"Stability of maintenance of male sterility and fertility restoration in pigeonpea [*Cajanus cajan* (L.) Millsp.] under different environments"

THESIS

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MASTER OF SCIENCE
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By

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CERTIFICATE – I

This is to certify that the thesis entitled **"Stability of maintenance of male sterility and fertility restoration in pigeonpea [*Cajanus cajan* (L.) Millsp] under different environments"** submitted in partial fulfillment of the requirement for the degree of **MASTER OF SCIENCE** in **AGRICULTURE (**Genetics & Plant Breeding**)** of Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior is a record of the bonafide research work carried out by **Mr. Sunil Chaudhari,** ID. No. **RA/SH/278/2007** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any degree or diploma has been published. All the assistance and help received during the course of the investigation has been acknowledged by the scholar.

Place: Sehore   **(Dr. A. N. Tikle)**

Date :   Chairman of the Advisory Committee

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This is to certify that the thesis entitled **"Stability of maintenance of male sterility and fertility restoration in pigeonpea [*Cajanus cajan* (L.) Millsp] under different environments"** submitted by **Mr. Sunil Chaudhari, ID. No. RA/SH/278/2007** to the Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior in partial fulfillment of the requirements for the degree of Master of Science in Agriculture (Genetics & Plant Breeding) has been accepted after evaluation by the external examiner and the Student's Advisory Committee after an oral examination on the same.

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CERTIFICATE - III

This is to certify that **Mr. Sunil Choudhary** has satisfactorily completed the research work related to this thesis entitled **"Stability of maintenance of male sterility and fertility restoration in pigeonpea [*Cajanus cajan* (L.) Millsp] under different environments".** His thesis contains results of original research work and it is of high standards to warrant its presentation to the examination. I also certify that neither the thesis nor its part, thereof, has been previously submitted by him for a degree at any other university.

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Acknowledgements

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Date   : June   2013   **(Sunil Chaudhari)**

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbol	Legend
<	Less than
>	Greater than
/	Per
&	And
@	At the rate of
^{o}C	Degree Celsius
C.D.	Critical Difference
cm	Centimeter
C.V.	Coefficient of Variation
D.F.	Degree of Freedom
et al.	Et alia (and others)
etc	Etcetera
Fig.	Figure (s)
g	Gram
ha	Hectare
i.e.	That is
K	Potassium
kg	Kilogram (s)
m	Meter (s)
N	Nitrogen
No.	Number (s)
NS	Non significant
P	Phosphorus
T	tones
R.H.	Relative humidity
S.Em.±	Standard error of mean
M.S.S.	Mean sum of square
Viz.	Videlicet (namely)
√	Square root
%	Percent
±	Plus or Minus

Dedicated
to
my
beloved parents
and
grand parents

INTRODUCTION

Chapter I
INTRODUCTION

Pigeonpea (*Cajanus cajan* (L). Millsp.) is an important grain legume crop of rainfed agriculture in the tropical and sub-tropical areas. Pigeonpea belongs to genus *Cajanus* of sub tribe *Cajaninae*, tribe Phaseoleae of the sub-family Papilionoideae under family Leguminosae and it is the only cultivated food crop of the *Cajaninae* sub tribe. The genus *Cajanus* comprises 32 species, most of which are found in India, Australia and one species is native to west Africa (van der Maesen, 1986). Pigeonpea is cultivated, either as the sole crop or inter crop with soybean, finger millet, sorghum, pearl millet, maize or with short duration legumes.

Botanically, pigeonpea is a perennial plant but cultivated as an annual crop. It is grown for its high protein seeds, which is consumed as a popular staple diet in predominantly vegetarian diet. It is mainly consumed in the form of *dal*. It plays an important role in food security, provide balanced diet and for alleviation of poverty because of its diverse usage as a food, fodder and fuel. The dry stalks are used for fuel and as wind breaks. The dried leaves, pod shells and seed coat are used as animal feed.
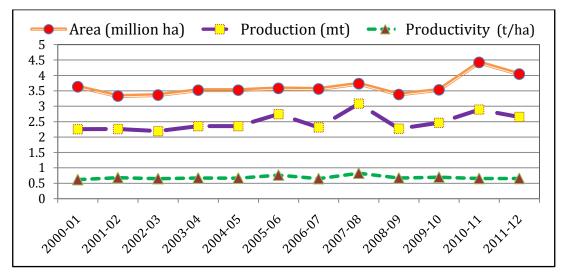
Pigeonpea is known with various names in different cultures such as red gram, arhar, tur, Angola pea, Congo pea, (van der Maesen 1986), tuvara (Achaya, 1998) etc. According to De Candolle (1904) and Krauss (1932), pigeonpea originated in Africa, from where it was introduced into West Indies, Brazil and India (Tothil, 1948). De (1974) believes that *Cajanus* species, together with the closely related *Atylosia* were first established in Northern India and on Deccan plateau 3,500 years ago. However, Vavilov (1926) concluded that large amount of variability with several wild relatives was available in Hindustan Centre and hence historians agree that the true origin of pigeonpea is India particularly in the Eastern Ghats (Vavilov, 1951; De, 1974; Royes 1976; van der Maesen, 1980, 1991).

It is cultivated worldwide on 5.83 million hectares (m ha) with an annual production of 4.40 million tons (mt) and average productivity of 754.9 kg ha^{-1} (Table 1.1). In India, pigeonpea is cultivated in 4.42 m ha with production of 2.86

mt and productivity of 647 kg ha^{-1} (FAOStat, 2011). The scenario of pigeonpea in India over a last decade for the area, production and productivity is presented in Fig. 1.1. In Madhya Pradesh, pigeonpea is cultivated on 0.53 m ha (13.24 %) with annual production of 0.33 mt (12.60 %) and productivity of 625 kg ha^{-1} in 2011-12 (IIPR, Kanpur, 2013).

Table 1.1 Global pigeonpea scenario (FAOStat 2011)

Region / country	Area (million ha)	Production (million tons)	Yield (kg ha^{-1})
World	5.836	4.405	754
Asia	5.072	3.713	732
India	4.420	2.860	647
Nepal	0.017	0.014	807
Myanmar	0.633	0.837	1322
Bangladesh	0.001	0.001	923
Philippines	0.001	0.001	1014
Central America	0.004	0.002	455
South America	0.002	0.001	787
Africa	0.724	0.658	907
Uganda	0.093	0.094	1024
Tanzania	0.288	0.272	946
Kenya	0.138	0.084	607
Malawi	0.190	0.195	1026

Fig.- 1.1 Area, production and productivity trend of pigeonpea in India from 2000 to 2012 (Source- AICRP on pigeonpea, IIPR Kanpur, 2013)

The phenomenon of male-sterility was recorded as early as by Kolreuter (1763) where the plants are unable to reproduce through natural means because of 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. Male-sterility has been successfully used for enhancing yield in a number of cereal and vegetable crops. In food legumes, this technology could never been used either due to non-availability of natural out-crossing system, or an efficient male-sterility system or both.

Pigeonpea is unique among legumes as its floral morphology allows both self as well as insect-aided cross pollination and it varies from place to place. However, most breeders in the past ignored this fact and handled pigeonpea as a self-pollinated crop as far as its breeding methodology was concerned. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), located at Patancheru (India), focused pigeonpea research on hybrid breeding through development of stable cytoplasmic male-sterility (CMS) system (Saxena *et al.,* 2005) and partial (25–70%) natural out-crossing ability (Saxena *et al.,* 1990).Pigeonpea is a partially cross-pollinated pulse and recent success in breeding a stable male-sterility system has allowed breeders to exploit hybrid vigour for increasing yields.

The discovery of genetic male sterility (GMS) coupled with the natural out crossing, opened the possibility of commercial utilization of heterosis in pigeonpea (Reddy *et al.,* 1978 and Saxena *et al.,* 1983). The GMS based world's first pigeonpea hybrid ICPH 8 was released by ICRISAT for cultivation in 1991 (Saxena *et al.,* 1992). Since no commercial hybrids were available in any pulse crop, the release of ICPH 8 was considered as a milestone in the history of breeding pulses. However, the hybrid seed production with a genetically determined male-sterile sibs, which account for 50 % of the population grown was a time and labour-intensive, involving 40-50 % of the seed production cost (Muthiah *et al.,* 1998). Inefficiency in eliminating the fertile sibs reduces quality of the hybrid seed. Further, the removal of 50 % of the population (Fertile sibs) also results in reduced yields. The experience with GMS hybrid technology has

conclusively demonstrated that in pigeonpea the exploitation of hybrid vigour was possible, if the seed production techniques were optimized (Saxena *et al.*, 1998; Rathnaswamy *et al.*, 1998a). Hence, it was felt that hybrid breeding could be revolutionized if the CGMS system (cytoplasmic genic male-sterility system) is exploited for hybrid breeding (Saxena *et al.*, 1998).

The first unsuccessful attempt to develop cytoplasmic genic male-sterile (CGMS) lines in pigeonpea by using the crossable wild relatives of pigeonpea was made by Reddy and Faris (1981). Ariyanayagam *et al.* (1995) and Saxena *et al.* (2004) reported *Cajanus sericeus* as the CGMS source. The first CGMS line GT 288A was developed by using *C. scarabaeoides* at Gujarat Agricultural University, S. K. Nagar, India (Tikka *et al.*, 1997) and at ICRISAT (Saxena and Kumar, 2003). Subsequently, other scientists have identified male-sterile lines from the interspecific crosses involving *C. volubilis* (Wanjari *et al.*, 2001), *C. acutifolius* (Rathnaswamy *et al.*, 1998b; Mallikarjuna and Saxena 2002), and *C. cajanifolius* (Saxena *et al.*, 2005b), while Mallikarjuna and Saxena (2005) reported a CMS source from a pigeonpea cultivar itself (*C. cajan*). The different cytoplasmic male sterility sources derived from wild relatives of pigeonpea are given in Table 1.1.

Hybrid technology has successfully been used to increase the yields. A new hybrid pigeonpea breeding technology is capable of substantially increasing the productivity of pigeonpea, and thus offering hope of pulse revolution in the country (Saxena and Nadarajan, 2010). The CGMS based hybrids in extra short, short and medium maturity groups have recorded grain yield superiority of 61 % over the best control cultivar in different locations across India (Saxena, 2008).

Meanwhile, ICRISAT developed a number of experimental hybrids and tested in multi-location trials. They also developed genetically diverse CMS lines and their fertility restorers for developing widely adaptable hybrids to different agro-ecological areas and cropping systems. Among the medium duration hybrids with A_4 cytoplasm, ICPH 2671 and ICPH 2740 are very promising in multi-location trials conducted for four years. During 2009, the best performing hybrid ICPH 2671 was evaluated in 1248 on-farm trials in four states of India (Saxena *et al.*, 2010). In these trials ICPH 2671, recorded 28.4 % yield superiority over local

checks in farmer's fields and, therefore, ICPH 2671 was released in Madhya Pradesh for commercial cultivation in 2010 (Saxena *et al.,* 2013). Likewise other hybrid ICPH 2740, also recorded 35.8 % superiority over the control was released for commercial cultivation in Andhra Pradesh by ICRISAT and Acharya N. G. Ranga Agriculture University, Hyderabad in 2012 (Saxena *et al.,* unpublished).

For effective utilization of a male-sterility system in hybrid breeding, it is important that the expression of both the male-sterility and its fertility restoration is stable over the years and locations. Looking to its prospects of development of new hybrids in pigeonpea, the present investigation is aimed with the following objectives:

➢ To evaluate the variability in fertility status of different hybrids and CMS lines.
➢ To study the stability of cytoplasmic genic male-sterile lines.
➢ To study the stability of fertility status of different hybrids under varying environmental conditions.
➢ Identification of stable fertile hybrids and sterile CMS lines.

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

S. No.	Wild relative	CMS System
1	*Cajanus sericeus* (Ariyanayagam *et al.*, 1995)	A_1
2	*Cajanus scarabaeoides* (Saxena and Kumar, 2003)	A_2
3	*Cajanus volubilis* (Wanjari *et al.,* 2001)	A_3
4	*Cajanus cajanifolius* (Saxena *et al.,* 2005)	A_4
5	*Cajanus cajan* (Mallikarjuna & Saxena, 2005)	A_5
6	*Cajanus lineatus*	A_6
7	*Cajanus platycarpus* (Mallikarjuna *et al.,* 2011)	A_7
8	*Cajanus reticulates* (Saxena *et al.,* unpublished)	A_8

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

REVIEW OF LITERATURE

CHAPTER II
REVIEW OF LITERATURE

The commercial exploitation of heterosis or hybrid vigour through the cultivation of hybrid cultivars is one of the landmark achievements of plant breeding. Ever since the two pioneering publications by George H. Shull about 100 years ago, in which he scientifically described heterosis and laid the foundation of modern hybrid breeding in maize. Development of male-sterile lines in pigeonpea opened new vista for commercial exploitation of heterosis. Recently, 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). The male-sterile lines developed need to study their stability for expression of male sterility and fertility restoration with their *per se* performance. Therefore, the present investigation was aimed to obtain information on development and stability of male sterility and fertility restoration over the environments. The literatures reviewed on related subjects are presented below.

2.1 Methods of stability analysis

The stability analysis gives an idea about the homeostasis of the material tested. The present study was carried out to evaluate the stability of the pigeonpea hybrids and CMS lines under varying environmental conditions to test whether there is any environmental effect on the performance of these hybrids and CMS lines for pollen fertility and sterility percentage respectively. The literature reviewed related to methods of stability analysis has been provided below.

2.1.1 Measurements of genotype × environment interaction

The genotype × environment interaction is a major challenge in obtaining a complete understanding of genetic control of variability. The study of genotype × environment interaction in biometrical aspects is important from the genetically

and evolutionary point of view. The phenotype has been conventionally defined as a linear function of the genotype, environment and interaction between them.

Grafius (1956) emphasized that the studies of individual yield component can lead to simplification in genetic explanation of yield stability. If characters associated with yield stability could be found, the breeder might effectively select for yield stability by selecting for these correlated characters. The genetic association of the component characters with yield should also be known.

Interaction will be absent when all the genotypes behave consistently in all environments or in other words their ranking does not change when subjected to different environments. Several workers (Finlay and Wilkinson, 1963; Perkins and Jinks, 1968; (a and b) and Johnson *et al.*, 1968) have attempted to measure the relationship between genotype and environment as well as interactions of genotype and the environment.

Finlay and Wilkinson (1963) developed a simple dynamic interpretation of varietal adoption to natural environments, which could provide a basis for formulation of broad biological concept of value to agronomist and the breeders. According to them an ideal variety may be defined as one with maximum yield potential in the most favorable environment with maximum phenotypic stability measured by regression coefficient.

The approaches of Finlay and Wilkinson (1963) and Tai (1971) are purely statistical and the components of this analysis have not been related to parameters in biometrical genetical model. Another approach is based on fitting of model, which specifies the contribution of genetic, environmental and genotype × environmental interactions to generation means and variances. This approach allows for contribution of additive, dominance and epistatic gene actions to the genetic and interaction components (Mather and Jones 1958, Jinks and Stevens 1959, and Bucio and Hills 1966). This approach was used to investigate genotype × environment interactions in *Nicotiana rustica.*

Eberhart and Russell (1966) improved upon the model of Finlay and Wilkinson (1963) by adding another stability parameter namely the deviation from regression (S^2di). Later Breese (1969) applied this approach to data on grasses and has discussed the utility of this technique in predicting relative performance

of genotype and hybrids over years, seasons and locations as well as to deduct differences in stability. He has shown that a major part of genotype × environment interaction could be explained by difference between linear responses as estimated by regression.

Perkins and Jinks (1968a) attempted to fill the gap between the two approaches by expressing the expectation of the statistical analysis in terms of standard model of genes, environmental action and genotype × environmental interactions and have extended the analysis to cover many inbred lines and crosses among them. Perkins and Jinks (1968b) have mentioned that prediction of the sole parameters can be made both across the environments and across the generations.

Breese (1969), Samuel *et al.* (1970) and Paroda and Hays (1971) stated that the linear regression should simply be considered as a measure of response of genotype, whereas deviation around the regression line is a measure of stability. They also pointed out that a genotype with a lowest deviation may be the most stable and vice-versa.

Shukla (1972) defined the stability variance of genotypes as its variance across environments after the main effects of environmental means have been removed. Since the genotype main effect is constant, the stability variance is thus based on the residual ($GEij + eij$) matrix in a two-way classification. The stability statistic is termed as "stability variance" (σ_i^2). The stability variance is the difference between two sums of squares, it can be negative, but negative estimates of variances are not uncommon in variance component problems. Negative estimates of variance may be taken as equal to zero as usual. Homogeneity of estimates can be tested using Shukla's (1972) model of stability based on stability variance.

Verma *et al.* (1978) proposed a technique which is consistent with the assumption that distinct sets of genes control linear sensitivity in contrasting environments. In the proposed analysis, the environments, first truncated at zero environmental index providing two subsets of environments, first comprising all the negative environmental indices (below average environment) and the second with the positive environmental indices (above average environment) as well as

the negative index with the smallest absolute value. Two response curves for the trial genotype are fitted separately for the two sets of graded environments.

Pooni and Jinks (1980) proposed the procedure which is more general in the sense that a pair of regression lines is fitted without the tacit assumption that the switch over between regressions takes place around the same environment value. This is achieved by refining the two phase regression techniques on the principle that significant part of the non-linearity results from thresholds in response of genotypes to change in environmental conditions. Here the environmental value at which a particular genotype changes its slope is determined as the point of intersection of a pair of best fitting straight lines, which allow for a considerable reduction in the residual mean square.

Becker and Leon (1988) suggested two different concepts of stability: static (biological) and dynamic (agronomical). With the static concept, a stable genotype possesses an unchanged performance regardless of variation in the environmental conditions. Thus, genotypic variance among environments is zero. With the dynamic concept, response of a genotype to environments is predictable. Thus, a stable genotype has no deviation from response to environments. Both concepts of stability are useful, but their application depends on the trait considered. For qualitative traits such as resistance to diseases or stress, the static concept of stability is useful. For quantitative traits such as yield, the dynamic concept of stability is useful.

Gauch (1988, 1992) has advocated the use of what he term AMMI analysis of yield trial data. In many cases this procedure has been showed to increase estimation accuracy since it fits additive main effects for genotypes and environment by an ordinary ANOVA procedure and then applies PCA to the matrix of residuals of that remain after the fitting of main effects. The interaction and error can be decomposed into N space PCA axes.

Recently Yan *et al.*, (2001) developed GGE-biplot method which provides more elegant and useful display of multi environment trial (MET) data. It effectively addresses both the issue of mega-environment differentiation and the issue of genotype selection for a given mega-environment based on mean yield and stability. It also allows environments to be evaluated just as well as

genotypes. In addition, it facilitates interpretation of GE as genotypic factor by environmental factor interactions (Yan and Hunt, 2001).

2.2 Development of CMS lines & their cyto-histological studies.

Both the genetic and cytoplasmic genic male-sterility systems were developed with help of wide hybridization technology. Newly developed CMS lines replaced the GMS lines. Some of the male-sterile lines developed were sensitive to environment. The literature reviewed related to development of CMS lines and their cyto-histological studies has been provided below.

Reddy *et al.* (1978) studied the microsporogenesis in six male-sterile lines of pigeonpea. The results indicated that microsporogenesis was similar in the male fertile and sterile plants at the pre-meiotic and post-meiotic stage. Tetrad formation was the latest stage observed in which fertile and sterile did not differ. Tetrads were released from the spore mother cell sac in the fertile plants; in sterile plants the tetrads were not released and they started degenerating by vacuolation was the prime cause of male sterility.

Dundas *et al.* (1981) studied microsporogenesis in genic male-sterile lines of pigeonpea and concluded that, in the sterile plants, pollen mother cell (PMC) degeneration occurred at the young tetrad stage with the rupturing of each nuclear membrane and callose of the outer cell wall. Conversely, in the fertile plants, microsporogenesis proceeded quickly from PMC to mature bi-nucleate pollen grains.

Reddy and Faris (1981) attempted first to develop cytoplasmic male sterility in pigeonpea using the crossable wild relatives of pigeonpea. They crossed *Cajanus scarabaeoides,* a wild species with fertile F_1 plants of *C. cajan × C scarabaeoides*. The resultant BC_1F_1 plants were fertile but in BC_1F_2 generation some male sterile segregants were identified.

Saxena *et al.* (1981) studied the cytology of a partially male-fertile line of pigeonpea and reported that in such plants the breakdown of the tapetum was irregular with inconsistency extent was the prime cause of production of fertile pollens by CMS lines.

Dundas *et al.* (1987) studied the meiotic behavior of hybrids of pigeonpea and two Australian native *Atylosia* species. They found that meiosis in the parental pigeonpea and *Atylosia* accessions appeared regularly, while that in the hybrids showed a lower frequency of ring bivalents at Metaphase 1st multivalents, univalents, rod bivalents, chromatin bridges, fragments, laggards and supernumerary microspores occurred in meiotic cells of hybrids.

Ariyanayagam *et al.* (1993) used several accessions of four wild *Cajanus* species as females and one as male in crosses with *Cajanus cajan*. They observed large differences in the level of cytoplasmic male-sterility (CMS) present in the different species and its transmission to successive generations. Sterile progeny were more frequent in crosses involving *C. sericeus, C. scarabaeoides* and *C. acutifolius* as female parents, compared with *C. albicans*. Furthermore, accessions within the three most promising species responded differently to selection for CMS PR4562 (*C. scarabaeoides*) and EC121208 (*C. sericeus*) responded well in triple crosses and backcrosses.

Rajni *et al.* (1993) searched sources of cytoplasmic male sterility, five species of *Atylosia*, two of *Rhynchosia* and two of *Flemingia* were crossed with nine varieties of *Cajanus cajan*. They reported one plant from the cross *C. cajan* cv. T21 x *A. mollis* was male sterile out of seven F_1 plants raised from those crosses. Analysis of PMC meiosis showed no separation of tetrads indicating that no pollen grains were formed. The plant was maintained by backcrossing with T21.

Ariyanayagam *et al.* (1994) studied the microsporogenesis of a cytoplasmic-genetic male sterile line of pigeonpea and reported that the development of sporogenous tissue and the progression into PMCs was normal. The tapetum showed signs of disintegration, suggesting that the development of the PMCs had progressed; the PMC developed into tetrads. Signs of meiotic failure appeared later when cytoplasm was seen to separate from the wall of the newly formed pollen grain; vacuolation then occurred and the pollen eventually became clear and unstained. Meiotic failure of the genetic-cytoplasmic male-sterile line occurred at a later stage than that reported for genetic male-sterile lines.

Katti *et al.* (1994) studied the persistence of callose and tapetum in the microsporogenesis of genic male-sterile lines of *Cajanus cajan*. They reported quite different reason for male-sterility than those reported by Dundas *et al.* (1981). They found that, the pre-meiotic development was identical in the anthers of both the lines, during post-meiotic stages, sterile anthers showed persistent callose and tapetum.

Pandey *et al.* (1994) found 15 obcordate leaf shape plants in a seed production plot of *Cajanus cajan* cv. EA_1. All had pollen sterility ranging from 80 to 100 %. Some flowers had two or more carpels. Those that had three had one bifid stigma and two which were free. Having bi- and tri-carpellate flowers it may increase the chances of two or three pods setting from the same flower. The exposed keel petals may encourage cross-pollination by wind and insects and, hence, pod setting. However, flower drop was very high causing a lack of pod setting in the early stages. Pod setting through open pollination increased with a rise in temperature.

Ariyanayagam *et al.* (1995) reported that the resultant BC_1 F_1 plants were fertile but some male sterile segregants were identified in BC_1F_2 generation. This male sterility was linked with female sterility and therefore, it was not pursued further they crossed *C. sericeus* with cultivated pigeonpea and the F_1 progeny was partially male sterile. The backcross $(BC_1F_1$-$BC_3F_1)$ populations were found segregating for male sterility.

Pelletier and Budar (1997) reported that nucleocytoplasmic 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.

Tikka *et al.* (1997) reported development of cytoplasmic-genic male-sterility in pigeonpea with the help of wide hybridization using *Cajanus scarabaeoides* as female parent. The F_1 was partially fertile, while they got

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completely sterile plants in the F_2 generation, to which they used as female parent and made crosses with four genotypes. They got 100 % sterile plants in the BC_1F_1 generation.

Verulkar and Singh (1997) observed a male sterile plant in the UPAS 120 cultivar of pigeonpea. The plant was about 5-7 days late flowering and had white translucent anthers with complete pollen sterility.

Ravikesavan *et al.* (1998) sprayed 100, 200 and 300 ppm gibberellic acid (GA) on flower buds of pigeon pea cultivar ICPL 87 and pollens were evaluated for sterility after three days. They found that 100 and 200 ppm GA resulted in 25.8 and 62.2% pollen sterility.

Rathnaswamy *et al.* (1999) crossed two wild species *C. cajanifolius* and *C. acutifolius* to the genic male-sterile lines of *Cajanus cajan* (ms co 5). All the F_1s of ms co 5 × *C. cajanifolius* were found to be fully fertile. The F_1 of ms co 5 × *C. acutifolius* were found to be partially sterile and they were backcrossed to ms co 5. They further found that the frequency of male-sterility varied from 40 – 90% and more plants were in 60–70 % range. Level of pollen fertility decreased during the initial flowering phase in September possibly due to high temperatures and low humidity.

Wanjari *et al.* (2000) reported that male-sterility derived from *Cajanus sericeus* × *Cajanus cajan* is actually a single dominant gene possibly acting in concert with a single recessive gene to mimic cytoplasmic male-sterility. They found a segregation ratio of 1:1 (fertile: sterile) in the F_3 sibs while a ratio of 3:1 (fertile: sterile) in the selfed progenies, which shows that this male-sterility is governed by monogenic recessive gene and that the male-sterile plants are homozygotes (ss). The fertile counterparts in the segregating sibs are heterozygotes (Ss).

Reddy *et al.* (2000) studied characterization of hybrids between *Cajanus cajan* × *C. reticulatus* var. *gradifolius*. The meiotic cells of the hybrid were found to have quadrivalents, trivalents, univalents and showed chromosome pairing as revealed by the increased number of rod bivalents per cell at Metaphase I and stickiness and precocious movement of chromosome to poles in the second division. In comparison to parents, the hybrid had fewer pods and seeds.

Saxena and Kumar (2001) reported a natural male-sterile mutant in the population of a short-duration pigeonpea (*C. cajan*) cv. ICPL 85010. This mutant is characterized by light yellow anthers of reduced size that are devoid of pollen grains. This mutant was crossed with two pigeonpea cultivars to study its inheritance. The results showed that this male sterility trait was genetic in origin and is controlled by a single recessive gene.

Sharangpani and Shirke (2001) conducted an experiment on the developmental stages in fertile and sterile anthers of pigeonpea cv. MS 3783 using histochemical staining techniques. They reported that the development of anthers up to the tetrad formation was similar in fertile and sterile plants. At the onset of the tetrad formation stage, however, significant variations were observed between the two sets of anthers. In sterile anthers, the callose wall remained persistent and the microspores did not separate even at the mature stage.

Wanjari *et al.* (2001) studied the cytoplasmic male sterility in pigeonpea hybrids carrying the cytoplasm from *Cajanus volubilis*. They reported that a completely sterile progeny having the highest frequency of sterile plants in BC_2, i.e. 36107-5 x 36108-20 was crossed with pigeonpea cultivars. The hybrids of the male parents ICPL-85030 and Akms 21 expressed complete sterility while the other crosses yielded partially fertile plants along with sterile pollens. Backcrossing of the male sterile hybrids of AKT-8811, Akms-21, and ICPL-85030 with their respective parents yielded completely sterile progenies.

Mallikarjuna and Saxena (2002) found that the interspecific hybrid seed obtained by cross between *Cajanus acutifolius* and *Cajanus cajan* were semi-shriveled. Very few seeds germinated to give rise to F_1 plants. Backcrossing the hybrid plants are obtained after recovering the aborting embryos in vitro. The BC_1 plants showed normal meiotic pairing, but had low pollen fertility. The reason for embryo abortion and low pollen fertility in spite of normal meiosis could be due to effects of wild species cytoplasm.

Saxena and Kumar (2003) developed a stable CMS system derived from a cross involving *C. scarabaeoides* and cultivated pigeonpea. Recently at ICRISAT, CMS lines were also developed using *C. sericeus* and *C. cajanifolius* as female parent. Identification of male sterile plants was also reported in

interspecific crosses with *C. volubilis* and *C. mollis*, to broaden the genetic base of newly established CMS lines. Agronomically superior and good combiners were selected and converted into CMS through backcross breeding programme.

Chauhan *et al.* (2004) studied an efficient and stable cytoplasmic genic male sterility system by using the cytoplasmic genic male sterile line GT-288 A/B along with fertility restoration mechanism. To identify perfect pollen fertility restorers, 543 derivative lines of the F_5 and F_6 populations of *C. scarabaeoides* x *C. cajan* and 1365 germplasm accessions were used as pollen parents. The promising pollen fertility restoring parents were advanced and purified through selection and selfing. A total of 18 fertility restorers were identified and characterized.

Mallikarjuna and Kalpana (2004) reported two types of CMS plants in pigeonpea, which were distinguished by anther morphology. The type I CMS had partially or totally brown and shriveled anthers and the process of microsporogenesis was inhibited at the pre-meiotic stage, while type II CMS plants had pale white shriveled anthers and the breakdown in microsporogenesis was at the post-meiotic stage after the formation of tetrads, which caused male-sterility of the plants. The cyto-genetic analysis between three cultivars of *Cajanus cajan* and four wild species of *Cajanus* showed normal meiosis in the parents but some meiotic abnormalities were observed in the F_1s indicating varying degrees of chromosomal and genetic differences between *C. cajan* and *C. acutifolius* (Jogendra Singh *et al.*, 2004).

Mallikarjuna and Saxena (2005) reported a cytoplasmic genic male-sterility system from cultivated pigeonpea cytoplasm. Here the wild species *C. acutifolius* has been used as one of the parent's maintained complete male-sterility. Cytological analysis revealed that both in the 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.

Singh *et al.* (2007) developed two CMS lines GT 288A using the cytoplasm of *Cajanus scarabaeoides* and 67A using the cytoplasm of *Cajanus*

sericeus through wide hybridization and diversified into several agronomic bases. For the diversification of these two CMS sources, desirable recurrent parents UPAS 120, ICPL 84023 and PA 163, having resistance to key biotic stresses along with good combining ability were selected. Individual plants of recurrent (male) parents used in crossing were selfed. Sterile plants resembling the recurrent parents were selected and crossed with the recurrent parent to get BC_2F_1 generation.

Thiyagarajan *et al.* (2008) developed cytoplasmic genetic male-sterile (CGMS) lines in pigeonpea, *viz.* CORG 990052 A and CORG 990047, by interspecific hybridization of *Cajanus cajan* and *C. scarabaeoides*. Restorers were identified and three CGMS-based pigeonpea hybrids were developed. The hybrid COPH 3 was found to be promising in Tamil Nadu State, India.

Mallikarjuna *et al.* (2011) successfully crossed *Cajanus platycarpus*, a distantly related wild species with cultivated pigeonpea using embryo rescue and tissue culture techniques. They reported that advance generation lines showed a wide range of desirable characters including cytoplasmic male sterility. A range of pigeonpea cultivars restored fertility and was maintained by few lines including cultivar ICPL 85010. Clasmogamous flowers were observed in the male sterile lines. They reported two mechanisms of male sterility existed, one was premeiotic, where PMCs did not form and in the second, although PMCs gave rise to pollen grains, they were either partially or totally sterile accompanied by nondehiscence of anther wall.

2.3 Stability studies on CMS lines

Pigeonpea

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 ms1 and ms2 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.

Mahiboobsa *et al.* (2011) studied 12 F_1'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 F_1 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 BC_4F_1 and BC_5F_1 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.* (2012) studied stability of three male sterile lines ICPA 2043, ICPA 2047 and ICPA 2092 derived from *Cajanus cajanifolius* (A_4) 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 A_4 cytoplasm exhibited stability throughout the crop season without any effect of increase or decrease in temperature, indicating male sterility in A_4 was independent of environment condition.

Makelo *et al.* (2013) studied the stability of pollen sterility of CMS lines under Kenyan conditions. Six CMS lines, with over 96 % cytoplasmic male sterility were sourced from ICRISAT India and evaluated for two seasons in 2009. They reported that two out of six CMS lines, ICPA 2039 and ICPA 2043 were most stable across the location with 99 and 100 % pollen sterility. Performance of two promising CMS lines under Kenyan conditions for pollen sterility was comparable to the result obtain from India can, therefore, be used in commercial hybrid breeding program.

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_1 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_1), ICSA701 (A_2), ICSA741 (A_3) and ICSA764 (A_4), 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.

2.4 Studies on fertility restoration in hybrids.

There are 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 genetics and stability of fertility restoration is necessary for improving the efficiency and quality of restorers used in hybrid pigeonpea breeding. The literature on fertility restoration in pigeonpea is briefly reviewed here under.

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_1 and F_2 generations and test cross progenies of fertile F_1 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>0.01, 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.

Saxena and Kumar (2003) studied the fertility restoration system in A_2 cytoplasm of pigeonpea. They developed the crosses between three CMS lines on the basis of A_2 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 as 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 genic male-sterile lines (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 pollen parent on stable cytoplasmic genic male sterile line GT-288A during *kharif* 1997 to 2003. The F_1 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.

Gangwar and Bajpai (2005) studied pollen fertility in F_3 generation of interspecific hybrids in pigeonpea and reported that all male and female parents

had complete pollen fertility (92.80-98.23 %). The hybrids of *C. cajan* x *C. cajanifolius* showed wide variation for pollen fertility (68.69-89.20 %) and the maximum fertility was seen in *C. cajan* x *C. scarabaeoides* (74.23-85.51 %). The poor fertility (8.02-36.50 %) was seen in segregants of *C. cajan* x *C. acutifolius.*

Lad and Wanjari (2005) reported that there may be many genes governing the fertility restoration in pigeonpea. They observed in segregating progenies a monogenic segregation pattern of 3 good : 1 poor dehiscence for pollen fertility percent. These progenies produced plants with 50-80% pollen fertility.

Singh and Bajpai (2005) studied the relative pollen fertility in interspecific crosses. They found that, *C. cajan* × *C. acutifolius* hybrid showed low pollen fertility in F_1 generation, whereas high pollen fertility was found in crosses utilizing *C. cajanifolius* and *C. scarabaeoides*. They also noticed moderate variation in size of pollen grains among the parents and their hybrids.

Saxena *et al.* (2005) studied that the tool of inter-specific hybridization can be used for the development of stable cytoplasmic genic male-sterility system in pigeonpea. They designated the CMS system as A_4, which is developed by an inter-specific cross between *Cajanus cajanifolius*, a wild relative of pigeonpea and a cultivar ICP 11501. Also they tested various testers for knowing fertility restoration and maintenance reaction. They found ICPH 2470 as a promising short-duration experimental hybrid, which exhibited 77.5 % advantage over the control cultivar UPAS 120.

Chaudhary *et al.* (2006) noticed a higher order of sterility in the hybrids of A_3 cytoplasm when cornered to other cytoplasmic hybrids. Fertility status of A_4 cytoplasm hybrids were in between A_2 and $A_3.$ From the fertility restoration studies it was concluded that the order of sterility in the diverse cytoplasm increased from A_1 to A_2 to A_4 to A_3.

Singh *et al.* (2006) studied two cytoplasmic genetic male sterile (CMS) lines of pigeonpea in BC_3F_1 namely, GT 288 A and CMS 1024 A along with their maintainers to confirm the nature of male sterility system. Pollen fertility test exhibited that only 50 and 35% plants of GT 288 A and B were completely male sterile and fertile, respectively, indicating that both A and B lines should be back crossed/selfed for a few more generations to obtain the perfect line. However,

CMS 1024A appeared to have a mutated gene (s) with varying degree of fertility and the lack of pod setting after selfing is due to heterostyly nature of the flower.

Wanjari *et al.* (2007) studied that individual hybrid of a set of 156 hybrid were tested for anther dehiscence and pollen fertility. Among 136 hybrids tested, 11 expressed high pollen fertility (> 80 %) in all the plants.

Dalvi *et al.* (2008a) reported the fertility restoration in cytoplasmic-nuclear male-sterile lines derived from three 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_1 cytoplasm), *C. scarabaeoides* (A_2 cytoplasm), and *C. cajanifolius* (A_4 cytoplasm) were crossed to seven pigeonpea cultivars in a line x tester mating scheme. Twenty-one F_1 hybrid combinations were planted in three environments. There was no effect of environment on the expression of fertility restoration. Pigeonpea cultivar ICPL 129-3 restored fertility in A_1 cytoplasm and maintained male sterility in the other two (A_2 and A_4) cytoplasm. Among crosses involving CMS line (of A_4 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 restorers in pigeonpea. All the F_1 plants in 5 crosses were fully fertile indicating the dominance of fertility restoring genes. Among the 5 crosses studied, three (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_2 generation and 1 fertile : 1 sterile in BC_1F_1 generation indicating the monogenic dominant nature of a single fertility restoring gene. The crosses ICPA 2039 x ICP 10650 segregated two dominant duplicate gene action with a ratio of 15 fertile : 1 sterile in F_2 and 3 fertile : 1 sterile in BC_1F_1, respectively. The cross ICPA 2039 x ICP 13991 had two complementary gene action of 9 fertile : 7 sterile in F_2 and 1 fertile : 3 sterile in BC_1F_1, respectively.

Nadrajan *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_1, A_2 and A_4. Among the three sources of male parents selected, restoration was maximum in the germplasm inbreds as compared to advanced breeding lines and cultivars indicating need for intensive exploration across genetically and geographically diverse genetic resources.

Ali (2009) evaluated seven hybrids in kharif 2005-06 for fertility, crop duration and grain yield and reported that all hybrids were 100 % fertile. Similarly, evaluation of nine hybrids during 2005-06 showed that four hybrids, namely GT 288 A x JBP 110, GT 290A x ICP 1763, 67A x ICP 9596 and Hy 28 BA x ICP 41 were 100% fertile.

Singh *et al.* (2009) reported that the formation of fertile pollen and its involvement in fertilization, pod formation and seed development was seriously affected or less in winter season (December – January) and an increase in temperature during spring season (February – March), however resulted in normal pod and seed development. Again in summer (April – May) fertilization, pod formation and seed development were decreased due to high temperature $>35^0$C.

Saxena *et al.* (2010a) 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_1s 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. All 35 F_1 plants of hybrid ICPA 2067 x ICP 12320 were male fertile indicated the dominance of fertility restoring genes. Out of 359 F_2 plants grown, 303 were found fertile whereas only 56 exhibited male sterility. This segregation fit well to a ratio of 13 fertile: 3 sterile (P = 0.01). In BC_1F_1 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 two dominant genes, with one basic and one inhibitory gene action for the determination of fertility restoration in ICPA 2067.

Lay and Saxena (2011) studied fertility restoration system in five CMS-based pigeonpea hybrids. They reported two hybrids 'ICPH 2671' and 'ICPH 2740' which had the same male parent but different females segregated in F_2 in the ratio of 12 fertile (F): 3 partial fertile (PF):1 sterile (S), and in BC_1F_1 generation as 2 fertile: 1 partial fertile: 1 sterile, suggesting that fertility restoration in these hybrids was controlled by digenic dominant epistatic interaction. The progenies derived from hybrid 'ICPH 3359' fitted well to an F_2 ratio of 9 F : 6 PF : 1 S, and 1 F : 2 PF : 1 S in BC_1F_1 generation, indicating the involvement of two major genes with incomplete dominant epistasis. Progenies of the other two hybrids 'ICPH 4012' and 'ICPH 4344' segregated in F_2 in the ratio of 9 F: 3 PF: 4 S and 1 F: 1 PF: 2 S in BC_1F_1 generations, suggesting that pollen fertility was controlled by digenic recessive epistatic gene action. They concluded that the fertility restoration of A_4 CMS system in pigeonpea was governed by two major genes but with different types of epistatic interactions in different crosses.

Saxena *et al.* (2011a) studied the inheritance of the obcordate leaf trait and its fertility restoration ability using 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 F_1 plants of the obcordate donor were fully male-fertile and had normal leaves suggested that the obcordate leaf trait was recessive and that fertility restoration was due to the effect of dominant gene (s).

Saxena *et al.* (2011b) studied one extra-early- (120 days), two early- (150 days), and two late maturing (180 days) pigeonpea hybrids to generate information on the genetics of fertility restoration of the A_4 CMS system. In the extra early maturing hybrids, pollen fertility was controlled by a single dominant gene, whereas in the early- and late-maturing hybrids, male fertility was 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. It was also concluded that

for breeding hybrids with stable fertility restoration, the presence of two dominant genes is essential.

Sawargaonkar *et al.* (2011) studied single crosses involving four CMS lines and three known fertility restorers to determine the genetics of fertility restoration in pigeonpea (*Cajanus cajan* L. Millsp.). They observed that the interaction of dominant nuclear genes of ICP 2766 with ICPA 2092 produced 100% fertile F_1 plant and showed complete dominance for fertility restoration. The restorer ICP 2766 when crossed with ICPA 2092 showed monogenic inheritance (3:1), while the of ICP 2766 × ICPA 2043 revealed digenic inheritance (15:1) of fertility restoration.

Kalaimagal *et al.* (2012) studied inheritance of fertility restoration in A_2 cytoplasm (*Cajanus scarabaeoides* (L.) Thouars) by utilizing two CMS lines, CORG 990052A and CORG 990047A crossed with three restorers viz., Co 1R, Co 2R and Co 3R. The study of F_2 generation indicated that the fertility restoration in CORG 990052A by Co 1R was governed by two independent dominant genes, while fertility restoration by Co 2R and Co 3R in CORG 990047A was governed by single dominant gene.

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

Lui 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 (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 × 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 F_1 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.

MATERIALS & METHODS

CHAPTER III
MATERIALS AND METHODS

The present investigation was carried out to determine the stability of male sterility of CMS lines and fertility restoration in CMS-based pigeonpea hybrids under different environmental conditions. The experimental materials used and the methods applied during the course of present investigation have been described below.

3.1 Study of stability of fertility restoration in hybrids.

3.1.1 Experimental materials

The experimental materials comprised of 12 genotypes, including 10 hybrids and two varietal checks (Table 3.1). The material was obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru (Andhra Pradesh) and evaluated at three different locations *viz.* Patancheru (Andhra Pradesh), Ranchi (Jharkhand) and Sehore (Madhya Pradesh). The Details of materials used in the investigation are given in Table 3.1.

3.1.2. Environmental conditions

The experimental material was sown at three locations in India *viz.* ICRISAT, Patancheru, Andhra Pradesh (E_1), GVT Farm, Department of Genetics and Plant Breeding, Birsa Agriculture University, Ranchi, Jharkhand (E_2) and Experimental Field of College of Agriculture, Sehore, Madhya Pradesh (E_3). The locations Patancheru, Ranchi and Sehore are designated as environment first, second and third (E_1, E_2 and E_3 environment) respectively. The details of environmental conditions of each location are given in Table 3.2.

3.1.3. Experimental design and sowings

The 10 promising hybrids developed by ICRISAT were evaluated along with two checks (Asha and Maruti) in Randomized Complete Block Design with two replications during *kharif* 2012. Six-row plots were planted with 4 m length with inter and intra-row spacing of 75 and 50 cm respectively.

Table 3.1: Salient characteristics of CMS-based pigeonpea hybrids and varieties used in study.

S. no.	Name of hybrids	Parentage	Maturity (days)	Plant height (cm.)	100 Seed mass (g)	Seed color
1	ICPH 2671	ICPA 2043 × ICPL 87119	165-175	230-260	11.0-11.5	DB
2	ICPH 2740	ICPA 2047 × ICPL 87119	180-185	210-230	10.4-11.3	B
3	ICPH 3933	ICPA 2078 × ICPL 87119	170-185	190-200	10.5-11.6	B
4	ICPH 2751	ICPA 2048 × ICPL 87119	175-185	210-240	11.0-11.8	B
5	ICPH 3477	ICPA 2047 × ICPL 20098	175-185	180-230	10.0-10.8	B
6	ICPH 3461	ICPA 2092 × ICPL 87119	170-185	230-250	10.2-10.5	B
7	ICPH 3762	ICPA 2092 × ICPL 20108	171-184	220-240	10.4-10.8	B
8	ICPH 4490	ICPA 2047 × ICPL 20126	175-190	200-220	11.2-12.0	B
9	ICPH 3491	ICPA 2048 × ICPL 20096	170-190	210-240	11.5-12.3	B
10	ICPH 3494	ICPA 2048 × ICPL 20093	180-195	210-230	11.0-12.0	B
11	Asha	C11 x ICP 1-6W3B	175-180	200-220	10.2-11.2	B
12	Maruti	ICP 8863	165-175	190-210	10.0-10.4	B

DB= Dark brown, B= Brown

The experimental materials were sown at three locations- ICRISAT, Patancheru (E_1) on June 29, 2012; GVT Farm, Department of Genetics and Plant Breeding, Birsa Agriculture University, Ranchi, (E_2) on July 11, 2012; and Experimental Field of College of Agriculture, Sehore, Madhya Pradesh (E_3) on August 16, 2012. To reduce competition between the crop and weeds for nutrient uptake, water absorption, and photosynthesis, two weedings were done at vegetative stage. Two irrigations were provided at the time of vegetative growth stage at Patancheru while the trail was maintained under rainfed conditions at Ranchi and Sehore. A basal fertilizer dose of 25:50:0 N:P:K kg/ha was applied uniformly in soil and three sprays of pesticides (acephate and spinosad at 1 kg ha^{-1} and 0.2 L ha^{-1}, respectively) at 10 days intervals to control pod borers (*Maruca vitrata* Fab. and *Helicoverpa armigera* Hub.) between flowering and podding stages and other agronomic practices were followed as per recommendations to keep the crop in good condition.

3.2. Study of stability of maintenance of male sterility of CMS lines.

3.2.1 Experimental materials

The experimental materials comprised of nine cytoplasmic male sterile (CMS) lines of pigeonpea, ICPA 2039, ICPA 2043, ICPA 2047, ICPA 2051, ICPA 2092, HyC3A, BRG1A, BRG3A and TTB7A. All these lines had *Cajanus cajanifolius* (A_4) cytoplasm. The lines ICPA 2039, ICPA 2043, ICPA 2047, ICPA 2051 and ICPA 2092 were developed at International Crops Research Institute for the Semi-Arid Tropics, Patancheru, and the other CMS lines HyC3A, BRG1A, BRG3A and TTB7A were developed by University of Agriculture Science, Bangalore, Karnataka. All these lines were evaluated for their stability of expression of male sterility under varying environmental conditions. The brief details of all CMS lines are given in Table 3.3.

Table 3.2 The details of geographical information of the three environments used in this study.

Particulars	Environments		
	E_1	E_2	E_3
Location	ICRISAT, Patancheru, Hyderabad.	Experimental farm, B.A.U., Ranchi	Experimental farm, R.A.K. College of Agriculture, Sehore
Latitude	17° 53'N	23° 17'N	23°12' N
Longitude	78° 27'E	85° 19'E	77°05' E
Altitude	545.0 m	625.0 m	498.77 m
Soil type	Medium black soil	Sandy soil	Medium black soil
Climatic zone	Moderate rainfall zone	sub-tropical	sub-tropical
Total rainfall	745.52 mm	984.7 mm	936.5 mm
Temperature Min. ^{o}C	7.6	1.8	6.1
Max. ^{o}C	38.6	32.5	35.5
Humidity Min. (%)	14.0	59.9	33.18
Max. (%)	98.0	85.3	98.42
Date of sowing	June 29, 2012	July 11, 2012	August 16, 2012
Date of harvesting	January 4, 2013	February 12, 2013	February 4, 2013

3.2.2. Environmental conditions

The experimental material was sown at experimental field of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Patancheru, Andhra Pradesh. To study on the stability of expression of male sterility, the experimental material was sown in three different dates- August 7, 2012, September 11, 2012, and October 18, 2012 to provide a range of environments. The details of environmental conditions of this location are given in Table 3.2.

Table 3.3: Details of cytoplasmic male sterile lines used for study.

S. No.	CMS Lines	Pedigree	Sources	Plant type
1.	ICPA 2039	(ICPW 29 x Pusa Ageti) x Pusa Ageti x Pusa Ageti x Pusa Ageti x Pusa Ageti x Pusa Ageti	ICRISAT-A Lines	DT
2.	ICPA 2043	(ICPA 2039 x ICPL 20176) x ICPL 20176 x ICPL 20176 x ICPL 20176 x ICPL 20176 x ICPL 20176	ICRISAT-A Lines	NDT
3.	ICPA 2047	(ICPA 2039 x ICPL 99050) x ICPL 99050 x ICPL 99050 x ICPL 99050 x ICPL 99050 x ICPL 99050	ICRISAT-A Lines	NDT
4.	ICPA 2051	(ICPA 2039 x ICP 5357) x ICP 5357 x ICP 5357 x ICP 5357 x ICP 5357 x ICP 5357	ICRISAT-A Lines	NDT
5.	ICPA 2092	(ICPA 2039 x ICPL 96058) x ICPL 96058 x ICPL 96058 x ICPL 96058 x ICPL 96058 x ICPL 96058	ICRISAT-A Lines	NDT
6.	HyC3A	Selection from Hyderabad (A.P.) material	U.A.S. Bangalore	NDT
7.	BRG1A	(Hy3C x Hosakote local) x Hosakote local x Hosakote local x Hosakote local x Hosakote local x Hosakote local	U.A.S. Bangalore	NDT
8.	BRG3A	(Hy3C x OGUK3) x OGUK3 x OGUK3 x OGUK3 x OGUK3 x OGUK3	U.A.S. Bangalore	NDT
9.	TTB7A	Local Selection	U.A.S. Bangalore	NDT

DT= Determinate, NDT= Non determinate

3.2.3. Experimental design and sowings

The cytoplasmic male sterile lines were evaluated in Randomized Complete Block Design with two replications on August 7, 2012, September 11, 2012 and October 18, 2012 respectively identified as first, second and third date of sowing. Two-row plots were planted with 4 m length with inter and intra row spacing of 75 and 30 cm respectively. Border rows were planted in all three dates of sowings 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. Two irrigations were provided in first date of sowing while because of less availability of moisture in soil, three irrigations were provided in second and third date of sowing.

3.3 Observations recorded

a) Cyto-histological observations

Assessment of pollen fertility and sterility in hybrids and CMS lines

Each plant of hybrids and CMS lines was tested for its pollen fertility/sterility status at the initial flowering stage. To identify fertility/sterility of pollen grains, 2 % aceto-carmine 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-carmine 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 hybrids and CMS lines were computed as follows.

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

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

b) Number of fertile and sterile plants

Numbers of fertile and sterile plants were counted on the basis of pollen fertility and sterility of individual plant. In hybrids, plants were categorized according to their fertility status, the plants showing >80 % pollen fertility were considered as fully male fertile and the plants which had 40-80 %, 10-39 % and <10% pollen fertility were considered as partial male fertile, partial male sterile, and complete male sterile plants respectively. While, in CMS lines plants were categorized according to their sterility status. The plants which showed >90 % pollen sterility were considered as complete male sterile and which had 61-90 %, 20-60 % and <20 % sterility were considered as partial male sterile, partial male fertile, and fully male fertile plants respectively (Khin Lay, 2011).

c) Plant fertility and sterility (%)

The total plant fertility and sterility percentage of different genotypes was classified into percentage of fully male fertile, partial male fertile, partial male sterile and complete male sterile plants. The per cent of fertility of each category was calculated on the basis of number of plants of that category found in each entry. The plant fertility/sterility (%) of these categories was calculated by the following formulae:

$$\text{Fully male fertile plants (\%)} = \frac{\text{No. of fully male fertile plants}}{\text{Total number of plants}} \times 100$$

$$\text{Partial male fertile plants (\%)} = \frac{\text{No. of partial male fertile plants}}{\text{Total number of plants}} \times 100$$

$$\text{Partial male sterile plants (\%)} = \frac{\text{No. of partial male sterile plants}}{\text{Total number of plants}} \times 100$$

$$\text{Complete male sterile plants (\%)} = \frac{\text{No. of complete male sterile plants}}{\text{Total number of plants}} \times 100$$

d) Plant stand

Plant stand for each genotype was taken by counting the total number of plants in net plot area (area excluding border rows and plants) at the time of recording pollen fertility data in hybrids.

3.4 Statistical analysis

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 ith genotype in the jth replication.

μ = general population mean

g_i = effect of ith genotype.

r_j = effect of jth 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.

$\sigma^2 g$ = genotypic variance

$\sigma^2 e$ = error variance

3.4.1. 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.

3.4.1.1 Mean:

Mean was calculated using following conventional formula

$$\bar{X} = \frac{\Sigma X}{N}$$

Where,

$\bar{X}$= simple mean

ΣX = summation of all the observation

N   = number of observation

3.4.1.2 Range:

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

3.4.1.3. Standard error of mean.

It was calculated as formula given below.

SEm± $= \sqrt{MSe/r}$

Where,

SE m± = standard error of mean

MSe = mean sum of square due to error

r = number of replication.

3.4.1.4. Standard error of differences.

It was calculated as formula given below

SEd ± $= \sqrt{2MSe/r}$

Where,

SEd± = standard error of differences.

Mse = mean sum of square due to error.

r = number of replications.

3.4.1.5. Critical difference:

It was measured as formula mentioned below.

CD = SEd X t value at 5% level of significance.

Where,

CD = critical difference

SEd= standard error of difference

T = table value at 5% probability level of error df.

3.4.2. Stability analysis

Stability analysis of hybrids and CMS lines was carried out for 5 characters under study. Three different approaches were adopted for estimating the stability parameters (a) conventional pooled analysis of variance (G x E interactions), (b) regression analysis of Eberhart and Russell (1966) and GGE biplot method of stability analysis (Yan *et al.,* 2000).

3.4.2.1 Pooled analysis of variance

The pooled analysis of variance was carried out as per the standard procedure given by Singh and Chaudhari (1985). The form of analysis of variance is given below.

Table 3.5 Analysis of variance for mean data

Source	df
Environments	(e-1)
Genotypes	(v-1)
Genotypes x Environment	(e-1) (v-1)
Pooled error	e (r-1) (v-1)

Where, e, v and r stand for number of environments, genotypes and replications respectively. The mean sum of squares due to genotypes and environments were tested against mean sum of squares due to genotype x environment. The mean sum of squares due to genotype x environment were tested against mean sum of squares for pooled error.

3.4.2.2 Stability model of Eberhart and Russell (1966)

The stability parameters are defined with the following model:

$$Y_{ij} = m + b_i I_j + d_{ij}$$

i = 1, 2 ..t and j = 1, 2... s.

Where,

Yij   = mean of ith variety in j^{th} environment

m   = mean of all the varieties over all the environments

bi   = the   regression   coefficient   of   the ith variety   on   the environmental index which measure   the response of this variety to varying environments.

Ij   = the environmental index which is defined as the deviation of the mean of all the varieties at a given location from the overall mean.

dij   = the deviation from regression of the ith variety at j^{th} environment.

$$
Ij \quad = \quad \frac{\Sigma_i Yij}{\Sigma v} \quad - \quad \frac{\Sigma_i \Sigma_j Yij}{n}
$$

With $\Sigma_i Ij$ = 0

a) Environmental index (Ij)

Ij   = $[(\Sigma_i Yij / g) - (\Sigma_i \Sigma_j Yij / ge)]$ With ΣIj = 0

Where,

Ij   =   environment index

Yij   =   summation of all the genotypes for j^{th} environment

g   =   number of genotypes

$\Sigma_i \Sigma_j Yij$ =   summation   of   all   the   genotypes   overall   the environments.

ge   =   number of genotypes × number of environments

b) Regression coefficient (bi)

The first stability parameter is a regression coefficient. The regression coefficient of the varietal mean on environmental index is estimated as:

$$bi \quad = \quad \Sigma jYij Ij \, / \, EI^2 j$$

Where,

$\Sigma jYij Ij$ = sum of the ith genotype x environmental index in j^{th} environment

$\Sigma I^2 j$ = as for environmental index

c) Deviation from regression

The performance of each genotype can be predicted by using estimate of parameter.

$$Yij \quad = \quad \overline{X}i + bi \, Ij$$

Where,

$\overline{X}i$ is the estimate of mean.

The deviations (Sij = Yij = Yij) are squared to provide an estimate of another stability parameter ($S^2 di$).

$$S^2 di \quad = \quad [\Sigma \sigma \, 2ij/(e-2) - S^2 \, e/r]$$

Where,

$S^2 \, e/r$ =   Estimate of the pooled error.

$\Sigma \sigma 2ij$ = $[\Sigma Y^2 ij = Y^2 \, i/e - (\Sigma jYij Ij)^2 \, / \, j I^2 j]$

The appropriate analysis of variance is given in following table. With this model the sum of squares due to environment and genotype x environment

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partitioned in environment (linear), genotype x environment (linear) and deviation from regression.

Table 3.6 Analysis of variance for stability parameter

Source of variation	df	Expectations of mean squares
Genotype (G)	(g-1)	$1/e \, \Sigma i \, Y$ - C.F. = $\Sigma Y^2 j$
Environment (E)	(e-1)	$1/g \, \Sigma j \, Y^2$ – C.F. = $\Sigma Y^2 j$
Genotype x Environment	(g-1) (e-1)	$\Sigma i \Sigma j Y^2 ij$ – C.F. = $\Sigma j – Y^2 j$
Environment (linear)	1	$1/g \, (\Sigma j \, Yijlj)^2 / \Sigma j l^2 j) \, Ms_2$
Genotype x Environment (linear)	(g-2)	$\Sigma i \, (\Sigma j \, Yijlj)^2 / \Sigma j l^2 j)$- Env. (linear)
Pooled deviation	g(e-2)	$\Sigma i \, \Sigma j \, S^2 ij \;\; Ms_3$
Genotype-g	(e-2)	$(jY^2 g-Y^2 g/e) – (jYg \, l^2 j) / jl^2 j = j^2 g)$
Pooled error	(r-1) (g-1)	

d) Test of significance

(a)   In order to test the significance of the difference among the variety means,

i.e. $H_0 = \mu_1 = \mu_2 = \mu_3 = \ldots \mu_n$

The appropriate 'F' test is defined as:

F = Ms_1 / Ms_3

(b)   To test that the varieties do not differ for their regression on the environmental

index, i.e. $H_0 = b_1 = b_2 = \ldots b_n$,

F = Ms_2 / Ms_3

Thus all the variances can be tested against pooled deviation means square (Ms3).

(c)   An appropriate test of the deviation from regression for each genotype can be obtained.

$F = [\Sigma i \Sigma j \sigma^2 ij / e\text{-}2] / Pooled\ error.$

The test of significance carried for the stability parameter, for phenotypic index and regression coefficients are as follows:

S.E.   = $\sqrt{\text{Error M.S.} / r\ e}$

F   =   Ij- µ / S.E.

Thus,

L.S.D. for Ij = S.E. x 't' at 0.05 per cent.

The hypothesis that any regression coefficient does not differ from unity can also be tested by 't' test. The S.E. and 't' for regression coefficient were calculated as follows:

S.E. (b)   = $[\text{deviation Ms} / \Sigma j\ Ij^2]^{1/2}$

't'   = b −1 / S.E.(b)

Thus, L.S.D. for b-1 = S.E. (b) x 't' at 0.05 per cent.

3.4.2.3 GGE Biplot analysis (Yan *et al.,* 2000)

To evaluates the phenotypic stability and adaptability, the GGE biplot analysis performed, considering the simplified model for two main components. In this approach, the effects of genotype (G) and genotype by environment (GE) were considered as random in the model. In this case, the best linear unbiased prediction (BLUP) of G and GE effects are calculated.

The components of genotypic variance, of the variance of GE interaction and residual were estimated by the method of restricted maximum likelihood (REML). For analysis of variance the software package SAS 9.2 version was used. GGE biplot software was used to explain relationship between genotype and locations graphical (Yan and Kang, 2003).

The model for a GGE biplot (Yan, 2002) based on singular value decomposition (SVD) of first two principal components is:

$$Y_{ij} - \mu\text{-}\beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij} \qquad\qquad [1]$$

where Y_{ij} is the measured mean (DBH) of genotype i in environment j, μ is the grand mean, β_j is the main effect of environment j, $\mu + \beta_j$ being the mean yield across all genotypes in environment j, λ_1 and λ_2 are the singular values (SV) for the first and second principal component (PC1 and PC2), respectively, ξ_{i1} and ξ_{i2} are eigenvectors of genotype i for PC1 and PC2, respectively, η_{j1} and η_{j2} are eigenvectors of environment j for PC1 and PC2, respectively, ε_{ij} is the residual associated with genotype i in environment j.

PC1 and PC2 eigenvectors cannot be plotted directly to construct a meaningful biplot before the singular values are partitioned into the genotype and environment eigenvectors. Singular-value partitioning is implemented by,

$$g_{i1} = \lambda_1{}^{f1}\xi_{i1} \text{ and eij} = \lambda_1{}^{1-f1}\eta_{1j} \qquad \textbf{[2]}$$

Where f_1 is the partition factor for PC1, Theoretically f_1 can be a value between 0 and 1, but 0.5 is most commonly used.
To generate the GGE biplot, the formulae [1] was presented as:

$$Y_{ij} - \mu - \beta_j = g_{i1}e_{1j} + g_{i2}e_{2j} + \varepsilon_{ij} \qquad \textbf{[3]}$$

If the data was environment-standardized, the common formula for GGE biplot was reorganized as follows:

$$Y_{ij} - \mu - \beta_j / s_j = \Sigma\, g_{i1}e_{1j} + \varepsilon_{ij} \qquad \textbf{[4]}$$

Where, s_j is the standard deviation in environment j, I =1, 2,…,k, g_{i1} and e_{1j} are PC1 scores for genotype i and environment j, respectively.

We used environment standardized model [4] to generate biplot of "which-won where". For the analysis of relationship between the trials, genotype and environment evaluation, we used unstandardized model [3]. The analyses were conducted and biplot generated using the "GGE biplot" software (Yan and Tinker, 2005).

RESULTS

CHAPTER IV
RESULTS

The present investigation entitled, **"Stability of maintenance of male sterility and fertility restoration in pigeonpea under different environments"** was conducted during *kharif* 2012 using 10 hybrids and two standard checks. The stability of fertility restoration was studied over three locations, while the stability of expression of male sterility was studied with nine CMS lines in different dates of sowing at ICRISAT. All the hybrids and checks were obtained from Pigeonpea Breeding Programme, ICRISAT, Patancheru while five out of nine CMS lines were obtained from ICRISAT and the other four CMS lines were obtained from University of Agriculture Science, Bangalore, Karnataka. The results obtained from the present investigations are presented as under.

4.1 Study on stability of CMS-based hybrids for fertility restoration.

4.1.1 Analysis of variance

The mean performance of genotypes (hybrids and checks) for each of the characters studied was analyzed statistically and the genotypic differences were found to be highly significant (P<0.01) for all the characters except for plant stand at each of three locations (Table 4.1). The mean genotypic values from different locations were subjected to pooled analysis. The result of pooled analysis of variance also revealed that genotypic differences were highly significant (P<0.01) for all traits except for plant stand. From pooled analysis of variance, it was observed that the individual environment effect was highly significant for all characters except partial male sterile plants (%), whereas the significant genotypic difference were found for all characters except plant stand. All characters except partial male sterile plants (%) and plant stand were found significant for G × E interaction when tested against pooled error (Table 4.2). The characters which had non-significant G × E interaction were not further analyzed for stability.

Table 4.1 Mean sum of squares for pollen fertility (%) and other characters of hybrids at three different locations.

Traits	PATANCHERU		RANCHI		SEHORE	
	Genotype	Error	Genotype	Error	Genotype	Error
Degree of freedom	11	11	11	11	11	11
Pollen fertility (%)	0.157**	0.002	0.115**	0.003	0.098**	0.002
Fully male fertile plants (%)	0.315**	0.007	0.279**	0.000	0.309**	0.002
Partial male fertile plants (%)	4.094**	0.188	3.035**	0.185	4.991**	0.049
Partial male sterile plants (%)	2.905**	0.446	3.821**	0.321	3.457**	0.208
Complete male sterile plants (%)	4.569**	0.040	1.858**	0.073	1.764**	0.290
Plant stand	2.405	2.678	0.985	1.303	2.223	1.405

*, ** = Significant at P≤ 0.05 and P≤ 0.01, respectively

Table 4.2 Analysis of variance over the location (F values)

Source of variation	df	Pollen fertility (%)	Fully fertile plants (%)	Partial fertile plants (%)	Partial sterile plants (%)	Complete sterile plants (%)	Plant stand
Replication	3	1.72	2.65	2.36	1.75	0.58	0.73
Genotype	11	145.61**	279.54**	74.75**	30.24**	53.76**	1.28
Environment	2	12.16**	47.13**	15.74**	1.12	18.59**	79.62**
Genotype × Environment	22	2.87*	14.67**	5.61**	0.56	6.34**	1.03

*, ** = Significant at P≤ 0.05 and P≤ 0.01, respectively

4.1.2. Mean performance of hybrids

The location-wise as well as pooled *per se* performance of hybrids and controls for pollen fertility (%) and other characters are given in Table 4.3 to 4.6. These were compared by using respective standard error of mean and coefficient of variation. The results related to individual characters are given below.

4.1.2.1 Pollen fertility (%)

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 48.2 to 98.4 %. Among the hybrids, ICPH 2740 recorded maximum pollen fertility (96.5 %) followed by ICPH 3933 (93.6 %) and ICPH 2671 (93.4 %), whereas the minimum pollen fertility was recorded in ICPH 3491 (48.2 %) followed by ICPH 3494 (55.1 %) (Table 4.3). At Ranchi, the variability for pollen fertility ranged from 58.0 to 98.5 %. Among the hybrids, ICPH 2671 (96.2 %) exhibited highest pollen fertility followed by ICPH 2740 (95.7 %) and ICPH 2751 (94.3 %). These values matched well with checks Asha (96.5 %) and Maruti (98.5 %). The minimum pollen fertility was recorded in ICPH 3494 (58.0 %) followed by ICPH 3491 (64.0 %) (Table 4.4). At Sehore, the variability for pollen fertility ranged from 62.1 to 98.1 %. Among hybrids, the maximum pollen fertility was recorded in ICPH 2671 (95.9 %) followed by ICPH 2740 (95.4 %) and ICPH 2751 (92.6 %). The hybrid ICPH 3494 (62.1 %) was recorded as minimum pollen fertility followed by ICPH 3491 (67.2 %). The checks Maruti (98.1 %) and Asha (97.6 %) recorded high pollen fertility at this location (Table 4.5). The pooled analysis over the locations showed that, the variability for pollen fertility in different hybrids ranged from 58.5 to 98.3 % across the locations. Among hybrids the maximum pollen fertility was recorded in ICPH 2740 (95.9 %) followed by ICPH 2671 (95.3 %) and ICPH 2751 (91.4 %). The minimum pollen fertility was recorded in ICPH 3494 (58.5%) followed by ICPH 3491 (60.1 %). Meanwhile all the hybrids except ICPH 3491 and ICPH 3494 recorded high pollen fertility across the locations indicating their fertility restoration was better over the locations (Table 4.6).

Table 4.3 *Per se* performance of CMS-based pigeonpea hybrids for pollen fertility (%) and other characters at Patancheru.

Genotypes	Pollen fertility (%)	Fully fertile plants (%)	Partial fertile plants (%)	Partial sterile plants (%)	Complete sterile plants (%)	Plant stand
ICPH 2671	93.4	100.0	0.0	0.0	0.0	24
ICPH 2740	96.5	100.0	0.0	0.0	0.0	24
ICPH 3933	93.6	100.0	0.0	0.0	0.0	24
ICPH 2751	86.4	89.5	8.5	1.6	0.0	24
ICPH 3477	83.0	85.5	6.1	1.6	6.1	24
ICPH 3461	90.0	98.9	1.6	0.0	0.0	24
ICPH 3762	83.4	80.2	15.6	3.3	0.0	23
ICPH 4490	81.1	83.6	12.2	4.2	0.0	24
ICPH 3491	48.2	46.8	14.8	17.0	21.3	24
ICPH 3494	55.1	56.3	12.5	12.5	18.7	24
Check						
Asha	97.7	100.0	0.0	0.0	0.0	22
Maruti	98.4	100.0	0.0	0.0	0.0	21
Mean	**86.87**	**93.11**	**4.06**	**2.00**	**1.74**	**23.29**
SEm±	**0.03**	**0.05**	**0.30**	**0.47**	**0.14**	**1.15**
LSD at 5%	**0.10**	**0.18**	**0.95**	**1.47**	**0.44**	**3.60**
CV (%)	**4.14**	**6.90**	**20.31**	**42.17**	**13.42**	**7.02**

Table 4.4 *Per se* performance of CMS-based pigeonpea hybrids for pollen fertility (%) and other characters at Ranchi.

Genotypes	Pollen fertility (%)	Fully fertile plants (%)	Partial fertile plants (%)	Partial sterile plants (%)	Complete sterile plants (%)	Plant stand
ICPH 2671	96.2	100.0	0.0	0.0	0.0	24
ICPH 2740	95.7	100.0	0.0	0.0	0.0	24
ICPH 3933	90.0	100.0	0.0	0.0	0.0	24
ICPH 2751	94.3	100.0	0.0	0.0	0.0	24
ICPH 3477	92.9	100.0	0.0	0.0	0.0	24
ICPH 3461	92.2	100.0	0.0	0.0	0.0	24
ICPH 3762	85.9	100.0	1.6	1.6	0.0	24
ICPH 4490	86.1	86.3	8.4	3.4	0.0	22
ICPH 3491	64.0	64.6	10.3	18.7	6.1	24
ICPH 3494	58.0	53.6	15.6	17.4	12.9	23
Check						
Asha	96.5	100.0	0.0	0.0	0.0	23
Maruti	98.5	100.0	0.0	0.0	0.0	24
Mean	**89.76**	**97.87**	**1.59**	**1.67**	**0.72**	**23.58**
SEm±	**0.04**	**0.01**	**0.30**	**0.40**	**0.19**	**0.80**
LSD at 5 %	**0.12**	**0.05**	**0.95**	**1.25**	**0.60**	**2.51**
CV (%)	**5.08**	**1.53**	**29.71**	**38.48**	**24.43**	**4.48**

Table 4.5 *Per se* performance of CMS-based pigeonpea hybrids for pollen fertility (%) and other characters at Sehore.

Genotypes	Pollen fertility (%)	Fully fertile plants (%)	Partial fertile plants (%)	Partial sterile plants (%)	Complete sterile plants (%)	Plant stand
ICPH 2671	95.9	100.0	0.0	0.0	0.0	21
ICPH 2740	95.4	100.0	0.0	0.0	0.0	20
ICPH 3933	89.8	100.0	0.0	0.0	0.0	20
ICPH 2751	92.6	100.0	0.0	0.0	0.0	19
ICPH 3477	90.7	100.0	0.0	0.0	0.0	20
ICPH 3461	90.6	100.0	0.0	0.0	0.0	19
ICPH 3762	84.6	92.5	5.1	1.8	0.0	20
ICPH 4490	86.2	93.2	6.8	0.0	0.0	22
ICPH 3491	67.2	52.8	23.7	18.3	3.4	19
ICPH 3494	62.1	51.0	19.6	14.5	14.1	21
Check						
Asha	97.6	100.0	0.0	0.0	0.0	18
Maruti	98.1	100.0	0.0	0.0	0.0	19
Mean	**89.48**	**97.14**	**2.31**	**1.29**	**0.65**	**19.54**
SEm±	**0.03**	**0.02**	**0.15**	**0.32**	**0.38**	**0.83**
LSD at 5 %	**0.10**	**0.09**	**0.49**	**1.00**	**1.19**	**2.61**
CV (%)	**4.26**	**3.01**	**13.25**	**33.96**	**50.22**	**6.06**

Table 4.6 Pooled performances of CMS-based pigeonpea hybrids for pollen fertility (%) and other characters, over the locations.

Genotypes	Pollen fertility (%)	Fully fertile plants (%)	Partial fertile plants (%)	Partial sterile plants (%)	Complete sterile plants (%)	Plant stand
ICPH 2671	95.3	100.0	0.0	0.0	0.0	23
ICPH 2740	95.9	100.0	0.0	0.0	0.0	23
ICPH 3933	91.2	100.0	0.0	0.0	0.0	23
ICPH 2751	91.4	98.7	1.7	0.4	0.0	22
ICPH 3477	89.2	98.2	1.3	0.4	1.3	23
ICPH 3461	91.0	99.8	0.4	0.0	0.0	22
ICPH 3762	84.6	94.1	6.3	2.2	0.0	22
ICPH 4490	84.5	88.1	9.0	2.1	0.0	23
ICPH 3491	60.1	55.0	15.8	18.0	8.9	22
ICPH 3494	58.5	53.6	15.8	14.7	15.1	22
Check						
Asha	97.3	100.0	0.0	0.0	0.0	21
Maruti	98.3	100.0	0.0	0.0	0.0	21
Mean	**88.74**	**96.29**	**2.58**	**1.65**	**1.00**	**22.14**
SEm±	**0.02**	**0.02**	**0.15**	**0.23**	**0.15**	**0.55**
CV (%)	**4.54**	**4.20**	**21.40**	**38.89**	**29.92**	**6.05**

4.1.2.2 Fully male fertile plants (%)

In pigeonpea, maximum proportion of fully male fertile plants is a desirable character for CMS-based hybrids. At Patancheru, the fully male fertile plants in different hybrids ranged from 46.8 to100 %. Among the hybrids ICPH 2671, ICPH 2740, and ICPH 3933 recorded 100 % fully male fertile plants followed by ICPH 3461 (98.9 %) and ICPH 2751 (89.5 %). The minimum proportion of fully male fertile plants was observed in ICPH 3491 (46.8 %) followed by ICPH 3494 (56.3 %) (Table 4.3). At Ranchi, the variability for fully male fertile plants within hybrids ranged from 53.6 to 100 %. The maximum (100 %) proportion of fully male fertile plants was observed in all the hybrids except ICPH 4490 (86.3 %), ICPH 3491 (64.6 %) and ICPH 3494 (53.6 %); whereas all other hybrids produced similar proportion of fully male fertile plants compared to both the checks Asha and Maruti (100 %) (Table 4.4). At Sehore, the fully male fertile plants ranged from 51 to 100 %. Among the hybrids ICPH 2671, ICPH 2740, ICPH 3933, ICPH 2751, ICPH 3477 and ICPH 3461 recorded 100 % fully male fertile plants followed by ICPH 4490 (93.2 %) and ICPH 3762 (92.6 %). The minimum proportion of fully male fertile plants was observed in ICPH 3494 (51.0 %) followed by ICPH 3491 (52.8 %) (Table 4.5). The pooled analysis showed that, the performance of hybrids for fully male fertile plants over the locations ranged between 53.7 to 100 %. Among the hybrids 100 % fully male fertile plants were recorded in ICPH 2671, ICPH 2740 and ICPH 3933 followed by ICPH 3461 (99.9 %) and ICPH 2751 (98.7 %) whereas, the minimum number of fully male fertile plants over the locations were recorded in ICPH 3494 (53.7 %) followed by ICPH 3491 (54.9 %). All the hybrids except ICPH 3494 and ICPH 3491 recorded high proportion of fully male fertile plants across the locations (Table 4.6).

4.1.2.3 Partial male fertile plants (%)

In CMS-based hybrids the restoration of fertility and its expression is depends on the genetic purity and restoration ability of the restorers. Therefore, to evaluate the proportion of partial male fertile plants in different hybrids is important to check either the restoration of fertility and its expression on different locations are good and stable or not. The absence of partial male fertile plants is

a desirable character for CMS-based hybrids. At Patancheru, the variability among hybrids for proportion of partial male fertile plants ranged from 0 to 15.9 %. The highest number of partial male fertile plants were recorded in ICPH 3762 (15.6 %) followed by ICPH 3491 (14.8 %) and ICPH 3494 (12.5%) while there were no partial male fertile plants in ICPH 2671, ICPH 2740 and ICPH 3933 which was similar to both the checks Asha and Maruti (Table 4.3). At Ranchi, the performance of hybrids for partial male fertile plants varied from 0 to 15.6 %. The highest number of partial male fertile plants recorded in ICPH 3494 (15.6 %) followed by ICPH 3491 (10.3 %) and ICPH 4490 (8.4 %); while there were no partial male fertile plants in hybrids ICPH 2671, ICPH 2740, ICPH 3933, ICPH 2751, ICPH 3477, ICPH 3461 and ICPH 3762. All these hybrids performed similar to both the checks for this character (Table 4.4). At Sehore, the partial male fertile plants ranged from 0 to 23.7 %. The maximum number of partial fertile plants were recorded in ICPH 3491 (23.7 %) followed by ICPH 3494 (19.6 %) and ICPH 4490 (6.8 %); while there were no partial male fertile plants in hybrids ICPH 2671, ICPH 2740, ICPH 3933, ICPH 2751, ICPH 3477 and ICPH 3461 (Table 4.5). The pooled analysis showed that, the partial male fertile plants ranged from 0 to 15.9 % across the locations and the highest number of partial male fertile plants were recorded in ICPH 3491 (15.9 %) followed by ICPH 3494 (15.8 %) and ICPH 4490 (9.0 %); while there were no partial male fertile plants in hybrids ICPH 2671, ICPH 2740 and ICPH 3933. Meanwhile all the hybrids except ICPH 3494, ICPH 3491 and ICPH 4490 were recorded non-significant proportion of partial male fertile plants indicating the restoration of fertility and its expression over the locations was better in these hybrids (Table 4.6).

4.1.2.4 Partial male sterile plants (%)

The absence of partial male sterile plants is also a desirable character for CMS-based hybrids in pigeonpea. At Patancheru, the partial male sterile plants produced by different hybrids ranged from 0 to 17.0 %. The maximum number of partial male sterile plants were recorded in ICPH 3491 (17.0 %) followed by ICPH 3494 (12.5 %) and ICPH 4490 (4.2 %) while there were no partial male sterile plants in hybrids ICPH 2671, ICPH 2740, ICPH 3933 and ICPH 3461 (Table 4.3).

At Ranchi, the variability for partial male sterile plants recorded in hybrids ranged from 0 to 18.7 %. The maximum number of partial male sterile plants were recorded in ICPH 3491 (18.7 %) followed by ICPH 3494 (17.4 %) and ICPH 4490 (3.4 %); while there were no partial male sterile plants in ICPH 2671, ICPH 2740, ICPH 3933, ICPH 2751, ICPH 3477 and ICPH 3461 which is similar to both the checks Asha and Maruti (Table 4.4). At Sehore, The highest number of partial male sterile plants were recorded in ICPH 3491 (18.3 %) followed by ICPH 3494 (14.5 %) and ICPH 3762 (1.8 %); while there were no partial male sterile plants in remaining all hybrids (Table 4.5). The pooled analysis revealed that, the variability for partial male sterile plants ranged from 0 to 18.0 %. The maximum number of partial male sterile plants were recorded in ICPH 3491 (18.0 %) followed by ICPH 3494 (14.7 %) and ICPH 3762 (2.2 %); while there were no partial male sterile plants in hybrids ICPH 2671, ICPH 2740, ICPH 3933 and ICPH 3461 which was similar to both the checks Asha and Maruti (Table 4.6).

4.1.2.5 Complete male sterile plants (%)

Fertility restoration and its expression are main requisites for development of hybrids based on cytoplasmic male sterility system. Therefore to check the fertility restoration and its stability over the environment it is important to evaluate the hybrids and their progenies either being fertile or sterile. Absence of complete male sterile plants is a desirable character for CMS-based hybrids. At Patancheru, the variability for complete male sterile plants in hybrids ranged from 0 to 21.3 %. The highest number of complete male sterile plants were recorded in ICPH 3491 (21.3 %) followed by ICPH 3494 (18.7 %) and ICPH 3477 (6.1 %); while there were no complete male sterile plants in other hybrids (Table 4.3). Similarly, at Ranchi the complete male sterile plants ranged from 0 to 12.9 % and maximum number of complete male sterile plants were recorded in ICPH 3494 (12.9 %) followed by ICPH 3491 (6.1 %) (Table 4.4). At Sehore, the variability for complete sterile plants recorded in different hybrids ranged from 0 to 14.1 %. The highest number of complete male sterile plants were recorded in ICPH 3494 (14.1 %) followed by ICPH 3491 (3.4 %); while there were no complete male sterile plants in other tested hybrids (Table 4.5). The pooled analysis showed that, the

complete male sterile plants in hybrids ranged between 0 to 15.1 %. The maximum number of complete male sterile plants were recorded in ICPH 3494 (15.1 %) followed by ICPH 3491 (8.9 %) and ICPH 3477 (1.3%); while there were no complete male sterile plants in all other hybrids indicating their better fertility restoration (Table 4.6).

4.1.2.6 Number of plants per plot

Plant stand is not very important character from breeding point of view but it provides valuable information about number of plants present in net plot area at the time of observations recorded. It also provides information related to number of plants survived till the harvesting. In present investigation the plant stand of individual genotype was recorded at the time of observing pollen fertility. At Patancheru, the plant stand of different hybrids ranged from 21 – 24. All the hybrids except ICPH 3762 (23) had maximum plant stand (24) with superiority over both the checks. The minimum plant stand was observed in Maruti (21) followed by Asha (22). All the hybrids were superior over both the checks for plant stand (Table 4.3). At Ranchi, the plant stand ranged from 22 to 24 and the maximum (24) plant stand was recorded in all hybrids except ICPH 2751, ICPH 4490, and ICPH 3494. The minimum plant stand was observed in ICPH 4490 (22) followed by ICPH 3494 (23) (Table 4.4). At Sehore, the highest plant stand was observed in ICPH 4490 (22) followed by ICPH 2671 (21) and ICPH 3494 (21). The minimum plant stand was observed in Asha (18) followed by Maruti (19). All the hybrids had superiority over both the checks for plant stand (Table 4.5). The pooled analysis showed that, the plant stand ranged from 21 to 23. The highest (23) plant stand was recorded in ICPH 2671, ICPH 2740 and ICPH 3933 followed by ICPH 3477 and ICPH 4490 (23) whereas both the checks Asha and Maruti (21) recorded as minimum plants stand. All the hybrids were superior to both the checks for plant stand across the locations (Table 4.6).
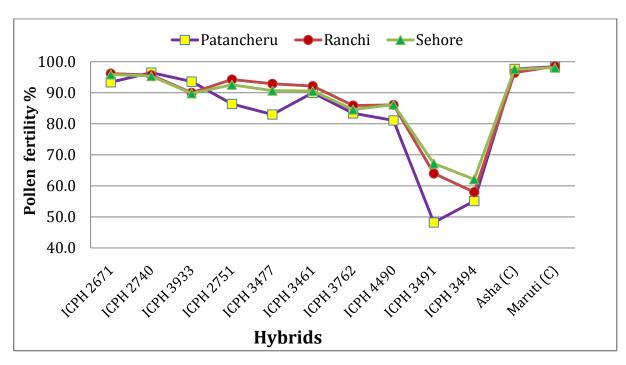
Fig. 4.1 The graph showing pollen fertility status of different CMS-based hybrids of pigeonpea at three different locations.

4.1.3 Stability analysis of hybrids by Eberhart and Russell's (1966) model of stability.

Development of a stable variety is one of the major objectives of all breeding programmes. Phenotypically stable varieties are usefully sought for commercial production of crop plants. In any breeding programme, it is necessary to screen and identify phenotypically stable genotypes, which could perform more or less uniformly under different environmental conditions. Several models have been proposed for stability analysis. According to Eberhart and Russell's (1966) model, a stable variety is one, which has above average mean yield, a regression coefficient of unity (bi=1) and non-significant mean square deviations from regression ($S^2di=0$). The high value of regression (bi>1) indicates that the variety is more responsive for input rich environment, while, low value of regression (bi<1) is an indication that the variety may be adopted in poor environment.

The stability analysis may be more meaningful when the material is tested under various environments. In the present study, a set of 10 hybrids with two checks were evaluated at three locations i.e*.* Patancheru, Ranchi and Sehore during *kharif* 2012. The performance of different genotypes in respect to various characters i.e*.* pollen fertility (%), fully male fertile plants (%), partial male fertile plants (%), partial male sterile plants (%) and complete male sterile plants (%) were studied for estimating stability and significance of genotype × environment interactions.

4.1.3.1 Analysis of variance

The pooled analysis of variance provides an estimate of genotype × environment interaction, which measures changes in rank and magnitude of fluctuations about the mean of different environments. The mean sum of squares due to genotypes were highly significant (P<0.01) for all the characters except for plant stand while the individual environment effect was significant for all characters except partial male sterile plants (%). The mean sums of squares due to genotype × environment interaction were significant for all the characters except partial male sterile plants (%) and plant stand. Thus, stability analysis was

carried out for all the traits except partial male sterile plants (%) and plant stand (Table 4.2).

Analysis of variance for stability (Table 4.7) revealed the existence of substantial variability among the genotypes for pollen fertility (%), fully male fertile plants (%), partial male fertile plants (%) and complete male sterile plants (%) showed that genotypic differences were highly significant for these characters. Significance of genotype × environmental interaction was found for all characters revealed that genotypes interacted significantly with environments (location). The partitioning of interaction showed that both the linear components of interaction (environment and genotype × environment linear) were highly significant. The phenotypic stability of genotypes was estimated by mean performance over the locations, regression coefficient (bi) and deviation from regression (S^2di).

Table 4.7 Analysis of variance (mean sum of square) for phenotypic stability for pollen fertility (%) and other characters of pigeonpea hybrids.

Source	df	Pollen fertility (%)	Fully fertile plants (%)	Partial fertile plants (%)	Complete sterile plants (%)
Genotype	11	0.179**	329.892**	5.264**	51.451**
Environment+ (Genotype × Environments)	24	0.004**	709.381**	0.488*	17.130**
Environment (Linear)	1	0.028**	15837.392**	2.950**	282.245**
Genotype × Environment (Linear)	11	0.006**	103.689**	0.582**	19.917**
Pooled deviation	12	0.001	3.932**	0.196	0.816
Pooled error	33	0.001	0.500	0.070	0.500

*, ** Significant at P≤ 0.05 and P≤ 0.01, respectively

4.1.3.2 Stability for individual characters

Analysis of stability of hybrids indicated that the differences in regression coefficient were found significant for most of the genotypes for all the characters. All the hybrids assessed with checks for their stability based on mean performance, regression coefficient (bi) and deviation from regression line (S^2di) for each character are presented in Table 4.8.

4.1.3.2.1 Pollen fertility (%)

All the hybrids recorded non-significant mean square deviation from regression (S^2di=0) for pollen fertility (%) indicating that the hybrids did not deviate across the locations for pollen fertility. The linear regression was observed non-significant (bi=1) for five hybrids. Among the hybrids, ICPH 2671 and ICPH 3461 were highly stable with high mean, non-significant regression coefficient (bi=1) and zero deviation from regression. Other two hybrids, ICPH 2740 and ICPH 3933 exhibited high mean pollen fertility with significant regression coefficient less than unity (bi<1) and non-significant deviation from regression (S^2di=0) indicating their above average stability and these hybrids can also perform in poor environmental conditions. All over, six hybrids performed above average mean while the other four were below the average mean (Table 4.8). The greater value of regression coefficient (bi>1) with non-significant mean square deviation from regression (S^2di=0) was observed in two hybrids ICPH 2751 and ICPH 3477 indicating that these hybrids showed positive interaction with favorable environment. Across the locations, the stable hybrids for pollen fertility were ICPH 2671 (mean=95.27, bi= 1.422, S^2di= 0.000), ICPH 3461 (mean=90.98, bi=0.630, S^2di=0.001), ICPH 2740 (mean= 95.87, bi= -0.532, S^2di= 0.000) and ICPH 3933 (mean=91.23, bi= -1.591, S^2di= 0.000). All other hybrids were unstable with either greater regression coefficient (bi>1) or below mean pollen fertility (Table 4.8).

Table 4.8 Estimates of stability parameters for pollen fertility (%) and other characters of CMS-based pigeonpea hybrids evaluated during 2012 rainy season

Genotypes	Pollen fertility (%)			Fully male fertile plants (%)			Partial male fertile plants (%)			Complete male sterile plants (%)		
	Mean	b_i (b_i=1)	S^2d_i (S^2d_i=0)	Mean	b_i (b_i=1)	S^2d_i (S^2d_i=0)	Mean	b_i (b_i=1)	S^2d_i (S^2d_i=0)	Mean	b_i (b_i=1)	S^2d_i (S^2d_i=0)
ICPH 2671	95.27	1.422	0.000	100.00	1.102	0.174	0.00	0.000	0.000	0.00	0.391*	0.133
ICPH 2740	95.87	-0.532*	0.000	100.00	1.102	0.174	0.00	0.000	0.000	0.00	0.391*	0.133
ICPH 3933	91.23	-1.591**	0.000	100.00	1.102	0.174	0.00	0.000	0.000	0.00	0.391*	0.133
ICPH 2751	91.39	2.856**	0.000	98.73	1.198**	5.698**	1.67	3.570*	0.378*	0.00	0.391*	0.133
ICPH 3477	89.22	3.192**	0.001	98.25	1.215**	8.399**	1.26	2.892	0.248	1.26	2.183**	6.315**
ICPH 3461	90.98	0.630	0.001	99.85	1.131*	0.203	0.40	1.130	0.038	0.00	0.391*	0.133
ICPH 3762	84.62	0.602	0.000	94.08	1.268**	17.253**	6.29	3.722**	0.004	0.00	0.391*	0.133
ICPH 4490	84.52	1.567	0.000	88.06	0.731**	10.62**	8.96	0.993	0.145	0.00	0.391*	0.133
ICPH 3491	60.11	3.543**	0.002	54.95	0.553**	3.21*	15.85	0.472	1.303**	8.92	3.627**	1.904
ICPH 3494	58.45	0.953	0.002	53.65	0.395**	0.929	15.77	-0.780	0.236	15.12	2.67**	0.369
Asha	97.29	-0.571**	0.001	100.00	1.102	0.174	0.00	0.000	0.000	0.00	0.391*	0.133
Maruti	98.31	-0.071	0.000	100.00	1.102	0.174	0.00	0.000	0.000	0.00	0.391*	0.133
Mean	**88.74**	**1.00**	**-**	**96.29**	**1.00**	**-**	**2.58**	**1.00**	**-**	**1.00**	**1.00**	**-**
SEm±	**0.02**	**0.50**	**-**	**1.40**	**0.05**	**-**	**0.31**	**0.89**	**-**	**0.64**	**0.23**	**-**

Where, b_i= Regression coefficient and S^2d_i = Deviation from regression

*, ** Significant at P≤ 0.05 and P≤ 0.01, respectively

4.1.3.2.2 Fully male fertile plants (%)

The main requisite for successful development of high yielding CMS-based hybrids is to evaluate their stability for fertility restoration under varying environmental conditions. The proportion of fully male fertile plants in different hybrids is an indicator of fertility restoration. The values of linear regression were non-significant (bi=1) for three hybrids while, the non-significant deviations from regression line (S^2di=0) were observed in five hybrids. The most stable hybrids for fully male fertile plants were ICPH 2671, ICPH 2740 and ICPH 3933 (mean=100, bi=1.102, S^2di=0.174) with similarly high mean, non-significant regression coefficient (bi=1) and non-significant deviation from regression (S^2di=0). One another hybrid ICPH 3461 (mean= 99.85, bi= 1.131, S^2di= 0.203) exhibited high mean with regression coefficient close to unity and non-significant deviation from regression line indicating that it was also stable over the locations. The hybrids ICPH 2751 (mean= 98.7, bi= 1.198, S^2di= 5.698) and ICPH 3477 (mean= 98.25, bi= 1.215, S^2di= 8.399) recorded greater performance to average mean with significantly greater regression coefficient (bi>1) and significant deviation from regression line (S^2di>0) showed that these hybrids deviated for proportion of fully male fertile plants which indicating their instability for fully male fertile plants. Likewise, hybrids ICPH 3762 (mean= 94.08, bi=1.268, S^2di= 17.253) and ICPH 4490 (mean= 88.06, bi=0.731, S^2di= 10.62) performed below average mean with significant deviation from regression line (S^2di>0) indicating these hybrids were also unstable for proportion of fully male fertile plants (%). Another two hybrids ICPH 3494 (mean= 53.65, bi=0.395, S^2di= 0.929) and ICPH 3491 (mean= 54.91, bi=0.553, S^2di= 3.210) recorded lowest mean with significant regression coefficient (bi<1) and deviation from regression line (S^2di>0) showed that these hybrids were also unstable with poor fertility restoration (Table 4.8).

4.1.3.2.3 Partial male fertile plants (%)

In pigeonpea, low mean performance and below average linear response are desirable for number of partial male fertile plants because higher number of partial male fertile plants indicate that the restoration of fertility in hybrid is not considerably good and stable. All the hybrids except ICPH 3491 and ICPH 2751

observed non-significant deviation from regression ($S^2di=0$) while the values of regression coefficient were found to be significant for ICPH 2751 and ICPH 3762 among all the hybrids. Six out of 10 hybrids recorded below the average mean, three of them (ICPH 2671, ICPH 2740 and ICPH 3933) had no partial male fertile plants across the locations with non-significant regression coefficient and deviation from regression ($S^2di=0$) showed that these hybrids were highly stable over the locations. Other two hybrids ICPH 3461 (mean=0.04 %, bi=1.130 and $S^2di=0.038$) and ICPH 3477 (mean=1.26 %, bi=2.892 and $S^2di=0.248$) performed below the average mean with non-significant regression coefficient and deviation from regression line indicating these hybrids were also good and stable for partial male fertile plants (%). The higher value of regression indicated that these hybrids would perform better under favorable conditions. Four hybrids were observed above average mean for partial male fertile plants which is undesirable for CMS-based hybrids. The hybrid ICPH 3491 (mean=15.85 %, bi=0.472 and $S^2di=1.303$) had highest mean with significant deviation from regression ($S^2di>0$) while ICPH 3494 (mean=15.77 %, bi= -0.780 and $S^2di=0.236$) recorded high mean with non-significant deviation from regression ($S^2di=0$) indicating that these two hybrids performed poor and unstable across the location. Five out of 10 hybrids were stable for partial male fertile plants with below mean performance, non-significant regression coefficient and deviation from regression (Table 4.8).

4.1.3.2.4 Complete male sterile plants (%)

Seven out of 10 hybrids had no complete male sterile plants with significant regression coefficient less than unity (bi<1) and non-significant mean square deviation ($S^2di=0$) indicating their above average stability for complete male sterile plants across the locations. Other three hybrids recorded high mean for complete male sterile plants as compared to average mean with significant regression coefficient greater than unity (bi>1) showed that these hybrids were unstable and more responsive in favorable environments. Two hybrids ICPH 3494 (mean=15.12 %, bi=2.670 and $S^2di=0.369$) and ICPH 3491 (mean=8.92 %, bi=3.627 and $S^2di=1.904$) observed higher proportion of complete male sterile plants with significantly greater regression coefficient (bi>1) indicating these

hybrids had poor fertility restoration and unstable for complete male sterile plants while ICPH 3477 (mean= 1.26 %, bi= 2.183 and S^2di= 6.315) recorded above average mean with significant regression coefficient (bi>1) and significantly greater deviation from regression ($S^2di>0$) indicating it was also unstable for complete male sterile plants (Table 4.8).

4.1.4 Stability analysis by GGE Biplot method (Yan *et al.,* 2000)

A phenotype is a result of genotype (G) and environment (E) components and the interactions (G × E) between them. Genotype × Environmental interaction complicates the process of selecting genotypes with superior performance. Consequently, METs (multi-environment trials) are widely used by plant breeders to evaluate the relative performance of genotypes for target environments (Delacy *et al.,* 1996). Numerous methods have been proposed to reveal patterns of G × E interaction, such as joint regression (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966; Perkins and Jinks, 1968), additive main effects and multiplicative interaction (AMMI, Gauch, 1992). These methods are commonly used to analyze METs data and have also been applied on G × E interaction.

GGE biplot analysis was recently developed to use some of the functions of these methods jointly. In total phenotypic variation, "E" explains most of the variation and "G" and "GE" are usually small (Yan, 2002). However, only the "G" and "G×E" interaction are relevant to cultivar evaluation particularly when G×E interaction is identified as repeatable (Hammer and cooper 1996). Hence, Yan *et al.* (2000) deliberately put two together and referred to the mixture as GGE. Following the proposal of Gabriel (1971), the biplot technique was used to display the GGE of a METs data, referred to as a GGE biplot (Yan, 2001; Yan *et al.*, 2000).

The data from multi environment trials are usually large, and their graphical presentation helps to understand the pattern involved in particular data set. The GGE biplot allows visual examination of the GE interaction pattern of multi environment trials data. To construct a meaningful biplot, PC1 and PC2 eigenvectors were plotted after partitioning of singular values into the genotype

and environment eigenvectors. Theoretically, the partitioning factors can take any value between 0 and 1. However, for this analysis, a value of 0.5 was used to give equal importance to both, the genotypes as well as environments.

The mean sum of square due to genotypes was highly significant for all the characters except for plant stand. The mean sum of squares due to genotype by environment interaction was significant for all the characters except partial sterile plants (%) and plant stand. Only those characters were analyzed for stability by GGE biplot which showed significant genotype × environmental interaction. The details of genotype code, name, parentage and source of 10 pigeonpea hybrids and two varietal checks used in present investigation are given below (Table 4.9)

Table: 4.9 Details of genotype code, name, parentage and source of 12 pigeonpea genotypes:

Genotype code	Genotype name	Parentage	Type of genotype	Source
1	ICPH 2671	ICPA 2043 × ICPL 87119	Hybrid	ICRISAT
2	ICPH 2740	ICPA 2047 × ICPL 87119	Hybrid	ICRISAT
3	ICPH 3933	ICPA 2078 × ICPL 87119	Hybrid	ICRISAT
4	ICPH 2751	ICPA 2048 × ICPL 87119	Hybrid	ICRISAT
5	ICPH 3477	ICPA 2047 × ICPL 20098	Hybrid	ICRISAT
6	ICPH 3461	ICPA 2092 × ICPL 87119	Hybrid	ICRISAT
7	ICPH 3762	ICPA 2092 × ICPL 20108	Hybrid	ICRISAT
8	ICPH 4490	ICPA 2047 × ICPL 20126	Hybrid	ICRISAT
9	ICPH 3491	ICPA 2048 × ICPL 20096	Hybrid	ICRISAT
10	ICPH 3494	ICPA 2048 × ICPL 20093	Hybrid	ICRISAT
11	Asha (check)	C11 x ICP 1-6W3B	Variety	ICRISAT
12	Maruti (check)	ICP 8863	Variety	ICRISAT

4.1.4.1 Pollen fertility (%)

The biplot analysis, as viewed the environment-vector of hybrids, has been shown in Fig 4.2. The results of PCA of GEI (genotype × environment interaction) showed that the first two principal components in the biplot explained 98.9 % of the total variation in GEI (Cooper and Delacy, 1994).

(a) Polygon view of GGE biplot analysis of MET data of pollen fertility (%)

The polygon view of a biplot is the best way to visualize the interaction patterns between genotypes and environments (Yan and Kang, 2003) to show the presence or absence of cross over GE interaction which is helpful in estimating the possible existence of different mega environments (Gauch and Zobel, 1997; Yan and Rajcan, 2002; Yan and Tinker, 2006). Visualization of the "which won where" pattern of MET data is necessary for studying the possible existence of different mega environments in the target environment (Gauch and Zobel, 1997; Yan *et al.*, 2000). Fig. 4.2 represents a polygon view of MET data for pollen fertility of different hybrids in this investigation. In this biplot, a polygon was formed by connecting the vertex of genotypes with straight lines and the rest of the genotypes placed within the polygon. The partitioning of GE interaction through GGE biplot analysis showed that PC1 and PC2 accounted for 97.88 and 2.02 % of GGE sum of squares, respectively, explaining a total of 99.89 % variation. The vertex genotypes in this study were 12, 11, 3, 10, 9, 5, and 4. These genotypes were the best or the poorest genotypes in some or all of the environments because they were farthest from the origin of the biplot (Yan and Kang, 2003). From the polygon view of biplot analysis of MET data in three locations, the genotypes were scattered in all four sections whereas the test environments positioned in two sections. The first section contains the test environments Ranchi and Sehore which had the genotype 12 as the winner and the second section contains the environments Patancheru with also the genotype 12 as the best. The vertex genotype 9 and 10 were not the good genotypes for pollen fertility in any environment (Fig. 4.2).

(b) Mean yield and stability performance of genotypes for pollen fertility

The ranking of 10 pigeonpea hybrids and the two standard check cultivars based on their mean pollen fertility and stability performance has been shown in Fig. 4.2. The genotypes more close to concentric circle indicates higher mean yield. The line which passes through the origin and is perpendicular to the AEA (Average Environment Axis) represents the stability of genotypes. Either direction away from the biplot origin on this axis indicates greater GE interaction and reduced stability. The genotypes on the right side of the perpendicular line (Fig. 4.2) performed greater than mean pollen fertility and the genotypes on the left side of this line had pollen fertility less than mean. For selection, the ideal genotypes are those with both high mean pollen fertility and high stability. In the biplot, they are close to the origin and have the shortest vector length from the AEA. The genotypes 12, 11, 1, 6 and 2 were highly stable with both high mean pollen fertility and shortest vector length from AEA. The genotypes with lower pollen fertility and greater vector length from AEA were 10 and 9 indicating their instability for pollen fertility whereas the genotypes with below to average pollen fertility and shorter vector length from AEA were 8 and 7 indicating that these hybrids were also unstable for pollen fertility across the locations. The genotype 11 (with relatively high performance) and 8 (with low performance) showed similar GE interaction. A breeder can also use Fig. 4.2 for selecting the genotypes with the best response to particular environments. For instance the genotype 11, 2 and 3 had the highest pollen fertility in Patancheru than other two locations and the genotype 4 and 5 performed well in Ranchi and Sehore than Patancheru showed their specific adaptability in these two environments.
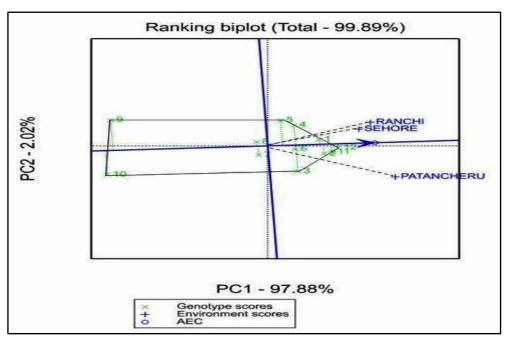
(c) Evaluation of genotypes for pollen fertility relative to an ideal genotype

An ideal genotype should have the highest mean performance and be absolutely stable (Yan and Kang, 2003). Such an ideal genotype is defined by having the greatest vector length of the high-yielding genotypes and with zero GEI (or highest stability), as represented by the concentric circle with an arrow pointing to it (Fig. 4.2). An ideal genotype, which is located at the center of the concentric circles, is the one that has both high mean performance and high

stability. Ideal genotype projection on the AEA X-axis is designed to be equal to the longest vector of all the genotypes. The ideal genotype is stable because its projection on the AEA Y-axis is near zero. A genotype is more favorable if it is closer to the ideal genotype. The genotype 12 was near to concentric circle hence it was an ideal genotype. Ranking of other genotypes based on the ideal genotype was 11>1>2> 6. In other words, the lower performing genotypes (9 and 10) were unfavorable because they are far from the ideal genotype (Fig 4.2). The relative contributions to the identification of desirable genotype for pollen fertility found in this study by the ideal genotype procedure of the GGE biplot are similar to those found in other stability studies (Samonte *et al.*, 2005; Fan *et al.*, 2007).

(d) Relationship among test environments

The summary of the interrelationships among the test environments has been provided in Fig. 4.2. The lines that connect the biplot origin and the markers for the environments are called environment vectors. The angle between the vectors of two environments is related to the correlation coefficient between them. The cosine of the angle between the vectors of two environments approximates the correlation coefficient between them (Kroonenberg, 1995; Yan, 2002). Acute angles ($<90^{0}$) indicate a positive correlation, obtuse angles ($<90^{0}$) a negative correlation and right angles ($=90^{0}$) indicate no correlation (Yan and Kang, 2003). A short vector may indicate that the test environment is not related to other environments. Based on the angles between environment vectors, all the three environments (Patancheru, Ranchi and Sehore) were positively correlated with each other because of acute angles ($<90^{0}$) formed between them.

Fig. 4.2 GGE biplot showing the ranking of genotypes for both mean pollen fertility and stability performance over the environments. The line passing through the biplot origin is called the average environment coordinate (AEC). Polygon view of G × E interaction for pollen fertility showed that the vertex genotype in each sector is the best genotype at environments whose markers fall into the respective sector. Environments within the same sector share the same winning genotype, and environments in different sectors have different winning genotypes. More close to concentric circle indicates higher mean yield.

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4.1.4.2 Fully male fertile plants (%)

The biplot analysis, as viewed the environment-vector of hybrids, has been shown in Fig 4.3. The results of PCA of GEI (genotype × environment interaction) showed that the first two principal components in the biplot explained 99.39 % (PC1= 95.89 %, PC2= 3.50 %) of the total variation in GEI (Cooper and Delacy, 1994).
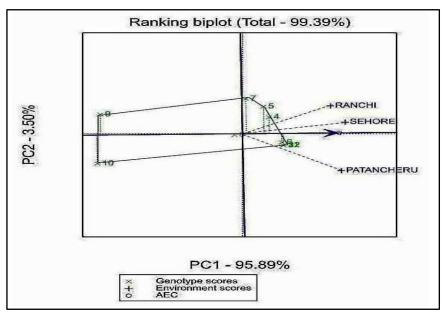
(a) Evaluation of genotypes based on GGE biplot

The position and perpendicular projection of genotypic points onto an environmental vector can be used to identify a genotype or genotypes having specific adaptation in that environment (Yan *et al.*, 2000). The genotypes that are farther along the positive direction of the vector tend to give higher performance, and are better adapted to those environments. The hybrids 1, 2, 3, and 6 had the highest and closely similar mean performance with shortest vector length from AEA and equal distant with all environments indicating that these hybrids were stable for fertility restoration across the locations. Among the hybrids, 7, 5 and 4 had high mean performance but away from AEA showed that these hybrids were unstable but they are near to Ranchi and Sehore indicating their specific adaptability to both the environments (Fig. 4.3). The genotype 10 and 9 observed performance below mean and had longest distance from the AEA indicating their instability and poor fertility restoration under all environments.

(b) Environment evaluation based on GGE biplot:

The pattern of environment in the biplot (Fig. 4.3) suggests that all the environments were clustered in a single group. In present study, as the angle between any two environments was less than 90^0, it suggested that GE was moderately small and these environments tend to discriminate among genotypes in a similar manner. The Sehore and Ranchi environment vectors positioned above the AEA with smaller angle ($<45^0$) between them indicated presence of positive correlation. Similarly, the Patancheru environment vector was found below the AEA and positively correlated with Sehore and Ranchi. It indicates that stability could be assessed by testing at either Sehore or Ranchi and Patancheru.
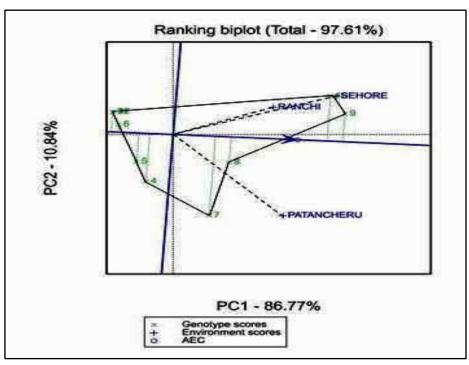
Patancheru had the longest environment vector which demonstrated more discriminating ability than other environments. The environments of Sehore and Ranchi were most discriminating along with smaller angle with the AEA and the genotypes nearer to these two environment vectors exhibited stability for fully fertile plants; whereas Patancheru had larger angle with AEA as compared to other two environments indicating that it was discriminating among all the environments.

Fig. 4.3 GGE biplot showing the ranking of genotypes for mean and stability performance for fully male fertile plants (%) over the locations

4.1.4.3 Partial male fertile plants (%)
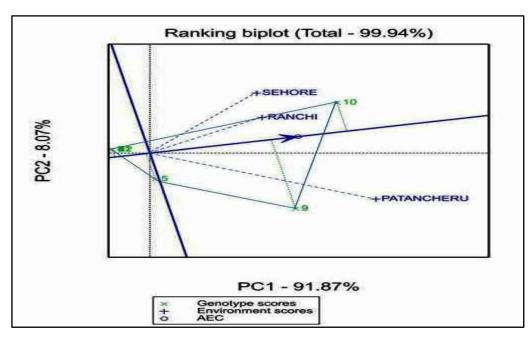
The GGE biplot ranking for stability and mean performance of genotypes for partial male fertile plants (%) has been presented in Fig. 4.4. The results of PCA of GEI (genotype × environment interaction) showed that the first two principal components in the biplot explained 97.61 % (PC1= 86.77 %, PC2= 10.84 %) of the total variation. Among the genotypes 9, 10, 8 and 7 had the high mean performance with longer vector length from AEA showed that these hybrids were unstable for partial male fertile plants. The hybrids 9 and 10 are near to Sehore and Ranchi indicating their highest performance in both the environments respectively (Fig 4.2), whereas hybrids 7 and 8 are positioned near to Patancheru indicating their higher performance in this environment. The hybrids 1, 2, 3, 6 and 5 had the low mean performance with shortest vector length from AEA indicating their stability for partial male fertile plants. The hybrid 4 had performed below the mean with greater vector length indicating it was unstable and performed more in Patancheru environment. The result revealed that the genotypes present in left side of perpendicular line with shorter vector length were the best and stable across the locations. The environments Ranchi and Sehore were positioned above the AEA with smallest angle (<45º) between them indicated presence of positive correlation between them. Similarly, the Patancheru environment vector was found below the AEA and positively correlated (<90º) with Ranchi and Sehore.

Fig 4.4 GGE biplot showing the ranking of genotypes for mean and stability performance for partial male fertile plants (%) over the locations.

4.1.4.4 Complete male sterile plants (%)

The GGE biplot ranking for stability and mean performance of genotypes for complete male sterile plants (%) has been presented in Fig. 4.5. The results of PCA of GEI showed that the two principal components in the biplot explained 99.94 % (PC1= 91.87 %, PC2= 8.07 %) of the total variation. All the genotype except 9 and 10 were observed below the population mean for proportion of complete male sterile plants. The hybrid 10 and 9 had highest mean for complete male sterile plants with greater vector length from AEA indicating their instability for complete male sterile plants and poor fertility restoration. The hybrid 10 was positioned near to Sehore and Ranchi indicate that this genotype performed more in both the locations whereas the genotype 9 was positioned near to Patancheru indicating that it was more responsive in Patancheru environment. The hybrids 1, 2, 3, 4, 6, 7, and 8 were highly stable across the locations with lower proportion of complete male sterile plants and shortest vector length from AEA. It also indicates their better fertility restoration across the locations. The genotype 5 was observed below the average mean with greater projection from AEA indicating its instability for complete male sterile plants across the location.

Fig. 4.5 GGE biplot showing the ranking of genotypes for mean and stability performance for complete male sterile plants (%) in hybrids over the locations.

4.2 Stability studies of CMS lines

The experiment for stability of CMS lines was conducted at Patancheru under three different dates of sowing (7th August, 11th September and 18th October) in 2012. The data collected individually from different sowings. In present investigation, a set of nine CMS lines were evaluated in three different dates of sowing during 2012 rainy season for stability analysis. The performance of different CMS lines in respect to different characters i.e. pollen sterility (%), complete male sterile plants (%), partial male sterile plants (%), partial male fertile plants (%) and fully male fertile plants (%). The data recorded on following characters showed that there was very small proportion of partial male sterile plants (%) and fully male fertile plants (%) found in only one CMS line while there were no partial male fertile plants recorded in all three sowings therefore the analysis could not be performed for these three characters. Other characters were statistically analyzed for estimating the stability and significance of genotype × environment interactions.

4.2.1 Analysis of variance

The mean performance of CMS lines for the characters studied was analyzed statistically and the genotypic differences were found to be significant (P<0.05) for pollen sterility (%) in all three dates of sowings. Whereas for complete sterile plants (%) significant genotypic differences were observed in first and second sowing and the non-significant genotypic difference was observed in third sowing (Table 4.10). The mean genotypic values from different locations were subjected to pooled analysis. The result of pooled analysis of variance reveled that genotypic differences were highly significant for both the characters. From pooled analysis of variance, it was seen that the genotypic differences were highly significant for both the characters whereas, the individual environment effects were highly significant only for pollen sterility (%). The result showed that there was significant G×E interaction for pollen sterility (%) while, it was observed non-significant for complete male sterile plants (%) hence it was not further analyzed for stability (Table 4.11).

Table 4.10 Mean sum of squares for pollen sterility (%) and other characters of CMS lines in three different dates of sowing.

Traits	I Sowing		II Sowing		III Sowing	
	Genotype	Error	Genotype	Error	Genotype	Error
Degree of freedom	8	8	8	8	8	8
Pollen sterility (%)	0.034**	0.001	0.021*	0.004	0.008**	0.001
Complete male sterile plants (%)	0.046**	0.004	0.026**	0.001	0.004	0.004

*, ** Significant at P≤ 0.05 and P≤ 0.01 respectively,
Note- The character partial male sterile plants (%), partial male fertile plants (%) and fully male fertile plants (%) could not be analyzed because most of the values were zero for these three characters.

Table 4.11 Analysis of variance over the different dates of sowing (F values)

Source of variation	df	Pollen sterility (%)	Complete male sterile plants (%)
Replication	3	2.63	1.12
Genotype	8	25.20**	19.57**
Environment	2	20.11**	2.42
Environment × genotype	16	2.66*	1.55

*, ** Significant at P≤ 0.05 and P≤ 0.01, respectively

4.2.2. Mean performance of CMS lines

The location wise as well as pooled *per se* performance of CMS lines for pollen sterility and other characters are given in Table 4.12 - 4.15. All the CMS lines were analyzed only for pollen sterility (%) and complete male sterile plants (%). 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 analyzed and the results of these characters are described on the basis of mean performance. The results according to individual characters are described below.

4.2.2.1 Pollen sterility (%)

Analysis of variance revealed that there was significant genotypic difference present among all the CMS lines for pollen sterility across the different dates of sowing. In first sowing, the variability for pollen sterility ranged from 93.3 to 100 %. The 100 % pollen sterility was recorded in BRG1A, HyC3A, BRG3A and TTB7A followed by ICPA 2092 (99.1 %) and ICPA 2039 (99.0 %) whereas, the minimum pollen sterility was recorded in ICPA 2047 (93.3 %) followed by ICPA 2051 (96.2 %) (Table 4.12). In second sowing, the variability for pollen sterility ranged from 94.9 to 100 %. Among the CMS lines BRG1A, HyC3A, BRG3A and TTB7A had 100 % pollen sterility followed by ICPA 2039 (99.9 %) and ICPA 2092 (99.5 %) while ICPA 2047 (94.9 %) recorded minimum pollen sterility (%) (Table 4.13). In third sowing, the 100 % pollen sterility was recorded in ICPA 2039, BRG3A, HyC3A, BRG1A and TTB7A followed by ICPA 2051 (99.9 %) and ICPA 2092 (99.8 %) while the minimum pollen sterility (%) recorded in ICPA 2047 (98.1 %) (Table 4.14). The pooled analysis revealed that, the pollen sterility ranged between 95.5 to 100 % across the different sowings. The maximum (100 %) pollen sterility recorded in CMS lines BRG3A, HyC3A, BRG1A and TTB7A showed that these lines were performed superior and unable to produce fertile pollen grains in all three different sowings whereas ICPA 2047 (95.7 %) had minimum pollen sterility across the sowings. All the CMS lines exhibited high (>95 %) pollen sterility (Table 4.15).

Table 4.12 *Per se* performance of CMS lines for pollen sterility (%) and other characters in first sowing (August 7, 2012).

CMS lines	Pollen sterility (%)	Complete sterile plants (%)	Partial sterile plants (%)	Partial fertile plants (%)	Fully fertile plants (%)
ICPA 2039	99.0	100.0	0.0	0.0	0.0
ICPA 2043	98.3	98.9	2.1	0.0	0.0
ICPA 2047	93.3	89.7	4.2	0.0	6.3
ICPA 2051	96.2	100.0	0.0	0.0	0.0
ICPA 2092	99.1	100.0	0.0	0.0	0.0
BRG3A	100.0	100.0	0.0	0.0	0.0
HyC3A	100.0	100.0	0.0	0.0	0.0
BRG1A	100.0	100.0	0.0	0.0	0.0
TTB7A	100.0	100.0	0.0	0.0	0.0
Mean	**99.16**	**99.72**	**0.69**	**0.00**	**0.69**
LSD at 5 %	**0.10**	**0.15**	**NA**	**NA**	**NA**
SEm±	**0.03**	**0.05**	**-**	**-**	**-**
CV (%)	**2.98**	**4.48**	**-**	**-**	**-**

NA= Not analyzed

Table 4.13 *Per se* performance of CMS lines for pollen sterility (%) and other characters in second sowing (September 11, 2012).

CMS lines	Pollen sterility (%)	Complete sterile plants (%)	Partial sterile plants (%)	Partial fertile plants (%)	Fully fertile plants (%)
ICPA 2039	99.9	100.0	0.0	0.0	0.0
ICPA 2043	99.2	100.0	0.0	0.0	0.0
ICPA 2047	94.9	93.9	2.1	2.1	2.1
ICPA 2051	99.1	100.0	0.0	0.0	0.0
ICPA 2092	99.5	100.0	0.0	0.0	0.0
BRG3A	100.0	100.0	0.0	0.0	0.0
HyC3A	100.0	100.0	0.0	0.0	0.0
BRG1A	100.0	100.0	0.0	0.0	0.0
TTB7A	100.0	100.0	0.0	0.0	0.0
Mean	**99.65**	**99.88**	**0.23**	**0.23**	**0.23**
LSD at 5 %	**0.14**	**0.07**	**NA**	**NA**	**NA**
SEm±	**0.04**	**0.02**	**-**	**-**	**-**
CV (%)	**4.03**	**1.88**	**-**	**-**	**-**

NA - Not analyzed

Table 4.14 *Per se* performance of CMS lines for pollen sterility (%) and other characters in third sowing (October 18, 2012)

CMS lines	Pollen sterility (%)	Complete sterile plants (%)	Partial sterile plants (%)	Partial fertile plants (%)	Fully fertile plants (%)
ICPA 2039	100.0	100.0	0.0	0.0	0.0
ICPA 2043	99.6	100.0	0.0	0.0	0.0
ICPA 2047	98.1	98.9	0.0	0.0	2.1
ICPA 2051	99.9	100.0	0.0	0.0	0.0
ICPA 2092	99.8	100.0	0.0	0.0	0.0
BRG3A	100.0	100.0	0.0	0.0	0.0
HyC3A	100.0	100.0	0.0	0.0	0.0
BRG1A	100.0	100.0	0.0	0.0	0.0
TTB7A	100.0	100.0	0.0	0.0	0.0
Mean	**99.88**	**99.97**	**0.00**	**0.00**	**0.23**
LSD at 5 %	**0.07**	**0.15**	**NA**	**NA**	**NA**
SEm±	**0.02**	**0.05**	**-**	**-**	**-**
CV (%)	**2.08**	**4.27**	**-**	**-**	**-**

NA - Not analyzed

Table 4.15 Pooled performances of CMS lines for pollen sterility (%) and other characters in three different dates of sowing in 2012.

CMS lines	Pollen sterility (%)	Complete sterile plants (%)	Partial sterile plants (%)	Partial fertile plants (%)	Fully fertile plants (%)
ICPA 2039	99.8	100.0	0.0	0.0	0.0
ICPA 2043	99.1	99.8	0.7	0.0	0.0
ICPA 2047	95.7	94.9	2.1	0.7	3.5
ICPA 2051	98.9	100.0	0.0	0.0	0.0
ICPA 2092	99.5	100.0	0.0	0.0	0.0
BRG3A	100.0	100.0	0.0	0.0	0.0
HyC3A	100.0	100.0	0.0	0.0	0.0
BRG1A	100.0	100.0	0.0	0.0	0.0
TTB7A	100.0	100.0	0.0	0.0	0.0
Mean	**99.62**	**99.88**	**0.31**	**0.08**	**0.39**
SEm±	**0.02**	**0.02**	**NA**	**NA**	**NA**
CV (%)	**3.13**	**3.73**	**-**	**-**	**-**

NA- Not analyzed

4.2.2.2 Complete male sterile plants (%)

For successful production of CMS-based hybrids, the stability of CMS lines for expression of male sterility is as much important as the stability of fertility restoration in hybrids therefore it is main requisite to evaluate the CMS lines for proportion complete male sterile plants (%). It provides an idea about number of complete male sterile plants produced by CMS lines which clearly indicates the status of CMS line for expression of male sterility. The results obtained from present investigation revealed that the mean performance of different CMS lines for complete male sterile plants in first sowing ranged from 89.7 to 100 %. All the CMS lines except ICPA 2043 and ICPA 2047 recorded 100 % complete male sterile plants whereas the CMS lines ICPA 2047 (89.7 %) recorded minimum proportion of complete male sterile plants followed by ICPA 2043 (98.9 %) (Table 4.12). In second sowing, the complete male sterile plants ranged from 93.9 to 100 %. All the CMS lines except ICPA 2047 recorded 100 % complete male sterile plants when sown in September month (Table 4.13). All the CMS line except ICPA 2047 had 100 % complete male sterile plants in third sowing (Table 4.14). The results obtained from pooled analysis revealed that the proportion of complete sterile plants in different CMS lines ranged between 94.9 to 100 %. All the CMS line except ICPA 2047 (94.9 %) and ICPA 2043 (99.8 %) recorded 100 % complete male sterile plants across the different dates of sowing indicating that these lines were highly stable for expression of male sterility (Table 4.15).

4.2.2.3 Partial male sterile plants (%)

For the quality seed production of hybrids, CMS lines should be stable for expression of male sterility and unable to produce fertile pollen grains. Evaluation of partial male sterile plants provides us information about number of partial male sterile plants produced by a CMS lines in different dates of sowing. All the CMS lines except ICPA 2043 (2.1 %) and ICPA 2047 (4.2 %) had no partial male sterile plants in first sowing (Table 4.12) whereas in second sowing, only ICPA 2047 had partial male sterile plants (2.1 %) and all other CMS lines were unable to produce partial male sterile plants (Table 4.13) while there were no partial male sterile

plants in all CMS lines of third sowing (Table 4.14). The results obtained from pooled analysis revealed that all the CMS lines except ICPA 2043 (0.7 %) and ICPA 2047 (2.1 %) recorded no partial male sterile plants across the different sowings showed that all the CMS lines exhibited complete male sterility (Table 4.15).

4.2.2.4 Partial male fertile plants (%)

All the CMS lines had non-significant proportion of partial male fertile plants across the different dates of sowing. Except ICPA 2047 in second sowing, the CMS lines had no partial male fertile plants in all three sowings (Table 4.12 to 4.14). The CMS line ICPA 2047 only produced 2.1 % partial fertile plants in second sowing (Table 4.13). Pooled analysis revealed the same result with ICPA 2047 which produced 0.7 % partial male fertile plants across the three sowings. As the absence of partial male fertile plants in CMS lines is a desirable character showed that all the CMS lines were stable for expression of male sterility (Table 4.15).

4.2.2.5 Fully male fertile plants (%)

A fully male fertile plant in CMS lines is undesirable character which indicates their poor performance for expression of male sterility. In present study, all CMS lines except ICPA 2047 were unable to produce fully male fertile plants in all three dates of sowing (Table 4.12 to 4.14). The highest proportion (6.3 %) of fully male fertile plants in first sowing was recorded in ICPA 2047 whereas it was recorded similar (2.1 %) proportion of fully male fertile plants in second and third date of sowing. The result of pooled analysis showed that all the CMS lines except ICPA 2047 (3.5 %) recorded no fully male fertile plants indicating that all these lines were unable to produce fertile plants and performed very well for expression of male sterility (Table 4.15).
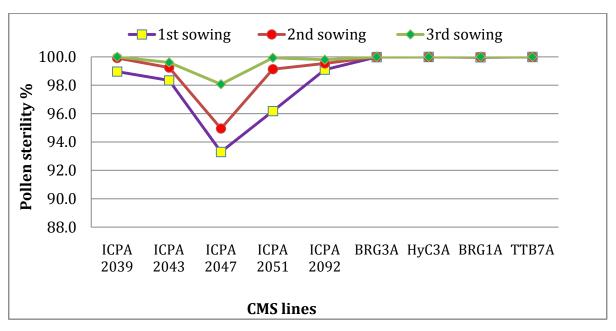
Fig. 4.6 The graph showing pollen sterility status of nine CMS lines of pigeonpea in three different dates of sowing.

4.2.3 Stability analysis of CMS lines by Eberhart and Russell's (1966) model

The stable CMS lines identified can be used as parents in the future breeding programmes for developing suitable high yielding hybrids with wider adaptability. Environment is a major factor which affects expression of male sterility of CMS lines. As the temperature and day length increased under short days, which result in decreased pollen sterility (%) and vice versa (Basha *et al.*, 2008). It is essential to have more precise information on the effect of temperature on male-sterility in order to utilize this CMS system in production of commercial hybrids. Therefore the objective of this study was to determine the influence of different month temperature on the expression of male-sterility of CMS lines of pigeonpea. The phenotypic stability of CMS lines for expression of male-sterility in different dates of sowing was estimated by mean performance over the locations (x), the regression coefficient (bi) and deviation from regression (S^2di).

4.2.3.1 Analysis of variance

The pooled analysis of variance provides an estimate of genotype × environment interaction, which measures changes in rank and magnitude of fluctuations about the mean of different sowings. The mean sum of square due to genotypes was highly significant for all the characters studied. The result showed that the individual environment effect was significant only for pollen sterility (%). The mean sum of squares due to genotype × environment interaction was significant for pollen sterility (%). Thus stability analysis was carried out only for this character.

Analysis of variance for stability revealed the existence of substantial variability among the genotypes for pollen sterility (%). Significance of genotype × environmental interaction revealed that genotypes interacted significantly with environments (different dates of sowing). The partitioning of interaction showed that both the linear components (environment and genotype × environment) were highly significant for this character (Table 4.16).

Table 4.16 Analysis of variance (mean sum of square) for phenotypic stability of CMS lines of pigeonpea for pollen sterility (%).

Source	df	Pollen sterility (%)
Genotype	8	0.027**
Environment+ (Genotype ×Environments)	18	0.003**
Environment (Linear)	1	0.030**
Genotype × Environment (Linear)	8	0.004**
Pooled deviation	9	0.000
Pooled error	24	0.001

*, ** Significant at P≤ 0.05 and P≤ 0.01, respectively

4.2.3.2 Stability for individual characters

All the CMS lines were assessed for their stability performance based on mean performance, regression coefficient (bi) and deviation from regression (S^2di) for pollen sterility (Table 4.17). The result of stability of CMS lines indicated that the regression coefficients were found significant in most of the CMS lines for pollen sterility.

4.2.3.2.1 Pollen sterility (%)

All the CMS lines were recorded non-significant mean square deviation from regression line (S^2di=0) for pollen sterility while the linear regression was observed significant for all CMS lines except ICPA 2043 and ICPA 2092. The CMS lines BRG3A, HyC3A, BRG1A and TTB7A exhibited highest pollen sterility with significant regression coefficient less than unity (bi<1) and non-significant (S^2di=0) deviation from regression indicating their above average stability and these lines can also perform in poor environmental conditions. Two out of five lines of ICRISAT had significant regression coefficient more than unity (bi>1) with non-significant mean square deviation from regression (S^2di=0) indicating their instability and positive interaction with the favorable environment. Over all six out of nine CMS lines had above average mean performance while two had performance around the average mean. The CMS line ICPA 2039 had high mean pollen sterility with regression coefficient close to unity and non-significant

deviation from regression (S^2di=0) indicating its stability for pollen sterility. Two CMS lines, ICPA 2043 and ICPA 2092 performed very close to average mean with non-significant regression coefficient and deviation from regression showed that these lines were also stable across the different dates of sowing. CMS lines ICPA 2047 and ICPA 2051 performed below the mean with significantly greater regression coefficient (bi>1) showed that these lines were unstable and more responsive to the favorable environments (Table 4.17).

Table 4.17 Estimates of stability parameters for pollen sterility (%) of CMS lines of pigeonpea evaluated during 2012-13 rainy season.

CMS lines	Pollen sterility (%)		
	Mean	bi (bi=1)	S^2di (S^2di=0)
ICPA 2039	99.8	1.684*	0.001
ICPA 2043	99.1	1.157	0.000
ICPA 2047	95.7	2.047**	0.002
ICPA 2051	98.9	2.974**	0.000
ICPA 2092	99.5	0.855	0.000
BRG3A	100.0	0.095**	0.000
HyC3A	100.0	0.000**	0.000
BRG1A	100.0	0.188*	0.000
TTB7A	100.0	0.000**	0.000
Mean	99.62	1.00	-
SEm±	0.011	0.27	-

Where, bi= Regression coefficient and S^2di = Deviation from regression
*, ** Significant at P= 0.05 and 0.01, respectively

4.2.4 Stability analysis by GGE Biplot method (Yan *et al.,* 2000)

Performance and stability of CMS lines were visualized graphically through GGE biplot (Fig. 4.7). The line with single arrow head is the AEC abscissa which passes through the biplot origin and marker for average environment and pointing towards higher mean values. The perpendicular line to the AEA passing through the biplot origin is referred as an indicator of stability. The greater absolute length of the projection of a cultivar showed less stable it is. Furthermore, the average yield of genotypes is approximated by the projections of their markers to the AEC abscissa (Kaya *et al.,* 2006).

The pooled analysis of variance provides an estimate of genotype × environment interaction, which measures change in rank and magnitude of fluctuations about the mean of different environments. The mean sum of square due to genotypes was highly significant for both the characters. The mean sum of square due to genotype by environment interaction was significant only for pollen sterility (Table 4.11). Therefore the GGE biplot analysis was performed only for pollen sterility to assess the stability of CMS lines for this character.
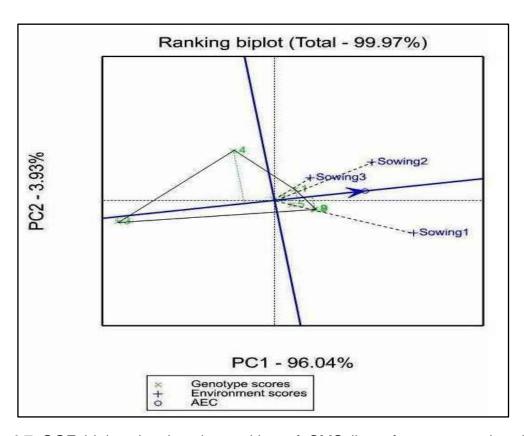
Table: 4.18 Details of genotype code, name, plant type and source of nine CMS lines of pigeonpea:

Genotype code	Genotype name	Plant type	Source
1	ICPA 2039	DT	ICRISAT, Patancheru
2	ICPA 2043	NDT	ICRISAT, Patancheru
3	ICPA 2047	NDT	ICRISAT, Patancheru
4	ICPA 2051	NDT	ICRISAT, Patancheru
5	ICPA 2092	NDT	ICRISAT, Patancheru
6	BRG3A	NDT	U.A.S., Bangalore
7	HyC3A	NDT	U.A.S., Bangalore
8	BRG1A	NDT	U.A.S., Bangalore
9	TTB7A	NDT	U.A.S., Bangalore

DT- Determinate, NDT- Non-determinate

4.2.3.2.1 Pollen sterility (%)

The ranking of nine CMS lines of pigeonpea based on their mean pollen sterility and stability performance has been shown in Fig. 4.7. The ideal genotypes are those with both high mean performance for pollen sterility and high stability. The first two principal components PC1 (96.04 %) and PC2 (3.93 %) explained total 99.97 % variation for pollen sterility. The CMS lines 6, 7, 8, and 9 observed very close to AEC with shortest vector from the AEA indicating these lines were highly stable for pollen sterility across the different sowings. The CMS lines 1, 5 and 2 performed high pollen sterility with shorter vector length from AEA indicating their stability for male sterility. The genotypes on the left side of the perpendicular line had pollen sterility below average mean. The genotype with below average performance with highest vector length from AEA was 4 whereas the genotype with lowest pollen sterility and shorter vector length from AEA stability was 3 (ICPA 2047) indicating these two CMS lines were unstable for pollen sterility. Breeders can also use GGE biplot (Fig. 4.7) for selecting the CMS lines with the best response to particular sowing. For instance, all the CMS lines had the highest performance for pollen sterility when they were sown in October (third sowing) whereas the CMS lines observed so far from first sowing indicating that the expression of male sterility of CMS lines was less in first sowing as compare to second and third sowings. All the three dates of sowing were positively correlated with each other because acute angles (<45°) were formed between them. The first sowing was most discriminating among all three sowings with longest environment vector while second and third sowings had high performing environment with shortest environment vectors and near to AEC. Meanwhile seven out of nine CMS lines were stable for expression of male pollen sterility and it was observed that there was significant influence of varying environments on pollen sterility.

Fig. 4.7 GGE biplot showing the ranking of CMS lines for mean and stability performance for pollen sterility (%) over the different dates of sowing.

Fully male fertile
(pollen fertility >80%)

Fig. 4.8 Microscopic view of pollen grains produced by fully male fertile plant

Partial male fertile
(pollen fertility 40 – 80 %)

Fig. 4.9 Microscopic view of pollen grains produced by partial male fertile plant

Partial male sterile
(pollen sterility 10-39 %)

Fig. 4.10 Microscopic view of pollen grains produced by partial male sterile plant

Complete male sterile
(pollen fertility <10%)

Fig. 4.11 Microscopic view of pollen grains produced by complete male sterile plant

B line   A line

Fig. 4.12 Morphology of anthers of male sterile line (right) and its fertile maintainer (left)

DISCUSSION

Chapter V
Discussion

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.

Cytoplasmic nuclear male sterility (CMS) system is ideal for commercial hybrid seed production of pigeonpea. The expression of CMS is controlled by genetic factors that are carried by the female parents and retained over generations. The male sterile (A) line with 'sterile' cytoplasm and homozygous recessive (*frfr*) nuclear genes is maintained by its counterpart male-fertile maintainer (B) line that carries a normal (fertile) cytoplasm, and the same homozygous recessive (*frfr*) nuclear genes. To produce male fertile hybrids, the A-line is crossed with a male fertility restorer (R) line, which carries normal cytoplasm and dominant nuclear alleles (*FrFr*) for fertility restoration. Hence, the hybrid breeding technology based on this system involves three parents; a male sterile (A) line, its maintainer (B) line and a fertility restorer (R) line.

As no CMS system could be found in pigeonpea germplasm, efforts were made to breed for this trait by placing pigeonpea genome into the cytoplasm of its related wild species. Using this mechanism Saxena *et al.* (2005) developed a stable cytoplasmic male sterility system in pigeonpea. It was developed by crossing *Cajanus cajanifolius* as female parent with a pigeonpea cv. 'ICP 28' as a male parent and designated as A_4 CMS system. The development of this stable CMS system in pigeonpea has opened new vistas for breeding commercial hybrids. Pigeonpea is the only food legume where commercial CMS-based

hybrids are now available (Saxena *et al.,* 2013 and Saxena *et al.,* Unpublished). This CMS system facilitates easy and cost effective seed production of pigeonpea hybrids and their parents because it can reduce production costs by eliminating extra labours. In order to take advantage of this CMS-based hybrid technology, it is essential to breed new high yielding hybrids and CMS lines with diverse genetic backgrounds and stable fertility restoration.

Therefore, present experiments were conducted to generate information on the stability of nine CMS lines for expression of male sterility and also to know the stability of fertility restoration in hybrids. In the present investigations, the major objective was to assess the performance of different hybrids for fertility restoration across the locations and CMS lines for expression of male sterility over the different dates of sowing. The interaction with environment and stability parameters for pollen fertility and sterility with other characters were estimated using Eberhart and Russell's (1966) and GGE biplot (Yan *et al.,* 2000) methods. The findings of present research are discussed here with appropriate sub headings.

5.1. Stability of hybrids for fertility restoration

Genotype × environment interaction is of major importance to the plant breeder in developing stable genotypes that will perform well under diverse environment conditions. If stability of performance or the ability to show a minimum interaction with the environment is a genetic characteristic, then preliminary evaluation could be planned to identify the stable genotypes. For development of high yielding CMS-based hybrids, it is essential to test new hybrids and CMS lines for their stability by growing them in number of environments.

5.1.1 Analysis of variance

The analysis of variance revealed that the genotypic differences among the entries were highly significant (P<0.01) for all the characters except for plant stand at all three locations i.e. Patancheru, Ranchi and Sehore. It indicating the presence of substantial genetic variation among the hybrids for pollen fertility and

other characters selected for the study. The pooled analysis of variance also revealed that the genotypic differences were highly significant for all traits except for plant stand whereas, the individual environment effects were highly significant for all characters except partial male sterile plants (%).

5.1.2 Mean performance of hybrids

Pollen fertility is an important character to assess the restoration of pollen fertility which is the basic need for the successful seed set in hybrids. The extent of pollen fertility among the hybrids ranged between 58.5 to 98.3 % across the locations. High values of pollen fertility indicates the higher fertility restoration and the vice versa. Among the hybrids, ICPH 2740 had highest pollen fertility at Patancheru, while ICPH 2671 recorded the maximum pollen fertility at the other two locations. All the hybrids except ICPH 3494 and ICPH 3491 exhibited high (>80 %) pollen fertility over the locations. Similarly, Wanjari *et al.* (2007) also reported high (>80 %) pollen fertility in different hybrid combinations. Among the hybrids tested, ICPH 2671, ICPH 2740, ICPH 3933, ICPH 2751 and ICPH 3461 have common male parent (ICPL 87119) but different female parents, and all these hybrids recorded >90 % pollen fertility across the locations. It indicated that the parent ICPL 87119 is a best fertility restorer and it can be utilized in further hybrid breeding programmes. Hybrids ICPH 3491 (ICPA 2048 × ICPL 20096) and ICPH 3494 (ICPA 2048 × ICPL 20093) have same female parent but different male parents and they exhibited least pollen fertility (50-60 %) across the locations. Similarly, Singh and Bajpai (2005), Saxena (2005) and Nadrajan *et al.* (2008) reported many hybrids with variable fertility restoration.

The pooled performances of hybrids for all the characters studied in the three environments are presented in Tables 4.3 to 4.6.

Among the hybrids, ICPH 2671, ICPH 2740 and ICPH 3933 produced 100 % fully male fertile plants followed by ICPH 3461 (99.9 %) and ICPH 2751 (98.7 %). This indicated that these hybrids expressed complete fertility restoration over the locations, whereas the minimum numbers of fully male fertile plants over the locations were recorded in ICPH 3494 and ICPH 3491, indicating its poor performance with partial fertility restoration. All the hybrids except ICPH 3494 and

ICPH 3491 exhibited high proportion of fully male fertile plants across the locations indicating their better fertility restoration. Similarly, Nadrajan *et al.* (2008) also reported the many hybrid combinations with better fertility restoration.

High proportion of partial male fertile plants in CMS-based hybrids indicates poor fertility restoration. In the present investigation two out of 10 hybrids (ICPH 3491 and ICPH 3494) recorded highest (15.8 %) proportion of partial male fertile plants indicating its poor fertility restoration. There were no partial male fertile plants in hybrids ICPH 2671, ICPH 2740 and ICPH 3933 indicating its perfect fertility restoration while other three hybrids ICPH 2751, ICPH 3477 and ICPH 3461 recorded very low proportions of partial male fertile plants indicating that these hybrids were highly fertile. Similar results were reported by Kalaimagal *et al.* (2008) and Masood Ali (2009).

The absence of partial male sterile plants is a desirable character for CMS-based hybrids of pigeonpea. The variability for partial male sterile plants recorded in different hybrids ranged between 0 to 15.9 % at Patancheru; 0 to 15.6 % at Ranchi; and 0 to 23.7 % at Sehore. Four hybrids ICPH 2671, ICPH 2740, ICPH 3933 and ICPH 3461 had no partial male sterile plants across the locations showed that these hybrids had superior fertility restoration. Another two hybrids, ICPH 2751 and ICPH 3477 recorded very little proportion of partial male sterile plants at Patancheru while they did not produce partial male sterile plants at Sehore and Ranchi. The maximum number of partial male sterile plants were recorded in ICPH 3491 followed by ICPH 3494. All the hybrids except ICPH 3494 and ICPH 3491 did not produce any significant number of partial male sterile plants indicating that the expression of fertility restoration was better across the locations.

The performance of different hybrids for complete male sterile plants over the locations ranged between 0 to 15.1 %. The highest proportion of complete male sterile plants was recorded in ICPH 3494 (15.1 %) followed by ICPH 3491 (8.9 %) indicating that these hybrids were unable to express complete fertility restoration while complete male sterile plants were not recorded in other hybrids.

Fertility restoration and its expression are prime requisites for development of CMS-based hybrid technology. The highest number of complete male sterile as well as partial male sterile plants were recorded in both the hybrids (ICPH 3494 and ICPH 3491) indicated that the fertility in these two hybrids was partially restored. All the hybrids except ICPH 3494 and ICPH 3491 did not produce complete male sterile plants across the locations indicating their stability for fertility restoration. Saxena *et al.* (2004) also reported many CMS-based cross combinations with partial fertility restoration.

5.1.3 Stability analysis of hybrids by Eberhart and Russell's (1966) model.

Information on genotype × environment interaction helps in the breeding of stable genotypes. Eberhart and Russell (1966) emphasized the need of considering both the linear (bi) and non-linear (S^2di) components of interaction in judging the stability of a genotype.

5.1.3.1 Analysis of variance for stability

The pooled analysis of variance for stability of pollen fertility and other characters showed significant differences among the genotypes under study (Table 4.7). The interaction between genotype and environment including linear was highly significant for all characters. Existence of real genotypic differences for regression over environmental means was revealed by highly significant variance due to environment (linear).

Genotype with unit regression coefficient and non-significant or least deviation from regression (S^2di) is considered as average stable one. When this is associated with high mean, genotypes have above average stability, but when the mean is low, genotypes are considered as poorly adapted. Regression values above unity (bi>1) reflect increasing sensitivity of the genotypes to environmental change (below average stability) and greater specificity of adaptation to favorable environments. Regression coefficient less than unity (bi<1) indicate greater resistance to environmental change (above average stability) and therefore, increasing specificity to poor environments.

The analysis of stability of hybrids revealed that the linear components of genotype × environment interaction (bi) as well as non-linear component (S^2di) for the characters studied were non-significant for most of the genotypes. The mean performances of the hybrids with stability parameters for different traits are presented in Table 4.8.

5.1.3.2 Stability of individual characters

5.1.3.2.1 Pollen fertility (%)

The most stable hybrids for pollen fertility across the locations were ICPH 2671, ICPH 2740, ICPH 3933 and ICPH 3461 with higher mean, non-significant regression coefficient (bi=1) and non-significant deviation from regression (S^2di=0). The hybrids ICPH 2751 and ICPH 3477 had regression coefficient greater than unity indicated their instability and these hybrids were more responsive to the favorable environments while ICPH 2740, ICPH 3933 and ICPH 3461 had regression coefficient less than unity (bi<1) indicated their above average stability and adaptation also in poor environmental conditions. Four out of 10 hybrids were stable over the environment with non-significant deviation from regression (S^2di=0), unit regression coefficient (bi=1) and performance above average mean indicating that there was no effect of environment on expression of fertility restoration in these hybrids (Table 5.1). Similar findings were earlier reported by Dalvi *et al*. (2008a) where, there was no effect of environment on expression of fertility restoration.

Table 5.1 Stable (S) and unstable (US) hybrids over the locations for pollen fertility (%) and other characters by Eberhart and Russell's model.

Genotype	Pollen fertility (%)	Fully male fertile plants (%)	Partial male fertile plants (%)	Complete male sterile plants (%)
ICPH 2671	S	S	S	S
ICPH 2740	S	S	S	S
ICPH 3933	S	S	S	S
ICPH 2751	US	US	US	S
ICPH 3477	US	US	S	US
ICPH 3461	S	S	S	S
ICPH 3762	US	US	US	S
ICPH 4490	US	US	US	S
ICPH 3491	US	US	US	US
ICPH 3494	US	US	US	US
Asha (check)	S	S	S	S
Maruti (check)	S	S	S	S

5.1.3.2.2 Fully male fertile plants (%)

The most stable hybrids with high mean, unit regression coefficient and non-significant deviation from regression line for fully male fertile plants were ICPH 2671, ICPH 2740, ICPH 3933 and ICPH 3461. Two hybrids ICPH 2751 and ICPH 3477 had significant regression coefficient more than unity (bi>1) and significant deviation from regression (S^2di>0) indicating that these hybrids were unstable across the locations and positive interaction with favorable environments. Eight out of 10 hybrids performed better for fully male fertile plants across the locations and four of them were showed stability for fertility restoration across the locations (Table 5.1). Saxena *et al.* (2011) also reported many hybrid combinations with stable fertility restoration.

5.1.3.2.3 Partial male fertile plants (%)

The hybrids ICPH 2671, ICPH 2740 and ICPH 3933 exhibited above average stability (no partial male fertile plants, bi<1 and S^2di=0) for partial male fertile plants. The other two hybrids ICPH 3461 and ICPH 3477 had performance lower than mean, non-significant regression coefficient and deviation from

regression indicating these hybrids were also stable for partial male fertile plants. The hybrid ICPH 3762 and ICPH 2751 performed above average mean with greater regression coefficient (bi>1) and non-significant deviation from regression (S^2di=0) indicating their instability over the locations and more responsive to the favorable environments. The hybrids ICPH 3494 and ICPH 3491 exhibited highest number of partial male fertile plants with minimum pollen fertility across the location indicating that these hybrids were unstable with partial fertility restoration (Table 5.1).

According to Saxena *et al.* (2011b) the fertility restoration in A_4 CMS lines of pigeonpea is controlled by either one or two dominant fertility restoring genes. The visual differences for pollen load and fertility among the hybrid plants is linked to the number of fertility-restoring genes present in an individual. The hybrid plants with both the fertility-restoring genes, the pollen load in the floral buds was similar to that of pure-line cultivars. On the contrary, when a single fertility restoring gene was present in the hybrids, they produced relatively fewer pollen grains and their fertility restoration was unstable in diverse environments. In this context, it is concluded that eight hybrids showed high pollen fertility and stability for fertility restoration at three different locations may have two dominant fertility-restoring genes while other two hybrids ICPH 3494 and ICPH 3491 produced lowest pollen fertility with unstable partial fertility restoration may have single fertility-restoring gene (Dalvi *et al.*, 2008a). Similarly, Bodden (1977) also identified certain wheat (*Triticum aestivum* L.) lines with poor pollen production, and they concluded that the restorer parents with a single gene were responsible for their poor pollinating capacity. Tang *et al.* (2007) also observed partially fertile plants in sorghum [*Sorghum bicolor* (L.) Moench] population that segregated for fertility-restoring alleles and they reported that full pollen fertility in a genotype essentially results from the presence of all the major and minor genes simultaneously. The previous studies on genetics of fertility restoration in pigeonpea suggested that hybrids with two dominant genes produced a greater pollen load and expressed greater stability as compared with those carrying a single dominant gene and it was concluded that for breeding hybrids with stable

fertility restoration, the presence of two dominant genes is essential (Saxena *et al.,* 2011b; Dalvi 2008a).

5.1.3.2.4 Complete male sterile plants (%)

All the hybrids except ICPH 3494, ICPH 3491 and ICPH 3477 exhibited absence of complete male sterile plants across the environments with regression coefficient less than unity (bi<1) and non-significant deviation from regression indicating their above average stability for complete male sterile plants. It also indicates that the fertility restoration in these hybrids was better over the locations. The hybrid ICPH 3477 performed above mean for complete male sterile plants with regression coefficient greater than unity (bi>1) and significant deviation from regression (S^2di>0) indicating its instability and more responsive to favorable environmental conditions. Two hybrids ICPH 3494 and ICPH 3491 recorded higher proportion of complete male sterile plants with significant regression coefficient (bi>1), indicating that these hybrids were also unstable for complete male sterile plants with partial fertility restoration. As the possibility of two or more genes for fertility restoration, the male parents of both the hybrids may not be having all the genes which are responsible for restoration of pollen fertility and this led to the expression of partial fertility restoration in these hybrids. Plant to plant crosses accompanied with selection may be helpful to identify the perfect restorer from segregating lines (Dalvi *et al.,* 2008a).

5.1.4 Stability analysis by GGE biplot method

According to GGE interpretation, an ideal test environment should be both discriminating and representative. An 'ideal' environment probably does not exist in reality but can be used as a reference point. From this study, it can be seen that Ranchi is the closest to the ideal environment, and therefore, is the most desirable among the three environments studied. The GGE biplot analysis is more efficient than the Eberhart and Russell's method because GGE biplot explains higher proportions of the sum of squares of the G × E interaction and is more informative with regard to environments and cultivar performance.

5.1.4.1 Pollen fertility (%)

The partitioning of GE interaction through GGE biplot analysis showed that both the principal components explained a total of 99.89 % variation. The hybrids ICPH 2671 (1), ICPH 2740 (2), ICPH 3933 (3) and ICPH 3461 (6) showed both high mean and stability for pollen fertility across the locations. The genotype Maruti (12) was near to concentric circle showing their higher performance for pollen fertility among all the genotypes hence it was an ideal genotype. Four out of 10 hybrids were stable over the locations (Fig. 4.2). The acute angles ($<90^0$) between environment vectors showed that all the three environments (Patancheru, Ranchi and Sehore) were positively correlated with each other. Sawargaonkar *et al.* (2011) were earlier used the GGE biplot method to estimate the stability of hybrids for seed yield and related traits.

5.1.4.2 Fully male fertile plants (%)

The results of PCA of GEI in biplot showed that the first two principal components in the biplot explained 99.39 % (PC1= 95.89 % and PC2= 3.50 %) of the total variation. The hybrids ICPH 2671 (1), ICPH 2740 (2), ICPH 3933 (3) and ICPH 3461 (6) had maximum proportion of fully male fertile plants with shortest vector length from AEA indicating their higher stability for fertility restoration. All the three environments were found to be positively correlated with smaller angle ($<45^0$) between them. The environments of Sehore and Ranchi were most discriminating along with smaller angle with the AEA and the genotypes nearer to these two environments exhibited higher stability for fully male fertile plants at these two locations.

5.1.4.3 Partial male fertile plants (%)

The hybrids ICPH 2671 (1), ICPH 2740 (2), ICPH 3933 (3) and ICPH 3461 (6) had no partial male fertile plants in all environments with shortest vector length from AEA indicating their higher stability for partial male fertile plants and expression of fertility restoration followed by ICPH 3477 (5). Five out of 10 hybrids were stable with absence or little proportion of partial male fertile plants and shorter vector length from AEA. The hybrid ICPH 3762 (7) had the longest vector length and near to Patancheru location indicating its instability and more

responsive at Patancheru as compared to Ranchi and Sehore. The results revealed that the genotypes which are positioned left side of perpendicular line in Fig 4.4 were the best with higher stability.

5.1.4.4 Complete male sterile plants (%)

The results showed that all the hybrids except ICPH 3491 (9), ICPH 3494 (10) and ICPH 3477 (5) observed absence or least proportion of complete male sterile plants with shorter vector length from AEA indicating that these hybrids were highly stable and better for fertility restoration over the locations. The hybrids ICPH 3494 (10) observed highest proportion of complete male sterile plants followed by ICPH 3491 (9) with longest vector length from AEA indicated that this hybrids were unstable with poor fertility restoration. The results obtained from both the methods (GGE biplot and Eberhart and Russell's) were similar for all the characters but the GGE biplot analysis provided additional information's related to correlation between environments and genotypes, and information related to 'which won where' is helpful for breeder to select the stable genotypes for specific location (Gauch and Zobel, 1997; Yan *et al*., 2000).

5.2 Stability of CMS lines

Development of cytoplasmic nuclear male sterile lines at cellular level

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 microsporogenesis 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).

Cytoplasmic male-sterility system has played an important role in exploiting hybrid vigor for enhancing productivity in field crops as well as in horticultural crops (Saxena, 2005). There are eight CMS systems developed so far in pigeonpea, the A_4 system derived by crossing of *Cajanus cajanifolius*, a wild relative of pigeonpea and a cultivar (Saxena *et al.*, 2005) is the best because it is stable across environments and has a large number of fertility restorers (Saxena *et al.*, 2006). According to De (1974), *C. cajanifolius* resembles cultivated types in most morphological traits. Therefore, the male sterile lines derived from A_4 cytoplasm are the best among those identified so far and found to be highly stable male sterile lines across environments and years (Saxena *et al.,* 2005; Dalvi *et al.,* 2008a; Saxena 2008; and Sawargaonkar 2010) and never showed any morphological deformity. In order to take advantage of this CMS hybrid technology, it is essential to breed promising hybrid parents and information related to their stability.

The environmental influences on male-sterility were noted for several plant species. Pigeonpea is a short day plant with the late maturing type's having a strict day-length requirement for induction of flowering. The phenological responses in pigeonpea are influenced by photoperiod and temperature that have played a major role in evolution of the various crop production systems that have been established. The photoperiod sensitive reaction in pigeonpea germplasm is not only linked to days to flowering but also to the amount of pollen grains produced (Wallis *et al.,* 1981). The objective of this study was to determine the influence of temperature in different months on the expression of male sterility of CMS lines by growing them in different dates of sowing. In present experiment

nine male sterile lines were planted during 2012 in three different dates at International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh.

5.2.1 Analysis of variance

The genotypic differences were significant ($P<0.05$) for pollen sterility (%) in all three dates of sowings whereas for complete male sterile plants (%) significant genotypic differences observed in first and second dates of sowing and the non-significant genotypic difference found in third date of sowing. The result of pooled analysis of variance also revealed that genotypic differences were found to be significant for all traits.

5.2.2 Mean performance of CMS lines

The CMS lines BRG3A, HyC3A, BRG1A and TTB7A recorded 100 % pollen sterility indicate that these lines were performed superior and unable to produce fertile pollen grains in all three different dates of sowing. Among the A-lines of ICRISAT, ICPA 2039 (99.8 %), ICPA 2092 (99.5 %) and ICPA 2043 (99.1 %) recorded highest pollen sterility over the different dates of sowing. All the CMS lines performed well with high (>95 %) pollen sterility across the different dates of sowing. Similar results were earlier reported by Dalvi (2007), Sawargaonkar *et al.* (2012a) and Makelo *et al.* (2013) in CMS lines of pigeonpea.

The proportion of complete sterile plants produced by different CMS lines over the different dates of sowing ranged from 94.9 to 100 %. All the CMS lines except ICPA 2047 and ICPA 2043 observed 100 % complete male sterile plants, exhibited their complete and stable expression of male-sterility in all three dates of sowing. The CMS line ICPA 2043recorded 99.8 % complete male sterile plants indicating that it also expressed complete male sterility. Sawargaonkar *et al.* (2012a) and Makelo *et al.* (2013) also reported the similar results in their studies where the CMS lines were stable for expression of male sterility.

All the CMS lines except ICPA 2043 (2.1 %) and ICPA 2047 (4.2 %) had no partial male sterile plants in first sowing, whereas in second sowing the partial male sterile plants observed only in ICPA 2047 and all other CMS lines were not

produced partial male sterile plants. All the CMS lines did not produce partial male sterile plants in the third sowing. Meanwhile, all the CMS lines except ICPA 2047 (2.1 %) were recorded as no partial male sterile plants across the different sowings. The cause of such partial male sterile plants in CMS lines was reported by Saxena *et al.* (1981). According to them, the irregularities in breakdown of the tapetum with an inconsistent extent is the prime cause of production of partial male fertile/sterile plants in CMS lines and the site of degeneration of the tapetal tissues in different flowers and plants. The microsporogenesis of the male sterile plants was not examined in this study, but it was likely that both pollen development and its release processes would be defective.

The CMS line ICPA 2047 was only observed to produce partial male fertile plants in second sowing, while all other CMS lines had no partial male fertile plants across the dates of sowing indicating that these lines exhibited stable male-sterility. The CMS line ICPA 2047 produced 0.7 % partial male fertile plants across the three sowings which was not significant and considerable amount of partial male fertile plants showed that it was also stable for expression of male sterility.

All the CMS lines except ICPA 2047 were unable to produce fully male fertile plants in all the three dates of sowing indicating that all these lines were stable for expression of male sterility. The CMS line ICPA 2047 recorded significantly inconsiderable proportion of fully male fertile plants showed that this line was also stable for male sterility. Individual plant to plant crosses may be helpful to identify the perfect maintainer. All the nine male sterile lines derived from A_4 cytoplasm exhibited stability throughout the different dates of showing without any effect of increase or decrease in temperature, indicating male sterility in A_4 system was independent of environment condition. Saxena *et al*. (2005) also found that the CMS lines derived from A_4 cytoplasm were stable when tested under varying environmental conditions at Patancheru.

5.2.3 Stability of CMS line by Eberhart and Russell's (1966) method.

As the expression of CMS requires two different genetic factors, one of each in cytoplasm and nucleus, to come together in a single cell; the frequency of spontaneously occurring mutants simultaneously in both the entities (i.e., nucleus and cytoplasm) is quite low. The influence of environment (temperature and/or photoperiod) on CMS controlling nuclear *fr* and *Fr* genes is more prominent. This may lead to instability of the expression of male sterility and its fertility restoration. Such unstable expressions are also some times influenced by the genetic background of an individual. Commercial use of CMS requires highly stable male sterility; to ensure genetically pure F_1 hybrid seed, therefore it is essential to have more precise information on the effect of temperature on male sterility in order to utilize A_4 CMS system in production of commercial hybrids. The objective of this study was to determine the influence of different dates of sowing on the expression of male sterility of different CMS lines.

5.2.3.1 Analysis of variance

Analysis of variance for stability revealed the existence of substantial variability among the genotypes for pollen sterility (%). Significance of genotype × environmental interaction revealed that genotypes interacted significantly with environments (different dates of sowing). The partitioning of interaction showed that both the linear components (environment and genotype × environment) were highly significant for pollen sterility. It was seen that the individual environment effects and interactions were highly significant for pollen sterility (%) hence the stability analysis was carried out for pollen sterility.

5.2.3.2 Stability of individual characters

5.2.3.2.1 Pollen sterility (%)

The non-significant deviation from regression line ($S^2di=0$) for pollen sterility (%) was observed for all the CMS lines indicating their stability across the different dates of sowing while the linear regression was observed significant either greater or less than unity for all CMS lines except ICPA 2043 and ICPA

2092. The CMS lines BRG3A, HyC3A, BRG1A and TTB7A exhibited 100 % pollen sterility with significant regression coefficient less than unity (bi<1) and non-significant ($S^2di=0$) deviation from regression indicating their above average stability and these lines can also perform in poor environmental conditions. The lines ICPA 2047 and ICPA 2051 had regression coefficient more than unity (bi>1) with non-significant deviation from regression line ($S^2di=0$) indicate that these CMS lines were unstable and more responsive to the favorable environment. Seven out of nine CMS lines were highly stable across the different sowings with non-significant deviation from regression line ($S^2di=0$), unit regression coefficient (bi=1) and performance above or close to average mean (Table 5.2). Similarly, Sawargaonkar *et al.* (2012a) reported the stability of CMS lines for expression of male sterility under different month temperature. Makelo *et al.* (2013) also reported the similar results for stability of CMS lines under different environmental conditions. Zhang *et al.* (2007) reported the stability of CMS lines of wheat in different dates of sowing and Goral *et al.* (2006) reported the stability of CMS lines of wheat using method of stability analysis based on regression coefficient.

Table 5.2 Stable (S) and unstable (US) CMS lines over the different dates of sowing for pollen sterility (%) by Eberhart and Russell's model.

Genotype	Pollen sterility (%)
ICPA 2039	S
ICPA 2043	S
ICPA 2047	US
ICPA 2051	US
ICPA 2092	S
BRG1A	S
HyC3A	S
BRG3A	S
TTB7A	S

5.2.4 Stability of CMS lines by GGE Biplot method (Yan *et al.,* 2000)

4.2.4.1 Pollen sterility (%)

The CMS lines BRG3A (6), HyC3A (7), BRG1A (8), and TTB7A (9) were observed very close to AEC with shortest vector from the AEA indicates that these lines were highly stable. Among the A-lines of ICRISAT, ICPA 2039 (1), ICPA 2092 (5) and ICPA 2043 (2) recorded high pollen sterility from average mean with shorter vector length from AEA indicating these lines were also stable. Another CMS lines ICPA 2051 (4) performed below the average mean with greater vector length from AEA indicating its instability for pollen sterility over the different dates of sowing. All the CMS lines had highest pollen sterility when they were sown in October month (third sowing) whereas the CMS lines were observed so far from first sowing indicating that the expression of male sterility in CMS lines was less in first sowing as compared to second and third sowings. It also indicates the influence of temperature on expression of male sterility. The CMS lines of first sowing were flowered on last week of November to first week of December when the temperature was lower by 12^0 C under shorter day length which induced production of fertile pollens in some CMS lines. Saxena (2009) reported the similar result where shortening of day lengths and reduction in temperature induced male fertility, while high temperature and longer days maintained male sterility.

5.3 Implications of present study in hybrid breeding

Stability analysis of hybrids for fertility restoration and CMS lines for expression of male sterility provides guideline for use of different restorer's and CMS lines in development of new commercially adopted hybrids for enhancement of yield. The promising stable hybrids ICPH 2671, ICPH 2740, ICPH 3933, ICPH 3461, and ICPH 2751 recorded high degree of fertility restoration. This is due to the presence of a common male parent ICPL 87119 in them having all the genes responsible for fertility restoration. Thus, it may be crossed with another CMS lines to breed new high yielding hybrids. These hybrids must be tested over the locations to confirm the stability for yield performance also. Following are the breeding implications of present study:

1. For pollen fertility and the proportion of fully male fertile plants, the hybrids ICPH 2671, ICPH 2740, ICPH 3933, ICPH 2751, ICPH 3461 and ICPH 3762 were superior over the locations. The hybrids ICPH 2671 and ICPH 2740 exhibited superiority with stable yield performance have already been released for commercial cultivation. The other promising hybrids for fertility restoration may also be released with further heterosis and stability studies for seed yield and related traits.

2. The hybrids showing stability for fertility restoration need to be tested for yield across more diverse environments.

3. The data on expression of male sterility of CMS lines may be used for diversification of CMS lines and for the development of new heterotic cross combinations.

4. The hybrids showing segregation for fertility restoration may be improved further with plant-to-plant crosses to select the perfect maintainer and restorer genotypes.

5. To confirm the restoration of a genotype for the particular cytoplasm, isogenic male-sterile lines may be used.

6. The results of stability of male sterility of CMS lines will help in further studies on combining ability with diverse restorer lines.

7. 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.

8. 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.

SUMMARY, CONCLUSION & SUGGESTIONS FOR FURTHER WORK

Chapter-VI
Summary, conclusion and suggestions for further work

The present investigation entitled "Stability of maintenance of male sterility and fertility restoration in pigeonpea (*Cajanus cajan* (L.) Millsp.) under different environments" was carried out to evaluate information on stability of cytoplasmic male sterile lines for expression of male sterility and also it was aimed to study the stability of fertility restoration in hybrids developed at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru (Andhra Pradesh). To study the stability of fertility restoration, a set of 10 hybrids and two standard varietal checks obtained from ICRISAT, Patancheru were evaluated at three different locations *viz.* ICRISAT, Patancheru (Andhra Pradesh) (17º53'N, 78º27'E), Birsa Agriculture University, Ranchi (Jharkhand) (23º17'N, 85º19'E) and College of Agriculture, Sehore (Madhya Pradesh) (23º12'N, 77º05'E). All these materials were evaluated in Randomized Complete Block Design (RCBD) with two replications during *kharif* 2012. Six row plots were planted with inter and intra row spacing of 75 and 50 cm respectively. Nine CMS lines derived from *Cajanus cajanifolius* (A_4) cytoplasm were also planted at ICRISAT in Randomized Complete Block Design (RCBD) with two replications in 2012 to study their stability for expression of male sterility in varying environmental conditions. To provide the varying environmental conditions, the CMS lines were planted under three different dates *viz.* August 7, 2012, September 11, 2012 and October 18, 2012 named as first, second and third dates of sowing respectively. Five out nine CMS lines were obtained from ICRISAT; and other four CMS lines were obtained from University of Agriculture Science, Bangalore, Karnataka. Observations were recorded from all the plants at the initial flowering stage on pollen fertility (%), fully male fertile plants (%), partial male fertile plants (%), partial male sterile plants (%), complete male sterile plants (%) and plant stand. Two most reliable methods of stability analysis, one is Eberhart and Russell's (1966) model based on regression coefficient and another graphical presentation of stability of genotypes called as GGE biplot method (Yan *et al.,* 2000) were used to estimate the stability of hybrids and CMS lines. The highlights of the results are summarized below.

A) *Per se* performance of hybrids

1. The hybrids ICPH 2671 and 2740 had highest pollen fertility across the locations indicating these hybrids were best among all hybrids.

2. Eight out of 10 hybrids recorded high (>80 %) pollen fertility showed their better fertility restoration across the locations.

3. The hybrids ICPH 3494 and ICPH 3491 exhibited lower pollen fertility over the locations with minimum proportion of fully male fertile plants indicating their poor fertility restoration.

4. The hybrids ICPH 2671, ICPH 2740 and ICPH 3933 had 100 % fully male fertile plants across the locations, whereas other three hybrids ICPH 2751, ICPH 3477 and ICPH 3461 performed more than 98 % fully male fertile plants showed their complete fertility restoration. These hybrids had no partial male fertile, partial male sterile or complete male sterile plants over the locations.

5. The hybrids ICPH 3491 and ICPH 3494 had highest proportion of partial male fertile, partial male sterile and complete male sterile plants at all three locations showed that these hybrids had poor fertility restoration.

B) *Per se* performance of CMS lines

1. The CMS lines BRG3A, HyC3A, BRG1A and TTB7A had 100 % pollen sterility in all three different sowings showed that these lines were unable to produce fertile pollens in all three dates of sowing.

2. Four out of five CMS lines of ICRISAT showed more than 99 % pollen sterility across the different dates of sowing. Meanwhile, all the CMS lines expressed high (>95%) pollen sterility over the different sowings.

3. Eight out of nine CMS lines had 100 % complete male sterile plants in all three dates of sowing and unable to produce partial male sterile, partial male fertile and fully male fertile plants.

C) Stability of hybrids for fertility restoration

i) Stability analysis by Eberhart and Russell's method.

1. The hybrids ICPH 2671, ICPH 2740, ICPH 3933 and ICPH 3461 showed high mean for pollen fertility with regression coefficient ($b_i=1$) and deviation from regression line ($S^2d_i=0$), which indicates their higher stability for pollen fertility under different environmental conditions.

2. Four out 10 hybrids, ICPH 2671, ICPH 2740, ICPH 3933 and ICPH 3461 recorded 100 % fully male fertile plants with unit regression coefficient and non-significant deviation from regression ($S^2di=0$) indicating their above average stability for fertility restoration.

3. The highly stable hybrids which had no partial male fertile, partial male sterile and complete male sterile plants with regression coefficient below unity (bi<1) and zero deviation from regression line were ICPH 2671, ICPH 2740, ICPH 3933 and ICPH 3461.

4. The results of the present study indicated that the hybrids ICPH 2671, ICPH 2740, ICPH 3933, ICPH 3461, ICPH 2751, ICPH 3762 and ICPH 4490 observed to have better fertility restoration. Four hybrids identified stable for fertility restoration can be used for commercial cultivation with yield superiority in future.

ii) Stability analysis by GGE biplot method

1. The hybrids ICPH 2671 (1), ICPH 2740 (2), ICPH 3933 (3) and ICPH 3461 (6) showed both high mean and stability for pollen fertility across the locations and the other hybrids were unstable for pollen fertility with low performance over the locations or greater vector length from AEA.

2. The hybrids ICPH 2671 (1), ICPH 2740 (2), ICPH 3933 (3) and ICPH 3461 (6) had maximum proportion of fully male fertile plants with shortest vector length from AEA indicating their high stability for fully male fertile plants and exhibited complete fertility restoration over the locations.

3. The most stable hybrids for fertility restoration were ICPH 2671 (1), ICPH 2740 (2), ICPH 3933 (3) and ICPH 3461 (6) which exhibited no partial male fertile as well as complete male sterile plants with shortest vector length from AEA.

4. The most unstable hybrids for fertility restoration were ICPH 3494 (10) and ICPH 3491 (9) with lowest pollen fertility and fully male fertile plants with highest proportion of partial male fertile, partial male sterile and complete male sterile plants over the locations with longest vector length from AEA and farthest from AEC.

D) Stability of CMS lines

i) Stability of CMS lines by Eberhart and Russell's model

1. The CMS lines ICPA 2043, ICPA 2039, ICPA 2092, BRG1A, BRG3A, HyC3A and TTB7A were highly stable across the different dates of sowing with more than 99 % pollen sterility, significant regression coefficient less than unity (bi<0) and non-significant deviation from regression ($S^2di=0$).

2. Seven out of nine CMS lines were stable for pollen sterility and can be effectively utilized in the development of high yielding hybrids.

3. The non-significant G × E interaction was found for complete male sterile plants (%) showed that all the CMS lines were stable for expression of male sterility.

ii) Stability of hybrids by GGE biplot method

1. Seven out of nine CMS lines were highly stable with the shorter vector length from AEA and near to AEC.

2. All CMS lines performed well in all three different dates of sowing and expressed complete male sterility. Comparatively all the CMS lines were expressed highest sterility in third sowing as compared to first and second sowings. The result from GGE biplot analysis showed that all three sowing dates were positively correlated because acute angle ($<45^0$) is formed between them.

Conclusion

Stability analysis of hybrids and CMS lines showed that there was no effect of environment on both expression of male sterility and fertility restoration. There was significant genotypic differences were observed for all characters. The significant genotype x environmental interaction was observed for four characters of hybrids and it was observed significant only for pollen sterility (%) in CMS lines. Analysis of variance for stability of hybrids and CMS lines revealed that both the linear component of variation were significant for all the characters.

The results revealed that GEI was an important source of variation for pollen fertility and sterility. The results obtained from both the methods of stability analysis showed that GGE biplot analysis is more efficient than Eberhart and Russell's method of

stability analysis because its biplots were effective enough for visualizing the response patterns of genotypes and environments. GGE Biplot method is adequate to explain the genotype × environment interactions, providing results that are consistent with the classic methods based on regression coefficient. The discriminating power × representativeness view of GGE biplot was effective in evaluating test environment, which is not possible with Eberhart and Russell's model of stability analysis. The results obtained from present investigations concluded that significant variability for pollen fertility and sterility was present among the hybrids and CMS lines. Four out of 10 hybrids performed better pollen fertility (%) and fully male fertile plants across the locations showed their stability for fertility restoration and there was no effect of environment on expression of fertility restoration in these hybrids. All CMS lines were highly stable for expression of male sterility across the different sowings and it was concluded that there was no effect of different sowing dates and temperature on expression of male sterility of these CMS lines.

Suggestions for further work

Information on stability of male sterility of CMS lines and fertility restoration in hybrids is very important and helpful in planning of future breeding programmes. On the basis of present investigation, breeding repercussions and suggestions have been made for further works are given below:

- ➢ A stable hybrid for expression of fertility restoration across the environments stable for seed yield and vice versa. So these stable hybrids can be tested for further yield performance and promising ones can be released.
- ➢ A stable male sterile line when crossed with stable male parent produced stable hybrids and vice versa. So more importance have to be given to these stable CMS lines of pigeonpea in hybrid breeding programme to develop stable high yielding hybrids under marginal environment conditions.
- ➢ The information generated on stability of fertility restoration will help to know the selection of parents which can be utilized in further transfer of fertility restorer genes into elite backgrounds.
- ➢ Stability of fertility restoration revealed that the environmental influence was cross-specific and depends on the nuclear background of CMS line and fertility

restorer. The data on fertility restoration may be used for diversification of CMS lines.

➢ As the hybrids belong to male parent ICPL 87119 showed the highest pollen fertility and complete fertility restoration. Therefore it can be exploited in further hybrid breeding programmes.

➢ The stability of CMS lines should be evaluated on different locations for expression of male sterility to ensure their stability on various locations also.

➢ The genetic purity of parental lines used in hybrid breeding programme should be increased to restore complete fertility.

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APPENDICES

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

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

Date dd/mm/yyyy	Rain fall (mm)	Minimum Temperature (ºC)	Maximum Temperature (ºC)	Rel. Humidity at 07:17 (%)	Rel. Humidity at 14:17 (%)
1-Jul-2012	0.0	22.6	32.5	51	87
2-Jul-2012	0.0	22.6	29.8	69	87
3-Jul-2012	0.0	22.2	29.5	64	83
4-Jul-2012	0.0	22.3	30.2	64	81
5-Jul-2012	0.0	23.6	31.8	53	77
6-Jul-2012	0.0	23.8	32.7	53	76
7-Jul-2012	13.0	20.8	30.4	59	90
8-Jul-2012	0.0	22.7	30.7	66	84
9-Jul-2012	0.2	22.0	32.6	55	92
10-Jul-2012	0.4	21.7	31.2	55	93
11-Jul-2012	0.0	22.8	33.2	50	85
12-Jul-2012	0.0	22.5	33.7	50	84
13-Jul-2012	0.0	23.8	33.6	51	83
14-Jul-2012	30.2	21.0	32.7	63	98
15-Jul-2012	11.5	21.5	29.0	71	98
16-Jul-2012	16.9	21.6	28.4	74	97
17-Jul-2012	3.6	22.0	27.5	81	92
18-Jul-2012	5.6	21.9	31.3	67	93
19-Jul-2012	10.8	20.8	29.2	71	91
20-Jul-2012	0.6	22.0	27.4	77	91
21-Jul-2012	71.0	20.8	28.0	78	98
22-Jul-2012	1.8	21.6	26.0	90	92
23-Jul-2012	0.0	21.8	27.8	78	85
24-Jul-2012	0.0	22.0	31.3	61	91
25-Jul-2012	0.0	22.4	30.4	67	87
26-Jul-2012	19.0	21.0	28.8	77	91
27-Jul-2012	0.0	22.4	27.6	73	87
28-Jul-2012	0.0	22.0	27.7	73	90
29-Jul-2012	14.2	21.5	30.8	61	88
30-Jul-2012	0.4	21.8	29.8	64	90
31-Jul-2012	0.0	22.0	29.2	66	85
1-Aug-2012	4.8	20.9	28.2	68	91
2-Aug-2012	0.4	21.0	29.9	62	91
3-Aug-2012	5.4	20.8	29.2	69	91
4-Aug-2012	1.0	21.8	27.5	92	91
5-Aug-2012	0.0	22.0	28.0	76	88

6-Aug-2012	0.8	22.4	29.4	75	88
7-Aug-2012	0.0	22.0	30.2	66	88
8-Aug-2012	1.5	21.9	31.2	60	90
9-Aug-2012	1.4	21.5	31.3	61	88
10-Aug-2012	2.4	21.4	30.4	65	90
11-Aug-2012	1.0	22.0	30.1	65	90
12-Aug-2012	7.8	21.0	30.2	63	91
13-Aug-2012	2.6	21.2	29.6	65	91
14-Aug-2012	0.0	20.7	30.2	66	86
15-Aug-2012	0.0	21.4	30.5	58	86
16-Aug-2012	0.0	22.2	30.4	64	82
17-Aug-2012	0.0	22.2	30.0	58	91
18-Aug-2012	0.8	22.0	30.8	54	93
19-Aug-2012	1.0	22.0	30.2	60	83
20-Aug-2012	0.0	22.3	28.4	71	91
21-Aug-2012	0.0	22.9	31.3	62	85
22-Aug-2012	0.0	22.0	28.4	67	92
23-Aug-2012	0.0	22.3	30.8	55	87
24-Aug-2012	0.0	23.2	31.8	58	88
25-Aug-2012	0.0	22.5	28.8	73	92
26-Aug-2012	8.4	21.9	30.4	95	91
27-Aug-2012	22.6	21.0	27.2	74	98
28-Aug-2012	7.4	21.5	28.2	82	91
29-Aug-2012	22.8	21.5	29.8	77	91
30-Aug-2012	0.0	22.6	30.2	72	91
31-Aug-2012	2.6	22.5	28.0	80	92
1-Sep-2012	0.0	22.2	29.0	77	91
2-Sep-2012	0.0	22.7	29.4	71	90
3-Sep-2012	18.4	21.5	30.7	68	95
4-Sep-2012	9.0	21.9	26.8	84	95
5-Sep-2012	0.6	21.4	27.7	80	91
6-Sep-2012	0.0	22.5	28.5	75	87
7-Sep-2012	0.0	21.5	30.0	65	93
8-Sep-2012	0.0	22.2	29.9	62	88
9-Sep-2012	0.0	22.3	30.3	66	91
10-Sep-2012	0.0	22.5	29.2	69	91
11-Sep-2012	10.8	21.3	27.4	81	93
12-Sep-2012	2.4	21.9	29.2	69	95
13-Sep-2012	2.4	22.0	29.0	65	97
14-Sep-2012	0.0	21.2	30.0	61	91
15-Sep-2012	0.0	20.5	30.3	58	93
16-Sep-2012	0.0	21.5	29.6	63	86
17-Sep-2012	0.0	22.5	29.0	63	88
18-Sep-2012	0.0	22.1	28.1	72	91
19-Sep-2012	0.0	21.7	29.8	62	95

20-Sep-2012	0.0	22.2	30.5	66	95
21-Sep-2012	0.0	22.2	32.2	56	98
22-Sep-2012	6.6	21.7	30.2	70	95
23-Sep-2012	2.6	21.5	30.2	65	98
24-Sep-2012	0.0	21.0	29.2	69	89
25-Sep-2012	0.0	21.7	31.0	61	95
26-Sep-2012	0.0	20.6	30.5	62	98
27-Sep-2012	0.0	20.6	31.3	56	98
28-Sep-2012	0.0	20.9	31.5	55	93
29-Sep-2012	0.0	20.7	31.4	44	95
30-Sep-2012	5.6	21.6	31.3	56	97
1-Oct-2012	4.8	22.0	31.0	64	95
2-Oct-2012	29.4	21.5	29.2	71	98
3-Oct-2012	17.4	21.5	27.2	83	97
4-Oct-2012	21.2	20.5	28.0	75	98
5-Oct-2012	0.0	21.7	29.2	68	91
6-Oct-2012	0.2	22.3	30.7	71	98
7-Oct-2012	0.5	21.0	30.2	84	93
8-Oct-2012	0.0	20.0	31.2	58	96
9-Oct-2012	0.0	18.2	31.4	47	93
10-Oct-2012	0.0	16.5	30.8	50	94
11-Oct-2012	0.0	16.1	31.6	38	92
12-Oct-2012	0.0	15.3	31.2	37	90
13-Oct-2012	0.0	15.6	31.4	36	94
14-Oct-2012	0.0	14.6	31.0	38	92
15-Oct-2012	0.0	14.9	31.7	35	92
16-Oct-2012	0.0	15.0	31.8	30	90
17-Oct-2012	0.0	15.0	31.8	33	90
18-Oct-2012	0.0	14.7	31.8	36	94
19-Oct-2012	0.0	15.6	31.5	40	98
20-Oct-2012	0.0	19.6	30.7	44	87
21-Oct-2012	0.3	21.0	27.8	68	95
22-Oct-2012	0.0	19.9	29.8	59	86
23-Oct-2012	0.0	20.0	29.9	58	98
24-Oct-2012	0.0	19.0	30.3	52	96
25-Oct-2012	0.0	19.2	30.3	49	96
26-Oct-2012	0.0	19.0	31.2	41	96
27-Oct-2012	0.0	16.7	30.6	52	96
28-Oct-2012	0.0	16.9	30.3	43	94
29-Oct-2012	0.0	16.6	31.0	41	63
30-Oct-2012	0.0	14.3	29.8	32	86
31-Oct-2012	0.0	13.6	28.2	38	90
1-Nov-2012	0.4	21.0	29.8	47	84
2-Nov-2012	26.6	21.0	23.5	91	98
3-Nov-2012	11.0	21.0	24.0	95	96

4-Nov-2012	0.2	21.4	26.3	84	97
5-Nov-2012	0.0	18.0	28.8	65	98
6-Nov-2012	0.0	17.0	29.4	50	96
7-Nov-2012	0.0	17.9	30.0	49	98
8-Nov-2012	0.0	18.5	30.2	50	94
9-Nov-2012	0.0	18.5	30.2	48	86
10-Nov-2012	0.0	15.7	26.2	58	98
11-Nov-2012	0.0	15.4	29.2	45	96
12-Nov-2012	0.0	13.5	29.0	42	94
13-Nov-2012	0.0	12.7	28.7	57	93
14-Nov-2012	0.0	11.5	28.8	28	93
15-Nov-2012	0.0	11.3	28.8	51	93
16-Nov-2012	0.0	9.6	28.2	37	88
17-Nov-2012	0.0	8.1	26.8	35	95
18-Nov-2012	0.0	7.9	27.2	33	92
19-Nov-2012	0.0	8.9	27.3	34	95
20-Nov-2012	0.0	13.0	30.7	48	98
21-Nov-2012	0.0	14.9	29.8	37	96
22-Nov-2012	0.0	21.4	30.8	54	90
23-Nov-2012	0.0	18.6	29.2	57	98
24-Nov-2012	0.0	18.0	30.0	52	98
25-Nov-2012	0.0	17.5	29.8	52	98
26-Nov-2012	0.0	17.9	30.0	54	96
27-Nov-2012	0.0	17.0	30.4	48	98
28-Nov-2012	0.0	16.0	29.2	51	98
29-Nov-2012	0.0	15.3	29.6	44	94
30-Nov-2012	0.0	15.4	29.9	47	96
1-Dec-2012	0.0	14.9	29.8	42	96
2-Dec-2012	0.0	16.9	30.3	34	98
3-Dec-2012	0.0	12.5	29.7	42	98
4-Dec-2012	0.0	14.0	29.4	41	89
5-Dec-2012	0.0	16.4	29.0	47	96
6-Dec-2012	0.0	16.5	28.7	52	90
7-Dec-2012	0.0	15.5	30.5	55	92
8-Dec-2012	0.0	16.4	32.2	39	96
9-Dec-2012	0.0	15.4	31.6	41	92
10-Dec-2012	0.0	14.8	33.2	33	82
11-Dec-2012	0.0	16.3	33.2	31	70
12-Dec-2012	0.0	15.0	32.6	33	79
13-Dec-2012	0.0	14.7	32.4	35	94
14-Dec-2012	0.0	16.4	31.4	40	96
15-Dec-2012	0.0	15.0	30.8	33	94
16-Dec-2012	0.0	12.7	29.2	36	96
17-Dec-2012	0.0	13.5	29.2	39	96
18-Dec-2012	0.0	11.0	29.0	44	95

19-Dec-2012	0.0	10.5	28.9	32	95
20-Dec-2012	0.0	10.0	28.8	39	95
21-Dec-2012	0.0	11.4	28.9	36	98
22-Dec-2012	0.0	12.5	28.6	44	91
23-Dec-2012	0.0	11.5	29.2	44	91
24-Dec-2012	0.0	12.4	28.7	30	98
25-Dec-2012	0.0	12.2	27.8	45	98
26-Dec-2012	0.0	8.7	28.4	28	95
27-Dec-2012	0.0	8.5	28.7	23	90
28-Dec-2012	0.0	7.6	28.0	23	85
29-Dec-2012	0.0	10.9	29.4	38	91
30-Dec-2012	0.0	18.9	29.0	52	84
31-Dec-2012	0.0	21.0	28.8	62	85
1-Jan-2013	0.0	19.3	30.4	59	93
2-Jan-2013	0.0	19.4	32.0	56	96
3-Jan-2013	0.0	19.4	32.7	45	87
4-Jan-2013	0.0	18.4	33.2	42	91
5-Jan-2013	0.0	16.9	32.7	40	94
6-Jan-2013	0.0	16.5	33.5	32	94
7-Jan-2013	0.0	16.1	31.5	44	96
8-Jan-2013	0.0	18.5	31.2	43	91
9-Jan-2013	0.0	17.3	30.3	45	85
10-Jan-2013	0.0	12.9	28.8	51	89
11-Jan-2013	0.0	11.9	29.6	37	93
12-Jan-2013	0.0	9.5	28.9	29	88
13-Jan-2013	0.0	8.5	29.4	22	88
14-Jan-2013	0.0	9.9	31.5	28	83
15-Jan-2013	0.0	12.2	32.6	26	83
16-Jan-2013	0.0	13.3	32.0	25	96
17-Jan-2013	0.0	14.0	31.8	27	98
18-Jan-2013	0.0	12.3	30.1	33	98
19-Jan-2013	0.0	14.1	29.9	31	98
20-Jan-2013	0.0	13.2	29.5	38	93
21-Jan-2013	0.0	12.7	29.2	38	93
22-Jan-2013	0.0	17.7	29.7	38	94
23-Jan-2013	0.0	15.8	29.8	39	94
24-Jan-2013	0.0	17.3	29.3	40	90
25-Jan-2013	0.0	18.5	30.2	39	91
26-Jan-2013	0.0	17.4	29.8	40	92
27-Jan-2013	0.0	16.9	30.0	44	90
28-Jan-2013	0.0	17.8	30.6	41	92
29-Jan-2013	0.0	16.1	29.9	40	90
30-Jan-2013	1.0	17.9	30.4	42	94
31-Jan-2013	0.0	15.1	28.2	47	96

APPENDIX II

Weakly weather data during the crop season recorded at Ranchi, Jharkhand 2012-13.

Latitude: 23°17'N   Longitude: 85°19'E   Altitude: 625 m

Standard weak	Rain fall (mm)	Minimum Temperature (ºC)	Maximum Temperature (ºC)	Rel. Humidity at 07:17 (%)	Rel. Humidity at 14:17 (%)
27	43.8	20.1	32.5	59.9	84.1
28	33.6	20.5	29.9	69.6	85.3
29	89.2	20.4	28.6	67.4	82.9
30	65.5	20.3	28.8	71.9	82.1
31	125.1	21.1	29.3	71.9	81.1
32	76.8	19.9	28.0	71.6	81.3
33	169.2	20.4	28.8	71.4	83.3
34	4.5	20.7	30.5	71.5	82.0
35	4.2	21.1	31.2	71.2	82.0
36	92.1	20.0	28.3	71.7	82.6
37	62.9	21.7	31.1	72.3	82.7
38	75.1	21.3	30.3	72.4	82.3
39	0.0	19.3	29.9	72.4	83.6
40	0.0	19.1	31.1	68.4	83.0
41	14.3	17.2	28.2	71.1	82.4
42	0.0	15.1	28.1	70.7	81.1
43	0.0	17.5	29.5	72.3	83.6
44	16.5	11.7	25.0	71.7	83.9
45	22.5	12.8	26.0	70.7	82.0
46	0.0	7.4	24.2	70.6	84.4
47	0.0	9.8	24.9	70.0	84.6
48	0.0	6.5	24.0	71.0	85.1
49	0.0	6.8	24.5	71.7	83.7
50	17.4	11.9	19.6	73.6	83.3
51	0.0	6.3	22.9	72.1	83.1
52	0.0	1.8	19.5	70.0	82.3
1	0.0	6.8	22.9	71.6	83.3
2	0.0	2.5	19.7	72.1	83.4
3	0.0	9.7	27.0	59.9	85.1
4	0.0	4.4	21.9	70.4	82.9
5	0.0	5.7	22.8	67.3	83.3
6	2.2	9.2	24.6	62.6	82.6
7	14.0	10.3	23.2	66.3	82.6

APPENDIX III

Weakly weather data during the crop season recorded at Sehore, Madhya Pradesh, 2012-13.

Latitude: 23°12'N   Longitude: 77°05' E   Altitude: 499 m

Standard weak	Rain fall (mm)	Minimum Temperature (°C)	Maximum Temperature (°C)	Average Rel. Humidity (%)
26	9.0	27.6	35.5	46.2
27	50.0	26.4	33.1	68.6
28	93.0	25.5	31.0	71.5
29	5.0	25.1	31.1	79.9
30	101.0	22.5	25.4	92.7
31	99.0	19.6	26.8	88.8
32	166.0	20.7	26.0	91.3
33	140.0	20.7	22.1	89.8
34	87.0	25.0	29.4	91.2
35	53.0	22.1	28.4	88.4
36	98.0	21.8	28.4	85.9
37	32.0	22.6	28.3	85.3
38	0.0	21.7	29.7	79.4
39	3.0	20.8	30.7	71.4
40	0.0	17.9	32.5	56.2
41	0.0	17.2	32.6	51.2
42	0.0	16.8	31.1	48.3
43	0.0	15.7	31.6	46.3
44	0.0	15.1	28.2	46.6
45	0.0	15.3	30.5	50.0
46	0.0	14.4	29.7	50.8
47	0.0	13.3	28.4	51.9
48	0.0	13.6	26.5	55.5
49	0.0	11.9	27.8	53.6
50	0.0	27.2	26.7	56.0
51	0.0	7.4	24.6	59.1
52	0.0	6.6	23.2	51.4
1	0.0	6.9	22.0	59.5
2	0.0	6.3	23.3	51.0
3	0.0	10.1	23.9	58.6
4	0.5	6.1	20.9	52.9
5	0.0	10.2	25.3	54.0

VITA

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