

# **IDENTIFICATION OF FERTILITY RESTORERS AND MAINTAINERS IN PIGEONPEA [*Cajanus cajan* (L.) Millspaugh]**

By

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This is to certify that the thesis entitled “**Identification of fertility restorers and maintainers in pigeonpea [*Cajanus cajan* (L.) Millspaugh]**” submitted in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) IN THE SUBJECT OF PLANT BREEDING & GENETICS** of the faculty of Agriculture, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, is genuine record of *bona fide* research work carried out by **Mr. Sudhir Kumar** under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been fully acknowledged.

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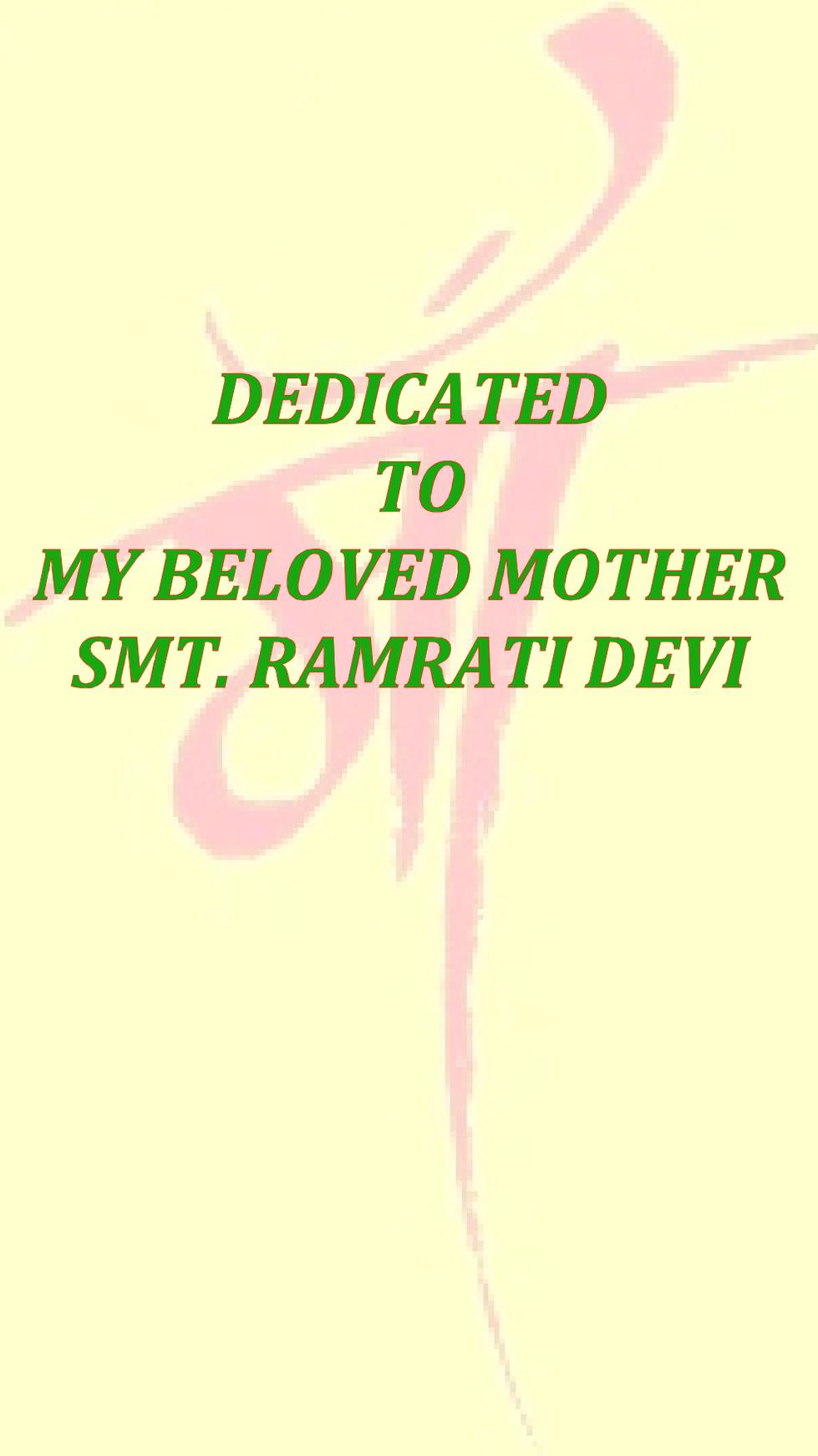
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***Sudhir Kumar***

## LIST OF SYMBOLS AND ABBREVIATIONS

Symbol	Legend
<	Less than
>	Greater than
/	Per
&	And
@	At the rate of
%	Percent
±	Plus or Minus
°C	Degree Celsius
C.D.	Critical Difference
cm	Centimeter
C.V.	Coefficient of Variation
d.f.	Degree of Freedom
<i>et al.</i>	Et alia (and others)
etc	Etcetera
Fig.	Figure (s)
g	Gram
ha	Hectare
i.e.	That is
kg	Kilogram (s)
M.S.S.	Mean sum of square
No.	Number (s)
NS	Non-significant
R.H.	Relative humidity
S.Em. <sub>±</sub>	Standard error of mean
Viz.	Videlicet (namely)
%	Percent



***DEDICATED  
TO  
MY BELOVED MOTHER  
SMT. RAMRATI DEVI***

# CHAPTER I

## *INTRODUCTION*



# INTRODUCTION

---

Pigeonpea [*Cajanus cajan* (L.) Millspaugh], is a short-lived perennial member of family Fabaceae and it is invariably cultivated as annual crop. Pigeonpea is an often cross-pollinated (20-30%) crop with  $2n = 2x = 22$  diploid chromosome number and genome size of  $1C = 858$  Mbp. Pigeonpea is commonly known as red gram, tur, arhar, tuvarica, congobean (Van der Maesen, 1986), thogari in India, 'Nandolo' in Malawi, 'Gandul' in Puerto Rico, 'Mu Dou' in China and 'Quinchocho' in Venezuela. India is considered as the native of pigeonpea (Van der Maesen, 1980) because of its natural genetic variability available in the local germplasm and the presence of its wild relatives in the country.

Pigeonpea is a hardy, widely adapted, and drought tolerant crop. It has a range of maturity which helps in its adaption in a wide range of environments and cropping systems. It is mainly grown as intercrop with other short duration cereals such as sorghum (*Sorghum bicolor*), pearl millet (*Pennisetium glaucum*), maize (*Zea mays*), and legumes such as soybean (*Glycine max*), greengram (*Vigna radiata*) and groundnut (*Arachis hypogaea*).

Being a pulse, pigeonpea enriches soil through symbiotic nitrogen fixation, releases soil-bound phosphorous, recycles the soil nutrients, and adds organic matter and other nutrients that make pigeonpea an ideal crop for sustainable agriculture (Saxena, 2008). It is chiefly grown for its seeds which are consumed either as dry splits (dal) or as a green vegetable.

Globally, it is cultivated on 5.32 million hectares (m ha) with an annual production of 4.32 million tonnes (m t) and mean productivity of  $813.2 \text{ kg ha}^{-1}$  (FAO, 2012). Pigeonpea is grown in over 50 countries, but 77.5% of its area is confined to India. Myanmar (0.64 m ha) and Malawi (0.19 m ha) are the other important pigeonpea growing countries. In India, pigeonpea is cultivated on 3.86 m ha with production of 2.65 m t and productivity of  $686.5 \text{ kg ha}^{-1}$  (FAO, 2012). It is mainly grown in the states of Andhra Pradesh (0.31 M ha,  $326.8 \text{ kg ha}^{-1}$ ), Gujarat (0.25 M ha,  $681 \text{ kg ha}^{-1}$ ), Maharashtra (0.85 M ha,  $608 \text{ kg ha}^{-1}$ ), Uttar Pradesh (0.48 M ha,  $1149.5 \text{ kg ha}^{-1}$ ) and Madhya Pradesh (0.43 M ha,  $761.0 \text{ kg ha}^{-1}$ ). These six states accounts for over 85% of total pigeonpea area (IIPR, Kanpur, 2013) and production in India and their average

pigeonpea grown area for the last six decades is presented in Fig. 1.1. In spite of high importance in domestic nutrition of Indian population and dedicated research efforts, the productivity of pigeonpea in the last few decades has remained constant at around 700 kg ha<sup>-1</sup> (www.faostat.org).

This is a matter of concern that the majority of the Indian population is vegetarian and their protein source directly depends on pulses. In order to meet this requirement, the Indian Government annually imports about 0.5 to 0.6 m. tons of pigeonpea mainly from Myanmar and southern and eastern Africa (Saxena and Nadarajan, 2010).

To promote the pigeonpea production, genetic improvement of pigeonpea was emphasized by researchers for more than five decades and a number of cultivars were developed from hybridization programmes and selection of landraces (Singh *et al.*, 2005). However, the progress in the genetic improvement of yield potential has been limited and the improved cultivars enhanced the productivity of the crop to some extent.

Therefore, an alternative breeding approach such as hybrid technology, which has been profitably used in a number of cereals, fruits and vegetable crops was attempted in pigeonpea to enhance the yield. The hybrid breeding technology has demonstrated quantum yield jump in various cereal (Alexandratos, 2001), vegetable (Rai and Rai, 2006) and fruit (Kuznecov, 1966) crops. The commercial exploitation of hybrids is known to be directly linked to the ease with which their hybrid seeds could be produced and delivered economically to farmers. In most food legumes, the absence of natural cross-fertilization is the major bottleneck in exploiting hybrid vigour at commercial scale because their flower structure forces high level of self-pollination (Saxena *et al.*, 1992).

The development of commercial hybrid pigeonpea programme was innovated at ICRISAT in collaboration with ICAR (Indian Council of Agricultural Research). In 1974, a source of genetic male-sterility (GMS) was identified. As a consequence, a genetic male-sterility based pigeonpea hybrid ICPH 8 was released in 1991 in India (Saxena *et al.*, 1992). It is considered a milestone in the history of crop breeding as ICPH 8 is the first ever commercial hybrid released in any food legume in the world. This hybrid, however, could not be commercialized due to its high seed cost and difficulties in maintaining the genetic purity. This development provided the most

important information on the role of partial natural out-crossing in large-scale hybrid seed production. This component is essential for commercial exploitation of hybrid vigour in pigeonpea (Saxena and Nadarajan, 2010). Natural out-crossing in pigeonpea was first reported by Howard *et al.* (1919). The out-crossing in this crop is mediated by a variety of insects (Onim, 1981) and wind does not play any role in this event (Kumar and Saxena, 2001). Bhatia *et al.* (1981) reported 24% natural out-crossing in pigeonpea at Patancheru. This level of out-crossing was found sufficient to maintain male-sterile lines and also to produce F1 hybrid seeds.

Due to the limitation of large-scale hybrid seed production in GMS-based hybrids, the development of cytoplasmic-nuclear male-sterility (CMS) became imperative. To develop a CMS system, it is believed that the interaction between wild cytoplasm and cultivated nuclear genome would produce male sterility effect. The different cytoplasmic male sterility sources derived from wild relatives of pigeonpea are given in Table 1.1. Of these, A2 and A4 systems derived from crosses involving wild relatives of pigeonpea and cultivated types have shown promise because of their stability under various agro-climatic conditions and availability of good maintainers and fertility restorers (Saxena and Nadarajan, 2010). By using A2 cytoplasm, a hybrid GTH-1 was released by ICAR for commercial cultivation in Gujarat state. It demonstrated 57.40% yield superiority over the best GMS hybrid AKPH 4101 (1183 kg ha<sup>-1</sup>) and 32% superiority over the best local variety GT 101 (1330 kg ha<sup>-1</sup>). This hybrid is early (140 days) in maturity.

ICRISAT developed a number of experimental hybrids and tested in multi-location trials. They also developed genetically diverse male-sterile lines and their fertility restorers for developing widely adaptability hybrids to different agro-ecological areas and cropping systems. Among the short duration hybrids ICPH 2433, which is based on A4 cytoplasm, recorded highest yield of 2419 kg ha<sup>-1</sup> and it exhibited high levels of hybrid vigour over all the local controls.

Since the scope of increasing pigeonpea area is limited, increasing its productivity is a viable option. In this context, the most reliable and proven approach is the hybrid breeding. This was possible due to successful breeding of a stable cytoplasmic nuclear male-sterility (CMS) system (Saxena *et al.*, 2005) in pigeonpea. The hybrids exhibited considerable superiority (20-25%) in terms of yield over the control cultivar, suggesting that significant yield advances are possible by adopting

hybrids technology in pigeonpea (Saxena and Nadarajan, 2010). To sustain the achievements of this breakthrough, it is essential that according to local need, superior hybrids are made available from time-to-time to farmers of different regions and to achieve this, breeding of suitable hybrids parents is a pre-requisite. To develop high yielding hybrids, identification of lines with fertility restorer and maintainer along with important yield contributing traits is of prime importance. Thus, the present research work was undertaken with following objectives:

1. To identify the fertility restorer (R-line) and maintainer (B-line) in pigeonpea genotypes.
2. To estimate the heterosis.
3. To estimate the effectiveness of CMS-lines.

Table 1.1: List of CMS sources derived from different wild relatives of pigeonpea

S. No.	Wild relative	CMS System
1	<i>Cajanus sericeus</i> (Ariyanayagam <i>et al.</i> , 1995)	A1
2	<i>Cajanus scarabaeoides</i> (Saxena and Kumar, 2003)	A2
3	<i>Cajanus volubilis</i> (Wanjari <i>et al.</i> , 2001)	A3
4	<i>Cajanus cajanifolius</i> (Saxena <i>et al.</i> , 2005)	A4
5	<i>Cajanus cajan</i> (Mallikarjuna & Saxena, 2005)	A5
6	<i>Cajanus lineatus</i>	A6
7	<i>Cajanus platycarpus</i> (Mallikarjuna <i>et al.</i> , 2011)	A7
8	<i>Cajanus reticulatus</i> (Saxena <i>et al.</i> , unpublished)	A8

Source: Saxena *et al.* 2010 and Mallikarjuna N. 2012.

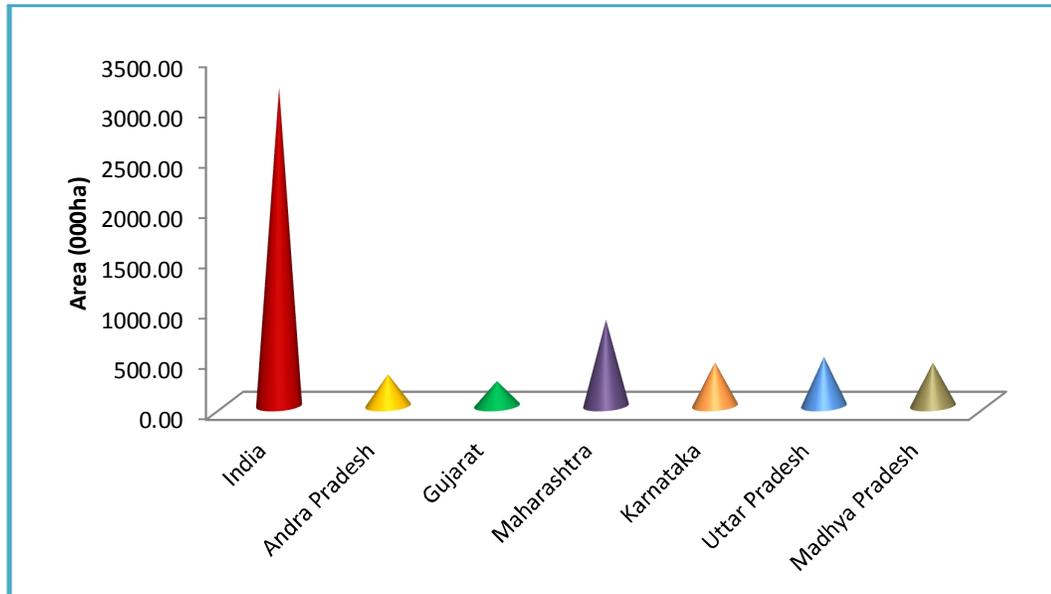


Fig. 1.1: Average area of pigeonpea in major growing states of India for the last six decades (Source- AICRP on pigeonpea, IIPR Kanpur, 2013)

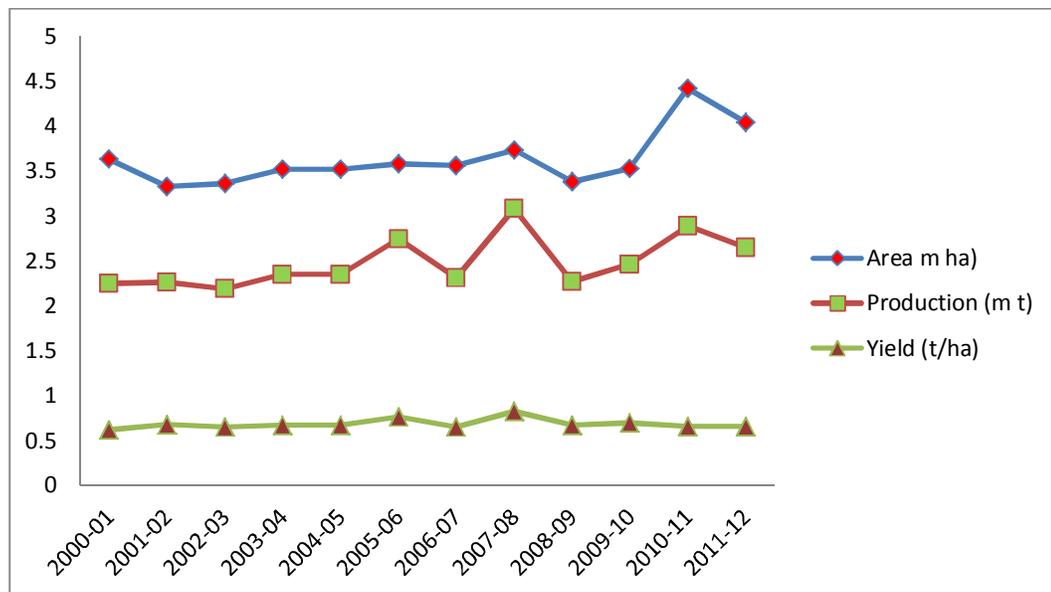


Fig. 1.2: Area, production and productivity of pigeonpea in India (Source- AICRP on pigeonpea, IIPR Kanpur, 2013)

# CHAPTER 2

## *REVIEW OF LITERATURE*



## REVIEW OF LITERATURE

---

Development of male-sterile lines in pigeonpea opened new vista for commercial exploitation of heterosis. ICRISAT developed a hybrid pigeonpea breeding technology based on cytoplasmic male-sterility (CMS) and insect-aided natural out-crossing systems (Saxena *et al.*, 2006). So far, over 1500 experimental hybrids have been tested and promising hybrids with yield advantages of 25 to 156% over the best inbred variety (Kandalkar 2007, Saxena and Nadarajan 2010). To sustain the achievements of this breakthrough, it is essential that according to local need, superior hybrids are made available to farmers of different regions, and to achieve this, breeding of suitable hybrids parents is a pre-requisite, i.e. male sterile line(A-line), maintainer line(B-line) and restorer line(R-line). The male-sterile lines developed; need to study their stability for expression of male sterility and fertility restoring ability of restorer lines with the performance of their hybrids in terms of heterosis.

The literature on different aspects of the present study has been reviewed, summarized and presented under the following heads:

- 2.1 Cytoplasmic-nuclear male-sterility, its maintainer and restorer in pigeonpea.
- 2.2 Heterosis in pigeonpea.
- 2.3 Effectiveness of CMS-lines.

### **2.1 Cytoplasmic-nuclear male-sterility, its maintainer and restorer in pigeonpea.**

By the various approaches contemplated to break the existing yield barriers in pigeonpea to feed the increasing population, hybrid technology is considered as one of the promising, sustainable and eco-friendly technologies. Impressive progress and success made by ICRISAT in this regard has encouraged the global pigeonpea production and productivity by adopting the CMS-based hybrid technology. Presence of exploitable hybrid vigour, availability of cytoplasmic nuclear male sterility and fertility restoration system and sound seed production techniques are the pre-requisites for the success of any hybrid breeding programme. In the exploitation of heterosis from potential crosses, the level of fertility restoration would likely be the key for added yield advantages. Therefore, a precise understanding of the fertility restoration is necessary for improving the efficiency and quality of restorers used in hybrid

pigeonpea breeding. The literature on Cytoplasmic-nuclear male-sterility (CMS), its maintenance, and fertility restoration in pigeonpea is briefly reviewed here:

Reddy and Faris (1981) reported first attempt to develop cytoplasmic male sterility in pigeonpea using the crossable wild relatives of pigeonpea. They crossed *Cajanus scarabaeoides*, a wild species with fertile F<sub>1</sub> plants of *C. cajan* x *C. scarabaeoides*. The resultant BC<sub>1</sub> F<sub>1</sub> plants were fertile but In BC<sub>1</sub>F<sub>2</sub> generation some male sterile segregants were identified.

Saxena *et al.* (1983) reported the inheritance of the B15B male sterile/fertile character. For the study of male sterile/fertile, male sterile plants of B15B were crossed with cultivars 3D8 103, QPL- 1, and Royes. The F<sub>1</sub> and F<sub>2</sub> generations and test cross progenies of fertile F<sub>1</sub> plants crossed to male sterile B15B were classified for male fertility. The results fitted a 3 fertile: 1 sterile ratio in all cases (all P>001, most P>0.05). The test cross progenies were of limited size but each fitted a 1: 1 ratio. These results suggested that B15B male sterility/fertility was conditioned by a single recessive/dominant gene.

Ariyanayagam *et al.* (1995) reported that the resultant BC<sub>1</sub> F<sub>1</sub> plants were fertile but in BC<sub>1</sub>F<sub>2</sub> generation some male sterile segregants were identified. This male sterility was linked with female sterility and therefore, it was not pursued further. They crossed *C. sericeus* with cultivated pigeonpea and found the F<sub>1</sub> progeny was partially male sterile. The backcross (BC<sub>1</sub>F<sub>1</sub>BC<sub>3</sub>F<sub>1</sub>) population was found segregating for male sterility.

Tikka *et al.* (1997) encouraged with the initial success in developing CMS at ICRISAT, six ICAR centers, viz. Indian Institute of Pulses Research (9 species), Indian Agricultural Research Institute (1 species), Gujarat Agriculture University (2 species), Punjabrao Agriculture Vidyapeeth (2 species), Tamil Nadu Agriculture University (2 species), and Punjab Agricultural University (2 species) also joined to develop CMS lines through intraspecific crosses. At Gujarat Agricultural University a CMS lines using *C. scarabaeoides* cytoplasm was developed.

Pelletier and Budar (1997) reported that Nucleo-cytoplasmic male sterilities are binary genetic systems driven by mitochondrial, maternally inherited genes that induce male sterility and a female phenotype and which are overcome by nuclear restorers of fertility. They contribute to the reproductive biology and evolution of natural

populations and are valuable tools for the commercial production of hybrid seeds in crops. For species with no natural form of cytoplasmic male sterility, such sterility can in some cases be introduced from different, but related, species through sexual crosses or somatic hybridization. Somatic hybridization is the only technique currently available for manipulating plant mitochondrial genomes. Recent successes in plastid transformation have opened up entirely new perspectives for the engineering of cytoplasmic male sterilities in trans-plastomic plants.

Saxena and Kumar (2003) studied the fertility restoration system in A<sub>2</sub> cytoplasm in pigeonpea. They developed the crosses between 3 CMS lines on the basis of A<sub>2</sub> cytoplasm with 14 diverse pigeonpea lines. Among these, five crosses had 94 to 100% fertility restoration and these parents need to be preserved to use directly in breeding high yielding restorer lines. Six crosses were male-sterile and from this group one or two crosses can be selected to develop maintainer by backcrossing to diversify the genetic base of the CMS system. The remaining three crosses segregated for partial fertility and such pollinators need to improve their genetic purity for fertility restoration ability.

Chauhan *et al.* (2004) studied fertility restoration in cytoplasmic genetic male-sterile (CGMS) of pigeonpea derived from *C. scarabaeoides*. To identify perfect pollen fertility restorers, 543 derivative lines of *C. scarabaeoides* x *C. cajan* and 1365 germplasm accessions were used as male parent on stable cytoplasmic genic male sterile line GT- 288A during *kharif* 1997 to 2003. The F<sub>1</sub> progenies of all the crosses were evaluated during *kharif* 1998 to 2003 for their pollen fertility. The promising pollen fertility restoring parents were advanced and purified through selfing. Finally, eighteen fertility restorers were identified and characterized.

Chase (2004) reported that mitochondrial function depends on the coordinate action of nuclear and mitochondrial genomes. The genetic dissection of these interactions presents special challenges in obligate aerobes, because the viability of these organisms depends on mitochondrial respiration. The plant trait cytoplasmic male sterility (CMS) is determined by the mitochondrial genome and is associated with a pollen sterility phenotype that can be suppressed or counteracted by nuclear genes known as restorer of fertility genes.

Singh and Bajpai (2005) found that heterosis is being the present communication reports the relative pollen fertility and morphology in interspecies crosses of pigeonpea. *C. cajan* x *C. acutifolius* hybrid showed low pollen fertility in crosses utilizing *C. cajanifolius* and *C. scarabaeoides*. Moderate variation (37.45) was noticed in size of pollen grains among the parents and their hybrid *Cajanus acutifolius* had the smallest pollen size.

Mallikarjuna and Saxena (2005) in pigeonpea report a new source of CMS developed by using the cultivated pigeonpea as the female parent and one of its wild relative *Cajanus acutifolius* as the pollen donor. This is the first report in pigeonpea where CMS has been developed using the cytoplasm of cultivated pigeonpea. Several pure line cultivars of pigeonpea restored pollen fertility whereas cv. HPL 24 partially maintained male sterility. The wild species *C. acutifolius* used as one of the parents, maintained complete sterility. Cytological analysis revealed that both in male sterile as well as the fertile floral buds, meiosis proceeded normally till the tetrad stage. However in the male sterile genotypes during the formation of tetrads, the pollen mother cell (PMC) wall did not dissolve to release the tetrads unlike in the fertile genotypes and this major event was found to be responsible for male sterility.

Souframanien *et al.* (2006) in order achieve breakthrough in pigeonpea productivity, exploited heterosis using the male sterility system based on cytoplasmic nuclear interactions. Production of hybrids using a cytoplasmic male sterility (CMS) system involves the use of a male sterile line (A line), a maintainer line (B line) and a restorer line (R line).

Wanjari *et al.* (2007) used highly fertile individual pigeonpea plants from potential fertility restorer lines to develop hybrids with CMS lines in the background of *Cajanus scarabaeoides* cytoplasm. A set of 136 hybrids was grown in plots in Maharashtra, India, along with AKT 8811 as the control. The hybrid plants were evaluated for anther dehiscence and pollen fertility. The hybrids are showing more than 80% pollen fertility in all the plants.

Kalaimagal *et al.* (2008) reported in any hybrid breeding program, identification of potential restorers forms an important part. Therefore, 80 cross combinations with both lines A were developed and tested for their restorability. One restorer CORG 9904 was identified for CORG 990052 A, with 96.2–98.6% fertility restoration, and 2

restorers, viz. ICPL 84031 and ICPL 90028, were identified for CORG 990047 A, with 90.2–98.2% and 91.0–96.0% fertility restoration, respectively. Although the CMS source was the same, these lines A did not share a common restorer, but this may be due to differential inter-genomic or cytoplasmic–genomic interactions. Also within a hybrid combination the plants showed differential fertility restoration, and this may be due to the heterogeneity within the restorer.

Dalvi *et al.* (2008a) reported the fertility restoration in cytoplasmic-nuclear male sterile lines derived from 3 wild relatives of pigeonpea. To study the fertility restoration of the CMS lines, three cytoplasmic-nuclear male-sterile (CMS) lines derived from *C. sericeus* (A<sub>1</sub> cytoplasm), *C. scarabaeoides* (A<sub>2</sub> cytoplasm), and *C. cajanifolius* (A<sub>4</sub> cytoplasm), were crossed to seven pigeonpea cultivars in a line x tester mating scheme. Twenty-one F<sub>1</sub> hybrid combinations were planted in three environments. There was no effect of environments on the expression of fertility restoration. Pigeonpea cultivar ICPL 129-3 restored fertility in A<sub>1</sub> cytoplasm and maintained male sterility in the other 2 (A<sub>2</sub> and A<sub>4</sub>) cytoplasms. Among crosses involving CMS line (of A<sub>4</sub> cytoplasm) ICPA 2039 one hybrid combination was male-sterile and another male fertile. The remaining five combinations segregated for male-fertility (66–84% fertility restoration). Such testers can easily be purified for use in hybrid breeding programmes by selfing and single-plant selection for 2–3 generations.

Dalvi *et al.* (2008b) studied the genetics of fertility restoration in a CMS line ICPA 2039 and its five fertility restores in pigeonpea. All the F<sub>1</sub> plants in 5 crosses were fully fertile indicating the dominance of fertility restoring genes. Among the 5 crosses studied, 3 (ICPA 2039 x ICPL 12320, ICPA 2039 x ICPL 11376, and ICPA 2039 x HPL 24-63) segregated in a ratio of 3 fertile : 1 sterile in F<sub>2</sub> generation and 1 fertile : 1 sterile in BC<sub>1</sub>F<sub>1</sub> generation indicating the monogenic dominant nature of a single fertility restoring gene. The crosses ICPA 2039 x ICP 10650 segregated two dominant duplicated gene action with a ratio of 15 fertile : 1 fertile in F<sub>2</sub> and 3 fertile : 1 sterile in BC<sub>1</sub>F<sub>1</sub>, respectively. The rest cross ICPA 2039 x ICP 13991 had two complementary gene action of 9 fertile : 7 sterile in F<sub>2</sub> and 1 fertile : 3 sterile in BC<sub>1</sub>F<sub>1</sub>, respectively.

Nadrajana *et al.* (2008) studied the extent of fertility restoration for various cytoplasmic sources across germplasm lines, advanced breeding lines and cultivars. One hundred and sixty eight CGMS based hybrids were synthesized by adopting L x T

mating design with 12 CGMS lines and 14 testers. The hybrids were tested for fertility restoration by observing the pollen fertility status. The results indicated that 19 hybrids were restored out of 168 crosses evaluated accounting to 11.3%. The extent of restoration varied from 9.5 to 14.3% across the three cytoplasmic sources *viz.*, A<sub>1</sub>, A<sub>2</sub> and A<sub>4</sub>. Among the three sources of male parents selected, restoration was higher in the germplasm inbreds as compared to advanced breeding lines and cultivars indicating need for intensive exploration across genetically and geographically diverse genetic resources.

Saxena *et al.* (2010) reported the development of cytoplasmic–nuclear male sterility, its inheritance, and fertility restoration for potential use in hybrid pigeonpea breeding. They searched wide diversity of fertility restores and male-sterility maintainers to produce heterotic hybrids for diverse environments. Among 251 F<sub>1</sub>s evaluated, 30 (12.0%) maintained male sterility, 23 (9.2%) restored fertility, and 198 (78.9 %) segregated for male-fertility and sterility traits due to heterozygosity within germplasm accessions. In genetic of fertility restoration studies, all 35 F<sub>1</sub> plants of hybrid ICPA 2067 x ICP 12320 were male fertile indicating the dominance of fertility restoring genes. Out of 359 F<sub>2</sub> plants grown, 303 were fertile whereas only 56 exhibited male sterility. This segregation fit well to a ratio of 13 fertile : 3 sterile ( $P = 0.01$ ). In BC<sub>1</sub>F<sub>1</sub> generation out of 175 plants, 121 were male fertile and 54 had male-sterile anthers, which showed a good fit for a 3 fertile:1 sterile ( $P = 0.01$ ) ratio. These results suggested the presence of 2 dominant genes, with one basic and one inhibitory gene action for the determination of fertility restoration in ICPA 2067.

Saxena *et al.* (2011) reported the genetics of fertility restoration in A<sub>4</sub>-based cytoplasm based on diverse maturing hybrids of pigeonpea. They observed that the fertility restoration of extra-early-maturing hybrid (ICPA 2089 x PHR 31) was governed by mono gene with the segregation ratio of 3 fertile : 1 sterile in F<sub>2</sub> and 1 fertile : 1 sterile in BC<sub>1</sub>F<sub>1</sub> while early-maturing hybrids ICPA 2039 x ICPR 2438 and ICPA 2039 x ICPR 2447 were governed by digenic duplicate dominant ratio of 15 fertile : 1 sterile in F<sub>2</sub> and 3 fertile : 1 sterile in BC<sub>1</sub>F<sub>1</sub>. Similarly, late-maturing hybrid ICPA 2043 x ICPR 2671 and ICPA 2043 x ICPR 3497 were also governed by two duplicate dominant genes. It was also observed that hybrids with two dominant genes produced a greater pollen load and expressed greater stability as compared with those carrying a single dominant gene.

Saxena *et al.* (2011) studied the inheritance of the obcordate leaf trait and its fertility restoration ability of the obcordate leaf line ICP 5529. The crosses were made between four CMS-lines (ICPA 2089, ICPA 2047, ICPA 2048 and ICPA 2049) and ICP 5529. All the F1 plants of the obcordate donor were fully male-fertile and had normal leaves, suggesting that the obcordate leaf trait was recessive and that fertility restoration was due to the effect of dominant gene(s).

Since hybrid pigeonpea breeding technology was the first and new among the legumes, there was limited literature to review. Hence, the available literature on genetics of fertility restoration in other CMS based hybrid crops such as maize, rice and wheat were briefly reviewed hereunder.

### **Maize**

Chen and Liu (1991) studied 24 maize cross combinations from 2 CMS lines and 5 restorer lines with C, E, S and T type cytoplasm groups with 4 sowing dates at 3 sites for evaluating the stability of male fertility restoration of maize hybrids. They reported that there were highly significant differences in fertility restoration existed among the cross combinations and among cytoplasm groups. Fertility restoration of hybrids varied with genetic background and environment.

Liu and Chen (1992) studied the effect of ecological and climatic conditions on the restoration of fertility in 24 maize cross combinations. They reported that effects of the restorer varied significantly with genetic background and environment. Cool environments with a high level of moisture were more favourable for restoration of male fertility than high temperatures and dry conditions. Levels of fertility restoration were stable across different sowing dates. It is suggested that restorability tests and selection of restorers should be conducted before CMS hybrids are entered into production.

Weider *et al.* (2009) evaluated 22 CMS versions of modern European maize hybrids under 17 environments in Switzerland, France, and Bulgaria with two or three sowing dates, in 2005 and 2006. They reported both stable and unstable male sterile lines in all three CMS types. T-cytoplasm hybrids were the most stable, while hybrids developed from S-cytoplasm showed partial restoration of fertility while C-cytoplasm was similar to T-cytoplasm regards to maintaining the male sterility. Climatic factors, especially air temperature, evapotranspiration and water vapor during the 10 days

before anthesis as well as during anthesis, were correlated positively or negatively with the partial reversion to male fertility of CMS hybrids, indicated an interaction between genetic and climatic factors.

### **Rice**

Sarkar *et al.* (2003) studied the effect of the environment on fertility restoration exhibited by rice hybrids derived from cytoplasmic male sterile lines with different genetic backgrounds under 10 different environments in eight locations of India. The stability analysis on fertility restoration, yield and other attributes revealed that fertility restoration in hybrids from different CMS lines was highly sensitive to the changes in the environment with gradual delay of sowing dates. Estimates of stability parameters showed that the hybrids were unstable over the environments for both fertility restoration and grain yield, with the exception of PRH 3. A linear (predictable) response was shown by nearly all hybrids for all characters, as revealed by a significant genotype x environment interaction (linear) variance, though part of the variation was unpredictable in nature as shown by significant pooled deviation values.

### **Wheat**

Esimbekova (1990) evaluated the variation in the trait due to genotype-environment interaction in a 3-year study of 23 F1 wheat hybrids bred by top crosses using cytoplasmic male sterility from *Triticum timopheevii*. Environmental effects accounted for 67.4 % of the variation. The material studied was divided into 3 groups on the basis of response, with <5 %, 5-10 % and >10 % variation of the genotype under contrasting environmental conditions. The first group was the most promising for breeding hybrid varieties, since the fertility restoration of this group was stable over the years.

Zhan *et al.* (2005) studied K-type hybrids with 2 sterile lines (A) and some restorer lines (R) to evaluate the easy restoration of fertility (ERF), stability of fertility restoration, and the effects of sowing time and method on the degree of restoration. They reported that the degree of restoration of fertility in most of the K-type hybrids varied significantly between the years. The hybrids characterized by high degrees of restoration also showed stability in fertility restoration. The sowing date had significant effects, whereas the sowing method had slight effects on the degree of restoration in K-

type hybrids. Abnormally high temperature from ear emergence to anthesis was the major environmental factors affecting the stability of the degree of restoration.

## 2.2 Heterosis in pigeonpea:

“Heterosis” is defined quantitatively as an upward deviation of the mid-parent, based on the mean values of the two parents (Johnson and Hutchinson, 1993). Heterosis may be positive or negative. Depending upon the breeding objectives, both positive and negative heterosis is useful for crop improvement. In general, positive heterosis is desired for yield and negative heterosis for maturity. Heterosis is expressed in three ways, depending on the criteria used to compare the performance of a hybrid. The three ways are: mid-parent, standard variety and better parent heterosis. However, from the plant breeder’s viewpoint, better parent (heterobeltiosis, Fanseco and Peterson, 1968) and/or standard variety (standard heterosis) are more effective but for economic point of view standard heterosis is most important. Exploitation of heterosis in agriculture provides enhancing food security. Pigeonpea is a partially cross pollinated crop and the plants express strong heterosis in their F<sub>1</sub> hybrids. These led to the conclusion of the presence of significant heterosis in pigeonpea, which could be exploited commercially by developing F<sub>1</sub> hybrids. Solomon *et al.* (1957) were the first to report hybrid vigour in pigeonpea in 10 inter-varietal crosses. Subsequently, a number of reports have been published on hybrid vigour for yield and yield components. Chauhan *et al.* (2008) reported 19.9 to 26.1 % heterosis for yield in pigeonpea, and it was related to an increase in the number of pods plant<sup>-1</sup>, pod length, and seed size. Recently, 25 to 156% of seed yield over the best inbred variety have been reported by Kandalkar (2007) and Saxena and Nadarajan (2010). The world’s first cytoplasmic male sterility (CMS) based pigeonpea hybrid Pushkal (ICPH 2671) had broken the yield barrier that has plagued Indian agriculture for the past five decades (Saxena, 2009). Heterosis in pigeonpea is briefly reviewed here:

Solomon *et al.* (1957) were the first to report a study of heterosis in pigeonpea. Hybrid vigour up to a maximum of 24.51% in grain yield, 13.04% for plant height, 9.6% for pod length were obtained in some of the crosses under his study. However, the fact that the best yielding hybrid had not been able to out yield the yielding type involved in one or more of the crosses.

Shrivastava *et al.* (1976) reported the heterosis in pigeonpea. They studied the heterosis in 17 F<sub>1</sub> hybrid combinations involving 14 genotypes of pigeonpea. Heterotic effects were analysed for yield, its components and some growth factors. Mean heterosis of 67% was obtained for yield, 96% for secondary branches and 80% for number of pods per plant. In general, medium x medium, low x medium crosses resulted in high heterotic performance and indicated that genetic diversity was the key to obtaining hybrid vigour.

Patel *et al.* (1991) reported high degree of standard heterosis for various morpho-physiological traits in short and medium duration genetic male-sterility based pigeonpea hybrids. Short duration hybrid, MS Prabhat x DL 78-1 showed 71.9% standard heterosis and it was due to significant and positive heterosis for morpho-physiological traits such as plant height, harvest index, per day productivity and reproductive period. Hybrid MS 3A x ICPL 8504 in medium group had highest heterosis (74.90%) over standard variety S 5 and BDN 2, respectively. In medium duration group, delayed flowering, taller plant height and high per day productivity was observed to be the causes of high heterotic response for seed yield plant<sup>-1</sup>.

Patel and Patel (1992) reported heterosis in 30 hybrids derived from six lines and five testers in pigeonpea for yield and important yield contributing traits. Maximum heterosis response over better parent was obtained for number of pods plant<sup>-1</sup> (169.31%) and it was followed by seed yield plant<sup>-1</sup> (136.49%). None of the hybrids exhibited significant heterobeltiosis in any direction for pod length and seeds pod<sup>-1</sup>.

Gumber and Singh (1996) studied the phenomenon of heterosis in pigeonpea crosses involving genotypes of three different growth habits (DT: determinate; SDT: semi-determinate, and IDT: indeterminate). They observed heterosis over better parent was from -16.3 to 19.3% for seed yield plant<sup>-1</sup>, 36.0 to 78.0% for plant height and -4.0 to 20.30% for pods plant<sup>-1</sup>. They also indicated that, the cross combinations involving parents of different growth habits expressed greater heterosis while the cross combinations involving parents of similar growth habit (DT x DT or IDT x IDT) exhibited low heterosis over better parent.

Kumar and Srivastava (1998) studied heterosis in relation to combining ability in a line x tester mating design involving three male sterile lines and 12 male fertile lines of long duration pigeonpea for yield and its components. Heterosis over better parent

for seed yield ranged from -77.91 to 110.07 %. Pods plant<sup>-1</sup> and primary branches plant<sup>-1</sup> contributed substantially towards the expression of heterosis for seed yield. The observed gene action was predominantly non-additive for the characters studied.

Hooda *et al.* (1999) provided the information on heterosis of pigeonpea in seven yield-related traits in the parents and 40 hybrids from a 4 line × 10 tester crosses. Maximum heterosis over the best standard check (Manak) was obtained for the pods plant<sup>-1</sup> in crosses Qms1 × TAT10 (38.1%), Qms1 × H88-22 (32.9%) and MS Prabhat (DT) × H88- 43 (28.9%). For seed yield plant<sup>-1</sup>, a good magnitude of heterosis ranging from 21.1 to 28.9% was observed.

Khorgade *et al.* (2000) reported the heterosis over the mid-parent and control cultivar (BDN 2) in 24 pigeon pea hybrids. Significant heterosis was observed for seven quantitative characters studied. Significant heterosis over the mid-parent and control cultivar was recorded for seed yield plant<sup>-1</sup> in the hybrids AKMS 11 x AKT 9221, AKMS 11 x C11, and AKMS 21 x C11.

Chandirakala and Raveendran (2002) reported the heterosis for yield and yield components in 30 pigeonpea hybrids. Crosses with MS Prabhat DT showed marked heterosis for number of pods plant<sup>-1</sup>, number of clusters plant<sup>-1</sup>, 100-grain weight, and grain yield plant<sup>-1</sup>. Significant negative heterosis over mid, better, and standard parents were observed in MS Prabhat DT x ICPL 88009 and MS CO 5 x ICPL 88009 for days to 50% flowering, and in MS Prabhat DT x ICPL 87104, MS Prabhat DT x ICPL 89020, MS Prabhat DT x ICPL 90012, and MS CO 5 x ICPL 87104 for plant height.

Lohithaswa and Dharmaraj (2003) studied heterosis for yield and yield attributes. Observations were recorded for 12 quantitative characters. Non-additive gene effects were predominant for all characters, except for days to 50% flowering, 100-seed weight and protein content, for which additive gene action was predominant. The heterosis values when considered alone were misleading as there was no correspondence with *per se* performance.

Sekhar *et al.* (2004) studied the heterosis in 36 early maturing pigeonpea hybrids involving 3 male sterile lines and 12 pollinator lines. Three crosses [QMS-1 x Sel 90307, QMS-1 x Sel 90311 and MS Prabhat (NDT) x Sel 90214] exhibited 51.3 to 171.6% heterosis for seed yield plant<sup>-1</sup> over the standard check and better parent,

respectively. Among the tested materials, the best five hybrids exceeded 40% standard heterosis for seed yield and its components.

Yadav and Singh (2004) reported the heterosis of pigeonpea in yield and its related traits. In their research finding, 20 to 49.8 % of standard heterosis for primary branches plant<sup>-1</sup> was expressed in all the hybrids except msUPAS 120 x Pant A 134. For seed pod<sup>-1</sup>, significant positive heterosis was observed in seven hybrids. Number of pods plant<sup>-1</sup> expressed up to 203.9% of standard heterosis. The highest standard heterosis for 100-seed weight was 12.1% in UPAS 120 x Pant A 169. The range of standard heterosis for grain yield over standard variety was -46.03 to 180%.

Wankhade *et al.* (2005) investigated the amount of heterosis for seed yield and its components by using three genetic male sterile lines (females) and eight testers (males) in a line x tester mating design. The heterosis study was observed for most of the traits, except plant height. The cross AKMS 11 x AKT 9221 showed highest seed yield plant<sup>-1</sup> and exhibited high heterosis (63.19%) and useful heterosis over BDN 2 (83.34%). The mean squares due to parents and crosses were highly significant for all the characters.

Aher *et al.* (2006) reported the range of heterosis for MP and BP was from 3.25 to 2.25% and 2.50 to 10.50% for days to maturity, -1.10 to 3.15% and 2.9 to 2.4% for number of primary branches plant<sup>-1</sup>, and -0.95 to 3.35% and -3.0 to 2.5% for secondary branches plant<sup>-1</sup>. For number of pods plant<sup>-1</sup>, significant and positive heterosis over mid-parent and better parent was observed in BDN-2 × BDN-201. Heterosis over mid-parent and better parent ranged from -1.65 to 3.60% and -3.30 to 3.20%, respectively, for number of seeds per pod. Heterosis for 100-seed weight was from -0.51 to 0.22% and -1.97 to 0.03% for mid-parent and better parent, respectively. For grain yield plant<sup>-1</sup>, the range of heterosis over better was -20.66 to 23.79%.

Baskaran and Muthiah (2006) reported the magnitude of relative heterosis, heterobeltiosis and standard heterosis of 18 hybrids derived for seed yield and yield attributing characters. Significant positive heterotic effect over mid-parent, better parent and standard control (CO 5) was recorded for seed yield plant<sup>-1</sup> in hybrid VBN 1 x ICPL 83027 (81.74%, 66.57% and 68.36%) followed by CO 5 x ICPL 83027 (24.46%, 23.80% and 25.13%) and CORG 9904 × ICPL 83027 (56.47%, 17.77% and 19.03%).

Banu *et al.* (2007) investigated the relative heterosis and heterobeltiosis in 45 pigeonpea hybrids on days to 50% flowering, maturity, plant height, number of branches plant<sup>-1</sup>, number of clusters plant<sup>-1</sup>, number of pods plant<sup>-1</sup>, number of seeds pod<sup>-1</sup>, pod length and 100-seed weight and single plant yield. ICP 13201 × CO 5 was the best with the maximum heterosis for most of the yield attributing characters, followed by ICP 11961 x ICP 7118 and ICP 11961 x CO 5, which showed higher heterobeltiosis and relative heterosis for most of the yield-attributing characters.

Wanjari *et al.* (2007) evaluated the heterosis in a set of 136 CMS-based pigeonpea hybrids in the background of A2 cytoplasm along with AKT 8811 as the control. Heterosis over male parent and the control was investigated. Among the 136 hybrids, 11 expressed high pollen fertility (>80%) in all the plants. The hybrids characterized by high pollen fertility varied in terms of heterosis. Six hybrids showed positive heterosis.

Hershey (2007) from ICRISAT released the world's first pigeonpea hybrids based on the cytoplasmic male sterility system. The hybrids developed at ICRISAT have shown 30 to 150% yield advantage. The hybrids also produce 30.40% more root mass that makes them more drought resistant. The adoption of hybrid technology has been adopted by the seed producers and at present 22 private and 3 public seed companies have adopted the technology. In 2007, a total of 250,000 kg of hybrid seed is being produced. This will bring about 50,000 ha land under hybrid cultivation.

Dheva *et al.* (2008a) reported the heterosis in CMS based pigeonpea hybrids. The highest heterosis is observed for the character such as number of pods plant<sup>-1</sup> (79.43%) followed by grain yield plant<sup>-1</sup> (68.06%) and plant height (37.89%) over the better parent in desirable positive direction. The highest heterosis over the better parent observed for the character days to 50% flowering (-23.84%) followed by days to maturity (-16.94%) in desirable negative directions.

Dheva *et al.* (2009) reported that thirty eight hybrids which were obtained from crossing new cytoplasmic male sterile and restorer lines were grown during *kharif* 2007-08 in Akola, Maharashtra, India, to determine the heterosis over the standard control for the characters number of pods per plant and grain yield per plant. The heterosis in positive direction for number of pods per plant varied from 1.95 to 79.19% and for grain yield per plant from 0.97% to 59.68 over the control. The crosses

AK1202A x AKPR219, GT33A x AKPR297 and GT288A x AKPR192 showed higher heterosis over the control for number of pods per plant and grain yield per plant.

Dheva *et al.* (2008b) evaluated the heterosis in CMS based hybrid pigeonpea. They studied on 31 hybrids showing fertility more than 80% which are evaluated for the heterosis over the male parent, better parent and standard check. Among these, three hybrids showed heterosis more than 40% for number of pods and grain yield plant<sup>-1</sup>. The range of heterosis over check for number of pods per plant is 0.84 to 87.68 % and 0.72 to 57.35% for grain yield.

Kumar and Krishna (2008) reported the heterosis in pigeonpea over superior and economic parent (T-7) for 13 quantitative characters in pigeonpea. Eight hybrids KA-1 x KA32-1, K35 x Banda Palera, KA-1 x Banda Palera, KA26-8 x Banda Palera, KA26-8 x KA32-1, T7 x Banda Palera, K9125(B) x Banda Palera, and KA108 x KA32-1 were judged to be promising for grain yield plant<sup>-1</sup> on the basis of their high heterosis response, *per se* performance.

Patel and Tikka (2008) reported the heterosis for yield and yield components in 45 hybrids and 18 parental genotypes of pigeonpea. For number of pods plant<sup>-1</sup>, 10 and 20 hybrids recorded significant positive heterosis over the better parent and control, respectively. Eight hybrids were superior over the better parent with respect to number of seeds pod<sup>-1</sup>. Only two hybrids over the better parent and one hybrid over the control showed significant positive heterosis over the better parent for protein content. For seed yield, 2 hybrids exhibited positive heterosis over the better parent. Hybrid MS 3783 x BSMR 853 (97.54%) recorded the highest positive heterobeltiosis.

Saxena *et al.* (2009) reported that male sterility has been successfully used for enhancing yield in a number of cereal and vegetable crops. In food legumes, this technology could never be used either due to non-availability of natural outcrossing system, or an efficient male sterility system or both. Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a partially cross pollinated food legume and recent success in breeding a stable male sterility system has allowed breeders to exploit hybrid vigour for increasing yields. The cytoplasmic-nuclear male sterility (CMS) based hybrids have recorded 28.4% yield superiority over local checks in farmer's fields. This study besides summarizing the reports of all the genetic and CMS systems, also discusses the

prospects of utilizing these male sterility systems in commercial hybrid breeding programmes.

Bhavani and Bhalla (2009) analyzed the heterotic effects in 20 hybrid pigeonpea combinations involving five diverse parents belonging to different maturity groups (early, medium and late) for yield and its components. The average heterosis was maximum for yield plant<sup>-1</sup>, followed by pods plant<sup>-1</sup> and number of fruit bearing branches. Comparatively, the other yield components showed low average heterosis values. In general, early x late and medium x late combinations resulted in high heterosis for yield.

Dheva *et al.* (2009) reported the heterosis in 31 hybrids for the heterosis over the male parent, better parent and standard check. The three hybrids showed heterosis more than 40% for the number of pods and grain yield plant<sup>-1</sup>, respectively. The highest standard heterosis is observed for the number of pods plant<sup>-1</sup> followed by grain yield plant<sup>-1</sup>. The range of heterosis over check for the number of pods plant<sup>-1</sup> is 0.84 to 87.68% and the heterosis over check for the character grain yield plant<sup>-1</sup> ranged from 0.72 to 57.35% in desirable direction.

Kumar *et al.* (2009) reported the heterosis of pigeonpea for yield and its component traits. Significant and positive heterosis over better parent and standard check for seed yield plant<sup>-1</sup> in four crosses was accompanied by significant and high positive heterosis for number of primary branches plant<sup>-1</sup>, number of pods plant<sup>-1</sup>, number of pod clusters plant<sup>-1</sup> and 100 seed weight. This study suggested that heterosis for yield should be through component trait heterosis. Hybrid vigour of individual yield components may have additive or synergistic effect on the yield.

Phad *et al.* (2009) reported the heterosis in pigeonpea by using 60 crosses in four different environments. The top 10 cross combinations recorded significant positive standard heterosis for number of secondary branches plant<sup>-1</sup>, whereas nine cross combinations recorded standard heterotic effect for plant spread, number of primary branches plant<sup>-1</sup> and number of pods plant<sup>-1</sup>. Significant positive standard heterosis was recorded in seven cross combinations for harvest index, two cross combinations for plant height and only one cross combination for 100-seed weight. On the basis of pooled mean, the top 10 cross combinations showed superiority in different environments.

Sarode *et al.* (2009) estimated the heterosis in long duration pigeonpea for yield and yield traits using five lines and three testers. The maximum standard heterosis was recorded in the cross Pusa 9 x Bahar (52.11%), followed by Pusa 9 x ICPL 84023 (44.17%) and DA 11 x Bahar (42.03%) for number of pods plant<sup>-1</sup>. Hybrid Pusa 9 x Bahar exhibited maximum economic heterosis (55.32%) for 100-seed weight, number of seeds pod<sup>-1</sup>, pods plant<sup>-1</sup> and number of primary and secondary branches.

Chandirakala *et al.* (2010) studied the heterosis, heterobeltiosis and standard heterosis in 30 GMS based pigeonpea hybrids. Among these, 13 hybrids exhibited significant and positive heterosis over all the three bases of estimation. The two hybrids showed highly significant and positive heterosis over MP, BP and standard check. The proportion of hybrids exhibiting significant heterotic effect for grain yield with genetic male sterile line MS Prabhat DT was greater as compared to lines, MS Prabhat NDT and MS CO5.

Shoba and Balan (2010) studied the magnitude of heterosis in 27 early maturing hybrids. They observed standard heterosis for single plant yield varied from -25.0 (CORG 990047 A x ICPL 87) to 325% (MS CO 5 x PA 128). The promising hybrids, CORG 990047 A x APK 1 manifested heterosis for days to 50% flowering (56.3%), days to maturity (92.47%), plant height (113.0%), number of pods plant<sup>-1</sup>(106.0%), seed protein content (22.71%) and single plant yield (40.0%). MS CO5 x ICPL 83027 had significant standard heterosis for plant height (98.38%), number of branches plant<sup>-1</sup>(128.2%), number of pods plant<sup>-1</sup>(110.0%), number of seeds pod<sup>-1</sup> (4.50%) and single plant yield (70.0%).

Lay *et al.* (2011) reported the heterosis in CMS-based pigeonpea hybrids. They evaluated fifteen ICRISAT's pigeonpea hybrids in Myanmar at three locations. Hybrids ICPH 2671, ICPH 2673, ICPH 2740 and ICPH 3497 were found stable over the three environments and produced 30.4 to 41.7% standard heterosis. Hybrid ICPH 3461 was found suitable for one environment with 42.0% standard heterosis. In 36 on-farm trials, hybrid ICPH 2671 was 11.9 to 53.1% superior in yield over the control. The other promising hybrid ICPH 2740 also exhibited 70.0% standard heterosis in an on-farm trial.

### 2.3 Effectiveness of CMS-lines.

In pigeonpea, several stable CMS systems have been developed (Saxena and Kumar, 2003; Mallikarjuna and Saxena, 2005; Saxena *et al.* 2005). The line ICPW29, an accession of *Cajanus cajanifolius* (Haines) (Van der Maesen) (A4 cytoplasm), a wild relative of pigeonpea was crossed with cultivated type and after seven backcrosses developed the CMS line ICPA2039. It was found to be a highly stable male sterile line across environments and years and never showed any morphological deformity (Dalvi *et al.*, 2008; Saxena *et al.*, 2010b). The CMS line ICPA2039 has been used to develop other CMS lines with resistance to diseases, with various maturity periods and with adaption to diverse environments of India (Saxena, 2008).

Environmental conditions are known to influence the expression of nuclear and cytoplasmic male sterility genes in some crops, whereby sterility and fertility changes depend on day length and/or temperature (Janska and Mackenzie, 1993). Ariyanayagam *et al.* (1995), using sensitive pigeon pea genotypes, established that short day length and low temperatures induce male fertility, while high temperatures and longer days maintain male sterility. In CMS cotton (*Gossypium* spp.), wind velocity, air temperature, global radiation, and pan evaporation have been shown to influence expression of male sterility two to three weeks before anthesis (Marshall *et al.*, 1974; Weider *et al.*, 2009). In rape seed (*Brassica napus* L.), day-night temperatures of 22 to 16°C resulted in stability of sterility while day-night temperatures of 30 to 24°C promoted anther development (Fan and Stefansson, 1986). On the other hand, CMS onion (*Allium cepa* L.) had more mature pollen at low temperature (Peterson and Foskett, 1953).

As in any breeding programme for pigeon pea, a desirable CMS line should possess yield components that have a direct positive effect on yield. These include; days to 50% flowering, primary branches, plant height, number of pods per plant and seed mass (Lal and Raina, 2002; Egbe and Vange, 2008; Bhadru, 2010). Favourable yield components will enable availability of sufficient number of flowers for pollination hence high seed yields.

The objective of this study was to evaluate stability of cytoplasmic male sterile lines. The use of highly stable CMS will reduce the cost of hybrid seed production by eliminating the task of emasculation. The aim of this study was to investigate the

stability of pollen sterility of CMS lines used in mating programme. Stability of CMS-lines in pigeonpea is briefly reviewed here:

Rao *et al.* (1996) studied pod and seed set by hand pollination in greenhouse condition. The result showed that 23 potted plants raised from progeny expressed 100 % pollen sterility and remaining plant showed 5-9% pollen sterility because of several constraints. Pollination studies using hand and natural out crossing suggest that CMS plants were female fertile and were capable of an acceptable level of pod set under natural pollination.

Vanniarajan *et al.* (1996) studied eight male sterile lines of the  $ms_1$  and  $ms_2$  types raised in four different environments. Anthers were embedded, sectioned and stained for analysis of pollen formation. All lines were stable under all environments and failed to produce fertile pollens.

Basha *et al.* (2008a) studied the effect of environment factors on inducing male sterility in four environmental sensitive male sterile lines (ESMS) of pigeon pea, namely PSMS 4-2, PSMS 5-16, PSMS 7-4 and PSMS 8-25. The data were recorded on per cent pollen sterility and other yield contributing traits. Environment is a major factor in inducing male sterility in ESMS. The temperature and day-length hours decreased under short days which results in the increase of per cent pollen sterility and vice versa. These two factors are interdependent in respect to expression of photo thermo sensitive male sterility hybrid seed production. PSMS 4-2 and PSMS 8-25 have shown 100% pollen sterility during December.

Basha *et al.* (2008b) evaluated 18 new cytoplasmic male-sterile lines in pigeonpea. Data were recorded for yield, yield attributing characters and pollen sterility. They reported that CMS lines flowered early as compared to their maintainers at both the seasons. Similarly, plant height of the corresponding maintainers was more than the CMS lines. Among CMS lines, pollen sterility was 100% in five lines, namely ICPMS 2004-1, ICPMS 2004, ICPMS 2006, ICPMS 2024 and CMS 288.

Kalaimagal *et al.* (2008) A total of 100 seeds of CORG 990052 A and CORG 990047 A each were supplied to various research stations of the Tamil Nadu Agricultural University (viz. Regional Research Station, Paiyur; Agricultural Research Station, Pattukkottai; Agricultural Research Station, Bhavanisagar; Agricultural Research Station, Virinjapuram; and National Pulses Research Centre, Vamban) for

multilocation trials and evaluated in 2003. In all the locations, plants of both lines were 100% male-sterile without pollen production, inferring that they were stable over locations.

Mahiboobsa *et al.* (2011) studied 12 F1's under the insect proof condition with an objective of transferring the male sterility into the back ground of locally adopted cultivars for developing stable male sterile lines. The 100 per cent male sterility was found in two F1 combinations *viz.*, GT-288A x Maruti and ICPA-2078 x WRP-1. In further back crossing, sterile back ground of recurrent parent genome can be recovered along with male sterility. The study of BC4F1 and BC5F1 generations revealed that there was no single cross which exhibited 100 per cent male sterility or not even near to 99 per cent male sterility. There was no drastic improvement in male sterility from the previous generations to present.

Sawargaonkar *et al.* (2011) studied the single crosses involving four CMS lines (A4 cytoplasm) and three known fertility restorers were studied to determine the genetics of fertility restoration. To determine the pollen fertility, five fully developed floral buds were collected randomly from each plant and the anthers were squashed in 2% aceto-carmin solution. The pollen fertility of each plant was studied in three microscopic fields under light microscope. The fully stained pollen grains were identified as fertile, while the empty or partially stained pollen grains were considered as sterile.

Sawargaonkar *et al.* (2012) studied stability of three male sterile lines ICPA 2043, ICPA 2047 and ICPA 2092 derived from *Cajanus cajanifolius* (A4) cytoplasm. The study of stability of CMS lines under different month temperature revealed that the male sterility was ranged from 84-100 % in ICPA 2043, from 94-100% in ICPA 2047 and from 93 -100% in ICPA 2092. All the three male sterile lines derived from A4 cytoplasm exhibited stability throughout the crop season without any effect of increase or decrease in temperature, indicating male sterility in A4 was independent of environment condition.

Makelo *et al.* (2013) reported that six CMS lines (ICPA2043, ICPA2039, ICPA2091, ICPA2050, ICPA2042 and ICPA2101), with over 96% cytoplasmic male sterility, and their maintainers were sourced from ICRISAT India and evaluated for two seasons in 2009 in a screen house at Katumani and Kiboko and in an isolated field at

Leldet Nakuru. Two CMS lines, ICPA2043 and ICPA2039 were the most stable across sites with 100 and 99% pollen sterility, respectively.

### **Other crops**

Since hybrid pigeonpea breeding technology was the first and new among the legumes, there was limited literature to review on stability of CMS lines for their sterility. Hence, the available literature on stability of CMS lines in other CMS based crops such as wheat, rice, sorghum and sunflower were briefly reviewed hereunder.

### **Wheat**

Arya *et al.* (2003) studied the stability of wheat cytoplasmic male sterile (CMS) lines, WH 416 A-1 (CMS 1), HD 2329 A (CMS 2), HD 2160 A (CMS 3) and WH 416 A-2 (CMS 4) along with the ability of the restorer lines CDWR 9591, CM 159, Zhan 742, CM 233, WH 595 and Raj 3765 to restore the fertility of CMS 1 and 4. They reported that all the CMS lines were stable under the different environments. Fertility restoration was highest (80.6%) in CMS 4 pollinated by WH 595.

Goral *et al.* (2006) studied the stability of male sterility of 6 triticale lines and that of restoring fertility of 15 F<sub>1</sub> hybrids, obtained from crossing three male sterile lines with five genotypes in several environments. The stability was assessed by a regression coefficient and deviation from regression of grain number per ear and restoration index of individual genotypes to the environment means. They reported that cms Salvo and cms 19 (bi close to zero) were the most stable male sterile inbreds. The restoration index of individual hybrids ranged from 50 to 100% in various environments. The hybrids with cultivars Lamberto and Krakowiak as pollinators exhibited the highest fertility and bi below one or close to one. The studies show that the fertility restoration should be assessed in different environments.

Zhang *et al.* (2007) analyzed the stability of sterility based on the data of 7-year experiments, in which C49S was sown on different dates. They reported that C49S showed sterility variation from complete sterility to semi-sterility to normal fertility and the variation pattern appeared to be stable throughout the 7 years. In Chongqing, China, C49S was completely sterile or highly sterile when sown before October, semi-sterile when sown between November 10 and November 30, and fertile when sown after December 10. The abnormal temperatures in a few years influenced the fertility

expression of this line. It is concluded that C49S can be used in the multiplication of the male sterile line or in hybrid seed production by adjusting its sowing date.

## Rice

Wu and Yin (1992) studied the stability of 3 transferable light-sensitive and temperature-sensitive male-sterile lines of indica rice. They reported that the lines in which transfer from fertility to male sterility, was temperature dependent, was stable and unaffected by sowing date and site. The An Nong and HengNong populations showed greater stability than did W6154S. The period of illumination had a slight effect on fertility in HengNongS1 and W6154S, but there was no such effect in An NongS1.

Sarial and Singh (1999) studied cytoplasmic male sterility (CMS) breakdown and relative stability of CMS lines for their sterility. Twelve CMS lines were evaluated over two seasons at Delhi. They found that the CMS lines PMS2A, PMS3A and PMS 10A were completely pollen sterile, had zero spikelet fertility and were highly stable while PMS 5A and IR 58025A were stable. The remaining CMS lines studied were unstable.

Sawant *et al.* (2006) studied the relative stability for sterility, agronomic characters and floral traits influencing outcrossing of 20 cytoplasmic male sterile lines (CMS) in rice. They reported that the CMS lines IR 58025A, IR 68886A, IR 68901A, IR 69628 A, PMS 2 A, IR 54755 A, G 46 A, D 297 A and IR 66707 A were completely pollen sterile and had zero spikelet fertility and were highly stable, while IR 68897 A, IR 68899 A, IR 70907 A, IR 71564 A, IR 68885 A were stable. The remaining CMS lines were unstable.

Umadevi *et al.* (2010) evaluated a total of 74 CMS lines in rice and their maintainers for morphological and floral characters influencing out crossing rate. Out of these CMS lines, 42 CMS lines were completely pollen sterile. For all the CMS lines spikelet fertility ranged from 0.51 to 4.55%. The medium duration CMS lines *viz.*, COMS 13, COMS 15, IR 68281, ICR 6626, DRR 7, RTN 6, RTN 13 and PMS 17 were found promising for the characters *viz.*, pollen sterility(%) and medium duration favorable for out-crossing during seed production of A x B and A x R combinations. These CMS lines offer scope for utilizing in the development of three line hybrids with high yield in rice.

## **Sorghum**

Shinde *et al.* (2006) computed the photo-thermo-sensitivity for male sterile lines on the basis of photoperiod sensitivity and seed setting percentage in sorghum. The male sterile that differed by lower magnitude of photoperiod sensitivity and recorded higher seed set percentage were considered as photo-thermo-insensitive. On the other hand the male sterile line that differed by higher magnitude of photoperiod sensitivity and recorded lower seed set percentage was considered as photo-thermo-sensitive. They found that male sterile line 1409A was found to be most photo-thermo-insensitive for all seasons.

Shivanna and Rahiman (2006) studied four male sterile lines of sorghum from diverse sources, i.e. 296A (A<sub>1</sub>), ICSA701 (A<sub>2</sub>), ICSA741 (A<sub>3</sub>) and ICSA764 (A<sub>4</sub>), to check the stability of these male sterile lines for male sterility expression. Observations on seed set under selfed condition and days to 50% flowering were recorded. They reported that all four lines tested did not produce seeds across all three seasons. This indicates that all male sterile lines are stable in expression of sterility and can be effectively utilized in the development of hybrids.

## **Sunflower**

Reddy *et al.* (2004) studied the stability of cytoplasmic male sterile lines of newly-developed cytoplasmic sources in sunflower. To identify the most stable sources among the newly-developed cytoplasmic sources, all the CMS lines were sown at 2-month intervals from June 1999 to April 2000 in Hyderabad, Andhra Pradesh. They reported that ANL-2 and PEF-1 was unstable as it produced fertile pollens during April, June, August and December whereas no fertile pollen was produced during October. GIG-1, PET-1 and PET-2 did not produce fertile pollen in all planting dates and were regarded as stable sources. Pollen and seed fertility was highest in the April-sown crop. High temperature and marginally low relative humidity prevailed during March, April, May and June indicating that the effect of high temperature and low relative humidity were the causes for breakdown of male sterility in sunflower.

# CAPTER III

## *MATERIALS AND METHODS*



# MATERIALS AND METHODS

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The present investigations were carried out to obtain information on fertility restorer (R-line), maintainer (B-line), effectiveness of CMS lines and estimation of heterosis in pigeonpea [*Cajanus cajan* (L.) Millsp.] genotypes.

## 3.1 Germplasm

Germplasm used in the present study consists of five cytoplasmic-nuclear male sterile (CMS) lines, viz., ICPA 2092, ICPA 2078, ICPA 2048, ICPA 2047 and ICPA 2043 which were derived from A<sub>4</sub> cytoplasm of *Cajanus cajanifolius*, and 11 male lines of pigeonpea, such as ICPL 87119, ICPL 20093, ICPL 20096, ICPL 20098, ICPL 20106, ICPL 20108, ICPL 20111, ICPL 20123, ICPL 20126, ICPL 20129 and ICPL 20186, were derived from diverse inbred lines. These all CMS-lines and male lines were developed at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The brief details of all CMS lines and male lines are given (Table 3.2 and 3.3).

In *kharif* 2012-13, a total of 20 hybrids were generated by manually pollination of these five cytoplasmic-nuclear male sterile (CMS) lines with 11 male lines and sufficient quantity of crossed seeds were produced (Table 3.1). Similarly studies for effectiveness of these CMS lines were also quantified by using 2% aceto-carmin solution. 20 F<sub>1</sub> hybrids along with two standard checks, Asha and Maruti were sown in *kharif* 2013-14 (Table 3.4).

## 3.2 Experimental sites, cultural practices, & weather conditions

The experimental site is located at ICRISAT, Patancheru, Andhra Pradesh at an altitude of 545 m above sea level, latitude of 17° 53' N and longitude of 78° 27' E. The soil of the experimental site was black and classified as Vertisols. During early vegetative stage spray of herbicide Stomp @ 3 l ha<sup>-1</sup> was carried out to control weeds and a spray of Rogor @ 1 l ha<sup>-1</sup> was applied to control the sucking pest aphids. During reproductive stages two sprays of Acephate @ 1 l ha<sup>-1</sup>, three sprays of Lannate @ 1 l ha<sup>-1</sup> and single spray each of Nuvan and Nuvacron @ 1 l ha<sup>-1</sup> were used to control Maruca while two sprays of Spinosad @ 0.2 l ha<sup>-1</sup> was used to control Helicoverpa. The crop was irrigated at critical stages such as vegetative and pod filling stage. The

weeds were controlled manually at various crop growth stages as per the intensity of the weeds. The mean meteorological data recorded during the crop growth period such as rainfall, temperature and relative humidity are presented in Appendix I and II.

Table 3.1: Number of pollinations carried out and the seeds set in the hybrids generated

<b>Sl. No.</b>	<b>Hybrid</b>	<b>Pollinations</b>	<b>Number of seeds</b>
1.	ICPA 2078 x ICPL 87119	110	188
2.	ICPA 2092 x ICPL 20106	325	174
3.	ICPA 2092 x ICPL 20186	350	170
4.	ICPA 2092 x ICPL 20123	390	165
5.	ICPA 2092 x ICPL 87119	350	160
6.	ICPA 2092 x ICPL 20108	370	161
7.	ICPA 2047 x ICPL 87119	400	152
8.	ICPA 2047 x ICPL 20098	310	160
9.	ICPA 2047 x ICPL 20108	300	165
10.	ICPA 2047 x ICPL 20126	310	171
11.	ICPA 2047 x ICPL 20111	300	161
12.	ICPA 2047 x ICPL 20129	310	170
13.	ICPA 2048 x ICPL 87119	310	162
14.	ICPA 2048 x ICPL 20093	370	170
15.	ICPA 2048 x ICPL 20106	150	176
16.	ICPA 2048 x ICPL 20096	125	182
17.	ICPA 2048 x ICPL 20098	130	172
18.	ICPA 2048 x ICPL 20108	300	155
19.	ICPA 2048 x ICPL 20111	310	161
20.	ICPA 2043 x ICPL 87119	320	170

Table 3.2: Descriptions of the cytoplasmic male sterile (CMS) lines used in crossing programme

Sl. No.	Entry	Pedigree	Days to		Plant height (cm)	Seeds pod <sup>-1</sup>	100- seed weight (g)	Seed colour	% Disease reaction in nursery	
			flower	mature					Wilt	SM
	<b>CMS Lines</b>									
1.	ICPA 2043	ICPA 2043 (ICPA 2039 x ICPL 20176) x ICPL 20176	114	162	198	4.1	10.0	Brown	19	-
2.	ICPA 2047	ICPA 2047 (ICPA 2039 x ICPL 99050) x ICPL 99050	122	165	242	3.9	10.8	Brown	-	-
3.	ICPA 2048	ICPA 2048 (ICPA 2039 x ICPL 99052) x ICPL 99052	123	168	235	4.2	12.9	Brown	-	-
4.	ICPA 2078	ICPA 2078 (ICPA 2039 x ICPL 118) x ICPL 118	103	146	132	4.4	13.7	Brown	-	-
5.	ICPA 2092	ICPA 2092 (ICPA 2039 x ICPL 96058) x ICPL 96058	120	167	220	4.2	9.7	Light Brown	11	-

Where, SM = sterility mosaic disease; Source: Pigeonpea Breeding department, ICRISAT, Patancheru, (Andhra Pradesh)

Table 3.3: Descriptions of the male parental lines used in hybridization

Genotype	Pedigree	Days to		Plant height (cm)	Seeds pod <sup>-1</sup>	100-seed weight (g)	Seed colour	% Disease reaction in nursery	
		flower	mature					Wilt	SM
<b>Male lines</b>									
ICPL 87119	C11 x ICP 1-6W3B	122	172	228	3.4	10.6	Brown	-	-
ICPL 20093	ICPL 87119 x ICP 13831 (Inbred)	124	169	232	3.2	11.3	Brown	8	-
ICPL20096	ICPL 87119 x ICP 12746 (Inbred)	123	162	228	3.8	10.8	Brown	15	-
ICPL 20098	ICPL 87119 x ICP 12746 (Inbred)	130	174	235	4.2	14.3	Light Brown	-	-
ICPL 20106	MS 3783 x ICPL 87119 (IPH 487 Inbred)	128	175	215	3.6	10.4	Cream	86	49
ICPL 20108	MS 3783 x ICPL 87119 (IPH 487 Inbred)	122	165	235	4.3	11.4	Cream	-	-
ICPL20111	MS 3783 x ICPL 87119 (IPH 487 Inbred)	125	166	245	3.7	9.1	Light Brown	11	4
ICPL 20123	MS 3783 x ICPL 87119 (IPH 487 Inbred)	122	168	228	3.9	10.8	Brown	-	-
ICPL 20126	MS 3783 x ICPL 87119 (IPH 487 Inbred)	121	168	230	3.8	10.6	Brown	-	-
ICPL 20129	MS 3783 x GAUT 85-19 (GUPH 1126 Inbred)	139	184	210	3.7	13.0	Light Brown	-	-
ICPL 20186	ICP 10928 selection	145	188	242	3.7	9.4	Light Brown	28	6

Where, SM = sterility mosaic disease; Source: Pigeonpea Breeding department, ICRISAT, Patancheru, (Andhra Pradesh)

### 3.3 Study of effectiveness of CMS lines

It is the study of sterility level of cytoplasmic-nuclear male sterile (CMS) lines for the effective utilization in hybrid production.

#### 3.3.1 Pattern of sowings

To study on the effectiveness of male sterility of CMS lines, the experimental material was sown on August 7, 2012, under insect-proof selfing net. The cytoplasmic male sterile lines were evaluated in two-row plots of 4 m length with inter and intra row spacing of 75 and 30 cm respectively. Border rows were planted to increase the precision of study and reduce border effect. All the agronomic practices were followed in these CMS lines as per also practiced for hybrids to keep the crop in good condition.

#### 3.3.2 Methodology for study of effectiveness of CMS lines

Each plant of CMS lines was tested for its pollen fertility/sterility status at the initial flowering stage. To identify fertility/sterility of pollen grains, 2% aceto-carmin solution was used. Five well developed flower buds were collected randomly from different parts of each plant at the time of anthesis (9-10 AM). From each bud, the anthers were collected on a glass slide and crushed with a drop of 2% aceto-carmin stain and examined under a light microscope. The count of sterile and fertile pollen grains in 10x microscopic fields was noted, five such microscopic fields were examined under each slide. The round and well stained pollen grains were counted as fertile while shriveled hyaline and unstained pollen grains were scored as sterile. The means for all the microscopic fields were worked-out and the proportion of fertile and sterile pollens was expressed in percentage on total in individual plants. The mean value of pollen fertility/sterility of all plants was considered as pollen fertility/sterility (%) for that genotype. Based on the number of stained and unstained pollen grains, the fertility and sterility status of CMS lines were computed as follows:

$$\text{Pollen sterility}(\%) = \frac{\text{No. of sterile (unstained, shrivelled hyaline) pollens}}{\text{Total number of pollen grains examined}} \times 100$$

### 3.4. Methodology for study of heterosis

Heterosis is expressed as a percent deviation of F1 hybrids from its mid parent value. Heterobeltiosis is estimated as the percent deviation of the F1 hybrid from that of the parent with greater or more desirable expression and standard heterosis as percent deviation of hybrid over standard variety. Here we specially deal standard heterosis. The experimental materials comprised of 20 F<sub>1</sub> hybrids and two standard checks. Two popular varieties, Asha and Maruti were used as standard checks. The experiment was sown on 1 July, 2013. The list of the hybrids and checks used for the study of heterosis is given in Table 3.4.

$$\text{Percent heterosis over standard check (SC) variety, Asha/Maruti} = \frac{\overline{F_1} - \overline{SC}}{\overline{SC}} \times 100$$

Where,

$$\overline{F_1} = \text{mean value of the } F_1$$

$$\overline{SC} = \text{mean value of the standard check variety, Asha/Maruti}$$

#### 3.4.1 Experimental design and layout (For evaluation of F1 with two checks)

All 20 Pigeonpea hybrids with two checks (Maruti and Asha) were planted in a randomized block design (RBD) with two replications. The each plot size was 10.2 m<sup>2</sup> with row to row and plant to plant spacing of 75 cm and 30 cm respectively.

Table 3.5: List of genotypes along with two checks for study of standard heterosis

<b>Sl. No.</b>	<b>Hybrid</b>
1.	ICPA 2078 x ICPL 87119
2.	ICPA 2043 x ICPL 87119
3.	ICPA 2047 x ICPL 87119
4.	ICPA 2047 x ICPL 20098
5.	ICPA 2047 x ICPL 20108
6.	ICPA 2047 x ICPL 20126
7.	ICPA 2092 x ICPL 87119
8.	ICPA 2092 x ICPL 20108
9.	ICPA 2048 x ICPL 87119
10.	ICPA 2048 x ICPL 20093
11.	ICPA 2047 x ICPL 20111
12.	ICPA 2047 x ICPL 20129
13.	ICPA 2048 x ICPL 20106
14.	ICPA 2048 x ICPL 20096
15.	ICPA 2048 x ICPL 20098
16.	ICPA 2048 x ICPL 20108
17.	ICPA 2048 x ICPL 20111
18.	ICPA 2092 x ICPL 20106
19.	ICPA 2092 x ICPL 20186
20.	ICPA 2092 x ICPL 20123
21.	Asha (check)
22.	Maruti (check)

### **3.4.2 Collection of Data**

Five competitive plants were randomly selected for recording observations on each hybrid and standard checks. The details of the observations recorded are as follows:

- **Days to initiation of first flower**  
Number of days taken from sowing to first flowering in a plot was recorded.
- **Days to 50% flowering**  
Number of days taken from sowing to flowering of 50% plants in a plot was recorded.
- **Percent sterility/fertility**  
Pollen fertility/sterility status at the initial flowering stage was recorded.
- **Days to maturity**  
Days required from sowing to 75% maturity were recorded.
- **Plant height (cm)**  
Height of plants from ground level to the tip of the plant was recorded at maturity.
- **Number of primary branches plant<sup>-1</sup>**  
Total number of pod bearing branches on the main stem of a plant was counted.
- **Number of secondary branches plant<sup>-1</sup>**  
Total number of pod bearing branches per plant on primary branches of a plant was counted.
- **Total number of pods plant<sup>-1</sup>**  
Total number of pods containing the seeds on each plant was counted.
- **Seeds pod<sup>-1</sup>**  
Seeds from randomly selected ten pods from each plant were counted and the average seeds pod<sup>-1</sup> was estimated.
- **100-Seed weight (g)**  
Fully grown 100 seeds of each entry were collected randomly and weighed on electronic balance.
- **Yield plant<sup>-1</sup> (g)**  
Grains of the selected plants were harvested and threshed separately. Grain weight was taken after thorough drying in the sun.

➤ **Seed yield plot<sup>-1</sup> (g)**

From all the plants in the plot area except the border plants, dry grains were harvested and threshed. Grain weights were recorded after thorough sun drying.

➤ **Seed yield (kg ha<sup>-1</sup>)**

The seed yield kg ha<sup>-1</sup> was estimated based on the plot area.

### 3.4.3 Methodology for identification of fertility restorers and maintainers

Five competitive plants were randomly selected for recording observations for identification of fertility restorers and maintainers on each hybrid and standard checks in both replications. Five plants of each hybrids and checks were tested for its pollen fertility/sterility status at the initial flowering stage. To identify fertility/sterility of pollen grains, 2% aceto-carmin solution was used. Five well developed flower buds were collected randomly from different parts of each plant at the time of anthesis (9 -10 AM). From each bud, the anthers were collected on a glass slide and crushed with a drop of 2% aceto-carmin stain and examined under a light microscope. The count of sterile and fertile pollen grains in 10x microscopic fields was noted, five such microscopic fields were examined under each slide. The round and well stained pollen grains were counted as fertile while shriveled hyaline and unstained pollen grains were scored as sterile. The means for all the microscopic fields were worked-out and the proportion of fertile and sterile pollens was expressed in percentage on total in individual plants. The mean value of pollen fertility/sterility of all five plants was considered as pollen fertility/sterility (%) for that genotype.

Based on the fertility/sterility data, the hybrids (F<sub>1</sub> progenies) will be classified in to one of the following three categories:

**Case I : When all the plants in a progeny will fully male-fertile:** The male parent of such cross may be classified as ‘restorer’ and its male counterpart plant may be used in the active hybrid breeding programme.

**Case II: When all the plants in a progeny will fully male-sterile:** The male parent of such a cross may be classified as ‘maintainer’ and it may be preserved as recurrent parent for backcrossing.

**Case III: When the F<sub>1</sub> progeny will segregate for male-sterility and fertility:** Such progeny is difficult to handle and may be given due consideration if sufficient number

of maintainers will not be available and will be preserved for use in the active hybrid breeding programme.

Based on the number of stained and unstained pollen grains, the fertility and sterility status of hybrids were computed as follows.

$$\text{Pollen fertility}(\%) = \frac{\text{Number of fertile (round and stained) pollens}}{\text{Total number of pollen grains examined}} \times 100$$

$$\text{Pollen sterility}(\%) = \frac{\text{No. of sterile (unstained, shrivelled) pollens}}{\text{Total number of pollen grains examined}} \times 100$$

### 3.5 Statistical analyses

The following statistical techniques were used to analyze the data collected from the above mentioned experiments.

The data was subjected to analysis of variance as per the method described by Fisher and Yates (1974).

The model for experimental design used in randomized block design can be expressed as follows.

$$P_{ij} = \mu + g_i + r_j + e_{ij}$$

Where,

$P_{ij}$  = phenotypic effect of  $i^{\text{th}}$  genotype in the  $j^{\text{th}}$  replication.

$\mu$  = general population mean.

$g_i$  = effect of  $i^{\text{th}}$  genotype.

$r_j$  = effect of  $j^{\text{th}}$  replication.

$e_{ij}$  = error associated with the experiment.

Table 3.4: the skeleton for analysis of variance for randomized complete block design

Source of variation	D .F.	Sum of squares	Mean sum of squares
Replication	(r-1)	SSr	$\sigma^2 r$
Genotypes	(g-1)	SSg	$\sigma^2 e + r \sigma^2 g$
Error	(r-1)(g-1)	SSE	$\sigma^2 e$
Total	(rg-1)		

Where,

r = number of replications.

g = number of genotypes.

$\sigma^2g$  = genotypic variance.

$\sigma^2e$  = error variance.

### 3.5.1. Test of significance

The mean sum of squares for genotypes and replications were tested against the error mean sum of squares for calculating F values which were compared with tabulated F value at 5 and 1 percent level of significance.

#### 3.5.1.1 Mean:

Mean was calculated using following conventional formula

$$\bar{X} = \frac{\sum_{i=1}^n x_i}{N}$$

Where,

$\bar{X}$  = simple mean.

$\sum_{i=1}^n x_i$  = summation of all the observation.

N = number of observation.

#### 3.5.1.2 Range:

It is the range of lowest and highest values of each trait taken in the observations.

#### 3.5.1.3. Standard error of mean:

It was calculated as formula given below.

$$SEm_{\pm} = \sqrt{MSe/r}$$

Where,

SE  $m_{\pm}$  = standard error of mean.

MSe = mean sum of square due to error.

r = number of replication.

**3.5.1.4. Standard error of differences:**

It was calculated as formula given below.

$$\mathbf{SEd}_{\pm} = \sqrt{2MSe/r}$$

Where,

$SEd_{\pm}$  = standard error of differences.

MSe = mean sum of square due to error.

r = number of replications.

**3.5.1.5. Critical difference:**

It was measured as formula mentioned below.

**CD** = SEd x t at 5% level of significance.

Where,

CD = critical difference.

SEd = standard error of difference.

t = table value at 5% probability level of error d.f.

**3.5.2 Significance of standard heterosis**

It was tested by using t-test (Wynne *et al.*, 1970).

$$t = (\overline{F}_{ij} - \overline{SC}_{ij} / \sqrt{3(\delta^2 E)/8})$$

Where,

$\overline{F}_{ij}$  = the mean of the ij<sup>th</sup> F<sub>1</sub> cross

$\overline{SC}_{ij}$  = the standard check value for the ij<sup>th</sup> cross

$\delta^2 E$  = estimate of error variance.

# CHAPTER IV

## *EXPERIMENTAL FINDINGS*



## EXPERIMENTAL FINDINGS

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The present investigation entitled, “Identification of fertility restorers and maintainers in Pigeonpea [*Cajanus cajan* (L.) Millsp.]” was conducted using five female parents (A-lines) and 11 male parents. A set of 20 hybrids were developed by crossing the parents in *kharif* 2012-13. These hybrids were evaluated at Patancheru during *kharif* 2013-14 to study their fertility restoration and hybrid vigour in F<sub>1</sub> generation. Observations were recorded on yield and yield contributing characters such as days to initiation of first flower, days to 50% flowering, percent sterility/fertility, days to maturity, plant height (cm), number of primary branches plant<sup>-1</sup>, number of secondary branches plant<sup>-1</sup>, number of pods plant<sup>-1</sup>, seeds pod<sup>-1</sup>, 100-seed weight (g), seed yield plant<sup>-1</sup> (g), seed yield plot<sup>-1</sup> (g) and seed yield (kg ha<sup>-1</sup>). The effectiveness of expression of male sterility was also studied with five CMS lines at ICRISAT, Patancheru during *kharif* 2012-13. The results from the present investigation are described here under:

### 4.1 Effectiveness of CMS lines

Male-sterility in plants is a phenomenon where the individuals are unable to reproduce through natural means due to their defective male-reproductive parts. Such plants reproduce only when fertile pollen from other plants is placed on the stigmatic surface of the male-sterile flowers through any mechanical means such as deliberate manual efforts, wind, or insects. In present experiment five male sterile lines(CMS) of pigeonpea, ICPA 2092, ICPA 2078, ICPA 2048, ICPA 2047 and ICPA 2043 were planted during *kharif* 2012-13 at ICRISAT, Patancheru, under insect-proof selfing net.

#### 4.1.1 Mean performance of CMS lines for pollen sterility %

All the CMS lines were analyzed for pollen sterility (%). The data recorded for partial male sterile plants (%), partial male fertile plants (%) and fully male fertile plants (%) were zero for most of the CMS lines therefore these characters could not be considered. The mean performance of CMS lines for pollen sterility are given in Table 4.1

**4.1.2 Pollen sterility (%)**

The pollen sterility (%) ranged from 99.60 to 95.69 %. The highest pollen sterility was recorded in ICPA 2092(99.60 %) followed by ICPA 2043(99.01 %), ICPA 2048(97.2%), ICPA 2047(96.30%) and ICPA 2078(95.69%). The graph showing pollen sterility status of five CMS lines of pigeonpea in Fig. 4.1

Table 4.1: *Per se* performance of CMS lines for pollen sterility (%)

Sl. No.	CMS Line	Pollen Sterility %
1.	ICPA 2043	99.01
2.	ICPA 2047	96.30
3.	ICPA 2048	97.20
4.	ICPA 2078	95.69
5.	ICPA2092	99.60

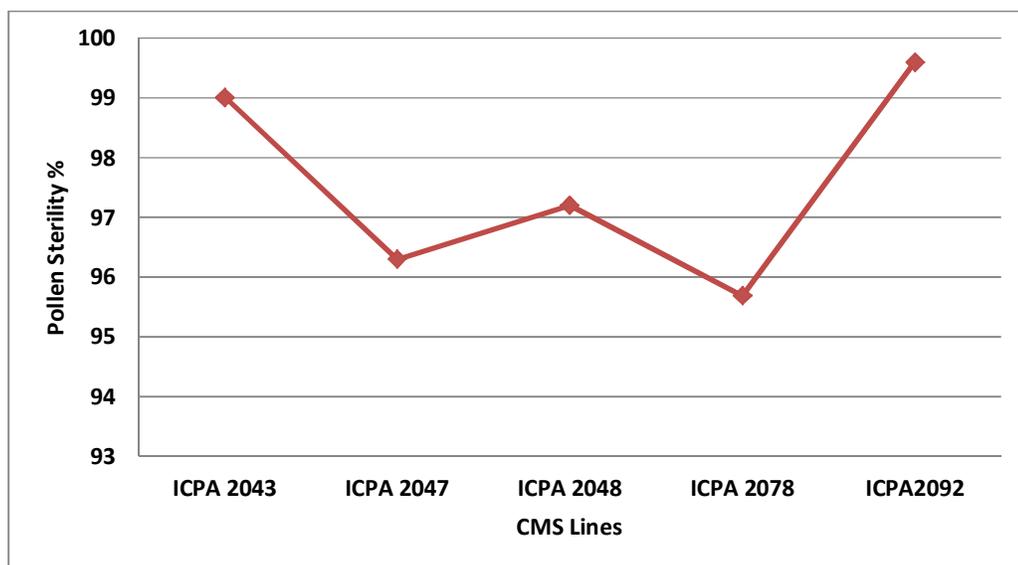


Fig. 4.1: The graph showing pollen sterility status of five CMS lines of pigeonpea.

#### 4.2 Analysis of Variance

The analysis of variance (ANOVA) F<sub>1</sub> hybrids and standard varieties is presented in Table 4.2. The ANOVA showed that the mean sum of squares were significant for all characters except for plant height. These results indicated highly significant genotypic differences in all the F<sub>1</sub> hybrids and standard checks.

Table 4.2: Analysis of variance for yield and related traits in pigeonpea hybrids

Sl. No.	Source	Replication	Treatment	Error
1.	d.f.	1	21	21
2.	Days to 1st flower	0.09	53.09**	9.85
3.	Days to 50% flower	20.45	55.66**	11.45
4.	Days to maturity	3.27	25.41*	10.18
5.	Plant height(cm)	17.82	221.37	197.96
6.	No. of prim. branches plant <sup>-1</sup>	0.23	23.60*	9.18
7.	No. of sec. branches plant <sup>-1</sup>	9.09	17.31*	8.21
8.	Pods plant <sup>-1</sup>	173.41	2615.99*	1133.93
9.	Seeds pod <sup>-1</sup>	0.03	0.08*	0.04
10.	100-seed weight(gm)	0.06	1.81*	0.22
11.	Yield plant <sup>-1</sup> (gm)	0.15	336.93*	127.20
12.	Yield plot <sup>-1</sup> (gm)	10832.68	180953.14**	335.23
13.	Yield(kg ha <sup>-1</sup> )	10412.04327	173926.51**	322.21
14.	Pollen fertility %	10.33	171.773**	10.47

Where, \*, \*\* = significant at 5% and 1% level, respectively

### 4.3 Mean Performance of Hybrids and Checks

The performance of all the tested materials was good for plant growth. However, there was variation in temperature and rainfall during *kharif* 2013-14 leading to differences in the flowering response of the genotypes. A total of 20 hybrids were evaluated in *kharif* 2013-14 along with two standard check varieties, Asha and Maruti. Mainly comparison was done on best check Asha. The *per se* performance for all the characters studied of F<sub>1</sub>s and standard checks are presented in Table 4.3a-4.3b.

#### 4.3.1 Days to 1st flowering

Among the twenty hybrids, ICPA 2043 x ICPL 87119 (105 days) was the earliest to 1st flower followed by ICPA 2078 x ICPL 87119 (109 days), ICPA 2047 x ICPL 20108 (115 days) and ICPA 2047 x ICPL 20126 (118 days). Two hybrids ICPA 2078 x ICPL 87119 (105 days) and ICPA 2078 x ICPL 87119 (109 days) showed significant negative difference over both mean performance (118 days) and check Asha (119 days) for days to 1st flowering. The check Asha took 119 days to 1<sup>st</sup> flower while Maruti 106 days. The range of 1<sup>st</sup> flowering was from 105 (ICPA 2043 x ICPL 87119) to 123 (ICPA 2048 x ICPL 20106) days, out of 22 genotypes, while general mean 118 days.

#### 4.3.2 Days to 50% flowering

Among the twenty hybrids, ICPA 2043 x ICPL 87119 (118 days) was the earliest to 50% flowering followed by ICPA 2078 x ICPL 87119 (122 days), ICPA 2047 x ICPL 20108 (124 days), ICPA 2047 x ICPL 87119 (125 days) and these four showed significantly low over check Asha (133 days). Only one hybrid ICPA 2043 x ICPL 87119 (118 days) showed significant negative difference over mean performance (128 days) for days to 50% flowering. The check Asha and Maruti took 133 days and 112 days to 50% flowering respectively. The range of days to 50% flowering was from 118 (ICPA 2043 x ICPL 87119) to 134 days (ICPA 2048 x ICPL 20106), out of 22 genotypes, while general mean was 128 days.

#### 4.3.3 Days to maturity

Among the twenty hybrids, ICPA 2078 x ICPL 87119 (184 days) and ICPA 2092 x ICPL 87119 (184 days) were the earliest to mature followed by ICPA 2043 x ICPL 87119 (185 days). No any hybrid showed significant difference over mean

performance and check Asha (185 days) against days to maturity. The range of days to maturity was from 172 (Maruti) to 191 (ICPA 2048 x ICPL 20093) days, while general mean was 186 days.

#### **4.3.4 Plant height (cm)**

Among the hybrids, ICPA 2078 x ICPL 87119 (195.0 cm) was shortest followed by ICPA 2047 x ICPL 87119 (199.5 cm) and ICPA 2092 x ICPL 87119 (201.0 cm). Only two hybrids, ICPA 2048 x ICPL 20108 (233 cm) and ICPA 2048 x ICPL 20098 (229.0 cm) showed positive significance difference over Asha (198.0 cm). The range of plant height was from 195.0 (ICPA 2078 x ICPL 87119) to 233.0 (ICPA 2048 x ICPL 20108) cm, out of 22 genotypes, while general mean was 212.2 cm.

#### **4.3.5 Number of primary branches plant<sup>-1</sup>**

Among the 20 hybrids, number of primary branches plant<sup>-1</sup> was maximum in ICPA 2048 x ICPL 87119 (24.30) followed by ICPA 2092 x ICPL 20108 (22.1) and ICPA 2092 x ICPL 20186 (20.0). Six hybrids, ICPA 2048 x ICPL 87119 (24.30), ICPA 2092 x ICPL 20108 (22.10), ICPA 2092 x ICPL 20186 (20.0), ICPA 2047 x ICPL 20098 (20.0), ICPA 2048 x ICPL 20098 (19.60) and ICPA 2047 x ICPL 20126 (18.8) showed significantly high number of primary branches plant<sup>-1</sup> with respect to check Asha (11.6). Only one hybrid, ICPA 2048 x ICPL 87119 (24.30) showed significantly high number of primary branches with respect to men value (16.37). The range of number of primary branches plant<sup>-1</sup> from 10.1 (ICPA 2047 x ICPL87119) to 24.30 (ICPA 2048 x ICPL 87119), out of 22 genotypes, while general mean was 16.37.

#### **4.3.6 Number of secondary branches plant<sup>-1</sup>**

Among the 20 hybrids, number of secondary branches plant<sup>-1</sup> was maximum in ICPA 2078 x ICPL 87119 (24.5) followed by ICPA 2047 x ICPL 20126 (23.0), ICPA 2047 x ICPL 20108 (22) and ICPA 2048 x ICPL 20096 (21.4). Two hybrids, ICPA 2092 x ICPL 20123 (11.4) and ICPA 2092 x ICPL 20108 (14.6) showed significantly low with respect to check Asha (20.8). One hybrid, ICPA 2092 x ICPL 20123 (11.4) showed significantly low with respect to mean value (19.08). The range of number of secondary branches plant<sup>-1</sup> from 11.4 (ICPA 2092 x ICPL 20123) to 24.5 (ICPA 2078 x ICPL 87119), out of 22 genotypes, while general mean was 19.08.

#### **4.3.7 Number of pods plant<sup>-1</sup>**

Among the 20 hybrids, number of pods plant<sup>-1</sup> was maximum in ICPA 2092 x ICPL 20108 (341.2), followed by ICPA 2047 x ICPL 20108 (310.9), ICPA 2047 x ICPL 20126 (307.7) and ICPA 2048 x ICPL 87119(306.2). Only one hybrid, ICPA 2092 x ICPL 20108 (341.2) was significantly high over mean performance. No any hybrids significantly differ from check Asha (272.0). The range of number of pods plant<sup>-1</sup> from 207.20 (ICPA 2047 x ICPL 20098) to 341.2 (ICPA 2092 x ICPL 20108), out of 22 genotypes, while general mean was 257.4.

#### **4.3.8 Seeds pod<sup>-1</sup>**

Among the 20 hybrids, number of Seeds pod<sup>-1</sup> was highest in ICPA 2078 x ICPL 87119 (4.25) followed by ICPA 2048 x ICPL 20108 (3.87), ICPA 2043 x ICPL 87119 (3.84) and ICPA 2048 x ICPL 20093 (3.81). Six hybrids, ICPA 2078 x ICPL 87119 (4.25), ICPA 2048 x ICPL 20108 (3.87), ICPA 2043 x ICPL 87119 (3.84), ICPA 2048 x ICPL 20093 (3.81), ICPA 2047 x ICPL 20126 (3.8) and ICPA 2048 x ICPL 20106 (3.79) were significantly high over check Asha (3.36) and ICPA 2078 x ICPL 87119 (4.25) against mean value (3.67) for this trait. The range of number of Seeds pod<sup>-1</sup> from 3.36 (Asha) to 4.25 (ICPA 2078 x ICPL 87119), out of 22 genotypes, while general mean was 3.67.

#### **4.3.9 100-seed weight (g)**

Among the 20 hybrids, 100-seed weight (g) was maximum in ICPA 2048 x ICPL 20093 (12.38 g) followed by ICPA 2048 x ICPL 20106 (12.14 g), ICPA 2048 x ICPL 87119 (11.96 g) and ICPA 2078 x ICPL 87119 (11.91 g). Four hybrids, ICPA 2048 x ICPL 20093(12.38 g), ICPA 2048 x ICPL 20106 (12.14 g), ICPA 2048 x ICPL 87119 (11.96 g) and ICPA 2078 x ICPL 87119 (11.91 g) were significantly high over general mean (10.75g) for 100-seed weight. Seven hybrids, ICPA 2048 x ICPL 20093 (12.38 g), ICPA 2048 x ICPL 20106 (12.14 g), ICPA 2048 x ICPL 87119 (11.96 g), ICPA 2078 x ICPL 87119 (11.91 g), ICPA 2048 x ICPL 20108 (11.47 g), ICPA 2047 x ICPL 20126 (11.21 g) and ICPA 2043 x ICPL 87119 (11.09 g) were significantly high over check Asha (10.12 g). The range of 100-seed weight from 8.4 g (ICPA 2092 x ICPL 20186) to 12.38 g (ICPA 2048 x ICPL 20093), out of 22 genotypes; while general mean was 10.75 g.

#### **4.3.10 Seed yield plant<sup>-1</sup> (g)**

Among the 20 hybrids, seed yield plant<sup>-1</sup>(g) was maximum in ICPA 2047 x ICPL 20126 (115.73 g) followed by ICPA 2047 x ICPL 20108 (107.95 g), ICPA 2092 x ICPL 20108 (106.96 g) and ICPA 2048 x ICPL 20106 (106.41 g). Four hybrids, ICPA 2047 x ICPL 20126 (115.73 g), ICPA 2047 x ICPL 20108 (107.95 g), ICPA 2092 x ICPL 20108 (106.96 g) and ICPA 2048 x ICPL 20106 (106.41 g) were significantly high with respect to check Asha (82.13 g). Only one hybrid, ICPA 2047 x ICPL 20126 (115.73 g) was significantly high over general mean (91.16 g). The range of seed yield plant<sup>-1</sup> from 64.26g (Maruti) to 115.73g (ICPA 2047 x ICPL 20126), out of 22 genotypes, while general mean was 91.16 g.

#### **4.3.11 Seed yield plot<sup>-1</sup>(g)**

Out of 22 genotypes Seed yield plot<sup>-1</sup>(g) was maximum in ICPA 2047 x ICPL 20126 (2688.07g) followed by ICPA 2047 x ICPL 20108 (2551.10g), ICPA 2092 x ICPL 20108 (2514.39g), ICPA 2092 x ICPL 20186 (2463.03g), ICPA 2078 x ICPL 87119 (2345.85g), ICPA 2048 x ICPL 20098 (2308.12g) and ICPA 2048 x ICPL 87119 (2229.67g) showed significantly high Seed yield plot<sup>-1</sup>(g) over check Asha (1669.03g) and general mean (2095.91g) both. The range of seed yield plot<sup>-1</sup> from 1409.63g (Maruti) to 2688.07g (ICPA 2047 x ICPL 20126), while general mean was 2095.91 g.

#### **4.3.12 Seed yield (kg ha<sup>-1</sup>)**

Out of 22 genotypes Seed yield (kg ha<sup>-1</sup>) was maximum in ICPA 2047 x ICPL 20126 (2635.36 kg ha<sup>-1</sup>) followed by ICPA 2047 x ICPL 20108 (2501.07 kg ha<sup>-1</sup>), ICPA 2092 x ICPL 20108 (2465.09 kg ha<sup>-1</sup>), ICPA 2092 x ICPL 20186 (2414.74 kg ha<sup>-1</sup>), ICPA 2078 x ICPL 87119 (2299.85 kg ha<sup>-1</sup>), ICPA 2048 x ICPL 20098 (2262.86 kg ha<sup>-1</sup>) and ICPA 2048 x ICPL 87119 (2185.95 kg ha<sup>-1</sup>) showed significantly high Seed yield(kg ha<sup>-1</sup>) over check Asha (1636.30kg ha<sup>-1</sup>) and general mean (2095.91 kg ha<sup>-1</sup>) both. The range of seed yield (kg ha<sup>-1</sup>) varies from 1381.99 kg ha<sup>-1</sup> (Maruti) to 2635.36 kg ha<sup>-1</sup> (ICPA 2047 x ICPL 20126).

**4.3.13 Pollen sterility/fertility (%)**

The variability for pollen fertility ranged from 59.22 to 99.76%. Among the hybrids, ICPA 2047 x ICPL 20108 recorded maximum pollen fertility (98.50%) followed by ICPA 2078 x ICPL 87119 (98.05%) and ICPA 2092 x ICPL 87119 (97.72%), whereas the minimum pollen fertility was recorded in ICPA 2048 x ICPL 20096 (59.22%) followed by ICPA 2047 x ICPL 20129 (74.46%). Seven hybrids, ICPA 2048 x ICPL 20096 (59.22%), ICPA 2047 x ICPL 20129 (74.46%), ICPA 2048 x ICPL 20106 (86.28%), ICPA 2092 x ICPL 20186 (87.12%), ICPA 2047 x ICPL 20111 (88.20%), ICPA 2048 x ICPL 20093 (88.43%) and ICPA 2092 x ICPL 20106 (89.14%) showed significantly low pollen fertility over check Asha. Two hybrids, ICPA 2048 x ICPL 20096 (59.22%) and ICPA 2047 x ICPL 20129 (74.46%) were also showed significantly low pollen fertility over general mean (89.96%). Among the all genotypes (hybrids and checks), Asha recorded maximum pollen fertility (99.76) followed by Maruti (99.58%). The pollen sterility ranged from 40.78 to 0.24 % (Table 4.5). Among the hybrids, ICPA 2048 x ICPL 20096 recorded maximum pollen sterility (40.78 %) followed by ICPA 2047 x ICPL 20129 (25.54 %) and ICPA 2048 x ICPL 20106 (13.72 %).

Table 4.3a: Per se performance of pigeonpea hybrids and checks for yield and yield related traits at Patancheru, kharif 2013-14

Entry name	Days to 1 <sup>st</sup> flower	Days to 50% flower	Days to maturity	Plant height(cm)	No. of primary branches plant <sup>-1</sup>	No. of secondary branches plant <sup>-1</sup>
ICPA 2078 x ICPL 87119	109	122	184	208.5	15.1	24.5
ICPA 2043 x ICPL 87119	105	118	185	211.0	15.7	20.5
ICPA 2047 x ICPL 87119	119	125	186	199.5	10.1	19.1
ICPA 2047 x ICPL 20098	121	127	188	204.0	20.0	19.8
ICPA 2047 x ICPL 20108	115	124	185	219.0	14.3	22.6
ICPA 2047 x ICPL 20126	118	128	186	209.0	18.8	23.0
ICPA 2092 x ICPL 87119	118	127	184	201.0	15.2	15.5
ICPA 2092 x ICPL 20108	121	128	187	217.0	22.1	14.6
ICPA 2048 x ICPL 87119	119	129	186	206.0	24.3	18.8
ICPA 2048 x ICPL 20093	121	132	191	195.0	16.7	18.1
ICPA 2047 x ICPL 20111	123	131	187	214.0	17.5	17.5
ICPA 2047 x ICPL 20129	121	131	187	216.5	14.6	21.3
ICPA 2048 x ICPL 20106	123	134	191	218.0	13.3	18.7
ICPA 2048 x ICPL 20096	122	130	188	219.5	16.8	21.4
ICPA 2048 x ICPL 20098	120	130	187	229.0	19.6	19.5
ICPA 2048 x ICPL 20108	121	132	185	233.0	16.0	19.0
ICPA 2048 x ICPL 20111	119	130	186	226.0	13.0	16.6
ICPA 2092 x ICPL 20106	120	130	188	217.0	14.0	21.0
ICPA 2092 x ICPL 20186	122	133	187	210.5	20.0	18.8
ICPA 2092 x ICPL 20123	122	132	188	220.5	17.7	11.4
Maruti (Check)	106	112	172	197.0	13.8	17.3
Asha (check)	119	133	185	198.0	11.6	20.8
<b>Mean</b>	118	128	186	212.23	16.37	19.08
<b>Range</b>	105-123	112-134	172-191	195-233	10.10-24.30	11.40-24.50
<b>S.Em<sub>±</sub></b>	2.22	2.39	2.26	9.95	2.14	2.03
<b>CD (5%)</b>	6.53	7.04	6.63	-	6.3	5.96
<b>CV (%)</b>	2.66	2.65	1.72	6.63	18.51	15.02

Table 4.3b: Per se performance of pigeonpea hybrids and checks for yield and yield related traits at Patancheru, kharif 2013-14

Hybrids	Pods plant <sup>-1</sup>	Seeds pod <sup>-1</sup>	100 seed- weight(gm)	Yield plant <sup>-1</sup> (gm)	Yield plot <sup>-1</sup> (gm)	Yield (kg ha <sup>-1</sup> )	Pollen fertility %
ICPA 2078 x ICPL 87119	247.0	4.25	11.91	99.47	2345.85	2299.85	98.05
ICPA 2043 x ICPL 87119	233.0	3.84	11.09	101.58	2098.30	2057.15	97.71
ICPA 2047 x ICPL 87119	251.3	3.72	10.72	85.06	1905.51	1868.15	97.44
ICPA 2047 x ICPL 20098	207.2	3.71	10.80	89.92	2112.96	2071.53	96.75
ICPA 2047 x ICPL 20108	310.9	3.52	10.88	107.95	2551.10	2501.07	98.5
ICPA 2047 x ICPL 20126	307.7	3.80	11.21	115.73	2688.07	2635.36	91.74
ICPA 2092 x ICPL 87119	247.0	3.38	10.10	84.73	1951.61	1913.34	97.72
ICPA 2092 x ICPL 20108	341.2	3.67	10.19	106.96	2514.39	2465.09	95.78
ICPA 2048 x ICPL 87119	306.2	3.57	11.96	95.14	2229.67	2185.95	92.62
ICPA 2048 x ICPL 20093	244.2	3.81	12.38	89.37	2106.46	2065.15	88.43
ICPA 2047 x ICPL 20111	265.0	3.53	9.45	83.43	1961.24	1922.78	88.20
ICPA 2047 x ICPL 20129	213.6	3.76	10.98	91.44	2130.93	2089.14	74.46
ICPA 2048 x ICPL 20106	230.0	3.79	12.14	106.41	2007.89	1968.52	86.28
ICPA 2048 x ICPL 20096	214.3	3.74	11.05	88.32	2069.05	2028.48	59.22
ICPA 2048 x ICPL 20098	246.4	3.72	10.94	97.97	2308.12	2262.86	93.75
ICPA 2048 x ICPL 20108	242.1	3.87	11.47	79.56	1910.49	1873.03	93.75
ICPA 2048 x ICPL 20111	249.3	3.50	10.10	77.26	1813.31	1777.75	91.82
ICPA 2092 x ICPL 20106	287.2	3.59	11.02	82.71	1942.73	1904.64	89.14
ICPA 2092 x ICPL 20186	291.9	3.58	8.40	104.73	2463.03	2414.74	87.12
ICPA 2092 x ICPL 20123	233.3	3.57	9.82	71.4	1669.03	1636.30	93.32
Maruti (Check)	222.0	3.37	9.84	64.26	1409.63	1381.99	99.76
Asha (check)	272.0	3.36	10.12	82.13	1920.87	1883.21	99.58
<b>Mean</b>	257.4	3.67	10.75	91.16	2095.91	2054.82	91.42
<b>Range</b>	207.20-341.20	3.36- 4.25	8.40-12.38	64.26-115.73	1409.63-2688.07	1381.99-2635.36	59.22-99.76
<b>S.Em<sub>±</sub></b>	23.81	0.14	0.33	7.98	12.94	12.69	2.29
<b>CD (5%)</b>	70.03	0.41	0.97	23.45	38.07	37.32	6.73
<b>CV (%)</b>	13.08	5.33	4.32	12.37	0.87	0.87	3.54

#### **4.4 Study on heterosis**

Heterosis refers to the superiority of F<sub>1</sub> hybrid over its parents in one or more characters. The term hybrid vigour is generally used as synonym for heterosis. It is believed that increased vigour in plant growth and a higher grain yield are usually expressed in the first filial generation. Heterosis may be either positive or negative. Depending upon breeding objectives, both positive and negative heterosis is useful for crop improvement. The positive heterosis is desirable for seed yield while negative heterosis for early maturity. A study of on heterosis is necessary to explore possibility of the exploiting the vigour in the CMS-based pigeonpea hybrids at commercial level. Heterosis is expressed in three ways, mid-parent, standard variety and better parent heterosis. Standard variety heterosis is more effective. Saxena *et al.* (2006) reported 50 to 100% of standard heterosis in medium duration pigeonpea hybrids over the local popular varieties. Kandalkar (2007) also reported up to 156% of standard heterosis for grain yield in CMS-based medium duration pigeonpea hybrids.

In the present investigation, different levels of heterosis were measured in hybrids as percent increase or decrease over the standard checks for different traits (Table 4.4a-4.4b). In the present study two popular varieties Asha and Maruti were used as standard checks. The standard heterosis of the hybrids was compared with the varieties based on their duration to maturity in relation to Asha and Maruti. The research findings for different traits are described below:

##### **4.4.1 Days to 1st flowering**

All the twenty hybrids were compared with both Maruti and Asha for estimating standard heterosis. Two hybrids, ICPA 2043 x ICPL 87119 (-11.81%) and ICPA 2078 x ICPL 87119 (-8.02%) were showed significant negative standard heterosis over Asha and its range was -11.81(ICPA 2043 x ICPL87119) to 3.80% (ICPA 2048 x ICPL 20106) against Asha for days to 1st flowering. The range of standard heterosis over Maruti was -0.95 (ICPA 2043 x ICPL 87119) to 16.59% (ICPA 2048 x ICPL 20106) and no any hybrids were showed significant negative standard heterosis over Maruti for days to 1st flowering.

#### **4.4.2 Days to 50% flowering**

All the twenty hybrids were compared with both Maruti and Asha for estimating standard heterosis. Nine hybrids, ICPA 2043 x ICPL 87119 (-11.28%), ICPA 2078 x ICPL 87119 (-8.27%), ICPA 2047 x ICPL 20108 (-7.14%), ICPA 2047 x ICPL 87119 (-6.02%), ICPA 2047 x ICPL 20098 (-4.89%), ICPA 2092 x ICPL 87119 (-4.51%), ICPA 2047 x ICPL 20126 (-3.76%), ICPA 2092 x ICPL 20108 (-3.76%) and ICPA 2048 x ICPL 87119 (-3.38%) were showed significantly low with respect to Asha and its range was -11.28 (ICPA 2043 x ICPL 87119) to 0.75% (ICPA 2048 x ICPL20106) over Asha for days to 50% flowering. The range of standard heterosis over Maruti was 5.36% (ICPA 2043 x ICPL 87119) to 19.64% (ICPA 2048 x ICPL 20106) and no any hybrids were showed significant negative standard heterosis over Maruti for days to 50% flowering.

#### **4.4.3 Days to maturity**

Among the 20 hybrids, no any hybrids were showed significant negative standard heterosis over both checks (Asha and Maruti). The range was -0.54 (ICPA 2078 x ICPL 87119) to 3.24% (ICPA 2048 x ICPL20093) over Asha for days to maturity. The range of standard heterosis over Maruti was 6.98 (ICPA 2078 x ICPL 87119) to 11.05% (ICPA 2048 x ICPL 20093).

#### **4.4.4 Plant height(cm)**

Among the twenty hybrids, ten hybrids ICPA 2048 x ICPL 20108 (18.27%) , ICPA 2048 x ICPL 20098 (16.24%), ICPA 2048 x ICPL 20111 (14.72%), ICPA 2092 x ICPL 20123 (11.93%), ICPA 2048 x ICPL 20096 (11.42%), ICPA 2047 x ICPL 20108 (11.17%), ICPA 2048 x ICPL 20106 (10.66%), ICPA 2092 x ICPL 20106 (10.15%), ICPA 2092 x ICPL 20108 (10.15%) and ICPA 2047 x ICPL 20129 (9.90%) were showed significant standard heterosis over the check variety Maruti while one hybrid ICPA 2048 x ICPL 20093 (-1.02%) showed negative heterosis for plant height. Ten hybrids, ICPA 2048 x ICPL 20108 (17.68%), ICPA 2048 x ICPL 20098(15.66%), ICPA 2048 x ICPL 20111 (14.14%), ICPA 2092 x ICPL 20123 (11.36%), ICPA 2048 x ICPL 20096 (10.86%), ICPA 2047 x ICPL 20108 (10.61%), ICPA 2048 x ICPL 20106 (10.10%), ICPA 2092 x ICPL

20106 (9.60%), ICPA2092 x ICPL 20108 (9.60%) and ICPA 2047 x ICPL 20129 (9.34%) were showed significant standard heterosis over the check variety Asha while one hybrid ICPA 2048 x ICPL 20093 (-1.52%) showed negative heterosis for plant height.

#### **4.4.5 Number of primary branches plant<sup>-1</sup>**

Out of twenty, the six hybrids, ICPA 2048 x ICPL 87119 (76.09%), ICPA 2092 x ICPL 20108 (60.14%), ICPA 2092 x ICPL 20108 (44.93%), ICPA 2047 x ICPL 20098 (44.93%), ICPA 2048 x ICPL 20098 (42.03%) and ICPA 2047 x ICPL 20126 (36.23%) were showed significant positive standard heterosis over Maruti for this trait with the range of -26.81 (ICPA 2047 x ICPL 87119) to 76.09% (ICPA 2048 x ICPL 87119). Twelve hybrids, ICPA 2048 x ICPL 87119(109.48%), ICPA 2092 x ICPL 20108 (90.52%), ICPA 2092 x ICPL 20186 (72.41%), ICPA 2047 x ICPL 20098 (72.41%), ICPA 2048 x ICPL 20098 (68.97%), ICPA 2047 x ICPL 20126 (62.07%), ICPA 2092 x ICPL 20123 (52.59%), ICPA 2047 x ICPL 20111 (50.86%), ICPA 2048 x ICPL 20096 (44.83%), ICPA 2048 x ICPL 20093 (43.97%), ICPA 2048 x ICPL 20108 (37.93%) and ICPA 2043 x ICPL 87119 (35.34%) were showed significant positive standard heterosis over Asha for this trait with the range of -12.93 (ICPA 2047 x ICPL 87119) to 109.48% (ICPA 2048 x ICPL 87119).

#### **4.4.6 Number of secondary branches plant<sup>-1</sup>**

Among the twenty hybrids, ICPA 2092 x ICPL 20123 (-34.10%) showed significant negative standard heterosis over Maruti while ICPA 2092 x ICPL 20123 (-45.19%), ICPA 2092 x ICPL 20108 (-29.81%), ICPA 2092 x ICPL 87119 (-25.48%), and ICPA 2048 x ICPL 20111 (-20.19%) showed significant negative standard heterosis over Asha. Out of 20 hybrids, ICPA 2078 x ICPL 87119 (41.62%), ICPA 2047 x ICPL 20126 (32.95%), ICPA 2047 x ICPL 20108 (30.64%), ICPA 2048 x ICPL 20096 (23.70%), ICPA 2047 x ICPL 20129 (23.12%) and ICPA 2092 x ICPL 20106 (21.39%) showed significant standard heterosis over Maruti whereas ICPA 2078 x ICPL 87119 (17.79%) showed significant standard heterosis over Asha.

**4.4.7 Pods plant<sup>-1</sup>**

Seven hybrids ICPA 2092 x ICPL 20108 (53.69%), ICPA 2047 x ICPL 20108 (40.05%), ICPA 2047 x ICPL 20126 (38.60%), ICPA 2048 x ICPL 87119 (37.93%), ICPA 2092 x ICPL 20186 (31.49%), ICPA 2092 x ICPL 20106 (29.37%) and ICPA 2047 x ICPL 20111(19.37%) showed significant positive heterosis over standard check Maruti. Only one hybrid, ICPA 2092 x ICPL 20108 (25.44%) showed significant positive heterosis over standard check Asha. The range of standard heterosis from -6.67% (ICPA 2047 x ICPL 20098) to 53.69% (ICPA 2092 x ICPL 20108) against Maruti while -23.82% (ICPA 2047 x ICPL 20098) to 25.44% (ICPA 2092 x ICPL 20108) against Asha.

**4.4.8 Seeds pod<sup>-1</sup>**

All the 20 hybrids showed positive heterosis over standard checks Maruti and Asha for this trait. Twelve hybrids, ICPA 2078 x ICPL 87119 (26.11%), ICPA 2048 x ICPL 20108(14.84%), ICPA 2043 x ICPL 87119 (13.95%), ICPA 2048 x ICPL 20093 (13.06%), ICPA 2047 x ICPL 20126 (12.76%), ICPA 2048 x ICPL 20106 (12.46%), ICPA 2047 x ICPL 20129 (11.57%), ICPA 2048 x ICPL 20096 (10.98%), ICPA 2048 x ICPL 20098 (10.39%), ICPA 2047 x ICPL 87119 (10.39%), ICPA 2047 x ICPL 20098 (10.09%) and ICPA 2092 x ICPL 20108 (8.90%) showed significant positive heterosis over standard check Maruti. The range of standard heterosis was from 0.30% (ICPA 2092 x ICPL 87119) to 26.11% (ICPA 2078 x ICPL 87119) against Maruti. Twelve hybrids, ICPA 2078 x ICPL 87119 (26.49%), ICPA 2048 x ICPL 20108 (15.18%), ICPA 2043 x ICPL 87119 (14.29%), ICPA 2048 x ICPL 20093 (13.39%), ICPA 2047 x ICPL 20126 (13.10%), ICPA 2048 x ICPL 20106 (12.80%), ICPA 2047 x ICPL 20129 (11.90%), ICPA 2048 x ICPL 20096 (11.31%), ICPA 2048 x ICPL 20098 (10.71%), ICPA 2047 x ICPL 87119 (10.71%), ICPA 2047 x ICPL 20098 (10.42%) and ICPA 2092 x ICPL 20108 (9.23%) showed significant positive heterosis over standard check Asha. The range of standard heterosis was from 0.60% (ICPA 2092 x ICPL 87119) to 26.49% (ICPA 2078 x ICPL 87119) against Asha.

**4.4.9 100 seed weight (g)**

Among the 20 hybrids, fourteen of them exhibited significant positive standard heterosis over both checks (Maruti and Asha) for 100 seed weight. Hybrids ICPA 2092 x ICPL 20108 (3.56%), ICPA 2048 x ICPL 20111 (2.64%) and ICPA 2092 x ICPL 87119 (2.64%) showed non-significant positive standard heterosis over Maruti whereas ICPA 2092 x ICPL 20108 (0.69%) also showed non-significant positive standard heterosis over Asha. The range of standard heterosis for Maruti was from -14.63 (ICPA 2092 x ICPL 20186) to 25.81% (ICPA 2048 x ICPL 20093) and for Asha it was from -17.0 (ICPA 2092 x ICPL 20186) to 22.33% (ICPA 2048 x ICPL 20093).

**4.4.10 Seed yield plant<sup>-1</sup> (g)**

All the 20 hybrids showed positive standard heterosis over Maruti. Hybrids ICPA 2047 x ICPL 20126 (80.10%), ICPA 2047 x ICPL 20108 (67.99%), ICPA 2092 x ICPL 20108 (66.45%), ICPA 2048 x ICPL 20106 (65.59%), ICPA 2092 x ICPL 20186 (62.98%), ICPA 2043 x ICPL 87119 (58.08%), ICPA 2078 x ICPL 87119 (54.79%), ICPA 2048 x ICPL 20098 (52.46%), ICPA 2048 x ICPL 87119 (48.05%), ICPA 2047 x ICPL 20129 (42.32%), ICPA 2047 x ICPL 20098 (39.93%), ICPA 2048 x ICPL 20093 (39.08%), ICPA 2048 x ICPL 20096 (37.44%), ICPA 2047 x ICPL 87119 (32.37%), ICPA 2048 x ICPL 20111 (31.85%), ICPA 2047 x ICPL 20111 (29.83%), ICPA 2092 x ICPL 20106 (28.71%) and ICPA 2048 x ICPL 20108 (23.81%) showed significant positive standard heterosis over Maruti. Eight hybrids ICPA 2047 x ICPL 20126 (40.91%), ICPA 2047 x ICPL 20108 (31.44%), ICPA 2092 x ICPL 20108 (30.23%), ICPA 2048 x ICPL 20106 (29.56%), ICPA 2092 x ICPL 20186 (27.52%), ICPA 2043 x ICPL 87119 (23.68%), ICPA 2078 x ICPL 87119 (21.11%) and ICPA 2048 x ICPL 20098(19.29%) showed significant positive standard heterosis over Asha. The range of standard heterosis for Maruti was from 11.11 (ICPA 2092 x ICPL 20123) to 80.10% (ICPA 2047 x ICPL 20126) and for Asha it was from -13.06 (ICPA 2092 x ICPL 20123) to 40.91% (ICPA 2047 x ICPL 20126).

**4.4.11 Seed yield plot<sup>-1</sup>(g)**

All 20 hybrids showed significant positive standard heterosis over Maruti whereas 16 hybrids showed positive standard heterosis over Asha. The range of standard heterosis over Maruti was from 15.54(ICPA 2092 x ICPL 20123) to 47.55% (ICPA 2047 x ICPL 20126) and for Asha it was from -15.09 (ICPA 2092 x ICPL 20123) to 28.54% (ICPA 2047 x ICPL 20126).

**4.4.12 Pollen fertility (%)**

All the 20 hybrids showed negative standard heterosis over both checks (Maruti and Asha) for pollen fertility %. The range of standard heterosis over Maruti was from -40.64% (ICPA 2048 x ICPL 20096) to -1.26% (ICPA 2047 x ICPL 20108) and for Asha it was from -40.53% (ICPA 2048 x ICPL 20096) to -1.08% (ICPA 2047 x ICPL 20108). Seven hybrids ICPA 2047 x ICPL 20108 (-1.26%), ICPA 2078 x ICPL 87119 (-1.71%), ICPA 2092 x ICPL 87119 (-2.04%), ICPA 2043 x ICPL 87119 (-2.05%), ICPA 2047 x ICPL 87119 (-2.33%), ICPA 2047 x ICPL 20098 (-3.02%) and ICPA 2092 x ICPL 20108 (-3.99%) were showed non-significant negative standard heterosis over Maruti. Seven hybrids ICPA 2047 x ICPL 20108 (-1.08%), ICPA 2078 x ICPL 87119 (-1.54%), ICPA 2092 x ICPL 87119 (-1.87%), ICPA 2043 x ICPL 87119 (-1.88%), ICPA 2047 x ICPL 87119 (-2.15%), ICPA 2047 x ICPL 20098 (-2.84%) and ICPA 2092 x ICPL 20108 (-3.82%) also showed non- significant negative standard heterosis over Asha.

Table 4.4a Standard heterosis in CMS-based pigeonpea hybrids at Patancheru, *kharif* 2013-14

Sl. No.	Genotype	Days to 1st flower		Days to 50% flower		Days to maturity		Plant height(cm)		No. of primary branches plant <sup>-1</sup>		No of secondary branches plant <sup>-1</sup>	
		SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2
1	ICPA 2078 x ICPL 87119	3.32	-8.02**	8.93**	-8.27**	6.98**	-0.54	5.84	5.30	9.42	30.17	41.62**	17.79*
2	ICPA 2043 x ICPL 87119	-0.95	-11.81**	5.36**	-11.28**	7.27**	-0.27	7.11	6.57	13.77	35.34*	18.50	-1.44
3	ICPA 2047 x ICPL 87119	12.32**	0.00	11.61**	-6.02**	7.85**	0.27	1.27	0.76	-26.81	-12.93	10.40	-8.17
4	ICPA 2047 x ICPL 20098	14.69**	2.11	12.95**	-4.89**	9.01**	1.35	3.55	3.03	44.93**	72.41**	14.45	-4.81
5	ICPA 2047 x ICPL 20108	9.00**	-2.95	10.27**	-7.14**	7.56**	0.00	11.17*	10.61*	3.62	23.28	30.64**	8.65
6	ICPA 2047 x ICPL 20126	11.37**	-0.84	14.29**	-3.76*	8.14**	0.54	6.09	5.56	36.23*	62.07**	32.95**	10.58
7	ICPA 2092 x ICPL 87119	11.85**	-0.42	13.39**	-4.51**	6.98**	-0.54	2.03	1.52	10.14	31.03	-10.40	-25.48**
8	ICPA 2092 x ICPL 20108	14.22**	1.69	14.29**	-3.76*	8.43**	0.81	10.15*	9.60*	60.14**	90.52**	-15.61	-29.81**
9	ICPA 2048 x ICPL 87119	12.80**	0.42	14.73**	-3.38*	8.14**	0.54	4.57	4.04	76.09**	109.48**	8.67	-9.62
10	ICPA 2048 x ICPL 20093	14.22**	1.69	17.86**	-0.75	11.05**	3.24**	-1.02	-1.52	21.01	43.97**	4.62	-12.98
11	ICPA 2047 x ICPL 20111	16.11**	3.38*	16.96**	-1.50	8.43**	0.81	8.63	8.08	26.81	50.86**	1.16	-15.87
12	ICPA 2047 x ICPL 20129	14.69**	2.11	16.52**	-1.88	8.43**	0.81	9.90*	9.34*	5.80	25.86	23.12*	2.40
13	ICPA 2048 x ICPL 20106	16.59**	3.80*	19.64**	0.75	10.76**	2.97**	10.66*	10.10*	-3.62	14.66	8.09	-10.10
14	ICPA 2048 x ICPL 20096	15.17**	2.53	16.07**	-2.26	9.01**	1.35	11.42*	10.86*	21.74	44.83**	23.70*	2.88
15	ICPA 2048 x ICPL 20098	13.27**	0.84	15.63**	-2.63	8.43**	0.81	16.24**	15.66**	42.03**	68.97**	12.72	-6.25
16	ICPA 2048 x ICPL 20108	14.22**	1.69	17.41**	-1.13	7.56**	0.00	18.27**	17.68**	15.94	37.93*	9.83	-8.65
17	ICPA 2048 x ICPL 20111	12.32**	0.00	16.07**	-2.26	8.14**	0.54	14.72**	14.14**	-5.80	12.07	-4.05	-20.19*
18	ICPA 2092 x ICPL 20106	13.27**	0.84	16.07**	-2.26	9.01**	1.35	10.15*	9.60*	1.45	20.69	21.39*	0.96
19	ICPA 2092 x ICPL 20186	15.17**	2.53	18.75**	0.00	8.72**	1.08	6.85	6.31	44.93**	72.41**	8.67	-9.62
20	ICPA 209 x ICPL 20123	15.64**	2.95	17.86**	-0.75	9.30**	1.62	11.93*	11.36*	28.26	52.59**	-34.10**	-45.19**

Where, \*, \*\* = significant at 5% and 1% level, respectively. SH1-Standard heterosis against Maruti variety, SH2- Standard heterosis against Asha variety

Table 4.4b Standard heterosis in CMS-based pigeonpea hybrids at Patancheru, *kharif* 2013-14

Sl. No.	Genotype	Pods plant <sup>-1</sup>		Seeds pod <sup>-1</sup>		100-seed weight (gm)		Yield plant <sup>-1</sup> (gm)		Yield plot <sup>-1</sup> (gm)		Pollen fertility %	
		SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2
1	ICPA 2078 x ICPL 87119	11.26	-9.19	26.11**	26.49**	21.04**	17.69**	54.79**	21.11*	39.91**	18.12**	-1.71	-1.54
2	ICPA 2043 x ICPL 87119	4.95	-14.34	13.95**	14.29**	12.70**	9.58**	58.08**	23.68*	32.82**	8.46**	-2.05	-1.88
3	ICPA 2047 x ICPL 87119	13.20	-7.61	10.39**	10.71**	8.94**	5.93*	32.37**	3.57	26.02**	-0.81	-2.33	-2.15
4	ICPA 2047 x ICPL 20098	-6.67	-23.82**	10.09*	10.42*	9.76**	6.72*	39.93**	9.48	33.29**	9.09**	-3.02	-2.84
5	ICPA 2047 x ICPL 20108	40.05**	14.30	4.45	4.76	10.57**	7.51*	67.99**	31.44**	44.74**	24.70**	-1.26	-1.08
6	ICPA 2047 x ICPL 20126	38.60**	13.13	12.76**	13.10**	13.92**	10.77**	80.10**	40.91**	47.56**	28.54**	-8.04**	-7.87**
7	ICPA 2092 x ICPL 87119	11.26	-9.19	0.30	0.60	2.64	-0.20	31.85**	3.17	27.77**	1.57**	-2.04	-1.87
8	ICPA 2092 x ICPL 20108	53.69**	25.44**	8.90*	9.23*	3.56	0.69	66.45**	30.23**	43.94**	23.60**	-3.99	-3.82
9	ICPA 2048 x ICPL 87119	37.93**	12.57	5.93	6.25	21.54**	18.18**	48.05**	15.84	36.78**	13.85**	-7.16**	-6.99**
10	ICPA 2048 x ICPL 20093	10.00	-10.22	13.06**	13.39**	25.81**	22.33**	39.08**	8.82	33.08**	8.81**	-11.36**	-11.20**
11	ICPA 2047 x ICPL 20111	19.37*	-2.57	4.75	5.06	-3.96	-6.62*	29.83*	1.58	28.13**	2.06	-11.59**	-11.43**
12	ICPA 2047 x ICPL 20129	-3.77	-21.46**	11.57**	11.90**	11.59**	8.50*	42.30**	11.34	33.85**	9.86**	-25.36**	-25.23**
13	ICPA 2048 x ICPL 20106	3.60	-15.44	12.46**	12.80**	23.37**	19.96**	65.59**	29.56**	29.80**	4.33**	-13.51**	-13.36**
14	ICPA 2048 x ICPL 20096	-3.47	-21.21*	10.98**	11.31**	12.30**	9.19**	37.44**	7.54	31.87**	7.16**	-40.64**	-40.53**
15	ICPA 2048 x ICPL 20098	10.99	-9.41	10.39**	10.71**	11.18**	8.10**	52.46**	19.29*	38.93**	16.78**	-6.02**	-5.85**
16	ICPA 2048 x ICPL 20108	9.05	-10.99	14.84**	15.18**	16.57**	13.34**	23.81*	-3.13	26.22**	-0.54	-6.02**	-5.85**
17	ICPA 2048 x ICPL 20111	12.30	-8.35	3.86	4.17	2.64	-0.20	20.23	-5.93	22.26**	-5.93**	-7.96**	-7.79**
18	ICPA 2092 x ICPL 20106	29.37**	5.59	6.53	6.85	11.99**	8.89**	28.71*	0.71	27.44**	1.13	-10.65**	-10.48**
19	ICPA 2092 x ICPL 20186	31.49**	7.32	6.23	6.55	-14.63**	-17.00**	62.98**	27.52**	42.77**	22.01**	-12.67**	-12.51**
20	ICPA 209 x ICPL 20123	5.09	-14.23	5.93	6.25	-0.20	-2.96	11.11	-13.06	15.54**	-15.09**	-6.46**	-6.29**

Where, \*, \*\* = significant at 5% and 1% level, respectively.

SH1-Standard heterosis against Maruti variety, SH2- Standard heterosis against Asha variety

### **4.3 Identification of fertility restorers and maintainers in hybrids**

Based on the fertility/sterility % in the F1 progenies, the male parent of such cross may be classified as restorer/maintainer. The mean performance of genotypes (hybrids and checks) for Pollen fertility % and sterility % were studied. The mean genotypic values from different genotypes are given in Table 4.5.

#### **4.3.1 Pollen fertility % in hybrids and checks**

Pollen fertility (%) is an important character to evaluate the restoration of fertility and amount of viable pollens produced by particular hybrid which is a basic need for the successful production of high yielding CMS-based hybrids of pigeonpea. At Patancheru, the variability for pollen fertility ranged from 59.22 to 99.76% (Table 4.3). Among the hybrids, ICPA 2047 x ICPL 20108 recorded maximum pollen fertility (98.50%) followed by ICPA 2078 x ICPL 87119 (98.05%) and ICPA 2092 x ICPL 87119 (97.72%), whereas the minimum pollen fertility was recorded in ICPA 2048 x ICPL 20096 (59.22%) followed by ICPA 2047 x ICPL 20129 (74.46%). Among the all genotypes (hybrids and checks), Asha recorded maximum pollen fertility (99.76%) followed by Maruti (99.58%) (Fig.4.2)

#### **4.3.2 Pollen sterility % in hybrids and checks**

When all the plants in a progeny will fully male-sterile, the male parent of such a cross may be classified as maintainer. The pollen sterility ranged from 40.78 to 0.24% (Table 4.3). Among the hybrids, ICPA 2048 x ICPL 20096 recorded maximum pollen sterility (40.78 %) followed by ICPA 2047 x ICPL 20129 (25.54%) and ICPA 2048 x ICPL 20106 (13.72%), whereas the minimum pollen sterility was recorded in ICPA 2047 x ICPL 20108 (1.5%) followed by ICPA 2078 x ICPL 87119 (1.95%). Among the all genotypes (hybrids and checks), Asha recorded minimum pollen sterility (0.24%) followed by Maruti (0.42%) (Fig.4.3). All 11 male lines with their fertility restoration capacity are given in Table 4.6.

Table 4.5: *Per se* performance of CMS-based pigeonpea hybrids with two checks for pollen fertility (%) and sterility (%) at Patancheru.

<b>Sl. No.</b>	<b>Hybrids</b>	<b>Pollen fertility (%)</b>	<b>Pollen sterility (%)</b>
1.	ICPA 2078 x ICPL 87119	98.05	1.95
2.	ICPA 2043 x ICPL 87119	97.71	2.29
3.	ICPA 2047 x ICPL 87119	97.44	2.56
4.	ICPA 2047 x ICPL 20098	96.75	3.25
5.	ICPA 2047 x ICPL 20108	98.50	1.50
6.	ICPA 2047 x ICPL 20126	91.74	8.26
7.	ICPA 2092 x ICPL 87119	97.72	2.28
8.	ICPA 2092 x ICPL 20108	95.78	4.22
9.	ICPA 2048 x ICPL 87119	92.62	7.38
10.	ICPA 2048 x ICPL 20093	88.43	11.57
11.	ICPA 2047 x ICPL 20111	88.20	11.80
12.	ICPA 2047 x ICPL 20129	74.46	25.54
13.	ICPA 2048 x ICPL 20106	86.28	13.72
14.	ICPA 2048 x ICPL 20096	59.22	40.78
15.	ICPA 2048 x ICPL 20098	93.75	6.25
16.	ICPA 2048 x ICPL 20108	93.75	6.25
17.	ICPA 2048 x ICPL 20111	91.82	8.18
18.	ICPA 2092 x ICPL 20106	89.14	10.86
19.	ICPA 2092 x ICPL 20186	87.12	12.88
20.	ICPA 2092 x ICPL 20123	93.32	6.68
21.	Asha (check)	99.76	0.24
22.	Maruti (check)	99.58	0.42

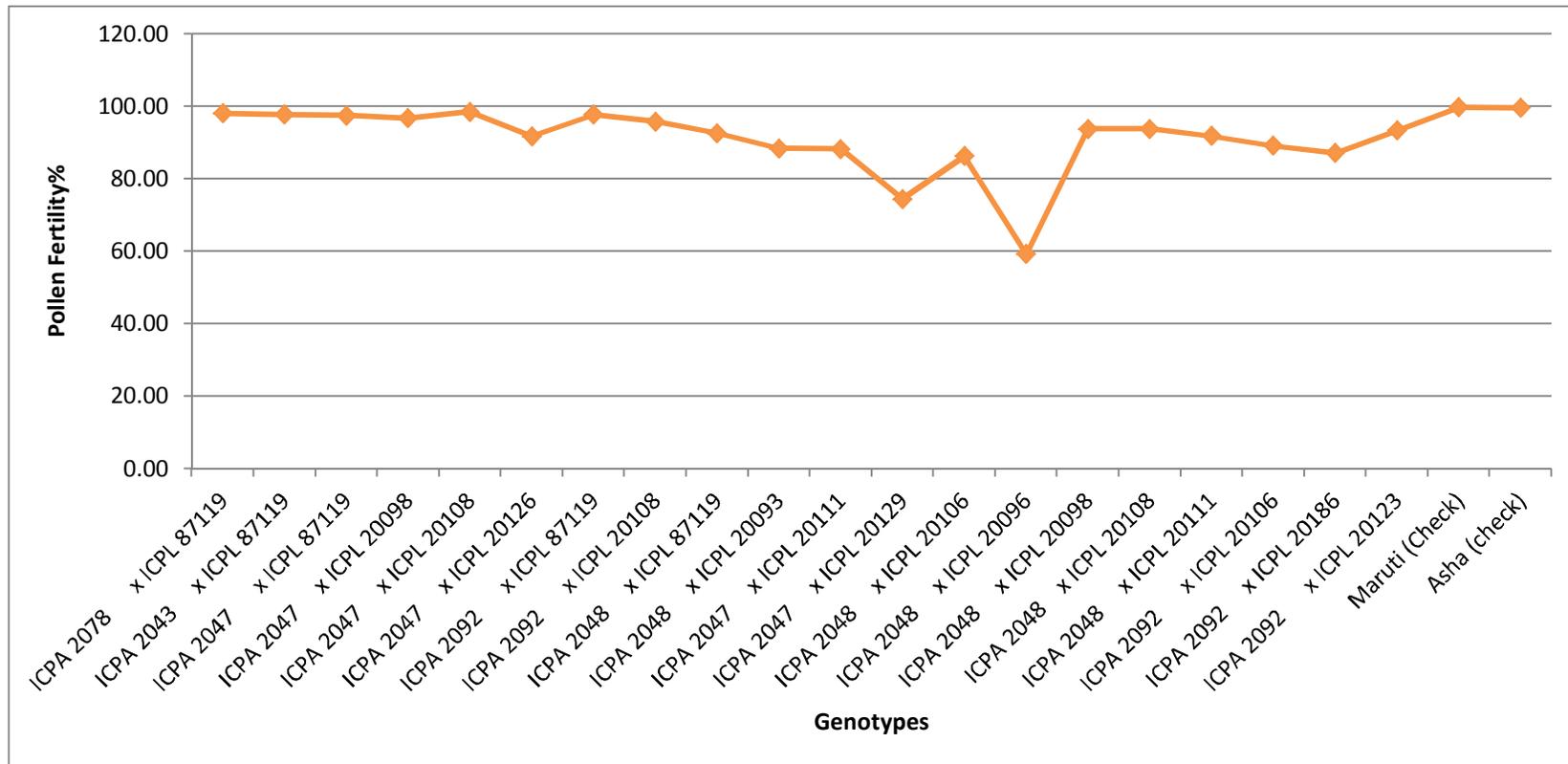


Fig. 4.2: The graph showing pollen fertility status of different CMS-based hybrids and checks of pigeonpea.

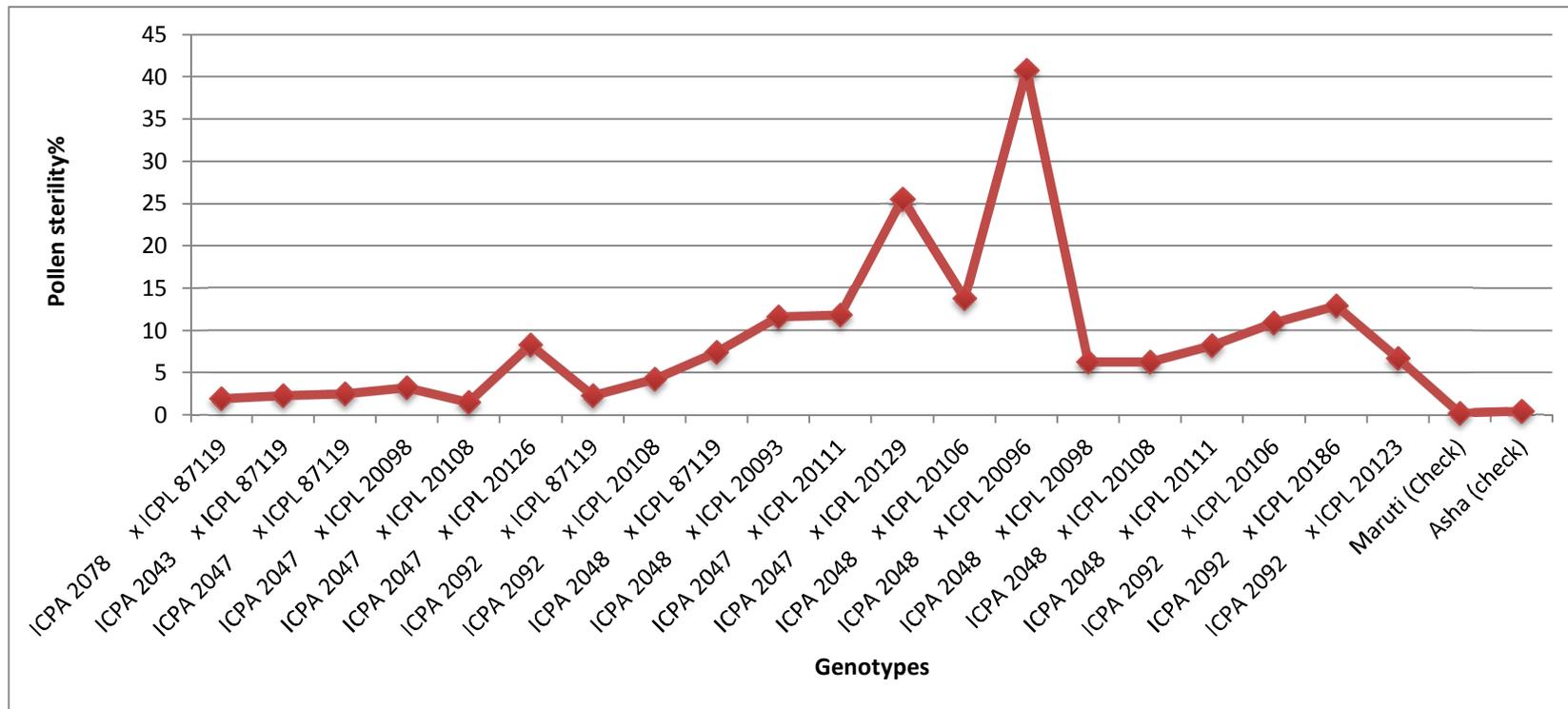


Fig. 4.3: The graph showing pollen sterility status of different CMS-based hybrids and checks of pigeonpea.

Sl. No.	Male lines	ICPA 2047 Fertility restoration (%)	ICPA 2048 Fertility restoration (%)	ICPA 2092 Fertility restoration (%)	ICPA 2078 Fertility restoration (%)	ICPA 2043 Fertility restoration (%)
1	ICPL 87119	97.44	92.62	97.72	98.05	97.71
2	ICPL 20093	-	88.43	-	-	-
3	ICPL 20096	-	59.22	-	-	-
4	ICPL 20098	96.75	93.75	-	-	-
5	ICPL 20106	-	86.28	89.14	-	-
6	ICPL 20108	98.5	93.75	95.78	-	-
7	ICPL20111	88.2	91.82	-	-	-
8	ICPL 20123	-	-	93.32	-	-
9	ICPL 20126	91.74	-	-	-	-
10	ICPL 20129	74.46	-	-	-	-
11	ICPL 20186	-	-	87.12	-	-

Table 4.6: male lines with their fertility restoration capacity

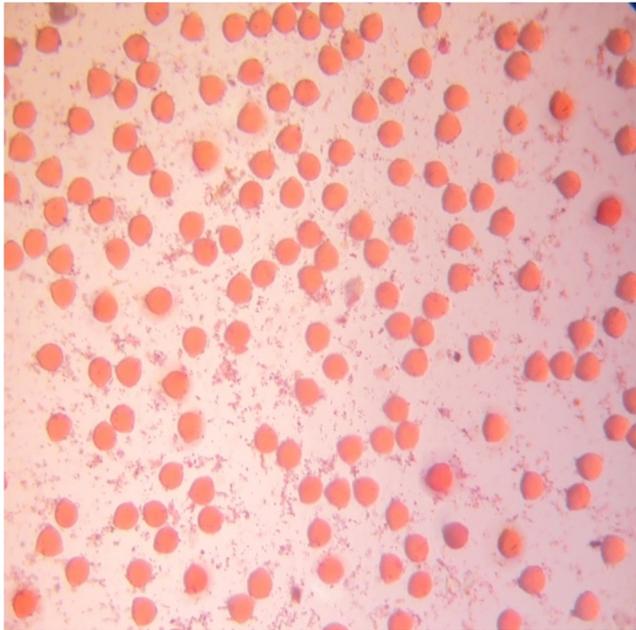


Fig. No.4.4: Microscopic view of pollen grains produced by fully male fertile plant.

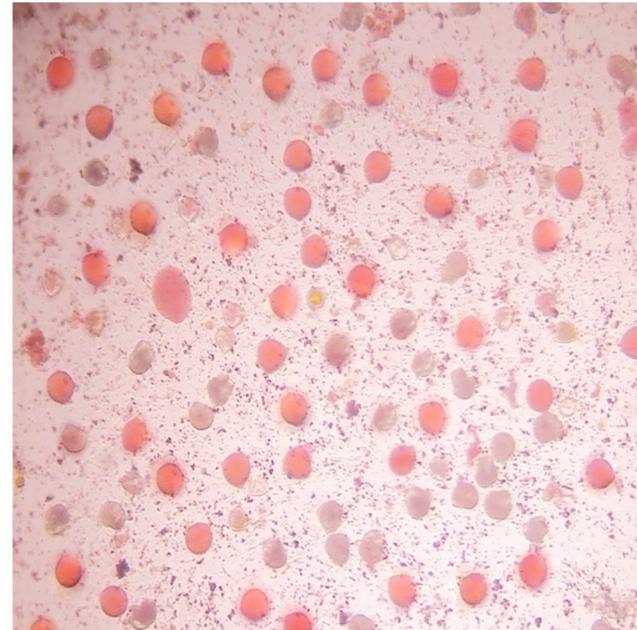


Fig. No.4.5: Microscopic view of pollen grains produced by partial male fertile plant.

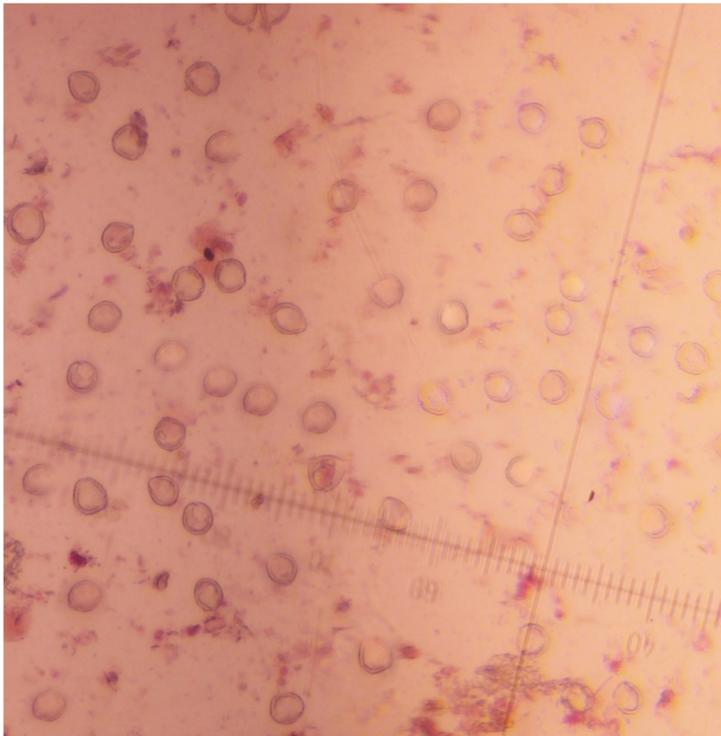


Fig. No.4.6: Microscopic view of pollen grains produced by fully male sterile plant.

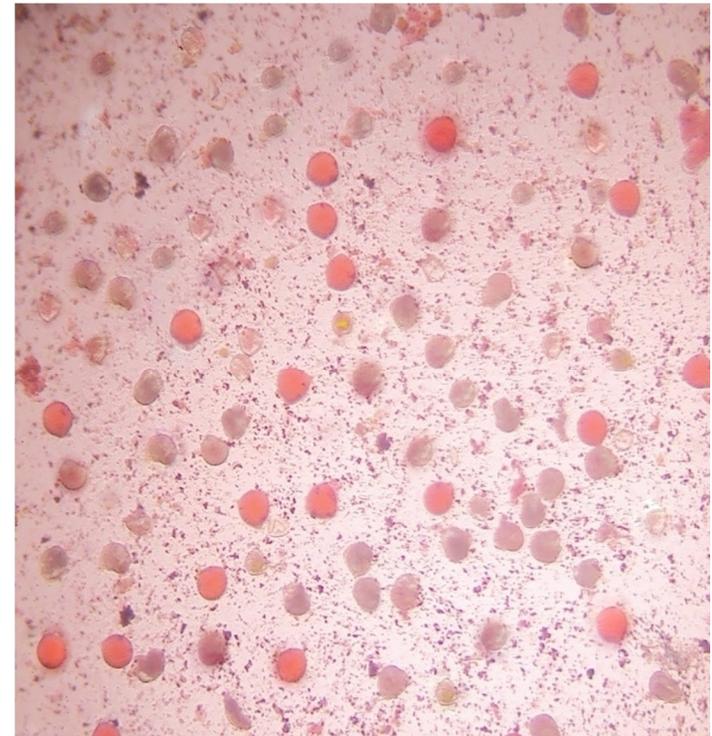


Fig. No.4.5: Microscopic view of pollen grains produced by partial male sterile plant.

# CHAPTER V

## *DISCUSSION*



## DISCUSSION

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The genetic male-sterility system (GMS) was identified in pigeonpea in late seventies that was controlled by a single recessive gene (Reddy *et al.*, 1978) but the hybrids from this material were not accepted by seed industries due to inherent problems associated with GMS system. The other type of major reproductive abnormality leading to male sterility is caused by interaction of specific nuclear and cytoplasmic genetic factors. In most cases, the recessive nuclear genes interact with specific genetic factors housed in the cytoplasm of a cell and make an individual's anthers non-functional leading to male-sterility. Such plants produce fertile pollen when the recessive nuclear genes are replaced by their dominant counterparts or the cytoplasmic male sterility causing factors by fertility inducing genetic factors.

Pigeonpea is the most important pulse crop and development of hybrids at commercial level is the specific feature (Saxena *et al.*, 2013). In the present investigation, 22 genotypes, comprising of 20 hybrids and two standard varietal checks were studied to estimate the standard heterosis for yield, yield attributes and pollen fertility/sterility%. Present experiments were also includes effectiveness of five CMS lines. In the present investigations, the major objective was to assess the performance of different hybrids for fertility restoration and CMS lines for expression level of male sterility.

### ➤ **Mean performance of CMS lines for pollen sterility %**

Several efforts were made to understand the cause of cytoplasmic male sterility at cellular level in pigeonpea. The cytological studies of male-sterile as well as male fertile floral buds showed normal meiosis with 11 chromosomes at each pole at anaphase and visible abnormalities were rare. The PMC's (Pollen Mother Cells) of the male-sterile plant becomes shriveled and the major reason for shriveled PMC's in male sterile plants is breakdown of tapetum layer, which not only gives support but also provides nutrition to PMC's. However, during growth of the tetrads, unlike in fertile plants, the normal event of dissolution of PMC wall was inhibited and the tetrads remained enclosed within the persistent tetrad wall. Consequently, the further growth of tetrads ceased and they lost their cell contents leading to

premature abortion of the pollen grains, but they remained together till the flowers opened. Hence, the degeneration of pollen grains at the late tetrad stage was identified as the prime cause for the manifestation of the male sterility in pigeonpea. Breakdown of micro-sporogenesis due to persistent tapetum was also observed in the genetic male sterility system reported by Reddy *et al.* (1978). However in the cytoplasmic male sterile pigeonpea during the formation of tetrads, the PMC wall did not dissolve to release the tetrads is a major cause found to be responsible for male sterility. This was confirmed based on studies by Wallis *et al.* (1981), Dundas *et al.* (1981), Dundas *et al.* (1982), Saxena *et al.* (1983), Katti *et al.* (1994), and Mallikarjuna and Saxena (2005).

In the present study, highest pollen sterility was recorded in ICPA 2092(99.60 %) followed by ICPA 2043 (99.01 %), ICPA 2048 (97.2%), ICPA 2047(96.30%) and ICPA 2078 (95.69%). All the CMS lines performed well with high (>95 %) pollen sterility. Similar results were earlier reported by Dalvi (2007), Sawargaonkar *et al.* (2012a) and Saxena *et al.* (2005) in CMS lines of pigeonpea. Makelo *et al.* (2013) also reported that six CMS lines (ICPA 2043, ICPA 2039, ICPA 2091, ICPA 2050, ICPA 2042 and ICPA 2101), with over 96% cytoplasmic male sterility.

#### ➤ **Analysis of Variance**

The studies on analysis of variance among 22 genotypes for fourteen characters revealed that the genotypes differed significantly for all the characters except for plant height (Table 4.1). It indicating the presence of substantial genetic variation among the hybrids for yield and yield contributing traits selected for the study.

#### ➤ **Mean Performance of Hybrids and Checks**

Maturity duration is a very important factor that determines the adaptation of varieties to various agro-ecological conditions and cropping systems (Sharma *et al.* 1981). In the present investigation ICPA 2043 x ICPL 87119 required minimum number of days to 1<sup>st</sup> flowering and it was significantly earlier than check Asha, while ICPA 2048 x ICPL 20106 required maximum number of days to 1<sup>st</sup> flowering. *The per se* performance of hybrids are presented in Tables 4.3a - 4.3b for all the characters studied.

Among 22 genotypes, ICPA 2043 x ICPL 87119 required minimum number of days to 50% flowering and it was significantly earlier over mean performance and check Asha, while ICPA 2048 x ICPL 20106 required maximum number of days to 50% flowering. Pigeonpea is considered as quantitative short-day flowering plant (Summerfield and Roberts 1985), *i.e.*, onset of flowering is hastened as the day-length reduces. Moreover, both low and high temperature may also delay flowering in pigeonpea (Whiteman *et al.* 1985).

Out of 22 genotypes, check Maruti was the earliest to mature, while ICPA 2048 x ICPL 20093 exhibited maximum number of days to mature. Most of the tested materials showed delay in maturity. It may be due to interaction between the effect of temperature and photo-period. Bright and dry days are favourable for fertilization, while cloudy, damp weather results in excessive flower drop (Howard *et al.*, 1919, Mahta and Dave, 1931). During the crop season, the extended rainfall and cloudy conditions occurred and there was a serious flower drop resulting in delayed maturity. Maturity duration is a very important factor that determines the adaptation of varieties to various agro-ecological conditions and cropping systems (Sharma *et al.* 1981).

All the hybrids were taller than both the checks (Asha and Maruti) except ICPA 2078 x ICPL 87119. In present study all the genotypes are non-determinate type for plant height. The range of plant height was from 195.0 to 233.0 cm, while general mean was 212.2 cm. Plant height is influenced by maturity duration, photoperiod, and environment (Reddy, 1990). It can be substantially increased through prolongation of the vegetative phase by exposure to long-day conditions. Pigeonpea was used in different ways in remote areas such as domestic fuel, to construct huts, and fences. Hence, plant height is also an important character to considered for plant selection.

Most of the hybrids were showed high number of primary and secondary branches per plant with respect to check Asha. The range of number of primary branches plant<sup>-1</sup> varies from 10.1 to 24.30, while general mean was 16.37. In over 8000 world germplasm accessions the average number of primary branches at harvest time ranged from 2.3 to 66 with a mean of 13.2 (Remanandan *et al.*, 1988). In pigeonpea, the plant grows slowly and primary branches start appearing from the

6<sup>th</sup> to 10<sup>th</sup> nodes. Varieties differ greatly in the number and angle of their branches when grown at fairly wide plant-to-plant spacings (Reddy, 1990).

Among the hybrids, ICPA 2092 x ICPL 20108 had the highest number of pods plant<sup>-1</sup>. The range of number of pods plant<sup>-1</sup> varies from 207.20 to 341.2, while general mean was 257.4. Among 22 genotypes, all hybrids showed higher mean performance for number of seeds pod<sup>-1</sup> over checks Maruti and Asha. The range of number of Seeds pod<sup>-1</sup> varies from 3.36 (Asha) to 4.25 (ICPA 2078 x ICPL 87119), while general mean was 3.67. Similar results were reported by Mahajan *et al.* (2007), Vanniarajan *et al.* (2000). Most of the hybrids performed good for 100 seed weight and Seed yield plant<sup>-1</sup>. 100-seed weights (g) were maximum in ICPA 2048 x ICPL 20093 and it was bold seeded. Among 22 genotypes, 16 hybrids showed better performance for seed yield plot<sup>-1</sup> and seed yield (kg/ha) over both checks (Asha and Maruti).

The extent of pollen fertility among the hybrids ranged between 59.22 to 99.76 %. High values of pollen fertility indicates the higher fertility restoration and the vice versa. Similarly, Singh and Bajpai (2005), Saxena (2005) and Nadrajan *et al.* (2008) reported many hybrids with variable fertility restoration. All the hybrids except ICPA 2048 x ICPL 20096 and ICPA 2047 x ICPL 20129 exhibited high (>80 %) pollen fertility. Similarly, Wanjari *et al.* (2007) also reported high (>80 %) pollen fertility in different hybrid combinations.

#### ➤ **Study on heterosis**

Heterosis breeding aims to exploit the phenomenon of hybrid vigour to increase yield potential of the crop. Presence of stable male sterility and effective fertility restorer systems are prerequisite to produce large quantity of hybrid seeds. The expression of heterosis also depends on the diversity among the parental lines used for the development of the hybrids (Arunachalam, 1981). The present study was aimed to study fertility restoration and standard heterosis in 20 hybrids.

Early flowering and maturity is one of the desirable traits in hybrid pigeonpea as it helps in escaping drought. In the present study 18 hybrids showed early 50% flowering over check Asha but the maturity was delayed due to flower drop on account of rainfall. The significant negative heterosis for earliness was earlier reported by Chaudhari (1979), Singh *et al.* (1989) and Pandey and Singh

(2002) for flowering and maturity. Wankhade *et al.* (2005) reported significant negative heterosis in the hybrids based on genetic male-sterility system for days to 50% flower. In present investigation two hybrids, ICPA 2078 x ICPL 87119 and ICPA 2043 x ICPL 87119 showed significant negative standard heterosis over check Asha for days to 1<sup>st</sup> flowering. Sarode *et al.* (2009) showed significant negative heterosis for this trait in long duration pigeonpea. Significant negative heterosis in CMS based hybrids was reported by Kandalkar (2007), Shoba and Balan (2010) and Sameer kumar *et al.* (2012).

Similarly in the present study for plant height, 19 hybrids showed positive standard heterosis over both checks and only one hybrid showed negative standard heterosis over both checks. Among the twenty hybrids, ten hybrids ICPA 2048 x ICPL 20108, ICPA 2048 x ICPL 20098, ICPA 2048 x ICPL 20111, ICPA 2092 x ICPL 20123, ICPA 2048 x ICPL 20096, ICPA 2047 x ICPL 20108, ICPA 2048 x ICPL 20106, ICPA 2092 x ICPL 20106, ICPA 2092 x ICPL 20108 and ICPA 2047 x ICPL 20129 were showed significant standard heterosis over both the checks (Maruti and Asha) while one hybrid ICPA 2048 x ICPL 20093 showed negative heterosis for plant height. Solomon *et al.* (1957), Singh (1971), Sharma *et al.* (1973), Veeraswamy *et al.* (1973), Chaudhari (1979) and Jain and Saxena (1990) also reported similar results. However, negative standard heterosis for plant height was reported by Pandey and Singh (2002) in pigeonpea which is desirable in the context of breeding dwarf genotypes.

The present study showed both positive and negative standard heterosis for number of primary branches plant<sup>-1</sup> and also for number of secondary branches plant<sup>-1</sup>. Range of standard heterosis over Maruti varies from -26.81 (ICPA 2047 x ICPL 87119) to 76.09% (ICPA 2048 x ICPL 87119) for number of primary branches plant<sup>-1</sup>. Similar results were reported by Shoba and Balan (2010) in CMS/GMS based pigeonpea hybrids. In present study highest standard heterosis for number of secondary branches plant<sup>-1</sup> was shown by ICPA 2078 x ICPL 87119 (41.62%). Shrivastava *et al.* (1976) reported that 96% heterosis for secondary branches per plant.

The results of the present investigation showed that heterosis for grain yield was due to more number of pods plant<sup>-1</sup>, which is in agreement with reports of Singh (1971), Veeraswamy *et al.* (1973), Shrivastava *et al.* (1976) Chaudhari (1979),

Patel and Patel (1992), Pandey and Singh (2002), Kandalkar (2007) and Phad *et al.* (2009b). The present study also reported standard heterosis for seeds pod<sup>-1</sup>. All the hybrids showed positive standard heterosis for seeds pod<sup>-1</sup> over both the checks (Asha and Maruti). Similarly, significant positive heterosis for seeds pod<sup>-1</sup> was reported by Wankhade *et al.* (2005).

The results of the present investigation showed that standard heterosis for grain yield was due to more 100-seed weight and most of the hybrids showed positive standard heterosis for this trait. In pigeonpea positive standard heterosis for 100-seed weight was reported by Chaudhari (1979), Reddy *et al.* (1979), Manivel *et al.* (1999), Deshmukh *et al.* (2001), Wankhade *et al.* (2005) and Kandalkar (2007).

Yadav and Singh (2004), Sekhar *et al.* (2004), Wankhade *et al.* (2005) and Phad *et al.* (2009b) recorded positive standard heterosis for seed yield plant<sup>-1</sup> in pigeonpea. Similar reported in present investigation. The positive heterosis could be useful for further exploitation of hybrid breeding in pigeonpea (Wanjari *et al.*, 2007).

The positive and high magnitude of heterosis for grain yield plant<sup>-1</sup> may be attributed to one or more yield contributing characters (Chandirakala *et al.*, 2010). Similar results were also found in the present study and yield is contributed by all the characters. The range of standard heterosis, for seed yield was 15.54 to 47.55% over Maruti and -15.09 to 28.54% against Asha. All 20 hybrids showed negative standard heterosis for pollen fertility% against both checks. Heterosis for seed yield in hybrid pigeonpea were depends upon all yield contributing characters including pollen fertility%. So for fully exploitation of heterosis, hybrid with good pollen fertility is needed. Similar result reported by Wanjari *et al.* (2007)

➤ **Identification of fertility restorers and maintainers in hybrids**

Based on the fertility/sterility % in the F<sub>1</sub> progenies, the male parent of such cross may be classified as restorer/maintainer. In present investigation 20 hybrids were synthesised by using 11 male lines. All male lines were acts as restorer, but two male lines (ICPL 20096 and ICPL 20129) showed lower restoration effect. ICPL 87119 showed more than 97% (97.44 to 98.05%) pollen fertility restoration effect in four CMS lines (ICPA 2078, ICPA 2092, ICPA 2047, and ICPA 2043) and classified as good restorer for these CMS lines. In present study, out of 11 male lines, six lines were

showed more than 90% fertility restoration in different CMS lines. Three male lines (ICPL 20106, ICPL 20093 and ICPL 20186) showed partial fertility restoration (89.14 to 86.28%) and such pollinators need to improve their genetic purity for fertility restoration ability by selfing and single-plant selections. Two male lines, ICPL 20096(59.22%) and ICPL 20129(74.46%) had lower level of fertility restoration can be selected to develop maintainer by backcrossing to diversify the genetic base of the CMS system. Chauhan *et al.* (2004), Tikka *et al.* (1997), Wanjari *et al.* (2007), Kalaimagal *et al.* (2008), and Dalvi *et al.* (2008a) reported similar result. Similarly, Saxena and Kumar (2003) studied the fertility restoration system in A<sub>2</sub> cytoplasm in pigeonpea. They developed the crosses between 3 CMS lines with 14 diverse pigeonpea lines. Among these, five crosses had 94 to 100% fertility restoration and these parents need to be preserved to use directly in breeding high yielding restorer lines. To produce heterotic hybrids for diverse environments it is important to have ample genetic diversity in both A- and R-lines. Therefore, we need to start a hybrid breeding program for search of new fertility restorers and male-sterility maintainers is very impotent.

➤ **Future Line of Work:**

The following attempts may be taken up are given to widen the scope of present investigations.

1. Hybrids could be exploited for the development of stable and about 30-35% more yield compare to varieties used as check Maruti and Asha.
2. The stability of male-sterile lines must be tested in controlled environments to know the factors affecting the stability of CMS lines and making it sensitive to environment.
3. The efforts made to know the stability of CMS lines may help in identifying the stable CMS lines. This study may be extended with more number of male-sterile lines with different cytoplasmic sources for genetic diversification.
4. The results of effectiveness of male sterility of CMS lines will help in further studies on combining ability with diverse restorer lines.
5. The hybrids showing stability for fertility restoration need to be tested for yield across more diverse environments.
6. Locally adopted genotypes may be studied to identify suitable maintainer as well as restorer lines.

# CHAPTER VI

## *SUMMARY AND CONCLUSIONS*



## SUMMARY AND CONCLUSIONS

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The present investigation entitled, “Identification of fertility restorers and maintainers in Pigeonpea [*Cajanus cajan* (L.) Millsp.]” was aimed to find out the effectiveness of CMS lines with identification of its restorers/maintainers. The study was also aimed to evaluate the extent of standard heterosis in cytoplasmic-nuclear male-sterility (CMS) based pigeonpea hybrids. For this study, 20 pigeonpea hybrids were developed by crossing CMS lines with diverse inbreds. All the 20 hybrids along with standard varieties as check were planted in a randomized complete block design with two replications at ICRISAT, Patancheru in *kharif* 2013-14. Each F<sub>1</sub> and standard checks (Asha and Maruti) were grown in plot size of 10.2 m<sup>2</sup> (net plot size). The inter- and intra-row spacing was kept at 75 cm and 30 cm, respectively. Observations were recorded on five competitive plants in each plot of F<sub>1</sub> and standard checks. The observations were recorded on yield and yield contributing characters that included days to 1<sup>st</sup> flowering, days to 50% flowering, days to maturity, plant height (cm), number of primary branches plant<sup>-1</sup>, number of secondary branches plant<sup>-1</sup>, number of pods plant<sup>-1</sup>, seeds pod<sup>-1</sup>, 100 seeds weight (g), seed yield plant<sup>-1</sup> (g), seed yield plot<sup>-1</sup>, seed yield (kg ha<sup>-1</sup>) and pollen fertility/sterility %. The salient results of the study and conclusion drawn from the experiment are summarized below:

- Out of five CMS lines, ICPA2092 and ICPA 2043 showed more than 99 % pollen sterility. Meanwhile, all the CMS lines expressed high (>95%) pollen sterility.
- The hybrids ICPA 2047 x ICPL 20108 recorded highest pollen fertility (98.50 %).
- ICPA 2047 x ICPL 20126 had maximum yield (2635.36 kg ha<sup>-1</sup>) indicating this hybrid was best among all hybrids.
- Eighteen out of 20 hybrids recorded high (>80 %) pollen fertility showed their better fertility restoration.
- Out of 11 male lines, nine were restore fertility more than 80% and classified as restorer for corresponding CMS lines.

- Two male lines, ICPL 20096 and ICPL 20129 showed lower level of fertility restoration effect for corresponding CMS lines.
- The desirable traits in pigeonpea to escape drought and moisture stress conditions are earliness in flowering and maturity. Therefore, negative heterosis for these traits is desirable. The hybrid ICPL 2043 × ICPL 87119 ranked first for higher negative standard heterosis over both checks, indicating exploitable hybrid vigour for early flowering. However, it exhibited positive standard heterosis for maturity against check Maruti but negative over Asha.
- Positive heterosis for grain yield was noticed which may be attributed by one or more yield components. Out of 20 hybrids, 16 were showed positive standard heterosis against best check Asha.

#### ❖ CONCLUSION

The results obtained from present investigations may be concluded as CMS lines used for synthesis of 20 hybrids showed high level of male sterility and highly effective. Out of 11, nine male pollinator genotypes performed good fertility restoration while two male lines showed lower level of restoration. These 9 male lines may be used as fertility restorer. Significant variability for pollen fertility was present among the hybrids. Yield point of view, most of the hybrids showed positive standard heterosis for yield and it was up to 59.93%. So over all most of the hybrids and its component showed good impact in terms of production of hybrid seeds and yield potential of pigeonpea hybrid.

Pigeonpea is known to have tremendous genetic variability but breeders have not been able to exploit it for the genetic enhancement of yield. In the last 50 years more than 100 pure line pigeonpea varieties have been released in India alone (Singh *et al.* 2005), but the national productivity has remained stagnant around at low levels. A number of genetic studies conducted on this subject in pigeonpea revealed that a considerable extent of non-additive genetic variance is also present in the crop which was never utilized in breeding high yielding cultivars (Saxena and Sharma, 1990) and invariably additive genetic variances were utilized for the enhancement of yield and other related traits. Successful breeding of a CMS system

has given a golden opportunity to breeders to utilize non-additive genetic variation for increasing pigeonpea productivity.

Even after centuries of cultivation and natural selection pigeonpea continues to remain a wild plant with its unique characteristics such as perennial and indeterminate growth habit, low harvest index, and photo - thermal sensitivity. Its ability to survive and produce food even under high stress conditions has helped subsistence farmers and even today pigeonpea is considered as the most ideal crop of small-holding rainfed farmers. In recent years, pigeonpea production has undergone significant growth and it is primarily attributed to the development of new disease resistant varieties. Since the demand for pigeonpea is ever increasing and the scope for area expansion is limited, the attention is now focusing on increasing its yield potential. The pure line variety breeding programme that was implemented decades ago did not produce desired results and the crop yields remained consistently low. The development of CMS system and natural cross pollination has opened a new option to breeding hybrids in pigeonpea for enhanced yield potential. So hybrid technology has potential to enhance the yield of pigeonpea.

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# APPENDICES



## APPENDIX I

Monthly weather data during the crop season recorded at ICRISAT, Patancheru, 2012-13

Latitude: 17°53'N, Longitude: 78°27' E, Altitude: 545 m

Year	Month	Rain	Max Temp (in °C)	Min Temp (in °C)	Rel Humidity1 at 07:17(in%)	Rel Humidity2 at 14:17 (in%)
2012	June	280.79	34.79	24.17	72	46
2012	July	199.19	30.15	22.04	89	66
2012	August	94.70	29.69	21.82	90	68
2012	September	58.39	29.77	21.67	93	66
2012	October	73.79	30.40	17.99	93	51
2012	November	38.20	28.72	15.79	95	51
2012	December	0.00	29.85	13.67	92	39
2013	January	1.00	30.60	15.38	92	39
2013	February	10.09	31.06	16.34	86	34

Where mm-millimeter

Source: Meteorological department, ICRISAT, Patancheru, (Andhra Pradesh)

## APPENDIX II

Daily weather data during the crop season recorded at ICRISAT, Patancheru, 2013-14

Latitude: 17°53'N, Longitude: 78°27' E, Altitude: 545 m

Date (mm/dd/yyyy)	Rain (in mm)	MaxTemp (in °C)	Min Temp (in °C)	Rel Humidity1 at 07:17(in %)	Rel Humidity2 at 14:17 (in %)
6/1/2013	0.0	38.0	23.6	78	31
6/2/2013	0.0	34.2	23.5	72	54
6/3/2013	1.4	37.4	22.9	78	43
6/4/2013	4.8	37.5	21.6	92	42
6/5/2013	0.0	34.2	24.0	80	47
6/6/2013	1.4	35.7	21.0	91	44
6/7/2013	0.0	33.7	24.3	73	45
6/8/2013	0.0	34.8	24.2	77	44
6/9/2013	0.8	35.0	23.4	77	43
6/10/2013	0.0	34.2	23.0	76	48
6/11/2013	28.8	33.6	21.4	87	45
6/12/2013	3.2	32.5	22.3	85	53
6/13/2013	5.4	29.8	21.4	84	68
6/14/2013	1.8	27.5	21.2	88	71
6/15/2013	1.2	25.4	21.0	88	89
6/16/2013	0.0	29.7	22.8	84	61
6/17/2013	0.0	32.8	22.5	79	50
6/18/2013	0.0	32.3	22.4	81	51
6/19/2013	0.0	33.6	22.2	82	52
6/20/2013	0.0	34.6	23.2	76	49
6/21/2013	2.2	34.8	22.6	73	42
6/22/2013	0.0	34.2	23.9	78	45
6/23/2013	15.2	33.2	21.7	87	56
6/24/2013	7.0	31.0	21.6	87	59
6/25/2013	19.8	26.3	20.0	95	84
6/26/2013	0.0	27.2	21.7	90	70
6/27/2013	9.2	30.8	21.6	90	87
6/28/2013	0.0	33.2	22.0	82	49
6/29/2013	0.0	33.2	22.9	76	44
6/30/2013	0.2	33.0	22.2	84	53
7/1/2013	0.0	31.8	23.0	82	52
7/2/2013	0.0	32.4	22.2	80	53
7/3/2013	8.2	33.0	20.5	93	53
7/4/2013	1.8	29.4	22.0	85	76
7/5/2013	0.0	30.8	21.0	88	63
7/6/2013	2.0	32.0	22.0	84	50
7/7/2013	0.0	31.5	22.0	88	56

Appendix

7/8/2013	0.0	30.2	22.2	87	62
7/9/2013	0.2	30.0	21.9	87	75
7/10/2013	1.2	31.0	21.5	88	68
7/11/2013	11.4	27.3	20.7	95	74
7/12/2013	78.7	23.8	20.0	98	88
7/13/2013	5.2	24.2	21.2	91	90
7/14/2013	0.0	29.2	21.6	88	68
7/15/2013	0.0	30.0	22.0	87	64
7/16/2013	6.4	30.8	20.5	90	56
7/17/2013	22.8	24.5	20.0	93	88
7/18/2013	2.8	25.0	21.5	88	88
7/19/2013	7.2	25.7	20.5	95	82
7/20/2013	5.0	23.6	22.3	90	91
7/21/2013	0.0	27.7	21.6	90	74
7/22/2013	8.2	28.8	20.9	93	67
7/23/2013	52.5	24.5	20.6	95	92
7/24/2013	0.5	25.0	21.4	85	81
7/25/2013	0.0	28.4	21.7	87	72
7/26/2013	0.0	28.6	21.6	85	71
7/27/2013	4.0	29.3	21.5	90	67
7/28/2013	0.0	30.0	21.9	91	68
7/29/2013	0.0	29.2	21.4	85	65
7/30/2013	0.0	29.5	21.2	85	61
7/31/2013	8.6	29.1	21.0	95	62
8/1/2013	13.0	22.8	20.5	93	93
8/2/2013	0.0	25.4	21.0	91	82
8/3/2013	0.0	28.7	21.0	85	70
8/4/2013	0.0	30.5	20.5	93	59
8/5/2013	7.0	29.8	20.5	95	63
8/6/2013	30.8	24.2	20.5	96	90
8/7/2013	1.4	26.4	21.5	91	80
8/8/2013	0.0	29.4	22.0	92	66
8/9/2013	0.0	28.2	21.9	90	76
8/10/2013	0.0	30.0	20.4	88	64
8/11/2013	0.2	29.9	21.4	88	63
8/12/2013	20.2	29.8	21.2	95	63
8/13/2013	8.2	27.8	21.2	97	75
8/14/2013	0.0	28.2	21.9	90	75
8/15/2013	23.8	28.8	21.8	97	72
8/16/2013	23.6	26.3	21.6	98	89
8/17/2013	13.8	27.0	21.0	96	80
8/18/2013	6.0	27.6	21.3	91	79
8/19/2013	12.4	29.3	21.2	95	71
8/20/2013	0.0	28.8	20.3	90	68

Appendix

8/21/2013	0.8	27.3	20.0	88	69
8/22/2013	0.0	29.5	20.6	86	56
8/23/2013	0.0	27.8	21.5	85	69
8/24/2013	0.0	27.8	19.8	86	68
8/25/2013	1.8	30.0	21.6	91	60
8/26/2013	1.0	29.0	20.8	91	66
8/27/2013	0.0	27.6	21.6	85	66
8/28/2013	0.0	28.5	22.0	91	67
8/29/2013	0.2	28.4	21.2	95	80
8/30/2013	0.6	29.8	21.2	95	76
8/31/2013	0.0	30.5	22.2	95	65
9/1/2013	0.0	29.4	21.0	95	73
9/2/2013	0.0	30.7	21.2	93	65
9/3/2013	0.0	29.8	21.2	95	70
9/4/2013	0.0	30.2	21.0	90	69
9/5/2013	0.0	29.2	21.0	91	69
9/6/2013	0.0	30.4	20.6	91	61
9/7/2013	0.0	30.5	21.5	91	64
9/8/2013	12.0	31.2	22.0	97	56
9/9/2013	39.1	29.4	19.8	98	53
9/10/2013	4.5	26.8	22.0	98	93
9/11/2013	1.8	29.3	21.8	97	83
9/12/2013	0.0	30.5	21.6	92	64
9/13/2013	0.0	30.8	21.6	97	60
9/14/2013	0.0	31.6	22.2	95	63
9/15/2013	0.0	30.8	21.8	91	77
9/16/2013	42.6	31.5	21.4	98	65
9/17/2013	5.0	30.2	22.2	93	80
9/18/2013	34.6	30.0	20.6	97	66
9/19/2013	58.0	27.8	21.5	98	83
9/20/2013	51.0	29.4	21.0	97	77
9/21/2013	29.0	29.0	20.2	91	78
9/22/2013	0.0	29.5	20.5	91	65
9/23/2013	0.0	29.8	20.8	91	62
9/24/2013	0.0	30.5	19.8	91	63
9/25/2013	0.0	29.8	20.8	91	64
9/26/2013	0.0	30.4	20.8	91	57
9/27/2013	0.0	31.4	21.6	91	58
9/28/2013	0.0	31.5	21.2	90	59
9/29/2013	0.0	31.4	21.0	86	58
9/30/2013	0.0	30.8	21.2	88	55
10/1/2013	2.0	29.0	21.2	95	68
10/2/2013	0.0	30.7	22.2	97	63
10/3/2013	22.4	28.8	21.2	95	74

Appendix

10/4/2013	0.0	30.0	21.5	90	72
10/5/2013	0.0	29.4	19.4	90	62
10/6/2013	0.0	29.4	22.4	88	67
10/7/2013	0.0	30.0	22.2	97	72
10/8/2013	0.0	31.2	22.2	92	61
10/9/2013	14.0	31.4	21.2	95	56
10/10/2013	47.0	30.0	20.0	95	72
10/11/2013	0.0	30.0	20.5	93	65
10/12/2013	8.4	28.5	21.8	95	87
10/13/2013	0.0	28.2	20.5	95	71
10/14/2013	0.0	30.2	20.8	98	72
10/15/2013	0.0	31.2	21.4	95	60
10/16/2013	0.0	31.2	20.4	88	57
10/17/2013	0.0	31.4	20.4	95	51
10/18/2013	5.8	31.8	21.4	88	54
10/19/2013	0.0	31.2	17.4	93	43
10/20/2013	0.0	31.2	17.6	92	41
10/21/2013	0.0	30.5	20.6	91	51
10/22/2013	3.8	30.2	21.4	95	54
10/23/2013	5.4	28.2	21.4	97	77
10/24/2013	38.4	26.6	20.6	98	80
10/25/2013	16.4	24.6	21.0	98	90
10/26/2013	43.4	23.3	21.2	98	95
10/27/2013	0.2	26.3	21.4	96	90
10/28/2013	0.0	28.4	18.8	96	74
10/29/2013	0.0	29.7	18.6	96	58
10/30/2013	0.0	30.2	16.0	90	50
10/31/2013	0.0	29.2	16.0	98	47
11/1/2013	0.0	29.6	19.6	93	50
11/2/2013	0.0	29.5	20.6	93	59
11/3/2013	0.0	30.4	19.6	93	60
11/4/2013	0.0	30.5	18.8	96	57
11/5/2013	0.0	29.6	16.8	93	54
11/6/2013	0.0	28.4	15.0	94	48
11/7/2013	0.0	28.0	15.2	86	45
11/8/2013	0.0	28.8	15.6	96	47
11/9/2013	0.0	28.2	15.2	94	54
11/10/2013	0.0	28.6	15.2	92	57
11/11/2013	0.0	28.2	13.2	94	46
11/12/2013	0.0	28.4	11.4	95	39
11/13/2013	0.0	28.6	12.5	91	32
11/14/2013	0.0	27.3	11.5	91	34
11/15/2013	0.0	27.2	10.7	95	34
11/16/2013	0.0	27.2	10.2	93	34

Appendix

11/17/2013	0.0	28.2	18.4	78	42
11/18/2013	0.0	28.8	16.5	89	49
11/19/2013	0.0	28.5	15.5	94	51
11/20/2013	0.0	29.3	11.5	91	33
11/21/2013	0.0	29.5	11.0	95	41
11/22/2013	0.0	29.6	14.8	94	38
11/23/2013	0.0	28.8	20.8	88	57
11/24/2013	18.7	23.2	21.0	91	97
11/25/2013	0.0	27.3	18.7	98	71
11/26/2013	0.0	31.0	18.5	94	56
11/27/2013	0.0	30.2	13.4	91	46
11/28/2013	0.0	29.4	11.6	93	31
11/29/2013	2.0	27.0	17.5	91	43
11/30/2013	0.0	23.4	16.5	92	85
12/1/2013	0.0	28.4	16.5	98	59
12/2/2013	0.0	29.7	18.4	98	53
12/3/2013	0.0	28.5	19.8	95	64
12/4/2013	0.0	27.8	16.4	96	64
12/5/2013	0.0	28.5	13.5	94	41
12/6/2013	0.0	27.7	11.6	91	41
12/7/2013	0.0	26.6	8.8	97	36
12/8/2013	0.0	26.6	8.4	97	35
12/9/2013	0.0	27.7	6.8	97	36
12/10/2013	0.0	28.4	7.0	95	22
12/11/2013	0.0	28.8	7.5	95	22
12/12/2013	0.0	29.3	9.8	90	31
12/13/2013	0.0	28.4	8.9	93	34
12/14/2013	0.0	28.8	8.8	97	39
12/15/2013	0.0	27.8	8.2	95	32
12/16/2013	0.0	28.8	8.0	95	30
12/17/2013	0.0	28.4	8.2	92	29
12/18/2013	0.0	28.2	8.4	97	29
12/19/2013	0.0	27.3	8.6	97	38
12/20/2013	0.0	29.4	9.0	98	35
12/21/2013	0.0	29.2	13.0	91	32
12/22/2013	0.0	27.6	15.8	77	44
12/23/2013	0.0	26.6	12.8	96	45
12/24/2013	0.0	25.8	12.0	98	49
12/25/2013	0.0	25.8	11.8	91	51
12/26/2013	0.0	27.2	11.0	93	40
12/27/2013	0.0	26.4	10.0	95	46
12/28/2013	0.0	27.0	11.0	98	46
12/29/2013	0.0	27.6	14.2	96	44
12/30/2013	0.0	26.6	14.0	90	50

*Appendix*

12/31/2013	0.0	26.0	15.4	88	45
1/1/2014	0.0	27.0	16.4	90	49
1/2/2014	0.0	26.0	11.4	98	49
1/3/2014	0.0	27.2	11.0	98	45
1/4/2014	0.0	28.2	10.8	95	39
1/5/2014	0.0	28.2	13.8	98	43
1/6/2014	0.0	30.4	14.4	96	38
1/7/2014	0.0	29.2	16.0	96	48
1/8/2014	0.0	28.3	13.2	98	41
1/9/2014	0.0	28.4	13.0	98	40
1/10/2014	0.0	29.2	15.8	92	43
1/11/2014	0.0	29.4	17.2	89	37
1/12/2014	0.0	28.8	17.0	85	50
1/13/2014	0.0	30.5	14.0	92	38
1/14/2014	0.0	28.2	14.2	88	38
1/15/2014	0.0	28.2	14.0	89	43
1/16/2014	0.0	29.7	14.4	94	38
1/17/2014	0.0	29.5	15.8	87	37
1/18/2014	0.0	28.7	16.0	92	43
1/19/2014	0.0	28.0	18.4	87	49
1/20/2014	0.0	28.8	14.0	96	43
1/21/2014	0.0	29.0	15.0	92	45
1/22/2014	0.0	28.0	16.8	92	41
1/23/2014	0.0	27.2	17.6	85	51
1/24/2014	0.0	27.5	15.6	94	52
1/25/2014	0.0	28.2	15.0	90	45
1/26/2014	0.0	28.6	17.0	81	45
1/27/2014	0.0	28.4	14.6	94	45
1/28/2014	0.0	26.9	12.6	87	49
1/29/2014	0.0	28.4	16.8	76	39
1/30/2014	0.0	27.8	15.0	87	45
1/31/2014	0.0	28.0	12.5	91	40

Where mm-millimeter

Source: Meteorological department, ICRISAT, Patancheru, (Andhra Pradesh)

# BIHAR AGRICULTURAL UNIVERSITY, SABOUR

## Department of Plant Breeding & Genetics

Title of the Thesis	:	<b>Identification of fertility restorers and maintainers in pigeonpea [<i>Cajanus cajan</i> (L.) Millspaugh].</b>
Name of the student	:	<b>Sudhir Kumar</b>
Registration Number	:	<b>M/PBG/87/BAC/2012-13</b>
Chairman Advisory Committee	:	<b>Dr. P.K. Singh</b> Univ. Prof.-cum-Chief Scientist Bihar Agricultural University, Sabour
Degree Programme	:	<b>M. Sc.(Ag.)</b>
Major subject	:	<b>Plant Breeding and Genetics</b>
Minor subject	:	<b>Plant Pathology</b>
Year	:	<b>2014</b>
Total Page of Thesis	:	<b>74 + xii (Bibliography)</b>

## ABSTRACT

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An investigation was carried out at ICRISAT, Patancheru, Hyderabad, during *kharif* season of 2013-14 to study identification of the fertility restorers (R-line) and maintainers (B-line) in pigeonpea [*Cajanus cajan* (L.) Millsp.] genotypes and standard heterosis in pigeonpea hybrids. A total of 20 hybrids were generated by hand pollinating five CMS-lines with 11 male lines from diverse sources during 2012-13 *kharif* season. In *kharif* 2012-13 also study was done for effectiveness of these five CMS lines. The study confirmed the existence of high level of male sterility (99.60 to 95.69%) in all five CMS lines used in crossing programme. The studies on fertility restoring capacity of 11 male lines on these five CMS lines were ranged from 59.22 to 98.50% and ICPL 87119 was one of the best restorer. Among 22 genotypes, ICPA 2043 x ICPL 87119 required minimum number of days to 50% flowering and it was significantly earlier over mean performance and check Asha. Pigeonpea is a considered as quantitative short-day flowering plant *i.e.*, onset of flowering is hastened as the day-length reduces. Moreover, both low and high temperature may also delay flowering in pigeonpea. Out of 22 genotypes, check Maruti was the earliest to mature, while ICPA 2048 x ICPL 20093 exhibited maximum number of days to mature. All the hybrids were taller than both the checks (Asha and Maruti) except ICPA 2078 x ICPL

87119. Most of the hybrids were showed high number of primary and secondary branches per plant with respect to check Asha. Among the hybrids, ICPA 2092 x ICPL 20108 had the highest number of pods plant<sup>-1</sup>. Including 22 genotypes, all hybrids showed higher mean performance for number of sees pod<sup>-1</sup> over checks Maruti and Asha. Most of the hybrids performed good for 100 seed weight and Seed yield plant<sup>-1</sup>. In this study, positive heterosis for grain yield plant<sup>-1</sup> was noticed which may be attributed to one or more yield components. For grain yield plant<sup>-1</sup>, 18 hybrids showed significant positive standard heterosis over Maruti and eight hybrids showed significant positive standard heterosis over Asha.

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## Biography

**Mr. SUDHIR KUMAR** S/O Shri ABHRAN YADAV was born on 5<sup>st</sup>, January 1986 at Village - Belhi, Post: Parva, P.S.- Jaynagar and Distt. -Madhubani, (Bihar). He accomplished his primary and middle school education in native place & H.S.C. (10<sup>th</sup>) from High School Jaynagar and H.S.S.C. (12<sup>th</sup>) education from C.M. Science Collage, Darbhanga (Bihar).

He qualified ICAR exam and then he joined **Bidhan Chandra Krishi Viswavidyalaya**, Nadia (West Bengal) in 2008 and passed B.Sc. (Agri.) examination in 2012 with first division (7.77 OGPA). Then, he cracked the BAU,Sabour PG exam and joined Bihar Agricultural University, Sabour in 2012 for the Post-Graduation Programme in Agriculture, Plant Breeding and Genetics.

He completed the thesis research work from **ICRISAT** as **Research Scholar** in 2012-14 and finally he completed his post graduate degree programme from **Bihar Agricultural University**, Sabour(Bihar) with first division in 2014 (8.22 OGPA).