  (30.49%),  $d$  (13.17%), and  $i$  (10.96%) in this character for explaining the variation (Table 46).

### **Number of seeds per pod**

#### ***ICP 28 x ICPW 94***

$F_1$  mean ( $4.30 \pm 0.133$ ) for number of seeds per pod was greater than ICP 28 ( $3.25 \pm 0.13$ ) and ICPW 94 ( $3.95 \pm 0.214$ ). The  $F_2$  mean ( $3.95 \pm 0.029$ ) was less than ICPW 94, and  $F_1$  but greater than ICP 28 means. The  $BC_1F_1$  ( $ICP\ 28 \times F_1$ ) mean ( $2.89 \pm 0.045$ ) was less than ICP 28, ICPW 94,  $F_1$ , and  $F_2$ . The  $F_3$  mean ( $4.89 \pm 0.045$ ) was greater than the means of ICP 28, ICPW 94,  $F_1$ ,  $F_2$  and  $BC_1F_1$  (Table 43).

Variance in  $F_1$  (0.178) was similar to the variance in ICP 28 (0.18) but less than ICPW 94 (0.36). Variance in  $F_2$  (0.21) was greater than the variance in ICP 28, and  $F_1$  but less than that in ICPW 94. Variance in  $BC_1F_1$  (0.122) was less than ICP 28, ICPW 94,  $F_1$ , and  $F_2$ . Variance in  $F_3$  (0.408) was greater than variances in ICPW 94, ICP 28,  $F_1$ ,  $F_2$  and  $BC_1F_1$  (Table 44).

The estimates of  $d$ ,  $h$ ,  $i$  and  $l$  were significant (Table 45) with the maximum contribution from  $l$  (75.8%) followed by  $h$  (9.08 %),  $i$  (8.26 %), and  $d$  (6.81 %) in explaining the variation in this character (Table 46).

#### ***ICP 28 x ICPW 130***

$F_1$  mean ( $3.60 \pm 0.133$ ) was less than ICP 28 ( $3.84 \pm 0.139$ ) and ICPW 130 ( $5.6 \pm 0.133$ ).  $F_2$  mean ( $3.20 \pm 0.039$ ) was less than ICP 28, ICPW 130, and  $F_1$ . The  $BC_1F_1$  ( $2.80 \pm 0.072$ ) was less than ICP 28, ICPW 130,  $F_1$ , and  $F_2$ . The  $F_3$  mean (4.23

$\pm 0.025$ ) was greater than ICP 28,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  but less than ICPW 130 (Table 47).

Variance in  $F_1$  (0.178) was equal to the variance in ICPW 130 (0.178) but less than that in ICP 28 (0.194). Variance in  $F_2$  (0.376) was greater than ICP 28, ICPW 130, and  $F_1$ . Variance in  $BC_1F_1$  (0.288) was greater than in ICP 28, ICPW 130, and  $F_1$  but less than  $F_2$ . Variance in  $F_3$  (0.128) was less than variance in ICP 28, ICPW 130,  $F_1$ ,  $F_2$  and  $BC_1F_1$  (Table 48).

The estimates of  $h$ ,  $i$  and  $l$  were significant but the estimates of  $d$  (additive) was non-significant (Table 49), however, the maximum contribution was from  $h$  (65.06 %), followed by  $l$  (28.47 %) in explaining the variation in this character (Table 46).

#### ***ICP 26 x ICPW 125***

$F_1$  mean ( $3.50 \pm 0.163$ ) was less than means of ICP 26 ( $3.60 \pm 0.163$ ) and ICPW 125 ( $5.40 \pm 0.163$ ).  $F_2$  mean ( $2.50 \pm 0.091$ ) was less than ICP 26, ICPW 125, and  $F_1$  means. The  $BC_1F_1$  mean ( $2.69 \pm 0.039$ ) was less than ICP 26, ICPW 125, and  $F_1$  but greater than  $F_2$ . The  $F_3$  ( $3.3 \pm 0.03$ ) was less than ICPW 125, ICP 26, and  $F_1$  but greater than  $F_2$ , and  $BC_1F_1$  means (Table 50).

Variance in  $F_1$  (0.266) was equal to the variance in ICP 26 (0.267) and ICPW 125 (0.267). Variance in  $F_2$  (0.465) was greater than the variances in ICP 26, ICPW 125, and  $F_1$ . Variance in  $BC_1F_1$  (0.328) was greater than the variance in ICP 26, ICPW 125, and  $F_1$  but less than that in  $F_2$ . Variance in  $F_3$  (0.209) was less than that in ICP 26, ICPW 125,  $BC_1F_1$ ,  $F_1$ , and  $F_2$  (Table 51).

The estimates of  $d$ ,  $h$ ,  $i$  and  $l$  were significant (Table 52), however, the maximum contribution was from  $l$  (53.37 %) followed by  $h$  (42.77 %),  $d$  (3.46 %), explaining the variation in this character (Table 46).



## Number of primary branches

### *ICP 28 x ICPW 94*

$F_1$  mean ( $7.23 \pm 0.257$ ) number of branches was less than ICP 28 ( $9.20 \pm 0.133$ ) and ICPW 94 ( $9.40 \pm 0.163$ ).  $F_2$  mean ( $6.68 \pm 0.036$ ) was less than ICP 28, ICPW 94 and  $F_1$  means. The  $BC_1F_1$  (ICP 28 x  $F_1$ ) ( $9.66 \pm 0.163$ ) was greater than ICP 28, ICPW 94 and  $F_1$  but less than  $F_2$ . The  $F_3$  mean ( $6.09 \pm 0.089$ ) was less than ICP 28, ICPW 94,  $F_2$ ,  $F_1$ , and  $BC_1F_1$  (Table 43).

Variance in  $F_1$  (0.661) was greater than the variance in ICP 28 (0.178) and in ICP 94 (0.267). Variance in  $F_2$  (1.33) was greater than the variance in ICP 28, ICPW 94, and  $F_1$ . Variance in  $BC_1F_1$  (1.626) was greater than the variances in ICP 28, ICPW 94,  $F_2$ , and  $F_1$ . Variance in  $F_3$  (1.629) was greater than variances in ICP 28, ICPW 94,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  (Table 44).

The estimates of  $d$ ,  $h$ ,  $i$  and  $l$  were significant (Table 45) the maximum contribution was from  $h$  (50.49 %) followed by  $d$  (43.70 %),  $l$  (5.8 %) in explaining the variation in this character (Table 46).

### *ICP 28 x ICPW 130*

$F_1$  mean ( $8.30 \pm 0.249$ ) was less than the means of ICP 28 ( $9.22 \pm 0.139$ ) and ICPW 130 ( $10.20 \pm 0.133$ ).  $F_2$  mean ( $7.3 \pm 0.106$ ) was less than ICP 28, ICPW 130, and  $F_1$ . Mean in  $BC_1F_1$  ( $8.15 \pm 0.046$ ) was less than the means of ICP 28, ICPW 130, and  $F_1$  but greater than  $F_2$ . The  $F_3$  mean ( $4.3 \pm 0.04$ ) was less than the means of ICP 28, ICPW 130,  $F_1$ ,  $F_2$  and  $BC_1F_1$  (Table 47).

Variance in  $F_1$  (0.622) was greater than the variances in ICPW 130 (0.178) but less than in ICP 28 (0.658). Variance in  $F_2$  (2.808) was greater than variances in ICP 28, ICPW 130, and  $F_1$ . Variance in  $BC_1F_1$  (1.121) was less than  $F_2$  but greater than the variances in  $F_1$ , ICP 28 and ICPW 130. Variance in  $F_3$  (1.365) was greater

than  $F_1$ , ICP 28, and ICPW 130 and  $BC_1F_1$  variances but less than variance in  $F_2$  (Table 48)

The estimates of  $d$ ,  $h$ ,  $i$  and  $l$  were significant (Table 49) with maximum contribution was from  $i$  (52.59 %) followed by  $h$  (27.03 %),  $l$  (19.38 %), and  $d$  (1.19 %) in explaining the variation in this character (Table 46).

#### ***ICP 26 x ICPW 125***

$F_1$  mean ( $5.7 \pm 0.213$ ) was less than ICPW 125 ( $7.6 \pm 0.163$ ) but greater than ICP 26 ( $3.2 \pm 0.133$ ).  $F_2$  mean ( $4.9 \pm 0.036$ ) was less than ICPW 125, and  $F_1$  but greater than ICP 26. The  $BC_1F_1$  mean ( $3.7 \pm 0.013$ ) was less than ICPW 125,  $F_1$  and  $F_2$  but greater than ICP 26. The  $F_3$  ( $4.3 \pm 0.04$ ) was less than ICPW 125,  $F_2$  and  $F_1$  but greater than ICP 26, and  $BC_1F_1$  (Table 50).

Variance in  $F_1$  (0.456) was greater than the variance in ICP 26 (0.178) and ICPW 125 (0.267). Variance in  $F_2$  (1.174) was greater than the variances in ICP 26, ICPW 125, and  $F_1$ . Variance in  $BC_1F_1$  (0.137) was less than the variances in ICP 26, ICPW 125,  $F_2$ , and  $F_1$ . Variance in  $F_3$  (1.307) was greater than the variances in ICP 26, ICPW 125,  $BC_1F_1$ ,  $F_1$ , and  $F_2$  (Table 51)

The estimates of  $d$ ,  $i$  and  $l$  were significant but  $h$  was non- significant (Table 52) with the maximum contribution from  $d$  (44.69 %) followed by  $i$  (26.84 %) and  $l$  (21.49 %) in explaining the variation in this character (Table 46)

#### **Number of secondary branches**

#### ***ICP 28 x ICPW 94***

$F_1$  mean ( $20.61 \pm 0.180$ ) number of secondary branches was greater than ICP 28 ( $12.40 \pm 0.267$ ) and ICPW 94 ( $17.40 \pm 0.163$ ). The  $F_2$  mean ( $13.92 \pm 0.059$ ) was greater than ICP 28 but less than the means of ICPW 94, and  $F_1$  means. The  $BC_1F_1$  (ICP 28 x  $F_1$ ) mean ( $6.16 \pm 0.150$ ) was less than the means of ICP 28, ICPW 94,  $F_1$ ,

and  $F_2$ . The  $F_3$  mean ( $12.91 \pm 0.095$ ) was less than the means of ICPW 94,  $F_1$ , and  $F_2$  but greater than ICP 28 and  $BC_1F_1$  (Table 43).

Variance in  $F_1$  (1.32) was greater than the variance in ICP 28 (0.711) and ICPW 94 (0.267). Variance in  $F_2$  (3.87) was greater than the variance in ICP 28, ICPW 94 and  $F_1$ . Variance in  $BC_1F_1$  (2.573) was greater than the variance in ICP 28, ICPW 94 and  $F_1$  but less than the variance in  $F_2$ . Variance in  $F_3$  (3.828) was greater than variance in ICP 28, ICPW 94,  $F_1$ , and  $BC_1F_1$  but less than that in  $F_2$  (Table 44).

The estimates of  $h$ ,  $i$  and  $l$  were significant but  $d$  was non - significant (Table 45) with the maximum contribution from  $l$  (58.49 %) followed by  $i$  (28.25 %), and  $h$  (13.22 %), in explaining the variation in this character (Table 46).

#### ***ICP 28 x ICPW 130***

The  $F_1$  mean ( $17.5 \pm 0.249$ ) was greater than ICP 28 ( $4.61 \pm 0.139$ ) and ICPW 130 ( $16.50 \pm 0.133$ ).  $F_2$  mean ( $3.9 \pm 0.601$ ) was less than the means of ICP 28, ICPW 130 and  $F_1$ . The  $BC_1F_1$  mean ( $8.69 \pm 0.049$ ) was less than ICPW 130, and  $F_1$  but greater than ICP 28 and  $F_2$  means. The  $F_3$  mean ( $8.7 \pm 0.04$ ) was equal to  $BC_1F_1$  but was less than, ICPW 130 and  $F_1$  and greater than ICP 28 and  $F_2$  means (Table 47).

Variance in  $F_1$  (1.622) was greater than in ICPW 130 (1.178) and in ICP 28 (1.194). Variance in  $F_2$  (6.808) was greater than the variance in ICP 28, ICPW 130, and  $F_1$ . Variance in  $BC_1F_1$  (5.121) was less than variance in  $F_2$  but greater than in ICP 28, ICPW 130, and  $F_1$ . The  $F_3$  (6.523) was greater than the variance in ICP 28, ICPW 130,  $F_1$ , and  $BC_1F_1$  but less than  $F_2$  (Table 48).

The estimates of  $d$ ,  $h$ ,  $i$  and  $l$  were significant (Table 49) with the maximum contribution from  $d$  (67.29 %) followed by  $l$  (30.38 %),  $i$  (2.09 %) in explaining the variation in this character (Table 46).

### **ICP 26 x ICPW 125**

Mean of  $F_1$  ( $12.60 \pm 0.452$ ) was equal to the mean of ICPW 125 ( $12.60 \pm 0.163$ ) but greater than ICP 26 ( $4.40 \pm 0.16$ ). The  $F_2$  mean ( $6.7 \pm 0.122$ ) was greater than the mean of ICP 26 but less than the means of ICPW 125 and  $F_1$ .  $BC_1F_1$  mean ( $8.69 \pm 0.169$ ) was less than ICPW 125, and  $F_1$  but greater than ICP 26 and  $F_2$ . The  $F_3$  ( $9.27 \pm 0.072$ ) was less than ICPW 125, and  $F_1$  but greater than ICP 26,  $F_2$  and  $BC_1F_1$  (Table 50).

Variance in  $F_1$  (2.044) was greater than variance in ICP 26 (0.820), and ICPW 125 (1.267). Variance in  $F_2$  (4.57) was greater than the variance in ICP 26, ICPW 125, and  $F_1$ . Variance in  $BC_1F_1$  (5.865) was greater than variance in ICP 26, ICPW 125,  $F_2$ , and  $F_1$ . Variance in  $F_3$  (1.302) was greater than the variance in ICP 26, ICPW 125, but less than  $F_2$ ,  $BC_1F_1$  and  $F_1$  (Table 51).

The estimates of  $d$  (,  $h$ ,  $i$  and  $l$  were significant (Table 52) with the maximum contribution from  $d$  (80.48 %) followed by  $l$  (14.98 %) and  $i$  (2.37 %) in explaining the variation in this character (Table 46).

### **100 - seed weight**

#### **ICP 28 x ICPW 94**

$F_1$  mean ( $5.53 \pm 0.112$ ) 100 – seed weight was greater than ICPW 94 ( $2.95 \pm 0.002$ ) but less than ICP 28 ( $11.05 \pm 0.015$ ). The  $F_2$  mean ( $4.96 \pm 0.016$ ) was greater than the means of ICPW 94 but less than the mean of ICP 28 and  $F_1$ . The  $BC_1F_1$  (ICP 28 x  $F_1$ ) ( $7.49 \pm 0.067$ ) was less than ICP 28 but greater than the mean of ICPW 94,  $F_1$  and  $F_2$ . The  $F_3$  mean ( $4.32 \pm 0.042$ ) was less than ICP 28,  $F_1$  and  $BC_1F_1$  but greater than ICPW 94 and  $F_2$  (Table 43).

Variance in  $F_1$  (0.12) was greater than the variances in ICP 28 (0.022) and ICPW 94 (0.01). The  $F_2$  (0.162) was greater than the variances of ICP 28, ICPW 94,

and  $F_1$ . Variance in  $BC_1F_1$  (0.276) was greater than ICP 28, ICPW 94,  $F_1$ , and  $F_2$ .  $F_3$  variance (0.36) was greater than ICP 28, ICPW 94,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  (Table 44).

The estimates of d, h, l and i were significant (Table 45) maximum contribution from was from d (95.10 %) followed by h (4.67 %) and i and l being non- significant in explaining the variation in this character (Table 46).

### ***ICP 28 x ICPW 130***

$F_1$  mean ( $5.97 \pm 0.119$ ) was less than ICP 28 ( $11.06 \pm 0.014$ ) but greater than ICPW 130 ( $2.33 \pm 0.001$ ).  $F_2$  mean ( $5.50 \pm 0.052$ ) was less than ICP 28, and  $F_1$  but greater than ICPW 130 means.  $BC_1F_1$  ( $3.73 \pm 0.075$ ) mean was less than ICP 28,  $F_1$  and  $F_2$  but greater than ICPW 130. The  $F_3$  mean ( $7.70 \pm 0.060$ ) was less than ICP 28 but greater than ICPW 130,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  (Table 47).

The  $F_1$  variance (0.412) was greater than the variances of ICPW 130 (0.014) and ICP 28 (0.042). The variance in  $F_2$  (0.675) was greater than ICP 28, ICPW 130, and  $F_1$ . Variance in  $BC_1F_1$  (0.316) was less than  $F_1$  and  $F_2$  but greater than ICP 28 and ICPW 130. Variance in  $F_3$  (0.729) was greater than ICP 28, ICPW 130,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  (Table 48).

The estimates of d, h, i and l were significant (Table 49) with the maximum contribution was from d (98.59 %) but the contributions of l, h, and i were non-significant in explaining the variation in this character (Table 46).

### ***ICP 26 x ICPW 125***

$F_1$  mean ( $4.52 \pm 0.172$ ) was less than ICP 26 ( $11.151 \pm 1.414$ ) but greater than ICPW 125 ( $2.54 \pm 0.002$ ). The  $F_2$  mean ( $3.9 \pm 0.271$ ) was greater than ICPW 125 but less than ICP 26 and  $F_1$  means. The  $BC_1F_1$  mean ( $7.63 \pm 0.259$ ) was less than ICP 26 but greater than ICPW 125,  $F_1$ , and  $F_2$ . The  $F_3$  ( $7.70 \pm 0.053$ ) was less than ICP 26 but greater than ICPW 125,  $F_1$ ,  $F_2$  and  $BC_1F_1$  (Table 50).

Variance in  $F_1$  (0.030) was greater than variance in ICP 26 (0.026) but less than in ICPW 125 (0.044). The  $F_2$  variance (0.073) was greater than the variances in  $F_1$ , ICP 26 and ICPW 125. Variance in  $BC_1F_1$  (0.067) was greater than ICP 26 and ICPW 125 and  $F_1$  but less than and  $F_2$ . Variance in  $F_3$  (0.740) was greater than variances in ICP 26, ICPW 125,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  (Table 51).

The estimates of d, h, i, were significant but l was non - significant (Table 52) from maximum contribution in explaining the variation was from d (98.56 %) followed by h (2.56 %) but the contributions of l and i were non- significant (Table 46).

## Harvest Index

### *ICP 28 x ICPW 94*

$F_1$  mean ( $5.41 \pm 0.259$ ) harvest index was less than mean of ICPW 94 ( $6.23 \pm 0.129$ ) and ICP 28 ( $18.24 \pm 1.255$ ). The  $F_2$  mean ( $5.27 \pm 1.259$ ) was less than ICP 28, ICPW94 and  $F_1$  means.  $BC_1F_1$  ( $F_1 \times$  ICP 28) mean ( $10.24 \pm 2.563$ ) was greater than ICPW 94,  $F_1$ , and  $F_2$  but less than ICP 28. The  $F_3$  mean ( $5.12 \pm 1.256$ ) was less than the means of ICP 28, ICPW 94,  $F_1$ ,  $F_2$  and  $BC_1F_1$  (Table 43).

Variance in  $F_1$  (0.278) was less than variance in ICP 28 (0.42) but greater than ICPW 94 (0.11). The  $F_2$  variance (2.56) was greater than variances in ICP 28, ICPW 94 and  $F_1$ . Variance in  $BC_1F_1$  (1.45) was less than  $F_2$  but greater than the variance in ICP 28, ICPW 94 and  $F_1$ . The  $F_3$  (2.56) was equal to  $F_2$  but greater than the variances in ICP 28,  $BC_1F_1$ , ICPW 94, and  $F_1$  (Table 44).

The estimates of d, h, i and l were significant with the maximum contribution (Table 45) from d (94.56 %) followed by h (3.56 %) but the contribution of i and l being non – significant in explaining the variation (Table 46).

### ***ICP 28 x ICPW 130***

$F_1$  mean ( $8.46 \pm 1.259$ ) was less than the means of ICP 28 ( $18.23 \pm 1.259$ ) but greater than ICPW 130 ( $7.41 \pm 2.598$ ).  $F_2$  mean ( $7.92 \pm 0.865$ ) was less than ICP 28 and  $F_1$  but greater than ICPW 130.  $BC_1F_1$  ( $F_1 \times$  ICP 28) mean ( $10.42 \pm 1.256$ ) was less than ICP 28 but greater than ICPW 94,  $F_1$ , and  $F_2$ .  $F_3$  mean ( $7.14 \pm 1.256$ ) was less than the mean of ICP 28, ICPW 94,  $F_1$ ,  $F_2$ , and  $BC_1F_1$  (Table 47).

Variance in  $F_1$  (0.682) was greater than ICP 28 (0.486) and ICPW 130 (1.316).  $F_2$  (1.589) was greater than variance in ICP 28, ICPW 130, and  $F_1$ .  $BC_1F_1$  (1.256) was greater than ICP 28, ICP 130 and  $F_1$ , but less than  $F_2$ . Variance in  $F_3$  (1.26) was greater than ICP 28, ICPW 130, and  $F_1$ , but less than  $F_2$  and  $BC_1F_1$  (Table 48).

The estimates of  $d$ ,  $h$ ,  $i$  and  $l$  were significant (Table 49) with the maximum contribution from  $d$  (98.0%) in explaining the variation in this character (Table 46).

### ***ICP 26 x ICPW 125***

$F_1$  mean ( $6.39 \pm 0.956$ ) was less than ICP 26 ( $17.42 \pm 0.126$ ) but greater than ICPW 125 ( $5.41 \pm 1.255$ ).  $F_2$  mean ( $5.41 \pm 0.256$ ) was less than ICP 26 and  $F_1$  but equal to ICPW 125 mean.  $BC_1F_1$  ( $8.46 \pm 0.549$ ) mean was less than ICP 26 but greater than ICPW 125,  $F_1$ , and  $F_2$  means.  $F_3$  mean ( $5.59 \pm 0.563$ ) was less than the mean of ICP 26,  $F_1$  and  $BC_1F_1$  but greater than ICPW 125 and  $F_2$  (Table 50).

Variance in  $F_1$  (1.158) was greater than the variance in ICP 26 (0.089) and ICPW 125 (0.124). Variance in  $F_2$  (4.456) was greater than the variance in ICP 26, ICPW 125, and  $F_1$ . Variance in  $BC_1F_1$  (4.512) was greater than the variance in ICP 26, ICPW 125,  $F_1$ , and  $F_2$ . Variance in  $F_3$  (3.112) was greater than the variances in ICP 26, ICPW 125 and  $F_1$  but less than  $F_2$ , and  $BC_1F_1$  (Table 51).

The estimates of  $d$ ,  $h$ ,  $i$  were significant but  $l$  was non – significant (Table 52) with maximum contribution in explaining the variation was from  $d$  (97.28 %) followed by  $h$  (2.54 %) in explaining the variation in this character (Table 46).

## **Means and variances for different characters in four crosses**

In addition to the above three crosses, the means and variances were calculated and the heritability of different characters was determined in ICP 26 x ICPW 94, ICP 28 x ICPW 125, ICP 26 x ICPW 130, ICP 28 x ICPW 141 crosses.

### ***ICP 26 x ICPW 94***

Means and variances for various characters are presented in Table 53 and 54.

#### **Days to flowering**

The  $F_1$  plants mean ( $53.40 \pm 0.367$ ) number of days to flower was less than ICP 26 ( $56.80 \pm 0.163$ ) but greater than mean of ICPW 94 ( $53.20 \pm 0.533$ ).  $F_2$  mean ( $52.1 \pm 0.269$ ) was less than the means of ICP 26, ICPW 94 and  $F_1$  means respectively.

Variance in ICP 26 (0.67) was less than ICPW 94 (2.844). Variance in  $F_1$  (1.344) was greater than the variance in ICP 26 but less than ICPW 94.  $F_2$  variance (18.02) was greater than ICP 26, ICPW 94, and  $F_1$  variances.

#### **Days to maturity**

The  $F_1$  mean ( $96.80 \pm 0.307$ ) number of days to maturity was less than ICP 26 ( $103.0 \pm 0.211$ ) but greater than ICPW 94 ( $85.60 \pm 1.12$ ).  $F_2$  mean ( $94.2 \pm 0.293$ ) was less than the means of ICP 26 and  $F_1$  but greater than ICPW 94.

Variance in ICP 26 (0.444) was less than ICPW 94 (12.71).  $F_1$  variance (0.944) was greater than the variance in ICP 26 but less than ICPW 94.  $F_2$  variance (21.344) was greater than ICP 26, ICPW 94 and  $F_1$  variances.



**Table - 53: Means values for various characters of ICP 26 x ICPW 94**

<b>Character</b>	<b>ICP 26 mean <math>\pm</math>SE</b>	<b>ICPW 94 mean <math>\pm</math>SE</b>	<b>F<sub>1</sub> mean <math>\pm</math>SE</b>	<b>F<sub>2</sub> mean <math>\pm</math>SE</b>
Days to flowering (No.)	56.80 $\pm$ 0.163	53.20 $\pm$ 0.533	53.40 $\pm$ 0.367	52.1 $\pm$ 0.269
Days to maturity (No.)	103.00 $\pm$ 0.211	85.60 $\pm$ 1.127	96.80 $\pm$ 0.307	94.2 $\pm$ 0.293
Leaf length (cm)	6.94 $\pm$ 0.034	4.84 $\pm$ 0.016	5.20 $\pm$ 0.056	1.51 $\pm$ 0.024
Leaf width (cm)	3.58 $\pm$ 0.013	2.60 $\pm$ 0.021	3.20 $\pm$ 0.026	2.91 $\pm$ 0.045
Pod length (cm)	5.18 $\pm$ 0.099	2.50 $\pm$ 0.021	3.67 $\pm$ 0.033	.53 $\pm$ 0.024
Pod width (cm)	0.62 $\pm$ 0.013	0.54 $\pm$ 0.016	0.80 $\pm$ 0.016	.76 $\pm$ 0.011
Pod Bearing length (cm)	4.79 $\pm$ 0.185	17.80 $\pm$ 0.827	9.5 $\pm$ 0.335	9.1 $\pm$ 0.514
No .of Locules per pod	4.00 $\pm$ 0.211	4.00 $\pm$ 0.211	4.20 $\pm$ 0.163	.74 $\pm$ 0.050
No .of seed per pod	3.60 $\pm$ 0.163	3.80 $\pm$ 0.133	4.30 $\pm$ 0.153	.43 $\pm$ 0.039
No .of Primary branches	3.20 $\pm$ 0.133	9.40 $\pm$ 0.163	5.20 $\pm$ 0.348	.89 $\pm$ 0.057
No .of Secondary branches	4.40 $\pm$ 0.163	17.40 $\pm$ 0.163	18.40 $\pm$ 0.636	13.8 $\pm$ 0.135
100 seed weight (g)	11.16 $\pm$ 0.002	2.95 $\pm$ 0.002	4.44 $\pm$ 0.040	3.9 $\pm$ 0.059
Harvest index	16.25 $\pm$ 0.586	4.56 $\pm$ 0.412	5.26 $\pm$ 0.256	.69 $\pm$ 0.598

**Table 54: Variance and heretability for various characters of ICP 26 x ICPW 94**

Character	Variance in ICP 26	Variance in ICPW 94	Variance in F <sub>1</sub>	Variance in F <sub>2</sub>	enviromen tal variance	Genotypic variance	Broad sense heritability (%)
Days to flower (No.)	0.67	2.84	1.34	18.02	1.62	16.40	91.02
Days to maturity (No.)	0.32	12.71	0.94	21.34	4.66	16.69	78.17
Leaf length (cm)	0.01	0.05	0.03	0.41	0.03	0.38	92.11
Leaf width (cm)	0.01	0.01	0.01	0.51	0.01	0.50	98.18
Pod length (cm)	0.08	0.01	0.01	0.15	0.03	0.12	79.33
Pod width (cm)	0.02	0.03	0.02	0.03	0.02	0.01	23.23
Pod Bearing length (cm)	1.12	3.61	1.12	65.78	1.95	63.83	97.03
No .of Locules per pod	0.32	0.44	0.27	0.63	0.34	0.28	45.08
No .of seed per pod	0.27	0.36	0.23	0.39	0.29	0.11	27.06
No .of Primary branches	0.23	0.27	1.21	0.82	0.57	0.25	30.45
No .of Secondary branches	0.57	0.67	4.04	4.54	1.63	2.92	64.20
100 seed weight (g)	0.01	0.01	0.02	0.89	0.01	0.88	98.65
Harvest Index	0.03	0.11	0.23	1.52	0.12	1.40	91.95

### **Leaflet length**

The  $F_1$  mean ( $5.20 \pm 0.056$ ) leaflet length was in between the means of ICP 26 ( $6.94 \pm 0.034$ ) and ICPW 94 ( $4.84 \pm 0.016$ ) but the  $F_2$  mean ( $1.51 \pm 0.024$ ) was less than ICP 26, ICPW 94, and  $F_1$  means.

Variance in  $F_1$  (0.311) was greater than the variances in ICP 26 (0.011) and ICPW 94 (0.003).  $F_2$  variance (0.41) was greater than the variances in ICP 26, ICPW 94, and  $F_1$ .

### **Leaflet width**

The  $F_1$  mean ( $3.20 \pm 0.026$ ) leaflet width was between the means of ICP 26 ( $3.58 \pm 0.013$ ) and ICPW 94 ( $2.60 \pm 0.021$ ). The  $F_2$  mean ( $2.91 \pm 0.045$ ) was less than the mean of ICP 26 and  $F_1$  but greater than ICPW 94.

Variance in  $F_1$  (0.001) was equal to variances in ICP 26 (0.001) and ICPW 94 (0.001). The variance in  $F_2$  plants (0.512) was greater than the variances in ICP 26, ICPW 94, and  $F_1$  variance.

### **Pod length**

The  $F_1$  mean ( $3.67 \pm 0.033$ ) was pod length between the means of ICP 26 ( $5.18 \pm 0.099$ ) and ICPW 94 ( $2.50 \pm 0.021$ ). The  $F_2$  mean ( $2.53 \pm 0.024$ ) was less than the mean of ICP 26 and  $F_1$  but greater than ICPW 94.

Variance in  $F_1$  (0.011) was less than the variance in ICP 26 (0.08) but greater than ICPW 94 (0.001). The variance in  $F_2$  plants (0.15) was greater than the variances in ICP 26, ICPW 94 and  $F_1$  variance.

### **Pod width**

The  $F_1$  mean ( $0.80 \pm 0.016$ ) pod width was greater than the means of ICP 26 ( $0.62 \pm 0.013$ ) and ICPW 94 ( $0.54 \pm 0.016$ ). The  $F_2$  mean ( $0.76 \pm 0.011$ ) was less than  $F_1$ , but greater than the mean of ICP 26 and ICPW 94.

Variance in  $F_1$  (0.02) was equal to the variance in ICPW 94 (0.03) but greater than ICP 26 (0.02). The variance in  $F_2$  plants (0.033) was greater than the variances in ICP 26, ICPW 94 and  $F_1$ .

### **Pod bearing length**

The  $F_1$  mean ( $9.5 \pm 0.335$ ) pod bearing length was between ICP 26 ( $4.79 \pm 0.185$ ) and ICPW 94 ( $17.80 \pm 0.827$ ). The  $F_2$  mean ( $9.10 \pm 0.514$ ) was greater than the mean of ICP 26 but less than the means of ICPW 94 and  $F_1$ .

Variance in  $F_1$  (1.122) was less than the variance in ICPW 94 (3.61) but greater than ICP 26 (1.128). The variance in  $F_2$  plants (65.778) was greater than the variance in ICP 26, ICPW 94 and  $F_1$ .

### **Number of locules per pod**

The  $F_1$  mean ( $4.20 \pm 0.163$ ) number of locules per pod was but greater than the means of ICP 26 ( $4.00 \pm 0.211$ ) and ICPW 94 ( $4.00 \pm 0.211$ ). The  $F_2$  mean ( $2.74 \pm 0.05$ ) was less than the means of ICP 26, ICPW 94, and  $F_1$ .

Variance in  $F_1$  (0.267) was less than the variances in ICP 26 (0.32) and ICPW 94 (0.444). The variance in  $F_2$  plants (0.63) was greater than the variances in ICP 26, ICPW 94 and  $F_1$ .

### **Number of seeds per pod**

The  $F_1$  mean ( $4.3 \pm 0.15$ ) number of seeds per pod was greater than the means of ICP 26 ( $3.6 \pm 0.163$ ) and ICPW 94 ( $3.8 \pm 0.133$ ). The  $F_2$  mean ( $2.43 \pm 0.039$ ) was less than the means of ICP 26, ICPW 94, and  $F_1$ .

Variance in  $F_1$  (0.233) was less than the variances in ICP 26 (0.266) but greater than ICPW 94 (0.36). The variance in  $F_2$  plants (0.49) was greater than the variances in ICP 26, ICPW 94 and  $F_1$  variance.

### **Number of Primary branches**

The  $F_1$  mean ( $5.21 \pm 0.348$ ) number of primary branches was between the means of ICP 26 ( $3.2 \pm 0.133$ ) and ICPW 94 ( $9.4 \pm 0.163$ ). The  $F_2$  mean ( $4.89 \pm 0.057$ ) was less than the means of ICPW 94 and  $F_1$  but greater than ICP 26.

Variance in  $F_1$  (1.21) was greater than the maximum ICP 26 (0.23) and ICPW 94 (0.27). The variance in  $F_2$  plants (0.82) was greater than the variances in ICP 26 and ICPW 94.

### **Number of secondary branches**

The  $F_1$  mean ( $18.40 \pm 0.636$ ) mean number of secondary branches was greater than the means of ICP 26 ( $4.4 \pm 0.163$ ) and ICPW 94 ( $17.4 \pm 0.163$ ). The  $F_2$  mean ( $13.8 \pm 0.14$ ) was greater than ICP 26 but less than ICPW 94 and  $F_1$  means.

Variance in  $F_1$  (4.044) was greater than the variances in ICP 26 (0.57) and ICPW 94 (0.67). The variance in  $F_2$  plants (0.54) was greater than the variances ICP 26, ICPW 94 and  $F_1$  variance.

### **Seed weight**

The  $F_1$  mean ( $4.44 \pm 0.040$ ) seed weight was less than ICP 26 ( $11.16 \pm 0.002$ ) but greater than ICPW 94 ( $2.95 \pm 0.002$ ). The  $F_2$  mean ( $3.90 \pm 0.059$ ) was less than the means of ICP 26, and  $F_1$  but greater than ICPW 94.

Variance in  $F_1$  (0.016) was greater than the variances in ICP 26 (0.001) and ICPW 94 (0.001). The variance in  $F_2$  plants (0.888) was greater than the variances in ICP 26, ICPW 94 and  $F_1$ .

### **ICP 28 x ICPW 125**

Means and variances of the following characters are presented in Table 55 and 56.

#### **Days to flower**

The  $F_1$  mean ( $71.6 \pm 0.22$ ) number of days to flower was less than the mean of ICPW 125 ( $74.70 \pm 0.15$ ) but greater than ICP 28 ( $66.40 \pm 0.163$ ).  $F_2$  mean ( $70.9 \pm 0.168$ ) was less than the means of ICPW 125, and  $F_1$  means but greater than mean of ICP 28.

**Table – 55: Means values for various characters of ICP 28 x ICPW 125**

Character	ICP 28 mean $\pm$ SE	ICPW 125 mean $\pm$ SE	F <sub>1</sub> mean $\pm$ SE	F <sub>2</sub> mean $\pm$ SE
Days to flowering (No.)	66.40 $\pm$ 0.163	74.70 $\pm$ 0.152	71.6 $\pm$ 0.22	70.9 $\pm$ 0.168
Days to maturity (No.)	97.81 $\pm$ 0.442	112.60 $\pm$ 0.427	103.30 $\pm$ 0.597	99.0 $\pm$ 0.271
Leaf length (cm)	6.36 $\pm$ 0.045	5.34 $\pm$ 0.030	5.27 $\pm$ 0.139	1.4 $\pm$ 0.023
Leaf width (cm)	2.12 $\pm$ 0.013	1.98 $\pm$ 0.033	2.22 $\pm$ 0.036	2.1 $\pm$ 0.035
Pod length (cm)	5.48 $\pm$ 0.039	2.54 $\pm$ 0.015	3.79 $\pm$ 0.038	3.7 $\pm$ 0.037
Pod width (cm)	0.54 $\pm$ 0.016	0.69 $\pm$ 0.010	1.12 $\pm$ 0.033	0.83 $\pm$ 0.031
Pod Bearing length (cm)	4.50 $\pm$ 0.224	14.10 $\pm$ 0.407	12.3 $\pm$ 0.578	1.72 $\pm$ 0.424
No .of Locules per pod	3.80 $\pm$ 0.133	4.70 $\pm$ 0.153	4.40 $\pm$ 0.163	2.44 $\pm$ 0.043
No .of seed per pod	3.80 $\pm$ 0.133	5.40 $\pm$ 0.163	4.40 $\pm$ 0.163	2.47 $\pm$ 0.044
No .of Primary branches	9.20 $\pm$ 0.133	7.70 $\pm$ 0.153	7.30 $\pm$ 0.213	7.0 $\pm$ 0.017
No .of Secondary branches	2.40 $\pm$ 0.267	12.70 $\pm$ 0.153	13..90 $\pm$ 0.433	6.7 $\pm$ 0.068
100 seed weight (g)	11.05 $\pm$ 0.018	2.54 $\pm$ 0.001	5.66 $\pm$ 0.049	5.1 $\pm$ 0.172
Harvest index	18.25 $\pm$ 0.569	5.62 $\pm$ 0.236	5.26 $\pm$ 1.256	4.12 $\pm$ 0.856

**Table - 56: Variance and heretability for various characters of ICP 28 x ICPW 125**

Character	Variance in ICP 28	Variance in ICPW 125	Variance in F <sub>1</sub>	Variance in F <sub>2</sub>	Environmental variance	Genotypic variance	Broad – sense Heritability (%)
Days to flower (No.)	0.27	1.33	2.32	9.76	1.31	8.45	86.61
Days to maturity (No.)	1.96	3.82	1.56	4.79	2.45	2.34	48.92
Leaf length (cm)	0.02	0.02	0.01	0.53	0.02	0.51	96.85
Leaf width (cm)	0.02	0.02	0.03	0.07	0.02	0.05	66.67
Pod length (cm)	0.02	0.03	0.02	0.34	0.02	0.32	93.14
Pod width (cm)	0.03	0.02	0.01	0.11	0.02	0.09	81.82
Pod Bearing length (cm)	2.50	1.60	1.12	5.78	1.74	4.04	69.89
No. of Locules per pod	0.18	0.27	0.62	2.81	0.36	2.45	87.31
No. of seed per pod	0.18	0.27	2.44	4.73	0.96	3.77	79.63
No. of Primary branches	0.18	0.18	0.27	0.38	0.21	0.17	44.74
No. of Secondary branches	0.71	0.27	0.46	4.56	0.48	4.08	89.47
100 seed weight (g)	0.02	0.01	0.03	0.67	0.02	0.65	97.01
Harvest Index	0.25	0.21	0.35	4.26	0.27	3.99	93.66



Variance in ICPW 125 (0.259) was less than the variances in ICP 28 (0.266). Variance in  $F_1$  (0.489) was greater than the variances in ICP 28 and ICPW 125.  $F_2$  variance (5.647) was greater than ICP 28, ICPW 125 and  $F_1$  variances.

### **Days to maturity**

The  $F_1$  mean ( $103.30 \pm 0.597$ ) number of days to maturity was between ICP 28 ( $97.81 \pm 0.442$ ) and ICPW 125 ( $112.60 \pm 0.427$ ).  $F_2$  mean ( $99.0 \pm 0.271$ ) was greater than the mean of ICP 28 but less than ICPW 125 and  $F_1$ .

Variance in ICPW 125 (1.822) was less than the variance in ICP 28 (1.955). Variance in  $F_1$  (3.567) was greater than the variance in ICP 28 and ICPW 125.  $F_2$  variance (14.817) was greater than ICP 28, ICPW 125 and  $F_1$ .

### **Leaflet length**

The  $F_1$  mean ( $5.27 \pm 0.139$ ) leaflet length was less than the means of ICP 28 ( $6.36 \pm 0.045$ ) and ICPW 125 ( $5.34 \pm 0.030$ ).  $F_2$  mean ( $1.40 \pm 0.023$ ) was less than the means of ICP 28, ICPW 125, and  $F_1$ .

Variance in ICPW 125 (0.009) was less than the variances in ICP 28 (0.020). Variance in  $F_1$  (0.124) was greater than the variances in ICP 28 and ICPW 125.  $F_2$  variance (0.156) was greater than ICP 28, ICPW 125, and  $F_1$  variances.

### **Leaflet width**

The  $F_1$  mean ( $2.22 \pm 0.036$ ) leaflet width was greater than the means of ICP 28 ( $2.12 \pm 0.013$ ) and ICPW 125 ( $1.98 \pm 0.033$ ).  $F_2$  mean ( $2.1 \pm 0.035$ ) was greater than ICPW 125 but was equal to the mean of ICP 28 but less than  $F_1$  means.

Variance in ICP 28 (0.002) was less than the variance in ICPW 125 (0.010). Variance in  $F_1$  (0.013) was greater than the variances in ICP 28 and ICPW 125.  $F_2$  variance (0.250) was greater than ICP 28, ICPW 125, and  $F_1$  variances.

### Pod length

The  $F_1$  mean ( $3.79 \pm 0.038$ ) pod length was between ICP 28 ( $5.48 \pm 0.039$ ) and ICPW 125 ( $2.54 \pm 0.015$ ).  $F_2$  mean ( $2.37 \pm 0.037$ ) was less than the means of ICP 28, ICPW 125, and  $F_1$ .

Variance in ICP 28 (0.015) was greater than the variance in ICPW 125 (0.002). Variance in  $F_1$  (0.014) was less than the variance in ICP 28 but greater than ICPW 125.  $F_2$  variance (0.276) was greater than ICP 28, ICPW 125, and  $F_1$  variances.

### Pod width

The  $F_1$  mean ( $1.12 \pm 0.033$ ) pod width was greater than ICP 28 ( $0.54 \pm 0.016$ ) and ICPW 125 ( $0.69 \pm 0.010$ ).  $F_2$  mean ( $0.83 \pm 0.031$ ) was less than  $F_1$  mean, but greater than ICP 28 and ICPW 125.

Variance in ICP 28 (0.002) was greater than the variance in ICPW 125 (0.001). Variance in  $F_1$  (0.011) was greater than the variance in ICP 28 and ICPW 125.  $F_2$  variance (0.195) was greater than ICP 28, ICPW 125, and  $F_1$  variances.

### Pod bearing length

The  $F_1$  mean ( $12.30 \pm 0.578$ ) pod bearing length was in between ICP 28 ( $4.50 \pm 0.224$ ) and ICPW 125 ( $14.10 \pm 0.407$ ).  $F_2$  mean ( $11.72 \pm 0.424$ ) was greater than ICP 28 but less than ICPW 125, and  $F_1$  mean.

Variance in ICP 28 (0.50) was less than ICPW 125 (1.655). Variance in  $F_1$  (3.344) was greater than ICP 28 and ICPW 125.  $F_2$  variance (36.123) was greater than ICP 28, ICPW 125, and  $F_1$  variances.

### **Number of locules per pod**

The  $F_1$  mean ( $4.40 \pm 0.163$ ) number of locules per pod was in between ICP 28 ( $3.8 \pm 0.13$ ) and ICPW 125 ( $4.7 \pm 0.15$ ).  $F_2$  mean ( $2.4 \pm 0.04$ ) was less than ICP 28, ICPW 125, and  $F_1$  mean.

Variance in ICP 28 (0.178) was less than ICPW 125 (0.233). Variance in  $F_1$  (0.267) was greater than ICP 28 and ICPW 125.  $F_2$  variance (0.374) was greater than ICP 28, ICPW 125, and  $F_1$  variances.

### **Number of seeds per pod**

The  $F_1$  mean ( $4.4 \pm 0.163$ ) number of seeds per pod was between ICP 28 ( $3.8 \pm 0.133$ ) and ICPW 125 ( $5.40 \pm 0.163$ ).  $F_2$  mean ( $2.47 \pm 0.044$ ) was less than ICP 28, ICPW 125, and  $F_1$  mean.

Variance in ICP 28 (0.178) was less than ICPW 125 (0.267). Variance in  $F_1$  (0.267) was equal to ICPW 125 but greater than ICP 28.  $F_2$  variance (0.388) was greater than ICP 28, ICPW 125, and  $F_1$  variances.

### **Number of primary branches**

The  $F_1$  mean ( $7.3 \pm 0.21$ ) number of primary branches was less than ICP 28 ( $9.2 \pm 0.13$ ) and ICPW 125 ( $7.7 \pm 0.15$ ).  $F_2$  mean ( $7.0 \pm 0.02$ ) was less than the means of ICP 28, ICPW 125, and  $F_1$ .

Variance in ICP 28 (0.178) was less than the variances in ICPW 125 (0.233). Variance in  $F_1$  (1.456) was greater than the variances in ICP 28 and ICPW 125.  $F_2$  variance (2.059) was greater than ICP 28, ICPW 125, and  $F_1$  variances.

### **Number of secondary branches**

The  $F_1$  mean ( $13.90 \pm 0.433$ ) number of secondary branches was greater than ICP 28 ( $2.4 \pm 0.27$ ) and ICPW 125 ( $12.7 \pm 0.15$ ).  $F_2$  mean ( $6.7 \pm 0.07$ ) was less than ICPW 125 and  $F_1$  mean, but greater than ICP 28.

Variance in ICP 28 (0.711) was greater than the variance in ICPW 125 (0.233). Variance in  $F_1$  (1.878) was greater than ICP 28 and ICPW 125.  $F_2$  variance (3.936) was greater than the variances in ICP 28, ICPW 125, and  $F_1$  variances.

#### **100-seed weight (g)**

The  $F_1$  mean ( $5.66 \pm 0.049$ ) 100- seed weight was less than ICP 28 ( $11.05 \pm 0.018$ ) but greater than ICPW 125 ( $2.54 \pm 0.001$ ).  $F_2$  mean ( $5.1 \pm 0.172$ ) was less than ICP 28, and  $F_1$  but greater than ICPW 125 mean.

Variance in ICP 28 (0.002) was greater than the variance in ICPW 125 (0.000). Variance in  $F_1$  (0.025) was greater than the variance in ICP 28 and ICPW 125.  $F_2$  variance (5.92) was greater than ICP 28, ICPW 125, and  $F_1$  variances

#### **ICP 26 x ICPW 130**

Means of the following characters are presented in Table 57 and variances in Table 58.

#### **Days to flower**

The  $F_1$  mean ( $61.6 \pm 0.65$ ) number of days to flower was in between ICP 26 ( $57.0 \pm 0.26$ ) and ICPW 130 ( $69.8 \pm 0.83$ ).  $F_2$  mean ( $59.7 \pm 0.09$ ) was greater than ICP 26, but less than ICPW 130, and  $F_1$  means respectively.

Variance in ICP 26 (0.667) was less than ICPW 130 (0.844). Variance in  $F_1$  (4.273) was greater than ICP 26 but less than ICPW 94.  $F_2$  variance (23.289) was greater than ICP 26, ICPW 130, and  $F_1$  variances.

**Table - 57: Means values for various characters of ICP 26 x ICPW 130**

<b>Character</b>	<b>ICP 26 mean <math>\pm</math> SE</b>	<b>ICPW 130 mean <math>\pm</math> SE</b>	<b>F<sub>1</sub> mean <math>\pm</math> SE</b>	<b>F<sub>2</sub> mean <math>\pm</math> SE</b>
Days to flowering (No.)	57.00 $\pm$ 0.258	69.80 $\pm$ 0.827	61.6 $\pm$ 0.654	59.7 $\pm$ 0.305
Days to maturity (No.)	103.00 $\pm$ 0.179	123.40 $\pm$ 0.805	108.73 $\pm$ 1.175	106.7 $\pm$ 0.262
Leaf length (cm)	7.00 $\pm$ 0.037	5.51 $\pm$ 0.038	.52 $\pm$ 0.052	4.62 $\pm$ 0.059
Leaf width (cm)	3.60 $\pm$ 0.011	3.18 $\pm$ 0.039	.73 $\pm$ 0.020	1.51 $\pm$ 0.027
Pod length (cm)	5.31 $\pm$ 0.088	2.21 $\pm$ 0.038	.41 $\pm$ 0.060	2.53 $\pm$ 0.224
Pod width (cm)	0.60 $\pm$ 0.015	0.73 $\pm$ 0.015	.77 $\pm$ 0.020	0.76 $\pm$ 0.011
Pod Bearing length (cm)	5.70 $\pm$ 0.335	18.40 $\pm$ 0.859	0.20 $\pm$ 0.426	9.3 $\pm$ 0.05
No .of Locules per pod	4.10 $\pm$ 0.179	5.20 $\pm$ 0.133	.82 $\pm$ 0.128	2.66 $\pm$ 0.042
No .of seed per pod	3.60 $\pm$ 0.163	5.20 $\pm$ 0.133	.64 $\pm$ 0.159	2.48 $\pm$ 0.044
No .of Primary branches	3.30 $\pm$ 0.152	10.21 $\pm$ 0.133	.27 $\pm$ 0.248	8.0 $\pm$ 0.067
No .of Secondary branches	4.40 $\pm$ 0.152	16.30 $\pm$ 0.133	17.3 $\pm$ 0.248	9.44 $\pm$ 0.067
100 seed weight (g)	11.00 $\pm$ 0.002	2.95 $\pm$ 0.002	5.6 $\pm$ 0.044	5.1 $\pm$ 0.059
Harvest index	17.25 $\pm$ 0.254	5.64 $\pm$ 0.259	4.35 $\pm$ 0.225	4.59 $\pm$ 0.546

**Table -58: Variance and heritability for various characters of ICP 26 x ICPW 130**

Character	Variance in ICP 26	Variance in ICPW 130	Variance in F <sub>1</sub>	Variance in F <sub>2</sub>	Environmental variance	Genotypic variance	Broad -sense heritability (%)
Days to flower (No.)	0.667	0.889	4.273	23.289	1.94	21.35	91.66
Days to maturity (No.)	0.322	6.489	13.818	17.233	6.88	10.36	60.09
Leaf length (cm)	0.014	0.416	0.028	0.874	0.15	0.72	82.53
Leaf width (cm)	0.010	0.024	0.044	0.141	0.03	0.12	81.56
Pod length (cm)	0.078	0.016	0.036	0.150	0.04	0.11	71.11
Pod width (cm)	0.020	0.012	0.024	0.033	0.02	0.01	43.43
Pod Bearing length (cm)	1.122	4.622	1.818	64.776	2.52	62.25	96.11
No. of Locules per pod	0.322	0.178	0.164	0.448	0.22	0.23	50.59
No. of seed per pod	0.267	0.178	0.255	0.476	0.23	0.24	50.98
No. of Primary branches	0.233	0.178	0.618	1.112	0.34	0.77	69.15
No. of Secondary branches	0.569	1.178	0.618	11.253	0.79	10.46	92.99
100 seed weight (g)	0.012	0.014	0.016	0.888	0.01	0.87	98.42
Harvest Index	0.025	0.442	0.149	0.856	0.21	0.65	76.01

### **Days to maturity**

The  $F_1$  mean ( $108.7 \pm 1.18$ ) number of days to maturity was greater than ICP 26 ( $103.0 \pm 0.18$ ) but less than ICPW 130 ( $123.4 \pm 0.81$ ).  $F_2$  mean ( $106.7 \pm 0.31$ ) was less than  $F_1$ , and ICPW 130 means but greater than the means of ICP 26.

Variance in ICP 26 (0.322) was less than the variance in ICPW 130 (6.489). Variance in  $F_1$  (13.818) was greater than the variances in ICP 26 and ICPW 94.  $F_2$  variance (17.232) was greater than ICP 26, ICPW 130, and  $F_1$  variances.

### **Leaflet length**

The  $F_1$  mean ( $5.52 \pm 0.052$ ) leaflet length was equal to ICPW 130 ( $5.51 \pm 0.038$ ) but less than ICP 26 ( $7.00 \pm 0.037$ ).  $F_2$  mean ( $4.62 \pm 0.059$ ) was less than ICP 26, ICPW 130, and  $F_1$  means respectively.

Variance in ICP 26 (0.014) was equal to ICPW 130 (0.014). Variance in  $F_1$  (0.028) was greater than ICP 26 and ICPW 130.  $F_2$  variance (0.874) was greater than ICP 26, ICPW 130, and  $F_1$  variances.

### **Leaflet width**

The  $F_1$  mean ( $2.73 \pm 0.020$ ) leaflet width was less than ICP 26 ( $3.60 \pm 0.011$ ) and ICPW 130 ( $3.18 \pm 0.039$ ).  $F_2$  mean ( $1.51 \pm 0.027$ ) was less than ICP 26, ICPW 130, and  $F_1$  means respectively.

Variance in ICP 26 (0.001) was less than the variance in ICPW 130 (0.015). Variance in  $F_1$  (0.004) was less than ICPW 130 but greater than ICP 26.  $F_2$  variance (0.141) was greater than ICP 26, ICPW 130, and  $F_1$  variances.

### **Pod length**

The  $F_1$  mean ( $3.41 \pm 0.060$ ) pod length was in between ICP 26 ( $5.31 \pm 0.088$ ) and ICPW 130 ( $2.21 \pm 0.038$ ).  $F_2$  mean ( $2.53 \pm 0.224$ ) was less than ICP 26, and  $F_1$  but greater than ICPW 130 means respectively.

Variance in ICP 26 (0.078) was greater than ICPW 130 (0.014). Variance in  $F_1$  (0.036) was less than ICP 26 but greater than ICPW 130.  $F_2$  variance (0.149) was greater than ICP 26, ICPW 130, and  $F_1$  variances.

### **Pod width**

The  $F_1$  mean ( $0.77 \pm 0.020$ ) pod width was greater than ICP 26 ( $0.60 \pm 0.015$ ) and ICPW 130 ( $0.73 \pm 0.015$ ).  $F_2$  mean ( $0.76 \pm 0.011$ ) was less than  $F_1$  mean but greater than ICP 26 and ICPW 130 respectively.

Variance in ICP 26 (0.002) was equal to ICPW 130 (0.002). Variance in  $F_1$  (0.004) was greater than ICP 26 and ICPW 130.  $F_2$  variance (0.033) was greater than ICP 26, ICPW 130, and  $F_1$  variances.

### **Pod bearing length**

The  $F_1$  mean ( $10.20 \pm 0.426$ ) pod bearing length was greater than mean of ICP 26 ( $5.70 \pm 0.335$ ) but less than ICPW 130 ( $18.40 \pm 0.859$ ) mean.  $F_2$  mean ( $19.31 \pm 0.051$ ) was greater than ICP 26 ICPW 130 and  $F_1$  means respectively.

Variance in ICP 26 (1.122) was less than the variance in ICPW 130 (7.378). Variance in  $F_1$  (0.164) was less than ICP 26 and ICPW 130.  $F_2$  variance (64.776) was greater than the variances in ICP 26, ICPW 130, and  $F_1$  variances.



### **Number of locules per pod**

The  $F_1$  mean ( $3.8 \pm 0.13$ ) number of locules per pod was in between ICP 26 ( $4.1 \pm 0.18$ ) and ICPW 130 ( $5.2 \pm 0.13$ ).  $F_2$  mean ( $2.7 \pm 0.04$ ) was less than ICP 26, ICPW 130, and  $F_1$  means respectively.

Variance in ICP 26 (0.322) was greater than the variances in ICPW 130 (0.178). Variance in  $F_1$  (0.164) was less than the variances in ICP 26 and ICPW 130.  $F_2$  variance (0.448) was greater than ICP 26, ICPW 130, and  $F_1$  variances.

### **Number of seeds per pod**

The  $F_1$  mean ( $3.6 \pm 0.16$ ) number of seeds per pod was equal to ICP 26 ( $3.6 \pm 0.16$ ) but less than ICPW 130 ( $5.2 \pm 0.13$ ).  $F_2$  mean ( $2.5 \pm 0.04$ ) was less than ICP 26, ICPW 130, and  $F_1$  means respectively.

Variance in ICP 26 (0.267) was greater than the variances in ICPW 130 (0.178). Variance in  $F_1$  (0.255) was less than ICP 26 but greater than the variances in ICPW 130.  $F_2$  variance (0.476) was greater than ICP 26, ICPW 130, and  $F_1$  variances.

### **Number of primary branches**

The  $F_1$  mean ( $8.3 \pm 0.25$ ) number of primary branches was greater than ICP 26 ( $3.3 \pm 0.15$ ) but less than ICPW 130 ( $10.2 \pm 0.13$ ).  $F_2$  mean ( $8.0 \pm 0.07$ ) was less than ICPW 130, and  $F_1$  means but greater than ICP 26 mean respectively.

Variance in ICP 26 (0.233) was greater than the variances in ICPW 130 (0.178). Variance in  $F_1$  (0.618) was greater than the variances in ICP 26 and ICPW 130.  $F_2$  variance (1.112) was greater than ICP 26, ICPW 130, and  $F_1$  variances.

### **Number of secondary branches**

The  $F_1$  mean ( $17.3 \pm 0.25$ ) number of secondary branches was greater than ICP 26 ( $4.4 \pm 0.15$ ) and ICPW 130 ( $16.3 \pm 0.13$ ).  $F_2$  mean ( $3.8 \pm 0.07$ ) was less than ICPW 130, ICP 26, and  $F_1$  means respectively.

Variance in ICP 26 (0.233) was less than the variance in ICPW 130 (1.178). Variance in  $F_1$  (2.618) was greater than the variances in ICP 26 and ICPW 130.  $F_2$  variance (3.112) was greater than ICP 26, ICPW 130, and  $F_1$  variances.

### **100 – Seed weight**

The  $F_1$  mean ( $5.6 \pm 0.04$ ) 100-seed weight was less than ICP 26 ( $11.00 \pm 0.002$ ) but greater than ICPW 130 ( $2.95 \pm 0.002$ ).  $F_2$  mean ( $5.1 \pm 0.06$ ) was less than ICP 26 and  $F_1$  but greater than ICPW 130 means, respectively.

Variance in ICP 26 (0.002) was greater than ICPW 130 (0.001). Variance in  $F_1$  (0.007) was greater than ICP 26 and ICPW 130.  $F_2$  variance (0.885) was greater than ICP 26, ICPW 130, and  $F_1$  variances.

### ***ICP 28 x ICPW 141***

Means of the following characters are presented in Table 59 and variances in Table 60.

### **Days to flower**

The  $F_1$  mean ( $60.1 \pm 0.48$ ) number of days to flower was less than ICP 28 ( $66.4 \pm 0.16$ ) and ICPW 141 ( $69.0 \pm 0.37$ ).  $F_2$  mean ( $59.1 \pm 0.20$ ) was less than  $F_1$  mean but less than ICP 28 and ICPW 141 means respectively.

Variance in ICP 28 (0.267) was less than the variances in ICPW 141 (1.33). Variance in  $F_1$  (2.322) was greater than the variance in ICP 28 and ICPW 141.  $F_2$  variance (9.764) was greater than ICP 28, ICPW 141, and  $F_1$  variances.

**Table - 59: Means values for various characters of ICP 28 x ICPW 141**

Character	ICP 28 mean $\pm$ SE	ICPW 141 mean $\pm$ SE	F <sub>1</sub> mean $\pm$ SE	F <sub>2</sub> mean $\pm$ SE
Days to flowering (No.)	66.40 $\pm$ 0.163	69.00 $\pm$ 0.365	60.10 $\pm$ 0.482	59.12 $\pm$ 0.198
Days to maturity (No.)	97.80 $\pm$ 0.442	104.60 $\pm$ 0.618	96.50 $\pm$ 0.394	95.1 $\pm$ 0.139
Leaf length (cm)	6.36 $\pm$ 0.045	4.72 $\pm$ 0.049	5.22 $\pm$ 0.029	3.71 $\pm$ 0.046
Leaf width (cm)	2.12 $\pm$ 0.013	1.70 $\pm$ 0.042	3.06 $\pm$ 0.052	1.32 $\pm$ 0.017
Pod length (cm)	5.48 $\pm$ 0.039	2.18 $\pm$ 0.025	3.93 $\pm$ 0.047	2.90 $\pm$ 0.037
Pod width (cm)	0.54 $\pm$ 0.016	0.68 $\pm$ 0.025	0.77 $\pm$ 0.021	0.72 $\pm$ 0.006
Pod Bearing length (cm)	5.90 $\pm$ 0.233	17.40 $\pm$ 0.400	14.3 $\pm$ 0.335	13.1 $\pm$ 0.152
No. of primary branches	5.20 $\pm$ 0.133	4.40 $\pm$ 0.163	6.3 $\pm$ 0.249	3.0 $\pm$ 0.106
No. of secondary branches	6.40 $\pm$ 0.267	34.40 $\pm$ 0.163	20.3 $\pm$ 0.494	6.81 $\pm$ 0.138
No. of locules / pod	3.80 $\pm$ 0.133	5.20 $\pm$ 0.133	4.40 $\pm$ 0.163	2.82 $\pm$ 0.039
No. of pods / pod	3.90 $\pm$ 0.133	4.90 $\pm$ 0.163	4.40 $\pm$ 0.213	2.37 $\pm$ 0.035
100 seed weight (g)	11.05 $\pm$ 0.014	2.07 $\pm$ 0.005	4.54 $\pm$ 0.027	3.92 $\pm$ 0.052
Harvest index	18.21 $\pm$ 0.568	5.48 $\pm$ 0.253	5.63 $\pm$ 0.536	5.14 $\pm$ 0.569

**Table 60: Variance and heretability for various characters of ICP 28 x ICPW 141**

Character	Variance in ICP 28	Variance in ICPW 141	Variance in F <sub>1</sub>	Variance in F <sub>2</sub>	Environmental variance	Genotypic variance	Broad- sense Heritability (%)
Days to flower (No.)	0.27	0.18	0.49	5.65	0.31	5.34	94.49
Days to maturity (No.)	1.96	3.55	3.57	14.82	3.03	11.79	79.58
Leaf length (cm)	0.02	0.02	0.09	0.11	0.04	0.07	60.30
Leaf width (cm)	0.02	0.01	0.01	0.25	0.02	0.23	93.76
Pod length (cm)	0.02	0.07	0.01	0.28	0.03	0.24	87.68
Pod width (cm)	0.03	0.02	0.01	0.19	0.02	0.17	88.89
Pod Bearing length (cm)	2.50	1.67	3.34	36.12	2.50	33.62	93.07
No .of Locules per pod	0.18	0.18	0.27	0.37	0.21	0.17	44.47
No .of seed per pod	0.18	0.27	0.27	0.39	0.24	0.15	38.30
No .of Primary branches	0.18	0.27	0.46	0.92	0.30	0.62	67.35
No .of Secondary branches	0.71	1.27	0.89	3.84	0.96	2.88	75.06
100 seed weight (g)	0.02	0.04	0.02	5.92	0.03	5.89	99.49
Harvest Index	0.25	0.12	0.36	5.68	0.24	5.44	95.69

### **Days to maturity**

The  $F_1$  mean ( $96.5 \pm 0.39$ ) number of days to maturity was less than ICP 28 ( $97.8 \pm 0.44$ ) and ICPW 141 ( $104.6 \pm 0.62$ ).  $F_2$  mean ( $95.1 \pm 0.14$ ) was less than ICP 28, ICPW 141, and  $F_1$  means respectively.

Variance in ICP 28 (1.956) was less than the variances in ICPW 141 (3.822). Variance in  $F_1$  (1.556) was less than ICP 28 and ICPW 141.  $F_2$  variance (4.797) was greater than ICP 28, ICPW 141 and  $F_1$  variances.

### **Leaflet length**

The  $F_1$  mean ( $5.22 \pm 0.029$ ) leaflet length was in between ICP 28 ( $6.36 \pm 0.045$ ) and ICPW 141 ( $4.72 \pm 0.049$ ).  $F_2$  mean ( $3.71 \pm 0.046$ ) was less than ICP 28, ICPW 141, and  $F_1$  means respectively.

Variance in ICP 28 (0.020) was less than the variances in ICPW 141 (0.024). Variance in  $F_1$  (0.008) was less than ICP 28 and ICPW 141.  $F_2$  variance (0.533) was greater than ICP 28, ICPW 141 and  $F_1$  variances.

### **Leaflet width**

The  $F_1$  mean ( $3.06 \pm 0.052$ ) leaflet width was greater than ICP 28 ( $2.12 \pm 0.013$ ) and ICPW 141 ( $1.70 \pm 0.042$ ).  $F_2$  mean ( $1.33 \pm 0.017$ ) was less than ICP 28, ICPW 141, and  $F_1$  means respectively.

Variance in ICP 28 (0.002) was less than ICPW 141 (0.018). Variance in  $F_1$  (0.027) was greater than ICP 28 and ICPW 141.  $F_2$  variance (0.072) was greater than ICP 28, ICPW 141, and  $F_1$  variances.

### **Pod length**

The  $F_1$  mean ( $3.93 \pm 0.047$ ) pod length was in between the means of ICP 28 ( $5.48 \pm 0.039$ ) and ICPW 141 ( $2.18 \pm 0.025$ ).  $F_2$  mean ( $2.90 \pm 0.037$ ) was less than ICP 28 and  $F_1$ , but greater than ICPW 141 means respectively.

Variance in ICP 28 (0.015) was greater than ICPW 141 (0.006). Variance in  $F_1$  (0.022) was greater than ICP 28 and ICPW 141.  $F_2$  variance (0.337) was greater than ICP 28, ICPW 141, and  $F_1$  variances.

### **Pod width**

The  $F_1$  mean ( $0.77 \pm 0.021$ ) was greater than ICP 28 ( $0.54 \pm 0.016$ ) and ICPW 141 ( $0.68 \pm 0.025$ ).  $F_2$  mean ( $0.72 \pm 0.006$ ) was less than  $F_1$ , but greater than ICPW 141 and ICP 28 means respectively.

Variance in ICP 28 (0.002) was less than ICPW 141 (0.006). Variance in  $F_1$  (0.004) was greater than ICP 28 but less than ICPW 141.  $F_2$  variance (0.012) was greater than ICP 28, ICPW 141 and  $F_1$  variances.

### **Pod bearing length**

The  $F_1$  mean ( $14.3 \pm 0.34$ ) pod bearing length was greater than means of ICP 28 ( $5.9 \pm 0.23$ ) but less than ICPW 141 ( $17.4 \pm 0.40$ ).  $F_2$  mean ( $13.1 \pm 0.15$ ) was greater than ICP 28 but less than the mean of ICPW 141 and  $F_1$  respectively.

Variance in ICP 28 (2.50) was greater than the variances in ICPW 141 (1.60). Variance in  $F_1$  (1.12) was less than the variance in ICP 28 and ICPW 141.  $F_2$  variance (5.78) was greater than ICP 28, ICPW 141, and  $F_1$  variances.

### **Number of locules per pod**

The  $F_1$  mean ( $4.4 \pm 0.16$ ) number of locules per pod was greater than ICP 28 ( $3.8 \pm 0.13$ ) but less than ICPW 141 ( $5.2 \pm 0.13$ ).  $F_2$  mean ( $2.8 \pm 0.04$ ) was less than the mean of ICP 28, ICPW 141 and  $F_1$  respectively.

Variance in ICP 28 (0.18) was less than the variances in ICPW 141 (0.27). Variance in  $F_1$  (0.62) was greater than ICP 28 and ICPW 141.  $F_2$  variance (2.81) was greater than ICP 28, ICPW 141, and  $F_1$  variances.

### **Number of seeds per pod**

The  $F_1$  mean ( $4.4 \pm 0.21$ ) number of seeds per pod was greater than ICP 28 ( $3.9 \pm 0.13$ ) but less than ICPW 141 ( $4.9 \pm 0.16$ ).  $F_2$  mean ( $2.4 \pm 0.04$ ) was less than the mean of ICP 28, ICPW 141 and  $F_1$  respectively.

Variance in ICP 28 (0.18) was less than ICPW 141 (0.27). Variance in  $F_1$  (2.44) was greater than ICP 28 and ICPW 141.  $F_2$  variance (4.73) was greater than ICP 28, ICPW 141 and  $F_1$  variances.

### **Number of primary branches**

The  $F_1$  mean ( $6.3 \pm 0.25$ ) number of primary branches was greater than the means of ICP 28 ( $5.2 \pm 0.13$ ) and ICPW 141 ( $4.4 \pm 0.16$ ).  $F_2$  mean ( $9.3 \pm 0.11$ ) was greater than mean of ICP 28, ICPW 141 and  $F_1$  respectively.

Variance in ICP 28 (0.18) was equal to variance in ICPW 141 (0.18). Variance in  $F_1$  (0.27) was greater than ICP 28 and ICPW 141 variances.  $F_2$  variance (0.38) was greater than ICP 28, ICPW 141, and  $F_1$  variances.

### **Number of secondary branches**

The  $F_1$  mean ( $20.3 \pm 0.49$ ) number of secondary branches was greater than the mean of ICP 28 ( $6.4 \pm 0.27$ ) but less than mean of ICPW 141 ( $34.4 \pm 0.16$ ).  $F_2$

mean ( $6.8 \pm 0.14$ ) was greater than mean of ICP 28 but less than ICPW 141 and  $F_1$  means.

Variance in ICP 28 (0.71) was greater than ICPW 141 (0.27). Variance in  $F_1$  (0.46) was greater than ICP 28 and ICPW 141.  $F_2$  variance (4.56) was greater than ICP 28, ICPW 141 and  $F_1$  variances.

### 100 – Seed weight

The  $F_1$  mean ( $4.54 \pm 0.027$ ) 100 – seed weight was greater than the mean of ICPW 141 ( $2.07 \pm 0.005$ ) but less than mean of ICP 28 ( $11.05 \pm 0.014$ ).  $F_2$  mean ( $3.92 \pm 0.052$ ) was greater than mean of ICPW 141 but less than ICP 28 and  $F_1$  means.

Variance in ICP 28 (0.02) was greater than variance in ICPW 141 (0.01). Variance in  $F_1$  (0.03) was greater than ICP 28 and ICPW 141.  $F_2$  variance (0.67) was greater than ICP 28, ICPW 141 and  $F_1$  variances.

### Harvest Index

The  $F_1$  mean ( $5.63 \pm 0.536$ ) was greater than the mean of ICPW 141 ( $5.48 \pm 0.253$ ) but less than the mean of ICP 28 ( $18.21 \pm 0.568$ ).  $F_2$  mean ( $5.14 \pm 0.569$ ) was less than the mean of ICP 28, ICPW 141 and  $F_1$ .

Variance in ICP 28 (0.25) was greater than ICPW 141 (0.21). Variance in  $F_1$  (0.35) was greater than ICP 28 and ICPW 141.  $F_2$  variance (4.26) was greater than ICP 28, ICPW 141 and  $F_1$  variances.

### Heritability

Heritability was calculated for different characters on the population obtained from seven crosses involving four *C. scrabaeoides* parents (ICPW 94, ICPW 125, ICPW 130 and ICPW 141) and two Pigeonpea varieties (ICP 26 and ICP 28).



Heritability values for all characters are presented in Tables 44, 48, 51, 54, 56, 58 and 60

For days to flower variance in  $F_2$  population was greater than the variances in both the parents and their  $F_1$ s in all the four crosses involving ICP 28 parent and three crosses involving the ICP 26 parent. Broad sense heritability for days to flower ranged from 79.32 % in ICP 28 x ICPW 94 to 96.05 % in ICP 28 x ICPW 130 and from 88.26 % in ICP 26 x ICPW 125 to 91.66 % in ICP 26 x ICPW 130.

For days to maturity, the variance in  $F_2$  population was greater than the variances in both the parents and their  $F_1$ s for all the four crosses involving ICP 28 parent and three crosses involving ICP 26 parent. Broad sense heritability, for days to maturity, ranged, from 48.42 % in ICP 28 x ICPW 125 to 79.58 % in ICP 28 x ICPW 141 and from 60.09 % in ICP 26 x ICPW 130 to 90.81 % in ICP 26 x ICPW 125.

For leaflet length, the variance in  $F_2$  population was greater than the variances in both the parents and their  $F_1$ s for all the four crosses involving ICP 28 and three crosses involving the ICP 26. Broad sense heritability for leaflet length ranged from 60.30 % in ICP 28 x ICPW 141 to 96.85 % in ICP 28 x ICPW 125 and from 80.65 % in ICP 26 x ICPW 125 to 92.11 % in ICP 26 x ICPW 94 .

For leaflet width, the variance in  $F_2$  population was greater than the variances in both the parents and their  $F_1$ s for all the four crosses involving ICP 28 parent and three crosses involving the ICP 26. Broad sense heritability ranged from 55.56 % in ICP 28 x ICPW 130 to 93.76 % in ICP 28 x ICPW 141 and from 81.56 % in ICP 26 x ICPW 130 to 98.18 % in ICP 26 x ICPW 94.

For pod length, the variance in  $F_2$  population was greater than the variances in both the parents and their  $F_1$ s for all the four crosses involving ICP 28 parent and three crosses involving the ICP 26 parent. Broad sense - heritability ranged from 55.00 % in ICP 28 x ICPW 130 to 93.14 % in ICP 28 x ICPW 141 and from 70.25 % in ICP 26 x ICPW 125 to 79.33 % in ICP 26 x ICPW 94.

For pod width, the variance in  $F_2$  population was greater than the variances in both the parents and their  $F_1$ s for all the four crosses involving ICP 28 parent and three crosses involving the ICP 26 parent. Broad sense heritability for pod width ranged from 68.93 % in ICP 28 x ICPW 94 to 88.89 % in ICP 28 x ICPW 141 and from 43.43 % in ICP 26 x ICPW 130 to 89.82 % in ICP 26 x ICPW 125.

For pod bearing length, the variance in  $F_2$  population was greater than the variances in both the parents and their  $F_1$ s for all the four crosses involving ICP 28 parent and three crosses involving the ICP 26 parent. Broad sense heritability for pod bearing length ranged from 69.89 % in ICP 28 x ICPW 125 to 93.09 % in ICP 28 x ICPW 141 and from 73.63 % in ICP 26 x ICPW 125 to 97.03 % in ICP 26 x ICPW 94.

For number of locules per pod, the variance in  $F_2$  population was greater than the variances in both the parents and their  $F_1$ s for all the four crosses involving ICP 28 parent and three crosses involving the ICP 26 parent. Broad sense heritability ranged from 44.47 % in ICP 28 x ICPW 141 to 87.31 % in ICP 28 x ICPW 125 and from 38.81 % in ICP 26 x ICPW 125 to 50.59 % in ICP 26 x ICPW 130.

For number of seeds per pod, the variance in  $F_2$  population was greater than the variances in both the parents and their  $F_1$ s for all the four crosses involving ICP 28 parent and three crosses involving the ICP 26 parent. Broad sense heritability ranged from 38.30 % in ICP 28 x ICPW 141 to 79.63 % in ICP 28 x ICPW 125 and from 27.06 % in ICP 26 x ICPW 94 to 50.98 % in ICP 26 x ICPW 130.

For number of primary branches, the variance in  $F_2$  population was greater than the variances in both the parents and their  $F_1$ s for all the four crosses involving ICP 28 parent and three crosses involving the ICP 26 parent. Broad sense heritability ranged from 63.75 % in ICP 28 x ICPW 141 to 82.62 % in ICP 28 x ICPW 130 and from 30.45 % in ICP 26 x ICPW 94 to 74.11 % in ICP 26 x ICPW 125.

For number of secondary branches, the variance in  $F_2$  population was greater than the variances in both the parents and their  $F_1$ s for all the four crosses involving

ICP 28 parent and three crosses involving the ICP 26 parent. Broad sense - heritability ranged from 75.06 % in ICP 28 x ICPW 141 to 89.47 % in ICP 28 x ICPW 125 and from 64.20 % in ICP 26 x ICPW 94 to 92.99 % in ICP 26 x ICPW 130 .

For 100- seed weight, the variance in  $F_2$  population was greater than the variances in both the parents and their  $F_1$ s for all the four crosses involving ICP 28 parent and three crosses involving the ICP 26 parent. Broad sense heritability ranged from 66.55% in ICP 28 x ICPW 125 to 77.04% in ICP 28 x ICPW 130 and from 54.79% in ICP 26 x ICPW 125 to 88.76% in ICP 26 x ICPW 94.

For harvest index, the variance in  $F_2$  population was greater than the variances in both the parents and respective  $F_1$ 's for all the four crosses involving ICP 28 parent and three crosses involving the ICP 26 parent. Broad sense heritability for harvest weight ranged from 63.14% in ICP 28 x ICPW 141 to 76.56% in ICP 28 x ICPW 94 and from 68.65% in ICP 26 x ICPW 94 to 73.92% in ICP 26 x ICPW 125.

## **Inheritance of qualitative characters**

The inheritance pattern of the qualitative characters (Plant growth habit, leaflet shape, seed strophiole, seed mottleness, pod hairiness) has been determined based on the results obtained from the seven interspecific crosses (ICP 28 X ICPW 94, ICP 28 X ICPW 125, ICP 28 X ICPW 130, ICP 28 X ICPW 141, ICP 26 X ICPW 94, ICP 28 X ICPW 125 and ICP 28 X ICPW 141).

### ***Plant growth habit***

The seven different  $F_1$  hybrids obtained from the seven different crosses involving different accessions of *C. scarabaeoides* and *C. cajan* varieties, exhibited semi- spreading plant habit, an intermediate type between the erect plant habit of *C. cajan* and spreading habit of *C. scarabaeoides*. The  $F_2$  population of all crosses involving ICP 28 and ICP 26, the cultivated parents, with the wild *C. scarabaeoides* accessions, a good fit was observed for the 1: 2: 1 segregation ( $\chi^2 = 7.66$ ;  $P = 0.01$ -

0.005) to erect: intermediate: spreading (Fig. 28) respectively, suggests that a single gene governs plant habit and is partially dominant/codominant nature (Table 61)

### ***Stem color***

The stems of F<sub>1</sub> hybrids, in all the seven crosses, had a mixed stem color, between the green color of wild accessions and the purple of *C. cajan*. The F<sub>2</sub> ratio in these crosses showed a good fit for 1:2:1, to green : mixed : purple ( $\chi^2 = 0.846$ ;  $P = 0.25 - 0.45$ ) suggests the incomplete dominance of the gene controlling the stem color (Table 62).

### ***Leaflet shape***

The F<sub>1</sub> hybrids of all the seven crosses had an intermediate leaflet shape, between the obovate leaflet of the *C. scarabaeoides* and lanceolate leaflet of *C. cajan*. F<sub>2</sub> ratio in these crosses showed a good fit for 1:2:1 to obovate: intermediate: lanceolate ( $\chi^2 = 0.650$ ;  $P = 0.10-0.25$ ) respectively, suggests the partial / co-dominance nature of a single gene (Table 63).

### ***Seed Mottleness***

The F<sub>1</sub> mottled seed of all crosses indicated that the mottleness of *C. scarabaeoides* is dominant over the nonmottled nature of *C. cajan*. In all the seven crosses, the F<sub>2</sub> data contributed a good fit of 9:7 ratio ( $\chi^2 = 0.077$ ;  $P = 0.75-0.90$ ) indicating the involvement of two complementary genes in the expression of the mottleness (Table 64).

### ***Seed strophiole***

Seed strophiole is present in *C. scarabaeoides* accessions and absent in the *C. cajan* varieties. Strophioles were present in all the F<sub>1</sub> hybrid seeds. The F<sub>2</sub> population gave a good fit for 13 (strophioled): 3 (non-strophioled) ( $\chi^2 = 1.859$ ;  $P = 0.10-0.25$ ) indicating the inhibitory gene action governing the expression of this character (Table 65).

**Table 61: Segregation of the F<sub>2</sub> plants for plant habit**

Cross	<i>C.cajan</i>	Wild	F <sub>1</sub>	Total No. of plants in F <sub>2</sub>	Segregation of F <sub>2</sub> plants			$\chi^2$ value (1: 2: 1)	P value
					E	I	S		
ICP 28 x ICPW 94	E	S	I	469	120	240	109	0.79	0.25 - 0.50
ICP 28 x ICPW 125	E	S	I	251	61	136	54	2.11	0.10 - 0.25
ICP 28 x ICPW 130	E	S	I	246	55	130	61	1.08	0.25 - 0.50
ICP 28 x ICPW 140	E	S	I	250	62	125	63	0.01	0.95 - 0.97
ICP 26 x ICPW 94	E	S	I	241	50	130	61	2.19	0.10 - 0.25
ICP 26 x ICPW 125	E	S	I	252	58	135	59	1.04	0.25 - 0.50
ICP 26 x ICPW 130	E	S	I	261	49	152	60	5.33	0.025 - 0.05
Total				1970	454	1050	466	7.66	0.010 - 0.005

E = Erect; S = Spreading; I = Intermediate;  $\chi^2$  heterogeneity = 4.89; 1 P value = 0.025-0.050

**Table 62 : Segregation of the F<sub>2</sub> plants for stem color**

Cross	<i>C.cajan</i>	Wild	F <sub>1</sub>	Total No. of F <sub>2</sub> plants	Segregation in the F <sub>2</sub>			$\chi^2$ value (9:7)	P value
					P	M	G		
ICP 28 X ICPW 94	P	G	M	469	115	239	115	0.223	0.50-0.75
ICP 28 X ICPW 125	P	G	M	251	62	130	59	0.505	0.50-0.75
ICP 28 X ICPW 130	P	G	M	246	57	130	60	0.763	0.25-0.50
ICP 28 X ICPW 141	P	G	M	250	66	119	65	0.917	0.25-0.50
ICP 26 X ICPW 94	P	G	M	241	56	119	66	0.866	0.25-0.50
ICP 26 X ICPW 125	P	G	M	252	60	129	63	0.214	0.50-0.75
ICP 26 X ICPW 130	P	G	M	261	60	136	67	0.448	0.50-0.75
Total				1970	476	1002	492	0.846	0.25-0.45

P = Purple; G = Green; M = Mixed;  $\chi^2$  heterogeneity = 3.090 P value = 0.05-0.10

**Table 63: Segregation of the F<sub>2</sub> plants for Leaflet shape**

Cross	<i>C.cajan</i>	Wild	F <sub>1</sub>	Total No. of F <sub>2</sub> plants	Segregation in F <sub>2</sub> Population			$\chi^2$ value (1: 2: 1)	P value
					L	I	O		
ICP 28 X ICPW 94	L	O	I	469	115	237	117	0.074	0.75-0.90
ICP 28 X ICPW 125	L	O	I	251	61	132	58	0.771	0.10-0.25
ICP 28 X ICPW 130	L	O	I	246	53	131	62	0.886	0.25-0.50
ICP 28 X ICPW 141	L	O	I	250	61	127	62	0.136	0.50-0.75
ICP 26 X ICPW 94	L	O	I	241	65	120	56	0.508	0.25-0.50
ICP 26 X ICPW 125	L	O	I	252	59	130	63	0.381	0.50-0.75
ICP 26 X ICPW 130	L	O	I	261	60	136	65	0.649	0.005-0.010
Total				1970	476	1013	481	0.650	0.10-0.25

L = lanceolate; O = Obovate; I = Intermediate;  $\chi^2$  heterogeneity = 2.755; P value = 0.05- 0.10

**Table 64: Segregation of the F<sub>2</sub> plants for mottleness of seed**

Cross	<i>C.cajan</i>	Wild	F <sub>1</sub>	Total No. of F <sub>2</sub> plants	Segregation in the F <sub>2</sub>		$\chi^2$ value (9:7)	P value
					M	UM		
ICP 28 X ICPW 94	UM	M	M	469	260	209	0.125	0.50- 0.75
ICP 28 X ICPW 125	UM	M	M	251	138	113	0.115	0.50- 0.75
ICP 28 X ICPW 130	UM	M	M	246	142	104	0.216	0.50- 0.75
ICP 28 X ICPW 141	UM	M	M	250	140	110	0.006	0.75 - 0.90
ICP 26 X ICPW 94	UM	M	M	241	133	108	0.078	0.75 - 0.90
ICP 26 X ICPW 125	UM	M	M	252	136	116	0.004	1.000
ICP 26 X ICPW 130	UM	M	M	261	149	112	0.122	0.50-0.75
Total				1970	1102	868	0.077	0.75-0.90

UM = unmottled; M = mottled;  $\chi^2$  heterogeneity = 0.589; P value = 0.05- 0.10

**Table 65: Segregation of the F<sub>2</sub> plants for strophiole on seed**

Cross	<i>C.cajan</i>	Wild	F <sub>1</sub>	Total No. of plants in F <sub>2</sub>	Segregation in F <sub>2</sub>		$\chi^2$ value (13:3)	P value
					Present	Absent		
ICP 28 X ICPW 94	A	P	P	469	378	91	0.131	0.50-0.75
ICP 28 X ICPW 125	A	P	P	251	200	51	0.405	0.25-0.50
ICP 28 X ICPW 130	A	P	P	246	200	46	0.000	1.00
ICP 28 X ICPW 141	A	P	P	250	199	51	0.377	0.50-0.75
ICP 26 X ICPW 94	A	P	P	241	192	49	0.373	0.50-0.75
ICP 26 X ICPW 125	A	P	P	252	200	52	0.612	0.25-0.50
ICP 26 X ICPW 130	A	P	P	261	208	53	0.407	0.50-0.70
Total				1970	1577	393	1.859	0.10-0.25

A = Absent; P = Present;  $\chi^2$  heterogeneity = 0.446

**Table 66: Segregation of the F<sub>2</sub> plants for pod hairiness**

Cross	<i>C.cajan</i>	Wild	F <sub>1</sub>	Total No. of F <sub>2</sub> plants	Segregation in F <sub>2</sub> plants		$\chi^2$ value (3:1)	P value
					P	G		
ICP 28 x ICPW 94	G	P	P	469	348	121	0.143	0.50-0.75
ICP 28 x ICPW 125	G	P	P	251	182	69	0.579	0.25-0.50
ICP 28 x ICPW 130	G	P	P	246	180	66	0.438	0.50-0.75
ICP 28 x ICPW 141	G	P	P	250	181	69	0.934	0.25-0.50
ICP 26 x ICPW 94	G	P	P	241	174	67	1.008	0.25-0.50
ICP 26 x ICPW 125	G	P	P	252	181	71	1.354	0.10-0.25
ICP 26 x ICPW 130	G	P	P	261	190	71	0.674	0.25-0.50
Total				1970	1436	534	1.191	0.25-0.50

P = Pubescent; G = Glabrous;  $\chi^2$  heterogeneity = 3.852 P value = 0.050-0.100

## **Pod hairiness**

*C. scarabaeoides* accessions had hairy pods compared to the pods of *C. cajan* varieties (Fig. 31). In all the  $F_1$  hybrids the hairiness of pods was dominant over the non-hairy nature of the Pigeonpea pods. The  $F_2$  data, on all the seven crosses, showed a good fit of 3 (hairy): 1 (non-hairy) ( $\chi^2 = 1.192$ ;  $P = 0.25-0.50$ ), indicating that a single dominant gene controls the pod hairiness (Table 66).

## **Inheritance of trichome A, B, C and D**

Inheritance pattern of density and types of trichomes was studied in two crosses ICP 28 x ICPW 94 and ICP 26 x ICPW 125. Density on pods of *C. scarabaeoides*,  $F_1$  and  $F_2$  pods are shown in Figure 31.

Density of trichome A (glandular hair) (Fig. 21) on pigeonpea (ICP 28 and ICP 26) pods was higher than the density on *C. scarabaeoides* (ICPW 94 and ICPW 125) pods. Density of trichome A on  $F_1$  pods was similar to the *C. scarabaeoides* pods in both the crosses. In  $F_2$  generation, the mean density of trichome A was greater than the mean of *C. scarabaeoides* accessions, ICPW 94 and / or ICPW 125, but less than the *C. cajan* genotypes ICP 26 and / or ICP 28. In the backcross progeny ( $BC_1F_1$ ), the mean density of trichome A was in between the densities of *C. scarabaeoides* parent and the pigeonpea parent, but greater than the  $F_1$  and  $F_2$  means in both crosses (ICP 28 x (ICP 28 x ICPW 94) and (ICP 26 x (ICP 26 x ICPW 125)). (Table 67)

The  $F_1$  pods in both the crosses ICP 28 x ICPW 94 and ICP 26 x ICPW 125 resembled the *C. scarabaeoides* pods indicating the dominance of the features of wild parent. Further, the 250  $F_2$  plants screened in each cross, segregated to give a good fit of 3 (low density): 1 (high density) in cross ICP 28 x ICPW 94 ( $\chi^2 = 0.033$ ;  $P = 0.75-0.90$ ) (Table 68) and in cross ICP 26 x ICPW 125 ( $\chi^2 = 0.432$ ;  $P = 0.50-0.70$ ), indicating that the low density of trichome A is controlled by of a single dominant gene (Table 69). Further, the 75 plants screened in the backcross



**Fig. 31. Electronmicrographs of pod wall surface**

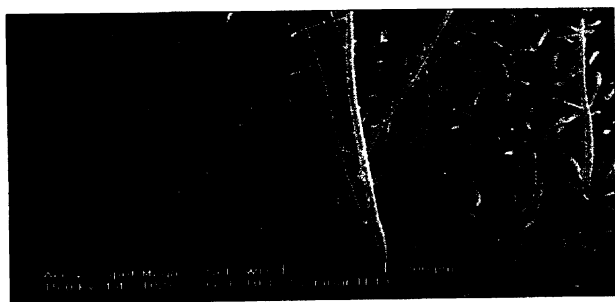
- a)*        *C. scarabaeoides* (ICPW 94)
- b)*        F<sub>1</sub> hybrid (ICP 28 x ICPW 94)
- c)*        F<sub>2</sub> segregant (ICP 28 x ICPW 94)



**a**



**b**



**c**

**Fig: 31**

generation gave a good fit of 1 (low density): 1 (high density) in cross ICP 28 x ICPW 94 ( $\chi^2 = 0.654$  P = 0.25-0.50) and in cross ICP26 x ICPW125 ( $\chi^2 = 0.334$ ; P = 0.50 - 0.75) (Table 69). Overall, both the crosses also fitted well to a 3 (low density): 1 (high density) ratio in  $F_2$  ( $\chi^2 = 0.384$ ; P = 0.50 - 0.75) and 1: 1 ratio in the backcross generation ( $\chi^2 = 0.027$ ; P = 0.75 - 0.90) confirming a monogenic inheritance (Table 70). The  $\chi^2$  due to heterogeneity was non-significant in both the crosses,  $F_2$  ( $\chi^2 = 0.015$ ; P = 0.90-0.95) and their respective backcross generations ( $\chi^2 = 0.961$ ; P = 0.25 -0.50), indicating similarity of segregation in both the crosses.

Density of trichome B (glandular hair) (Fig. 20) was more on the pods of *C. scarabaeoides* accessions than on the pigeonpea pods. The  $F_1$  mean, was more the than mean of *C. cajan*, ICP 28, but less than the mean of *C. scarabaeoides*, ICPW 94 in cross ICP 28 x ICPW 94. However, in cross ICP 26 x ICPW 125, the  $F_1$  mean was equal to the mean of ICPW 125 and greater than ICP 26 mean. In the  $F_2$  generation, the mean density of trichome B was higher than the means of ICP 26 and ICP 28, but less than the ICPW 94 and ICPW 125 means. In the backcross generation (ICP 28 x (ICP 28 x ICPW 94) and (ICP 26 x (ICP 26 x ICPW125) crosses, the mean density of trichome B was greater than the *C. cajan* mean but less than the means of *C. scarabaeoides*,  $F_1$  and  $F_2$  (Table 67) .

$F_1$  pods in both the interspecific crosses resembled the *C. scarabaeoides* pods, having higher density of trichome B. Further, the pods of 250  $F_2$  plants in each cross gave a good fit of 1 (low density): 3 (high density) in cross ICP 28 x ICPW 94 ( $\chi^2 = 1.181$  P = 0.10-0.25) ( Table 68) and in cross ICP 26 x ICPW 125 ( $\chi^2 = 0.133$  P = 0.50-0.75) (Table 69) . Further, 75 plants of the backcross progeny screened in each cross showed a good fit of 1 (low density) : 1 (high density) in cross ICP 28 x ICPW 94 ( $\chi^2 = 0.654$  P =0.25-0.05) and in cross ICP 28 x ICPW 125 ( $\chi^2 = 0.654$  P = 0.25-0.50) (Tables 68 and 69) . Overall, both the crosses gave a good fit of 1 (low density): 3 (high density) ratio in  $F_2$  ( $\chi^2 = 0.266$ ; P = 0.50 - 0.75 ) and 1(low density): 1(high density) ratio in the backcross generation ( $\chi^2 = 0.000$ ; P = 0.995 - 1.00 ) confirming a monogenic inheritance

Table - 67: Mean values for type and density of different trichome in segregating generations of two interspecific crosses

Cross	Means $\pm$ SE			
	Trichome A	Trichome B	Trichome C	Trichome D
<b>ICP 28 x ICPW 94</b>				
ICP 28	5.69 $\pm$ 1.000*	2.42 $\pm$ 0.542	31.76 $\pm$ 1.471	2.74 $\pm$ 0.542
ICPW 94	0.49 $\pm$ 0.216	6.49 $\pm$ 0.501	174.20 $\pm$ 10.291	10.21 $\pm$ 1.132
F <sub>1</sub> (ICP 28 x ICPW 94)	0.90 $\pm$ 0.639	4.07 $\pm$ 0.623	163.35 $\pm$ 13.472	9.61 $\pm$ 0.962
F <sub>2</sub> (ICP 28 x ICPW 94)	0.87 $\pm$ 1.031	3.56 $\pm$ 1.812	129.71 $\pm$ 62.503	8.73 $\pm$ 3.341
BC <sub>1</sub> F <sub>1</sub> (ICP 28 x F <sub>1</sub> )	2.09 $\pm$ 1.700	3.00 $\pm$ 2.673	94.54 $\pm$ 64.781	5.01 $\pm$ 2.941
<b>ICP 26 x ICPW 125</b>				
ICP 26	4.39 $\pm$ 0.641	0.81 $\pm$ 0.112	25.54 $\pm$ 1.124	2.26 $\pm$ 0.593
ICPW 125	0.49 $\pm$ 0.195	7.57 $\pm$ 0.824	157.23 $\pm$ 6.192	5.85 $\pm$ 0.593
F <sub>1</sub> (ICP 26 x ICPW 125)	0.66 $\pm$ 0.105	7.69 $\pm$ 0.761	155.57 $\pm$ 2.742	5.83 $\pm$ 0.432
F <sub>2</sub> (ICP 26 x ICPW 125)	1.32 $\pm$ 1.128	5.26 $\pm$ 2.812	111.17 $\pm$ 54.791	3.31 $\pm$ 1.708
BC <sub>1</sub> F <sub>1</sub> (ICP 26 x F <sub>1</sub> )	2.79 $\pm$ 1.433	3.09 $\pm$ 2.730	104.76 $\pm$ 61.209	5.17 $\pm$ 3.001

\* = density based on mean of observations on 5 pods

Table - 68:  $\chi^2$  test for trichome density  $F_2$  and backcross,  $F_1$  plants of ICP 28 x ICPW 94

	Population size	Low density	High density	Excepted ratio	$\chi^2$ value	P- value
<b>Trichome A</b>						
ICP 28	10	-	10	-	-	-
ICPW 94	10	10	-	-	-	-
$F_1$ (ICP 28 x ICPW 94)	10	10	-	-	-	-
$F_2$ (ICP 28 x ICPW 94)	250	186	64	3:1	0.033	0.75-0.90
$BC_1F_1$ (ICP 28 x $F_1$ )	75	41	34	1:1	0.654	0.25-0.50
<b>Trichome B</b>						
ICP 28	10	10	-	-	-	-
ICPW 94	10	-	10	-	-	-
$F_1$ (ICP 28 x ICPW 94)	10	-	10	-	-	-
$F_2$ (ICP 28 x ICPW 94)	250	70	180	1:3	1.181	0.10-0.25
$BC_1F_1$ (ICP 28 x $F_1$ )	75	41	34	1:1	0.654	0.25-0.50
<b>Trichome C</b>						
ICP 28	10	10	-	-	-	-
ICPW 94	10	-	10	-	-	-
$F_1$ (ICP 28 x ICPW 94)	10	-	10	-	-	-
$F_2$ (ICP 28 x ICPW 94)	250	69	181	1:3	1.204	0.25-0.50
$BC_1F_1$ (ICP 28 x $F_1$ )	75	39	36	1:1	0.123	0.50-0.75
<b>Trichome D</b>						
ICP 28	10	10	-	-	-	-
ICPW 94	10	-	10	-	-	-
$F_1$ (ICP 28 x ICPW 94)	10	-	10	-	-	-
$F_2$ (ICP 28 x ICPW 94)	250	69	181	1:3	0.901	0.25-0.50
$BC_1F_1$ (ICP 28 x $F_1$ )	75	39	36	1:1	0.121	0.50-0.70

**Table - 69:  $\chi^2$  test for trichome density  $F_1$ ,  $F_2$  and backcross of ICP 26 x ICPW 125**

	Population size	Low density	High density	Excepted	$\chi^2$ value	P value
<b>Trichome A</b>						
ICP 26	10	-	10	-	-	-
ICPW 125	10	10	-	-	-	-
$F_1$ (ICP 26 x ICPW 125)	10	10	-	-	-	-
$F_2$ (ICP 26 x ICPW 125)	250	183	67	3:1	0.432	0.50-0.75
$BC_1F_1$ (ICP 26 x $F_1$ )	75	35	40	1:1	0.334	0.50-0.75
<b>Trichome B</b>						
ICP 26	10	10	-	-	-	-
ICPW 125	10	-	10	-	-	-
$F_1$ (ICP 26 x ICPW 125)	10	-	10	-	-	-
$F_2$ (ICP 26 x ICPW 125)	250	60	190	1:3	0.133	0.50-0.75
$BC_1F_1$ (ICP 26 x $F_1$ )	75	34	41	1:1	0.654	0.25-0.50
<b>Trichome C</b>						
ICP 28	10	10	-	-	-	-
ICPW 125	10	-	10	-	-	-
$F_1$ (ICP 26 x ICPW 125)	10	-	10	-	-	-
$F_2$ (ICP 26 x ICPW 125)	250	73	177	1:3	2.352	0.10-0.25
$BC_1F_1$ (ICP 26 x $F_1$ )	75	38	37	1:1	0.012	0.90-0.95
<b>Trichome D</b>						
ICP 26	10	10	-	-	-	-
ICPW 125	10	-	10	-	-	-
$F_1$ (ICP 26 x ICPW 125)	10	-	10	-	-	-
$F_2$ (ICP 26 x ICPW 125)	250	91	159	1:3	17.328	-
$BC_1F_1$ (ICP 26 x $F_1$ )	75	41	34	1:1	0.654	0.25-0.50

(Table 69). The  $\chi^2$  due to heterogeneity was non-significant in both the crosses  $F_2$  ( $\chi^2 = 1.048$ ;  $P = 0.25-0.50$ ) and backcross generations ( $\chi^2 = 1.308$ ;  $P = 0.25-0.50$ ), indicating similarity in the segregation of both crosses (Table 70).

Density of trichome C (non glandular trichome) (Fig. 20 and 21) was higher on the *C. scarabaeoides*, ICPW 94 and ICPW 125, pods than on the pigeonpea, ICP 26 and ICP 28, pods. Pods of  $F_1$  hybrid plants resembled the *C. scarabaeoides* pods a the mean greater than the pigeonpea mean, but less than the *C. scarabaeoides* mean. The  $F_2$  mean was less than the *C. scarabaeoides* and  $F_1$  means but greater than the pigeonpea mean in both crosses. Mean of backcross progeny was greater than pigeonpea, ICP 26 and ICP 28 mean but less than *C. scarabaeoides*, ICPW 94 and ICPW 125,  $F_1$  and  $F_2$  means in both the interspecific crosses. (Table 67).

Pods on  $F_1$  plants resembled *C. scarabaeoides* pods, in having a high density of trichome C. In the  $F_2$  generation, the 250 plants of both the crosses segregated to give a good fit of 1(low density): 3 (high density), in cross ICP 28 x ICPW 94 ( $\chi^2 = 1.204$ ;  $P = 0.25-0.50$ ) (Table 68) and in cross ICP 26 x ICPW 125 ( $\chi^2 = 2.352$ ;  $P = 0.10-0.25$ ) (Table 69). Further, 75 plants in the backcross progeny segregated to give a good fit of 1 (low density): 1 (high density) in cross ICP 28 x ICPW 94 ( $\chi^2 = 0.123$ ;  $P = 0.50-0.75$ ) and in cross ICP 26 x ICPW 125 ( $\chi^2 = 0.012$ ;  $P = 0.90 - 0.95$ ). Overall, both the crosses also fitted well to a 1 (low density): 3 (high density) ratio in  $F_2$  ( $\chi^2 = 3.083$ ;  $P = 0.05 - 0.10$ ) and 1(low density): 1(high density) ratio in the backcross generation ( $\chi^2 = 0.104$ ;  $P = 0.50 - 0.75$ ) confirming a monogenic inheritance (Table 69). The  $\chi^2$  due to heterogeneity was non-significant in both the crosses in  $F_2$  ( $\chi^2 = 0.473$ ;  $P = 0.25-0.50$ ) and backcross generations ( $\chi^2 = 0.031$ ;  $P = 0.75-0.90$ ), indicating similarity in the segregation of both the crosses (Table 70).

Density of trichome D ( non-glandular trichome) (Fig. 20 and 21) was higher on the pods of *C. scarabaeoides*, ICPW 94 and ICPW 125, accessions than on the pigeonpea, ICP 26 and ICP 28 pods. Mean of  $F_1$  was greater than the pigeonpea

**Table - 70: Density of different types of trichomes in the F<sub>1</sub>, F<sub>2</sub> and backcross generation**

Cross	Generatio	Total No. of pods	Low density	High density	Segregation	$\chi^2$ value	P value
<b>Trichome A</b>							
ICP 28 x ICPW 94	F <sub>2</sub>	250	183	67	3:1	0.43	0.50-0.75
ICP 26 x ICPW 125	F <sub>2</sub>	250	186	64	3:1	0.03	0.50-0.75
Total		500	369	131	3:1	0.38	0.50-0.75
$\chi^2$ Heterogeneity = 0.015 P value = 0.90-0.95							
ICP 28 x ICPW 94	BC <sub>1</sub>	75	35	40	1:1	0.33	0.50-0.75
ICP 26 x ICPW 125	BC <sub>1</sub>	75	41	34	1:1	0.65	0.25-0.50
Total		150	76	74	1:1	0.02	0.75-0.90
Heterogeneity = 0.961 P value = 0.25-0.50							
<b>Trichome B</b>							
ICP 28 x ICPW 94	F <sub>2</sub>	250	70	180	1:3	1.18	0.10-0.50
ICP 26 x ICPW 125	F <sub>2</sub>	250	60	190	1:3	0.13	0.50-0.75
Total		500	130	370	1:3	0.26	0.50-0.75
$\chi^2$ Heterogeneity = 1.048 P value = 0.25-0.50							
ICP 28 x ICPW 94	BC <sub>1</sub>	75	41	34	1:1	0.65	0.25-0.50
ICP 26 x ICPW 125	BC <sub>1</sub>	75	34	41	1:1	0.65	0.25-0.50
Total		150	75	75	1:1	0.00	0.995-1.0
$\chi^2$ Heterogeneity = 1.308 P value = 0.25-0.50							
<b>Trichome C</b>							
ICP 28 x ICPW 94	F <sub>2</sub>	250	69	181	1:3	1.20	0.25-0.50
ICP 26 x ICPW 125	F <sub>2</sub>	250	73	177	1:3	2.35	0.10-0.25
Total		500	142	358	1:3	3.08	0.05-0.10
$\chi^2$ Heterogeneity = 0.473 P value = 0.25-0.50							
ICP 28 x ICPW 94	BC <sub>1</sub>	75	39	36	1:1	0.12	0.50-0.75
ICP 26 x ICPW 125	BC <sub>1</sub>	75	38	37	1:1	0.01	0.90-0.95
Total		150	77	73	1:1	0.10	0.50-0.75
$\chi^2$ Heterogeneity = 0.031 P value = 0.75-0.90							
<b>Trichome D</b>							
ICP 28 x ICPW 94	F <sub>2</sub>	250	69	181	1:3	0.90	0.25-0.50
ICP 26 x ICPW 125	F <sub>2</sub>	250	91	159	1:3	17.32	<0.005
Total		500	160	340	1:3	13.06	<0.005
$\chi^2$ Heterogeneity = 5.162 P value = 0.010-0.025							
ICP 28 x ICPW 94	BC <sub>1</sub>	75	39	36	1:1	0.121	0.50-0.70
ICP 26 x ICPW 125	BC <sub>1</sub>	75	41	34	1:1	0.654	0.25-0.50
Total		150	80	70	1:1	0.625	0.50-0.75
$\chi^2$ Heterogeneity = 0.15 P value = 0.50-0.75							



mean but less than the *C. scarabaeoides* mean in cross ICP 28 x ICPW 94, and equal to *C. scarabaeoides* mean in ICP 26 x ICPW 125. The  $F_2$  mean was less than the  $F_1$  and *C. scarabaeoides* means but greater than the pigeonpea mean in both crosses, ICP 28 x ICPW 94 and ICP 26 x ICPW 125. The mean in backcross population was greater than the means of  $F_1$ ,  $F_2$  and *C. cajan* parent but less than *C. scarabaeoides* parent in both ICP 28 x ICPW 94 and ICP 26 x ICPW 125 crosses (Table 67).

The  $F_1$  plants had pods with high density of trichome D, like the *C. scarabaeoides* parent. The  $F_2$  generation segregated into 1 (low density): 3 (high density) in cross ICP 28 x ICPW 94 ( $\chi^2=0.901$ ;  $P = 0.25-0.50$ ), but in cross ICP 26 x ICPW 125 ( $\chi^2=17.328$ ;  $P = <0.001$ ) did not give a good fit for the 1 (low density) : 3 (high density) (Tables 68 and 69). Further, 75 plants in the backcross generation gave a good fit for 1 (low density): 1 (high density) in cross ICP 28 x ICPW 94 ( $\chi^2=0.121$ ;  $P = 0.50 - 0.70$ ) and in cross ICP 26 x ICPW 125, ( $\chi^2=0.654$ ;  $P = 0.25-0.50$ ). Overall, both the crosses fitted well to a 1 (low density): 3 (high density) ratio in  $F_2$  ( $\chi^2 = 13.067$ ;  $P = <0.005$ ) and 1(low density): 1(high density) ratio in the backcross generation ( $\chi^2 = 0.625$ ;  $P = 0.50 - 0.75$ ) confirming a monogenic inheritance (Table 70). The  $\chi^2$  due to heterogeneity was non-significant in both the crosses in  $F_2$  ( $\chi^2 = 5.162$ ;  $P = 0.01 - 0.025$ ) and backcross generations ( $\chi^2=0.15$ ;  $P = 0.50 - 0.75$ ), indicating similarity in the segregation of both the crosses (Table 70).

## Genetic basis of podborer resistance

Parents,  $F_1$ ,  $F_2$ ,  $F_3$  and backcross generations were screened in field, under multi-choice conditions for podborer resistance. Various reproductive parts attacked by podborer are shown in Figure 32. Cultivated pigeonpea (ICP 28 and ICP 26) and accessions of *C. scarabaeoides* (ICPW 94, ICPW 125 and ICPW 130) differed for bud, flower and pod damage, and number of eggs and larvae on the inflorescence. In a damage rating scale of 1-5; 1-2 scale were rated as resistant and 3-5 were scored as susceptible based on the pod damage. All the three *C. scarabaeoides* parents had no pod damage and were rated as 1, whereas, ICP 28 and ICP 26 parents showed

56.65% and 65.45% pod damage respectively with a damage rating of 5. The  $F_1$  hybrid plants had a mean damage rating of  $1.5 \pm 0.01$  in ICP 28 x ICPW 94,  $1.6 \pm 0.03$  in ICP 28 x ICPW 130 and  $1.8 \pm 0.01$  in ICP 26 x ICPW 125, and were classified as resistant. In each cross, a population of 250  $F_2$  plants was screened. The mean damage rating was  $3.5 \pm 1.21$  in cross ICP 28 x ICPW 94,  $3.84 \pm 1.12$  in cross ICP 28 x ICPW 130 and  $3.72 \pm 1.24$  in cross ICP 26 x ICPW 125. In each  $BC_1F_1$  the 75 plants screened exhibited a mean damage rating of  $4.2 \pm 0.95$  in ICP 28 x ICPW 94 cross,  $3.94 \pm 0.69$  in ICP 28 x ICPW 130 cross and  $4.1 \pm 0.89$  in ICP 26 x ICPW 125 cross.

Out of 250  $F_2$  plants screened for podborer resistance, in a cross ICP 28 x ICPW 94, 185 were found resistant and 65 plants were susceptible giving a good fit of 3 (resistant): 1 (susceptible) ( $\chi^2 = 0.133$ ;  $P = 0.50 - 0.75$ ) (Table 67). In the backcross generation, out of the 112 plants, 53 were found to be resistant and 59 were susceptible, giving a good fit for 1 (resistant): 1 (susceptible) ( $\chi^2 = 0.322$ ;  $P = 0.50 - 0.75$ ) (Table 71). In  $F_3$  generation, out of 116 progenies, only 56 showed segregation. Of the remaining 60 non-segregating progenies, 32 were resistant and 28 susceptible. This gave a good fit for 1 non-segregating resistant: 2 segregating: 1 non-segregating susceptible ( $\chi^2 = 0.414$ ;  $P = 0.75 - 0.90$ ). Further, the 56  $F_3$  segregating progenies gave a good fit for 3 resistant: 1 susceptible individually as well as overall ( $\chi^2 = 1.937$ ;  $P = 0.10 - 0.25$ ) (Table 72). The  $\chi^2$  due to heterogeneity ( $\chi^2 = 11.10$ ;  $P = 0.995 - 1.000$ ) suggested that these progenies were highly homogenous in segregation. The  $F_2$  generation and  $F_3$  segregating progenies were also homogenous ( $\chi^2 = 0.947$ ;  $P = 0.25 - 0.50$ ) (Tables 73 and 74).

In ICP 28 x ICPW 130 cross, out of 250  $F_2$  plants screened, 183 were found resistant and 67 were susceptible which showed a good fit for 3 resistant: 1 susceptible ( $\chi^2 = 0.432$ ;  $P = 0.50 - 0.75$ ) (Table 71). In  $BC_1F_1$  generation, out of a total 106 plants, 51 were resistant and 55 were susceptible showing a good fit of 1 (resistant): 1 (susceptible) ratio ( $\chi^2 = 0.151$ ;  $P = 0.50 - 0.75$ ) (Table 72). In  $F_3$

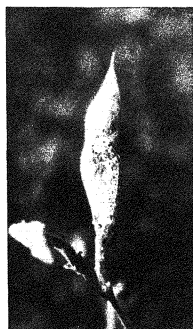
Fig. 32: Different reproductive plant parts attacked by podborer  
(*Helicoverpa armigera*)



a



b



c



d



e



f



g

Fig: 32

**Table - 71: Resistance/susceptibility against pod borer in different generations.**

Cross	Population size	Resistant	Susceptible	Expected ratio	$\chi^2$ value	P value
<b>Field screening</b>						
<b>ICP 28 x ICPW 94</b>						
ICP 28	10	-	10	-	-	-
ICPW 94	10	10	-	-	-	-
F <sub>1</sub> (ICP 28 x ICPW 94)	10	10	-	-	-	-
F <sub>2</sub> (ICP 28 x ICPW 94)	250	185	65	3:1	0.133	0.50-0.75
BC <sub>1</sub> F <sub>1</sub> (ICP 28 x F <sub>1</sub> )	112	53	59	1:1	0.322	0.50-0.75
<b>ICP 28 x ICPW 130</b>						
ICP 28	10	-	10	-	-	-
ICPW 130	10	10	-	-	-	-
F <sub>1</sub> (ICP 28 x ICPW 130)	10	10	-	-	-	-
F <sub>2</sub> (ICP 28 x ICPW 130)	250	183	67	3:1	0.432	0.50-0.75
BC <sub>1</sub> F <sub>1</sub> (ICP 28 x F <sub>1</sub> )	106	51	55	1:1	0.151	0.50-0.75
<b>ICP 26 x ICPW 125</b>						
ICP 26	10	-	10	-	-	-
ICPW 125	10	10	-	-	-	-
F <sub>1</sub> (ICP 26 x ICPW 125)	10	10	-	-	-	-
F <sub>2</sub> (ICP 26 x ICPW 125)	216	158	58	3:1	0.395	0.50-0.75
BC <sub>1</sub> F <sub>1</sub> (ICP 26 x F <sub>1</sub> )	75	36	39	1:1	0.121	0.50-0.75
<b>Laboratory screening (No- Choice ) conditions</b>						
<b>ICP 28 x ICPW 94</b>						
ICP 28	10	-	10	-	-	-
ICPW 94	10	10	-	-	-	-
F <sub>1</sub> (ICP 28 x ICPW 94)	10	10	-	-	-	-
F <sub>2</sub> (ICP 28 x ICPW 94)	250	184	66	3:1	0.261	0.50-0.75
BC <sub>1</sub> F <sub>1</sub> (ICP 28 x F <sub>1</sub> )	75	36	39	1:1	0.121	0.50-0.75

**Table - 72: Segregation of F<sub>3</sub> families into resistant and susceptible plants**

Cross	No. F <sub>3</sub> Families	Segregation of F <sub>3</sub> families			Expected ratio	$\chi^2$ value	P value
		Resistant	Segregated	Susceptible			
ICP 28 x ICPW 94	116	32	56	28	1:2:1	0.414	0.75-0.90
ICP 28 x ICPW 130	120	28	62	30	1:2:1	0.200	0.90-0.95
ICP 26 x ICPW 125	96	26	48	22	1:2:1	0.250	0.75-0.90

**Table - 73: Segregation of 56 F<sub>3</sub> families for pod borer resistance in cross ICP 28 x ICPW 94**

S.No	F <sub>3</sub> family No.	No. of plants	Observed		Excepted		$\chi^2$ value	P value
			Resistant	Susceptible	Resistant	Susceptible		
1	1	20	14	6	15.00	5.00	0.267	0.500-0.750
2	2	14	11	3	10.50	3.50	0.024	0.750-0.900
3	3	16	13	3	12.00	4.00	0.146	0.500-0.750
4	5	17	14	3	12.75	4.25	0.255	0.500-0.750
5	8	20	14	6	15.00	5.00	0.267	0.500-0.750
6	9	18	15	3	13.50	4.50	0.389	0.500-0.750
7	10	19	16	3	14.25	4.75	0.544	0.250-0.500
8	11	11	9	2	8.25	2.75	0.087	0.750-0.900
9	14	14	11	3	10.50	3.50	0.024	0.750-0.900
10	15	15	12	3	11.25	3.75	0.067	0.250-0.500
11	16	16	13	3	12.00	4.00	0.146	0.500-0.750
12	17	12	10	2	9.00	3.00	0.194	0.500-0.750
13	18	20	16	4	15.00	5.00	0.117	0.500-0.750
14	19	20	15	5	15.00	5.00	0.000	1.000
15	20	24	19	5	18.00	6.00	0.222	0.500-0.750
16	21	20	15	5	15.00	5.00	0.000	1.000
17	22	15	13	2	11.25	3.75	0.689	0.250-0.500
18	25	16	13	3	12.00	4.00	0.146	0.500-0.750
19	26	14	11	3	10.50	3.50	0.024	0.750-0.900
20	27	17	13	4	12.75	4.25	0.152	0.500-0.750
21	28	15	12	3	11.25	3.75	0.067	0.750-0.900
22	29	16	13	3	12.00	4.00	0.146	0.500-0.750
23	30	17	14	3	12.75	4.25	0.255	0.500-0.750
24	31	18	14	4	13.50	4.50	0.019	0.900-0.950
25	32	15	13	2	11.25	3.75	0.272	0.500-0.750
26	33	16	11	5	12.00	4.00	0.146	0.500-0.750
27	35	17	11	6	12.75	4.25	0.961	0.250-0.500
28	36	18	14	4	13.50	4.50	0.019	0.900-0.950
29	39	16	11	5	12.00	4.00	1.333	0.100-0.250
30	40	12	8	4	9.00	3.00	0.193	0.500-0.750
31	41	15	9	6	11.25	3.75	1.800	0.100-0.250
32	43	14	11	3	10.50	3.50	0.024	0.750-0.900
33	44	18	15	3	13.50	4.50	0.389	0.500-0.750
34	46	19	16	3	14.25	4.75	0.544	0.250-0.500
35	48	16	12	4	12.00	4.00	0.000	0.750-0.900
36	49	14	11	3	10.50	3.50	0.024	0.500-0.750
37	50	15	13	2	11.25	3.75	0.272	0.500-0.750
38	52	18	15	3	13.50	4.50	0.389	0.750-0.900
39	53	15	12	3	11.25	3.75	0.067	0.500-0.750
40	54	16	13	3	12.00	4.00	0.146	0.250-0.500
41	59	14	9	5	10.50	3.50	0.692	0.500-0.750
42	60	17	14	3	12.75	4.25	0.255	0.750-0.900
43	61	18	13	5	13.50	4.50	0.074	0.750-0.900

Cont..

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S.No	F <sub>1</sub> families No.	No. of plants	Observed		Excepted		$\chi^2$ value	P value
			Resistant	Susceptible	Resistant	Susceptible		
44	63	15	11	4	11.25	3.75	0.023	0.750-0.900
45	64	20	15	5	15.00	5.00	0.000	1.000
46	68	20	16	4	15.00	5.00	0.117	0.500-0.750
47	69	15	11	4	11.25	3.75	0.023	0.750-0.900
48	70	16	11	5	12.00	4.00	0.083	0.750-0.900
49	96	9	7	2	6.75	2.25	0.111	0.500-0.750
50	99	7	5	2	5.25	1.75	0.048	0.250-0.500
51	105	15	11	4	11.25	3.75	0.023	0.750-0.900
52	106	11	9	2	8.25	2.75	0.023	0.750-0.900
53	107	11	9	3	9.00	3.00	0.000	1.000
54	108	15	11	4	11.25	3.75	0.023	0.750-0.900
55	111	14	9	5	10.50	3.50	0.692	0.250-0.500
56	113	16	11	5	12.00	4.00	0.023	0.750-0.900
Total		892	687	205	669	223	1.937	0.100-0.250

$\chi^2$  heterogeneity = 11.099 P value = < 0.005

**Table - 74: Segregation of 62 F<sub>3</sub> families for pod borer resistance in ICP 28 x ICPW 130**

S.No	F <sub>3</sub> family No. no.	No. of plants	Observed		Excepted		$\chi^2$ value	P value
			Resistant	Susceptible	Resistant	Susceptible		
1	2	25	19	6	18.75	6.25	0.013	0.900-0.950
2	3	24	19	5	18.00	6.00	0.222	0.500-0.750
3	5	22	17	5	16.50	5.50	0.061	0.750-0.900
4	6	25	19	6	18.75	6.25	0.013	0.900-0.950
5	7	22	17	5	16.50	5.50	0.061	0.750-0.900
6	9	21	16	5	15.75	5.25	0.016	0.900-0.950
7	11	20	14	6	15.00	5.00	0.267	0.500-0.750
8	13	14	11	3	10.50	3.50	0.024	0.750-0.900
9	15	15	12	3	11.25	3.75	0.067	0.750-0.900
10	16	18	14	4	13.50	4.50	0.019	0.900-0.950
11	17	19	15	4	14.25	4.75	0.052	0.750-0.900
12	21	14	11	3	10.50	3.50	0.024	0.750-0.900
13	25	15	12	3	11.25	3.75	0.067	0.750-0.900
14	26	16	13	3	12.00	4.00	0.146	0.500-0.750
15	31	14	11	3	10.50	3.50	0.024	0.750-0.900
16	33	12	10	2	9.00	3.00	0.194	0.500-0.750
17	39	14	11	3	10.50	3.50	0.024	0.250-0.500
18	45	15	12	3	11.25	3.75	0.067	0.250-0.500
19	51	15	12	3	11.25	3.75	0.067	0.750-0.900
20	53	12	10	2	9.00	3.00	0.194	0.500-0.750
21	56	14	11	3	10.50	3.50	0.024	0.750-0.900
22	59	25	19	6	18.75	6.25	0.013	0.900-0.950
23	61	20	14	6	15.00	5.00	0.267	0.500-0.750
24	66	21	16	5	15.75	5.25	0.016	0.900-0.950
25	69	21	17	4	15.75	5.25	0.206	0.500-0.750
26	71	20	16	4	15.00	5.00	0.117	0.500-0.750
27	77	21	16	5	15.75	5.25	0.016	0.900-0.950
28	79	22	17	5	16.50	5.50	0.061	0.750-0.900
29	80	21	16	5	15.75	5.25	0.016	0.900-0.950
30	81	20	16	4	15.00	5.00	0.117	0.500-0.750
31	84	15	12	3	11.25	3.75	0.067	0.750-0.900
32	86	14	11	3	10.50	3.50	0.024	0.750-0.900
33	87	17	14	3	12.75	4.25	0.491	0.250-0.500
34	89	18	14	4	13.50	4.50	0.019	0.900-0.950
35	91	15	12	3	11.25	3.75	0.067	0.750-0.900
36	92	16	14	2	12.00	4.00	0.146	0.500-0.750
37	93	14	11	3	10.50	3.50	0.024	0.750-0.900
38	94	17	13	4	12.75	4.25	0.196	0.500-0.750
39	95	15	12	3	11.25	3.75	0.067	0.750-0.900
40	96	18	14	4	13.50	4.50	0.019	0.900-0.950
41	97	19	15	4	14.25	4.75	0.052	0.750-0.900
42	100	20	16	4	15.00	5.00	0.117	0.500-0.750
43	101	21	17	4	15.75	5.25	0.206	0.500-0.750
44	102	16	13	3	12.00	4.00	0.146	0.500-0.750

Cont..



**Cont.....**

S.No	F <sub>3</sub> Family No.	No. of plants	Observed		Expected		$\chi^2$ value	P value
			Resistant	Susceptible	Resistant	Susceptible		
45	103	15	12	3	11.25	3.75	0.067	0.750-0.900
46	104	14	11	3	10.50	3.50	0.024	0.750-0.900
47	105	15	12	3	11.25	3.75	0.067	0.750-0.900
48	106	14	11	3	10.50	3.50	0.024	0.750-0.900
49	107	12	10	2	9.00	3.00	0.194	0.500-0.750
50	108	12	10	2	9.00	3.00	0.194	0.500-0.750
51	109	15	12	3	11.25	3.75	0.066	0.250-0.500
52	110	14	11	3	10.50	3.50	0.024	0.750-0.900
53	111	15	13	2	11.25	3.75	0.689	0.250-0.500
54	112	14	11	3	10.50	3.50	0.024	0.750-0.900
55	113	12	10	2	9.00	3.00	0.194	0.500-0.750
56	114	15	13	2	11.25	3.75	0.689	0.250-0.500
57	115	25	19	6	18.75	6.25	0.013	0.900-0.950
58	116	17	13	4	12.75	4.25	0.137	0.500-0.750
59	117	21	15	6	15.75	5.25	0.143	0.500-0.750
60	118	20	14	6	15.00	5.00	0.067	0.750-0.900
61	119	19	15	4	14.25	4.75	0.052	0.750-0.900
62	120	18	13	5	13.50	4.50	0.074	0.750-0.900
Total	1079	846	233	809.25	269.75	6.676	0.005-0.010	

$\chi^2$  heterogeneity = 0.182 P value = 0.50 - 0.75

generation, out of 120 progenies, only 62 segregated, and of the remaining 58 non-segregating progenies, 28 were resistant and 30 families were susceptible, a ( $\chi^2 = 0.200$  ;  $P = 0.90-0.95$ ) (Table 72). Overall, 62  $F_3$  segregating families did not show good fit for 3 (resistant): 1 (susceptible) with a  $\chi^2 = 6.68$  ( $P = 0.005 - 0.010$ ) but exhibited good fit individually (Table 75). The heterogeneity value ( $\chi^2 = 0.182$  ;  $P = 0.90-0.95$ ) suggests that the population was homogenous for the segregation. The  $F_2$  and  $F_3$  segregating progenies were heterogeneous at a  $\chi^2$  value of 2.935 ( $P = 0.050-0.100$ ) (Table 74).

In a crosses of ICP 26 x ICPW 125, 216  $F_2$  plants segregated into 158 resistant and 58 susceptible, showing a good fit for 3: 1 ratio with ( $\chi^2 = 0.395$  ;  $P = 0.50 - 0.75$ ) (Table 71). In  $BC_1F_1$  the 75 plants segregated into 1 resistant: 1 susceptible with a ( $\chi^2 = 0.121$  ;  $P = 0.50-0.75$ ). Further, the 96  $F_3$  families segregated into 26 resistant, 48 segregating progenies and 22 susceptible giving a good fit for 1 non- segregating resistant: 2 Segregating: 1 non- segregating susceptible, with a ( $\chi^2 = 0.250$  ;  $P = 0.75-0.90$ ) (Table 72). Further, the segregating  $F_3$  progenies gave a good fit for 3 (resistant): 1 (susceptible) progenies ( $\chi^2 = 4.406$ ;  $P = 0.025 - 0.050$ ) (Table 76) with a heterogeneity of ( $\chi^2 = 0.155$ ;  $P = 0.50-0.75$ ) suggesting that the segregating  $F_3$  population was homogenous. The  $F_2$  and  $F_3$  segregating progenies were heterogeneous at a ( $\chi^2 = 2.268$ ;  $P = 0.050-0.100$ ) (Table 77).

In addition to the field screening; the parents,  $F_1$ ,  $F_2$  and  $BC_1F_1$  plants of a cross ICP 28 x ICPW 94, were also screened, in the laboratory, for podborer resistance under no – choice conditions. Plants were given a damage rating based on the scale from 0-9. Plants with 0 – 3 damage rating were classified resistant and in the scale of 4 – 9 were susceptible. ICP 28, the susceptible parent, recorded a mean damage rating of  $6.25 \pm 1.064$  and the resistant parent, ICPW 94 with a damage rating of  $0.42 \pm 0.116$ .  $F_1$  plants were resistant to podborer attack and recorded a damage rating of  $0.72 \pm 0.511$ , while the mean damage rating of  $2.50 \pm 1.400$  in  $F_2$  population and  $3.31 \pm 1.957$  in  $BC_1F_1$  plants were recorded. In  $F_2$  population of 250 plants, 184 were found to be resistant and 66 were susceptible, with a good fit of 3

**Table - 75: Segregation of 48 F<sub>3</sub> families for podborer resistance in cross ICP 26 x ICPW 125**

S. No	No. of F <sub>3</sub> families observed	Total No. of plants	Observed		Expected		$\chi^2$ value	P value
			Resistant	Susceptible	Resistant	Susceptible		
1	5	25	19	6	18.75	6.25	0.013	0.975-0.95
2	6	24	18	6	18.00	6.00	0.014	0.975-0.95
3	8	22	17	5	16.50	5.50	0.061	0.75-0.90
4	7	25	19	6	18.75	6.25	0.013	0.975-0.95
5	9	22	17	5	16.50	5.50	0.061	0.75-0.90
6	10	21	16	5	15.75	5.25	0.016	0.75-0.90
7	12	20	14	6	15.00	5.00	0.266	0.50-0.75
8	14	14	11	3	10.50	3.50	0.024	0.75-0.90
9	15	15	12	3	11.25	3.75	0.067	0.75-0.90
10	16	18	14	4	13.50	4.50	0.019	0.975-0.95
11	19	19	15	4	14.25	4.75	0.052	0.75-0.90
12	20	14	11	3	10.5	3.5	2.317	0.10-0.25
13	22	15	12	3	11.25	3.75	0.067	0.75-0.90
14	24	16	13	3	12.00	4.00	0.146	0.50-0.75
15	25	14	11	3	10.5	3.5	0.024	0.75-0.90
16	26	12	10	2	9.00	3.00	0.443	0.50-0.75
17	28	14	12	2	10.50	3.50	0.449	0.25-0.50
18	29	15	12	3	11.25	3.75	0.688	0.005-0.01
19	32	15	12	3	11.25	3.75	0.067	0.75-0.90
20	35	12	10	2	9.00	3.00	0.193	0.75-0.90
21	39	14	11	3	10.50	3.50	0.023	0.75-0.90
22	40	25	19	6	18.75	6.25	0.013	0.975-0.95
23	43	20	14	6	15.00	5.00	0.117	0.50-0.75
24	44	21	16	5	15.75	5.25	0.016	0.75-0.90
25	45	21	17	4	15.75	5.25	0.206	0.75-0.90
26	49	20	16	4	15.00	5.00	0.117	0.50-0.75
27	50	21	16	5	15.75	5.25	0.016	0.750-0.90
28	52	22	17	5	16.50	5.50	0.061	0.750-0.90
29	55	21	16	5	15.75	5.25	0.016	0.750-0.90
30	58	20	16	4	15.00	5.00	0.117	0.50-0.75
31	59	15	12	3	11.25	3.75	0.067	0.750-0.90
32	60	14	11	3	10.50	3.50	0.023	0.750-0.90
33	62	17	13	4	12.75	4.25	0.248	0.50-0.75
34	64	18	14	4	13.50	4.50	0.019	0.750-0.90
35	67	15	12	3	11.25	3.75	0.067	0.50-0.750
36	68	16	12	4	12.00	4.00	0.891	0.25-0.50
37	70	14	11	3	10.50	3.50	0.023	0.75-0.90
38	72	17	13	4	12.75	4.25	0.019	0.75-0.90
39	73	15	12	3	11.25	3.75	0.067	0.50-0.75
40	77	18	14	4	13.50	4.50	0.019	0.75-0.90
41	78	19	15	4	14.25	4.75	0.052	0.50-0.75
42	82	20	16	4	15.00	5.00	0.167	0.50-0.75
43	86	21	17	4	15.75	5.25	0.206	0.50-0.75
44	89	16	13	3	12.00	4.00	0.146	0.50-0.75
45	90	15	12	3	11.25	3.75	0.067	0.50-0.75
46	92	14	11	3	10.50	3.50	0.023	0.75-0.90
47	93	15	12	3	11.25	3.75	0.067	0.50-0.75
48	96	14	11	3	10.50	3.50	0.023	0.75-0.90
	Total	850	664	186	637.5	212.5	4.406	0.025-0.050
			$\chi^2$ heterogeneity = 0.155		P value = 0.50-0.75			

(resistant): 1 (susceptible) ( $\chi^2 = 0.261$ ;  $P = 0.50 - 0.75$ ) (Table 71). Further, the backcross population of 75 plants segregated into 36 (resistant): 39 (susceptible) giving a good fit for 1: 1:: resistant : susceptible with a ( $\chi^2 = 0.121$  ; $P = 0.50 -0.75$ ) (Table 71).

### **Correlation between trichomes and resistance to podborer**

Density of trichome A was positively correlated to the densities of trichome B and D in all three crosses (ICP 28 x ICPW 94, ICP 28 x ICPW 130 and ICP 26 x ICPW 125), but was significantly correlated negatively to the density of trichome C in ICP 28 x ICPW 94 ( $r = -0.19$ ), ICP 28 x ICPW 130 ( $r = -0.15^*$ ) and ICP 26 x ICPW 125 ( $r = -0.19^{**}$ ). Density of trichome A was positively correlated with percent bud damage in two crosses ICP 28 x ICPW 94 and ICP 28 x ICPW 130, and was significantly correlated positively in ICP 26 x ICPW 125 ( $r = 0.19^{**}$ ). Highly significant positive correlation was observed between density of trichome A and percent flower and pod damage in all the three crosses, ICP 28 x ICPW 94 ( $r = 0.09$  and  $r = 0.25^{**}$ ), ICP 28 x ICPW 130 ( $r = 0.35^{**}$  and  $r = 0.16^{**}$ ) and in ICP 26 x ICPW 125 ( $r = 0.34^{**}$  and  $r = 0.45^{**}$ ) (Table 78). Density of trichome A was positively correlated to number of eggs and larvae on the inflorescences of  $F_2$  plants.

Density of trichome B was positively correlated to the densities of trichome C and D, percent bud , flower and pod damage, number of eggs and larvae, on the inflorescence, in all three crosses.

Density of trichome C was significantly positively correlated with the density of trichome D in crosses ICP 28 x ICPW 94 ( $r = 0.15^*$ ) and ICP 26 x ICPW 130 ( $r = 0.11^*$ ) and was positively correlated in ICP 28 x ICPW 130. Highly significant negative correlation was observed between density of trichome C and percent bud, flower and pod damage in ICP 28 x ICPW 94 ( $r = -0.51^{**}$ ,  $-0.53^{**}$  and  $-0.22^{**}$ ), in ICP 28 x ICPW 130 ( $r = -0.46^{**}$ ,  $-0.41^{**}$  and  $-0.59^{**}$ ) and in ICP 26 x ICPW 125 (

**Table - 76: - Resistant and Susceptible Plants against Podborer in F<sub>2</sub> and Segregating F<sub>3</sub> Progenies**

Gene -ration	Total No. of plants	No. of plants Segregating		$\chi^2$ value (3:1)	P value
		Resistant	Susceptible		
ICP 28 x ICPW 94					
F <sub>2</sub>	250	185	65	0.133	0.500-0.750
F <sub>3</sub>	892	687	205	1.937	0.100-0.250
Total	1142	872	270	1.123	0.250-0.500
$\chi^2$ heterogeneity				0.947	0.250-0.500
ICP 28 x ICPW 130					
F <sub>2</sub>	250	183	67	0.432	0.500-0.750
F <sub>3</sub>	1079	846	233	6.676	0.005-0.010
Total	1329	1029	300	4.173	0.025-0.050
$\chi^2$ heterogeneity				2.935	0.050-0.100
ICP 26 x ICPW 125					
F <sub>2</sub>	216	158	58	0.395	0.050-0.750
F <sub>3</sub>	850	664	186	4.406	0.025-0.050
Total	1066	822	244	2.533	0.100-0.250
$\chi^2$ heterogeneity				2.268	0.050-0.100

**Table - 77: Resistant and Susceptible Plants against Podborer in F<sub>2</sub>, BC<sub>1</sub> F<sub>1</sub> and Segregating F<sub>3</sub> Progenies**

Generation	Cross	Total No. of plants	No. of resistant plants	No. of susceptible plants	$\chi^2$ value (3:1)	P value
F <sub>2</sub>	ICP 28 x ICPW 94	250	185	65	0.133	0.500-0.750
F <sub>2</sub>	ICP 28 x ICPW 130	250	183	67	0.432	0.500-0.750
F <sub>2</sub>	ICP 26 x ICPW 125	216	158	58	0.395	0.050-0.750
Total		716	526	190	0.901	0.250-0.500
<b>Heterogeneity</b>					<b>0.059</b>	<b>0.75-0.90</b>
F <sub>3</sub>	ICP 28 x ICPW 94	892	687	205	1.937	0.100-0.250
F <sub>3</sub>	ICP 28 x ICPW 130	1079	846	233	6.676	0.005-0.010
F <sub>3</sub>	ICP 26 x ICPW 125	850	664	186	4.406	0.025-0.050
Total		2821	2197	624	3.005	0.025-0.050
<b>Heterogeneity</b>					<b>10.014</b>	<b>0.005-0.001</b>
					$\chi^2$ (1:1)	
BC <sub>1</sub> F <sub>1</sub>	ICP 28 x ICPW 94	112	53	59	0.322	0.500-0.750
BC <sub>1</sub> F <sub>1</sub>	ICP 28 x ICPW 130	106	51	55	0.151	0.500-0.750
BC <sub>1</sub> F <sub>1</sub>	ICP 26 x ICPW 125	75	36	39	0.121	0.500-0.750
Total		293	140	153	0.576	0.250-0.500
<b>Heterogeneity</b>					<b>0.018</b>	<b>0.90-0.95</b>

**Table 78: Correlations between the densities of different Trichome types with the percent damage in F<sub>2</sub> populations of the interspecific crosses**

		Density of Trichome A (no./mm <sup>2</sup> )	Density of Trichome B (no./mm <sup>2</sup> )	Density of Trichome C (no./mm <sup>2</sup> )	Density of Trichome D (no./mm <sup>2</sup> )	% Bud damage	% Flower damage	% Pod damage	Number of eggs
Density of Trichome B (no./mm <sup>2</sup> )	Cross 1 Cross 2 Cross 3	0.09 0.08 0.09							
Density of Trichome C (no./mm <sup>2</sup> )	Cross 1 Cross 2 Cross 3	-0.09 -0.15* -0.19**	0.04 0.03 0.04						
Density of Trichome D (no./mm <sup>2</sup> )	Cross 1 Cross 2 Cross 3	0.08 0.07 0.03	0.06 0.05 0.04	0.15* 0.10 0.11					
% Bud damage	Cross 1 Cross 2 Cross 3	0.12 0.04 0.19**	0.05 0.07 0.06	-0.51** -0.46** -0.49**	-0.19** -0.06 -0.07				
% Flower damage	Cross 1 Cross 2 Cross 3	0.09 0.35** 0.34**	0.02 0.04 0.02	-0.53** -0.41** -0.35**	-0.06 -0.09 -0.28**	0.51** 0.22** 0.19**			
% Pod damage	Cross 1 Cross 2 Cross 3	0.25** 0.16* 0.45**	0.05 0.02 0.03	-0.22** -0.59** -0.74**	-0.10 -0.06 -0.17*	0.11* 0.14* 0.11	0.21** 0.35** 0.32**		
No. of eggs	Cross 1 Cross 2 Cross 3	0.09 0.18* 0.06	0.06 0.02 0.02	-0.42** -0.15* -0.21**	-0.06 -0.02 -0.05	0.11 0.12 0.15**	0.15* 0.08 0.09	0.54** 0.42** 0.59**	
No. of larvae	Cross 1 Cross 2 Cross 3	0.16* 0.07 0.14*	0.09 0.08 0.07	-0.15** -0.25** -0.09	-0.04 -0.05 -0.19*	0.15* 0.19* 0.15*	0.22** 0.21** 0.31**	0.25** 0.39** 0.35**	0.54** 0.26** 0.29**

• \*\* Significant at 1% level, \* Significant at 5% level  
• Cross 1 = ICP 28 x ICPW 94, Cross 2 = ICP 28 x ICPW 130 and Cross 3 = ICP 26 x ICPW 125

negatively correlated to the number of eggs and larvae on the inflorescences in crosses ICP 28 x ICPW 94 ( $r = -0.42^{**}$  and  $-0.15^{*}$ ), ICP 28 x ICPW 130 ( $r = -0.15^{*}$  and  $-0.25^{**}$ ) and in cross ICP 26 x ICPW 125 ( $r = -0.21^{**}$  and  $-0.09$ ).

Density of trichome D was significantly correlated negatively to the percent bud damage and flower damage in all three crosses, but was significantly negatively correlated to the percent pod damage in cross ICP 28 x ICPW 94 ( $r = -0.14^{**}$ ) and in cross ICP 26 x ICPW 125 ( $r = -0.19^{**}$ ) and negatively correlated in cross ICP 28 x ICPW 130. In all three crosses, the density of trichome D was negatively correlated to the number of eggs and larvae (Table 78).

Percent bud damage was significantly correlated positively to the percent flower and pod damage and number of eggs and larvae in all three crosses ICP 28 x ICPW 94 ( $r = 0.51^{**}$ ,  $0.11^{*}$ ,  $0.11^{*}$  and  $0.15^{**}$ ), ICP 28 x ICPW 130 ( $r = 0.22^{**}$ ,  $0.14^{*}$ ,  $0.12^{*}$  and  $0.19^{*}$ ) and ICP 26 x ICPW 125 ( $r = 0.19^{**}$ ,  $0.11^{*}$ ,  $0.15^{**}$ ,  $0.15^{**}$ ) (Table 78).

The percent flower damage was significantly correlated positively to the percent pod damage in all three crosses ( $r = 0.21^{**}$ ,  $0.35^{**}$  and  $0.32^{**}$ ), and positively correlated to the number of eggs and significantly correlated positively to the number of larvae in all three crosses ( $r = 0.22^{**}$ ,  $0.21^{**}$  and  $0.31^{**}$ ) (Table 78).

Percent pod damage was significantly positively correlated to the number of eggs and larvae present per inflorescence in crosses ICP 28 x ICPW 94 ( $r = 0.54^{**}$  and  $r = 0.25^{**}$ ), ICP 28 x ICPW 130 ( $r = 0.42^{**}$  and  $0.39^{**}$ ) and ICP 26 x ICPW 125 ( $r = 0.59^{**}$  and  $0.35^{**}$ ). Number of eggs per inflorescence was significantly positively correlated to the number of larvae in all three crosses ( $r = 0.54^{**}$ ,  $0.26^{**}$  and  $0.29^{**}$ ) (Table 78).

**Table 79: - Estimates of the A, C and D values for various morphological and agronomic characters in cross (ICP 28 x ICPW 94)**

	A	t-value	C	t-value	D	t-value
Days to flowering (50%)	-8.34±0.451	18.50*	0.75±0.868	49.15*	30.71±2.256	13.62*
Days to maturity (50%)	19.60±2.771	7.07*	40.22±1.464	27.46*	146.52±3.952	37.08*
Leaf length (cm)	-5.62±0.123	45.38*	-7.25±0.218	33.44*	-4.04±0.196	20.24*
Leaf width (cm)	-2.56±0.058	44.65*	-4.85±0.122	40.40*	-1.74±0.129	13.60*
Pod length (cm)	-3.05±0.102	29.99*	-4.19±0.177	23.37*	1.65±0.189	9.04*
Pod width (cm)	0.08±0.028	NS	0.86±0.094	9.01*	0.29±0.056	4.09*
Pod bearing length (cm)	-3.69±0.897	4.12*	-29.06±1.59	18.31*	54.74±1.956	28.18*
No. of locules /pod	-2.11±0.208	10.54*	-0.78±0.389	2.03*	0.65±0.275	2.27*
No. of seed per pod	-1.765±0.209	8.43*	-0.17±0.389	NS	4.54±0.318	14.42*
No. of primary branches	-10.69±0.441	24.23*	-5.34±0.535	10.04*	4.02±0.505	7.96*
No. of secondary branches	2.89±0.437	6.62*	5.67±0.576	9.85*	-13.57±0.421	32.33*
100 seed weight	-1.59±0.176	9.08*	-5.24±0.237	22.51*	-2.65±0.176	15.44*
Harvest index	-1.79±0.251	8.09*	-4.56±0.253	21.21*	-1.25±0.152	14.25*



**Table 80: Estimates of the A, C and D values for various morphological and agronomic characters in cross (ICP 28 x ICPW 130)**

	A	t value	C	t value	D	t value D
Days to flowering (50%)	5.79±0.792	7.39*	5.49±2.026	2.698*	14.86±1.448	10.26*
Days to maturity (50%)	49.02±2.561	19.38*	71.27±2.269	31.49*	47.09±2.118	22.20*
Leaf length (cm)	-5.71±0.165	34.55*	-10.91±0.188	57.91*	-3.23±0.186	17.31*
Leaf width (cm)	-2.21±0.075	30.07*	-5.48±0.094	58.41*	-1.32±0.129	10.26*
Pod length (cm)	-2.637±0.135	20.24*	-4.09±0.179	22.78*	2.67±0.184	14.65*
Pod width (cm)	0.14±0.039	3.89*	0.08±0.056	1.55*	0.42±0.036	11.61*
Pod bearing length (cm)	-9.29±0.854	10.99*	29.55±2.697	10.96*	46.34±2.413	19.20*
No. of locules /pod	-1.96±0.245	8.04*	-5.29±0.368	14.38*	1.65±0.235	6.89*
No. of seed per pod	-2.41±1.045	2.30*	-7.11±1.677	4.27*	1.72±1.567	1.49*
No. of primary branches	-1.12±0.304	3.69*	1.24±0.684	NS	-20.64±0.336	61.39*
No. of secondary branches	10.37±0.657	15.78*	14.86±0.898	16.52*	3.274±0.677	4.85*
100 seed weight	-9.56±0.195	49.68*	-9.67±0.318	30.51*	9.55±0.262	36.49*
Harvest index	-8.69±0.125	35.65*	-8.52±0.412	25.41*	9.25±0.254	32.56*

**Table 81:- Estimates of the A, C and D values for various morphological and agronomic characters in cross (ICP 26 x ICPW 125)**

	A	t value	C	t value	D	t value
Days to flowering (50%)	0.29 ± 0.792	NS	14.33 ± 1.09	13.13*	-6.59 ± 1.432	4.66*
Days to maturity (50%)	31.69 ± 1.53	20.72*	54.56 ± 1.42	38.52*	55.95 ± 1.452	39.25*
Leaf length (cm)	-6.98 ± 0.159	44.48*	-13.31 ± 0.165	81.52*	-1.51 ± 0.281	6.36*
Leaf width (cm)	-3.66 ± 0.789	46.24*	-5.38 ± 0.115	45.59*	-1.61 ± 0.129	12.62*
Pod length (cm)	-2.39 ± 0.168	14.56*	-11.38 ± 0.175	66.46*	6.52 ± 0.219	29.83*
Pod width (cm)	0.11 ± 0.033	3.16*	5.97 ± 0.186	32.56*	-2.58 ± 0.092	27.96*
Pod bearing length (cm)	-15.03 ± 0.966	15.55*	-8.78 ± 2.75	3.175*	40.64 ± 2.53	16.09*
No. of locules /pod	-2.92 ± 0.302	9.65*	-7.64 ± 0.435	17.75*	2.59 ± 0.258	9.081*
No. of seed per pod	-1.65 ± 0.277	5.85*	-5.98 ± 0.458	13.73*	-0.83 ± 0.288	2.94*
No. of primary branches	-1.52 ± 0.259	5.84*	-7.06 ± 0.481	14.68*	-1.06 ± 0.273	3.89*
No. of secondary branches	0.38 ± 0.806	NS	-11.32 ± 0.945	11.64*	4.56 ± 0.416	11.17*
100 seed weight	-1.69 ± 0.195	9.79*	-5.24 ± 0.237	22.51*	-2.65 ± 0.176	15.44*
Harvest index	-1.26 ± 0.156	8.52*	-4.56 ± 0.356	21.56*	-2.51 ± 0.245	14.21*

# DISCUSSION

## DISCUSSION

Sustained progress in purposeful plant breeding rests on the availability of genetic diversity, which refers to genome differences ranging from a single base pair to rearrangements of entire chromosomes. These variations in the genetic makeup, interacting with the environment, dictate the observable patterns of diversity shown by the multitude of living organisms. This genetic variation within and between species, generated by the process of mutation, sexual reproduction and selection, ensures its capacity in evolutionary change and ecological adaptation. Genetic diversity is also the basic raw material for developing improved genotypes aimed at maintaining and enhancing the productivity, stability and sustainability of agriculture.

Pigeonpea, an important pulse crop of semi – arid tropics, has long been considered a genetically diverse species. Breeding programmes have been helpful in the development of hybrids suitable for diverse agro- climatic conditions. However, the genetic upgradation is critically limited by the lack of adequate variability, especially for the pest and disease resistance. Studies, so far, on the mechanisms of inheritance for pod borer resistance and characters positively correlated to resistance are very limited.

Wild relatives of pigeonpea represent a potential genetic resource, which has not been explored in breeding, which could be used to effectively broaden the genetic base and enhance the pigeonpea breeding prospects. In view of this, the present investigation was undertaken to study the diversity among the wild pigeonpeas at the morphological, molecular and biochemical levels; and also to screen the accessions of *C. scarabaeoides* for pod borer resistance and to utilize the most resistant accessions in the breeding programs to introgress the pod borer resistant genes into cultivated background. Further, it is aimed at studying the genetic basis of qualitative and quantitative characters including mechanisms of resistance against pod borer and different types of trichomes.

## Diversity analysis

In the present investigation, diversity among 30 accessions of wild *C. scarabaeoides* and 6 cultivated varieties of pigeonpea, for different characters, has been studied at the morphological, molecular and biochemical levels.

### Morphological diversity analysis

Days to flower, pod width and number of locules per pod, among 13 traits studied, varied significantly in both the seasons, indicating the contribution of environmental influence governing these traits. Interaction between season and plant habit was also significant for days to flower, indicating that this trait was influenced by both the season and plant habit. Significant genotypic differences in all the traits except for days to maturity, leaf area, leaf dry weight and number of primary branches, indicated that accessions differed with each other significantly.

*C. scarabaeoides*, as a group, took more number of days to flower and mature than the *C. cajan* genotypes. However, the early flowering accessions of wild (ICPW 83, ICPW 86, ICPW 90, ICPW 96, ICPW 98 and ICPW 101) flowered earlier than the cultivated varieties. Heritability was high for all the traits studied, except for days to flower and number of seeds per pod, indicating that the environmental influence was very meager in explaining the expression of this trait.

The PcoA and dendrogram of morphological data effectively brought out the intraspecific differences among the accessions. Accessions of *C. scarabaeoides* formed a separate group from that of the *C. cajan* varieties. The early, mid and late flowering accessions formed different subclusters under the major *C. scarabaeoides* cluster. Accessions, which are highly resistant to podborer, formed a separate sub-cluster from the other moderately resistant accessions. A similar type of study in sorghum did not show intraspecific differences among the different accessions of a single species (Kamala, 2003).

## Molecular diversity analysis

Genetic diversity, generated by selection, mutation and sexual reproduction, rests on the genome changes ranging from a single base pair to rearrangements of the entire chromosomes. These nucleotide level changes are reflected in phenotypic differences among individuals, at increasingly higher levels of cellular organization, ranging from variations in amino acid sequences of proteins to morphological, physiological, chemical and behavioral characteristics. Classical methods of estimating genetic diversity and / or relatedness among groups of plants rely upon phenotypic (observable) traits. However, there are two disadvantages; the traits are subjected to environmental influences and the level of polymorphism (allelic variation) expressed could be limited. The deployment of environment neutral biochemical markers the isozymes, protein electrophoresis (Hunter and Markert, 1957) and molecular markers have circumvented these limitations by focusing directly on the variation at the level of genes, the DNA itself. The higher resolution of molecular markers make them a valuable tool for a variety of purposes, such as fingerprinting, facilitating the appropriate choice of parents for breeding programs, analyzing quantitative traits, location and detection of quantitative trait loci (QTLs), gene mapping, marker assisted selection, gene transfer, understanding evolutionary pathways and for assessing the genetic diversity of plant germplasm.

Classical methods of estimating genetic diversity, and / or relatedness, among plants have relied on the morphological (phenotypic) traits. The present study revealed a large phenotypic variability and variation for resistance to podborer. Analysis of quantitative traits helped to obtain broad differences among *C. scarabaeoides* accessions. However, the relationships at lower levels of biological organizations were not evident. For instance, the accessions belonging to three different flowering duration groups (early, medium and late) were grouped separately for almost all morphological and agronomic characters. But with the molecular markers, the differences within these subgroups could be better understood, though the basic grouping did not change. In order to better understand, the extent and distribution of diversity among the wild *Cajanus* species a subset of the accessions available at ICRISAT, were analyzed at the molecular levels using a). nine enzyme - maize mitochondrial DNA probe combinations, b) five AFLP primer combinations and c) ten SSR primer pairs.

## Diversity analysis using RFLP markers

Hybridization of total cellular DNA to defined mitochondrial DNA sequences facilitates the detection and characterization of organelle genomes without undertaking the complicated procedure of isolating purified DNA from small amounts of tissue. Thus, total DNA can provide suitable source for identifying mitochondrial gene in cases where the plant material is scarce. Studies in wheat (Timms and Scott, 1985) suggest that sequences related to *cox I* probes are not present in the nuclear genome. Lonsdale (1985) reported that the sequences homologous to the chloroplast genome were absent for the *cox I* probes used. However, in the present study, the possibility of cross hybridization between mitochondrial and non-mitochondrial probes, to a limited extent, cannot entirely be ruled out.

Evidences from the data, obtained from different sets of molecular markers, revealed the inherent relationships among different species of the wild belonging to the secondary gene pool, of the pigeonpea and the cultivated primary gene pool. The strong hybridization signals obtained with three mitochondrial DNA probes in all the 42 accessions belonging to four species reflect the high homology between the maize and pigeonpea mitochondrial DNA. Different sizes of bands were obtained with all the three multi-copy probes. Different relative intensities observed in some bands of *EcoRI* - *atp a* and *EcoRI* - *atp 6* combination suggest variation in the copy number of these genes. Sivaramakrishnan *et al.* (2001), in the assessment of genetic diversity, observed similar results in six wild *cajanus* species. Only one of the banding patterns obtained by *EcoRI* - *cox1* combination was shared among *C. scarabaeoides*, *C. sericeus* and *C. cajan* and none other enzyme probe combinations were shared between any of the species suggests the highly conserved nature of the *cox 1* genes. While two banding patterns each, were shared between these species for *EcoRI* - *atp 6* and *Hind III* - *atp a* and one each for *Hind III* - *atp 6* and *EcoRI* - *atp a*. Organellar genomes, such as mitochondrial genomes, were supposed to detect inter-specific variations more efficiently than the intra-specific variations. However in the present study that even intra-specific variation could be detected efficiently as exemplified in *C. scarabaeoides*. Similar results were reported in the intraspecific variation studies of *Sorghum bicolor*, *S. halepense* and *S. purpureoserium* (Kamala, 2003). Wang *et al.*, (1996) reported that, the attempts to

detect intraspecific differences in coding sequence of the mt genome have often proved unfruitful.

The PcoA of RFLP data placed the *C. scarabaeoides* accessions of Australia, India and Sri Lanka in 3 different sub- groups, based on geographic origin. This indicates the differences in the genetic makeup of these accessions. The use of mt. DNA probes RFLP marker for diversity analysis among Pigeonpea accessions of wild and cultivated pigeonpeas suggests the conserved nature of mitochondrial genome among the cereals and legumes (Sivaramakrishnan *et al*; 2001).

### **Diversity analysis using AFLP markers**

The AFLP markers revealed higher levels of polymorphism in the wild accessions *C. scarabaeoides*, *C. sericeus*, *C. reticulatus* (97%) compared to the cultivated genotypes of *C. cajan* (27%). The use of AFLP markers revealed high levels of polymorphism among the cultivated species of barley, maize, and millet ( Rao *et al.*, 1997; Cervera *et al.*, 1998; Law *et al.*, 1998) compared to the polymorphism exhibited by cultivated pigeonpeas in the present study. In contrast to the low levels of genetic variation observed among cultivated pigeonpea lines, the intraspecific variation among *C. scarabaeoides* accessions was significantly higher. This is evidenced by the larger diversity index values obtained from the AFLP profiles of cultivated and wild pigeonpea accessions. One of the accessions, ICPW 147 (*C. scarabaeoides*, India), showed very unique AFLP banding pattern in all the primer combinations, and grouped separately from the other Indian accessions. This accession is highly resistant to podborer, in the field-screening and is used in the crossing program for introgression of pod borer resistance gene into pigeonpea cultivars. PcoA of RFLP data revealed distinct groupings of all the four species. Accessions of *C. reticulatus* formed a distinct group in all the marker techniques. The separation of *C. reticulatus* from other species agrees well with the distinct morphological and phenoloical characteristics of this species, such as plant habit, days to flower and maturity and the country of origin.

Comparison of groupings obtained using different subsets of AFLP markers, with primer combinations, which generated more than 40 polymorphic bands were sufficient for classification of major groups in wild species of pigeonpea. The present study suggests, that



with appropriate reaction conditions and specific combination of selective primers, it is possible to yield a sufficient number of polymorphic bands to allow the meaningful comparison among cultivated and wild accessions and even within species. In some cases, a single primer combination was found to be capable of providing a sufficient number of data points (primer combination E-ACG M-CTC >50 polymorphic fragments) to distinguish between highly related individuals. Using multiple primer combinations, it was able to build data sets providing clear and conclusive relationships among the accessions and defining evolutionary relationships among species. Similar results were observed in *Nicotiana* (Nan Rex *et al.*, 2000). Approximately, one quarter of fragments amplified from the various accessions of *C. cajan* and *C. scarabaeoides* were polymorphic and provided sufficient resolution to distinguish closely related accessions. Interestingly, AFLP analysis indicates that accessions from different geographical locations with similar morphological characters and days to flowering tend to cluster based upon their profiles, supporting the fact that the traits have a genetically definable basis. Similar results were obtained in a study of morphological and molecular diversity analysis of cultivated and wild accessions of *Nicotiana* (Nan Rex *et al.*, 2000)

The groupings obtained by PCo Analysis are very much in agreement with the groupings derived from the dendrograms. Definite grouping, of different species, revealed the accurate resolution power of the marker system and its ability for its use in any of the interspecific linkage mapping.

### **Diversity analysis using SSRs**

Seven out of ten microsatellites have amplified the alleles in all the accessions of wild and cultivated, while two of the remaining three (CCB 3 and CCB 4) microsatellites have amplified alleles only in the cultivated genotypes. This might be because the microsatellites in *Cajanus* were designed based on the genome of cultivated accessions. Though the SSR markers were limited in number, yet they were highly polymorphic and revealed maximum diversity index among the accessions. The high diversity obtained with SSRs is consistent with their known characteristics –that they are more variable, and provide higher resolution and higher expected heterozygosity than the RFLPs, RAPDs or AFLPs (Pejei *et al.*, 1989;

Powell *et al.*, 1996, Taramino and Tingey, 1996). The high levels of polymorphism associated with SSRs are expected because of the unique mechanism responsible for generating SSR allelic diversity by replication slippage (Tautz and Renz, 1984; Tautz *et al.*, 1986) rather than by simple mutations, insertions or deletions.

The interspecific variation was very clear in accessions of Australian, Sri Lankan and Indian origin (Fig. 11 and 12). Some SSR primer pairs (primers 5 and 6) revealed higher levels of polymorphism within the cultivated types than the wild genotypes. RFLP analysis using mtDNA showed variation at both inter- and intra-specific level which was similar to the earlier observations made in pigeonpea (Sivaramakrishnan *et al.*, 2001) and sorghum (Kamala, 2003). In the present study, RFLP markers could bring out the intra-specific variation among the *C. scarabaeoides* accessions and also differences among the cultivated varieties of pigeonpea. RFLP markers were found to be more efficient in bringing out the variation among the wild and cultivated species of pigeonpea in contrast to AFLP and SSR markers. But the RFLP markers were confined to the mitochondrial genome and not to the whole pigeonpea genome unlike the other two markers.

### **Comparison of three molecular markers used in diversity analysis**

The analysis of data obtained by using RFLP, AFLP and SSR markers revealed significant differences among the four species. The major objective of the study was to determine the intraspecific variation among the *C. scarabaeoides* accessions, belonging to the secondary gene pool of pigeonpea. The species is easily crossable to cultivated and has many important features like high protein content, resistance to podborer and pod wasp. Only a few cultivated pigeonpeas were included to choose the most diverse parents based on the morphological molecular and resistance screening information. Very little information is available on the use of molecular variation at the inter- and intra- specific level among the wild and cultivated *Cajanus* species (Ratnaparkhe *et al.* 1999; Parani *et al.* 2000; Sivaramakrishnan *et al.* 2001).

The present investigation clearly demonstrates that the use of all three marker techniques to study the genetic diversity among the wild and cultivated pigeonpea genotypes is very appropriate. Though, the results were more or less similar in all the three-marker

analysis the SSRs were found to be more efficient than AFLP and RFLP markers. The study suggests that only two or three AFLP primer combinations (82-121 markers) would be sufficient to estimate the similarities accurately between the major groups. Further, little advantage would be gained from generating larger sets of data. In practice, that any accession can be fingerprinted using only two or three tracks of sequencing gel. However, this observation is limited to the material under study, and clearly more markers are needed for more closely related varieties, such as cultivar collections or for pedigree analysis. Scoring is a drawback in AFLP, as the number of bands produced makes the job very tedious and strenuous. While the co-dominant nature of microsatellite marker (SSRs) and the almost 100% transferability of the map positions makes them preferable to AFLP. More studies are required to compare the relative merits of the two approaches. In the present study, though SSR primers were designed based on the pigeonpea genome, the 10 primers used were able to bring out the diversity among the accessions. However, the RFLP assay requires large amount of DNA and frequent use of radioisotopes in detection method, makes it technically demanding, costly to characterize large number of samples. It is perhaps relevant to consider SSRs as the logical replacement of the RFLP, especially for the linkage studies and AFLP as more robust and productive replacement for RAPD technology. SSRs can be used to provide co-dominant anchor markers for mapping studies, but the development and application costs may hinder their application in large numbers needed to study large germplasm collections. It is likely that strategies utilizing the combination of two marker techniques might prevail in the coming years.

The results of this study conclude that in accessions of the same region have more genetic similarities than do populations of the same species derived from different geographical regions. Similar results were obtained in a study of conspecificity of foxtail millet and green foxtail millet (Prasada Rao *et al.*, 1987 and Wang 1995a).

The levels of polymorphism revealed by three techniques did not differ considerably, as all the marker techniques yielded highly polymorphic bands ranging from 93.5 % with RFLP, to 95.4% with AFLP to 100% with SSRs. Similar results were observed in wild and cultivated *Sorghum* species (Kamala, 2003), where the levels of polymorphism ranged from 60% in AFLPs, 80% in RFLPs and 100% in SSRs. Russel *et al.*, (1997) compared SSRs with

AFLPs in barley where the polymorphism was 49% in AFLPs and 100% in SSRs. Most of the studies with SSRs have revealed the highest level of polymorphism compared to other molecular markers (Wu and Tanksley 1993; Rus-Kortekas *et al.*, 1994; Saghai Maroof *et al.*, 1994; Morgante *et al.*, 1994; Salimath *et al.*, 1995; Maughan *et al.*, 1995; Powell *et al.*, 1996). Although, AFLPs do not offer high levels of polymorphism but they are the most efficient because they have the capacity to reveal many polymorphic bands in a single lane. In the present study, the average number of AFLP bands per lane, or per PCR, was 46 compared to a single band per lane of SSRs.

Gene diversity is a function of both allelic richness and allelic evenness. In this study, both allelic richness, where 177 out of 182 loci were found to be polymorphic. Most of the alleles were present in all accessions except in *C. reticulatus*. This could be due to less gene diversity in *C. reticulatus*. Low values of diversity index were also seen in *C. reticulatus* with SSR markers ( $H = 0.41$ ), compared to other species. This could be due to the involvement of only one accession in this species in this study.

In the present study, the three molecular markers revealed close proximity between *C. sericeus* and *C. scarabaeoides* with that of *C. cajan* compared to *C. reticulatus*. RFLP analysis of r DNA by Parani *et al.*, (2000) revealed a close proximity between *C. scarabaeoides* with *C. cajan* while *C. reticulatus* was more closer to *C. platycarpus* belonging to tertiary gene pool.

All the molecular marker techniques revealed inter- and intra- species differences in pigeonpea. A similar study in sorghum did not bring out such differences suggesting that not only the marker systems but also the crop can make the difference (Kamala, 2003). Three marker systems were equally efficient in placing 31 accessions of *C. scarabaeoides* from India, Sri Lanka, Australia, Myanmar, Indonesia and Philippines into separate groups with minor differences. In the major cluster, at the subspecies level the subgroups were based on the geographical origin and maturity (early, medium and late flowering genotypes). All accessions of *C. scarabaeoides* originating from Sri Lanka were grouped together and those from Australia were in a different cluster. The grouping of *C. scarabaeoides* accessions of India further differentiated into subgroups based on days to flowering (early, medium and

late) and also based on resistance to pod borer which was the criteria used in the initial selection of accessions.

The similarities between accessions from the same geographical origin and also on the resistance levels against podborer revealed by the morphological groupings were further confirmed by the molecular marker studies. The collective information can be used in the breeding programs, conservation of germplasm and management of genetic resources. Evaluation of landraces and wild relatives based on morphological, resistance related characters and biochemical characters along with molecular markers is critical to exploit the genetic potential for improvement of traits needed for pest resistance, protein content etc. Wild relatives represent a large proportion of the total genetic variation (Miller and Tanskley, 1990) and may not display characters of interest but it is likely that they possess alleles that can improve the character. Genetic diversity can be used to maximize the level of variation present in segregating population by intermating the accessions with greater genetic distance. The results of this study, together with the results of other morphological, biochemical and resistance related characters might help in the selection of the most diverse parents for podborer resistance related characters and greatly expand the genetic variation in pigeonpea improvement.

### **Biochemical diversity analysis**

In crop plants, resistance to insects can be mediated by a wide range of metabolic products; including lectins, proteinase and amylase inhibitors and secondary metabolites, like tannins, alkaloids, rotenoids volatiles etc. In the present study, the lectin content was more in the wild pods compared to the cultivated ones. Further, the pods at juvenile stage had more lectins than the immature pods, while the mature pods had no lectins indicating a decrease in the lectin levels with increasing pod maturity stages. ICPW 138 and ICPW 98 juvenile pods had almost three times the lectin content compared to its content, in the juvenile pods of *C. cajan* genotypes. Stage and genotype x stage interaction were highly significant indicating the significant differences among the genotypes and pod stages for the lectin content.

Similar results were obtained when the leaves and developing pods of two *Cajanus* species were assayed for lectin content. The lectin content was maximum in the juvenile pods followed by the immature pods while no lectins were noticed in mature pods (Sonali, 2001).

Trypsin inhibitors have been found to be effective insecticidal proteins. Maturity stage was found to be significant in *C. scarabaeoides* and *C. cajan* group. The inhibitor levels were higher in *C. scarabaeoides* accessions than in the cultivated genotypes. The mature pods of both in the cultivated and the wild accessions showed more trypsin inhibitor levels than the immature and juvenile pods. Unlike lectins, the levels of trypsin inhibitors increased with the increase in maturity levels of pods. However, Pichare and Kachole (1994) did not find any variability in the number of electrophoretic forms of proteinase inhibitors, among the 20 accessions of pigeonpea and ten related wild species analysed. No significant differences were observed in the trypsin and chymotrypsin levels in *H. armigera* tolerant and susceptible varieties. In the present investigation, both the lectins and trypsin inhibitors were found to be conferring resistance to the accessions; the lectins at the juvenile pod stage while the trypsin inhibitors at the mature pod stage.

## Trichome types and density

The five types of trichomes described in pigeonpea by Romies, (1997) have been found on the wild species *C. scarabaeoides* accessions. *C. scarabaeoides* accessions differed significantly in the densities of different types of trichomes (A, B, C and D) on pods. The density of trichome type C was the highest followed by the trichome type B and type D. The density of type C and D trichomes, the two non-glandular types, was 2 to 20 times higher on *C. scarabaeoides* pods than on the *C. cajan* pods. Similar observations were made on the trichome densities of *C. cajan*, *C. scarabaeoides* and *C. platycarpus* (Romies, 1997). Trichome type A was found on the pigeonpea pods but was almost absent in most of the *C. scarabaeoides* pods and even if they were present, the density was very less. Romies, (1997) have reported the complete absence of the trichome type A on *C. scarabaeoides* pods. Density of trichome types A, C and D varied significantly among the pods of different pigeonpea genotypes. Pods of pigeonpea had higher densities of trichome type C followed

by type D and A. Type B was the rarest among the pigeonpea genotypes examined. Similar observations were made on the density of trichomes in ICPL 87, a cultivated pigeonpea genotype ( Romies, 1997).

Exudates of glandular trichomes, such as type A, might act as deterrents against small, soft bodied insects (Obrycki, 1986; Peter *et al.*, 1995). But the type A exudates do not affect the hatchability of *H. armigera* eggs and it is not known whether they trap and kill *H. armigera* larvae (Shanower *et al.*, 1997). There is an indication that the exudates contain a feeding stimulant for *H. armigera* larvae (Shanower *et al.*, 1997).

The function of type B trichomes is not well known. However, Bisen and Sheldrake (1981) suggested that this trichome is the source of characteristic pigeonpea fragrance. The secretion by type B trichome is caused only when the cell wall is ruptured. This could be caused by a chewing insect, such as *H. armigera* larvae or by the abiotic factors such as high temperatures or low humidity (Ascensao *et al.*, 1995). Bisen and Sheldrake (1981) considered type E to be a developmental stage of the type B trichome. But in this study, no intermediate forms were found, indicating that type E is a separate trichome type. Morphologically similar trichome has been described in cowpea (Oghiakie *et al.*, 1992).

Type C, non- glandular trichomes on the pods of *C. scarabaeoides*, might be conferring resistance against *H. armigera*. The mortality of small larvae was significantly higher on the pods of *C. scarabaeoides* compared to the *C. cajan* or *C. platycarpus* (Shanower *et al.*, 1997). This may be due to the presence of much higher density of type C trichomes on *C. scarabaeoides*, which prevented the larvae from reaching the pod surface. They further stated that the distribution and size of trichomes on pigeonpea leaves were significantly different from those on the reproductive structures. In this study, type A glandular trichomes on some of the *C. scarabaeoides* accessions have been reported for the first time. The earlier studies conducted on eight accessions of *C. scarabaeoides* could not detect the presence of type A trichomes (Shanower *et al.*, 1997). There were no significant differences in different seasons, between the types and density of trichomes. However, Southwood (1986) stated that the season, plant habit and developmental variation can affect the ontogeny and expression of trichomes. The trichomes and their exudates, on pigeonpea

pod surface are likely to play an important role in the selection of host and oviposition behaviour by herbivores such as *H.armigera* (Renwick and Chew, 1994). The trichomes and their sticky exudates on the reproductive plant structures will also interfere with the searching behaviour of the arthropod enemies, especially small parasitoids such as *Trichogramma* spp. (Shanower, 1999). The significance of trichomes in plant defense system against herbivores, has long been recognized (Challahan, 1957; Beck, 1965, Levin, 1973; Norris and Kogan , 1980; Stipanivic, 1983; Jermy, 1984).

## **Podborer resistance**

The *C. scarabaeoides* accessions; ICPW 83, ICPW 94, ICPW 116, ICPW 125, ICPW 130 and ICPW 141 did not show bud, flower and pod damage and had no eggs or larvae on the inflorescences, whereas ICPW 147, ICPW 281 and ICPW 305 exhibited little pod damage and showed no eggs and larvae on the inflorescences were grouped as resistant genotypes which can be utilized in the breeding program for production of interspecific hybrids. The early flowering *C. scarabaeoides* accessions; ICPW 83 and ICPW 94; the medium flowering accessions, ICPW 116, ICPW 125, ICPW 130 and ICPW 141 can be utilized in the breeding program to yield early flowering, pod borer resistant hybrids. Sharma *et al.* (2001) screened several wild accessions of *C. scarabaeoides* and *C. sericues* and reported that the accessions; ICPW 83, ICPW 90, ICPW 94, ICPW 116, ICPW 125, ICPW 130, ICPW 137, ICPW 141, ICPW 152, ICPW 278, ICPW 280 and ICPW 281 exhibited less than 10% pod damage and had no eggs and larvae on the inflorescence.

Correlation among traits may result from pleiotropy or physiological associations among characters. Correlation coefficients indicate the degree and direction of association between different traits. They help in deciding a suitable selection criterion for the genetic improvement of complex associated characters. When two or more traits are considered, the correlation studies have been found to be useful in describing the associations, and often indicate useful selection indices. Correlation among the characters related to podborer resistance and some physical attributes of the pods and leaves prove to be important selection index for handling this very complicated menace of *Helicoverpa*.



The correlation studies indicate that the pods having high densities of trichome types B, C and D were more resistant than those having lower densities of these trichomes or with a higher density of trichome A. Early flowering accessions were less susceptible to pod borer attack than the late flowering accessions. Plants having more number of primary and secondary branches, small leaves, longer pods, lighter seeds, pod wall surface with higher density of the trichomes B, C and D were less damaged by podborer. However, virtually no significant correlation, between the plant traits and resistance to either of the two major diseases of chickpea, *Aschochyta blight* and *Fusarium wilt* was noticed (Singh *et al.*, 1983).

## Interspecific hybridisation

Interspecific crosses were made between the wild accessions of *C. scarabaeoides* and cultivated varieties of *C. cajan* to raise F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> generations to study the genetic basis of qualitative and quantitative traits including resistance against podborer and its related traits. Besides, the F<sub>1</sub> hybrids of these crosses were also backcrossed to the cultivated parents to obtain BC<sub>1</sub>F<sub>1</sub>, BC<sub>1</sub>F<sub>2</sub>, BC<sub>2</sub>F<sub>2</sub> and BC<sub>3</sub>F<sub>1</sub> seed to introgress the genes from the wild to cultivated genotypes. The five *C. scarabaeoides* accessions used as wild parents and two cultivated varieties of *C. cajan* genotypes used in the wide hybridization differed significantly for most of the characters studied.

The *C. scarabaeoides* parents flowered and matured earlier than *C. cajan* parents, except the parents involved in the cross ICP 26 x ICPW 141 where both the parents matured almost at the same time. Parents differed significantly for leaf length and width, pod length, number of secondary branches, 100- seed weight, seed protein, density of trichomes A, B, C and D in all the ten crosses. Differences among parents were non-significant for pod width in crosses ICP 28 x ICPW 94, ICP 26 x ICPW 94, ICP 26 x ICPW 116, ICP 26 x ICPW 125, ICP 26 x ICPW 130 and ICP 26 x ICPW 141. Parents involved in the crosses ICP 28 x ICPW 94, ICP 28 x ICPW 116, ICP 28 x ICPW 125, ICP 26 x ICPW 94, ICP 26 x ICPW 116, ICP 26 x ICPW 125, ICP 26 x ICPW 130 and ICP 26 x ICPW 141 did not differ significantly for number of locules per pod. Significant differences for seeds per pod were not observed in crosses ICP 28 x ICPW 94, ICP 28 x

ICPW 116, ICP 26 x ICPW 94 and ICP 26 x ICPW 116. In cross ICP 28 x ICPW 94 the parents did not differ significantly for number of primary branches but in all other crosses significant differences were observed in all the crosses for this character.

Direct crosses gave fertile seed in all the crosses attempted but the reciprocal crosses made with pigeonpea parent as the male and *C. scarabaeoides* parent as the female did not give many seeds. This might be because of inhibition of the fertilization by the cytoplasm of the wild. The hybrids were pollen rich and highly fertile.

## Heterosis and inbreeding depression

Heterosis is the manifestation of heterozygosity in  $F_1$ s compared to their homozygous parents. It occurs both in self and pollinated species and is often exploited to increase the yield potential of crop plants. The magnitude of heterosis encountered in any crop species is of paramount importance in deciding as to whether or not heterosis breeding is practical. Inbreeding depression refers to decrease in the fitness and vigour due to inbreeding. Inbreeding depression is due to the fixation of unfavorable recessive genes in the  $F_2$ , while in case of heterosis the favourable dominant genes of the other parent cover the unfavourable recessive genes of one parent line. Heterosis might be useful in breeding if  $F_1$  and  $F_2$  performance was indicative of superiority and if inbreeding depression was lacking (Singh, and Choudhary, 1996) (Tables 33 - 42).

Mid-parent heterosis for days to flower was positive in 3 crosses (2 crosses with ICP 28 and 1 crosses with ICP 26) and negative in 7 crosses (3 crosses with ICP 28 and 4 cross with ICP 26). The negative heterosis indicates that hybrids of crosses, ICP 28 x ICPW 94, ICP 28 x ICPW 130, ICP 28 x ICPW 141, ICP 26 x ICPW 94, ICP 26 x ICPW 116, ICP 26 x ICPW 130 and ICP 26 x ICPW 141, flower earlier than the parents. Better parent heterosis was positive in 8 crosses (3 crosses with ICP 28 and 5 cross with ICP 26) and was negative in two crosses (ICP 28 x ICPW 94 and ICP 28 x ICPW 141). Among the latter two crosses, the hybrids of the first cross flowered earlier than the wild parent while the hybrids of second cross flowered earlier than the cultivated parent. Inbreeding depression, for days to flower, was positive in all crosses. The trend for days to flower indicates the preponderance of additive gene action governing this trait, which could be due to the

fixation of unfavorable recessive genes in F<sub>2</sub> population. Positive mid- and better- parent heterosis was reported earlier for days to flower in the pigeonpea hybrids of a cross Mukta (Medium duration flowering) x UPAS (Short- duration flowering) by Singh *et al.*, (1983) and in crosses of GAUT 82 -99 x ICP 9175 by Patel *et al.* (1992).

Mid-parent heterosis, for days to maturity, was positive in 2 crosses (one with ICP 28 and another with ICP 26) and negative in 8 crosses (4 with ICP 28 and 4 with ICP 26). Negative heterosis for days to maturity was observed in all the crosses except the crosses involving two cultivated pigeonpea parents with ICPW 94. Though, the hybrids obtained in a cross with ICPW 94 flowered earlier than the earliest parent (wild), yet it matured later than the late maturing parent (cultivated) which may be due to the masking / modification of gene(s) for flowering by those for maturity in this hybrid. Better parent heterosis was positive in 5 crosses (3 with ICP 28 and 2 with ICP 26) and negative in 5 crosses (2 with ICP 28 and 3 with ICP 26). Inbreeding depression was positive in all the crosses. Significant negative or positive values of heterosis for days to maturity followed by the positive inbreeding depression suggests the role of additive and non - additive gene action in governing the expression of this character, which could be due to the fixation of unfavorable genes in F<sub>2</sub> population. Positive mid- and better- parent heterosis for days to maturity in crosses between Mukta (Medium duration flowering) x UPAS (Short- duration flowering) was reported by Singh *et al.*, (1983) and in crosses of GAUT 82 -99 x ICP 9175 by Patel *et al.*, (1992).

Mid-parent heterosis, for leaflet length, was positive in 2 crosses (1 with ICP 28 and 1 with ICP 26) and negative in 8 crosses (4 with ICP 28 and 4 with ICP 26). Better parent heterosis was negative in all the 10 crosses. For leaflet length, the inbreeding depression was positive in all crosses, suggesting the preponderance of non - additive gene action in governing this trait.

Mid-parent heterosis for leaflet width was positive in 8 crosses (5 crosses with ICP 28 and 3 crosses with ICP 26) and negative in 2 crosses with ICP 26. Better parent heterosis was positive in 4 crosses with ICP 28 and negative in 6 crosses (1 with ICP 28 and 5 with ICP 26). High positive heterosis followed by inbreeding depression suggests the

preponderance of additive gene action and the negative heterosis followed by inbreeding depression indicated the preponderance of non-additive gene effects governing this character.

Mid-parent heterosis, for pod length, was positive in 2 crosses with ICP 28 and negative in 8 crosses (3 with ICP 28 and 5 with ICP 26). Better parent heterosis was negative in all the 10 crosses. Inbreeding depression, for pod length, was positive in all the crosses. Mid- and better parent negative heterosis followed by inbreeding depression suggests the non-additive gene action governing the expression of this character. The inbreeding depression might be due to the fixation of unfavorable recessive genes from the wild parent. Expression of mid- and better parent heterosis was poor and in the undesired direction in cultivated pigeonpea crosses of GAUT - 135 x ICP 84010, GAUT 84-54 x ICP 84010, GAUT - 83-17 x ICPL 332, GAUT 87-19 x ICP 9229 (Patel *et al.*, 1992). Similar observations were also reported in cultivated pigeonpea crosses (Saxena *et al.*, 1980; Singh *et al.*, 1983).

For pod width, the mid- and better parent heterosis, and the inbreeding depression were positive in all the 10 crosses. High positive mid- and better parent heterosis followed by inbreeding depression suggests that additive genes govern the expression of pod width. The increase in vigour could be due to the masking of the unfavorable recessive genes of the wild with the dominant genes of cultivated pigeonpea for pod width. Saxena *et al.*, 1980, and Singh *et al.*, 1983, found similar observations in crosses between cultivated pigeonpea varieties.

Mid-parent heterosis, for pod bearing length, was positive in 3 crosses (1 with ICP 28 and 2 with ICP 26) and negative in 7 crosses (4 with ICP 28 and 3 with ICP 26). Better parent heterosis was negative in all the crosses followed by a positive inbreeding depression. The negative heterosis followed by positive inbreeding depression suggested that the trait is governed by non-additive genes. Negative heterosis was reported in the intraspecific crosses between pigeonpea varieties for this trait by Saxena *et al.* (1980).

Mid-parent heterosis, for number of locules per pod, was positive in 7 crosses (3 crosses with ICP 28 and 4 with ICP 26) and negative in 3 crosses ( 2 crosses in ICP 28 and 1 cross with ICP 26). Better parent heterosis was positive in 2 crosses (1 with ICP 28 and 1 with ICP 26) and negative in 8 crosses (4 crosses with ICP 28 and 4 crosses ICP 26). Inbreeding depression was positive in all the 10 crosses. Positive mid- parent heterosis and negative better parent heterosis followed by the positive inbreeding depression indicates the additive and non – additive gene actions respectively in the expression of this character. Negative heterosis was observed in the cultivated pigeonpea crosses for number of locules per pod by Saxena *et al.* (1980).

Mid-parent heterosis, for number of seeds per pod, was positive in 5 crosses (2 crosses with ICP 28 and 3 crosses with ICP 26) and negative in 5 crosses (3 crosses in ICP 28 and 2 crosses in ICP 26). Better parent heterosis was positive in 3 crosses (1 with ICP 28 and 2 with ICP 26) and negative in 7 crosses (4 crosses with ICP 28 and 3 with ICP 26). Inbreeding depression was positive in all the 10 crosses. Positive mid parent heterosis and negative better parent heterosis followed by the positive inbreeding depression indicates the additive and non – additive gene actions respectively in the expression of this character. Saxena *et al.* (1980) reported negative heterosis in pigeonpea x pigeonpea crosses for this trait.

Mid-parent heterosis, for number of primary branches, was positive in 2 crosses with ICP 26 and negative in 8 crosses ( 5 crosses with ICP 28 and 3 with ICP 26). Better parent heterosis was positive in ICP 28 x ICPW 94 cross and negative in 9 crosses ( 4 with ICP 28 and 5 with ICP 26). Inbreeding depression for number of primary branches was positive in all the crosses. Negative heterosis followed by inbreeding depression suggests the preponderance of the non- additive gene action in the expression of this trait.

Mid-parent heterosis, for number of secondary branches, was positive in all the 10 crosses and better parent heterosis was positive in 8 crosses (4 crosses with ICP 28 parent and 4 with ICP 26 parent) and negative in 2 crosses (1 with ICP 28 and another with ICP 26 ). Inbreeding depression was positive in all the crosses. Positive mid- and better parent heterosis followed by inbreeding depression suggests the preponderance of additive

gene action in controlling the expression of this trait. Saxena *et al.* (1980), reported positive heterosis in pigeonpea x pigeonpea crosses, for number of primary and secondary branches. Negative heterosis for number of primary branches and positive heterosis for number of secondary branches indicate that the hybrids had profuse branching compared to the parents used in the crossing programme. The branching trait which is significantly positively correlated with the yield and yield related traits could be an important selection criterion in the pigeonpea breeding programme for selection of high yielding plants.

Mid-parent heterosis, for 100-seed weight, was positive in cross ICP 28 x ICPW 116, and negative in 9 crosses (4 with ICP 28 and 5 with ICP 26). Better parent heterosis was negative in all the 10 crosses. The inbreeding depression was positive in all the ten crosses. Negative heterosis followed by inbreeding depression suggests the preponderance of non-additive gene action in the expression of the trait.

Mid-parent heterosis, for seed protein, was positive in cross ICP 28 x ICPW 94, and negative in 9 crosses (4 with ICP 28 and 5 with ICP 26). Better parent heterosis was negative in all the ten crosses. *C. scarabaeoides* seeds had higher protein content than the pigeonpea seeds, but none of the F<sub>1</sub> hybrids had better protein content than the parents, except the hybrids of a cross between ICP 28 x ICPW 94.

Mid-parent heterosis, for harvest index, was positive in cross ICP 28 x ICPW 116, and was negative in 9 crosses (4 with ICP 28 and 5 with ICP 26). Better parent heterosis was negative in all the 10 crosses. The inbreeding depression was positive in all the ten crosses. Negative heterosis followed by inbreeding depression suggests the preponderance of non-additive gene action in the expression of this trait.

In the present investigation, mid-parent heterosis was low for 100-seed weight and seed protein but was average to high for seed yield and harvest index. Heterosis for harvest index is generally higher than any of its components, since yield is the product of several component characters (Matzinger and Wersman, 1967). Yield represents the ultimate expression during the development of plant metabolism hence, increase in yield level does not necessarily result in a change of its components (Grafius, 1965). Hybrids of the crosses ICP 28 x ICPW 116, ICP 28 x ICPW 141 and ICP 26 x ICP 130, exhibited considerable

heterosis for 100- seed weight , harvest index, seed yield. Hence, these lines with marked superiority may be utilized in the development of commercial hybrids.

Mid-parent heterosis, for density of trichome A, was positive in 2 crosses with ICP 26 and negative in 8 crosses (5 with ICP 28 and 3 with ICP 26). Better parent heterosis was positive in cross ICP 26 x ICPW 130 but negative in 9 crosses (5 with ICP 28 and 4 with ICP 26).

Mid-parent heterosis, for density of trichome B, was positive in 9 crosses (5 crosses in ICP 28 and 4 crosses in ICP 26) and negative in cross ICP 26 x ICPW 125. Better parent heterosis was positive in 2 crosses (1 with ICP 28 and 1 with ICP 26) but negative in 8 crosses (4 crosses with ICP 28 and 4 with ICP 26).

Mid-parent heterosis, for density of trichome C, was positive in all the 10 crosses. Better parent heterosis was positive in cross ICP 28 x ICPW 125, and negative in the remaining 9 crosses. The trichomes on  $F_1$  hybrids were denser than on the *C. cajan* but less dense than on the *C. scarabaeoides* accessions (better parent).

Mid-parent heterosis, for density of trichome D, was positive in 8 crosses (5 crosses with ICP 28 and 3 with ICP 26) and negative in 2 crosses with ICP 26. Better parent heterosis was positive in cross ICP 28 x ICPW 116, and was negative in the remaining 9 crosses. The trichomes on  $F_1$  hybrids were denser than on the pigeonpea genotypes but less dense than on the *C. scarabaeoides* accessions.

Positive correlations have been observed in the segregating populations between number of primary and secondary branches and seed yield. Increased branching can exert a positive effect on the pod number which then exerts a positive, though indirect, effect on the seed yield (Benjamin, 1981). This correlation provides the breeders with an easily recognized trait (s) on which to apply selection pressure during the plant development. Significant correlations were observed between branching and seed yield in chickpea crosses (Choudhary and Khan, 1974; Bhal and Jain, 1977; Tomar *et al.*, 1982).

## **Genetic basis of quantitative characters**

The means and variances obtained for various quantitative characters were subjected to scaling test and five parameter model generation mean analysis to determine their genetic basis. Knowledge of genetic components of multigenic traits and the environmental effects is important for the choice of breeding methods, size of populations and intensity of selection. Besides estimates of genetic parameters, inbreeding depression, beyond  $F_2$  generation, indicates that dominance is not an important genetic variance component for yield in this crop. Knowledge of plant characteristics is essential for planning an effective breeding programme. This is useful in selection of individuals with adaptation to different agro- ecological zones. Measurement of genetic variability and understanding of inheritance of characters is of prime importance in pigeonpea to formulate a sound crop improvement program.

To understand the major gene effects and different gene interactions, five parameter model of generation mean analysis was applied to five generations (parents,  $F_1$ ,  $F_2$ ,  $F_3$  and one backcross population) to estimate different genetic parameters that account for variation for different characters under study. Information on the additive, dominance, additive x additive and dominance x dominance interactions could be obtained from the study. In the absence of second backcross generation, the j (dominance x additive) interaction could not be calculated. Results of the scaling test are presented in Tables 79 - 81.

### **Days to flowering**

The estimates of A, C and D scales deviated significantly from zero indicating the inadequacy of additive-dominance model to explain the variation for this character and presence of epistatic interaction effects in all the crosses except the scale A which was non-significant in cross (ICP 26 x ICPW 125). The estimates of additive, dominance, and dominance x dominance interactions were significant in all three crosses, whereas the additive x additive was significant in ICP 28 x ICPW 130 and ICP 26 x ICPW 125. The dominance and dominance x dominance gene effects expressed negatively for days to flowering indicate the presence of complementary epistasis in cross ICP 28 x ICPW 94 which suggests that simple pedigree breeding would be rewarding for bringing



improvement. However, in cross ICP 28 x ICPW 130 the dominance gene effects were negative and the dominance x dominance gene effects were positive indicating the duplicate epistasis. In cross, ICP 26 x ICPW 125 the dominance effects were positive and the dominance x dominance effects were negative indicating duplicate epistasis which suggests that the biparental crosses can be adapted for the improvement. In cross ICP 28 x ICPW 94, dominance was more significant than the additive gene effect and additive x additive gene action was insignificant. In cross ICP 28 x ICPW 130, dominance was more significant than the additive gene effect but in cross ICP 26 x ICPW 125 the additive gene effect was more significant than dominance. Sharma *et al.* (1973a), Dahiya and Brar (1977), Dahiya and Satija (1978), Gupta *et al.* (1981), Reddy *et al.* (1981b) reported additive gene action for days to flowering in cultivated pigeonpea crosses, and non-additive gene action was reported by Reddy *et al.*, (1981b). Additive and non-additive gene action for days to flowering was reported by Choudhary *et al.* (1980), Sidhu and Sandhu (1981) and Saxena *et al.* (1981b). Additive and non-additive gene actions were reported by Kidambi *et al.*, (1988), Salimath and Bhal (1989), Malhotra *et al.* (1993) and Jha *et al.* (1997) in chickpea.

### **Days to maturity**

The estimates of A, C and D scales deviated significantly from zero indicating the inadequacy of additive-dominance model to explain the variation for this character and presence of epistatic interaction in all three crosses. Estimates of additive, dominance, and additive x additive interactions were significant, for days to maturity, in all crosses, but in cross ICP 26 x ICPW 125 the dominance x dominance effect was non-significant. In crosses, ICP 28 x ICPW 94 and ICP 26 x ICPW 125, the dominance gene effects were negative and the dominance x dominance gene effects were positive indicating the presence of duplicate epistasis, which suggests that the biparental crosses can be adapted for improvement. In cross, ICP 28 x ICPW 130 dominance and dominance x dominance gene effects were negative indicating the presence of complementary epistasis, which suggests that simple pedigree breeding would be rewarding for improvement. In ICP 28 x ICPW 94 the additive x additive interaction was more significant than the dominance interaction. In ICP 28 x ICPW 130, dominance x dominance interaction was non-significant and the additive was more significant than the dominance and additive x additive gene actions. In

cross ICP 26 x ICPW 125, the dominance x dominance interaction was non - significant but additive x additive was more significant than the dominance gene action, which suggests that pedigree method of breeding should be followed for obtaining superior lines from segregating generations in the cross. Pandey (1972) and Sharma *et al.* (1972) reported additive gene action for days to maturity. Kapur (1977) and Sidhu and Sandhu (1981) reported both additive and non-additive gene action for days to maturity. Importance of both additive and dominance gene actions were reported for days to maturity by Kidambi *et al.* (1988) ; Salimath and Bhal (1989); and Jha *et al.* (1997).

### **Leaflet length**

The estimates of A, C and D scales significantly deviated from zero indicating the inadequacy of additive – dominance model to explain the maximum variation for this trait and indicates presence of epistatic interaction effects in three crosses. Estimates of additive, dominance x dominance and additive x additive interactions were significant in all three crosses. Dominance gene effect was significant only in ICP 28 x ICPW 94 and ICP 26 x ICPW 125 crosses but non-significant in a cross, ICP 28 x ICPW 130. The dominance and dominance x dominance gene effects were positive indicating the presence of complementary epistasis in cross ICP 28 x ICPW 94, which suggests simple pedigree breeding will be rewarding for bringing improvement. In crosses ICP 28 x ICPW 130 and ICP 26 x ICPW 125, the dominance gene effects were negative but the dominance x dominance gene effects were positive, indicating the presence of duplicate epistasis. Bi – parental crosses can be adapted for the improvement. In crosses, ICP 28 x ICPW 94, ICP 28 x ICPW 130 and ICP 26 x ICPW 125, the additive x additive interaction was more significant than the dominance in explaining variance for this trait which suggests that pedigree method of breeding could be followed for obtaining superior lines from segregating generations in the crosses.

### **Leaflet width**

The estimates of A, C and D scales significantly deviated from zero indicating the inadequacy of additive-dominance model in explaining the variation for this character. In the entire three crosses additive, dominance x dominance and additive x additive interactions

were significant. Dominance gene effects were significant only in ICP 28 x ICPW 94 and ICP 26 x ICPW 125 cross but non-significant in ICP 28 x ICPW 130. The dominance and dominance x dominance gene effects were positive indicating the presence of complementary epistasis in all the three crosses, suggesting that simple pedigree breeding will be rewarding for bringing improvements. In cross, ICP 28 x ICPW 94 d (70.4%); in cross ICP 28 x ICPW 130 and h (58.7%) and i (60.4%) in cross ICP 26 x ICPW 125, were maximum in explaining variance in this trait.

### **Pod length**

The estimates of A, C and D scales deviated significantly from zero indicating the inadequacy of the additive-dominance model to explain the variation for this character and the presence of epistatic interaction effects. In all the three crosses; additive, dominance and dominance x dominance and additive x additive interactions were significant. In all three crosses the dominance gene effects were negative and the dominance x dominance gene effects were positive indicating the duplicate epistasis, suggests that the bi-parental crosses can be adapted for the improvement. In crosses, ICP 28 x ICPW 94 and ICP 28 x ICPW 130, the additive gene effects were more significant than the dominance interactions, suggesting, that the pedigree method of breeding could be followed for obtaining superior lines from segregating generations in the crosses. In cross ICP 26 x ICPW 125 the dominance x dominance gene action was more significant in explaining the variation for this trait.

### **Pod width**

The estimates of A, C and D scales were significant in three crosses, except the estimates of A scale in cross ICP 28 x ICPW 94 which indicates the inadequacy of the additive-dominance model in explaining the variation for this trait. The additive, dominance, dominance x dominance and additive x additive gene actions were significant in all three crosses, except in cross ICP 28 x ICPW 94, where additive x additive type of interaction was non-significant. In crosses, ICP 28 x ICPW 94 and ICP 26 x ICPW 125 the gene effects of dominance were positive and that of dominance x dominance was negative, indicating the presence of duplicate epistasis. However, in cross ICP 28 x ICPW 130, the estimates of dominance was negative and dominance x dominance was positive indicating

the presence of duplicate epistasis in explaining the variation in the cross. The maximum contribution was additive x additive in cross ICP 26 x ICP 125, additive in cross ICP 28 x ICPW 130 and dominance x dominance in cross ICP 28 x ICPW 94 in explaining the maximum variation for this character, except the additive x additive which was non-significant in cross ICP 28 x ICPW 94.

### **Pod bearing length**

The estimates of A, C and D scales deviated significantly from zero indicating the inadequacy of additive-dominance model in explaining the variation this character in all the three crosses. The estimates of additive, dominance, dominance x dominance and additive x additive gene actions were significant in all three crosses indicating the presence of epistatic gene interaction in explaining variation in this character. In all three crosses, the gene effects of dominance were negative and dominance x dominance were positive indicating the duplicate epistasis interaction in this character. The maximum contribution of dominance x dominance (60.9%) in ICP 28 x ICPW 94, h (64.3%) in cross ICP 28 x ICPW 130 and d (61.8%) in cross ICP 26 x ICPW 125, in explaining the variation for this character. Sharma *et al.* (1973a) and Sharma (1981) reported additive gene action, whereas Pandey, (1972) and Reddy *et al.* (1979) reported non-additive gene action while, Kapur (1977), Sidhu and Sandhu (1981), Saxena *et al.* (1981b) and Reddy *et al.* (1981b) reported additive and non-additive gene action for pod bearing length in the intraspecific crosses of pigeonpea. Dahiwal and Gill, (1973); Katiyar, (1975); Gowda and Bahl, (1978) and Malhotra *et al.* (1983) reported additive gene action for pod bearing length in interspecific crosses of chickpea.

### **Number of locules per pod**

The estimates of A, C and D scales deviated significantly from zero, indicating the inadequacy of additive-dominance model, to explain the variation in this character in all the three crosses. The estimates of dominance, dominance x dominance and additive x additive gene actions were significant in all three crosses, except d (additive) which was significant only in crosses ICP 28 x ICPW 130 and ICP 26 x ICPW 125. In all three crosses, the gene effects of h were negative and l were positive indicating the duplicate epistatic interaction governing this character. The maximum contribution was from l (42.9%) in cross ICP 28 x

ICPW 94, from l (59.7%) in ICP 28 x ICP 130 and h (45.5%) in ICP 26 x ICPW 125 in explaining the variation for this character.

### **Number of seeds per pod**

The estimates of A, C and D scales deviated significantly from zero in two crosses, (ICP 28 x ICPW 130 and ICP 26 x ICPW 125), suggesting the inadequacy of the additive-dominance model in explaining the variation in this character. In cross, ICP 28 x ICPW 94, the estimate of C was non-significant suggesting the absence of dominance x dominance interaction in this cross to explain the variation. The estimates of h (dominance), l (dominance x dominance) and i (additive x additive) were significant in all crosses, but in ICP 28 x ICPW 130, the d (additive) effects were non-significant, explaining the presence of the epistatic interactions in this trait. In crosses, ICP 28 x ICPW 94, ICP 28 x ICPW 130, the gene effects of h were negative and l were positive, indicating the duplicate epistasis interaction in this character, but in cross ICP 26 x ICPW 125, the h and l gene effects were positive indicating the complementary epistasis (Tables 53, 54 and 55). The maximum contribution of l (75.9%) in cross ICP 28 x ICPW 94, h (65.1%) in ICP 28 x ICP 130 and l (53.37%) in cross ICP 26 x ICPW 125 in explaining the variation in these characters. Saxena *et al.* (1981b) and Mohamed *et al.* (1985) reported the additive gene actions for number of seeds per pod while Kapur (1977) and Venkateshwarulu and Singh (1982) reported both additive and non-additive gene action in pigeonpea x pigeonpea crosses. In chickpea, diallelic crosses, additive gene action, for yield and its related components was reported (Dahiwal and Gill, 1973; Katiyar, 1975; Gowda and Bhal, 1978; Malhotra *et al.*, 1983).

### **Number of primary branches**

Estimates of A, C and D values deviated significantly from zero indicating the inadequacy of additive – dominance model to explain the variation in this character, and the presence of inter- allelic interactions were effective in all three crosses. The estimates of d (additive), l (dominance x dominance) and i (additive x additive) interactions were significant in all three crosses, where as the h (dominance) was significant only in crosses ICP 28 x ICPW 94 and ICP 28 x ICPW 130. The h and l gene effects were positive in crosses, ICP 28 x ICPW 94 and ICP 26 x ICPW 125 for number of primary branches

indicate the presence of complementary epistasis, while in cross ICP 28 x ICPW 130 the *l* gene effect was negative and *h* gene effect was positive indicating duplicate epistasis. The maximum contribution for variation in this character was from *h* (50.49%) in cross ICP 28 x ICPW 94; *i* (52.56 %) in cross ICP 28 x ICPW 130 and of *d* (44.67%) in cross ICP 26 x ICPW 125. Chaudhari *et al.*, (1980) reported additive gene action for number of primary and secondary branches in the interspecific crosses between pigeonpea genotypes.

### **Number of secondary branches**

The estimates of *A*, *C* and *D* deviated significantly from zero indicating the inadequacy of the additive – dominance model to explain the variation for this character and the presence of epistatic interaction effects in all the crosses, except the scale *A* in cross, ICP 26 x ICPW 125. The estimates of *h* (dominance), *i* (additive x additive) and *l* (dominance x dominance) were significant in all three crosses whereas the *d* (additive) was significant in crosses ICP 28 x ICPW 130 and ICP 26 x ICPW 125. The *h* and *l* gene effects were positive in ICP 28 x ICPW 130, indicating the presence of complementary epistasis. The *h* gene effect was positive and *l* was negative in ICP 28 x ICPW 94 while in ICP 26 x ICPW 125 the *h* gene effect was negative and *l* was positive indicating the duplicate epistasis in both the crosses. The maximum contribution in expressing the variation, for this character was from *l* (58.49%) in ICP 28 x ICPW 94, *d* (67.28%) in ICP 28 x ICPW 130 and also (80.48%) in ICP 26 x ICPW 125. Chaudhari *et al.*, (1980) reported additive gene action for number of primary and secondary branches among the cultivated pigeonpea crosses.

### **100 - Seed weight**

Estimates of *A*, *C* and *D* values deviated significantly from zero indicating the inadequacy of additive – dominance model to explain the variation for this character, and presence of the inter- allelic interactions effective in all three crosses. The estimates of *d* (additive), *h* (dominance) and *i* (additive x additive) interactions were significant in all three crosses, whereas the *l* (dominance x dominance) was significant in two crosses ICP 28 x ICPW 94 and ICP 28 x ICPW 130 but non – significant in ICP 26 x ICPW 125. The *h* gene effect expressed negatively and *l* gene effects positively in all three crosses indicating the presence of duplicate epistasis. Maximum contribution was of *d* in all the three crosses.

Pandey (1972); Sharma *et al.* (1973a); Chaudhari *et al.* (1980) and Saxena *et al.* (1981b) reported additive gene action for grain yield. Laxman Singh and Pandey, (1974); Dahiya and Brar (1977), Sidhu and Sandhu, (1981) reported the non-additive gene action for yield and yield components. Sidhu and Sandhu (1981); Reddy *et al.* (1981b) and Venkateshwarulu and Singh (1982) reported both additive and non-additive gene actions.

## Harvest index

Estimates of A, C and D values deviated significantly from zero indicating the inadequacy of additive – dominance model to explain the variation in this character, and the presence of inter- allelic interactions in all three crosses. The estimates of d (additive), h (dominance) and i (additive x additive) interactions were significant in all three crosses, whereas the l (dominance x dominance) was significant in two crosses ICP 28 x ICPW 94 and ICP 28 x ICPW 130. The h gene effect was negative and l gene effects were positive in all three crosses indicating the presence of duplicate epistasis. The maximum contribution was from d in explaining the variation in this character. The importance of additive and non-additive gene actions, in explaining the variation in this character, was explained by Pandey (1972); Sharma *et al.* (1973a); Laxman Singh and Pandey (1974); Dahiya and Brar (1977); Chaudhari *et al.* (1980) and Saxena *et al.* (1981b), Sidhu and Sandhu (1981) in the pigeonpea x pigeonpea intraspecific crosses. In the diallelic crosses in chickpea, importance of both additive and non- additive gene action was reported by Singh *et al.* (1981); Singh and Bians, (1982); Singh and Paroda, (1983).

## Heritability

Heritability is the ratio of genetic variance to phenotypic variance (Singh, 1977) and expressed in percentages. It is a good index of transmission of characters from parents to the offspring (Falconer, 1989). The knowledge of heritability helps the plant breeder in predicting the behavior of characters in succeeding generations and to make desirable selections. It depends on the variability present in the material and also on the environmental effects. Heritability estimates provide efficient selection criterion as they refer to the proportion of phenotypic variance which is a reflection of the genetic variance. A high heritability estimate suggests, that the character concerned can be easily selected in the test

environment. However, the heritability estimate is valid for a given population, and the environment in which it was obtained. Therefore, it is difficult to generalize heritability estimates from one population to another (Tables 44, 48, 51, 54, 56, 58 and 60).

The variances in parents, *C. cajan* and *C. scarabaeoides*, and  $F_1$ s were lower for all the characters. The variances in  $F_2$  population, for all the characters, were higher than both the parents, indicating segregation for all the characters in all the crosses. In the absence of a second backcross inference cannot be drawn on the dominance of characters from one parent.

The results indicate the existence of sufficient variability between the parents for the characters studied for the effective utilization. The more diversity among parents, the greater the chances of recovering desirable recombinants. Thus, crop improvement depends on the magnitude of genetic variability in the base population. This variability can be easily utilized if the heritability of these characters is high.

High values of broad-sense heritability for days to flower, indicates that the environmental effects influence the character the least. High broad-sense heritability value for days to flower was reported by Munoz and Abrahms (1971), Khan and Rachei (1972), Pandey (1972), Sharma *et al.* (1973b), Kumar and Reddy (1982) and Patel *et al.*, (1992) among the cultivated pigeonpea crosses but medium heritability was reported by Rubaihayo and Onim (1975) and Dahiya and Brar (1977). Rao *et al.*, (1994) and Sabaghpour (2000) reported high broad and narrow sense heritability values in chickpea diallelic crosses.

Medium to high heritability values were observed in the interspecific hybrids of crosses involving ICP 28 with wild genotypes and high heritability in the hybrids of crosses of ICP 26 with wild genotypes, which indicates that the characters were not influenced by the environment and could be used as selection criteria for selecting early flowering and maturing hybrids. Similar results were reported by Kumar and Reddy (1982) and Sidhu *et al.* (1985). However, these results are in contrast to those of Sharma *et al.* (1973b) and Sidhu and Sandhu (1981) who reported low heritability values for days to maturity but medium heritability was reported by Dahiya and Satija (1978).



Medium to high heritability was seen, for pod length and width, in crosses of wild accessions with ICP 28; but medium heritability in crosses with ICP 26, indicating the influence of environment in controlling the expression of this character, medium broad sense heritability values were also reported by Dahiya and Brar (1977) and Sidhu and Sandhu (1981).

Broad sense heritability was medium to high, for pod bearing length, in the crosses of wild accessions, both with ICP 28 and ICP 26 which indicated the less influence of environment in controlling the expression of this character. Kumar and Reddy (1982), in the interspecific crosses between the wild and cultivated pigeonpea, reported medium heritability for pod bearing length.

Low heritability values, for number of locules per pod and number of seeds per pod, in the crosses of both ICP 28 and ICP 26 with wild accessions; and low to medium heritability was seen for 100- seed weight and harvest index, indicating that the yield and yield related components are under the environmental influence difficult to make selections based on these characters. Low heritability values were reported for number of seeds per pod, grain yield, 100- seed weight etc. by Munoz and Abrams (1971), Khan and Rachie (1972), Sharma *et al* (1973a), Sharma *et al.* (1973b), Rubaihayo and Onim (1975), Sidhu and Sandhu (1981), Kumar and Reddy (1982). Medium heritability values were reported by Munoz and Abrams (1971), Pandey (1972), Malhotra and Sodhi (1977) and Sidhu *et al.*, (1985) in the intraspecific cultivated pigeonpea crosses.

Though, additive and non- additive genetic variances are important for yield and its related components, yet the heritability estimates have been small which hinders the selection. The heritability estimates are very small and measurements are time consuming and difficult to make. Direct measurement of yield is likely to be a better approach in breeding for improvement in yield. Improved yields can be obtained by partitioning the biomass more into the economic yield than the vegetative component. Tall, erect, and compact plant types would allow increased plant density and possibly gives a greater yield per unit area.

Variance component estimates reveal little of mode of action of the genes involved. Studies being contemplated in this area should focus on the variance component estimates that are devoid of genotype x environmental interactions, and so improve their reliability and usefulness. Replicating the experiments in time and space can reduce the environmental affects on the genotype.

## **Genetic basis of qualitative characters**

The inheritance pattern of the following characters ; plant habit, stem color, leaflet shape, seed mottleness, strophioled seeds, pod hairiness, resistance against podborer and trichome density was studied (Tables 61-66).

### **Plant habit**

The F<sub>1</sub> hybrids were semi-spreading, suggesting the incomplete dominance of either genes in governing the character. Further, the F<sub>2</sub> ratio of 1 spreading: 2 semi – spreading: 1 erect, suggests that the plant growth habit is governed by a single gene with incomplete dominance. However, single dominant gene control of the growth habit was has been reported by in crosses of *C. cajan* with *C. cajanifolius*. Pundir and Singh (1985) reported the F<sub>1</sub>s with intermediate plant habit between erect and spreading growth habit and in the F<sub>2</sub> generation they observed a ratio of 1 erect: 1 spreading: 14 intermediate, suggested the two genes with partial dominance. Reddy *et al.* (1980) obtained 13 spreading: 3 erect in crosses of *C. scarabaeoides* with *C. cajan*, suggested the twining growth habit of *C. scarabaeoides* as controlled by two genes with epistatic gene action. This was also suggested by Kumar *et al.* (1985) in crosses involving Pant- 2 and *C. scarabaeoides* and Pundir and Singh (1985) in interspecific crosses between *C. sericeus* and *C. reticulatus* with *C. cajan*. Single dominant gene expression of the plant habit gave a ratio of 3 erect: 1 prostrate in the chickpea cross of BGM 417 x ponaflair which was confirmed by backcross progeny with a ratio of 1 erect: 1 prostrate. The results of F<sub>2</sub> and backcrosses suggested that a single recessive gene governs the prostrate growth habit Singh *et al.*, (1992).

## Stem colour

The F<sub>1</sub> hybrids had mixed stem color, between the purple and green color of the parents, suggesting the incomplete dominance of genes in governing the character. Further, the F<sub>2</sub> ratio of 1 purple: 2 mixed: 1 green, suggests that the plant growth habit is governed by a single gene with incomplete dominance. The control of stem color of *C. scarabaeoides* by a single partially dominant gene was earlier reported by (Reddy, 1973; Kumar *et al.*, 1985 and Pundir and Singh, 1985).

## Leaflet shape

Leaflet shape in F<sub>1</sub> hybrid was intermediate between the obovate leaflet shape of *C. scarabaeoides* and the lanceolate leaflet shape of *C. cajan* in all seven crosses interspecific crosses. F<sub>2</sub> population in all the seven crosses gave a good fit for 1 obovate: 2 intermediate: lanceolate suggested that the leaflet shape is governed by single gene with incomplete dominance. The control of obovate leaflet shape of *C. scarabaeoides* by a single partially dominant gene was earlier reported by (Reddy, 1973; Kumar *et al.*, 1985; Pundir and Singh, 1985). In crosses of *C. sericeus* with *C. cajan*, the F<sub>1</sub>s had an intermediate leaflet shape between the oblance ovate of *C. sericeus* and lanceolate of *C. cajan* in the F<sub>2</sub> generation suggests the incomplete dominance of this gene governing this characters (Singh., 2000).

## Seed mottleness

Nature of F<sub>1</sub> seeds in all the seven interspecific crosses was unmottled and segregated in F<sub>2</sub> population into a ratio of 9 mottled: 7 unmottled seed indicated the epistatic interaction in the expression of this character. The involvement of complementary genes in the expression of mottledness of the seed was reported earlier in the crosses between ICP 7035 x *C. scarabaeoides* and ICP – 6915 x *C. scarabaeoides* and however the data from the cross of ICP 6997 x *C. scarabaeoides* indicated duplicate epistatic interaction with a good fit of 15:1 ratio (Reddy *et al.*, 1980).

## Seed strophiole

The strophioled nature of *C. scarabaeoides* seeds was dominant over the non-strophioled seeded condition of pigeonpea cultivars was indicated by the strophioled seeds of F<sub>1</sub> hybrids. In F<sub>2</sub> generation, the ratio of 13 (strophioled): 3 (non-strophioled) indicates the inhibitory gene action. The inhibitory gene action was also reported in the crosses of ICP 6195 x *C. scarabaeoides* and ICP – 6997 x *C. sericeus* however, 9:7 ratio was observed in the crosses between ICP- 7035 x *C. scarabaeoides*, indicating the complementary gene action in the expression this character (Reddy *et al.*, 1980). Pundir and Singh (1985) reported the duplicate gene action, while inhibitory gene action was reported in the crosses of pigeonpea with *C. scarabaeoides*, *C. sericeus* and *C. albicans* (Reddy *et al.*, 1981a and Kumar *et al.*, 1985).

## Pod hairiness

The hairiness of pods in *C. scarabaeoides* accessions was dominant over the non- hairy nature of pigeonpea pods. The non - glandular hairs on the pods of wild accessions make the pods a non-preferring surface to the insects for oviposition. The F<sub>2</sub> ratio in all the seven crosses gave a good fit for 3 (hairy): 1 (non-hairy) pods, suggests the gene controlling hairiness was single and dominant over the non-hairiness. Similar observations were also made in the interspecific crosses of ICP – 6915 x *C. scarabaeoides*, however, the F<sub>2</sub> data gave a good fit for 13:3 ratio suggesting the inhibitory gene action in crosses of ICP – 6997 x *C. scarabaeoides* (Reddy *et al.*, 1980). Similar studies were also reported in the interspecific crosses between *C. scarabaeoides* x *C. cajan* and *C. sericeus* x *C. cajan*, suggesting the single dominant gene, designated as Hp, governing this trait (Pundir and Singh, 1985; Singh, 2000).

## Inheritance of podborer resistance

The polyphagous nature of podborer is a serious problem and is highly devastating in many countries. It attacks the reproductive parts; the buds, flowers and pods. The identification and transfer of gene (s) for pod borer resistance from the wild accessions to

cultivated background to create an inbuilt mechanism in the plants, is one of the major steps to control this devastating pest.

Significant correlations were observed between the density of non-glandular trichomes and the pod borer resistance. The highly significant positive correlations between the density of trichome types; C and D; and podborer resistance, indicates the selection of plants bearing pods with high density of C and D trichomes for resistance against podborer. However, it is important to select those segregants which have cultivated pigeonpea seeds and pod wall resembling that of *C. scarabaeoides*.

The *C. scarabaeoides* accessions; ICPW 94, ICPW 125, and ICPW 130 were the most resistant parents with no damage to the flowers and pods by *H. armigera*. The  $F_1$  hybrids in all three crosses, ICP 28 x ICPW 94, ICP 28 x ICPW 130 and ICP 26 x ICPW125, were almost like the *C. scarabaeoides* parents in resistance, with less than 5% damage, indicating that resistance was dominant over susceptibility. In  $F_2$  generation, a good fit for 3 resistant: 1 susceptible was observed, indicating the monogenic control of antixenosis component mechanism of resistance. The monogenic control of resistance was further confirmed by 1 (resistant) : 1 (susceptible) segregation in backcross generation ( $F_1$  x ICP 28 and  $F_1$  x ICP 26). In  $F_3$  generation, the expected ratio of 1 non-segregating resistant : 2 segregating : 1 non-segregating susceptible was recorded, which supported the monogenic control of resistance, in each of the three crosses individually and overall in three crosses. One hundred and sixty six segregating progenies in all three crosses (ICP 28 x ICPW 94 (56), ICP 28 x ICPW 130 (62) and ICP 26 x ICPW 125 (48)), in  $F_3$  generation gave a good fit for 3 resistant: 1 susceptible, individually, as well as overall. The segregating  $F_3$  families were also homogeneous in each of the three crosses individually as well as overall. Further, there was homogeneity in segregation in  $F_2$  generation and segregating  $F_3$  families in all the three crosses. This confirmed that the antixenosis mechanism of resistance to pod borer is controlled by dominant allele of a single gene in three interspecific crosses. However, the allelic relationships in these *C. scarabaeoides* parents are not known. Crosses between the *C. scarabaeoides* parents would reveal the allelic relations for pod borer resistance gene.

In the studies for antibiosis mechanism of resistance against podborer, the *C. scarabaeoides* accession (ICPW 94) was found to be more resistant, with no damage, compared to the *C. cajan* (ICP 28) which showed the maximum damage. The  $F_1$  plants were resistant to podborer attack indicating dominance of resistance over susceptibility. The  $F_2$  generation segregated into 3 resistant: 1 susceptible, indicating the antibiosis component of resistance mechanism was controlled by the dominant allele of a single gene. The monogenic control of antibiosis mechanism of resistance was further confirmed by the segregation ratio of 1 resistant : 1 susceptible, backcross generation. Verulkar *et al.*, (1997) observed similar results in the interspecific crosses, involving *C. cajan* and *C. scarabaeoides*, by dual choice arena test. They evaluated the parents,  $F_1$ ,  $F_2$  and the  $BC_1F_1$  population for podborer and pod wasp resistance and their results indicated that the antibiosis mechanism of resistance is governed by the dominant allele of a single gene for podborer resistance and by the recessive allele of a single gene for the pod wasp resistance.

### **Inheritance of trichomes**

The high density of erect non - glandular trichomes, predominantly on the pods of wild *C. scarabaeoides* accessions, confers a high level of resistance against podborer (Shanower *et al.*, 1997). This necessitated the need to search and utilize the cross compatible wild relatives of *Cajanus* to produce hybrids having pods with higher number of non-glandular trichomes. The genetic basis, governing the expression of non - glandular and glandular hairs has been investigated in the present study. There are no reports on the inheritance of trichome type and density in pigeonpea. The results obtained in the study clearly indicate that the high density trichome nature of the wild was dominant over the low density on the cultivated features. The hairiness of pods in *C. scarabaeoides* accessions was dominant over the non- hairy nature of pigeonpea pods. The non - glandular hairs on the pods of wild accessions make the pod a non-preferring surface for oviposition. The  $F_2$  ratios obtained in all the seven crosses gave a good fit for 3 (hairy): 1 (non-hairy) pods, suggests that the, hairiness is dominant over the non-hairiness and is governed by a single gene. Similar observations were made by Reddy *et al.* (1980) in the interspecific cross of ICP – 6915 x *C. scarabaeoides*, however, they have noticed the inhibitory gene action in the cross of ICP – 6997 x *C. scarabaeoides*, as the  $F_2$  data gave a good fit for 13: 3. Pundir and Singh

(1985) and Singh (2000) reported a single dominant gene, designated as Hp, governing this trait in the interspecific crosses between *C. scarabaeoides*, *C. sericeus* and *C. cajan*.

The hairiness of *C. scarabaeoides* pods was because of the presence of non-glandular trichomes types C and D. the density of trichome types; B, C and D is very high on the pods of *C. scarabaeoides* compared to the *C. cajan* pods. On the contrary, the density of trichome type A is very less on the pods of *C. scarabaeoides* compared to *C. cajan* pods. The study on the inheritance of these trichomes indicates that the *C. scarabaeoides* trichome features were dominant over the pigeonpea trichome features. Pods of F<sub>1</sub> plants had lower densities of type A, in all three crosses, similar to *C. scarabaeoides* pods. In F<sub>2</sub> generation, a good fit for 3 (low density): 1 (high density) for type A was observed, indicating the monogenic the density of trichome A and the dominance of its low density in *C. scarabaeoides* over the high density in *C. cajan*. Further, the monogenic control of this character was confirmed by the segregation in the backcross generation which gave a good fit for 1 (low density):1 (high density). The segregation in F<sub>2</sub> and BC<sub>1</sub>F<sub>1</sub> generations were homogenous in all three crosses.

The F<sub>1</sub> pods had higher densities of type B, similar to the pods of *C. scarabaeoides*. In F<sub>2</sub> generation, a good fit for 3 (high density): 1 (low density) was observed, indicating the monogenic dominance nature of the high density of trichome B. Further, this was confirmed by the ratio of 1(low density): 1 (high density) segregation observed in the backcross generation. The results suggest that the wild characters of glandular trichomes are dominant over the cultivated.

The non- glandular trichomes; C and D, were denser on the pods of *C. scarabaeoides* than on *C. cajan*. The pods of F<sub>1</sub> hybrids had higher densities of both types C and D, types, indicating the dominance of *C. scarabaeoides* features. In F<sub>2</sub> generation, a good fit for 3 (high density): 1 (low density), indicates the monogenic and dominance nature of high density of these trichomes. Further the segregation of 1 (high density) : 1 (low density) in the backcross generation confirmed the monogenic control of these two types of trichomes.

# SUMMARY



## SUMMARY

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is an important pulse crop sustaining the livelihood of resource poor farmers in the semi-arid tropics. Major advancement in crop improvement has resulted in the development of short statured, photo-insensitive, high yielding, high seed protein content, varieties suitable for diverse agro-climatic conditions. In spite of such a progress made in this crop, the productivity of cultivated pigeonpea continues to be constrained by various biotic and abiotic stresses. Insects are the most important biotic constraint to pigeonpea production worldwide, causing losses of more than US \$ 1000 million every year. More than 200 species of insects feed on pigeonpea, of which *Helicoverpa armigera*, *Maruca vitrata*, *Melanagromyza obtusa*, *Clavigralla* spp., *Nezara viridula* and *Callosobruchus* spp. are the most important (Lateef and Reed, 1992). Of these, legume podborer, *Helicoverpa armigera*, is the most destructive and notorious pest of the field crops (Lateef and Reed, 1992). *Helicoverpa* alone causes an estimated loss of US \$ 927 million in chickpea and pigeonpea, and possibly, over US\$ 2 billion on other crops worldwide. A conservative estimate is that over US\$ 1 billion are spent on insecticides to control this pest. In addition to huge economic losses caused by the pest, there are several indirect costs from the deleterious effects of pesticides on the environment and human health (Sharma *et al.*, 2001). Continuous use of insecticides and chemicals has led to the insecticide resistance in this species, which resulted in several crop failures. Therefore, host plant resistance plays an important role in the management of this pest. It offers a viable economic solution in this situation.

Wild *Cajanus* species, especially *C. scarabaeoides*, has been identified as a potential genetic source of resistance (Pundir and Singh, 1987; Saxena *et al.*, 1990; Shanower *et al.*, 1997) that has not been fully explored and exploited in pigeonpea breeding. There is also some evidence that this species has different mechanisms of resistance than those in the cultivated types. The genes from the wild relatives can be tapped through wide hybridization for use in the crop improvement to diversify the

basis of resistance to the pests. However, despite the availability of a wide array of wild sources of resistance, their utility in pigeonpea improvement has not been fully explored. *C. scarabaeoides* accessions are spread across India, Sri Lanka, Myanmar, Philippines, Australia and South America (Nene and Sheila, 1990). These wild accessions could be used to broaden the genetic base and provide alternate sources of resistance for the long-term control of major biotic/abiotic stresses. There is no report on the genetic basis of resistance, and resistance related characters, for pod borer resistance. With this in view, the present investigation was undertaken to study the morphological, molecular and biochemical diversity among wild accessions of *C. scarabaeoides*; to identify the physical and biochemical features which play an important role against podborer ; to identify the sources of resistance in wild accessions against podborer ; to introgress the pod borer resistance genes from wild accessions to the cultivated background through back crossing ; and to study the genetic basis of certain qualitative and quantitative traits, including the resistance against pod borer.

In the present investigation, thirty wild accessions of *C. scarabaeoides*, from six geographical locations, in and out side India, and six cultivated varieties of *C. cajan* were used. All the 36 genotypes could be clearly identified based on diagnostic morphological traits. Distinct genetic differences were observed for the quantitative traits (days to flowering and maturity, leaf area, leaf specific area, pod length and width, pod bearing length, number of locules per pod, number of seeds per pod, 100- seed weight, number of primary and secondary branches and seed protein) in 2000 and 2001 Kharif seasons. The large phenotypic variability obtained for the quantitative traits facilitated a clear distinction among accessions from different geographical locations indicating the existence of region specific adaptations. UPGMA dendrogram and PcoA analysis, based on the morphological traits, revealed hierarchical clustering of the accessions. *C. scarabaeoides* accessions were grouped into different sub - clusters based on the geographical origin. Indian, Sri Lankan, Australian, Myanmar and Philippines origin accessions formed separate sub - clusters.

Molecular diversity in 42 accessions, belonging to three wild species; *C. scarabaeoides*, *C. sericeus* and *C. reticulatus* ; and cultivated *C. cajan*, was assessed using (i) nine maize mitochondrial DNA probe-enzyme combinations, (ii) five AFLP primer combinations and (iii) ten SSR primer sets.

The studies revealed that all three molecular markers were informative in evaluating the genetic diversity in the wild relatives of pigeonpea. Differences between species could be resolved with all the three marker types. However, the intraspecific differences were more prominent with AFLP markers, and the four *cajanus* species, *C. scarabaeoides*, *C. sericeus*, *C. reticulatus* and *C. cajan*, formed distinct groups.

The RFLP profiles of mt DNA, AFLP and SSRs clearly differentiated the three wild and one cultivated species. In 42 accessions, the levels of polymorphism varied in all the three marker types ranging from 95.4 % for AFLPs and RFLPs to 100 % for SSRs. The diversity index values were very high for SSRs ( $H = 0.89$ ), followed by RFLPs ( $H = 0.85$ ) and AFLPs ( $H = 0.75$ ). Effective multiplex ratio was highest for AFLP markers which could uniquely fingerprint each accession. The distance matrix D, produced from the binary data was subjected to sequential agglomerative hierarchical cluster (SHAN) analysis using UPGMA (Unweighted pair group arithmetic mean) of NTSYS software. The relationship between accessions, as revealed by three molecular markers, was visualized through dendrograms. Differences between three dendrograms were tested by generating cophenetic values ( $r$ ) for each dendrogram, and the assembly of the cophenetic matrix for each marker type. A highly significant correlation ( $r = 0.96$ ) between the cophenetic matrix and the dendrogram was observed. This was further confirmed by a high stringent stress value (0.5 to 0.7) on MDS (Multi-dimensional scaling) scatter plot. The grouping pattern in the combined dendrogram (RFLP, AFLP and SSR) was similar to that obtained from the morphological data with a high cophenetic correlation ( $r = 0.97$ ).

UPGMA dendrogram and PCoA analysis based on the AFLP, RFLP and SSR markers revealed similar grouping of accessions. The four different species formed different major groups. *C. scarabaeoides* accessions belonging to the same geographical location grouped together. Accessions from India, Australia, Sri Lanka, Indonesia, Myanmar and Philippines formed different sub-clusters under the major *C. scarabaeoides* group. Intraspecific variation was effectively revealed by SSRs and AFLPs compared to the RFLP markers. The SSR markers further clustered the *C. scarabaeoides* accessions of Indian origin based on three maturity groups (early, medium and late flowering).

The accessions were also screened against pod borer under field conditions by tagging two inflorescences per plant and observations were recorded on the 5<sup>th</sup>, 7<sup>th</sup>, 11<sup>th</sup>, 21<sup>st</sup> and 31<sup>st</sup> day of tagging. ICPW 83, ICPW 94, ICPW 116, ICPW 125, ICPW 130 and ICPW 141 were found to be highly resistant among the *C. scarabaeoides* accessions screened based on no bud, flower and pod damage and no eggs and larvae per inflorescence. These early and medium flowering wild accessions, with high resistance to podborer can be effectively utilized in the breeding programme for producing podborer resistant varieties.

Biochemical analysis of total protein content by Lowry's method, trypsin inhibitors using BAPNA as a substrate and lectin by haemagglutination was carried out on all the thirty *C. scarabaeoides* accessions and six cultivated pigeonpeas. Total protein content was maximum in mature pods, followed by the immature and juvenile pods in both the wild and cultivated varieties. Mean protein content was higher in the wild pods than in the cultivated ones. The trypsin inhibitor levels were found to be high among the *C. scarabaeoides* accessions. Significant differences were not found between the juvenile and immature stages; however its content was maximum in mature pods compared to other two stages of pods. The lectin content significantly differed among different maturity stages of pod, with maximum lectin content in the juvenile stage followed by the immature stage. However, there was no lectin in the mature pods. Significant differences were observed for the lectin content among the *C. scarabaeoides* accessions. The lectin content was more in wild than the

cultivated varieties. ICPW 138 and ICPW 98 had the highest lectin content among the *C. scarabaeoides* accessions.

Trichomes play an important role in plant-insect interactions (Jeffree, 1986; David and Easwarmoorthy, 1988; Smith, 1989; Peter *et al.*, 1995). Therefore, the study was conducted to identify different types of trichomes and their distribution in cultivated pigeonpeas and *C. scarabaeoides* accessions. Ten pods were collected from each accession, in all the three replications, in both the seasons. The pods were examined under the light microscope at a magnification of 100x with an ocular measuring grid to identify different types of trichomes and also their distribution. The pods were also scanned under the Scanning Electron Microscope (SEM) using the methodology described by Reddy *et al.* (1995). Electron micrographs were taken with a JEOL JSM 35 CF. Five different types of trichomes; three glandular (A, B and E) and two non-glandular types (C and D) were found on the pods of wild accessions and pigeonpea varieties. Significant differences were observed among the density of four types of trichomes (A, B, C and D) on the pods of different wild and cultivated accessions. However, the density of trichome E could not be studied due to its very small size. Types B, C and D trichomes were present on pods of all the *C. scarabaeoides* accessions and *C. cajan*. Type A was absent in most of the *C. scarabaeoides* accessions and even if present in a few accessions their density was very low. Pods of *C. scarabaeoides* were more pubescent than the pods of *C. cajan* because of the higher density of types B, C and D trichomes. Density of trichome type C was significantly correlated negatively with the percentage bud, flower and pod damage and number of eggs and larvae per inflorescence. Significant seasonal variation for type and density of trichomes was not found in wild and cultivated genotypes.

Days to flower and maturity, leaflet length and width, pod length and width, pod bearing length, number of locules and seeds per pod, seed protein, density of trichomes A, B, C and D showed highest broad sense heritability. The 100 – seed weight showed medium heritability value. This suggests that these traits are less

affected by the season and therefore can be effectively used as selection criteria in breeding programmes.

In the interspecific hybridization studies, five *C. scarabaeoides* accessions (ICPW 94, ICPW 116, ICPW 125, ICPW 130 and ICPW 141) and two varieties of *C. cajan* (ICP 28 and ICP 26) were used as parents for the production of interspecific hybrids. Medium- and short-duration *C. scarabaeoides* accessions were used in the hybridization program based on their resistance to pod borer. Short-duration pigeonpea varieties, ICP 26 and ICP 28 (susceptible to pod borer), grown in India, were used as female parents and *C. scarabaeoides* as male parents. The parents differed significantly for all the morphological and agronomic characters. This study involved production and evaluation of hybrids for pod borer resistance.

In all, ten crosses were made, five with ICP 28 (ICP 28 x ICPW 94, ICP 28 x ICPW 116, ICP 28 x ICPW 125, ICP 28 x ICPW 130 and ICP 28 x ICPW 141) and five with ICP 26 (ICP 26 x ICPW 94, ICP 26 x ICPW 116, ICP 26 x ICPW 125, ICP 26 x ICPW 130 and ICP 26 x ICPW 141), in 1999-2000 Kharif season. Reciprocal crosses were also attempted with *C. cajan* as the male parent and *C. scarabaeoides* as the female parent. The t-test revealed significant differences among the parents used in the crossing program. The pollen viability test revealed that the hybrids showed 92 – 95 % viability. F<sub>1</sub> hybrids of seven of the ten crosses were selfed to produce F<sub>2</sub> plants during 2001 Kharif, and three out of the seven populations were further selfed to produce F<sub>3</sub> population in the consecutive years. The F<sub>1</sub> hybrids of these three crosses were also used in the backcrossing programme.

The F<sub>1</sub> plants were also screened for bud, flower and pod damage in field under multi-choice conditions for podborer resistance. The resistant F<sub>1</sub> hybrids of three crosses (ICP 28 x ICPW 94, ICP 28 x ICPW 130 and ICP 26 x ICPW 125), out of the ten F<sub>1</sub> crosses, were further backcrossed with *C. cajan* parent to produce the backcross generations. The BC<sub>1</sub>F<sub>1</sub> plants were screened in the next season for pod borer resistance in the field under multi-choice conditions. The resistant plants were again backcrossed to produce the next generation of backcrosses; BC<sub>2</sub> and BC<sub>3</sub>.

Mid- and better parent heterosis was studied for days to flower, days to maturity, leaflet length and width, pod length and width, number of locules per pod, number of seeds per pod, 100- seed weight, number of primary and secondary branches, density of trichomes A, B, C and D. Mid- and better parent heterosis values for days to flower and maturity indicated that the hybrids flowered and matured earlier than both the parents, had smaller leaves than the parents, had wider but short pods compared to parents, had less seed protein content but heavier seeds than the parents, had more number of primary but less number of secondary branches. Pods of hybrids had less number of trichome A but more number of trichomes B, C and D.

Parents,  $F_1$  and  $F_2$  populations of seven interspecific crosses were evaluated to study the inheritance pattern of qualitative (plant habit, stem color, leaflet shape, presence and absence of strophiole, mottleness of seed and pod hairiness) characters. The results indicated that the plant habit (erect, semi-spreading and spreading) is controlled by a single gene with incomplete/partial dominance, the leaflet shape (obovate, intermediate and lanceolate) was controlled by a single gene with incomplete / partial dominance. The seed mottleness is under the dominant epistatic gene interaction, presence and absence of strophiole on seed was under the inhibitory gene action and pod hairiness was controlled by a single dominant gene.

The means and variances obtained for various quantitative characters, in five generations (parents,  $F_1$ ,  $F_2$  and  $F_3$ ), and in three crosses (ICP 28 X ICPW 94, ICP 28 X ICPW 130 and ICP 26 X ICPW 125), were subjected to joint scaling test and five parameter model of generation mean analysis to determine their genetic basis. The joint scaling test (Cavalli, 1952) was conducted to obtain information on the nature of the gene effects involved in the quantitative characters (days to flowering, days to maturity, leaflet length, leaflet width, pod length, pod width, pod bearing length, number of locules per pod, number of seeds per pod, number of primary and secondary branches, 100-seed weight and harvest index). The parameters estimated were  $m$  (mean),  $d$  (pooled additive effects),  $h$  (pooled dominance effects),  $i$  (the pooled additive x additive epistatic effects), and  $l$  (pooled dominance x dominance

effects). In the absence of second backcross progeny, the *j* (pooled additive x dominance effects) component was not estimated.

In all the three crosses, estimates of *d* (additive) were significant for days to flower, pod length, pod bearing length, seeds per pod, number of primary branches, 100 - seed weight and harvest index; *h* (dominance) was significant for days to flower, days to maturity, leaflet width, pod bearing length, seeds per pod, locules per pod and number of primary branches. The estimates of *i* (additive x additive) were significant for days to maturity, leaflet length, leaflet width, pod width, locules per pod, number of secondary branches, while *l* (dominance x dominance) was significant for leaflet length, leaflet width, pod length, pod width, seeds per pod, locules per pod, number of primary and secondary branches.

In the cross, ICP 28 x ICPW 94; *h* and *l* effects expressed negatively for days to flower, and positively for leaflet length, leaflet width and number of primary branches indicating duplicate epistasis. The *h* and *l* effects expressed with opposite signs indicated complimentary epistasis for days to maturity, pod length, pod width, pod bearing length, number of locules per pod, number of seeds per pod, number of secondary branches, 100 - seed weight and harvest index .

In the cross, ICP 28 x ICPW 130; *h* and *l* effects expressed negatively for days to maturity, and positively for leaflet width and number of secondary branches indicating duplicate epistasis. The *h* and *l* effects expressed with opposite signs indicated complimentary epistasis for days to flower, leaflet length, leaflet width, pod length, pod bearing length, number of locules per pod, number of seeds per pod, number of primary branches, 100 - seed weight and harvest index.

In the cross, ICP 26 x ICPW 125; *h* and *l* effects expressed positively for leaflet width and number of seeds per pod and number of secondary branches indicating duplicate epistasis. The *h* and *l* effects expressed with opposite signs indicated complimentary epistasis for days to flower, days to maturity, leaflet length, pod length, pod width, pod bearing length, number of locules per pod, number of primary branches, 100 - seed weight and harvest index .



Variation in the population was explained with contributions of d, h, i and l for all the traits studied. The contribution of d in cross, ICP 28 x ICPW 94 was maximum for pod length (87.5%), 100 - seed weight (95.10%) and harvest index (94.56%); while h was maximum for pod width (48.82%) and number of primary branches (50.49%); i for days to flower (86.48%), days to maturity (47.77%), leaflet length (60.36%) and leaflet width (76.77%); and l for pod bearing length (60.85%), number of seeds per pod (75.83%), number of locules per pod (42.86%) and number of secondary branches (58.49%).

In the cross, ICP 28 x ICPW 130; contribution of d was maximum for days to maturity (69.30%), pod length (47.79%), number of secondary branches (67.28%), 100-seed weight (98.59%) and harvest index (98.32%); h for days to flower (57.48%), leaflet length (71.89%), leaflet width (58.75%), pod bearing length (64.27%) and number of seeds per pod (65.06%); i for pod width (70.51%) and number of primary branches (52.55%); and l for number of locules per pod (59.66%) in explaining the variation of these characters.

In the cross, ICP 26 x ICPW 125; contribution of d was maximum for days to flower (86.53%), leaflet width (70.43%), pod bearing length (61.85%), number of primary branches (44.67%), number of secondary branches (80.48%), 100 - seed weight (98.56%) and harvest index (97.28%); h for number of locules per pod (45.50%); i for days to maturity (55.75%) and leaflet length (65.78%), and l for pod length (77.73%), pod width (81.56%) and number of seeds per pod (53.37%).

The harvest index was explained with maximum contribution from d in crosses, ICP 28 x ICPW 94 (94.56), ICP 28 x ICWP 130 (98.00) and ICP 26 x ICWP 125 (97.28). Seed weight in all three crosses was explained with maximum contribution from d (95.104 to 98.61) in crosses ICP 28 x ICPW 94, ICP 26 x ICPW 125 and ICP 28 x ICPW 130 indicating that the additive effect was significant in all the crosses. The contribution of i and l were non-significant in crosses ICP 28 x ICPW 94 and ICP 26 x ICPW 125, while in cross ICP 28 x ICPW 130 only the i was non-significant for both harvest index and the seed weight.

Inheritance of type and density of trichomes, on the pod wall, was determined in two crosses; ICP 28 x ICPW 94 and ICP 26 x ICPW 125. Ten pods from each plant and ten plants in each parent ( $P_1$  and  $P_2$ ), ten from  $F_1$ , 250 plants of the  $F_2$  generation, and 75 from the  $BC_1F_1$  generation were observed for the type and density of trichomes in each cross. The data on type and density of trichomes types A, B, C and D on pods in parents,  $F_1$ ,  $F_2$ , and backcross generations in both the crosses were analyzed using  $\chi^2$ -test for goodness of fit. The low density of trichomes A and B, and high density of trichome C and D types were dominant over high density of A, B, and low density of C and D, respectively. The segregation pattern in  $F_2$  and backcross generations indicated that low density of trichome A and B types in both crosses, and high density of C type in both the crosses and D type only in one cross (ICP 28 x ICPW 94) was governed by the dominant allele of a single gene. The segregation pattern in  $F_2$  and backcross generations of the cross ICP 26 x ICPW 125 did not fit a single gene pattern, indicating presence of digenic or higher order interlocus interaction.

Parents,  $F_1$ ,  $F_2$  and  $BC_1$  populations of three crosses (ICP 28 x ICPW 94, ICP 28 x ICPW 130 and ICP 26 x ICPW 125) were evaluated in the field under multi-choice conditions for pod borer resistance. Percentage of bud, flower and pod damage and numbers of eggs and larvae per inflorescence were recorded. The plants of a cross ICP 28 x ICPW 94 were also screened under no-choice conditions in the laboratory for pod borer resistance. Pods of parents,  $F_1$ ,  $F_2$  and backcross generations were screened for antibiosis. The genetics of pod borer resistance for antixenosis and antibiosis mechanisms were determined. The segregation pattern of 3 (resistant): 1 (susceptible), in  $F_2$  generation, indicated that the antixenosis and antibiosis mechanisms of resistance are governed by the dominant allele of a single gene, indicating a simple inheritance.

Pigeonpea improvement was hitherto based on the variability present within the primary gene pool, as gene transfer from one variety to another can be easily achieved. The present study demonstrates that 30 wild accessions of *C. scarabaeoides*, evaluated for the first time, could be a potential and valuable

source of germplasm for pigeonpea improvement. Hence, large-scale morphological, molecular and biochemical diversity studies, on wild accessions of *C. scarabaeoides*, have been carried out for the first time. The identification of parents is an important prerequisite in any crop-breeding programme. Further, pigeonpea, for the first time, pod borer resistance gene (s) from the wild *C. scarabaeoides* were successfully introgressed into the cultivated background through backcross programme. Also, the genetic bases of different qualitative and quantitative characters, including resistance to podborer as well as resistance related traits such as trichome type and their densities have been determined. In the present study, sufficient genetic information has been generated for undertaking gene mapping besides F<sub>4</sub> and BC<sub>3</sub> populations that can be advanced to further generations for selecting desirable recombinants.

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† Original not seen